HAL open science

Apport analytique de la spectrométrie de masse haute résolution en QTOF pour l'identification et la prise en charge de l'exposition aux substances psychoactives

Ruben Goncalves

To cite this version:

Ruben Goncalves. Apport analytique de la spectrométrie de masse haute résolution en QTOF pour l'identification et la prise en charge de l'exposition aux substances psychoactives. Médecine humaine et pathologie. Université de Bordeaux, 2022. Français. NNT : 2022BORD0260 . tel-03883356

HAL Id: tel-03883356

https://theses.hal.science/tel-03883356v1

Submitted on 3 Dec 2022

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

THÈSE PRÉSENTÉE

POUR OBTENIR LE GRADE DE

DOCTEUR DE

L'UNIVERSITÉ DE BORDEAUX

École doctorale Sociétés, Politique, Santé Publique

Pharmacologie, option Pharmaco-épidémiologie, Pharmacovigilance

Par Ruben GONCALVES

APPORT ANALYTIQUE DE LA SPECTROMETRIE DE MASSE HAUTE RÉSOLUTION EN QTOF POUR L'IDENTIFICATION ET LA PRISE EN CHARGE DE L'EXPOSITION AUX SUBSTANCES PSYCHOACTIVES

Sous la direction de : Mathieu MOLIMARD

Soutenue le 7 octobre 2022

Membres du jury :

M. SAINT-MARCOUX, Franck	PU-PH, Université de Limoges	Rapporteur
Mme MICALLEF-ROLL, Joëlle	PU-PH, Université Aix-Marseille	Rapporteur
Mme CANAL-RAFFIN, Mireille	MCU-PH, Université de Bordeaux	Examinateur
M. MOLIMARD, Mathieu	PU-PH, Université de Bordeaux	Directeur
M. AKNOUCHE, Fréderic	Pharmacien Biologiste, Synlab Nice	Membre invité

REMERCIEMENTS

Aux membres du jury,

Monsieur le Professeur Franck Saint-Marcoux

Madame le Professeur Joëlle Micallef-Roll

Madame le Docteur Mireille Canal-Raffin

Monsieur le Docteur Fréderic Aknouche

Merci de me faire l'honneur d'avoir accepté de juger ce travail de thèse. Veuillez trouver ici l'expression de ma gratitude et de mon profond respect

À mon directeur de thèse,

Monsieur le Professeur Mathieu Molimard

Merci d'avoir accepté de diriger ce travail, pour vos précieux enseignements et pour le temps que vous m'avez accordé.

Au Laboratoire de Pharmacologie et Toxicologie du CHU de Bordeaux,

Merci pour votre disponibilité, vos conseils, vos enseignements, votre bonne humeur et tous ces bons moments passés ensemble.

Aux équipes avec lesquelles j'ai collaboré,

Madame le Docteur Amélie Daveluy et son équipe du CEIP-A

Monsieur le Docteur Antoine Loquet et son équipe

Madame le Docteur Camille Richeval et son équipe

À ma famille et mes amis,

À Miguel.

RÉSUMÉ

Dans le monde, environ 271 millions d'adultes, soit 5,5 % des personnes âgées de 14 à 64 ans, auraient déjà consommé des drogues illicites au cours de leur vie (UNODC, 2019). Le nombre de consommateurs augmente avec des usages souvent problématiques. En parallèle, le développement permanent de nouvelles molécules rend le diagnostic d'exposition difficile. Le manque de connaissances concrètes sur la composition et les dosages en principes actifs de ces produits pose un risque sanitaire important, voire mortel. Au cours de la dernière décennie, la spectrométrie de masse à haute résolution a suscité un intérêt croissant dans le domaine de la toxicologie analytique. L'objectif principal de la présente étude a été d'évaluer l'apport analytique de la spectrométrie de masse haute résolution en QTOF pour l'identification et la gestion de l'exposition aux substances psychoactives. Pour cela, nous avons comparé une méthode de criblage toxicologique par QTOF avec une méthode par spectrométrie infrarouge en analysant des produits provenant directement de consommateurs de drogues. En combinant des approches *in vivo*, *in silico* et *in vitro*, ainsi que d'autres méthodes comme la RMN et l'IR, nous avons évalué l'apport du QTOF pour l'identification des nouveaux produits de synthèse et la mise en évidence de nouveaux biomarqueurs d'exposition. Enfin, nous avons évalué son intérêt en pratique clinique pour l'identification de cannabinoïdes de synthèse dans les départements d'Outre-mer et en France métropolitaine. Ces différents apports sont particulièrement intéressants dans le domaine de la toxicologie analytique et clinique, notamment pour l'identification de nouvelles substances psychoactives et de nouveaux métabolites.

Mots clés : Spectrométrie de masse haute résolution ; LC-QTOF ; Substances psychoactives ; Nouveaux produits de synthèse ; Étude de métabolisme

Intitulé et adresse du laboratoire d'accueil

Bordeaux Population Health Research Center INSERM U1219,
Équipe « Pharmacoépidémiologie et impact des médicaments sur les populations ».
Université de Bordeaux
Site de Carreire, Bâtiment Pharmacie, quatrième tranche, troisième étage. Case 121
146 rue Léo Saignat
33076 Bordeaux Cedex, France

ABSTRACT

Globally, an estimated 271 million adults, or 5.5% of people aged 14-64, have used illicit drugs in their lifetime (UNODC, 2019). The number of consumers is increasing and their use is often problematic. At the same time, the constant development of new molecules makes the diagnosis of exposure harder. The lack of concrete knowledge about the composition and dosage of active compounds in these drugs poses a significant, even lethal, health risk. Over the last decade, high-resolution mass spectrometry has attracted increasing interest in the field of analytical toxicology. The main objective of the present study was to evaluate the analytical contribution of high-resolution mass spectrometry by QTOF for the identification and management of psychoactive substances exposure. For this purpose, we compared a QTOF screening method with an infrared spectrometric screening method by analysing products directly from drug users. By combining *in vivo, in silico* and *in vitro* approaches, as well as other methods such as NMR and IR, we evaluated the contribution of QTOF to the identification of new psychoactive substances and the discovery of new exposure biomarkers. Finally, we evaluated its interest in clinical practice for the identification of synthetic cannabinoids in the French overseas departments and in mainland France. These various contributions are particularly interesting in the field of analytical and clinical toxicology, especially for the identification of new psychoactive substances and new metabolites.

Keywords: High resolution mass spectrometry; LC-QTOF; drugs of abuse; New psychoactive substances; Metabolism study;

LISTE DES PRINCIPALES ABRÉVIATIONS

5HT : 5-hydroxytryptamine

AMM : Autorisation de Mise sur le Marché

ANSM : Agence Nationale de Sécurité du Médicament

ATR : Réflectance totale atténuée

BHD : Buprénorphine Haut Dosage

CAARUD : Centre d'Accueil et d'Accompagnement à la Réduction des risques pour les Usagers de Drogues

CAP-TV : Centre antipoison et toxicovigilance

CASF : Code de l'Action Sociale et des Familles

CAWG : Chemical Analysis Working Group

CBD : Cannabidiol

CCM : Chromatographie Sur Couche Mince

CCS : Collision cross section

CDC : Chloro-N, N-diméthylcathinone

CEC : Chloroéthcathinone

CEIP-A : Centre d'Évaluation et d'Information sur les Pharmacodépendances et Addictovigilance

CépiDc : Centre d'épidémiologie sur les causes médicales de Décès

CHU : Centre Hospitalo-Universitaire

CHM : Centre hospitalier de Mayotte

CPG-SM : Chromatographie en phase gazeuse couplée à la spectrométrie de masse

CS : Cannabinoïdes de Synthèse

CSAPA : Centres de Soins d'Accompagnement et de Prévention en Addictologie

CSP : Code de la Santé Publique

CYP : Cytochrome P450

DAT : Dopamine Transporter

DGS : Direction Générale de la Santé

DIMS : Drug Information and Monitoring System

DMT : Dimethyltryptamine

DOM : Département d'Outre-Mer

DRAMES : Décès en Relation avec l'Abus de Médicaments et de Substances

DTA : Décès Toxiques par Antalgiques

ECG : Électrocardiogramme

ELSA : Équipe de Liaison et de Soins en Addictologie

EMCDDA : European Monitoring Centre for Drugs and Drug Addiction ou Observatoire européen des drogues et des toxicomanies

ENa-CAARUD : Enquête Nationale dans les CAARUD

EWS : Early Warning System

FID : Free Induction Decay

FTIR : Spectroscopie infrarouge à transformée de Fourier

FWHM : Full Width at Half Maximum

GABA : Acide gamma-aminobutyrique

GC-MS : Chromatographie gazeuse couplée à la spectrométrie de masse

GST : Glutathion S transférase

GUS : General unknown screening

HLM : Microsome hépatique humain

HPLC-DAD : Chromatographie Liquide Haute Performance couplée à un détecteur type barrette de diode

HSH : Hommes ayant des relations Sexuelles avec d'autres Hommes

IMAO : Inhibiteurs de MonoAmine Oxydase

IR : Infrarouge

IV : Intraveineuse

JORF : Journal Officiel de la République Française

LC-QTOF : Chromatographie Liquide couplée à la spectrométrie de masse en temps de vol

LRMS : Low resolution mass spectrometry

LSD : Acide Lysergique

MDMA : 3,4-MéthylèneDioxy-Méthamphétamine

MILD&CA : Mission Interministérielle de Lutte contre les Drogues & les Conduites Addictives

MRP : Multidrug resistance proteins

MS : Mass spectrometry

MSI : Metabolomics Standards Initiative

MSO : Médicaments de Substitution aux Opiacés

MXE :Methoxetamine

MXPr : Methoxpropamine

NET : Noradrenaline Transporter

NMDA : N-Méthyl-D-Aspartate

NOS : Nouveaux opioïdes de synthèse

NPS : Nouveaux Produits de Synthèse

NSP : Nouvelles Substances Psychoactives

OFDT : Observatoire Français des Drogues et des Toxicomanies

OPPIDUM : Observation des Produits Psychotropes Illicites ou Détournés de leur Utilisation Médicamenteuse

OSCOUR : Organisation de la Surveillance Coordonnée des Urgences

PCP : Phénycyclidine

P-gp : Glycoprotéine P

QTOF : Quadrupole time of flight : quadripôle-temps de vol

RC : Research Chemicals

RDR : Réduction des Risques

RdRD : Réduction des Risques et des Dommages

RF : Radiation de radiofréquence

RMN : Résonance magnétique nucléaire

RPP : Reconnaissance Présomptive des Produits

SAP : Système d'alerte précoce

Se : Sensibilité

SERT : Serotonine Transporteur

SFC : Chromatographie sur fluide supercritique

SIDA : Syndrome d'ImmunoDéficience Acquise

SINTES : Système d'Identification National des Toxiques Et Substances

SNC : Système Nerveux Central

Spe : Spécificité

SPA : Substances Psychoactives

TEDI : réseau TransEuropéen d'Information sur les Drogues

THC : TetraHydro Cannabinol

TREND : Tendances Récentes et Nouvelles Drogues

TQ : Triple quadripôle

TSO : Traitement de Substitution aux Opiacés

UE : Union Européenne

UGT : UDP-glucuronosyltransférases

UNODC : United Nations Office on Drugs ou Office des Nations unies contre la drogue et le crime

UPLC-MS/MS : Chromatographie liquide Ultraperformante couplée à la spectrométrie de masse en tandem

VHB : virus de l'hépatite B

VHC : virus de l'hépatite C

VIH : Virus de l'Immunodéficience Humaine

LISTE DES FIGURES

Figure 1. Estimation de la consommation de drogues dans l'Union Européenne (d'après le « Rapport européen sur les drogues », EMCDDA 2022)..... 20

Figure 2. Maladies infectieuses liées aux drogues (d'après le « Rapport européen sur les drogues », EMCDDA 2022)..... 27

Figure 3. Roue des drogues (Drugs Wheel) d'après le CEIPA-A de Paris. Cercle intérieur : substances psychoactives classiques, Cercle extérieur : nouveaux produits de synthèse. 31

Figure 4. Données européennes sur les nouveaux produits de synthèse (d'après le « Rapport européen sur les drogues », EMCDDA 2022)..... 32

Figure 5. Nombres de NPS surveillés par le système d'alerte précoce de l'Union européenne, par catégorie (d'après le « Rapport européen sur les drogues », EMCDDA, 2022)..... 33

Figure 6. Trajectoire ionique en Orbitrap (d'après R. Thissen, 2014) 40

Figure 7. Schéma d'un instrument QTOF (d'après Waters®) 43

Figure 8. Schéma des principaux modes d'acquisition en spectrométrie de masse haute résolution (83) 45

LISTE DES TABLEAUX

Tableau 1. Estimation de la prévalence de l'usage de drogues en France et dans l'UE (d'après le « Rapport le rapport européen sur les drogues », EMCDDA 2022) 23

Tableau 2. Saisies de drogues de l'année 2020 en France et dans l'UE (adapté du « Rapport Européen sur les drogues », 2022, EMCDDA)..... 24

Tableau 3. Complications liées à la consommation de drogues en France et dans l'UE (adapté du « Rapport Européen sur les drogues », EMCDDA 2022)..... 29

TABLE DES MATIÈRES

REMERCIEMENTS **2**

RÉSUMÉ **3**

ABSTRACT **4**

LISTE DES PRINCIPALES ABRÉVIATIONS **5**

LISTE DES FIGURES **9**

LISTE DES TABLEAUX **9**

LISTE DES PUBLICATIONS **12**

LISTE DES COMMUNICATIONS **12**

INTRODUCTION GÉNÉRALE **15**

DONNÉES BIBLIOGRAPHIQUES **19**

I. La consommation de substances psychoactives, une problématique majeure de Santé publique *20*

A. Les drogues dites « classiques » 20

1. Données de consommation en France et dans l'UE 20
2. Conséquences de la consommation de substances psychoactives sur la santé publique 25

B. Les nouveaux produits de synthèse 30

1. Données de consommation en France et dans l'UE 30
2. Conséquences de la consommation de NPS sur la santé publique 34
3. Difficultés liées à l'analyse des NPS 35

II. La spectrométrie de masse haute résolution, un outil indispensable en toxicologie analytique ? *37*

A. Principes généraux de la spectrométrie de masse haute résolution 37

B. Principales technologies utilisées 40

1. Spectrométrie de masse à trappe orbitale (Orbitrap) 40
2. Spectrométrie de masse à temps de vol (TOF MS) 42

C. Acquisition des données 45

1. Balayage complet ou *Full scan* 45
2. Acquisition dépendante des données (DDA) 46
3. Acquisition indépendante des données (DIA) 47
4. *Parallel reaction monitoring – Multiple reaction monitoring (MRM)* 48

D. Traitement des données 49

1. Identification des composés 49
2. Choix des bibliothèques 50
3. Prédictions *in silico* 51
4. Métabolomique 51

E. Intérêt de la spectrométrie de masse haute résolution en toxicologie analytique : focus sur les substances psychoactives – Revue de la littérature n° 1 52

1. Introduction et présentation 52
2. Discussion 52

OBJECTIFS DE LA THÈSE **68**

TRAVAUX EXPÉRIMENTAUX **71**

I. Matériel et méthodes *72*
A. Identification de substances psychoactives par spectroscopie infrarouge à transformée de Fourier (FTIR) 72
B. Caractérisation d'une poudre par résonance magnétique nucléaire 73
C. Criblage toxicologique par UPLC-MS-QTOF 74
D. Étude du métabolisme des nouveaux produits de synthèse 75

II. Apport analytique de la spectrométrie de masse haute résolution en QTOF dans le cadre d'une comparaison de méthode – Publication n° 1 ..... *79*
A. Introduction et présentation 79
B. Discussion 80

III. Apport analytique de la spectrométrie de masse haute résolution en QTOF pour l'identification des nouveaux produits de synthèse : intérêt du couplage de méthodes et de l'étude de métabolisme – Publication n° 2 ..... *89*
A. Introduction et présentation 89
B. Discussion 90

IV. Apport clinique de la spectrométrie de masse haute résolution en QTOF, focus sur l'exposition aux cannabinoïdes de synthèse à Mayotte (Outre-mer) : Protocole HOMACHI – Publication n° 3.102
A. Introduction et présentation 102
B. Discussion 103

V. Application en pratique clinique, focus sur l'exposition aux cannabinoïdes de synthèse en France métropolitaine – Publication n° 4 ..... *120*
A. Introduction et présentation 120
B. Discussion 120

DISCUSSION GÉNÉRALE ET PERSPECTIVES **129**

CONCLUSION GÉNÉRALE **136**

RÉFÉRENCES **138**

ANNEXES **154**

I. Annexe 1 - Autres travaux, publications et communications sur le thème de la toxicologie clinique, analytique et médico-légale *155*

II. Annexe 2 – Protocole HOMACHI *233*

III. Annexe 3 – Protocole DRUGETOF mis en place dans le cadre de la thèse et en cours d'étude 236

IV. Annexe 4 – Protocole RuNPS mis en place dans le cadre de la thèse et en cours d'étude *246*

V. Annexe 5 – Fiche de renseignements pour la recherche de nouveaux produits de synthèse, mise en place dans le cadre de la thèse *250*

LISTE DES PUBLICATIONS

1. Revue de la littérature : **R. Goncalves**, R. Pelletier, A. Couette, T. Gicquel, B. Le Daré. « Suitability of high-resolution mass spectrometry in analytical toxicology: Focus on drugs of abuse », *Toxicol Anal Clin*., déc. 2021 ; S2352007821002183.

2. Publication n° 1 : **R. Goncalves**, K. Titier, V. Latour, A. Peyré, N. Castaing, A. Daveluy, M. Molimard. « Suitability of infrared spectroscopy for drug checking in harm reduction centres », *International Journal of Drug Policy*, Volume 88, 2021, 103037, ISSN 0955-3959.

3. Publication n° 2 : **R. Goncalves**, N. Castaing, C. Richeval, D. Ducint, K. Titier, E. Morvan, A. Grélard, A. Loquet, M. Molimard. « Methoxpropamine (MXPr) in powder, urine and hair samples: Analytical characterization and metabolite identification of a new threat », *Forensic Sci Int.,* avr. 2022 ; 333:111215.

4. Publication n° 3 : **R. Goncalves**, M. Labadie, S. Chouraqui, A. Peyré, N. Castaing, A. Daveluy, M. Molimard. « Involuntary MDMB-4en-PINACA intoxications following cannabis consumption: clinical and analytical findings », *Clin Toxicol.,* 1 déc. 2021 ; 1-6.

5. Publication n° 4 : **R. Goncalves**, A. Peyré, N. Castaing, T. Beeken, S. Olivier, P. Combe, G. Miremont-Salamé, K. Titier, M. Molimard, A. Daveluy. « Minors and young adult's hospitalizations after "chimique" consumption in Mayotte Island: which substances are involved? », *Therapies*, août 2022;S004059572200124X.

LISTE DES COMMUNICATIONS

Communication orale à un congrès international

1. **R. Goncalves**, V. Latour, A. Peyre, C. Mathieu, O. Capdebosq, L. Baron, N. Castaing, M. Molimard, A. Daveluy, K. Titier. « Étude de la mise en place du « *drug checking* » par spectrométrie infrarouge dans un CAARUD ». Colloque international Addictions Toxicomanies Hépatites SIDA (ATHS), Biarritz (France), 1-4 octobre 2019.

Communications orales à des congrès nationaux

1. **R. Goncalves**, V. Dumestre-Toulet. « Évolution des besoins depuis les recommandations de 2012 et intérêt de rechercher les Nouveaux Produits de Synthèse (NPS) dans les cas d'agitations inexpliquées ». Congrès annuel de la Société Française de Toxicologie Clinique (STC), Bordeaux (France), 16-18 septembre 2020.

2. **R. Goncalves**, D. Mété, A. Maillot, A. Peyré, S. Bastard, P. Combe, G. Miremont-Salamé, M. Molimard, N. Castaing, K. Titier, A. Daveluy. « La chimique dans les départements français de l'Océan Indien : importance d'un phénomène ». Congrès annuel de la Société Française de Toxicologie Analytique (SFTA), Grenoble (France), 9-11 décembre 2020.

3. **R. Goncalves**, D. Mété, A. Maillot, A. Peyré, S. Bastard, A. Youssouf, G. Brouessard, P. Combe, N. Castaing, G. Miremont-Salamé, M. Molimard, K. Titier, A. Daveluy. « Importance of the consumption of a new drug called chimique in the French departments of Indian Ocean (Mayotte and Reunion Island) ». E-congrès annuel de la Société Française de Pharmacologie et Thérapeutiques (SFPT), 15-16 juin 2021.

4. **R. Goncalves**. « Étude du métabolisme *in silico* des cannabinoïdes de synthèse – Cas du 5F-ADB ou 5FMDMB-PINACA à la Réunion ». Forum Clinique Waters, Paris (France), 15 octobre 2019.

5. **R. Goncalves**, N. Castaing, A. Loquet, C. Richeval, M. Molimard, K. Titier. « Identification and analytical characterization of recently emerged methoxpropamine (MXPr) in product and human urine samples ». Printemps des Jeunes Scientifiques de la Société Française de Toxicologie Analytique (SFTA), Paris (France), 10 décembre 2020.

6. **R. Goncalves**, R. Pelletier, A. Couette, T. Gicquel, B. Le Daré, « Suitability of high-resolution mass spectrometry in analytical toxicology: Focus on drugs of abuse ». Journée de formation de la Société Française de Toxicologie Analytique (SFTA) et de la Compagnie Nationale des Biologistes et Analystes Experts (CNBAE), Paris (France), 21 janvier 2021.

Communication affichée à un congrès international

1. **R. Goncalves**, V. Latour, A. Peyre, C. Mathieu, O. Capdebosq, L. Baron, N. Castaing, M. Molimard, A. Daveluy, K. Titier. « Étude de la mise en place du « *drug checking* » par spectrométrie infrarouge dans un CAARUD ». Colloque international Addictions Toxicomanies Hépatites SIDA (ATHS), Biarritz (France), 1-4 octobre 2019.

Communications affichées à des congrès nationaux

1. **R. Goncalves**, V. Dumestre-Toulet. « Évolution des besoins depuis les recommandations de 2012 et intérêt de rechercher les Nouveaux Produits de Synthèse (NPS) dans les cas d'agitations inexpliquées ». Congrès annuel de la Société Française de Toxicologie Clinique (STC), Bordeaux (France), 16-18 septembre 2020.

2. **R. Goncalves**, D. Mété, A. Maillot, A. Peyré, S. Bastard, P. Combe, G. Miremont-Salamé, M. Molimard, N. Castaing, K. Titier, A. Daveluy. « Importance de la consommation de « chimique » dans les départements français de l'Océan Indien ». Congrès annuel de la Société Française de Toxicologie Clinique (STC), Bordeaux (France), 16-18 septembre 2020.

3. **R. Goncalves**, D. Mété, A. Maillot, A. Peyré, S. Bastard, P. Combe, G. Miremont-Salamé, M. Molimard, N. Castaing, K. Titier, A. Daveluy. « La chimique dans les départements français de l'Océan Indien : importance d'un phénomène ». Congrès annuel de la Société Française de Toxicologie Analytique (SFTA), Grenoble (France), 9-11 décembre 2020.

4. **R. Goncalves**, D. Mété, A. Maillot, A. Peyré, S. Bastard, A. Youssouf, G. Brouessard, P. Combe, N. Castaing, G. Miremont-Salamé, M. Molimard, K. Titier, A. Daveluy. « Importance of the consumption of a new drug called chimique in the French departments of Indian Ocean (Mayotte and Reunion Island) ». E-congrès annuel de la Société Française de Pharmacologie et Thérapeutiques (SFPT), 15-16 juin 2021.

INTRODUCTION GÉNÉRALE

Les intoxications par les substances psychoactives (SPA) sont de plus en plus nombreuses en Europe et touchent des patients très jeunes, majoritairement entre 15 et 25 ans (1). De nombreux produits sont peu coûteux et facilement accessibles, notamment sur Internet (darknet). On observe par ailleurs une croissance importante du nombre de nouveaux produits de synthèse (NPS) dont la toxicité est inconnue ou peu connue : en 2020, 52 nouvelles substances ont été détectées pour la première fois en Europe (2). Du fait de cette émergence régulière et rapide, ces molécules, dans la grande majorité des cas, ne sont pas inscrites sur la liste des stupéfiants. Les intoxications avec ce type de produits peuvent être responsables de complications sévères entraînant des hospitalisations, voire des décès (3–6). Les services d'urgences sont de plus en plus confrontés à de nouvelles manifestations aiguës, dont les signes cliniques sont souvent hétérogènes et dont la prise en charge est mal connue (7,8). En France, l'ampleur réelle de la consommation de ces NPS est très peu documentée même si quelques publications en font état (9). En effet, il est très difficile de repérer les NPS car ces molécules ont des sites de vente de plus en plus accessibles et sécurisants augmentant leur disponibilité, surtout en milieu festif. Leur dangerosité est également souvent méconnue (9–11). Si on prend l'exemple des cannabinoïdes de synthèse (CS), ils ont été associés à de nombreuses intoxications aiguës et des décès (3). Un récent rapport de l'EMCDDA a attiré l'attention sur les problèmes croissants pour la santé et la sécurité générés par l'usage de ces substances (2). Les informations toutefois disponibles montrent que la prévalence de l'usage de CS est en augmentation, et que la toxicité potentielle de ces substances est considérable. Ces problèmes passent parfois inaperçus à cause d'un phénomène de contrebande facilitée et des défis analytiques que posent leurs détections. En effet, l'évolution constante des NPS sur le marché de drogues, et leurs diversités chimiques et fonctionnelles nécessitent le développement continu de techniques performantes d'identification et de dosage. L'objectif étant de les identifier chez les populations d'usagers, ou dans des échantillons non biologiques issus des saisies, et ce, pour permettre une prise en charge médicale adéquate dans le premier cas, ou une décision quant à leur contrôle dans le second.

Le toxicologue analyste est confronté à diverses problématiques associées à la détection ainsi qu'à la quantification des NPS et de leurs métabolites dans les échantillons biologiques. Il s'agit d'un réel défi en raison de la disponibilité limitée de standards industriels, du délai entre la disponibilité du produit sur le marché des drogues et l'obtention des standards et de leur coût. Il ne faut également pas oublier le temps nécessaire à la mise en place des techniques et à leur mise à jour. En raison du métabolisme important de ces produits, les molécules mères

sont rarement identifiées dans les urines. Dresen *et al.* (12) ont signalé très tôt l'importance de mettre au point des méthodes d'analyse pour détecter les molécules mères dans le sang. Cependant, ils ont surtout souligné qu'il était impératif de corréler les résultats des échantillons sanguins à leurs principaux métabolites urinaires pour identifier de nouveaux/meilleurs biomarqueurs. Le criblage toxicologique est généralement la première étape de toute investigation toxicologique ; il s'agit d'une étape clé qui devrait idéalement permettre l'identification de toute substance d'intérêt toxicologique, notamment des substances psychoactives. Depuis de nombreuses années déjà, la chromatographie et la spectrométrie de masse se sont imposées comme les méthodes analytiques de référence pour le criblage toxicologique (13). L'amélioration constante de ces technologies permet cependant de faire évoluer leur usage vers des procédures de plus en plus rapides, sensibles et fiables. Une des limites de ces techniques est la connaissance préalable des substances susceptibles d'être identifiées et de disposer de molécules de référence pour optimiser leur détection. Le couplage des systèmes chromatographiques avec des analyseurs haute résolution mesurant la masse exacte (temps de vol ou TOF, Orbitrap) ouvrent de nouveaux champs d'investigation au toxicologue analyste, en particulier les détecteurs hybrides (Q-TOF, Q-Orbitrap) (14). L'UPLC-Q-TOF constitue un outil analytique puissant qui, par mesure de la masse exacte des xénobiotiques, réalise un screening large en mode « full scan », c'est-à-dire non ciblé, avec une grande spécificité. Cette technologie Q-TOF est un moyen de rechercher et de caractériser de nouvelles molécules et leurs métabolites en l'absence de molécules de référence (15). Enfin, le couplage de l'UPLC-Q-TOF avec une extraction en ligne réduit considérablement le temps de préparation des échantillons et apporte de la robustesse au screening toxicologique (16).

Dans ce contexte, l'étude de l'identification et la prise en charge de l'exposition aux substances psychoactives avec la spectrométrie de masse haute résolution en Q-TOF apparaît primordiale.

DONNÉES BIBLIOGRAPHIQUES

I. La consommation de substances psychoactives, une problématique majeure de Santé publique

A. Les drogues dites « classiques »

1. Données de consommation en France et dans l'UE

En matière de drogues, nous faisons face aujourd'hui à une situation complexe, caractérisée par une disponibilité accrue et par une plus grande diversité des modes de consommation. La disponibilité et la consommation de drogues restent à des niveaux élevés dans l'Union européenne (UE) (Figure 1), bien qu'il existe des différences considérables entre les pays. Selon les estimations, environ 83,4 millions d'adultes (dont 50,5 millions d'hommes), soit 29 % des adultes âgés de 15 à 64 ans, auraient déjà fait usage d'une substance illicite (1). Le tableau 1 résume la prévalence de l'usage de drogues en France et dans l'UE d'après les dernières données de l'EMCDDA.

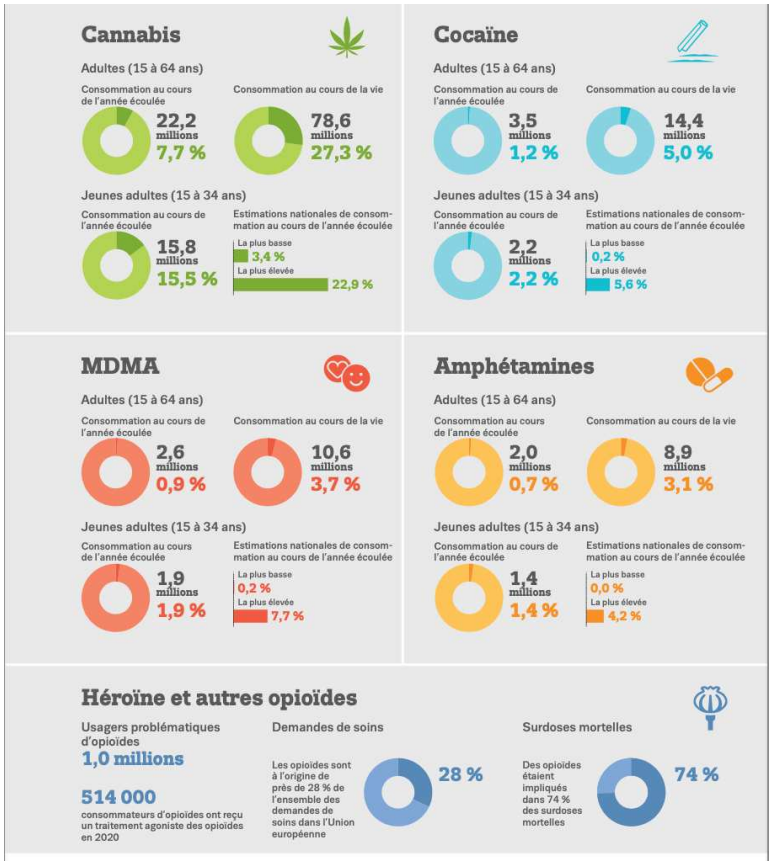


Figure 1. Estimation de la consommation de drogues dans l'Union Européenne (d'après le « Rapport européen sur les drogues », EMCDDA 2022)

Le cannabis reste la substance la plus consommée en France et en Europe, plus de 22 millions d'adultes européens ont déclaré en avoir consommé au cours de l'année écoulée (17). La France est la première consommatrice en termes de prévalence d'usage devant les autres membres de l'UE (3). En 2017, 45 % des adultes âgés de 18 à 64 ans déclarent avoir déjà consommé du cannabis au cours de leur vie en France. Un usage régulier est déclaré par 3,6 % de la population (17,19). En tant que stupéfiant le plus fréquemment signalé par le réseau hospitalier Euro-DEN Plus en 2020, le cannabis était impliqué dans 23 % des passages aux urgences pour toxicité aiguë (contre 27 % en 2019), généralement en présence d'autres substances (20).

Les stimulants constituent la deuxième catégorie la plus souvent mentionnée. Au cours de l'année passée, 3,5 millions d'adultes auraient consommé de la cocaïne, 2,6 millions de la MDMA et 2 millions des amphétamines. Dans l'ensemble, les indicateurs suggèrent que la disponibilité et l'usage de la cocaïne en Europe restent historiquement élevés. En 2020, un volume record de 213 tonnes de cocaïne a été saisi (Tableau 2). La cocaïne, deuxième cause de complications chez les patients admis en traitement pour la première fois, était également la deuxième substance la plus fréquemment signalée par les hôpitaux du réseau Euro-DEN Plus en 2020, présente dans 21 % des cas de toxicité aiguë. Le nombre de passages aux urgences liés à la cocaïne a diminué de 15 % entre 2019 et 2020 (1). Selon l'étude DRAMES (Décès en Relation avec l'Abus de Médicaments Et de Substances), la proportion des décès liés à la cocaïne a triplé entre 2013 et 2017 (de 9 à 26 % des décès) et dépasse pour la première fois les décès par l'héroïne (21,22).

Les enquêtes, qui regroupent l'amphétamine et la méthamphétamine, menées par 25 pays de l'UE entre 2016 et 2021, suggèrent que 1,4 million de jeunes adultes (15-34 ans) ont consommé des amphétamines au cours de l'année écoulée (1,4 % de cette tranche d'âge). L'amphétamine était la quatrième substance la plus fréquemment signalée par les hôpitaux du réseau Euro-DEN Plus en 2020, présente dans 13 % des passages aux urgences (1). Certaines données indiquent que la disponibilité et la consommation de méthamphétamine semblent augmenter, bien que le seuil de départ reste bas.

La disponibilité continue de comprimés de MDMA fortement dosés expose les usagers à des risques majeurs pour leur santé. Toutefois, certains signes indiquent que les niveaux de consommation de MDMA/ecstasy ont diminué en 2020, probablement du fait de la pandémie du Covid-19 qui a eu de grandes répercussions sur l'économie de la vie nocturne en Europe,

une crise qui s'est poursuivie en 2021 (23). Les autres données communiquées par Euro-DEN Plus concernent le nombre de passages aux urgences liés à la MDMA/ecstasy, qui ont diminué de moitié environ en 2020. Les enquêtes menées par 26 pays de l'UE entre 2015 et 2021 suggèrent que 1,9 million de jeunes adultes (de 15 à 34 ans) ont consommé de la MDMA/ecstasy au cours de l'année passée (1,9 % de cette tranche d'âge). Les estimations de la prévalence sont plus élevées chez les jeunes de 15 à 24 ans, dont 2,2 % (1,0 million) auraient consommé de la MDMA/ecstasy au cours de l'année précédente. La MDMA/ecstasy était la sixième drogue la plus fréquemment signalée par les hôpitaux du réseau Euro-DEN Plus en 2020, présente dans 6 % des cas de toxicité aiguë (1).

En ce qui concerne les opioïdes, environ un million d'Européens ont consommé de l'héroïne ou un autre opioïde illicite au cours de l'année écoulée. Bien que la prévalence de la consommation d'opioïdes soit plus faible que celle d'autres drogues, ceux-là représentent toujours la majeure partie des hospitalisations attribuées à la consommation de substances illicites. Cette situation est illustrée par la présence d'opioïdes, souvent en combinaison avec d'autres substances, qui étaient impliqués dans environ trois quarts des surdoses mortelles au sein de l'UE en 2020. L'usage d'opioïdes a été évoqué comme la principale raison pour laquelle 66 000 patients avaient entamé un accompagnement thérapeutique spécialisé en 2020, soit 28 % de l'ensemble des usagers européens admis en traitement. L'héroïne était la drogue posant le plus de problèmes pour 8 500 patients (77 %) des 11 200 admis en traitement pour la première fois et ayant déclaré qu'un opioïde spécifique constituait leur stupéfiant principal (24). Les opioïdes étaient impliqués dans environ 74 % des surdoses mortelles en Europe (25).

Il convient de noter que des cas de polyconsommation sont couramment observés dans les rapports toxicologiques concernant des décès présumés liés à l'usage de drogues. Nous constatons également une complexité considérablement accrue des modes de consommation de drogues, avec des médicaments, de nouvelles substances psychoactives non réglementées et d'autres, telles que la kétamine, le GBL/GHB ou encore le protoxyde d'azote, qui sont désormais associées à des troubles de l'usage dans certains pays (26).

Tableau 1. Estimation de la prévalence de l'usage de drogues en France et dans l'UE (d'après le « Rapport le rapport européen sur les drogues », EMCDDA 2022)

Drogue	Pays	Estimation de prévalence					Patients admis en traitement au cours de l'année						Patients suivant un traitement agoniste des opioïdes
		Année de l' estimation	Population générale			Population scolaire	Patients usagers en % des demandes de soins			% de patients usagers par injection (principal mode d'administration)			
			Vie, adultes (15-64 ans)		12 derniers mois, jeunes adultes (15-34 ans)	Vie, élèves (15-16 ans)	Tous les patients admis en traitement	Patients admis en traitement pour la première fois	Usagers admis en traitement, traités antérieurement	Tous les patients admis en traitement	Patients admis en traitement pour la première fois	Usagers admis en traitement, traités antérieurement	
			Nombre de cas pour 1 000 habitants	%	%	%	% (comptage)	% (comptage)	% (comptage)	% (comptage)	% (comptage)	% (comptage)	Comptage
Opioïde	France	2019	4,9-5,2				25,6 (9 562)	13,2 (1 229)	37,4 (5 863)	15,4 (1 282)	9,7 (109)	17,2 (888)	177 100
	UE	-	-				25,7 (72 449)	12,8 (15 688)	39,3 (57 128)	30,8 (16 702)	21,5 (2 520)	38,3 (17 078)	514 324
Cocaïne	France	2017	-	5,6	3,2	3	11,8 (4 424)	10,9 (1 014)	13,5 (2 114)	6,9 (276)	2,8 (26)	10,1 (196)	-
	UE	-	-	5,0	2,2	-	19,8 (55 780)	22,4 (27 318)	17,7 (25 738)	2,0 (983)	0,8 (184)	3,3 (736)	-
Amphé-tamines	France	2017	-	2,2	0,6	1	0,5 (201)	0,4 (37)	0,5 (86)	19,3 (32)	6,7 (2)	23,6 (17)	-
	UE	-	-	3,1	1,4	-	7,5 (21 209)	8,0 (9 734)	7,1 (10 267)	20,6 (2 765)	17,4 (1 113)	22,1 (1 406)	-
MDMA/ Ecstasy	France	2017	-	3,9	1,3	2	0,5 (169)	0,7 (62)	0,3 (54)	-	-	-	-
	UE	-	-	3,7	1,9	-	0,4 (1 076)	0,6 (695)	0,2 (313)	-	-	-	-
Cannabis	France	2017	-	44,8	21,8	23	56,6 (21 186)	69,7 (6 504)	43,7 (6 851)	-	-	-	-
	UE	-	-	23,7	15,5	-	34,3 (96 804)	45,7 (55 815)	21,3 (30 961)	-	-	-	-

Tableau 2. Saisies de drogues de l'année 2020 en France et dans l'UE (adapté du « Rapport Européen sur les drogues », 2022, EMCDDA)

Pays	Héroïne		Cocaïne		Amphétamines		MDMA, MDA, MDEA			Résine de cannabis		Herbe de cannabis		Plants de cannabis		
	Quantité saisie	Nombre de saisies	Quantité saisie	Nombre de saisies	Quantité saisie	Nombre de saisies	Quantité saisie		Nombre de saisies	Quantité saisie	Nombre de saisies	Quantité saisie	Nombre de saisies	Quantité saisie		Nombre de saisies
	Kg	Comptage	Kg	Comptage	Kg	Comptage	Comprimés	Kg	Comptage	Kg	Comptage	Kg	Comptage	Plants	Kg	
France	1 132	-	13 145	-	-	-	1 227 876	-	-	50 248	-	46 277	-	115 365	-	-
UE	5 125	18 499	212 574	64 406	23 387	30 711	4 720 121	1 025	12 616	584 319	86 483	155 359	240 097	2 756 284	50 181	11 912

2. Conséquences de la consommation de substances psychoactives sur la santé publique

Qui dit consommation récréative et occasionnelle ne dit pas consommation sans danger, bien au contraire. Le manque d'information sur les produits et les risques liés à leur consommation peuvent induire des prises de risque importantes, en particulier lorsqu'il s'agit d'usages expérimentaux ou de primoconsommations.

Les risques infectieux épidémiques les plus fréquents sont ceux d'infection au virus de l'hépatite B (VHB), de l'hépatite C (VHC) et de l'immunodéficience humaine (VIH) (Figure 2) (27). Ces risques touchent principalement les usagers de drogues qui ont recours à l'injection, mais également ceux qui en consomment par voie nasale « en sniff ». En 2016, la contamination par voie injectable représente 4 % des découvertes de séropositivité au VIH en France (Tableau 3).

Les autres risques infectieux correspondent à des complications somatiques faisant intervenir des agents qui ne présentent pas de potentiel épidémique majeur.

Parmi ces complications, on peut mentionner (28) :

- des infections locales au point d'injection : abcès, cellulite ;
- des septicémies ;
- des endocardites infectieuses ;
- des candidoses disséminées profondes ;
- des « poussières » : apparu dans les années 1970, le terme anglais « *cotton fever* » (fièvre du coton) définissait le tableau clinique caractéristique des usagers lors de la réutilisation de cotons pour filtrer les produits injectés. Le terme de « poussières » apparu en France dans les années 1990, désigne un ensemble de symptômes associés à l'usager de SPA par voie intraveineuse. Une « poussière » correspond à une manifestation fébrile (frissons, transpiration, céphalées) qui se produit juste après l'injection et spontanément résolutive en quelques heures. Cette réaction est due à l'introduction dans le sang soit d'un agent infectieux mort ou en faible quantité (bactériémie/fongémie transitoire), soit d'un agent pyrogène non infectieux (produit de coupe, excipient).

Les troubles sanitaires non infectieux sont principalement représentés par les accidents, les overdoses, les risques psychiatriques, les complications liées à l'injection, l'altération du capital veineux et les dangers liés à la prise simultanée de plusieurs drogues (polyconsommation).

Il existe un certain nombre de risques intrinsèques aux produits consommés, relatifs à leur composition, mais également à leur concentration en substances actives. Le nombre de décès par surdose en 2015 est de 236 chez les 15-49 ans (373 au total) selon le registre général de mortalité (OFDT). Près de 10 000 passages aux urgences en lien avec l'usage de drogues ont été dénombrés en 2015 en France (réseau Oscour : organisation de la surveillance coordonnée des urgences).

Parmi les complications liées à l'injection, on peut mentionner (4-6):

- l'injection en sous-cutanée (shoot raté) ou épanchement de la solution injectée dans les tissus environnants, pouvant conduire à la formation d'un granulome ou d'un abcès stérile ;
- les granulomes pulmonaires ;
- les micro-embolies entraînant un œdème du membre (main, avant-bras) ;
- l'obstruction veineuse qui peut se compliquer à plus ou moins long terme d'ulcères, d'infections locales (abcès, cellulite), de gangrènes ;
- la thrombose veineuse profonde susceptible de causer une embolie pulmonaire ou un syndrome post-phlébitique ;
- la vascularite.

Parmi les risques psychiatriques, nous pouvons évoquer les psychoses, les troubles anxieux, dépressifs, de l'humeur, etc.

La polyconsommation peut compliquer la prise en charge médicale et induire des risques supplémentaires. Les usagers peuvent parfois consommer plusieurs substances de manière simultanée ou rapprochée dans le temps, de telle façon que leur spectre d'action se chevauche. Plus d'un tiers des usagers en CAARUD aurait consommé au moins 5 substances différentes (ENa-CAARUD). L'association la plus fréquente est celle de l'alcool avec une ou plusieurs substances. Certains mélanges sont très risqués avec de possibles conséquences fatales ; c'est le cas, par exemple, des mélanges alcool/cocaïne (formation de cocaéthylène), alcool/kétamine ou de certains médicaments avec des substances psychoactives :

antidépresseurs (IMAOs)/MDMA (risque de syndromes sérotoninergiques), antidépresseurs (IMAOs)/cocaïne (risque de troubles cardiovasculaires sévères).

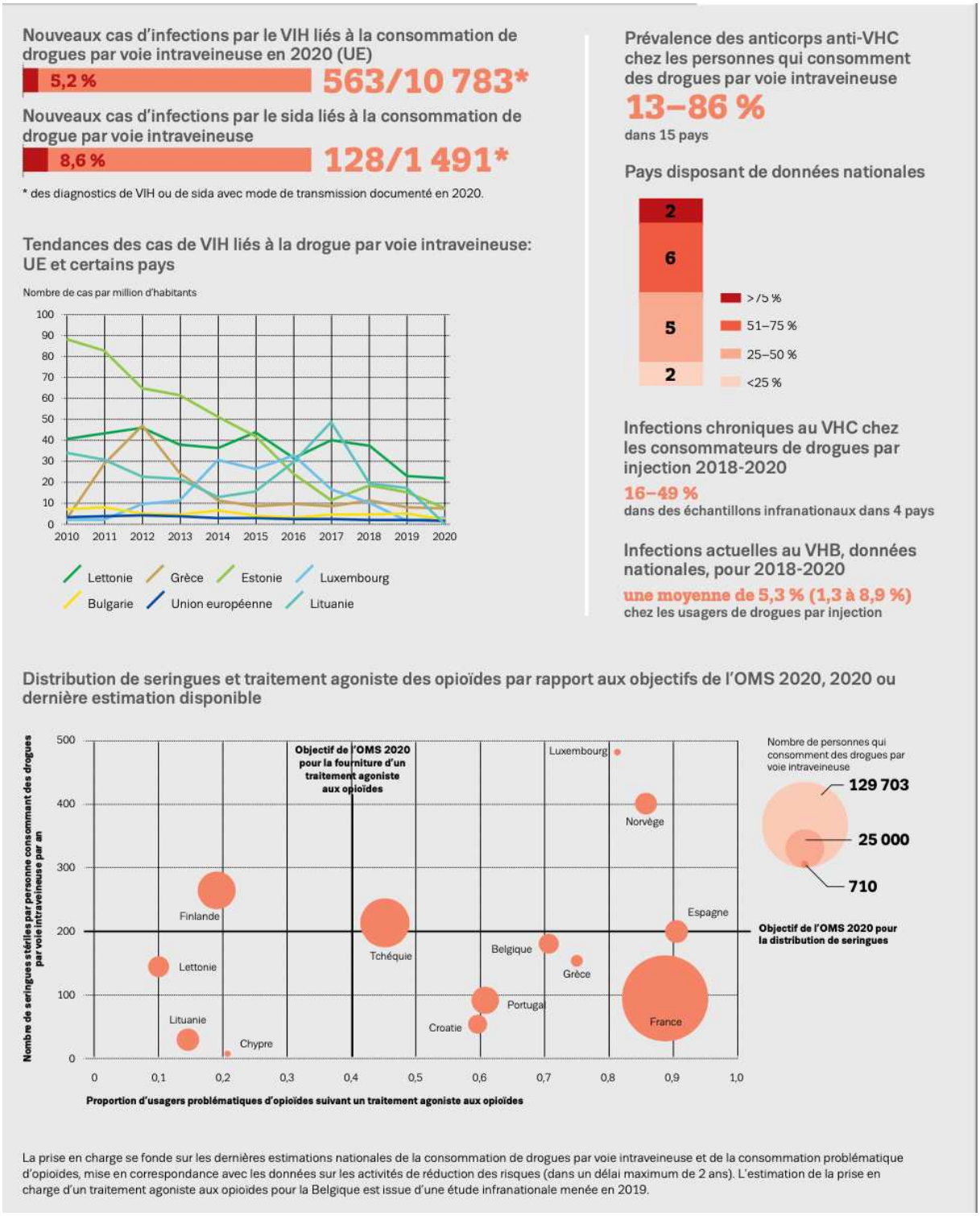


Figure 2. Maladies infectieuses liées aux drogues (d'après le « Rapport européen sur les drogues », EMCDDA 2022)

On estime qu'au moins 5 800 décès par surdose, impliquant des drogues illicites, sont survenus dans l'Union européenne en 2020, ce qui représente un taux de mortalité de 16,7 décès par million d'habitants pour la population adulte (1). La plupart de ces décès sont associés à une polyconsommation qui associe généralement des combinaisons d'opioïdes ou d'autres drogues illicites, de médicaments et d'alcool. Dans les rapports toxicologiques de certains pays sur les décès liés à l'usage de drogues, les benzodiazépines sont souvent mentionnées en combinaison avec d'autres drogues. On ignore généralement si ces médicaments ont été prescrits à l'individu à des fins thérapeutiques, mais il est probable que ce ne soit souvent pas le cas. Bien que les données soient difficiles à interpréter, elles suggèrent que les benzodiazépines peuvent avoir causé certains de ces décès ou y avoir contribué. Les opioïdes sont présents dans environ trois quarts de tous les décès liés à l'usage de drogues. Il convient également de noter que les opioïdes autres que l'héroïne, notamment la méthadone et, dans une moindre mesure, la buprénorphine, l'oxycodone et le fentanyl, ont été associés à une part importante des décès par surdose dans certains pays (32–34).

Le nombre de décès par surdose dans la tranche d'âge des 50 à 64 ans a augmenté de 82 % entre 2012 et 2020 (35). Cela reflète la tendance au vieillissement chez les consommateurs problématiques de drogues et, dans certains pays, l'augmentation des décès liés aux opioïdes délivrés sur ordonnance, souvent chez les femmes, peut-être liés à la gestion de la douleur et à l'usage détourné de médicaments. Il est donc de plus en plus nécessaire, d'une part, d'élaborer des stratégies qui répondent mieux aux besoins d'une population vieillissante souffrant de problèmes chroniques de santé et de drogues et, d'autre part, de mieux comprendre le lien entre la consommation d'opioïdes chez les groupes plus âgés et une santé dégradée. Cette problématique est d'autant plus importante avec l'arrivée inquiétante de nombreux produits de synthèse, notamment les nouveaux opioïdes de synthèse (NOS).

Tableau 3. Complications liées à la consommation de drogues en France et dans l'UE (adapté du « Rapport Européen sur les drogues », EMCDDA 2022)

Pays	Décès dus aux drogues			Cas de VIH diagnostiqués imputés à l'usage de drogue par injection (ECDC)	Estimation de l'usage de drogues par voie intraveineuse		Seringues distribuées par des programmes spécialisés
	Année	15-64 ans	Tous les âges				
		Comptage	Nombre de cas par million d'habitants (comptage)	Nombre de cas par million d'habitants (comptage)	Année de l'estimation	Nombre de cas pour 1 000 habitants	Comptage
France	2016	465	9 (391)	0,7 (50)	2019	3,1-3,3	12 572 530
UE	-	5 796	16,7 (3 904)	1,3 (563)	-	-	-

B. Les nouveaux produits de synthèse

1. Données de consommation en France et dans l'UE

Les nouveaux produits de synthèse (NPS) ou Nouvelles Substances Psychoactives (NSP) se définissent, par référence à la définition de l'Office des Nations Unies contre la Drogue et le Crime (UNODC), comme les substances naturelles ou synthétiques : ils sont consommés sous forme pure ou de préparation pour leurs effets psychoactifs, non contrôlés par la convention des Nations Unies de 1961 sur les stupéfiants ni celle de 1971 sur les psychotropes, mais peuvent constituer une menace pour la santé publique.

Le terme NPS privilégié par l'Observatoire Français des Drogues et des Toxicomanies (OFDT) correspond à l'acronyme anglais NPS (*New Psychoactive Substances*). Les NPS sont obtenus par des modifications chimiques sur les structures de base des stupéfiants classiques (dits « conventionnels ») tels que l'amphétamine, la 3,4-méthylène-dioxy-méthamphétamine (MDMA ou ecstasy), le fentanyl, la kétamine ou d'autres drogues, afin d'accentuer leurs effets, et d'échapper à la législation sur les stupéfiants ; les nouvelles substances, ayant des structures différentes des drogues classiques dont elles sont issues, ne sont donc ni listées comme stupéfiants ni facilement identifiables par les techniques d'analyse usuelles (Figure 3). Ces produits sont vendus via des sites Internet ou dans des magasins spécialisés sous des appellations ambiguës comme « produits chimiques de recherche » (*Research Chemicals RC*), « euphorisants légaux » (*legal highs*), « euphorisants végétaux » (*herbal highs*), « sels de bain », « engrais pour plantes », « encens », et sont étiquetés comme « impropres à la consommation humaine » (*Not for Human consumption*) afin de dissimuler leur vraie nature (2,9,10,36–38).

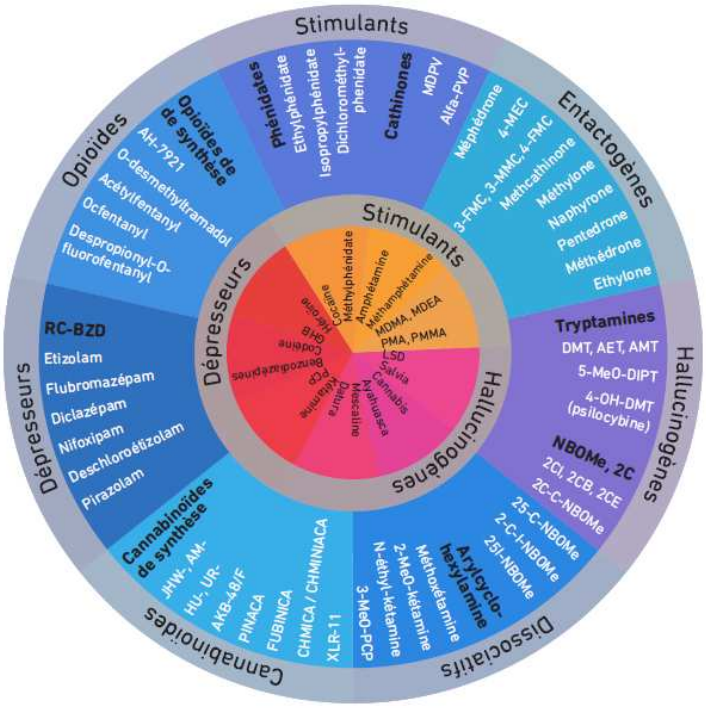


Figure 3. Roue des drogues (Drugs Wheel) d'après le CEIPA-A de Paris. Cercle intérieur : substances psychoactives classiques, Cercle extérieur : nouveaux produits de synthèse.

Du fait de la variété des produits concernés, de leurs noms et de leurs formes, l'estimation de la prévalence des NPS est complexe. Le marché des NPS est un marché mondial complexe et très dynamique car des substances peuvent apparaître puis disparaître rapidement, mais leur nombre en circulation reste relativement élevé. Fin 2021, l'EMCDDA surveillait environ 880 nouvelles substances psychoactives, dont 52 avaient été signalées pour la première fois en Europe en 2021 (1,2). En 2020, 65 % des matières saisies (3,3 tonnes) étaient des poudres de cathinone, dont le N-éthylhexdrone représentait un tiers, tandis que le 3-MMC et le 3-CMC représentaient chacun un quart. Le phénomène des NPS montre que presque tout ce qui a un potentiel psychoactif risque aujourd'hui d'apparaître sur le marché, souvent sous des étiquettes trompeuses, de sorte que les personnes qui utilisent ces substances peuvent ne pas savoir ce qu'elles consomment réellement. Un des exemples que nous étudierons dans ce travail est la falsification de produits à base de cannabis avec des cannabinoïdes de synthèse (CS) (39,40). Un autre phénomène inquiétant est la production croissante de drogues de synthèse en Europe. Un total de 224 nouveaux CS ont été détectés en Europe depuis 2008 et 15 de ces substances ont été signalées pour la première fois en 2021 (Figure 5). En 2020, les États membres de l'UE ont signalé 6 300 saisies, soit 236 kilogrammes de matières contenant des CS (Figure 5). Des décès impliquant des CS ont été signalés par trois pays en 2020 : l'Allemagne (n=9), la Hongrie (n=34) et la Turquie (n=49) (Figure 4 et 5) (1,3).

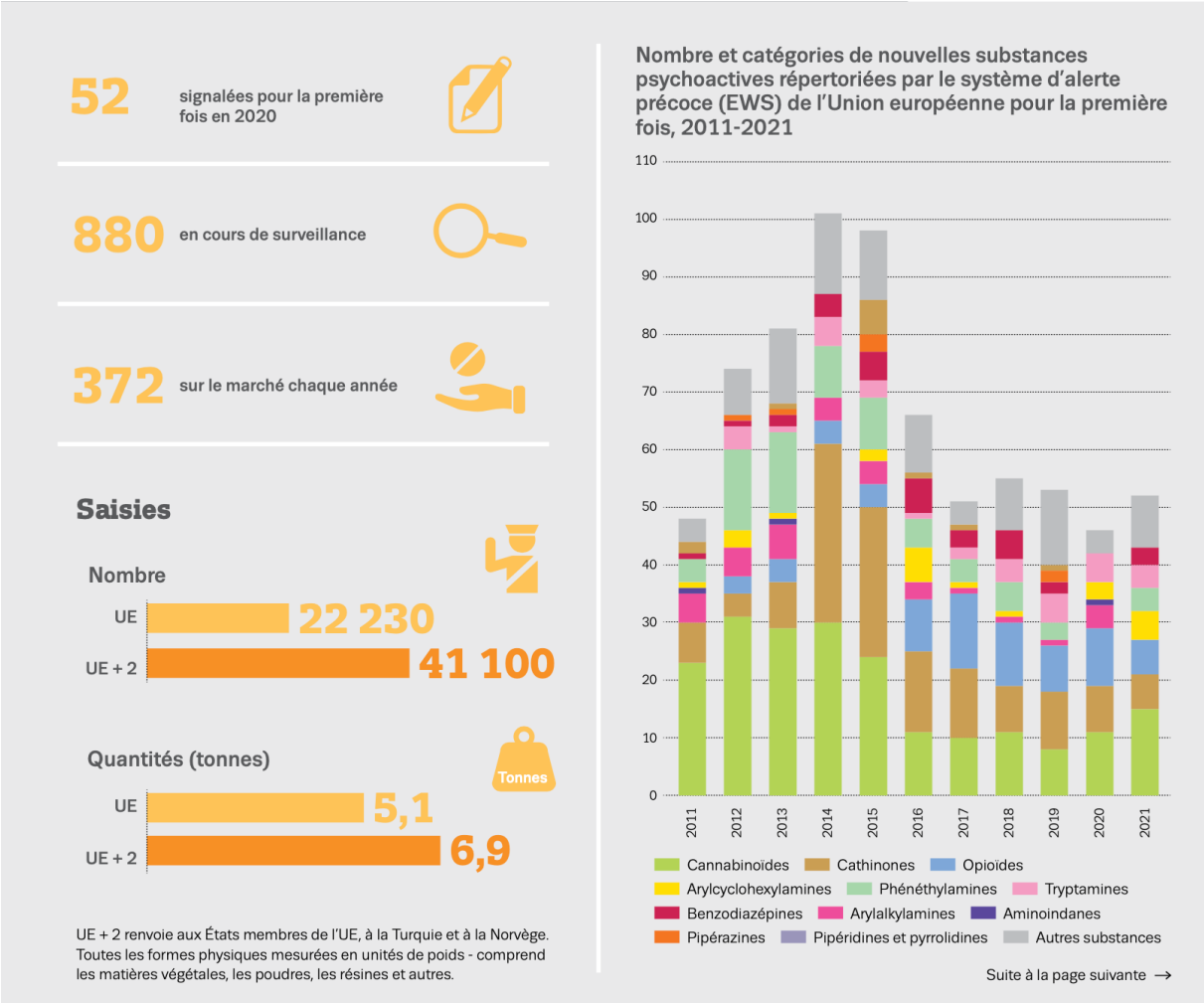


Figure 4. Données européennes sur les nouveaux produits de synthèse (d'après le « Rapport européen sur les drogues », EMCDDA 2022)

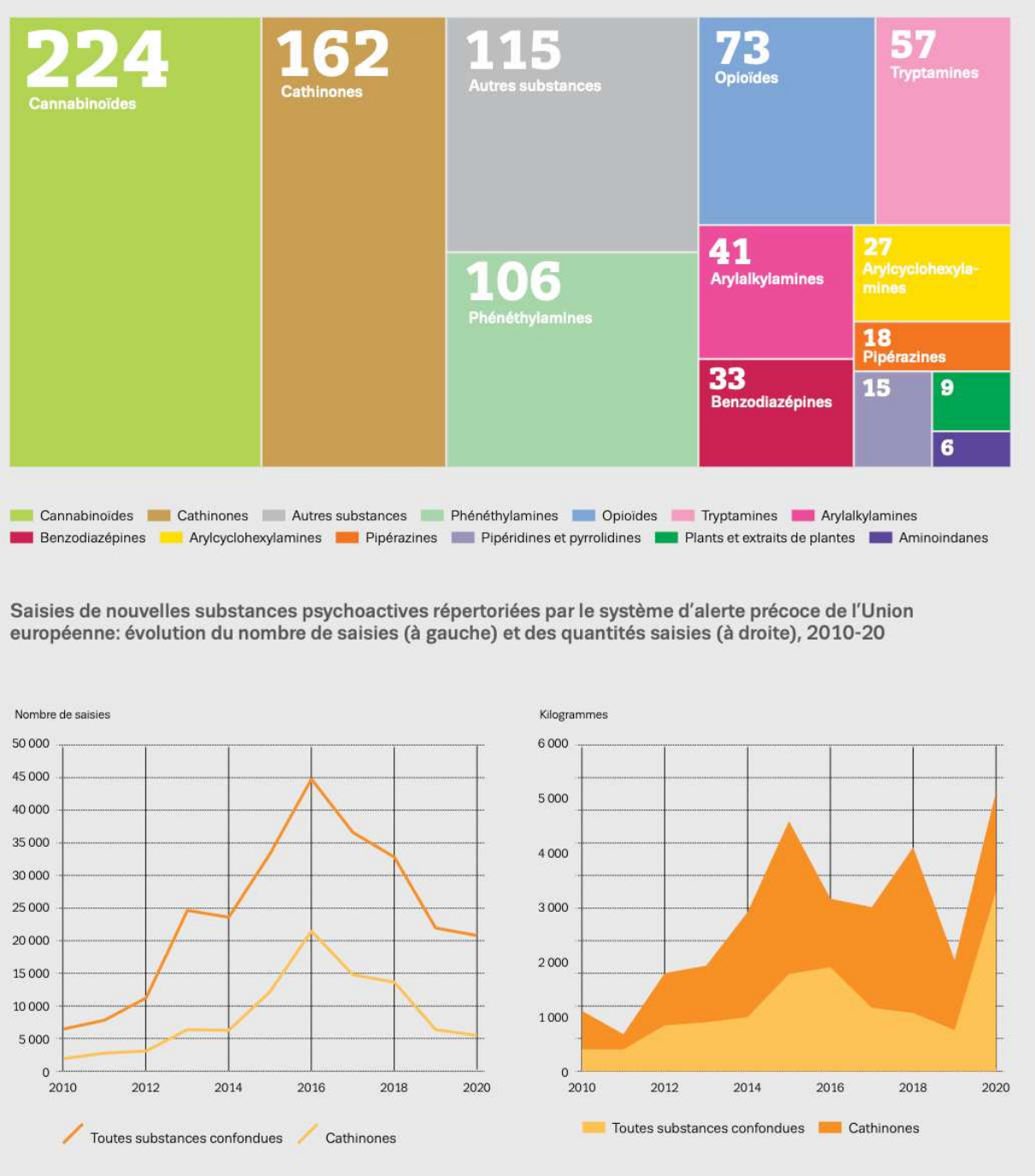


Figure 5. Nombres de NPS surveillés par le système d'alerte précoce de l'Union européenne, par catégorie (d'après le « Rapport européen sur les drogues », EMCDDA, 2022)

2. Conséquences de la consommation de NPS sur la santé publique

Les risques de complications liés à l'usage de NPS sont d'autant plus importants que leurs effets pharmacologiques sont souvent plus prononcés que ceux des molécules mères dont ils dérivent. De plus, les disparités entre le contenu réel des produits proposés à la vente et le contenu annoncé, en matière de quantité, qualité ou composition, sont à l'origine de plusieurs cas d'intoxications accidentelles, voire de décès (4,41–43).

Ces complications vont des convulsions à l'agitation, l'agressivité, la psychose (délire et paranoïa) aiguë ainsi qu'au développement potentiel de la dépendance (44–46). Les NPS sérotoninergiques (entactogènes et hallucinogènes) sont associés à des risques de syndrome sérotoninergique aigu, à l'hyperthermie, aux convulsions et à l'hyponatrémie. Les NPS dopaminergiques créent une forte dépendance et la toxicité aiguë comprend une stimulation prolongée, une insomnie, une agitation et une psychose.

D'autres symptômes peuvent apparaitre avec les cathinones de synthèse : tachycardie, douleurs thoraciques, hypertension artérielle, anomalies à l'ECG, mydriase, bruxisme, douleurs abdominales, vomissements, céphalées, œdème cérébral, tremblements, parkinsonisme, rhabdomyolyse, insuffisance rénale, vasoconstriction des extrémités (47,48). Plusieurs décès imputés aux dérivés de cathinones sont rapportés dans la littérature (49).

Les surdosages aux CS sont dominés par l'agitation, l'anxiété, la paranoïa, les rêves vifs et l'hallucination. Sur le plan somatique, il est possible d'observer une tachycardie, une hypertension, des convulsions, une perte de conscience, une dépression respiratoire, une rhabdomyolyse, une insuffisance rénale, et plus rarement un infarctus du myocarde (40,50,51). Plusieurs cas de décès ont été attribués aux CS seuls ou en association avec d'autres NPS (3).

Les nouveaux opioïdes de synthèse (NOS) provoquent un toxidrome pathognomonique des opiacés associant un trouble de conscience avec coma calme hypotonique, myosis bilatéral serré, bradypnée (fréquence respiratoire 12/min), bradycardie, hypotension, vomissements et constipation (32,42,52–54). Leurs effets peuvent être jusqu'à 10 000 fois plus puissants (par exemple, ceux du carfentanil) que ceux des opiacés classiques. Le risque de décès par dépression respiratoire sévère est majeur (42).

Les surdosages aux benzodiazépines de synthèse sont caractérisés par des troubles de la conscience et de la coordination, un coma calme hypotonique, des vertiges, une hypotension, et une amnésie antérograde. Le risque d'une dépression respiratoire sévère peut être majoré en cas d'association avec les opioïdes. Cependant, les décès attribués à cette classe de NPS demeurent rares (55).

3. Difficultés liées à l'analyse des NPS

La diversité des NPS et leur spectaculaire diffusion depuis leur réapparition sur le marché de drogues dans les années 2000 avaient incité le développement de nombreuses méthodes analytiques sensibles et fiables pour leur identification et quantification dans différentes matrices biologiques (sang, urine, salive, cheveux, etc.) ou non biologiques (poudres, liquides, plantes, etc.), dont le but était de permettre leur contrôle et de limiter leur dangerosité.

Classiquement, un dépistage par immunoanalyse est réalisé dans des laboratoires de biochimie ou de toxicologie non spécialisés sur des automates dotés d'une sensibilité et d'une rapidité largement suffisantes pour répondre à des situations d'urgence (par exemple, intoxications aigues). Cependant, ces techniques sont limitées au dépistage de drogues classiques (cocaïne, amphétamines, THC, opiacés) et de certains médicaments (par exemple, benzodiazépines, barbituriques, antidépresseurs), et ne permettent donc pas de détecter les NPS. De plus, elles ont l'inconvénient majeur de générer des résultats faussement positifs à cause des réactions croisées entre l'anticorps employé, et des composés endogènes ou exogènes présentant une similitude structurelle avec les composés d'intérêt, ce qui rend obligatoire la confirmation de tout résultat positif par une technique plus spécifique.

L'avènement des techniques séparatives telles que la chromatographie liquide (HPLC : chromatographie liquide haute performance) ou gazeuse (CPG : chromatographie en phase gazeuse), et leur couplage à la spectrométrie de masse simple (LC-MS, GC-MS) ou en tandem (LC-MS/MS, GC-MS/MS), ainsi que leur démocratisation ont permis de s'affranchir de certains problèmes liés aux techniques précédentes. Les spectromètres de masse sont considérés comme des balances de très haute précision pouvant identifier et doser une quantité de matière aussi infime que le yoctogramme (1 yoctogramme = 10^{-27} kg) par mesure des rapports de masse sur charge (ou m/z) des molécules d'intérêt (ex : NPS) soumises à un champ magnétique. Ces techniques ont révolutionné le champ de la toxicologie et le domaine

de l'analyse de traces en général, et ont permis d'analyser des matrices autrefois considérées comme trop complexes, tels que les cheveux.

Le spectre de masse correspond à l'empreinte spécifique d'une molécule, et présente d'une part, plusieurs fragments générés après la cassure de cette dernière et d'autre part, l'analyse ciblée des fragments les plus caractéristiques (d'où l'appellation « criblage » ou « screening ciblé »). Cette empreinte spectrale ne peut être partagée avec d'autres substances (à quelques exceptions près, dont les isomères de position), ce qui élimine la possibilité de tout faux-positif et confère une grande spécificité à ce type d'approche. Cependant, cette analyse dite « quadripolaire » (du nom de l'analyseur le plus employé), appelée aussi « spectrométrie de masse de basse résolution », nécessite l'établissement préalable d'une bibliothèque de spectres des molécules d'intérêt à l'aide de standards de référence achetés auprès des industriels. Le nombre de molécules recherchées par chaque laboratoire dépend donc de sa capacité à se procurer ces standards de référence, notamment ceux des NPS entrainant des problèmes de coûts (très élevé) et de disponibilité. On comprend maintenant pourquoi les différentes études évaluant la prévalence de NPS par cette approche analytique ciblée présentent une certaine hétérogénéité des résultats obtenus : en effet, elles sont directement conditionnées par le nombre et le type de molécules recherchées. Par conséquence, une sous-estimation du nombre de cas de NPS pouvant être identifiés doit être assumée de par la limite imposée par le nombre de composés contenus dans ces bibliothèques de spectres.

Plus récemment, une nouvelle approche de la spectrométrie de masse, dite « spectrométrie de masse haute résolution » ou HRMS (*High Resolution Mass Spectrometry*), basée sur le calcul de la masse exacte d'un nombre considérable de composés d'intérêt en toxicologie, a permis de s'affranchir de l'emploi des standards de référence. Des milliers de spectres de masse de haute résolution générés et conservés dans des bibliothèques peuvent être partagées en ligne (par exemple, sur HighResNPS®) et exploités gratuitement par tous les laboratoires qui disposent de cette technologie (56).

Les données scientifiques relatives à la pharmacologie et la toxicologie de ces nouveaux produits restent insuffisantes et des efforts sont à mener pour étudier leur mécanisme d'action, leur métabolisme et leur toxicité, afin que soient améliorées les connaissances dans ce domaine et que soit élaboré un plan d'action global de prévention.

II. La spectrométrie de masse haute résolution, un outil indispensable en toxicologie analytique ?

A. Principes généraux de la spectrométrie de masse haute résolution

Au cours des 40 dernières années, la toxicologie analytique a fait d'énormes progrès grâce aux évolutions technologiques : de la chromatographie en phase gazeuse couplée à la spectrométrie de masse (GC-MS), de la chromatographie en phase liquide couplée à la spectrométrie de masse (LC-MS), et plus récemment avec l'avènement de la spectrométrie de masse à haute résolution (HRMS). En 2010, la spectrométrie de masse à haute résolution était quasiment absente, même dans le milieu universitaire, mais aujourd'hui, grâce à une interface simplifiée et un coût moindre, la plupart des laboratoires de toxicologie possèdent leur propre spectromètre de masse Orbitrap ou à temps de vol (TOF). Le passage de la spectrométrie de masse à basse résolution (LRMS) au HRMS a non seulement amélioré la résolution des m/z mesurés (ce qui permet de déterminer la composition élémentaire des composés parents et des fragments), mais a également entraîné un profond changement dans la manière d'utiliser les spectromètres de masse. Les analyseurs de masse TOF et Orbitrap fonctionnent d'une manière différente du quadripôle, et sont utilisés en tant que spectromètres de masse hybrides (Q-TOF ou Q-Orbitrap). En outre, les développements technologiques ont donné lieu à la production de spectres MS^2 plus fiables et reproductibles, ce qui a permis l'augmentation de grandes bibliothèques de spectres de masse ainsi que de logiciels de traitement des données. Ces changements ont offert de nouveaux modes d'acquisition de données, plus adaptés à l'analyse de matrices complexes, et ont ouvert la voie au développement d'applications dans le domaine de la toxicologie (criblage, quantification, métabolisme, identification de biomarqueurs).

Depuis le développement des analyseurs de masse à temps de vol (TOF) en 1946 par Stephens, jusqu'aux premiers systèmes à triple quadripôle (TQ) dans les années 1980, ou l'analyseur de masse Orbitrap en 2000 par Makarov (57), différentes technologies et systèmes ont été développés avec un nombre assez similaire de champs d'application. En spectrométrie de masse, la résolution est une mesure de la capacité de l'instrument à distinguer deux pics de rapports masse/charge légèrement différents dans un spectre de masse. La mesure de la résolution par la méthode « *Full Width at Half Maximum* » (FWHM) permet de distinguer les systèmes LRMS modernes (TQ), avec une résolution d'environ 2 000 FWHM, des systèmes HRMS qui se définissent par une résolution allant jusqu'à 20 000 FWHM (58). Cette haute

sélectivité offre une plus grande certitude d'identification car elle permet la discrimination des composés isobares en MS^1 et l'identification du spectre en MS^2. De plus, l'acquisition d'un spectre MS^2 d'une molécule permet d'obtenir les masses précises des ions produits et leurs abondances isotopiques. Il s'agit d'un avantage majeur pour l'élucidation structurale et donc la découverte de nouvelles molécules. L'application est particulièrement intéressante pour la description de NPS et de leurs métabolites (59), pour la découverte de nouveaux biomarqueurs (60) et de molécules actives issues de plantes (61). En effet, certains systèmes HRMS bénéficient d'une nouvelle approche en MS^2, qui consiste en une gamme croissante d'énergies de collision (CE-spread) allant de valeurs faibles à élevées (62). En utilisant une telle approche, les spectres MS^2 acquis sont regroupés pour donner un spectre moyen (« hybride ») contenant tous les ions fils.

L'obtention d'une masse précise peut être très utile pour les applications ciblées et non ciblées. Dans le cas de l'analyse ciblée, l'avantage principal est le haut niveau de sélectivité, qui prévient le risque de faux positifs en excluant les interférences isobariques. La valeur ajoutée de l'analyse non ciblée est évidemment la possibilité d'une identification sans ambiguité et exhaustive des composés éventuellement présents dans un échantillon. Théoriquement, et contrairement au LRMS, le HRMS peut identifier un composé inconnu sans aucune information *a priori* sur sa structure ou ses voies de fragmentation. En effet, en MS^1, en utilisant un HRMS à balayage complet (*full scan*), chaque composé potentiellement présent dans un échantillon est théoriquement considéré et ses informations spectrales sont enregistrées (et peuvent être recherchées) en fonction de sa masse exacte. Les spectres MS^2 dérivés contiennent également des fragments spécifiques de chaque ion pseudo-moléculaire. Intuitivement, cela réduit de façon considérable l'incertitude de l'identification lorsqu'on compare un candidat à un spectre enregistré dans une bibliothèque (62). De plus, le fait de disposer d'informations précises sur chaque fragment est également très utile pour l'élucidation de la structure. Contrairement au LRMS, un criblage non ciblé par HRMS permet aussi une analyse rétrospective plus exhaustive et plus précise des acquisitions précédentes lorsqu'une analyse antérieure n'a pas réussi à identifier un composé détecté. C'est un réel avantage pour l'identification de nouvelles molécules comme les NPS (58). Par conséquent, le dépistage par HRMS est appliqué dans de nombreux domaines : la toxicologie clinique avec le *general unknown screening* (GUS), la toxicologie environnementale avec l'analyse d'échantillons de sol ou d'eau (63), la toxico-épidémiologie avec des applications pour la biosurveillance de l'exposome humain (62), ou la recherche de médicaments et de drogues illicites dans les eaux usées (14).

Les instruments HRMS de première génération étaient limités pour des applications qualitatives, alors que le LRMS était le *gold standard* pour la quantification dans les matrices biologiques (15). Cependant, les instruments les plus récents ont une sensibilité nettement améliorée. Ils offrent ainsi de meilleures performances de détection et de résolution (64). Actuellement, de nombreuses études soutiennent le fait que les systèmes HRMS peuvent maintenant défier les systèmes LRMS pour les applications quantitatives. Enfin, on peut dire que la sensibilité n'est pas systématiquement une limite pour l'HRMS. Une analyse au cas par cas est nécessaire pour évaluer si l'HRMS est adaptée ou non à une application quantitative.

L'exhaustivité théorique de l'approche HRMS produit une énorme quantité de données (ce qui implique des capacités de stockage accrues), jusqu'à 25 fois plus que celles produites par une approche LRMS classique (60). Ce phénomène est d'autant plus important lorsque la préparation des échantillons n'est pas optimale. Par conséquent, un traitement long et fastidieux des résultats par des analystes qualifiés est indispensable (58). En outre, il est nécessaire de recalibrer régulièrement le système avec des ions de référence pour maintenir un niveau élevé de précision de la masse et éviter les faux négatifs. Pour éviter cette opération chronophage, on peut proposer ce que l'on appelle une « calibration interne de la masse » qui consiste à calibrer pendant les acquisitions ; néanmoins, des phénomènes de suppression d'ions peuvent se produire à cause de la compétition en source entre les calibrants et les échantillons (65).

B. Principales technologies utilisées

1. Spectrométrie de masse à trappe orbitale (Orbitrap)

Le concept de piégeage orbital décrit par Kingdon en 1923 (66) a été appliqué dans le domaine de l'analyse par Alexander Makarov (57). Dans cette étude, un nouveau type d'analyseur de masse à piège électrostatique orbital a été présenté ; cet analyseur a ensuite été commercialisé sous la marque Orbitrap par Thermo Fisher Scientific®. Une conception spécifique du piège avec deux électrodes extérieures formant un tonneau, entourant une électrode centrale en forme de broche, a permis la production d'un champ électrostatique quadro-logarithmique. L'application d'une tension entre les électrodes intérieure et extérieure crée ce champ électrique linéaire. Lorsque les ions sont introduits tangentiellement dans l'analyseur de masse Orbitrap, ils subissent à la fois une attraction électrostatique et une force centrifuge, qui résultent de leur vitesse tangentielle. Ces éléments sont alors pressés vers l'électrode centrale en diminuant sa tension. Après l'arrêt de la baisse de tension, la trajectoire des ions devient une spirale stable. En raison de la forme des électrodes, dans la direction axiale, les ions sont forcés de s'éloigner de l'espace étroit pour se diriger vers l'espace plus large près de l'équateur, induisant des oscillations axiales harmoniques sans qu'aucune autre excitation ne soit nécessaire (67). La fréquence de rotation des ions étant dépendante de leurs énergies, angles et positions, les ions vont s'étendre rapidement sur la coordonnée angulaire et forment un anneau rotatif (figure 6).

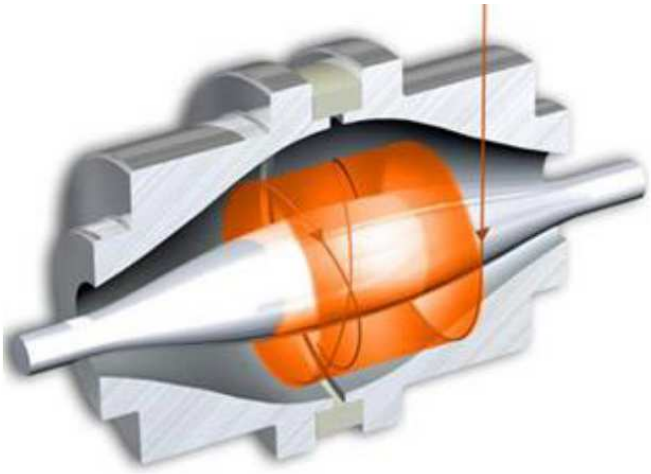


Figure 6. Trajectoire ionique en Orbitrap (d'après R. Thissen, 2014)

Les ions plus légers entrent dans l'analyseur de masse Orbitrap en premier formant un anneau rotatif d'une ellipticité différente de celle des ions plus lourds. Le mouvement ionique dans l'analyseur de masse Orbitrap est caractérisé par trois fréquences : la fréquence de rotation qui décrit le mouvement des ions autour de l'électrode centrale, la fréquence des oscillations radiales qui reflète le mouvement des ions entre les rayons maximum et minimum autour de l'électrode centrale, et la fréquence axiale qui décrit les oscillations harmoniques des ions le long de l'électrode centrale utilisée pour la détermination du m/z. L'oscillation harmonique le long de l'axe (la direction z) d'un anneau d'ions avec le même m/z, induit un courant alternatif sur les électrodes extérieures. Son amplitude est proportionnelle au nombre d'ions dans l'anneau, et il a la même fréquence que les oscillations axiales des ions dans l'anneau. Les électrodes extérieures agissent alors comme des plaques réceptrices qui détectent un courant, image de ces ions oscillants, ou transitoires. Le signal est amplifié, numérisé et transposé du domaine temporel au domaine fréquentiel à l'aide d'une transformée de Fourier (FT).

Pour pouvoir détecter et différencier des ions de masses très proches, les ions doivent diverger pendant le temps de détection, ce qui nécessite un grand nombre d'oscillations. Par conséquent, la résolution de l'analyseur de masse Orbitrap est proportionnelle au temps d'acquisition. Les oscillations des paquets d'ions restent stables si elles ne subissent pas de collisions avec les gaz résiduels. L'analyseur de masse Orbitrap travaille donc dans des conditions d'ultravide, de l'ordre de 10-10 mbar, qui garantissent des temps de transit d'environ une seconde ou plus, selon la taille de la molécule.

Les instruments quadripolaires-Orbitrap actuels, qui utilisent des analyseurs de masse Orbitrap à ultra-haut champ, peuvent atteindre une résolution allant jusqu'à 480 000 (FWHM) à m/z=200 pour le haut de gamme Orbitrap ExplorisTM 480 MS (Thermo Fisher Scientific®, Bremen, Allemagne), et jusqu'à 120 000 (FWHM) à m/z = 200 de résolution pour l'Orbitrap ExplorisTM 120 MS (Thermo Fisher Scientific®, Bremen, Allemagne). Les instruments quadripôles-Orbitrap actuels peuvent fonctionner avec des gammes de masse commençant à m/z = 40, et pouvant aller jusqu'à 3 000 pour l'Orbitrap Exploris 120 MS ou 8 000 pour d'autres instruments quadripôles-Orbitrap. La technologie quadripolaire-Orbitrap a également été adaptée à la chromatographie en phase gazeuse (GC). Les analyseurs de masse Orbitrap ont été utilisés dans les domaines de la toxicologie clinique et médico-légale pour les études du métabolisme des médicaments (68,69), la pharmacocinétique et la toxicocinétique des médicaments (70,71), le criblage (69,72–75) et les mesures quantitatives (76–81).

Les différents modes de balayage disponibles pour les instruments Orbitrap quadripolaires peuvent être non ciblés, comme l'acquisition *full scan*, et ciblés, comme le *single ion monitoring* (SIM) ou le MS^2 ciblé (82). Pour l'analyse quantitative, trois approches différentes peuvent être utilisées avec une sélectivité croissante : le balayage complet ou *full scan*, le SIM ou le MS^2 ciblé. Il est possible d'optimiser la quantification en combinant les trois modes d'acquisition. Les instruments à haute résolution permettent d'acquérir une quantité importante de données par rapport aux instruments à triple quadripôle. La taille d'un fichier va dépendre de nombreux paramètres, tels que le temps d'analyse et le type d'acquisition. Par conséquent, plus le type d'acquisition est complexe, plus la taille du fichier correspondant sera importante.

2. Spectrométrie de masse à temps de vol (TOF MS)

L'utilisation de la spectrométrie de masse haute résolution à temps de vol (TOF HRMS) a connu une croissance significative, notamment dans les domaines pharmaceutiques, biopharmaceutiques et cliniques. Pour soutenir cette progression, des améliorations ont été apportées aux performances analytiques et aux fonctionnalités des spectromètres de masse actuels, notamment en matière de résolution instrumentale, de sensibilité et de vitesse de balayage. Les progrès simultanés des logiciels de retraitement des données ont permis de réaliser des analyses ciblées et non ciblées à partir d'une seule acquisition MS.

Les instruments TOF d'aujourd'hui peuvent atteindre des valeurs de résolution allant de 20 000 à 60 000 dans des conditions réelles. Le temps de vol de chaque ion de m/z particulier est unique : déterminé par l'énergie (E) à laquelle un ion est accéléré, la distance (d) qu'il doit parcourir et sa masse (à proprement parler son rapport masse/charge), le temps de vol (t) commence lorsqu'une impulsion haute tension est appliquée à la plaque arrière de l'émetteur d'ions et se termine lorsque les ions concernés frappent le détecteur (Figure 7). Il existe deux formules bien connues qui s'appliquent à l'analyse du temps de vol. La première est la formule de l'énergie cinétique, c'est-à-dire l'énergie d'un objet (ou d'un ion) en mouvement, qui s'exprime par : $E = 1/2\ mv^2$, qui, résolue pour m, donne : $m = 2E/v^2$. La deuxième équation est la suivante : la vitesse (v) est égale à la distance (d) divisée par le temps (t) ou : $v = d/t$. En combinant la première et la deuxième équation, on obtient : $m = (2E/d^2)t^2$. Cela nous donne la relation de base du temps de vol. Pour une énergie (E) et une distance (d) données, la masse est proportionnelle au carré du temps de vol de l'ion. L'équation stipule que pour une énergie cinétique donnée, E, les petites masses auront des vitesses plus grandes, et les grandes masses

des vitesses plus petites. Par conséquent, les ions de faible masse arrivent plus tôt au détecteur.

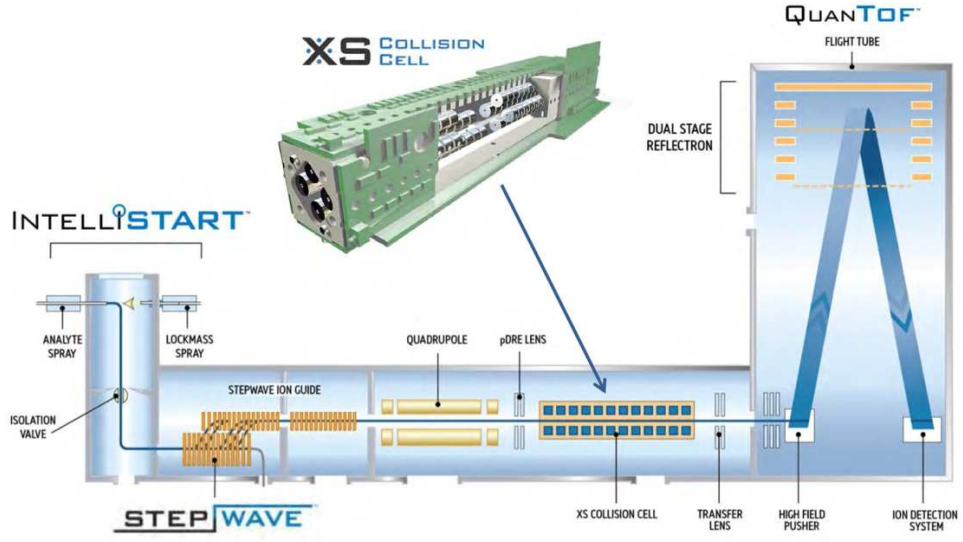


Figure 7. Schéma d'un instrument QTOF (d'après Waters®)

Lors de la conception d'un spectromètre de masse Q-TOF, de nombreux efforts sont déployés pour maintenir constantes les valeurs de l'énergie (déterminée par les hautes tensions) et de la distance parcourue par l'ion, afin qu'une mesure précise du temps de vol donne une valeur de masse précise. Comme ces paramètres sont maintenus constants, ils sont souvent combinés en une seule variable, A, comme suit : $m = A(t)^2$. Il s'agit d'une équation idéale, basée sur des temps de vol réels. En pratique, il y a un délai entre le moment où le système de commande envoie une impulsion de démarrage et le moment où la haute tension est effectivement présente sur les plaques de l'émetteur d'ions. Il y a également un délai entre le moment où un ion atteint la surface avant du détecteur, et celui où le signal généré par cet ion est numérisé par le système d'acquisition. Ces délais sont très courts, mais non négligeables. Comme le temps de vol réel ne peut pas être mesuré, il est nécessaire de corriger le temps mesuré, t_m, en soustrayant la somme des délais de démarrage et d'arrêt qui, lorsque additionnés, sont appelés t_0 : $t = t_m - t_0$. Par substitution, la formule de base qui peut être appliquée pour les mesures réelles devient : $m=A(t_m-t_0)^2$.

Pour effectuer la conversion du temps de vol mesuré, t_m, en masse, les valeurs de A et de t_0 doivent être déterminées, et un étalonnage est donc effectué. On analyse un mélange de composés dont les masses exactes sont connues avec une grande précision. On établit ensuite un tableau simple des temps de vol et des masses connues correspondantes.

Maintenant que m et t_m sont connus pour un certain nombre de valeurs dans la gamme de masses, l'ordinateur qui reçoit les données de l'instrument effectue les calculs pour déterminer A et t_0. À l'aide d'un algorithme intelligent, il essaie différentes valeurs de A et t_0 jusqu'à ce que le côté droit de l'équation d'étalonnage, $m = A(t_m - t_0)^2$, corresponde le plus possible au côté gauche de l'équation (m), pour les valeurs de masse du mélange d'étalons.

Bien que cette détermination initiale de A et t_0 soit très précise, elle ne l'est pas encore assez pour donner la meilleure précision de masse possible pour l'analyse par temps de vol. Ainsi, une deuxième étape d'étalonnage est nécessaire. Une fois que les coefficients d'étalonnage A et t_0 ont été déterminés, une comparaison est effectuée entre les valeurs de masse réelles des masses d'étalonnage et leurs valeurs calculées à partir de l'équation. Ces dernières ne s'écartent généralement que de quelques parties par million (ppm). Comme ces écarts sont faibles et relativement constants dans le temps, il est possible d'effectuer une deuxième correction pour obtenir un étalonnage de masse encore meilleur. Cela se fait par le biais d'une équation qui corrige les petits écarts sur l'ensemble de la plage de masse. Cette équation de correction est enregistrée dans le cadre de l'étalonnage de l'instrument. L'erreur de masse restante après cette méthode d'étalonnage en deux étapes, en négligeant tous les autres facteurs instrumentaux, est typiquement égale ou inférieure à 1 ppm sur la gamme des masses d'étalons.

Les améliorations apportées à l'instrumentation TOF ont conduit à son utilisation croissante dans un ensemble plus large d'applications. Les instruments TOF ont une large gamme de masses allant des rapports masse/charge de 10 m/z à 20 000 m/z. La taille des fichiers de données générés par l'HRMS TOF est variable en fonction des conditions d'acquisition mais est très souvent autour de centaines de mégaoctets (Mo) pour une analyse de trente minutes.

C. Acquisition des données

La figure 8 ci-après représente les différents modes d'acquisition en HRMS qui seront passés en revue dans cette partie :

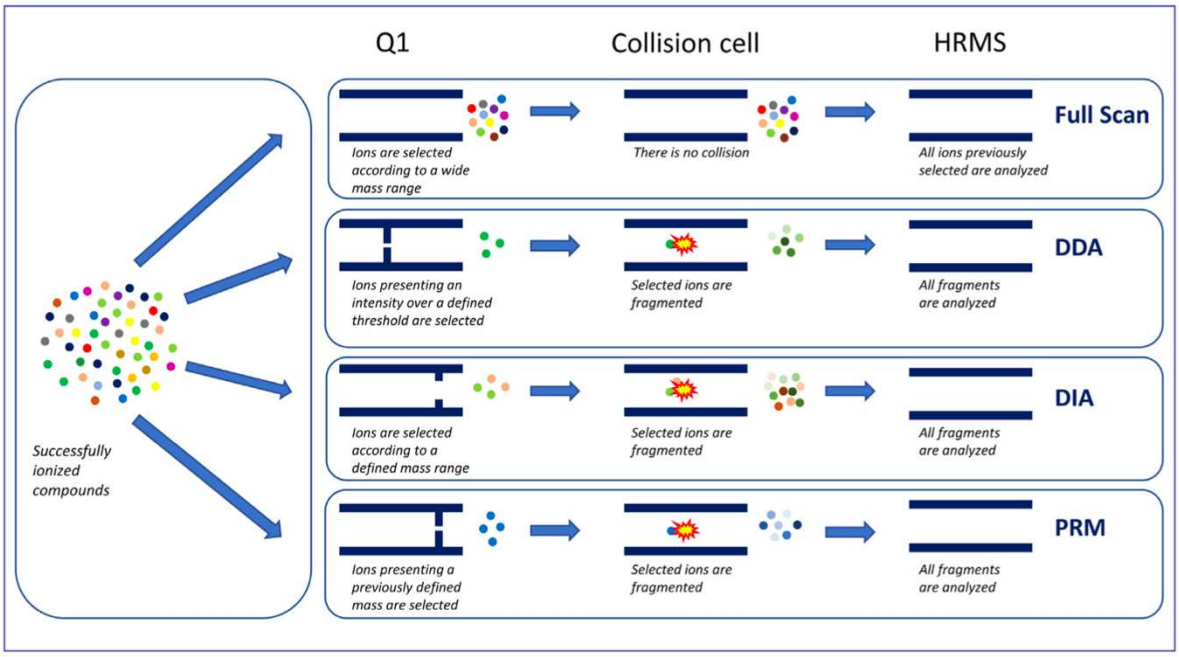


Figure 8. Schéma des principaux modes d'acquisition en spectrométrie de masse haute résolution (83)

1. Balayage complet ou *Full scan*

Pour les instruments à haute résolution, l'un des modes d'acquisition de base est celui à grande échelle, appelé « balayage complet » ou *full scan*. Par rapport à un triple quadripôle, le balayage complet, ayant un impact sur la sensibilité et la vitesse, est extrêmement utile pour les instruments à haute résolution, tant pour l'analyse quantitative que qualitative. Avec l'acquisition par balayage complet, aucune sélection n'est effectuée avant la collecte des données, et il n'y a aucune limitation du nombre d'analytes pouvant être étudiés. En fait, tout composé qui s'ionise et se trouve dans la plage de masse du balayage complet sera détecté. Cela présente des avantages considérables pour le criblage toxicologique notamment pour effectuer des analyses rétrospectives.

En outre, pour la quantification, l'analyse à balayage complet s'est avérée aussi sélective et sensible que l'analyse en *single reaction monitoring* (SRM) en triple quadripôle (15,67,84). L'acquisition par balayage complet en haute résolution permet d'étudier le profil isotopique

d'un composé et de l'utiliser pour l'élucidation de la structure, et donc pour l'identification (85). De plus, le balayage complet en acquisition haute résolution a l'avantage de générer deux points d'identification, ce qui est utile pour la confirmation (86). Ce type d'analyse fournit donc simultanément des informations quantitatives et qualitatives. En utilisant l'acquisition *full scan*, le développement de la méthode est beaucoup plus simple car il n'est pas nécessaire d'infuser des standards industriels. La sélection des molécules à analyser se fait après l'étape d'acquisition, ce qui offre des avantages significatifs pour les laboratoires de toxicologie d'urgence.

2. Acquisition dépendante des données (DDA)

Pour le l'étape de criblage, ainsi que pour l'analyse de confirmation, l'acquisition peut inclure des données MS^2. L'acquisition dépendante des données ou *data dependent acqquisition* (DDA) est un mode qui génère des données de fragmentation où la présence d'un composé d'intérêt, soit en balayage complet soit en balayage SIM, peut déclencher sa fragmentation. Le déclenchement peut être ciblé, basé sur une liste de composés à fragmenter, ou non ciblé, basé alors sur l'intensité du signal des ions dans le balayage complet. Le DDA non ciblé s'appuie uniquement sur l'acquisition du balayage complet avant le déclenchement des spectres MS^2 en fonction des données. Pour ce mode d'acquisition, l'instrument examine le spectre de masse du balayage complet et sélectionne les ions les plus intenses. Ensuite, ces ions sélectionnés seront filtrés par le quadripôle, fragmentés et analysés pour générer des spectres MS^2 en haute résolution. Pour les instruments Orbitrap, la méthode peut être configurée pour sélectionner le nombre d'ions qui seront fragmentés entre chaque balayage complet, et il est également possible de multiplexer les spectres MS^2. L'avantage de la méthode DDA non ciblée est qu'elle peut donner des informations MS^2 spécifiques pour des composés non ciblés. Cependant, cette méthode DDA est limitée lorsqu'il s'agit d'ions de faible intensité, car elle repose principalement sur l'intensité des composés dans le balayage complet. Différentes stratégies sont possibles pour améliorer les résultats de ce DDA non ciblé : la principale est d'exclure les composés les moins intéressants de la liste de fragmentation, moyennant une liste où sont définies la masse et éventuellement la fenêtre de temps de rétention du composé à exclure. Cette liste d'exclusion reste ciblée et doit être configurée dans la méthode de l'instrument. L'exclusion peut également être dynamique pendant l'analyse, les masses à peine

fragmentées pouvant être exclues pendant une période définie dans la méthode de l'instrument.

Une autre façon de gérer la faible intensité de certains ions dans le balayage complet est d'utiliser la DDA ciblée. Dans ce cas, le déclenchement de l'acquisition des spectres MS^2 exige que le composé d'intérêt donne une intensité de signal spécifiée, dans une certaine fenêtre de temps de rétention, et avec une précision de masse spécifique. L'inconvénient principal de cette méthode DDA concerne les composés inconnus. Une bibliothèque et une base de données commerciales de masse précise à haute résolution ont été générées sur la base de l'approche DDA avec plus de 2 000 molécules et plus de 3 000 de leurs métabolites (87). Cette approche a suscité un grand intérêt pour le criblage (88) et constitue aujourd'hui l'approche de base pour les criblages de routine par HRMS.

3. Acquisition indépendante des données (DIA)

L'acquisition indépendante des données ou *data independent acquisition* (DIA) est une méthode de détermination de la structure moléculaire dans laquelle tous les ions dans une plage m/z sélectionnée sont fragmentés et analysés. La DIA est devenue une technique établie dans la recherche « omique » (89). Elle est de plus en plus utilisée pour le dépistage des xénobiotiques, les métabolites de médicaments, les pesticides et d'autres substances toxiques (89–93).

Pendant de nombreuses années, la spectrométrie de masse en tandem (Triple Quad et QTRAP®) en DDA a été le *gold standard* du criblage en toxicologie médico-légale (94). Cependant, le suivi des MRM sur les spectromètres de masse triple-quadripôle n'est pas facilement adapté à l'identification de grands panels de composés car il repose sur le développement de méthodes ciblées. L'HRMS (dont les technologies Q-TOF et Orbitrap) a montré son utilité dans le *general unknown screening* (GUS) pour l'identification de composés non ciblés, réalisable uniquement à une masse précise en haute résolution. La compréhension des limites de la DDA a conduit à la recherche de méthodes alternatives d'acquisition de données pour le dépistage toxicologique par HRMS.

Aujourd'hui, on observe une évolution vers le HRMS incluant la possibilité de DIA (95). Plusieurs fabricants proposent différentes solutions de DIA sur le marché, dont les plus récentes sont vDIA de Thermo Fisher Scientific (96), MSE de Waters (97), tout Ion MS/MS (Agilent Technologies), Broadband CID (Bruker), MS/MS-DIA (Shimadzu) ou variable

window SWATH® de SCIEX (98). Le criblage non ciblé avec ces nouvelles méthodes DIA est sans biais (aucune cible préalable n'est nécessaire). Cette méthode offre la possibilité de détecter tous les analytes ionisés dans un échantillon et l'acquisition des spectres des ions fils pour chaque précurseur. Elle permet également une analyse rétrospective des données comme option supplémentaire. L'approche MSE utilisée dans le cadre de ce travail permet de collecter en continu un catalogue complet d'informations pour les ions parents et les ions fils en une seule analyse. Elle permet de collecter de façon alternative des spectres à basse et à haute énergie au cours de la même acquisition. La première fonction acquiert des données dans un état de basse énergie fournissant la masse exacte des ions parents. La seconde acquisition à haute d'énergie, fournit la masse exacte des ions du fils. Cette technique permet la détection / le dépistage rapide de composés par la recherche de leur masse exacte à partir de spectres complets générés en utilisant une énergie de collision faible. Il peut s'ensuivre une confirmation avec des standards de référence, ou une identification provisoire, en recherchant les masses exactes des ions fils dans les spectres acquis à une énergie de collision élevée (99–101).

4. *Parallel reaction monitoring – Multiple reaction monitoring (MRM)*

Le mode d'acquisition le plus sélectif est le MS^2 ciblé, également appelé « *parallel reaction monitoring* » ou PRM (Thermo Fisher Scientific), MRM (Agilent, Shimadzu), Tof-MRM (Waters) ou MRMHR (SCIEX). Il s'agit d'une technique où le quadripôle sélectionne la de l'ion parent dans une petite fenêtre de masse qui est ensuite fragmentée avec une énergie de collision spécifiée dans la cellule HCD (Orbitrap) ou la cellule de collision (QTOF), et pour laquelle tous les fragments sont ensuite détectés dans l'analyseur de masse (102,103). Ce mode est similaire à celui du balayage de l'ion fils dans les triples quadripôles, avec deux différences principales : les fragments sont acquis simultanément et à haute résolution, ce qui rend l'analyse encore plus sélective, et la sensibilité bien plus élevée, plus proche des modes SRM ou de la MRM avec les triples quadripôles. Les instruments HRMS permettent également le multiplexage d'expériences MS^2 ciblées.

D. Traitement des données

1. Identification des composés

Pour le criblage non ciblé, l'identification des composés est réalisée à l'aide d'outils logiciels et de bases de données. Une base de données de composés est une collection d'informations chromatographiques et spectrales utilisées afin d'identifier des composés dans un criblage toxicologique. L'HRMS fournit des informations importantes pour l'élucidation de la structure des composés, telles que la masse exacte, le schéma de fragmentation (masses des ions fils et leurs abondances relatives), la distribution isotopique, le temps de rétention (t_r) quand il est associé à une méthode de séparation, et la *collision cross section* (CCS) lorsqu'est utilisée la technologie de mobilité ionique. Une approche de criblage utilisant des bibliothèques de masse précises a été décrite pour la première fois par Gergov *et al*. en 2001 (104). Les auteurs ont construit leur propre bibliothèque basée sur des propriétés enregistrées avec un standard de référence et se sont servis de ces propriétés pour identifier les xénobiotiques dans les échantillons d'intérêt. En cas d'indisponibilité des standards, des biais d'identification peuvent survenir, conduisant à des faux positifs. Ojanperä *et al*., ont trouvé un taux de 39 % de faux positifs en criblant cinq échantillons d'urine avec une bibliothèque de 735 composés (105). En effet, selon la stratégie adoptée pour l'identification des composés, il existe différents niveaux/degrés de confiance. Un consensus sur ces niveaux a été proposé par le Chemical Analysis Working Group (CAWG) de la Metabolomics Standards Initiative (MSI) (106). Le niveau 1 est le plus haut niveau de confiance, définissant une identification sûre et définitive d'un composé : il est atteint lorsque deux ou plusieurs propriétés sont appariées avec des propriétés analysées dans les mêmes conditions analytiques. Il convient de noter que certains analytes nécessitent une prudence particulière en matière d'identification structurelle, comme le cas des isomères. Les niveaux 2 (élevé) et 3 (moyen) sont respectivement des caractéristiques supposées d'un composé ou d'une classe de composés. Ici, l'identification est basée sur la comparaison des données avec des bibliothèques publiques sans comparaison avec des standards dans les mêmes conditions analytiques. Comme décrit précédemment, ces méthodologies peuvent générer un nombre élevé d'erreurs d'identification et donc de faux positifs. Le niveau de confiance le plus bas (niveau 4) rassemble les composés inconnus. Bien que non identifiés et non classifiés, ils peuvent néanmoins être différenciés et quantifiés sur la base des données spectrales. En effet, la première étape de l'identification des caractéristiques est basée sur la comparaison des données avec les bases de données internes

ou externes existantes. Si aucune correspondance n'est possible, une élucidation de la structure *de novo* pourrait être effectuée sur la base de l'isolement de l'analyte associé à des prédictions *in silico* de ses caractéristiques.

2. Choix des bibliothèques

La construction d'une bibliothèque interne consiste à sélectionner et à acheter des standards disponibles dans le commerce afin d'obtenir leurs propriétés dans des conditions expérimentales prédéfinies qui seront utilisées pour l'analyse des échantillons. Même si ce type d'acquisition permet théoriquement d'identifier les composés avec le plus haut niveau de confiance, il est très vite couteux et chronophage si l'on considère le large panel de composés susceptibles d'être recherchés. Dans un échantillon d'intérêt, l'identification est alors réalisée en comparant les résultats expérimentaux à ceux obtenus à partir de standards et stockés dans la bibliothèque interne.

Contrairement aux bibliothèques internes, les bibliothèques externes ne contiennent que des informations sur les spectres de masse expérimentaux et/ou prédits, ainsi que sur la masse exacte. Pour cette raison, le temps de rétention ne peut pas être utilisé pour l'identification des composés. On les emploie largement car elles sont en libre accès et donc représente un gain de temps. Dans les deux cas, une très grande attention doit être accordée aux conditions dans lesquelles sont générées ces bases de données. Il faut préférer les conditions les plus proches des conditions expérimentales retenues. Par exemple, les bases de données peuvent permettre l'identification à partir de données de balayage complet (comme PubChem (107), Chemspider (108), entre autres), ou à partir de données MS/MS ou MSn (comme Massbank (109), mzCloud de Thermo Fisher Scientific). En effectuant l'identification avec des bibliothèques externes, un score de correspondance des propriétés pourrait être généré sur la base de la comparaison entre les données attendues et celles observées, ce qui permettrait de classer les candidats si nécessaire. Plus récemment, l'avènement des nouvelles bibliothèques partagées en toxicologie apporte de nombreux avantages qui seront abordés dans la revue de la littérature n°1 publiée dans le cadre de ce travail.

3. Prédictions *in silico*

Dans l'analyse toxicologique, il ne faut pas oublier que les métabolites des xénobiotiques peuvent être d'un grand intérêt et qu'ils doivent être correctement identifiés (110). Le problème majeur est qu'ils sont très souvent absents des bibliothèques (111). Dans ces cas, certains logiciels comme MetaSite, Metabolite Predict ou MetWorks, par exemple, sont capables de prédire le métabolisme *in silico* (112) permettant d'obtenir le t_r (113) ou le CCS (114). Cette prédiction est basée sur les principales réactions enzymatiques du métabolisme (hydroxylation, déshydrogénation, déméthylation, etc.). Les spectres théoriques sont générés pour chaque métabolite et ensuite comparés à ceux obtenus pour le composé non identifié.

On peut isoler la substance ou le métabolite d'intérêt par une analyse RMN pour l'élucidation de la structure (115) et une analyse LC-MS/MS combinée à la prédiction *in silico* des propriétés de la molécule (116). Cette approche peut être complétée par des outils d'interprétation comme les réseaux moléculaires. Cela permet de mieux élucider les processus de métabolisation, et placer les composants identifiés dans un contexte biologique plus complet.

4. Métabolomique

La métabolomique est une stratégie informatique qui peut aider à la visualisation et à l'interprétation des données complexes obtenues par l'analyse MS. Elle permet d'identifier les similitudes potentielles entre les spectres dans l'ensemble de données et les comparer à des molécules inconnues qui seraient apparentées (117). Cette approche est basée sur l'hypothèse que les molécules structurellement apparentées produisent des schémas de fragmentation similaires, et qu'elles sont donc liées au sein d'un réseau de fragments (118). Dans la métabolomique, les données HRMS sont reproduites sous forme de graphique, où chaque nœud représente un ion avec un spectre de fragmentation associé. Les liens entre les nœuds indiquent les similitudes entre les spectres. La comparaison des informations structurelles au sein du réseau donne lieu à la mise en évidence des molécules inconnues mais structurellement apparentées (119).

E. Intérêt de la spectrométrie de masse haute résolution en toxicologie analytique : focus sur les substances psychoactives – Revue de la littérature n° 1

1. Introduction et présentation

La spectrométrie de masse a une longue histoire dans les laboratoires de toxicologie. Depuis son développement dans les années 1990, la chromatographie liquide (LC), associée à la spectrométrie de masse (MS) ou à la spectrométrie de masse en tandem (MS/MS), est devenue une technique complémentaire de la chromatographie en phase gazeuse couplée à la spectrométrie de masse (GC-MS). Utilisée à l'origine principalement pour l'analyse qualitative et quantitative ciblée d'un nombre limité de composés, la recherche « non ciblée » a ouvert de nouvelles possibilités pour le dépistage toxicologique. Cela permet la détection et la quantification d'un grand nombre de molécules dans un échantillon de composition inconnue, notamment en toxicologie clinique et médico-légale. Les NPS représentent un grand défi pour les toxicologues car les standards de référence sont encore très coûteux ou non disponibles. La plupart de leurs métabolites sont également inconnus. Des techniques plus sophistiquées sont donc nécessaires pour s'affranchir de ces problèmes. Si la LC-MS/MS est capable de déterminer les caractéristiques spectrales d'un médicament donné, son manque de précision en masse ne lui permet parfois pas de distinguer deux molécules de masse identique ayant les mêmes fragments ; une technologie plus précise est donc nécessaire. Plus récemment, la MS haute résolution (HRMS) a montré sa supériorité par rapport aux autres méthodes en matière de spécificité et de flexibilité.

Ce premier article a pour objectif de passer en revue les applications et les innovations de l'HRMS en toxicologie humaine (avantages et limites), en particulier en toxicologie clinique et médico-légale, en se concentrant sur les SPA, y compris les NPS.

2. Discussion

L'HRMS dans le domaine de la toxicologie analytique a gagné du terrain ces dernières années, offrant une plateforme extrêmement fiable pour le dépistage ciblé et non ciblé de substances psychoactives et leurs métabolites. Les avantages de l'HRMS en toxicologie sont nombreux. En effet, l'HRMS donne lieu à la détermination de la masse exacte d'une molécule

avec une précision proche de 1 ppm (120). Cette résolution permet de séparer les pics de composés ayant des rapports masse sur charge (m/z) proches. Il est également possible d'attribuer une formule moléculaire exacte, ce qui limite le nombre de composés candidats. La sensibilité de l'HRMS est également beaucoup plus élevée que celle de la SM basse résolution (LRMS), ce qui est particulièrement utile dans les expertises médico-légales où la notion de seuil est importante. De plus, l'amélioration de la spécificité et des performances de séparation chromatographique de l'HRMS a permis de différencier des isomères ou des molécules ayant des masses exactes très proches, ce qui était auparavant délicat (121). Enfin, elle donne accès au développement de méthodes qualitatives et quantitatives.

Dans le cadre du dépistage des NPS, l'HRMS représente le *gold standard* grâce à sa capacité à acquérir des données MS à balayage complet, qui peuvent ensuite être traitées rétrospectivement (122,123). Cela consiste à cribler un très grand nombre de composés d'intérêt dans des échantillons complexes en utilisant leur formule moléculaire (et la masse exacte calculée qui en résulte) si aucun standard de référence n'est disponible (124). En fonction de la stratégie analytique choisie par le laboratoire, il existe plusieurs excellents outils gratuits en ligne pour aider à l'analyse des NPS, telles que les bibliothèques partagées. L'idée ici est d'alléger la charge d'un seul laboratoire et d'améliorer le savoir-faire collectif à travers l'utilisation et le partage de l'ensemble des données d'un groupe de pairs. En effet, employer des sources de données externes peut contribuer à la détection des nouveaux NPS émergents par les laboratoires du monde entier.

L'avènement des méthodes bio-informatiques au cours des dernières années a fait progresser les données LC-HRMS, notamment en ce qui concerne l'analyse approfondie du métabolisme des xénobiotiques (*in vivo* et *in vitro)* et la découverte de nouveaux marqueurs de consommation de ces substances. Dans ce contexte, de nouveaux outils bio-informatiques puissants apparaissent également, tels que les réseaux moléculaires ou la métabolomique, dans lesquels les étapes d'optimisation incluent le retraitement des données acquises (110,119,125).

Revue de la littérature n° 1

Suitability of high-resolution mass spectrometry in analytical toxicology: focus on drugs of abuse

Ruben Goncalves, Romain Pelletier, Aurélien Couette, Thomas Gicquel, Brendan Le Daré

Publié en janvier 2022 dans *Toxicologie Analytique et Clinique* (ToxAC)

Volume 34, Issue 1, Supplement, Pages 29-41

https://doi.org/10.1016/j.toxac.2021.11.006

Toxicologie Analytique & Clinique xxx (xxxx) xxx–xxx

ELSEVIER

Disponible en ligne sur
ScienceDirect
www.sciencedirect.com

Elsevier Masson France
EM|consulte
www.em-consulte.com

ORIGINAL ARTICLE

Suitability of high-resolution mass spectrometry in analytical toxicology: Focus on drugs of abuse

Ruben Goncalves[a,b,*], **Romain Pelletier**[c], **Aurélien Couette**[c], **Thomas Gicquel**[c,d], **Brendan Le Daré**[d,e]

[a] *Forensic and Clinical Toxicology Laboratory, CHU de Bordeaux, 33000 Bordeaux, France*
[b] *Inserm, BPH, U1219, University Bordeaux, 33000 Bordeaux, France*
[c] *Forensic and Clinical Toxicology Laboratory, CHU de Rennes, 35033 Rennes, France*
[d] *Inserm, INRAE, Institut NuMeCan (Nutrition, Metabolismes and Cancer), PREVITOX network, CHU de Rennes, University Rennes, 35033 Rennes, France*
[e] *Pharmacy Department, CHU de Rennes, 35033 Rennes, France*

Received 3 September 2021; received in revised form 26 November 2021; accepted 26 November 2021

KEYWORDS
High resolution mass spectrometry;
New psychoactive substances;
Drugs of abuse;
Drug metabolism;
Online libraries

Summary High-resolution mass spectrometry (HRMS) is now the method of choice in several toxicology contexts. This paper reviews HRMS approaches for research and application in various toxicology fields, focusing on drugs of abuse in clinical and forensic toxicology. Papers concerning HRMS applications in screening, quantification and metabolism of drugs of abuse in biological and non-biological samples were included. Specific applications for new psychoactive substances in contexts such as online libraries, bioinformatic tools (molecular networking) and methods combinations were also included.
© 2021 Société Française de Toxicologie Analytique. Published by Elsevier Masson SAS. All rights reserved.

DoA, Drugs of abuse; OFDT, French Observatory of Drugs and Drug Addiction; NPS, new psychoactive substances; RC, research chemicals; LC, liquid chromatography; MS, single-stage mass spectrometry; MS/MS, tandem mass spectrometry; GC-MS, gas chromatography coupled to mass spectrometry; HRMS, high-resolution mass spectrometry; QTOF-MS, quadrupole-coupled time-of-flight mass spectrometry; IA, immunoassay; SPE, Solid-phase extraction; LLE, liquid-liquid extraction; NCD, nitrogen chemiluminescence detection; FTIR, Fourier-transform infrared; NMR, nuclear magnetic resonance; MN, molecular networks.

* Corresponding author.
E-mail address: ruben.goncalves@chu-bordeaux.fr (R. Goncalves).

https://doi.org/10.1016/j.toxac.2021.11.006
2352-0078/© 2021 Société Française de Toxicologie Analytique. Published by Elsevier Masson SAS. All rights reserved.

Please cite this article as: R. Goncalves, R. Pelletier, A. Couette et al., Suitability of high-resolution mass spectrometry in analytical toxicology: Focus on drugs of abuse, Toxicologie Analytique & Clinique, https://doi.org/10.1016/j.toxac.2021.11.006

Introduction

Drugs of abuse (DoA) are psychoactive substances that affect mood or behavior and are consumed for non-medical purposes. Their use is likely to induce addiction and psychic or somatic disturbances. For these reasons, DoA are defined and legally regulated at the international level.

In France, ''the illicit transport, possession, supply, transfer, acquisition or use of narcotics'' is punishable by ten years' imprisonment and a fine of 7,500,000 euros according to Article 222-37 of the Penal Code (2002) [1]. It is also forbidden to drive under the influence of narcotics according to the French Highway Code, a rule that differs from one country to another [2]. In 2019, data from the French Observatory of Drugs and Drug Addiction (OFDT) reported different consumption levels depending on the substance used: cannabis (45% of experimenters, 11% of users in the year) and cocaine (1.6% of users in the year) are widely consumed in France. However, other illicit drugs are marginally used by adults (5% of experimenters use hallucinogenic mushrooms or MDMA/ecstasy) [3]. Classified at the international level by the Single Convention of 1961, DoA were transposed into French law by the decree of 22 February 1990. This classification includes cannabis (THC and its metabolites), cocaine (and its metabolite benzoylecgonine), opiates (opium, morphine, etc.), amphetamines (e.g. MDMA), and some of the new psychoactive substances (NPS) such as mephedrone and benzylpiperazine [4].

The detection and quantitation of DoA is of major importance from a forensic and public health point of view and represents an analytical challenge due to the wide variety of molecules used, particularly with the arrival of NPS. Since the 2000s, more and more NPS, also called research chemicals (RC), ''legal highs'', ''designer drugs'' and ''synthetic drugs'', have appeared on the drug market with the advent of the Dark Web. In general, only one function or chemical moiety differs from the original molecule, thus modifying its pharmacological properties. This makes the use of a molecule legal, simply because the derivative is not yet registered on the list of narcotic products [5]. These molecules are of great interest for toxicologists, even if the constant renewal of substances and the difficulty of obtaining reference materials make it difficult to analyze and detect them. Above all, by investigation their consumption, toxicologists can report information on these emerging molecules to national and international authorities.

Mass spectrometry has a long history in toxicology laboratories. Since its development in the 1990s, liquid chromatography (LC) coupled to single-stage mass spectrometry (MS) or tandem mass spectrometry (MS/MS) has evolved as a complementary technique to the gold standard of gas chromatography coupled to mass spectrometry (GC-MS). Originally used mainly for targeted qualitative and quantitative analysis of a limited number of compounds, the broad spectrum of scan types of modern LC-MS(/MS) have opened new possibilities for non-targeted toxicological screening. This allows the detection and quantification of a large number of molecules in a sample of unknown composition, particularly in clinical and forensic toxicology [6]. While LC-MS/MS is able to determine the characteristics of a given drug, its lack of mass accuracy sometimes does not allow it to distinguish two molecules of the same mass and with the same fragments, so a more accurate technology is needed.

More recently, high-resolution MS (HRMS) has proved superior to other methods in terms of specificity and flexibility, allowing the exact mass of the molecule to be determined with an accuracy close to 1 ppm mass resolution [7]. This resolution enables peaks of compounds with close m/z to be separated. It is also possible to assign an exact molecular formula, thus limiting the number of candidate compounds. The sensitivity of HRMS is also much higher than low resolution MS, which is particularly useful in forensic or roadside expertise cases where the notion of threshold is important. Furthermore, improvements in the specificity and chromatographic separation performance of HRMS have made it possible to differentiate isomers or molecules with very similar accurate masses, which was previously tricky [8].

In 2012, researchers showed that the chromatographic system influenced the mass accuracy of untargeted HRMS. In particular, they fund that high chromatographic separation power is essential to obtain high-quality HRMS data, as the mass accuracy and thus the number of detected compounds are strongly influenced by the co-eluting compounds [9]. This also demonstrated the need to optimize HRMS-based detection methods, especially when the results are for use in clinical or forensic settings. In this context, new powerful bioinformatics tools are also emerging such as molecular networks or metabolomics, since the optimization steps include the reprocessing of the acquired data [10,11]. Despite their great diversity, more and more databases used in HRMS for toxicological screening, listing NPS in particular, are emerging and allow faster detection, efficient medical management and retrospective data processing.

This paper reviews the applications and innovations of HRMS in human toxicology (advantages and limitations), especially in clinical and forensic toxicology focusing on DoA including NPS.

General applications for drugs of abuse including new psychoactive substances

The advent of LC-MS/MS has revolutionized DoA detection and HRMS goes one step further. In addition, HRMS has led to much more efficient screening methods both in terms of sensitivity and specificity, especially for forensic screening to identify drugs without a clinical context. NPS are also of great interest to toxicologists since the reference standards are still very expensive or not available and most of the metabolites are unknown. More sophisticated techniques are therefore required to solve these problems. In this section, we present the advantages of HRMS for the identifying and quantifying DoA including NPS.

Screening for drugs of abuse

Toxicological screening is a specific approach to analytical toxicology that allows the detection and simultaneous identification of a large number of compounds [12]. DoA screening is suitable for both forensic and clinical applications.

In the emergency hospital setting, the rapid delivery of results is an important prerequisite as an early diagnosis of intoxication may improve patient prognosis. Since urine is the major matrix for drug screening, immunoassay techniques predominate in most hospital laboratories because of their rapidity and ease of execution. However, only a few relevant molecules can be covered by currently available immunoassay techniques: classical DoA (THC, cocaine, opiates, MDMA and amphetamines are usually detected) while some drugs require specific chromatography coupled to mass spectrometry such as NPS due to their lack of commercial alternatives. Kronstrand et al. compared the performance of an immunoassay (IA) for synthetic cannabinoids with a recently developed confirmatory method using quadrupole-coupled time-of-flight mass spectrometry (QTOF-MS). The MS procedure proved superior, as new compounds and their metabolites can be quickly included and thus identified [13]. The advantage of HRMS over the still widely used IA techniques is its high flexibility, which allows toxicologists to react to the dynamic and ever-changing NPS market.

In forensic toxicology, screening should allow the identification of drugs without reference to any clinical context. The same biological matrices are used as in clinical toxicology, but the difference is that the data are used for judicial purposes. In addition, the application of analytical methods on post-mortem samples involves dealing with sometimes putrefied samples and variable matrices [14]. In this context, it is important to have comprehensive and specific screening methods such as LC-HRMS to identify new or rare drugs, or metabolites that can shed light on the investigation.

HRMS screening procedures are mainly qualitative and can be applied to most matrices, e.g. urine, serum, hair, or whole blood samples. Most chromatographic systems are coupled to an LC-QTOF or an Orbitrap. The main difference between most HRMS and MS/MS methods is that HRMS is flexible and cater for non-targeted screening. Previously, HRMS was considered best suited for multi-component targeting and the screening of unknowns. Nowadays, large-scale screening by HRMS is one of the most powerful and efficient analytical tools in the NPS field. HRMS accurate establishes the mass of analytes and their fragments, thereby allowing elucidation of their molecular formulas. The list of possible candidates can be narrowed down to one or a limited number of compounds, which can be further investigated in the available databases. In other words, the analysis can be performed initially without reference standards, because the overall information provided by HRMS (full-spectrum acquisition, precise mass of the (de)protonated molecule and relevant fragment ions, and isotopic profile) is relevant and allows the putative identification of the detected compounds [15]. The ability to screen without having standards a priori is very useful, as laboratories do not need to purchase all the reference standards prior to analysis, with the attendant problems of cost and expiration dates. Reference standards are needed for final confirmation, but only as a last step, when there is strong, well-founded evidence that the compound has been identified in the sample. The addition of new NPS into existing qualitative workflows is straightforward and only requires the availability of reference compounds. An advantage of HRMS accurate-mass full-spectrum acquisition is the possibility of performing retrospective analysis. Thus, while initial data inspection may involve a targeted approach, designer analogues that are unreported or unknown at the time of analysis can be targeted subsequently in post-acquisition data processing without the need for additional analysis. This is mainly the case for NPS, which are often absent from them [16].

HRMS has not changed the role that separative methods plays upstream of detection. Chromatography, whether liquid or gas, has pride of place in the final detection of compounds [16,17]. The way in which the molecules are derived, the solvents used and the preparation of the samples play an important role in the final detection, especially for isobaric substances. For example, the NPS beta-keto-2C-B ((2-amino-1-(4-bromo-2,5-dimethoxyphenyl)ethenone; exact mass: 274.0079 Da) and DOB (2,5-dimethoxy-4-bromamphetamine; exact mass: 274.0364 Da) can be differentiated by selecting the protonated masses of the precursors and by comparing the different elemental compositions of certain fragment ions [18,19].

Incorporating metabolites into urine screening procedures is more difficult [20]. Reference NPS metabolite standards are usually not immediately available and may have already been withdrawn from the market. Kinyua et al. presented a qualitative screening method also based on all-ion MS to allow the detection and tentative identification of NPS and their metabolites [21]. An HRMS method was also published for the simultaneous screening and confirmation of selected synthetic cannabinoids, cathinones, amphetamines, natural cannabinoids, opioids, cocaine, and other important DoAs, as well as their major metabolites in urine samples [22]. Solid-phase extraction (SPE) was used as sample preparation prior to ultra-high performance liquid chromatography and TOF-MS using fragmentation without precursor selection (all-ion MS/MS) to avoid loss of information. Application data were included for 50 authentic urine samples. However, particularly extensive sample preparation, such as SPE and liquid-liquid extraction (LLE), could be replaced by simple dilution or precipitation using these highly sensitive systems [23,24]. This approach is also contributive in judicial seizures, allowing the rapid identification of products and subsequent legal sanctions. Furthermore, the detection of impurities and cutting products is easier, sometimes revealing a ''fingerprint'' that allows the location of the manufacturers to be traced.

These techniques also have limitations such as the cost of instruments, the complexity of data processing software and the requirement of skilled analysts [25]. Other disadvantages of HRMS are the enormous size of the data, which can be a problem in terms of data storage. In addition, the examination of unknown HR data is laborious and time-consuming, and the analyst must be familiar with MS. Moreover, HRMS does not always provide additional knowledge. This is the case with already characterized molecules, for which detection and separation methods are robust. For example, roadside narcotics as defined by French legislation comprise only four families of well-known molecules: amphetamines, cocaine, cannabis, opiates, so HRMS is of little interest in this setting. HRMS systems will become more attractive not only as their cost decreases over time but also with the advent of more robust software that is easier to use for identifying new compounds. However, HRMS may hold promise in situations where a combination of screening

and quantification is required. Overall, HRMS appears to be the gold standard for non-targeted screening procedures because of its high flexibility, sensitivity, and selectivity.

Quantification of drugs of abuse

After DoA detection, their quantification allows the prediction of potential damage from intoxication, especially in blood. DoA should be quantified in most clinical and forensic cases of overdose. This is a prerequisite for evidence-based case interpretation, which can have a significant impact on clinical or forensic consequences and outcomes [26]. This is particularly the case in clinical and forensic toxicology, where blood concentrations can be compared to reference values in order to evaluate the degree of intoxication. Quantification also allows the kinetics of elimination to be assessed, a necessary step in the follow-up of acute intoxication or withdrawal. Currently, LC-MS/MS is the most widely used technique for (multi-target) quantification [27—29]. However, thanks to the high sensitivity it achieves by excluding all compounds with different elemental composition, LC-HRMS has become a powerful alternative for DoA quantification. Several recent studies have quantified DoA and/or their metabolites in biological samples using LC-HRMS with TOF or Orbitrap mass analyzers [30]. These are summarized in Table 1. Multi-analyte LC-HRMS approaches to DoA quantification allow for much easier method development without the need for extensive MS optimization for each analyte and a limitation on the number of compounds investigated. This is also the case for ''pharmaco-toxics'' such as buprenorphine and ketamine, where dosing techniques are now well established [31,32]. The Q-TOF detection and quantification of cathinones, hallucinogenic phenethylamines, and piperazines after salting-out assisted LLE with acetonitrile in whole blood was presented by Pasin et al. [33]. Identification was based on the precise masses of the molecular ion, the two ions produced, and the retention time and quantification performed over the range of 0.05—2 mg/L. Concheiro et al. described the simultaneous quantification of 28 synthetic cathinones and metabolites [34] as well as 40 novel psychoactive stimulants in urine by LC-HRMS [35]. A particularly interesting application for simultaneous identification and quantification of NPS in blood without the need for authentic reference standards was published by Ojanpera et al. [36]. They combined gas chromatography coupled with nitrogen chemiluminescence detection (NCD) and atmospheric pressure chemical ionization Q-TOF MS. The GC stream was split between NCD for single-calibration quantification, using the equimolar response of the detector to nitrogen, and Q-TOF MS for identification. This configuration showed promise for simultaneous qualitative and quantitative drug analysis in the absence of authentic reference standards.

Innovative applications for new psychoactive substances

HRMS online libraries

An analytical reference standard is essential in forensic and clinical screening procedures for unequivocal identification. Even if they are available, however, the cost of purchasing samples of each NPS is prohibitive, given their volume and diversity. Moreover, delays in the manufacture and/or delivery of these products are unavoidable, so newly emerging drugs may be overlooked. A full-scan screening workflow may be needed to keep the laboratory up to speed for evaluating new cases where new NPS drugs may be present in the sample(s) being analyzed. This consists of screening large lists of compounds of interest in complex samples using their molecular formula (and the resulting calculated exact mass) if no reference standards are available [49]. Regardless of how a laboratory chooses to handle the issue, there are several excellent free online tools to support NPS analysis, some of which are crowd- and open-sourced. One of the key ideas of crowd-sourced science is to alleviate the burden of a single lab and improve collective know-how by sharing user data and using the summed data of a group of peers. Hopefully, the implementation of crowd-sourcing science in NPS screening and intelligence will help to improve the quality and coverage of NPS analysis in forensic toxicology. Indeed, the use of external data sources can help laboratories around the world to detect emerging NPS by qualitative screening.

Query online compound databases and/or perform structure elucidation

In untargeted screening for NPS analysis, peak selection and filtering are performed on the full HRMS data to select a smaller number of peaks that may provide a clue about an NPS. In the untargeted approach, peak searching involves extracting all detectable peaks in a single analysis using a peak selection algorithm to generate a list of candidate masses that can be reliably used for identification. This shortlist of candidate masses can then be compared to existing MS libraries and/or used for mass spectral structure elucidation. The peak selection algorithm uses raw full-scan MS data (without prior knowledge of specific ions) to search for peaks that have a common elution profile. The masses are then grouped into compound masses, resulting in a list of candidate masses. The algorithm can apply user-defined compound filters, including blank subtraction, peak intensity and ion number to facilitate peak prioritization. This approach significantly reduces false positives and limits features that do not contain enough information to result in an identification attempt. The list of accurate candidate masses can then be subjected to molecular formula assignment and/or compared to a list of suspects, and the results can be confirmed using online MS data.

Acquire/purchase curated spectral databases for NPS

Purchasing a mass spectra database for NPS screening ensures that the screening data are accurate, collected systematically, and often accompanied by recommended LC and MS acquisition parameters and workflows for use in the intended contexts. This screening strategy does not rely on free online tools, but it is a very feasible alternative. HRMS screening libraries can be purchased, such as the ''LC-HRMS/MS Library of Drugs, Poisons, and Their Metabolites'' [50], the ''Mass Spectra of Designer Drugs'' [51], or the ''Wiley Registry of Tandem Mass Spectral Data, MS for ID''

Table 1 Examples of HRMS methods for drugs of abuse.

Analytes	Matrix	Sample preparation	HRMS technology	Qual/quant	Ref.
THC	Urine	LLE	Orbitrap	Quant	[8]
Multidrug including cocaine, amphetamines, and opiates	Blood	PP	MS^E-TOF	Quant	[37]
Methadone, Buprenorphine	Blood	LLE	Orbitrap	Quant	[38]
Ketamine	Hair	DPE	Orbitrap	Quant	[39]
> 2500 toxic compounds	Blood, urine	PP	QTOF	Qual	[40]
39 NPS	Urine	SALLE	QTOF	Quant	[41]
47 synthetic cannabinoid metabolites	Urine	SLE	QTOF	Quant	[42]
30 acidic and neutral pharmaceuticals	Blood	SPE	MS^E-TOF	Qual	[43]
30 common DoA	DBS	SLE	Orbitrap	Qual	[44]
Fentanyl and analogs	Oral fluid	SPE	QTOF	Quant	[45]
MDMA and phase II metabolites	Urine	SPE	Orbitrap	Quant	[46]
28 cathinones and metabolites	Urine	SPE	Orbitrap	Quant	[47]
Hallucinogens and opioids	Blood	LLE	Orbitrap (ThermoFisher)	Quant	[48]
LSD, GHB	No analytical method found in the literature				

LLE: liquid/liquid extraction; PP: protein precipitation; DPE: decontamination; pulverization, extraction; SALLE: Salting-out Liquid-Liquid Extraction; SLE: Solid-Liquid extraction; DBS: Dried blood spot; Qual: qualitative; Quant: quantitative.

[52]. Waters®, Thermo Fisher®, Agilent® and SCIEX® also provide spectral libraries tailored to their respective instruments.

Download crowd-sourced screening library

MS data from one instrument can be used as reference data on another MS device with comparable parameters for screening purposes [53]. For this reason, several users can share and use screening data. These data sources include data repositories such as the NPS Data Center [54], the RESPONSE database [55] or HRMS databases such as Massbank [56], MzCloud [57] or HighResNPS [58] (Table 2). The HighResNPS.com homepage was designed as a repository of HRMS data for NPS detection that can easily be converted into a screening library. The primary molecular identifier on HighResNPS is the InChIKey, which provides an unambiguous identifier, as opposed to a given name, SMILES string, or IUPAC name. Each data entry must include the protonated molecular ion and optionally one to three diagnostic fragment ions. Each user adds information about the analytical setup they use to generate their results. If some users have identical analytical setups, they can also use retention times with the MS data, which increases confidence in detection. HRMS identification of unknowns using HighResNPS does not distinguish between positional isomers, which could account for most of the incorrectly assigned NPS. In addition, HighResNPS.com has been used successfully to identify NPS in seizures [59], environmental samples [60], and for the retrospective analysis of post-mortem biological samples [61]. In the current setup, data quality in HighResNPS increases when multiple users add data on the same compound. Unlike the NPS datahub, user-entered data are not manually verified and classified. Diagnostic fragment ions are added to the database with the molecular formula and exact mass, reducing the risk of rounding errors or mistakenly adding exact masses. Two limitations of HighResNPS.com are the manual data curation and the lack of peer reviewing. Continuous curation is an advantage of the commercial databases, which have designated funds and labor for this. When a given NPS has multiple analytical entries in the database, the data self-validate, since different users with different standards are unlikely to report the same faulty data. Thus, if a positive hit is obtained after screening with HighResNPS, the user can further support identification by retrieving full HRMS spectra on MzCloud and/or additional information on NPS Data Hub, and ultimately obtain an unambiguous identification with an analytical reference standard. Note that when identification is based on HighResNPS screening data, it must always be confirmed using additional identification techniques, multiple analytical targets and/or analytical reference material.

Combination with other structural identification methods

The confirmation of NPS identification requires the development of a specific, high-quality, comprehensive method. Toxicology laboratories are therefore limited and must adapt to the material they can purchase and try to set up legally acceptable exchanges between colleagues. In addition to the difficulties associated with molecule characterization such as identification, quantification and purity, analytical

Table 2 Examples of crowed-sourced online libraries available for HRMS.

Online library	Type	References
General libraries		
Massbank	HRMS database public repository for small compounds (< 3000 Da)	[56]
MzCloud	HRMS database	[57]
NPS libraries		
NPS data hub	Web-based community driven	[54]
RESPONSE database	analytical data repository	[55]
HighResNPS	HRMS database	[58]

Table 3 Studies of HRMS methods combinations for NPS identification.

Context	Matrix	Analytes	HR-MS technique	Combined methods	Ref.
Seizure	Powders; crystals	NPSs	LC-Orbitrap	GC-MS; NMR	[62]
Seizure	Tablet powder	bk-2C-B	LC-Orbitrap	NMR	[19]
Seizure	Powder	deschloroketamine	LC-Orbitrap	GC-MS; NMR	[19]
Seizure	Powders	3-MeO-PCP; 3-MMC	LC-Orbitrap	sd-GC-FTIR	[70]
Seizure	41 powders	NPSs	GC-QTOF	NMR	[64]
Users	5 powders	SC	LC-QTOF	NMR; IR; GC-MS	[63]

NPS: new psychoactive substances; SC: synthetic cannabinoids.

methods used routinely are not always sufficient to firmly identify NPS, especially in cases of powders or e-liquids. It is therefore sometimes necessary to combine several analytical methods. A selection of studies dealing with combination methods is summarized in Table 3.

For example, the analysis of seized drugs depends on the available instrumental facilities. Traditionally, this includes a combination of analytical techniques such as GC-MS, LC-MS, Fourier-transform infrared (FTIR) spectroscopy and nuclear magnetic resonance (NMR) spectroscopy for confirmation and structural elucidation [62]. NMR is a physical phenomenon in which spin (most commonly 1H, 13C, 19F, 31P) placed in a strong magnetic field absorbs and re-emits electromagnetic radiation. This method not only makes it possible to identify a compound unambiguously: it also confirms the absence of other organic compounds and determines the purity of a product [63—66]. FTIR is gaining ground as the gold standard for fast non-destructive analysis and the detection of different compounds in complex matrices. When used alone, however, all these methods have limitations for the unequivocal identification of an unknown molecule [62]. For example, LC, which is the most widely used separative method, may not discriminate positional isomers (such as chloro-N, N-dimethylcathinone (CDC) and chloroethcathinone (CEC) or 3-MMC and mephedrone (4-MMC)) [59,67]. In LC-MS/MS, their retention time may be identical as well as their fragmentation pattern. It is therefore necessary to use another method, such as nuclear magnetic resonance (NMR) spectroscopy, GC-MS, infrared spectroscopy or supercritical fluid chromatography (SFC) [68] to discriminate the structures. The availability of GC/MS libraries helps greatly in the identification of new drugs. For relatively pure samples, a GC—MS analysis together with an FTIR-spectrum can lead to the full identification of the substance if both spectra give a match with existing data. Unfortunately, in most cases involving NPS, commercial GC-MS libraries do not contain novel analogues. In this case, HRMS is often used to perform MS/MS to evaluate collision-induced dissociation (CID) pathways for putative structural elucidation, followed by confirmation using NMR and/or IR spectroscopy (Fig. 1) [69]. The combination of results from HRMS and NMR with data obtained by MS, MS/MS and IR can help to elucidate the structures of unknown substances prior to the syntheses of ad hoc standards. Systematic use of the multi-methods approach allows the structural definition and successful confirmation of unknown samples thanks to powerful complementary analytical techniques, despite the lack of a reference standard (Table 4).

Contribution of HRMS in metabolism studies

New varieties of NPS are constantly emerging on the market yet data on their pharmacodynamics or pharmacokinetics are lacking. This is especially an issue for compounds that are extensively metabolized such as synthetic cannabinoids [71] and NBOMe [72], where the parent compound itself is not a reliable target. To increase the chances of detecting the consumption of these NPS to increase the detection window in biological matrices, the identification of NPS metabolites appears unavoidable so metabolism studies need to be done. This is the case for both clinical and forensic cases, especially in matrices known to accumulate metabolites such as bile and urine.

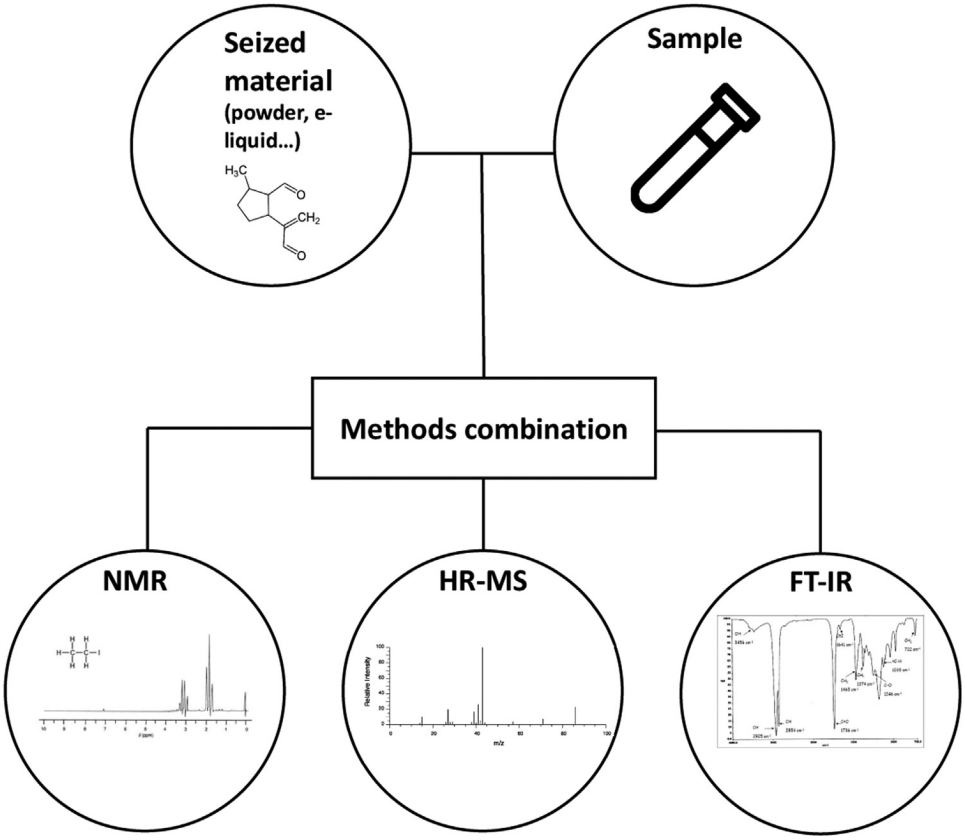


Figure 1. Illustration of methods combination with spectra.

Table 4 Methods for identifying unknown powders.

Step	Objective	Methods
1	Study of mass spectrometric behavior using electron impact ionization	GC-MS
2	Study of accurate molecular mass, raw formulas and isotopic clusters obtained without fragmentation	LC-HRMS (ESI+)
3	Applying different fragmentation voltages to study accurate fragment masses obtained to determine their structures	LC-HRMS (ESI+)
4	Molecular structure evaluation by molecular weight determination and significant fragment interpretation using these two methods	GC-MS/LC-HRMS (ESI+)
5	Isomer identification in absence of certified standard	NMR/FTIR

Study models

Before the analytical phase involving mass spectrometry, care must be taken in choosing the right model for characterizing metabolites, since this conditions the relevance of the subsequent steps. For ethical reasons, pharmacokinetic studies in humans are not usually carried out as soon as an NPS emerges, and animal experiments should be conducted parsimoniously. Consequently, several in vitro models for identifying the metabolism of NPS have been designed to anticipate the behavior of these xenobiotics *in vivo* [73].

Primary human hepatocytes (PHH) are considered the gold standard in in vitro metabolism studies, as they express relevant human enzyme clusters, co-substrates, and drug transporters [74,75]. It is now possible to explore the metabolism of numerous NPS such as cannabinoids [71,76], cathinones [77] NBOMe [78] or benzodiazepines [79]. However, their limited availability, high cost, variability in the expression of metabolic enzymes and the loss of their phenotype over time may limit their applicability.

Table 5 Bioinformatics tools used in xenobiotic metabolic exploration.

Software	Purpose	References
Metabolism prediction		
FAME 2	Prediction of sites of metabolism	[94]
SMARTCyp		[95]
SOMP		[96]
Xenosite		[97]
CYPReact		[98]
BioTransformer	Prediction of sites of metabolism and metabolite structures	[99]
MetaSite		[100]
META Ultra		[101]
GLORY		[102]
MetaTox	Web application for predicting structure and toxicity of xenobiotic metabolites	[103]
Tandem mass-spectrometry data processing and analysis		
MZMINE	Open-source software for mass-spectrometry data	[104]
OpenMS	processing, visualizing and analysis	[105]
Tandem mass-spectrometry data visualization		
SIRIUS 4	Turn tandem mass spectra into metabolite structure information	[106]
GNPS	Open-source software for visualizing tandem mass spectrometry data and facilitating identification and	[92]
METGEM	discovery	[107]
CYTOSCAPE	Open-source software for visualization and analysis of complex networks	[108]

Metabolism studies should be conducted in a fast, simple, cost-efficient and reliable way. Consequently, alternative stable cell sources have emerged, allowing NPS metabolism studies in pooled human liver microsomes (pHLM) [80—83], pooled human S9 fraction (pS9) [73,84] and differentiated HepaRG cells [83,85]. Interestingly, these three models have shown comparable results to PHH for identifying both major phase I and phase II metabolites [86]. The HepG2 human hepatoma cell line, another model capable of expressing metabolic enzymes, has also been used, although it produces a lower number and lower abundance of metabolites compared to HepaRG [87]. Data of in vitro model systems were also compared to human urinary metabolites [73]. Surprisingly, no 3D model seems to have been used to study NPS metabolism so far.

In recent years, zebrafish (*Danto reto*) larvae (embryos) have increasingly been used as a new in vivo model to study metabolism [73,85], and a recent study revealed the NPS metabolism of new cannabinoids by using zebrafish [88]. NPS metabolism in vivo investigations are frequently performed using authentic clinical or forensic cases [89,90]. They provide data that reflect the behavior of xenobiotics in the body and take into account the absorption, distribution, and elimination of the substance, which also underlines the relevance of urinary consumption markers. The importance of this work has even led some researchers to swallow NPS to study their pharmacokinetic parameters [91].

Bioinformatic tools

HRMS coupled with bioinformatics methods is very effective in identifying metabolites. Table 5 shows some examples of bioinformatics tools allowing: (i) the prediction of metabolism sites and metabolite structures; (ii) the processing and analysis of mass spectrometry data; and (iii) the visualization of mass spectrometry data. Recently, molecular networks (MN) have led to the organization and representation of tandem mass spectrometry (MS/MS) data in a graphical form [92]. MN visually represent chemical similiarity among analytes in an untargeted MS/MS analysis. Each node represents an ion and its associated fragmentation spectrum, and the links between the nodes indicate similarities between spectra (Fig. 2). Since some mass differences correspond to well known biotransformation reactions, it is possible to determine the structure of potential metabolites by information propagation. In addition, the MN approach offers valuable insights into drug metabolism by facilitating sample-to-sample comparison, and increases the number of metabolites compared to HRMS analysis without data reprocessing [93].

The Multimatrix approach provides a semi-quantitative visualization of molecule distribution in different samples. It has already shown its value in the analysis of drug metabolism, *in vivo* [90,109,110], in vitro [111], and in samples of suspected substances consumed, even when metabolites are poorly expressed [112]. Interestingly, MN combining interpretation of NPS metabolism data obtained in vivo and by different in vitro models would therefore be of particular interest in the future.

To go further, combined in silico analysis by creating MN of high-precision spectral data ($[M+H]^+$ and fragments) from different sources (in vitro and in vivo/in cadaver): (i) provides a solution for identifying unknown metabolites of NPS; and (ii) represents a visual model for interpreting and understanding toxicological results in the context of a death involving NPS.

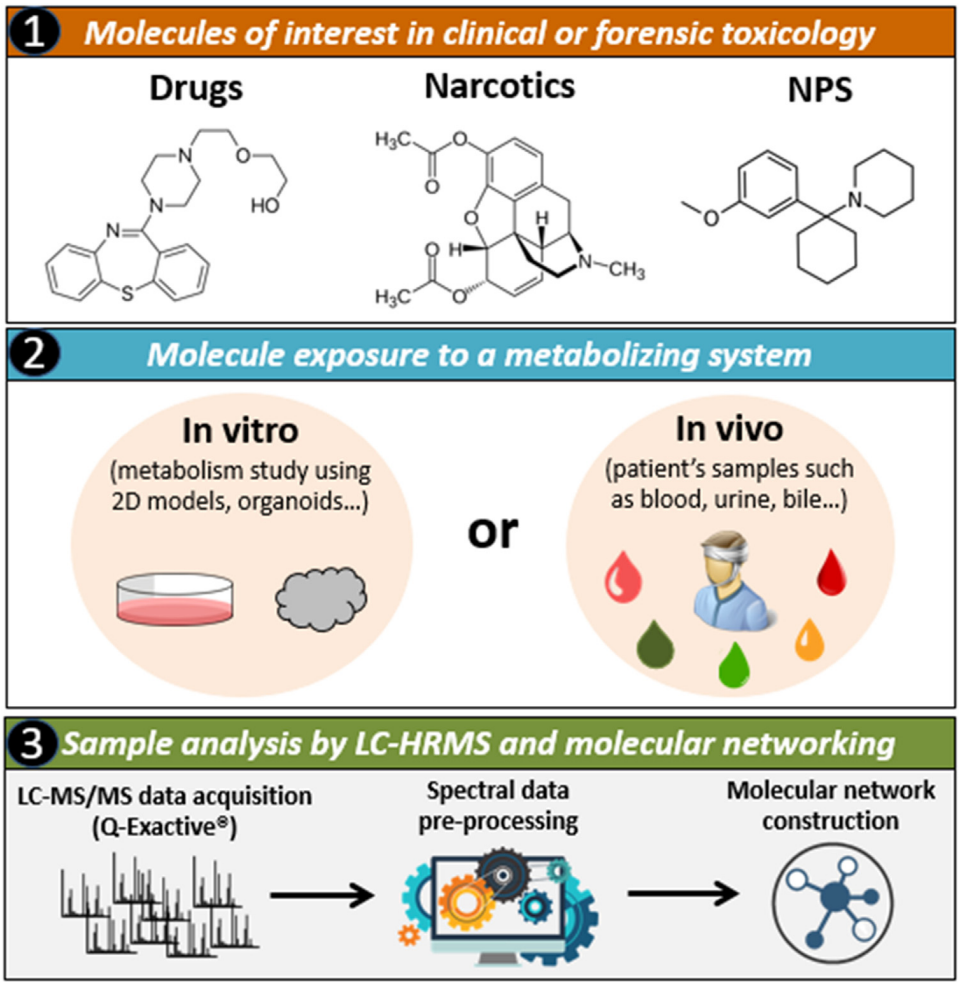


Figure 2. Principles of metabolism study using LC-HRMS and molecular networking.

Conclusion

HRMS in forensic drug chemistry and analytical toxicology has gained ground in recent years, providing a highly versatile analytical platform for the targeted and non-targeted screening of suspected metabolites. It is highly sensitive and specific for DoA identification and allows the development of qualitative and quantitative methods. It is clearly the gold standard for analyzing samples suspected of containing NPS thanks to its ability to acquire full-scan MS data that can then be assessed retrospectively. The advent of bioinformatics methods in recent years has taken LC-HRMS data one step further, allowing for the in-depth analysis of xenobiotic metabolism, both in vivo and in vitro, and leading to the discovery of new markers of consumption of these substances.

Disclosure of interest

The authors declare that they have no competing interest.

References

[1] Article 222-37 - Code pénal - Légifrance [Internet]. [cited 2021 Aug 20]. Available from: https://www.legifrance.gouv.fr/codes/article_lc/LEGIARTI000006417724.
[2] Article L235-1 - Code de la route - Légifrance [Internet]. [cited 2021 Feb 24]. Available from: https://www.legifrance.gouv.fr/codes/article_lc/LEGIARTI000031927915/2016-01-28/.
[3] Drogues et addictions, données essentielles [Internet]. [cited 2021 Aug 20]. Available from: https://www.ofdt.fr/BDD/publications/docs/DADE2019.pdf.
[4] Règlementation relative à l'addictovigilance - ANSM (Classement des produits stupéfiants et psychotropes au niveau international 2013) [Internet]. [cited 2021 Aug 20]. Available from: https://ansm.sante.fr/documents/reference/reglementation-relative-aux-differentes-vigilances/reglementation-relative-a-laddictovigilance.
[5] Madras BK. The growing problem of New Psychoactive Substances (NPS). In: Baumann MH, Glennon RA, Wiley JL, editors. Neuropharmacology of New Psychoactive Substances (NPS). Cham: Springer International Publishing; 2016. p. 1—18 [cited 2021 Aug 30. Current Topics in Behavioral Neurosciences; vol. 32].

[6] Remane D, Wissenbach DK, Peters FT. Recent advances of liquid chromatography—(tandem) mass spectrometry in clinical and forensic toxicology — An update. Clin Biochem 2016;49(13—14):1051—71.
[7] Knolhoff AM, Callahan JH, Croley TR. Mass accuracy and isotopic abundance measurements for HR-MS instrumentation: capabilities for non-targeted analyses. J Am Soc Mass Spectrom 2014;25(7):1285—94.
[8] Mohamed MK, Takyi-Williams J, Baudot B, Grobler A. Development and validation of a LC-HRMS method for the quantification of cannabinoids and their metabolites in human plasma. Eur J Pharm Sci 2021;159:105705.
[9] Croley TR, White KD, Callahan JH, Musser SM. The chromatographic role in high resolution mass spectrometry for non-targeted analysis. J Am Soc Mass Spectrom 2012;23(9):1569—78.
[10] Vincenti F, Montesano C, Di Ottavio F, Gregori A, Compagnone D, Sergi M, et al. Molecular networking: a useful tool for the identification of new psychoactive substances in seizures by LC—HRMS. Front Chem 2020;8:572952.
[11] Uawisetwathana U, Plaisen S, Arayamethakorn S, Jitthiang P, Rungrassamee W. Optimization of metabolite extraction and analytical methods from shrimp intestine for metabolomics profile analysis using LC-HRMS/MS. Metabolomics 2021;17(1):8.
[12] Guitton J, Gaulier J-M, Citterio-Quentin A, Abe E, Allibé N, Bartoli M, et al. Accreditation of the toxicological screening: recommendations of the SFBC-SFTA group. Ann Biol Clin (Paris) 2019;77(2):219—24.
[13] Kronstrand R, Brinkhagen L, Birath-Karlsson C, Roman M, Josefsson M. LC-QTOF-MS as a superior strategy to immunoassay for the comprehensive analysis of synthetic cannabinoids in urine. Anal Bioanal Chem 2014;406(15):3599—609.
[14] Drummer OH. Post-mortem toxicology. Forensic Sci Int 2007;165(2—3):199—203.
[15] Favretto D, Pascali JP, Tagliaro F. New challenges and innovation in forensic toxicology: focus on the ''New Psychoactive Substances.''. J Chromatogr A 2013;1287:84—95.
[16] Gundersen POM, Broecker S, Slørdal L, Spigset O, Josefsson M. Retrospective screening of synthetic cannabinoids, synthetic opioids and designer benzodiazepines in data files from forensic post mortem samples analysed by UHPLC-QTOF-MS from 2014 to 2018. Forensic Sci Int 2020;311:110274.
[17] Partridge E, Trobbiani S, Stockham P, Charlwood C, Kostakis C. A Case Study Involving U-47700. Diclazepam and flubromazepam-application of retrospective analysis of HRMS data. J Anal Toxicol 2018;42(9):655—60.
[18] Power JD, Kavanagh P, O'Brien J, Barry M, Twamley B, Talbot B, et al. Test purchase, identification and synthesis of 2-amino-1-(4-bromo-2, 5-dimethoxyphenyl)ethan-1-one (bk-2C-B). Drug Test Anal 2015;7(6):512—8.
[19] Frison G, Zamengo L, Zancanaro F, Tisato F, Traldi P. Characterization of the designer drug deschloroketamine (2-methylamino-2-phenylcyclohexanone) by gas chromatography/mass spectrometry, liquid chromatography /high-resolution mass spectrometry, multistage mass spectrometry, and nuclear magnetic resonance. Rapid Commun Mass Spectrom RCM 2016;30(1):151—60.
[20] Lee HK, Ho CS, Iu YPH, Lai PSJ, Shek CC, Lo Y-C, et al. Development of a broad toxicological screening technique for urine using ultra-performance liquid chromatography and time-of-flight mass spectrometry. Anal Chim Acta 2009;649(1):80—90.
[21] Kinyua J, Negreira N, Ibáñez M, Bijlsma L, Hernández F, Covaci A, et al. A data-independent acquisition workflow for qualitative screening of new psychoactive substances in biological samples. Anal Bioanal Chem 2015;407(29):8773—85.
[22] Sundström M, Pelander A, Angerer V, Hutter M, Kneisel S, Ojanperä I. A high-sensitivity ultra-high performance liquid chromatography/high-resolution time-of-flight mass spectrometry (UHPLC-HR-TOFMS) method for screening synthetic cannabinoids and other drugs of abuse in urine. Anal Bioanal Chem 2013;405(26):8463—74.
[23] Guale F, Shahreza S, Walterscheid JP, Chen H-H, Arndt C, Kelly AT, et al. Validation of LC—TOF-MS screening for drugs, metabolites, and collateral compounds in forensic toxicology specimens. J Anal Toxicol 2013;37(1):17—24.
[24] Beck O, Ericsson M. Methods for urine drug testing using one-step dilution and direct injection in combination with LC—MS/MS and LC—HRMS. Bioanalysis 2014;6(17):2229—44.
[25] Castaneto MS, Wohlfarth A, Desrosiers NA, Hartman RL, Gorelick DA, Huestis MA. Synthetic cannabinoids pharmacokinetics and detection methods in biological matrices. Drug Metab Rev 2015;47(2):124—74.
[26] Maurer HH. Demands on scientific studies in clinical toxicology. Forensic Sci Int 2007;165(2):194—8.
[27] Maurer HH, Meyer MR. High-resolution mass spectrometry in toxicology: current status and future perspectives. Arch Toxicol 2016;90(9):2161—72.
[28] Patteet L, Cappelle D, Maudens KE, Crunelle CL, Sabbe B, Neels H. Advances in detection of antipsychotics in biological matrices. Clin Chim Acta 2015;441:11—22.
[29] Meyer GM, Maurer HH, Meyer MR. Multiple stage MS in analysis of plasma, serum, urine and in vitro samples relevant to clinical and forensic toxicology. Bioanalysis 2016;8(5):457—81.
[30] Meyer MR, Helfer AG, Maurer HH. Current position of high-resolution MS for drug quantification in clinical & forensic toxicology. Bioanalysis 2014;6(17):2275—84.
[31] Orfanidis A, Gika H, Mastrogianni O, Krokos A, Theodoridis G, Zaggelidou E, et al. Determination of drugs of abuse and pharmaceuticals in skeletal tissue by UHPLC-MS/MS. Forensic Sci Int 2018;290:137—45.
[32] Ceccato A, Klinkenberg R, Hubert P, Streel B. Sensitive determination of buprenorphine and its N-dealkylated metabolite norbuprenorphine in human plasma by liquid chromatography coupled to tandem mass spectrometry. J Pharm Biomed Anal 2003;32(4—5):619—31.
[33] Pasin D, Cawley A, Bidny S, Fu S. Current applications of high-resolution mass spectrometry for the analysis of new psychoactive substances: a critical review. Anal Bioanal Chem 2017;409(25):5821—36.
[34] Concheiro M, Anizan S, Ellefsen K, Huestis MA. Simultaneous quantification of 28 synthetic cathinones and metabolites in urine by liquid chromatography-high resolution mass spectrometry. Anal Bioanal Chem 2013;405(29):9437—48.
[35] Concheiro M, Castaneto M, Kronstrand R, Huestis MA. Simultaneous determination of 40 novel psychoactive stimulants in urine by liquid chromatography-high resolution mass spectrometry and library matching. J Chromatogr A 2015;1397:32—42.
[36] Ojanperä I, Mesihää S, Rasanen I, Pelander A, Ketola RA. Simultaneous identification and quantification of new psychoactive substances in blood by GC-APCI-QTOFMS coupled to nitrogen chemiluminescence detection without authentic reference standards. Anal Bioanal Chem 2016;408(13):3395—400.
[37] Rosano TG, Na S, Ihenetu K, Swift TA, Wood M. Multidrug and metabolite quantification in postmortem blood by liquid chromatography-high-resolution mass spectrometry: comparison with nominal mass technology. J Anal Toxicol 2014;38(8):495—506.
[38] Feliu C, Konecki C, Binet L, Vautier D, Haudecoeur C, Oget O, et al. Quantification of methadone, buprenorphine, naloxone, opioids, and their derivates in whole blood by liquid chromatography-high-resolution mass spectrometry: analysis of their involvement in fatal forensic cases. J Chromatogr B Analyt Technol Biomed Life Sci 2020;1152:122226.

[39] Li R, Zhong K, Jiang J, Zhan Y, Chen X. Enantioselective determination of ketamine in dog plasma by chiral liquid chromatography-tandem mass spectrometry. Biomed Chromatogr BMC 2019;33(9):e4578.
[40] Broecker S, Herre S, Wüst B, Zweigenbaum J, Pragst F. Development and practical application of a library of CID accurate mass spectra of more than 2,500 toxic compounds for systematic toxicological analysis by LC-QTOF-MS with data-dependent acquisition. Anal Bioanal Chem 2011;400(1):101—17.
[41] Paul M, Ippisch J, Herrmann C, Guber S, Schultis W. Analysis of new designer drugs and common drugs of abuse in urine by a combined targeted and untargeted LC-HR-QTOFMS approach. Anal Bioanal Chem 2014;406(18):4425—41.
[42] Scheidweiler KB, Jarvis MJY, Huestis MA. Non-targeted SWATH acquisition for identifying 47 synthetic cannabinoid metabolites in human urine by liquid chromatography-high-resolution tandem mass spectrometry. Anal Bioanal Chem 2015;407(3):883—97.
[43] Dalsgaard PW, Rode AJ, Pedersen AJ, Rasmussen BS, Windberg CN, Linnet K. Screening of 30 acidic and neutral pharmaceuticals in whole blood by fully automated SPE and UPLC-TOF-MS(E.). Drug Test Anal 2013;5(4):254—8.
[44] Joye T, Widmer C, Morger Mégevand R, Longère S, Augsburger M, Thomas A. High-throughput qualitative and quantitative drug checking by MALDI HRMS. Front Chem 2020;8:695.
[45] Palmquist KB, Swortwood MJ. Quantification of fentanyl analogs in oral fluid using LC-QTOF-MS. J Forensic Sci 2021;66(5):1871—8.
[46] Schwaninger AE, Meyer MR, Huestis MA, Maurer HH. Development and validation of LC-HRMS and GC-NICI-MS methods for stereoselective determination of MDMA and its phase I and II metabolites in human urine. J Mass Spectrom JMS 2011;46(7):603—14.
[47] Concheiro M, Anizan S, Ellefsen K, Huestis MA. Simultaneous quantification of 28 synthetic cathinones and metabolites in urine by liquid chromatography-high resolution mass spectrometry. Anal Bioanal Chem 2013;405(29):9437—48.
[48] Caspar AT, Kollas AB, Maurer HH, Meyer MR. Development of a quantitative approach in blood plasma for low-dosed hallucinogens and opioids using LC-high resolution mass spectrometry. Talanta 2018;176:635—45.
[49] Krauss M, Singer H, Hollender J. LC-high resolution MS in environmental analysis: from target screening to the identification of unknowns. Anal Bioanal Chem 2010;397(3):943—51.
[50] Maurer/Meyer/Helfer/Weber LC-HR-MS/MS Library of Drugs, Poisons, and Their Metabolites | Wiley [Internet]. Wiley.com. [cited 2021 Aug 31]. Available from: https://www.wiley.com/en-us/Maurer+Meyer+Helfer+Weber+LC+HR+MS+MS+Library+of+Drugs%2C+Poisons%2C+and+Their+Metabolites-p-9783527343386.
[51] Wiley mass spectra of designer drugs; 2021 [cited 2021 Aug 31. Available from: https://www.sisweb.com/software/wiley-designer-drugs.htm].
[52] Wiley Registry of Tandem Mass Spectral Data, MS for ID | Wiley [Internet]. Wiley.com. [cited 2021 Aug 31]. Available from: https://www.wiley.com/en-us/Wiley+Registry+of+Tandem+Mass+Spectral+Data%2C+MS+for+ID-p-9781118037447.
[53] Oberacher H, Pavlic M, Libiseller K, Schubert B, Sulyok M, Schuhmacher R, et al. On the inter-instrument and inter-laboratory transferability of a tandem mass spectral reference library: 1. Results of an Austrian multicenter study. J Mass Spectrom JMS 2009;44(4):485—93.
[54] Urbas A, Schoenberger T, Corbett C, Lippa K, Rudolphi F, Robien W. NPS data hub: a web-based community driven analytical data repository for new psychoactive substances. Forensic Chem 2018;9:76—81.
[55] RESPONSE [Internet]. [cited 2021 Aug 31]. Available from: https://www.policija.si/apps/nfl_response_web/seznam.php.
[56] Horai H, Arita M, Kanaya S, Nihei Y, Ikeda T, Suwa K, et al. MassBank: a public repository for sharing mass spectral data for life sciences. J Mass Spectrom 2010;45(7):703—14.
[57] mzCloud DataViewer [Internet]. [cited 2021 Aug 31]. Available from: https://www.mzcloud.org/DataViewer#/Main/reference$4878/T8392%23Standard/Recalibrated/1461299.
[58] Mardal M, Andreasen MF, Mollerup CB, Stockham P, Telving R, Thomaidis NS, et al. HighResNPS.com: an online crowd-sourced HR-MS database for suspect and non-targeted screening of new psychoactive substances. J Anal Toxicol 2019;43(7):520—7.
[59] von Cüpper M, Dalsgaard PW, Linnet K. Identification of new psychoactive substances in seized material using UHPLC—QTOF-MS and an online mass spectral database. J Anal Toxicol 2020;44(9):1047—51.
[60] Diamanti K, Aalizadeh R, Alygizakis N, Galani A, Mardal M, Thomaidis NS. Wide-scope target and suspect screening methodologies to investigate the occurrence of new psychoactive substances in influent wastewater from Athens. Sci Total Environ 2019;685:1058—65.
[61] Gundersen POM, Broecker S, Slørdal L, Spigset O, Josefsson M. Retrospective screening of synthetic cannabinoids, synthetic opioids and designer benzodiazepines in data files from forensic post mortem samples analysed by UHPLC-QTOF-MS from 2014 to 2018. Forensic Sci Int 2020;311:110274.
[62] Strano Rossi S, Odoardi S, Gregori A, Peluso G, Ripani L, Ortar G, et al. An analytical approach to the forensic identification of different classes of new psychoactive substances (NPSs) in seized materials: analytical approach for the identification of NPSs in seizures. Rapid Commun Mass Spectrom 2014;28(17):1904—16.
[63] Bovens M, Bissig C, Staeheli SN, Poetzsch M, Pfeiffer B, Kraemer T. Structural characterization of the new synthetic cannabinoids CUMYL-PINACA, 5F-CUMYL-PINACA, CUMYL-4CN-BINACA, 5F-CUMYL-P7AICA and CUMYL-4CN-b7aica. Forensic Sci Int 2017;281:98—105.
[64] Bottinelli C, Revelut K, Hologne M, Gaillard Y, Bévalot F. GC-MS, GC-QTOF and NMR analyses to elucidate composition of 41 powders from an NPS collector. Toxicol Anal Clin 2019;31(4):275—82.
[65] Hays PA. Proton nuclear magnetic resonance spectroscopy (NMR) methods for determining the purity of reference drug standards and illicit forensic drug seizures. J Forensic Sci 2005;50(6):1342—60.
[66] Castaing-Cordier T, Ladroue V, Besacier F, Bulete A, Jacquemin D, Giraudeau P, et al. High-field and benchtop NMR spectroscopy for the characterization of new psychoactive substances. Forensic Sci Int 2021;321:110718.
[67] Cheng W-C, Wong W-C. Forensic drug analysis of chloro-N, N-dimethylcathinone (CDC) and chloroethcathinone (CEC): identification of 4-CDC and 4-CEC in drug seizures and differentiation from their ring-substituted positional isomers. Forensic Sci Int 2019;298:268—77.
[68] Kolderová N, Jurásek B, Kuchař M, Lindner W, Kohout M. Gradient supercritical fluid chromatography coupled to mass spectrometry with a gradient flow of make-up solvent for enantioseparation of cathinones. J Chromatogr A 2020;1625:461286.
[69] Evans-Brown M, Gallegos A, Francis W, Christie R, Cunningham A, Sekula J, et al. New psychoactive substances in Europe: an update from the EU Early Warning System, March 2015. Luxembourg: Publications Office; 2015 [cited 2019 Jul 28. Available from: http://dx.publications.europa.eu/10.2810/372415].
[70] Frison G, Zancanaro F, Frasson S, Quadretti L, Agnati M, Vlassich F, et al. Analytical characterization of 3-MeO-PCP and

3-MMC in seized products and biosamples: the role of LC-HRAM-Orbitrap-MS and solid deposition GC-FTIR. Front Chem 2020;8:618339.
[71] Diao X, Scheidweiler KB, Wohlfarth A, Pang S, Kronstrand R, Huestis MA. In vitro and in vivo human metabolism of synthetic cannabinoids FDU-PB-22 and FUB-PB-22. AAPS J 2016;18(2):455—64.
[72] Caspar AT, Brandt SD, Stoever AE, Meyer MR, Maurer HH. Metabolic fate and detectability of the new psychoactive substances 2-(4-bromo-2, 5-dimethoxyphenyl)-N- [(2-methoxyphenyl)methyl]ethanamine (25B-NBOMe) and 2-(4-chloro-2,5-dimethoxyphenyl)- N-[(2-methoxyphenyl)methyl]ethanamine (25C-NBOMe) in human and rat urine by GC—MS, LC—MS n, and LC—HR—MS/MS approaches. J Pharm Biomed Anal 2017;134:158—69.
[73] Richter LHJ, Herrmann J, Andreas A, Park YM, Wagmann L, Flockerzi V, et al. Tools for studying the metabolism of new psychoactive substances for toxicological screening purposes — A comparative study using pooled human liver S9, HepaRG cells, and zebrafish larvae. Toxicol Lett 2019;305:73—80.
[74] Anthérieu S, Chesné C, Li R, Guguen-Guillouzo C, Guillouzo A. Optimization of the HepaRG cell model for drug metabolism and toxicity studies. Toxicol In Vitro 2012;26(8):1278—85.
[75] Gerets HHJ, Tilmant K, Gerin B, Chanteux H, Depelchin BO, Dhalluin S, et al. Characterization of primary human hepatocytes, HepG2 cells, and HepaRG cells at the mRNA level and CYP activity in response to inducers and their predictivity for the detection of human hepatotoxins. Cell Biol Toxicol 2012;28(2):69—87.
[76] Wohlfarth A, Pang S, Zhu M, Gandhi AS, Scheidweiler KB, Liu H, et al. First metabolic profile of XLR-11, a novel synthetic cannabinoid, obtained by using human hepatocytes and high-resolution mass spectrometry. Clin Chem 2013;59(11):1638—48.
[77] Ellefsen KN, Wohlfarth A, Swortwood MJ, Diao X, Concheiro M, Huestis MA. 4-Methoxy-α-PVP: in silico prediction, metabolic stability, and metabolite identification by human hepatocyte incubation and high-resolution mass spectrometry. Forensic Toxicol 2016;34(1):61—75.
[78] Wohlfarth A, Roman M, Andersson M, Kugelberg FC, Diao X, Carlier J, et al. 25C-NBOMe and 25I-NBOMe metabolite studies in human hepatocytes, in vivo mouse and human urine with high-resolution mass spectrometry: metabolism of 25C- and 25I-NBOMe. Drug Test Anal 2017;9(5):680—98.
[79] Vikingsson S, Wohlfarth A, Andersson M, Gréen H, Roman M, Josefsson M, et al. Identifying metabolites of meclonazepam by high-resolution mass spectrometry using human liver microsomes, hepatocytes, a mouse model, and authentic urine samples. AAPS J 2017;19(3):736—42.
[80] Hong Y, Kim Y-H, Lee J-M, Yoo HH, Choi S-O, Kang MS. Characterization of in vitro phase I metabolites of methamnetamine in human liver microsomes by liquid chromatography-quadrupole time-of-flight mass spectrometry. Int J Legal Med 2021;135(4):1471—6.
[81] Larabi IA, Zerizer F, Ameline A, Etting I, Joseph D, Kintz P, et al. Metabolic profiling of deschloro-*N*-ethyl-ketamine and identification of new target metabolites in urine and hair using human liver microsomes and high-resolution accurate mass spectrometry. Drug Test Anal 2021;13:1108—17 [dta.3007].
[82] Richeval C, Gaulier J-M, Romeuf L, Allorge D, Gaillard Y. Case report: relevance of metabolite identification to detect new synthetic opioid intoxications illustrated by U-47700. Int J Legal Med 2019;133(1):133—42.
[83] Richeval C, Gicquel T, Hugbart C, Le Dare B, Allorge D, Morel I, et al. In vitro characterization of NPS metabolites produced by human liver microsomes and the HepaRG cell line using liquid chromatographyhigh resolution mass spectrometry (LC-HRMS) analysis: application to furanyl fentanyl. Curr Pharm Biotechnol 2018;18(10):806—14.
[84] Caspar AT, Westphal F, Meyer MR, Maurer HH. LC-high resolution-MS/MS for identification of 69 metabolites of the new psychoactive substance 1-(4-ethylphenyl-)-N-[(2-methoxyphenyl)methyl] propane-2-amine (4-EA-NBOMe) in rat urine and human liver S9 incubates and comparison of its screening power with further MS techniques. Anal Bioanal Chem 2018;410(3):897—912.
[85] Wagmann L, Frankenfeld F, Park YM, Herrmann J, Fischmann S, Westphal F, et al. How to study the metabolism of new psychoactive substances for the purpose of toxicological screenings — A follow-up study comparing pooled human liver S9, HepaRG Cells, and Zebrafish Larvae. Front Chem 2020;8:539.
[86] Richter LHJ, Maurer HH, Meyer MR. New psychoactive substances: studies on the metabolism of XLR-11, AB-PINACA, FUB-PB-22, 4-methoxy-α-PVP, 25-I-NBOMe, and meclonazepam using human liver preparations in comparison to primary human hepatocytes, and human urine. Toxicol Lett 2017;280:142—50.
[87] Richter LHJ, Flockerzi V, Maurer HH, Meyer MR. Pooled human liver preparations, HepaRG, or HepG2 cell lines for metabolism studies of new psychoactive substances? A study using MDMA, MDBD, butylone, MDPPP, MDPV, MDPB, 5-MAPB, and 5-API as examples. J Pharm Biomed Anal 2017;143:32—42.
[88] Yue L, Xiang P, Shen B, Xu D, Song F, Yan H. Metabolism of 4F-MDMB-BICA in zebrafish by liquid chromatography — high resolution mass spectrometry. Drug Test Anal 2021;13(6):1223—9.
[89] Ameline A, Greney H, Monassier L, Raul J, Kintz P. Metabolites to parent 3-MeO-PCP ratio in human urine collected in two fatal cases. J Anal Toxicol 2019;43(4):321—4.
[90] Pelletier R, Le Daré B, Grandin L, Couette A, Ferron P-J, Morel I, et al. New psychoactive substance cocktail in an intensive care intoxication case elucidated by molecular networking. Clin Toxicol 2021;0:1—4.
[91] Ameline A, Richeval C, Gaulier J-M, Raul J-S, Kintz P. Detection of the designer benzodiazepine flunitrazolam in urine and preliminary data on its metabolism. Drug Test Anal 2019;11(2):223—9.
[92] Wang M, Carver JJ, Phelan VV, Sanchez LM, Garg N, Peng Y, et al. Sharing and community curation of mass spectrometry data with global natural products social molecular networking. Nat Biotechnol 2016;34(8):828—37.
[93] Allard S, Allard P-M, Morel I, Gicquel T. Application of a molecular networking approach for clinical and forensic toxicology exemplified in three cases involving 3-MeO-PCP, doxylamine, and chlormequat. Drug Test Anal 2019;11(5):669—77.
[94] Šícho M, de Bruyn Kops C, Stork C, Svozil D, Kirchmair J. FAME 2: simple and effective machine learning model of cytochrome P450 regioselectivity. J Chem Inf Model 2017;57(8):1832—46.
[95] Olsen L, Montefiori M, Tran KP, Jørgensen FS. SMARTCyp 3.0: enhanced cytochrome P450 site-of-metabolism prediction server. Valencia A, editor. Bioinformatics 2019;35(17):3174—5.
[96] Rudik A, Dmitriev A, Lagunin A, Filimonov D, Poroikov V. SOMP: web server for in silico prediction of sites of metabolism for drug-like compounds. Bioinformatics 2015;31(12):2046—8.
[97] Zaretzki J, Matlock M, Swamidass SJ. XenoSite: accurately predicting CYP-mediated sites of metabolism with neural networks. J Chem Inf Model 2013;53(12):3373—83.
[98] Tian S, Djoumbou-Feunang Y, Greiner R, Wishart DS. CypReact: a software tool for in silico reactant prediction for human cytochrome P450 enzymes. J Chem Inf Model 2018;58(6):1282—91.

[99] Djoumbou-Feunang Y, Fiamoncini J, Gil-de-la-Fuente A, Greiner R, Manach C, Wishart DS. BioTransformer: a comprehensive computational tool for small molecule metabolism prediction and metabolite identification. J Cheminformatics 2019;11(1):2.
[100] Cruciani G, Carosati E, De Boeck B, Ethirajulu K, Mackie C, Howe T, et al. MetaSite: understanding metabolism in human cytochromes from the perspective of the chemist. J Med Chem 2005;48(22):6970—9.
[101] Klopman G, Dimayuga M, Talafous J. META. 1. A program for the evaluation of metabolic transformation of chemicals. J Chem Inf Comput Sci 1994;34(6):1320—5.
[102] de Bruyn Kops C, Stork C, Šícho M, Kochev N, Svozil D, Jeliazkova N, et al. GLORY: generator of the structures of likely cytochrome P450 Metabolites based on predicted sites of metabolism. Front Chem 2019;7:402.
[103] Rudik AV, Bezhentsev VM, Dmitriev AV, Druzhilovskiy DS, Lagunin AA, Filimonov DA, et al. MetaTox: web application for predicting structure and toxicity of xenobiotics' metabolites. J Chem Inf Model 2017;57(4):638—42.
[104] Pluskal T, Castillo S, Villar-Briones A, Oresic M. MZmine 2: modular framework for processing, visualizing, and analyzing mass spectrometry-based molecular profile data. BMC Bioinformatics 2010;11:395.
[105] Röst HL, Sachsenberg T, Aiche S, Bielow C, Weisser H, Aicheler F, et al. OpenMS: a flexible open-source software platform for mass spectrometry data analysis. Nat Methods 2016;13(9):741—8.
[106] Dührkop K, Fleischauer M, Ludwig M, Aksenov AA, Melnik AV, Meusel M, et al. SIRIUS 4: a rapid tool for turning tandem mass spectra into metabolite structure information. Nat Methods 2019;16(4):299—302.
[107] Olivon F, Elie N, Grelier G, Roussi F, Litaudon M, Touboul D. MetGem software for the generation of molecular networks based on the t-SNE algorithm. Anal Chem 2018;90(23):13900—8.
[108] Shannon P. Cytoscape: a software environment for integrated models of biomolecular interaction networks. Genome Res 2003;13(11):2498—504.
[109] Allard S, Allard P, Morel I, Gicquel T. Application of a molecular networking approach for clinical and forensic toxicology exemplified in three cases involving 3-MeO-PCP, doxylamine, and chlormequat. Drug Test Anal 2019;11(5):669—77.
[110] Le Daré B, Allard S, Bouvet R, Baert A, Allard P-M, Morel I, et al. A case of fatal acebutolol poisoning: an illustration of the potential of molecular networking. Int J Legal Med 2020;134(1):251—6.
[111] Le Daré B, Ferron P-J, Allard P-M, Clément B, Morel I, Gicquel T. New insights into quetiapine metabolism using molecular networking. Sci Rep 2020;10(1):19921.
[112] Allard S, Le Daré B, Allard P-M, Morel I, Gicquel T. Comparative molecular networking analysis of a Rauwolfia plant powder and biological matrices in a fatal ingestion case. Forensic Toxicol 2020;38(2):447—54.

OBJECTIFS DE LA THÈSE

Dans le monde, environ 271 millions d'adultes, soit 5,5 % des personnes âgées de 14 à 64 ans, auraient déjà consommé des drogues illicites au cours de leur vie (UNODC, 2019). À l'échelle européenne, cela représente 96 millions d'adultes, soit 29 % (EMCDDA, 2019). Le nombre de consommateurs avec des usages problématiques augmente. En parallèle, le développement permanent de nouvelles molécules rend le diagnostic d'exposition difficile. Par ailleurs, le manque de connaissance concrète sur la composition et les dosages en principes actifs de ces produits pose un risque sanitaire important, voire mortel. L'exposition aux substances psychoactives et notamment aux NPS devient donc une préoccupation majeure pour les autorités de santé.

Le plan national de mobilisation contre les dépendances 2018-2022 de la Mission interministérielle de la lutte contre les drogues et les conduites addictives (MILD&CA) s'articule autour de plusieurs axes principaux. Parmi leurs objectifs, on retrouve celui de renforcer les mécanismes de détection et d'alerte sur les nouvelles substances psychoactives, notamment les opioïdes de synthèse (Objectif 13.4), d'élargir les connaissances sur les usages et les trajectoires de consommation (Objectif 16.1), d'élargir les connaissances pour mieux prévenir, mieux réduire les risques et soigner (Objectif 16.2 et 16.3), de mobiliser dans les Outre-mer (Axe 6) (126). Dans son dernier rapport sur les drogues de 2022, l'Observatoire européen des drogues et des toxicomanies (EMCDDA) souligne également l'importance des sources de données toxicologiques et médico-légales en provenance des laboratoires pour que soient mieux comprises les tendances en matière de drogues et les menaces pour la santé.

L'objectif général de ce projet de thèse est d'étudier l'apport analytique de la spectrométrie de masse haute résolution en QTOF pour l'identification et la prise en charge de l'exposition aux substance psychoactives. Afin d'atteindre cet objectif, le projet a été découpé en 4 étapes de recherche :

- Étape 1 : Nous avons réalisé une **revue générale** des applications de la spectrométrie de masse haute résolution en toxicologie analytique avec un *focus* sur les substances psychoactives, afin de pouvoir aborder l'ensemble de ce travail de thèse (**Revue de la littérature n° 1**).
- Étape 2 : Nous avons évalué l'apport analytique de la spectrométrie de masse haute résolution en QTOF dans le cadre d'une comparaison de méthode d'identification de produits stupéfiants (échantillons non biologiques). Pour cela, nous avons étudié la

mise en place du *drug checking* par spectrométrie infrarouge dans un centre d'Accueil et d'Accompagnement à la Réduction des Risques des Usagers de Drogues (CAARUD). Ce travail nous a permis de déterminer l'apport du QTOF par comparaison avec une méthode infrarouge (**Article 1**).

- Étape 3 : Nous avons évalué l'apport analytique de la spectrométrie de masse haute résolution en QTOF comme outil analytique, pour améliorer l'identification des NPS et la mise en évidence de biomarqueurs d'exposition. Ce travail repose sur la combinaison des approches *in vivo*, *in silico* et *in vitro* ainsi que sur d'autres méthodes d'identification telles que la RMN et l'IR (**Article 2**).
- Étape 4 : Nous avons évalué l'apport en pratique clinique de la spectrométrie de masse haute résolution en QTOF pour l'identification des cannabinoïdes de synthèse dans les départements d'Outre-mer (cas de la « chimique » à Mayotte ; **Article 3**) et en France métropolitaine (**Articles 4**). Ce travail a permis de mettre en évidence l'apport du QTOF pour l'amélioration de la prise en charge des patients et, de façon plus globale, une amélioration des connaissances sur ces substances et de leurs dangers permettant d'établir des bases de réflexion pour les autorités sanitaires.

Ces études ont fait l'objet de **cinq articles scientifiques** acceptés dans des revues nationales ou internationales à comité de lecture. Elles ont également fait l'objet de **sept communications orales** et **quatre communications affichées** présentées dans des congrès nationaux ou internationaux. En parallèle, d'autres travaux ont été menés dans des champs de recherche proches, présentés en **Annexe 1**.

TRAVAUX EXPÉRIMENTAUX

I. Matériel et méthodes

A. Identification de substances psychoactives par spectroscopie infrarouge à transformée de Fourier (FTIR)

Brièvement, le principe de la spectroscopie infrarouge repose sur l'absorption de la lumière par la plupart des molécules dans la région de l'infrarouge du spectre électromagnétique et sa conversion en vibration moléculaire. Cette absorption correspond spécifiquement aux liaisons présentes dans la molécule. Avec un spectromètre, cette absorption du rayonnement infrarouge par le matériau de l'échantillon est mesurée en fonction de la longueur d'onde. Le résultat est un spectre qui donne une "empreinte chimique" distinctive qui peut être utilisée pour visualiser et identifier des échantillons organiques et inorganiques.

L'utilisation de la spectrométrie infrarouge en mode réflectance totale atténuée (ATR) ne nécessite pas de préparation d'échantillon : celui-ci est directement déposé sur le cristal pour l'analyse. L'étude menée dans l'article n° 1 comportait l'emploi d'un spectromètre infrarouge (ATR) modèle Alpha de chez Bruker®. Le système d'acquisition et de traitement des données était piloté depuis un ordinateur équipé du logiciel Opus® version 7.0. Les bibliothèques utilisées étaient les suivantes : ATR-LIB-FORENSICS-1-472-2.S, ATR-LIB-FORENSICS-2-472-2.S, ATR-LIB- FORENSICS-3-472-2.S, A TR-LIB-FORENSICS-4-472-2.S, Demolib.s01, GCL.S01, RAMDEMO.S01, SR.IDX, Tictac Drug Library 20170213.S ainsi qu'une bibliothèque « maison ». Les résultats ont été interprétés par les professionnels du CAARUD ayant reçu une formation par le fournisseur de l'appareil, et ont été transmis au laboratoire du CHU de Bordeaux via un fichier Excel®. Quant à l'étude menée dans l'article n° 2, nous avons eu recours à un spectromètre FTIR (Nicolet Summit (Thermo Fisher, MA, USA)) fonctionnant en mode ATR grâce à un dispositif FTIR Nicolet 6700. Les signaux ont été enregistrés entre 4000 et 500 cm-1 avec une résolution de 4 cm-1. L'acquisition des données spectroscopiques a été effectuée avec le logiciel OMNIC Paradigm (Thermo Fisher, MA, USA).

B. Caractérisation d'une poudre par résonance magnétique nucléaire

La résonance magnétique nucléaire (RMN) est fondée sur la mesure de l'absorption de la radiation de radiofréquence (RF) par un noyau atomique dans un champ magnétique fort. La spectroscopie par RMN constitue l'un des plus puissants instruments de détermination de la structure des espèces organiques et inorganiques. Cette technique est également utile pour la quantification des espèces absorbantes.

La RMN repose sur les propriétés magnétiques de certains noyaux atomiques tels que l'hydrogène (^{1}H), le carbone (^{13}C), le phosphore (^{31}P), le fluor (^{19}F), l'oxygène (^{17}O) ou l'azote (^{14}N). Ces noyaux ont la particularité d'être dotés d'un moment magnétique appelé également « spin », assimilable à un barreau aimanté. En l'absence d'un champ magnétique extérieur, les spins de ces atomes s'orientent de manière aléatoire à l'intérieur de l'échantillon. Dès qu'un champ magnétique intense est appliqué à l'échantillon, ces atomes s'orientent dans la direction du champ magnétique extérieur à l'image de l'aiguille d'une boussole dans le champ magnétique terrestre. La somme des champs magnétiques produits par les moments magnétiques constitue l'aimantation de l'échantillon qu'il est possible de mesurer par RMN. Pour mesurer cette aimantation, il faut modifier son orientation par rapport au champ magnétique externe en utilisant un apport d'énergie sous forme d'ondes radiofréquences créées par une bobine supraconductrice excitatrice. Mesuré en fonction du temps et appelé FID (*Free Induction Decay*) ou signal d'induction libre, ce signal acquis est traité informatiquement par transformée de Fourier pour obtenir un spectre RMN en fonction de la fréquence (Hz).

Un appareil de RMN se présente comme un cylindre constitué d'une succession de couches. La bobine supraconductrice utilisée pour créer un champ magnétique de plusieurs Teslas (100 000 fois le champ magnétique terrestre) doit être maintenue à une température très basse (-273° C). Pour cela, la bobine est placée dans une enceinte d'hélium liquide, entourée d'une zone de vide et ensuite d'une seconde enceinte contenant de l'azote liquide. Ces zones de vide agissent contre les déperditions de chaleur. L'échantillon est inséré dans sa rampe d'introduction au centre de l'appareil à température ambiante.

Pour obtenir un spectre RMN, l'échantillon est donc soumis à des impulsions de radiofréquence. Le signal émis par les noyaux en résonance revenus à leur état d'équilibre, est mesuré sous forme d'un courant induit dans une bobine. Grâce à un traitement mathématique,

un spectre RMN est alors généré. Classiquement, l'échantillon à analyser sous forme de poudre est mis en solution dans un solvant dépourvu de l'atome à analyser. Dans le cas de l'analyse du proton RMN (1H), les solvants couramment utilisés sont le méthanol deutéré ou chloroforme deutéré, respectivement CD3OD ou CDCl3. Un matériau supraconducteur permet de conduire un courant électrique sans perte de résistance électrique (de chaleur). Pour atteindre cette performance, il doit se conserver à des températures très faibles, entre -118° C et -279° C. Les chercheurs utilisent une autre échelle : le Kelvin (K) (-273 K = 0° C). Cette méthode est basée sur une référence électronique. En effet, un pic correspondant à un pseudo signal RMN (Pseudo-FID) produit par un générateur de signal électronique, est ajouté au sein du spectre du composé à quantifier.

C. Criblage toxicologique par UPLC-MS-QTOF

Dans cette étude, la technologie utilisée pour le criblage toxicologique est la chromatographie liquide ultra haute performance couplée à la spectrométrie de masse haute résolution en QTOF (UPLC-MS/QTOF). Il s'agit d'une technique d'analyse qualitative et quantitative. La séparation par chromatographie en phase liquide s'opère suivant les interactions entre les analytes entraînés par la phase mobile, et la phase stationnaire. La spectrométrie de masse est une technique physique d'analyse qui détecte et identifie des molécules d'intérêt par mesure de leur masse, et caractérise leur structure chimique. Son principe réside dans la séparation de molécules chargées (ions) en fonction de leur rapport masse/charge (m/z). Le screening non ciblé ou screening large consiste à rechercher et à identifier des molécules présentes dans un échantillon, en comparant leurs spectres à ceux d'une librairie ou base de données. La caractérisation repose sur la connaissance d'une formule brute (permettant de calculer la masse exacte), d'un temps de rétention chromatographique, et d'un ou plusieurs fragments spécifiques obtenus après collision. Cette approche en haute résolution présente l'avantage de retraiter rétrospectivement des données acquises en cas d'informations différées (informations sur les circonstances de l'intoxication, molécules dont les informations spectrales étaient initialement indisponibles, etc.).

Les conditions chromatographiques retenues dans le cadre de ce travail étaient les suivantes. Le système de chromatographie liquide ultra-performante (UPLC)-HRMS était constitué d'un système de classe Acquity I (Waters, États-Unis) couplé à un analyseur Xevo G2-XS

Quadrupole Time of flight (QTof) (Waters, États-Unis), utilisant l'ionisation par électronébulisation en mode positif et négatif, et contrôlé par le logiciel UNIFI 1.9. L'acquisition se fait en mode « full scan », appelé aussi balayage en mode MSe, dans une gamme de masse allant de 50 à 1 000 m/z. Ce mode effectue une double acquisition afin de collecter les informations de masses exactes, ions précurseurs et leurs fragments :

- une acquisition à faible énergie de collision (6 eV afin d'obtenir le spectre de masse de l'ion moléculaire protoné [M+H]+
- une acquisition à haute énergie de collision (rampe de 10 à 40 eV) dans le but de générer des fragments.

La séparation chromatographique a été réalisée sur une colonne ACQUITY HSS C18 (150×2 mm, 1,8 µm, Waters). Le gradient de phases mobiles était composé de solvants A (tampon formiate d'ammonium 5 mM, pH 3) et B (acétonitrile dans de l'acide formique à 0,1 % v/v). La colonne a été maintenue à 50° C et un gradient d'élution a été utilisé à un débit de 0,4 mL/min : 13 % de B maintenu jusqu'à 0,5 min, augmenté à 50 % à 10 min, puis à 95 % à 10,25 min, et enfin maintenu jusqu'à 12,25 min, retour à l'état initial en 0,25 min jusqu'à 17 min. Le volume d'injection était de 2 µL. Les données ont été analysées avec une base de données de masse exacte de Waters® contenant plus de 3 500 médicaments, NPS et métabolites pertinents sur le plan toxicologique, comprenant des données sur les molécules protonées, leur fragmentation et leur temps de rétention chromatographique. Les critères utilisés pour identifier les composés comprenaient une erreur de masse exacte 5 ppm sur l'ion parent ainsi que des ions fils et une tolérance de + /- 0,5 min sur le temps de rétention de l'ion parent.

D. Étude du métabolisme des nouveaux produits de synthèse

L'identification des métabolites revêt un intérêt majeur pour les analystes. Elle permet d'augmenter la fenêtre de détection d'une consommation de NPS et de renforcer la spécificité des analyses. En effet, pour la plupart des xénobiotiques, la demi-vie d'élimination des métabolites est très supérieure à celle de la molécule mère. D'autre part, dans le cas de l'analyse capillaire, la recherche des métabolites peut s'avérer très utile pour exclure une potentielle contamination externe des cheveux par la manipulation des produits ou par

l'environnement. Cependant, l'identification des métabolites n'est pas facile. Pour la grande majorité des NPS, très peu de données sont disponibles dans la littérature. Pour d'autres, ces données sont inexistantes. Une fois identifiés, les métabolites ne sont pas toujours disponibles à l'achat, et les standards existants sont très coûteux et longs à recevoir. Il est donc nécessaire de mettre en place des stratégies directes d'identification permettant au mieux de documenter les observations. Les NPS, comme la plupart des xénobiotiques, sont fréquemment hydrophobes et/ou possèdent des groupements chimiques réactifs. Afin de les rendre plus hydrophiles, pour être excrétés hors de l'organisme ou pour neutraliser leur groupement réactif, ils subissent un métabolisme *in vivo*. Le métabolisme des xénobiotiques se décompose en trois phases qui aboutissent à l'élimination des substances exogènes dans la bile et l'urine (127,128) :

- Phase I : Étape de fonctionnalisation (oxydation, réduction, hydrolyse) effectuée essentiellement par les cytochromes P450 (CYP). Les cytochromes responsables du métabolisme des xénobiotiques sont localisés principalement dans le réticulum endoplasmique des hépatocytes (129).

- Phase II : Étape de conjugaison Glutathion S transférase (GST), UDP-glucuronosyltransférases (UGT) catalysent les réactions de transfert, généralement après fonctionnalisation.

- Phase III : Les enzymes de phase III sont des transporteurs membranaires (Glycoprotéine-P (P- gp), *multidrug resistance proteins* (MRP) qui éliminent de la cellule les xénobiotiques ou leurs métabolites conjugués.

Les études de métabolisme font appel à différentes méthodes *in vivo*, *in vitro* ou *in silico*. L'objectif final étant de pouvoir recouper l'ensemble des données obtenues selon ces trois approches. La difficulté d'identification de nouveaux métabolites réside dans le choix du modèle d'étude (*in vivo* ou *in vitro*), mais surtout dans le développement d'outils analytiques fiables et performants, capables d'identifier de la façon la plus exhaustive possible tous les composés issus du métabolisme. Ces outils analytiques sont basés sur deux méthodologies complémentaires : 1) le développement d'une méthode de détection sensible et spécifique du composé étudié ; 2) le retraitement adéquat des données brutes acquises (souvent très fournies) afin d'identifier les spectres des métabolites potentiels et proposer des structures adéquates de ces derniers. Dans un premier temps, ces études ont été mises en place en collaboration avec le Centre Hospitalier Universitaire de Lille, qui a développé le protocole.

Compte tenu du potentiel de cette approche, le protocole a été appliqué et optimisé dans notre laboratoire.

L'approche *in silico* est basée sur les connaissances structurelles de la molécule mère et l'utilisation d'algorithmes (MetaboLynx) permettant de prédire les biotransformations de la molécule sur les groupes fonctionnels identifiés. À l'aide des données disponibles sur le métabolisme de substances apparentées, des biotransformations théoriques ont été créés (incluant la N-désalkylation et la glucuronidation) sur la base de la structure chimique des molécules. Le processus de profilage automatisé des métabolites a été réalisé par l'intermédiaire de l'outil logiciel d'interprétation de la fragmentation MassFragmentTM 4.1 (Waters), qui permet d'assigner les structures des métabolites à partir des modèles de fragmentation. Les critères utilisés pour identifier les composés ciblés comprenaient une erreur de masse exacte sur l'ion parent et sur les ions fils de 5 ppm et +/- 0,5 min de tolérance pour le temps de rétention de l'ion parent. Les données ont été analysées avec le logiciel Unifi (Waters, Manchester, UK).

Les méthodes *in vitro*, en particulier le modèle hépatique, sont fréquemment utilisées pour ces études de métabolisme. Elles incluent principalement l'utilisation de coupes d'organes, d'hépatocytes et de microsomes (130). *In vitro*, la production de métabolites peut être réalisée à partir d'incubations utilisant des microsomes hépatiques humains (HLMs). Le choix du modèle et des co-substrats permet l'identification de différentes voies métaboliques (phase I ou II). Dans ce travail, les études *in vitro* ont été réalisées en analysant les incubats de HLMs par chromatographie liquide avec détection par spectrométrie de masse à haute résolution (LC-HRMS), puis identifiés de manière rétro-spécifique dans des échantillons (131). Une fois décongelées, 50 µL de HLMs (2 mg de protéines/mL) dans du Tris-HCl-MgCl2 0,1 M (solution de MgCl2 10 mM et de Tris-HCl 100 mM) à pH 7,4 ont été préactivées par l'alaméthicine (à 0,1 mmol/L) dans de la glace. Ce mélange a été ajouté à un résidu sec de 50 µL de deux concentrations différentes (100 µM ou 500 µM) de la solution methanolique de la molécule. Cinquante microlitres d'un mélange de cofacteurs (5 mM UDPGA, 1,3 mM NADPH, 3,3 mM G6P et 0,5 U/mL G6PD) dans du Tris-HCl 0,1 M y ont ensuite été ajoutés. La réaction enzymatique a été réalisée à 37° C pendant 60 minutes et arrêtée par l'ajout de 100 µL de méthanol. Les surnageants de culture cellulaire ont été récupérés et congelés à - 20° C jusqu'à leur analyse. Les données acquises par LC-HRMS des incubats HLMs ont ensuite été recoupées avec les biotransformations prédites *in silico* (112). Les critères d'identification des

composés détectés comprenaient une erreur de masse exacte de 5 ppm sur l'ion parent et les ions fils et ± 0,25 min comme tolérance sur le temps de rétention de l'ion parent.

L'approche *in vivo* consiste à recueillir les échantillons biologiques (urine, sang ou cheveux) après l'administration d'un xénobiotique à l'animal ou l'homme. Le modèle animal ne peut donner qu'une idée du métabolisme à prévoir chez l'homme, puisque la nature et la répartition des enzymes impliquées dans le métabolisme des xénobiotiques diffèrent entre les espèces (132). Les échantillons ont été analysés au moyen de la méthode LC-HRMS décrite ci-dessus.

II. Apport analytique de la spectrométrie de masse haute résolution en QTOF dans le cadre d'une comparaison de méthode – Publication n° 1

A. Introduction et présentation

Le *drug checking* est un service destiné aux usagers de drogues, qui comprend une analyse de produit et un entretien individuel incluant un retour sur les résultats et des conseils en matière de réduction des risques. Il peut faire appel à différentes méthodes analytiques, mais peu d'études ont été réalisées afin de démontrer leur avantage en pratique courante. Dans leur revue des méthodes d'analyse de drogues (fondées sur les avantages théoriques), Harper *et al.* recommandent la spectrométrie de masse (LC-MS/MS et GC-MS) comme *gold standard* de l'analyse médico-légale (133). Dans les centres d'accueil et d'aide à la réduction des risques des usagers de drogues (CAARUD), la spectrométrie de masse n'est presque pas utilisée actuellement car elle nécessite un équipement coûteux et un personnel habilité. La spectroscopie IR peut potentiellement être bien adaptée à ce contexte en raison à la fois de la rapidité et de la fiabilité de ses résultats (133). Par exemple, l'IR a montré une bonne sensibilité et une bonne spécificité par rapport à la GC-MS pour l'identification du fentanyl (134). La spectroscopie IR est de plus en plus utilisée dans le *drugchecking*, même si peu d'études démontrent son intérêt (133–136). Il donc est important d'identifier les limites analytiques de l'IR dans ce contexte. Par rapport à la LC-MS/MS et à la GC-MS, la spectrométrie de masse à haute résolution (LC-HRMS) offre de nombreux avantage notamment en terme de spécificité (72). Cette technique permet une identification précise et exhaustive des composés et également leur quantification. À notre connaissance, aucune étude n'a comparé les performances analytiques de la spectroscopie IR par rapport à une méthode de référence telle que la spectrométrie de masse haute résolution dans le cadre du *drug checking* dans un CAARUD. L'objectif principal de ce travail était de comparer les résultats de l'analyse de produits issus des usagers par spectroscopie IR dans un centre de réduction des risques avec ceux obtenus en spectrométrie de masse haute résolution en QTOF. Les objectifs secondaires étaient de décrire la population des usagers de drogues demandant une analyse de produit, et de comparer les compositions supposées des produits achetés avec leurs compositions réelles.

B. Discussion

Cette étude est la première à évaluer l'utilisation de la spectroscopie IR en comparaison de la spectrométrie de masse haute résolution dans le contexte du *drug checking* à partir de drogues de rue en France. Elle représente également le premier partenariat entre un laboratoire hospitalo-universitaire et un CAARUD.

La spectroscopie IR a permis d'identifier avec précision le composé majoritaire contenu dans le produit ; il n'y a eu aucun faux positif et les faux négatifs étaient très peu nombreux. Ces résultats sont comparables à ceux retrouvés en comparant la spectroscopie IR à la GC-MS (136). En spectroscopie IR, des limites importantes ont été mises en évidence notamment pour l'analyse du cannabis (herbe ou résine), des composés sous forme de buvard et des composés présents en faible quantité et/ou dans des mélanges. Pour les composés présents en faible quantité, la spectroscopie IR n'a pu identifier que les principales substances actives (composés majoritaires) de l'échantillon. Parmi ces substances majoritaires, certaines n'ont pas été détectées car absents des bibliothèques utilisées (1,5 %, n=2), comme le 2C-E (nouvelle substance psychoactive) et la méthandiénone (anabolisant), tandis que d'autres étaient inférieurs à la limite de détection de la spectroscopie IR (2,2 %, n=3). Celles qui étaient en dessous de la limite de détection correspondaient à des composés minoritaires présents dans un mélange ; il peut notamment s'agir de contaminations lors de la préparation de l'échantillon par les dealers. Ces derniers ont pu augmenter le nombre de faux négatifs dans notre étude. Parmi les produits de coupe, à l'exception du paracétamol, de la caféine et de l'aspirine, la spectroscopie IR n'a pas permis de les identifier de manière fiable dans les échantillons. La noréphédrine, l'acétylleucine, la sélégiline, l'atropine, l'harpagoside et le milnacipran n'ont pas été identifiés par spectroscopie IR en raison de leur absence des bibliothèques. Pour les autres produits de coupe, l'absence d'identification est possiblement liée à une concentration inférieure à la limite de détection de l'appareil, soit une présence de manière hétérogène dans l'échantillon et donc indétectable sur la petite portion analysée. Les difficultés d'identification des spectres minoritaires peuvent être dues à une mauvaise interprétation des spectres IR obtenus. Il apparaît intéressant de rappeler aux professionnels de la réduction des risques qui utilisent la spectrométrie IR de l'importance de soustraire les spectres majoritaires afin d'isoler les spectres minoritaires. Il existe également un risque de biais d'interprétation, car les analyses ont été réalisées par des professionnels de la réduction des risques. Bien qu'ils aient tous reçu une formation de la part du fournisseur de l'instrument,

ils n'avaient pas l'expertise technique des professionnels de l'analyse. Nous supposons qu'un plus grand nombre de composés auraient pu être identifiés par des professionnels formés ; cependant, ce choix était intentionnel pour déterminer la performance de la spectroscopie IR dans des conditions réelles d'utilisation. Ce travail de collaboration entre les toxicologues hospitaliers et les travailleurs sociaux a donc permis d'améliorer l'interprétation des analyses IR. Pour certaines substances, le faible nombre d'échantillons rapportés n'a pas permis de réaliser des analyses statistiques. De plus, seules les substances apportées par les usagers de drogues ont pu être testés, les données ne sont donc pas exhaustives. Les composés trouvés ne sont pas nécessairement représentatifs de l'ensemble des usagers et des produits circulant sur le marché des drogues. Nous aurions pu tester des produits purs, mais nous avons voulu rester fidèles aux conditions réelles en utilisant directement des drogues de rue. En effet, ces produits sont très souvent hétérogènes ; d'un point de vue technique, il est plus difficile de les identifier par rapport aux substances pures. Certains d'entre eux sont actifs et nocifs à très faible dose, ce qui peut rendre la spectroscopie IR peu utile dans de nombreux contextes, comme dans le cas des cannabinoïdes de synthèse. La spectroscopie IR est également très sensible aux matrices dans lesquelles l'échantillon est présenté (par exemple, solvants, eau, plantes ou papier buvard).

En résumé, la spectroscopie IR présente un certain nombre d'avantages. Elle permet une détection rapide et fiable du composé majoritaire dans un échantillon ne nécessitant pas une formation approfondie de l'opérateur. Elle ne nécessite pas de solvants ni de préparation d'échantillon ; il s'agit donc d'un appareil facile à utiliser et dont le coût d'utilisation est faible. Elle utilise un processus non destructif qui permet une analyse ultérieure sur le même échantillon. Il s'agit d'un appareil hautement portable et donc facilement utilisable en milieu festif. Ces avantages offrent aux professionnels de la réduction des risques la possibilité de mieux diffuser leur message en utilisant un outil réactif et bien adapté à une clientèle urbaine qui achète souvent en petites quantités et consomme rapidement. Cependant, la spectroscopie IR présente des limites importantes pour l'identification de divers types d'échantillons, notamment le cannabis, les buvards, les mélanges et les produits présents en faible quantité. Dans ces cas, ainsi que dans les situations où les usagers de drogues rapportent des effets indésirables ou inhabituels, des analyses supplémentaires par spectrométrie de masse haute résolution sont nécessaires. La spectroscopie IR est donc une méthode prometteuse dans le contexte du *drug checking* mais doit faire l'objet d'études complémentaires qui définissent mieux son cadre et ses limites.

Publication n° 1

Suitability of infrared spectroscopy for drug checking in harm reduction centres

Ruben Goncalves, Karine Titier, Véronique Latour, Alexandre Peyré, Nadège Castaing, Amélie Daveluy, Mathieu Molimard

Publié en 2021 dans *International Journal of Drug Policy*

Volume 88, 103037, ISSN 0955-3959

https://doi.org/10.1016/j.drugpo.2020.10303

International Journal of Drug Policy 88 (2021) 103037

Contents lists available at ScienceDirect

International Journal of Drug Policy

journal homepage: www.elsevier.com/locate/drugpo

Research Paper

Suitability of infrared spectroscopy for drug checking in harm reduction centres

R. Goncalves [a,b,*], K. Titier [a], V. Latour [c], A. Peyré [d], N. Castaing [a], A. Daveluy [b,d], M. Molimard [a,b]

[a] CHU de Bordeaux, Laboratoire de Pharmacologie et Toxicologie, Place Amélie Raba Léon, F-33076 Bordeaux, Cedex, France
[b] Univ. Bordeaux, INSERM, BPH, U1219, 146 rue Léo Saignat 11, F-33076 Bordeaux, Cedex, France
[c] La Case, 36 rue Saint-James, F-33000 Bordeaux, France
[d] CHU de Bordeaux, Centre d'addictovigilance, Place Amélie Raba Léon, F-33076 Bordeaux, Cedex, France

ARTICLE INFO

Keywords:
Drug checking
Infrared spectroscopy
Harm reduction
High resolution mass spectrometry
QTOF

ABSTRACT

Drug checking is a service for people who use drugs that includes product analysis and an individual interview including results feedback and harm reduction counselling. It uses different analytical methods but few studies demonstrate their value in current practice. The main objective of this work is to compare the analytical performance of IR spectroscopy to laboratory reference method in the context of drug checking in a harm reduction centre. The secondary objectives are to carry out a description of the people who use drugs requesting a product analysis, and to compare the assumed compositions of products purchased with their real compositions. During 2018, all requests for drug testing analysis were included for on-site analysis by IR spectrometry in a harm reduction center and verified by the reference method (UPLC-HRMS) at Bordeaux University Hospital Center. Socioeconomic and product data were also collected. One hundred and thirty-six samples were collected. The results obtained with IR and UPLC-HRMS were compared. IR spectrometry results did not match with reference method in 8 % (n=11) of cases, corresponding to blotters, cannabis and some psychoactive substances present in mixture or in small quantities. Among the products collected, only 5.1 % (n=7) did not correspond to the declared product, either alone or with adulterants. The IR spectrometer allows a simple and rapid detection of at least one molecule, most often the one of interest. However, it is limited to powder and tablet type matrices and is not suitable for blotters, cannabis, mixed or low content substances for which high resolution mass spectrometry remains the reference method.

Introduction

In the past few years, the range of substances available has grown beyond the traditional drugs of abuse to include hundreds of new psychoactive substances. Final product composition and concentration can vary considerably among sources; they can include a number of adulterants and intermediates, exposing users to an uncontrolled health risk. Health authorities have begun proposing harm reduction strategies including drug checking service (Harper et al., 2017). This service includes a personalised analysis of products then provide individualised feedback of results (content, sometimes purity) and counselling which may include advice related to harm reduction. There is a wide variety of techniques that have been validated for drug identification and/or quantification. Each of these techniques has a variety of associated pros and cons that must be considered. Reagent testing have been banned in France since 2005 and various screening analytical methods have been proposed as components of these services, most commonly comprising thin layer chromatography and infrared (IR) spectroscopy in France (NDARC, 2017). Harper *et al.* in their overview of drug checking methods (based on theoretical advantages) recommend mass spectrometry (LC-MS/MS and GC-MS) as a gold standard in forensic drug analysis (Harper et al., 2017). In the context of harm reduction centres, mass spectrometry which requires expensive equipment, trained personnel and a long analysis time are not used currently. IR spectroscopy is potentially well-suited for applications such as drug checking in a harm reduction centre due to both the speed and reliability of the results; these features enable a timely response which may help to reduce the health risks associated with illicit drug use (Harper et al., 2017). For example, IR showed good sensitivity and specificity compared to GC-MS for fentanyl identification (Green et al., 2020). IR

* Corresponding author.
E-mail address: ruben.goncalves@chu-bordeaux.fr (R. Goncalves).

https://doi.org/10.1016/j.drugpo.2020.103037

0955-3959/© 2020 Elsevier B.V. All rights reserved.

spectroscopy is increasingly being used in this context, although the number of studies demonstrating its analytical value in drug checking practice remains limited (Harper et al., 2017, Green et al., 2020, Grobério et al., Dec 2015, Ti et al., 2020). It is important to identify IR analytical limitations in this context. Compared to LC-MS/MS and GC-MS, high resolution mass spectrometry (LC-HRMS) offers unique opportunities for sensitive analysis in analytical toxicology (Wu et al., 2012). This technique allows a precise and exhaustive identification (accuracy and reliability) of the compounds as well as their quantification. To the best of our knowledge, no studies have compared the analytical performance of IR spectroscopy to LC-QTOF reference method in the context of drug checking in a harm reduction centre.

The main objective of this work was to compare the results of product analysis by IR spectroscopy in a harm reduction centre with the results of high-resolution mass spectroscopy to evaluate the ability of an IR spectrometer to identify drugs in this context. The secondary objectives were to carry out a description of people who use drugs requesting a product analysis, and to compare the assumed compositions of products purchased with their real compositions.

Material and methods

We conducted a comparative retrospective study between on-site IR spectroscopy and ultraperformance liquid chromatography–high-resolution mass spectroscopy (UPLC-HRMS), which is the current reference method for drug analysis. Drug samples, along with surveys provided to people who use drugs, were collected from 1 January 2018 to 31 December 2018. Data collection and sample analysis by IR spectroscopy was carried out by professionals at the La Case harm reduction centre for people who use drugs in Bordeaux (France) during normal working hours. Each sample request was anonymized. After IR spectroscopy identification, the content result was given to each person during an individualised harm reduction interview. Subsequently, anonymized samples were sent for LC-QTOF analysis to the Pharmacology and Toxicology Laboratory of the Bordeaux University Hospital Centre.

All samples from people who use drugs who requested a product analysis between 1 January 2018 and 31 December 2018 were included in the study.

The data from surveys of people who use drugs reported here were routinely collected during their interactions at the harm reduction centre. Each patient requesting drug checking completed a survey: the first one upon initial contact with the centre, and the second or subsequent survey upon each request for drug checking. This survey was completed during an individual interview with a centre worker (e.g., social worker, educator, or nurse); it was filled out on the first visit to the harm reduction centre. This questionnaire focused primarily on the socioeconomic data of people who use drugs (e.g., nationality, age, sex, situation, social coverage, economic resources, and housing type). At each analysis request, a product survey was completed during a one-on-one interview with a harm reduction centre professional. The topics covered in this survey included information regarding the collection (i.e., date, person involved, and reason for analysis) and characteristics of the product collected (i.e., name and presumed content). The sample was generally collected after the end of the interview in an Eppendorf tube with the sample/questionnaire number.

Toxicological analysis

Infrared spectroscopy analysis does not require sample preparation. This protocol required the use of a Bruker Alpha model infrared spectrometer (ATR) controlled from a computer equipped with Opus software version 7.0. The UPLC-HRMS system consisted of an Acquity I class system (Waters, Milford, MA, USA) coupled to a Xevo XS G2 QTOF analyser (Waters) using positive and negative mode electrospray ionisation and controlled with Unifi 1.9 software. Quantification of MDMA, cocaine, amphetamine, THC, morphine, and codeine by UPLC–tandem mass spectrometry were performed using an Aquity UPLC system (Waters) coupled with a TQD detector and associated with the MassLynx software. Validation methods were assessed in accordance with the EMEA guideline on bioanalytical method validation (reference number EMEA/CHMP/EWP/192217/2009 Rev. 1). Technical details are available in online supplementary document.

Statistics

Quantitative variables were described by means ± standard deviations and medians, while qualitative variables were expressed as numbers (percentages). Positive predictive value (PPV), negative predictive value (NPV), sensitivity (se), specificity (spe) false negative (1-se) and false positive (1-spe) were calculated when sample numbers were ≥10 in order to be statistically relevant. Statistical analyses were conducted using SAS software (version 9.4, SAS Institute Inc., Cary, NC, USA).

Results

Between 1 January 2018 and 31 December 2018, 2142 people who use drugs were registered at the harm reduction centre, of which 51 (2.4%) requested a product analysis. The average age of people who use drugs requesting a product analysis (n=51) was 35 years (median, 33 years [19-55]; $n = 51$). The majority of people who use drugs were men (90.2%) of French nationality (88.2%), single (94.1%) and without children (88.2%) (sex ratio = 10.8). More than half reported living in precarious housing (62.7%), either on the street (homeless; 0.3%), in squats (58.8%), or in a car (3.6%). Forty-seven percent of the people who use drugs were either without resources or living on illegal resources. They were asked their reasons for requesting testing. 26% had doubt regarding composition, 26% had unusual side effects, 5% reported poor perceived effect, and the remaining 43% did not provide a reason for their testing request.

One hundred thirty-six of the 163 collected samples were available for method comparison. Of the 27 non-analysed samples, four were sent for analysis via the national drug checking system (French National Identification System for Drugs and Other Substances, SINTES), the others were destroyed before they could be sent to the laboratory (Figure 1). The different products brought for analysis exhibited classical presentation: cocaine, amphetamine, and heroin mainly in powder form; MDMA in tablet form (ecstasy); opium in paste form; and cannabis in herb or resin form. The samples were all solid or half-solid. No liquids were brought for analysis.

Of the 136 samples available for method comparison, 112 and 163 psychotropic drugs were identified by IR and laboratory analysis, respectively. The main drugs identified more than 10 times included cocaine ($n = 38$), MDMA ($n = 38$), amphetamine ($n = 17$), and heroin ($n = 12$) (Table 1). For IR spectroscopy, sensitivity ranged from 50% to 100% depending on the molecules; the worst sensitivity was observed for heroin (50%). The rate of false negative with IR spectrometry ranged as follow: heroin (50%), amphetamine (17.6%), MDMA (13.2%). There

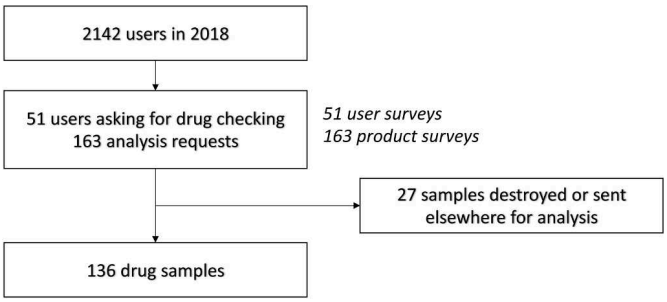


Fig 1. Flow chart (Period from 01/01/2018 to 31/12/2018)

Table 1
Comparison of main psychotropic drugs identification

Identified substance	Laboratory analysis *LC-QTOF (n)*	On-site analysis **IR (n)**	*IR PPV (%)*	*IR NPV (%)*	*IR Se (%)*	*IR Spe (%)*
Cocaine	**38**	**38**	**100**	**100**	**100**	**100**
MDMA	**38**	**33**	**100**	**93.2**	**86.8**	**100**
Amphetamine	**17**	**14**	**100**	**97.5**	**82.4**	**100**
Noscapine	**15**	**0**	**-**	**-**	**-**	**-**
Heroin	**12**	**6**	**100**	**98.5**	**50**	**100**
Opium	**8**	8	**-**	**-**	**-**	**-**
Ketamine	**5**	5	**-**	**-**	**-**	**-**
Codeine	**4**	0	-	-	-	-
THC	**4**	0	-	-	-	-
Morphine	**3**	3	-	-	-	-
4-CEC	**2**	2	-	-	-	-
Methamphetamine	**2**	1	-	-	-	-
MMC	**1**	1	-	-	-	-
Methoxetamine	**1**	1	-	-	-	-
2C-E	**1**	0	-	-	-	-
Alprazolam	**1**	0	-	-	-	-
Hydromorphone	**1**	0	-	-	-	-
Papaverine	**1**	0	-	-	-	-
LSD	**1**	0	-	-	-	-
Methandienone	**1**	0	-	-	-	-
Methcathinone	**1**	0	-	-	-	-
Thebaine	**1**	0	-	-	-	-
Zolpidem	**1**	0	-	-	-	-
Total	**159**	**112**	**-**	-	-	-

Legend: IR: InfraRed spectrometry; LC-DAD: liquid chromatography-diode array detection; GC-MS: gas chromatography-mass spectrometry; LC-MS/MS: liquid chromatography-tandem mass spectrometry; LC-QTOF: quadrupole time-of-flight mass spectrometry; se : sensitivity ; spe : specificity; PPV positive predictive value ; NPV: negative predictive value.

was no false negative with cocaine. The calculated specificities for these molecules were 100%. There were no instances of false positive identification with IR. None of the molecules in non-powder forms were able to be identified, including blotter (e.g., LSD or 2-CE), resin, and herbs (e.g., cannabis). The few samples of MDMA, amphetamine, and heroin not detected by IR were mixtures. IR was unable to distinguish among the opioids present in opium: morphine, thebaine, papaverine, and codeine. Table 2 shows the comparative data for adulterants and filler materials. Less than 25% of adulterants with pharmacological properties were identified by the IR spectroscopy method. Only caffeine (sensitivity of 63.2%), paracetamol (sensitivity of 84.6%), and acetylsalicylic acid were identified. Table 3 shows the main drugs along with their purities and associated adulterants. Among the main suspected products, amphetamine samples appeared to contain the most unexpected substances. One hundred twenty-nine of the 136 samples analysed (94.9%) had the psychoactive product composition expected. The remaining samples corresponded to: a sample of acetylsalicylic acid expected as cocaine; 4-CEC expected as 4-MEC; paracetamol expected as a unknown new psychoactive substance; methandienone expected to be MDMA; alprazolam expected to be mescaline; mixture of MDMA with methoxetamine expected as MDMA alone and 2C-E which was an unknown sample.

Discussion

This study is the first to assess the use of IR spectroscopy in comparison to high resolution mass spectrometry in the context of drug checking for drugs directly from the street market in France. It also represents the original partnership between a university hospital laboratory and a harm reduction centre for people who use drugs. This retrospective study is a method validation study with no return of LC-QTOF results to people who use drugs. Due to a lack of collection of pertinent information, the impact of drug checking on the behaviour of

Table 2
Comparison of adulterants identification

Identified substance	Laboratory analysis *LC-QTOF(n)*	On-site analysis **IR (n)**	*IR PPV (%)*	*IR NPV (%)*	*IR Se (%)*	*IR Spe (%)*
Levamisole	**20**	**0**	**-**	**-**	**-**	**-**
Caffeine	**19**	**12**	**100**	**94.4**	**63.2**	**100**
Paracetamol	**13**	**11**	**100**	**98.4**	**84.6**	**100**
Norephedrine	5	0	-	-	-	-
Acetyleucine	4	0	-	-	-	-
Domperidone	4	0	-	-	-	-
Hydroxyzine	4	0	-	-	-	-
Phenacetin	4	0	-	-	-	-
Ephedrine	3	0	-	-	-	-
Acetylsalicylique acid	2	1	-	-	-	-
Adrafinil	2	0	-	-	-	-
Pseudoephedrine	2	0	-	-	-	-
Selegiline	2	0	-	-	-	-
Atropine	1	0	-	-	-	-
Harpagoside	1	0	-	-	-	-
Lidocaine	1	0	-	-	-	-
Milnacipran	1	0	-	-	-	-
Nefopam	1	0	-	-	-	-
Tetramisol	1	0	-	-	-	-
Total	**90**	**24**	-	-	-	-

Legends: IR: InfraRed spectrometry; LC-DAD: liquid chromatography-diode array detection; GC-MS: gas chromatography-mass spectrometry; LC-MS/MS: liquid chromatography-tandem mass spectrometry; LC-QTOF: quadrupole time-of-flight mass spectrometry; se : sensitivity ; spe : specificity; PPV positive predictive value ; NPV: negative predictive value.

services users was beyond the scope of this paper.

In this study, we focused on people who use drugs who requested drug checking services, which corresponded to approximately 2% of the harm reduction centre population that year. This low proportion is consistent with the findings in previous reports (Tupper et al., 2018). In our population of people who use drugs, the average age was 35 years (median, 33 years [19-55]; $n = 51$); the vast majority were men (90.2%). People who use drugs who have substances assessed using SINTES since 2009 are typically between the ages of 18 and 69 (mean, 32 years) and are primarily men (78%) (OFDT 2019). In this study, more than half reported living in precarious housing (62.7%), compared with only 28.6% in the national survey (OFDT 2017). Concerning resources, 47% had no income or were living on illegal resources, compared to 21.5% in the national survey (OFDT 2017). People who use drugs asking for a drug checking in this harm reduction centre were not representative of national people who use drugs.

We had 163 requests for analyses in 2018, compared to 385 for the SINTES system at the national level in the same year. Because this study was limited to a single centre, the number of samples is considerable when compared to the national collection; however, our results may have been susceptible to selection bias because we were only interested in one harm reduction centre for people who use drugs in Bordeaux.

The drugs most commonly collected via the SINTES system in 2018 included cocaine, heroin, MDMA, and cannabis (OFDT 2019). In our study, the most frequently collected products were cocaine, MDMA, and amphetamine. The fact that less heroin and more MDMA were reported is inconsistent with the worse socioeconomic status of our population, compared with the national data. This could be explained because we only had solid samples in this study and heroin needs to be in liquid form to be injected. The average cocaine content found in our samples (61%) was comparable to that found in the literature (OFDT 2019). In contrast, a large majority of our samples contained another substance (76.3%), which was higher than the national average of 67%. Most of the adulterants identified were conventional substances, of which levamisole was the most common. In recent years, we have noted in Europe a

Table 3
Main drugs identified with purity and adulterants

	Cocaïne (n=38)	MDMA (n=38)	Amphetamine (n=17)	Heroin (n=12)	Opium (n=8)	Ketamine (n=5)	Cannabis (n=4)	Morphine (n=3)
Purity	65 % [3,2-98,5]; n=37	51 % [10-100]; n=35	51,4 % [9,9-87,7]; n=15	NA	Morphine: 24,7 % [20-30,8]; n=7; Codeine: 3,3%	NA	12,1 % [10,6-13,4]; n=4	56,7 % [26-100]; n=3
Samples adultered (%)	76,3 % (n=29)	18,4 % (n=7)	91,4 % (n=16)	66,7 % (n=8)	None	None	None	33,3 % (n=1)
Alduterants (n)	Levamisole (20); Hydroxyzine (4); Phenacetin (4); Acetyleucine (3); Paracetamol (3); Adrafinil (2); Harpagoside (2); Selegiline (2); Acetylsalicylique acid (1); Atropine (1); Caffeine (1); Lidocaine (1); Milnacipran (1); Nefopam (1); Tetramisol (1)	Domperidone (3); Caffeine (1); Paracetamol (1)	Caffeine (6); Norephedrine (5); Pseudoephedrine (1); acetyleucine (1)	Paracetamol (8); Caffeine (7); Domperidone (1)	None	None	None	Caffeine (1)

NA: non assesed

consistent trend towards increased purity in both drug checking samples and police seizures (EMCDDA 2019). The purity of the samples collected was as high as 98.5%, which is also consistent with European and national data.

The average MDMA content found in our samples was 54.3%. This level was noticeably lower than the level observed nationally, with an average of purity of 78% (OFDT 2019). MDMA in powder/crystal form is generally not mix and corresponds to the yield obtained during synthesis of the molecule. The majority of MDMA in our study was in tablet form (ecstasy), which may explain this difference (Vidal Giné et al., 2016). The average MDMA content per tablet in Europe is between 84 and 160 mg (EMCDDA 2019). Unfortunately, tablet strength could not be assessed in our study because we only had a portion of the tablet for analysis. The popularity of ecstasy tablets has been increasing in recent years, with greater purity levels leading to substantial risks of intoxication. Some MDMA samples were associated with amphetamine or new psychoactive substances (e.g., methoxetamine or methcathinone). Methoxetamine associated with MDMA was previously reported by SINTES (OFDT 2017) and is increasingly common as a substitute or adulterating agent in some samples. However, it was not possible to differentiate between sample contamination during dose preparation or intentional mixing in this study.

The average amphetamine content of our samples was twofold greater than the national and European averages (46% vs. 28%) (OFDT 2019, EMCDDA 2019). This level contrasts with the trend for reduced purity of these samples (EMCDDA 2019). Nearly all samples contained one or more compounds, consistent with previous findings (OFDT 2019, EMCDDA 2019). As in the national data, the primary adulterant identified was caffeine, along with more hazardous chemicals such as norephedrine, ephedrine, pseudoephedrine, methamphetamine, and MDMA.

The main substances identified by the laboratory method included cocaine, MDMA, and amphetamine, consistent with the findings in the European drug checking study (EMCDDA 2019). IR spectroscopy was able to accurately identify the primary compound; there were no false positives and very few false negatives. These results are comparable to those found for IR spectroscopy in comparison with GC-MS method (Ti et al., 2020). considerable limitations for IR spectroscopy were identified in its analysis of cannabis (herb or resin), compounds in blotter form, and compounds present in small quantities and/or in mixtures. For compounds presented in small quantities, IR spectroscopy was only able to identify the main active substances in the sample. For other compounds tested, some were not detected due to their absence from the standards library (1.5 %, n=2), such as 2C-E (new psychoactive substance) and methandienone (anabolic), while others were below the limit of detection for IR spectroscopy (2.2 %, n=3). The compounds below the limit of detection were mostly minority compounds present in a mixture; these were presumably contaminants which arose during preparation of the sample by dealers and may have affected the number of false negatives in our study.

With the exception of paracetamol, caffeine, and aspirin, IR spectroscopy was unable to reliably identify adulterants in samples submitted as mixtures. Norephedrine, acetylleucine, selegiline, atropine, harpagoside, and milnacipran were not identified by IR spectroscopy due to their absence from the standard library. For other substances, the absence of detection was not related to deficiencies in analytical standards, suggesting that that their contents were either below the limit of detection for the device or that they were heterogeneously present in the sample and therefore undetectable on the small portion analysed.

Difficulties in identifying minority spectra may be due to misinterpretation of the IR spectra obtained. This observation is a useful reminder to harm reduction professionals who use IR spectrometry regarding the importance of subtracting out the majority spectra, thereby isolating the minority spectra. There is also the potential for interpretation bias, because analyses were performed by harm reduction professionals. While all of them received training from the instrument supplier, they lacked the technical expertise of analytical professionals. We hypothesise that some more compounds could have been identified by trained professionals; however, this choice was intentional to determine the performance of IR spectroscopy under real-world conditions. This collaborative work between hospital toxicologists and social workers led to improved interpretation of the IR analyses on-site.

For some drugs, the small number of samples submitted prevented any meaningful statistical analyses. Moreover, only drugs brought by people who use drugs could be tested, so the data are not exhaustive. The aim of our study was to compare analytical methods. The compounds found are not necessarily representative of all patients and products circulating in the drug market. We could have tested pure products, but we wanted to remain true to real-world conditions with drugs submitted directly by people who use drugs. Indeed, these products are very often heterogeneous; from a technical perspective, it is more difficult to identify them, compared to pure substances. Some of them are active and harmful in very low doses which may make IR not particularly useful in many settings that are contending with a saturated fentanyl market for example.

IR spectroscopy is very sensitive to the matrices in which the sample is presented (e.g., solvents, water, plants, or blotter paper). Notably, the supplier claims that IR spectroscopy is able to differentiate THC from cannabidiol (Bruker 2019), but this differentiation was not achieved in our study. These samples are very often heterogeneous and contain high

water content, thereby limiting the accuracy of the instrument.

There are many variables to consider when choosing an analytical technique for drug checking in a harm reduction setting. First, the type of information provided to people who use drugs must be determined, as this decision will dramatically affect the choice of methodology. The necessary and available resources (financial and labour) for implementation of these methods should also be considered. In some studies, people who use drugs indicated that quantitative methods were preferable to other methods; however, the length of the analysis and the use of methods which ultimately destroyed the target substance were identified as significant limitations to the use of drug checking (Bardwell et al., 2019). People who use drugs prefer rapid, non-destructive, quantitative methods that are capable of detecting compounds present in small quantities. They also question the legal framework of the system and possible cost of the analysis (Barratt et al., 2018, Glick et al., 2019). Furthermore, it is important to consider the evolution of the drug market. Diversification of products in the drug market, with the presence of increasingly powerful products which are active in very low doses (e.g., fentanyloids), require very sensitive identification methods. The risks of false or partial information can affect people who use drugs and the device itself, which may lose credibility for drug checking. However, care is needed to avoid providing people who use drugs a false sense of security, warning them of potentially dangerous compounds which may have been missed, such as ocfentanyl heroin detected by a drug checking programme in Spain by LC-MS/MS (Quintana et al., 2017). In this context, the evaluation of techniques in real-world settings (as performed in this study) remains a vital step to ensure the validity of drug checking systems.

Among the products collected, only seven samples did not correspond to the declared product, either alone or with adulterants. In our study, the discordant substances were highly variable: acetylsalicylic acid, 4-CEC, paracetamol, methandienone, alprazolam, methoxetamine, and 2C-E. Replacement can generally be done with inert products such as certain excipients (e.g., lactose or mannitol), other products without major toxicity (e.g., aspirin), or molecules with similar effects and varying toxicity thresholds that can cause undesirable or unusual effects. Among the fraudulent samples identified in our study, only two presented a serious risk: 4-CEC in place of 4-MEC, and 2C-E which was an unknown sample. 4-CEC is part of the same family of substances as 4-MEC (cathinones), but there are limited data regarding its toxicity and potential adverse effects (Grifell et al., 2017). 2C-E is a member of the phenethylamine family, more specifically the "2C" family, a new hallucinogenic synthetic product for which one case of fatal toxic leukoencephalopathy and other cases of death have been described (Sacks et al., Oct 2012). Hallucinogenic phenethylamines (e.g., 2C-E) showed a similar negative profile to LSD but were rated as less pleasurable and less likely to be used again (Matthews et al., 2017). In case of intoxication, the symptoms are very variable, ranging from vomiting to respiratory distress and hallucinations (Iwersen-Bergmann et al., 2019, Papaseit et al., 2018). The findings presented here appear to be consistent with previous studies, with few studies identifying unexpected new psychoactive substances instead of conventional molecules (Caudevilla et al., 2016, ÁJ et al., 2017).

These two cases highlight the main interests of drug checking, in that they support the toxicovigilance interests of risk reduction advocates regarding the detection of new practices or substances and the direct interest for people who use drugs to ensure the relative safety of the product analysed (Giné et al., 2017, Butterfield et al., 2016). A similar study by the EMCDDA examining another European drug checking effort was recently presented in its latest report on drugs in 2020 (EMCDDA 2020).

Conclusions

IR spectroscopy has a number of advantages. It allows for rapid and reliable detection of the main drug in a sample with minimal operator training. It requires no solvents or sample preparation; thus, it is an easy-to-use device with a low cost of ownership. It uses a non-destructive process that allows further analysis with the same sample and is highly portable, enabling use at festivals and other events. These benefits allow harm reduction advocates to better spread their message by offering a reactive tool that is well adapted to an unstable urban clientele that buys in small quantities and consumes them quickly.

However, IR spectroscopy has important limitations for identification of various sample types, including cannabis, blotters, mixtures, and products present in low levels. In these instances, as well as in situations where people who use drugs report undesirable or unusual effects, additional analyses with high resolution mass spectroscopy are needed to better assess the composition of the substance.

This analysis is the first study in France to evaluate an on-site drug checking method by IR spectroscopy performed at a harm reduction centre, with a direct comparison to more sophisticated laboratory methods. This is an original work, which has not been the subject of previous studies. This study has highlighted the limits of IR spectroscopy in the context of on-site drug checking under real-world conditions at a harm reduction centre for people who use drugs, thereby allowing device users to better understand the limits of IR and aid in their harm reduction advice. Based on these results, other institutions will be able to better implement their own drug checking programs using similar devices. IR spectroscopy is therefore a promising method in the context of drug checking but must be the focus of further studies to better define its framework and limits.

Declaration of Competing Interest

None.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.drugpo.2020.103037.

References

Bardwell, G, Boyd, J, Tupper, KW, & Kerr, T (2019). "We don't got that kind of time, man. We're trying to get high!": Exploring potential use of drug checking technologies among structurally vulnerable people who use drugs. *Int J Drug Policy, 71*, 125–132. Sept.

Barratt, MJ, Bruno, R, Ezard, N, & Ritter, A (2018). Pill testing or drug checking in Australia: Acceptability of service design features: Designing a drug-checking service. *Drug Alcohol Rev, 37*(2), 226–236. February.

Bruker (2019). Differentiation of THC and CBD cannabis using FTIR [Accessed on 6 Oct 2019]. Available at: https://www.bruker.com/fileadmin/user_upload/8-PDF-Docs/OpticalSpectrospcopy/FT-IR/ALPHA/AN/AN_M157_Differenzierung_Cannabis_FTIR_EN.pdf.

Butterfield, R, Barratt, M, Ezard, N, & Day, R (2016). Drug checking to improve monitoring of new psychoactive substances in Australia. *Med J Aust, 204*(4), 144–145.

Caudevilla, F, Ventura, M, Fornís, I, Barratt, MJ, Vidal, C, lladanosa, CG, et al. (2016). Results of an international drug testing service for cryptomarket users. *Int J Drug Policy, 35*, 38–41. Sept.

EMCDDA (2019). European Drug Report 2019 [Accessed on 9 Sept 2019]. Available at: http://www.emcdda.europa.eu/system/files/publications/11364/20191724_TDAT19001FRN_PDF.pdf.

EMCDDA (2020). European Drug Report 2020 [Accessed on 9 Sept 2019]. Available at: https://www.emcdda.europa.eu/system/files/publications/13236/TDAT20001ENN_web.pdf.

Giné, CV, Vilamala, MV, Measham, F, Brunt, TM, Bücheli, A, Paulos, C, et al. (2017). The utility of drug checking services as monitoring tools and more: A response to Pirona et al. *Int J Drug Policy, 45*, 46–47. July.

Glick, JL, Christensen, T, Park, JN, McKenzie, M, Green, TC, & Sherman, SG (2019). Stakeholder perspectives on implementing fentanyl drug checking: Results from a multi-site study. *Drug Alcohol Depend, 194*, 527–532. January.

Green, TC, Park, JN, Gilbert, M, McKenzie, M, Struth, E, Lucas, R, et al. (2020). An assessment of the limits of detection, sensitivity and specificity of three devices for public health-based drug checking of fentanyl in street-acquired samples. *Int J Drug Policy., 77*, Article 102661, 1 March.

Grifell, M, Ventura, M, Carbón, X, Quintana, P, Galindo, L, Á, Palma, et al. (2017). Patterns of use and toxicity of new para-halogenated substituted cathinones: 4-CMC

(clephedrone), 4-CEC (4-chloroethcatinone) and 4-BMC (brephedrone). *Hum Psychopharmacol Clin Exp, 32*(3), e2621.
Grobério, TS, Zacca, JJ, ÉD, Botelho, Talhavini, M, & Braga, JWB (Dec 2015). Discrimination and quantification of cocaine and adulterants in seized drug samples by infrared spectroscopy and PLSR. *Forensic Sci Int, 257*, 297–306.
Harper, L, Powell, J, & Pijl, EM (2017). An overview of forensic drug testing methods and their suitability for harm reduction point-of-care services. *Harm Reduct J., 14*(1), 52, 31.
Iwersen-Bergmann, S, Lehmann, S, Heinemann, A, Schröder, C, Müller, A, Jungen, H, et al. (2019). Mass poisoning with NPS: 2C-E and Bromo-DragonFly. *Int J Legal Med., 133*(1), 123–129, 1 January.
Matthews, A, Sutherland, R, Peacock, A, Van Buskirk, J, Whittaker, E, Burns, L, et al. (2017). I like the old stuff better than the new stuff? Subjective experiences of new psychoactive substances. *Int J Drug Policy, 40*, 44–49.
NDARC (2017). Bulletin no. 24: Global review of drug checking services operating in 2017 | NDARC - National Drug and Alcohol Research Centre. [Accessed on 15 January 2019]. Available at: https://ndarc.med.unsw.edu.au/resource/bulletin-no-24-global-review-drug-checking-services-operating-2017.
OFDT (2017). Profils et pratiques des usagers des CAARUD en 2015 - Profiles and practices of harm reduction centres for people who use drugs in 2015. [Accessed on 3 July 2019]. Available at: https://www.ofdt.fr/publications/collections/periodiques/lettre-tendances/profils-et-pratiques-des-usagers-des-caarud-en-2015-tendances-120-octobre-2017/.
OFDT (2019). SINTES 2019. [Accessed on 13 Oct 2020]. Available at: https://www.ofdt.fr/BDD/sintes/LePointSINTES05.pdf.
Palma-Conesa, ÁJ, Ventura, M, Galindo, L, Fonseca, F, Grifell, M, Quintana, P, et al. (2017). Something New about Something Old: A 10-Year Follow-Up on Classical and New Psychoactive Tryptamines and Results of Analysis. *J Psychoactive Drugs., 49*(4), 297–305, 8 August.
Papaseit, E, Farré, M, Pérez-Mañá, C, Torrens, M, Ventura, M, Pujadas, M, et al. (2018). Acute Pharmacological Effects of 2C-B in Humans: An Observational Study. *Front Pharmacol, 9*.
Quintana, P, Ventura, M, Grifell, M, Palma, A, Galindo, L, Fornís, I, et al. (2017). The hidden web and the fentanyl problem: Detection of ocfentanil as an adulterant in heroin. *Int J Drug Policy, 40*, 78–83.
Sacks, J, Ray, MJ, Williams, S, & Opatowsky, MJ (Oct 2012). Fatal Toxic Leukoencephalopathy Secondary to Overdose of a New Psychoactive Designer Drug 2C-E ("Europa"). *Bayl Univ Med Cent Proc, 25*(4), 374–376.
Ti, L, Tobias, S, Lysyshyn, M, Laing, R, Nosova, E, Choi, J, et al. (2020). Detecting fentanyl using point-of-care drug checking technologies: A validation study. *Drug Alcohol Depend., 212*, Article 108006, 1 July.
Tupper, KW, McCrae, K, Garber, I, Lysyshyn, M, & Wood, E (2018). Initial results of a drug checking pilot program to detect fentanyl adulteration in a Canadian setting. *Drug Alcohol Depend, 190*, 242–245. Sept.
Vidal Giné, C, Ventura Vilamala, M, Fornís Espinosa, I, Gil Lladanosa, C, Calzada Álvarez, N, Fitó Fruitós, A, et al. (2016). Crystals and tablets in the Spanish ecstasy market 2000–2014: Are they the same or different in terms of purity and adulteration? *Forensic Sci Int, 263*, 164–168, 1 June.
Wu, AH, Gerona, R, Armenian, P, French, D, Petrie, M, & Lynch, KL (2012). Role of liquid chromatography–high-resolution mass spectrometry (LC-HR/MS) in clinical toxicology. *Clin Toxicol., 50*(8), 733–742, 1 Sept.

III. Apport analytique de la spectrométrie de masse haute résolution en QTOF pour l'identification des nouveaux produits de synthèse : intérêt du couplage de méthodes et de l'étude de métabolisme – Publication n° 2

A. Introduction et présentation

La méthoxpropamine (MXPr ou 3-MeO-2′-oxo-PCPr) est une drogue dissociative de la famille des arylcyclohexylamines et un homologue de la méthoxétamine (MXE), structurellement similaire à la 3-MeO-PCE, la kétamine et la deschlorokétamine (137). Cette drogue était à l'origine vendue en ligne comme une drogue de synthèse et a été identifiée pour la première fois en Europe en octobre 2019 au Danemark. La MXPr a été ensuite signalée en 2020 comme un NPS par l'Observatoire européen des drogues et des toxicomanies (EMCDDA) (138). Il existe peu de données dans la littérature sur la pharmacologie, le métabolisme et la toxicité de la MXPr. Comme ses analogues, à savoir la kétamine et la MXE, la MXPr pourrait agir comme un puissant antagoniste des récepteurs N-méthyl-D-aspartate (NMDA) (139). La MXPr bloquerait les récepteurs NMDA de manière dose-dépendante (140). La CI50 de la MXPr et de la MXE décroît dans l'ordre suivant : MXPr (1,647 µM) > MXE (0,841 µM). Étant donné que la MXPr présente des similitudes structurelles avec d'autres arylcyclohexylamines et a un effet antagoniste potentiel contre les récepteurs NMDA, une action dissociative et des effets indésirables similaires sont possibles en cas de surdosage. Des décès suite à la consommation de MXE ont été rapportés, entrainant son interdiction dans de nombreux pays (4,141,142). L'usage récréatif de la MXE peut provoquer une tachycardie, une confusion, une agitation, une ataxie, un nystagmus et un état dissociatif (142,143). Selon les *trip report* disponibles sur les forums de consommateurs, la MXPr est principalement consommée par voie nasale, mais les voies orale et rectale (*plug*) ont également été rapportées. Les usagers décrivent un rétrécissement du champ visuel, un myosis, un sentiment d'euphorie, des hallucinations pouvant aller jusqu'au « *M hole* », ce qui a conduit un usager à être hospitalisé (Psychoactif.org).

Nous rapportons la première identification de MXPr en France avec confirmation analytique. Le premier objectif était d'identifier et de caractériser la MXPr dans des échantillons d'urine, de cheveux et de poudre en combinant plusieurs méthodes analytiques : résonance magnétique nucléaire (RMN) à l'état liquide, spectroscopie infrarouge (IR) et spectrométrie

de masse à haute résolution couplée à la chromatographie liquide (LC-HRMS). Le second objectif était d'explorer le métabolisme *in silico*, *in vitro* et *in vivo* de la MXPr.

B. Discussion

Les méthodes analytiques de routine comme la LC-MS/MS ne sont pas toujours suffisantes pour identifier formellement les NPS, par exemple dans le cas d'isomères de position. Il est donc nécessaire d'utiliser des méthodes complémentaires, telle que la spectroscopie RMN ou IR pour pouvoir les distinguer (144,145). Nous avons utilisé la LC-HRMS, l'IR et la RMN afin d'identifier et de caractériser l'échantillon de poudre inconnu collecté chez un patient admis aux urgences du CHU de Bordeaux. La MXPr a pu être identifiée avec cette combinaison d'analyses. Aucune impureté organique significative n'a été identifiée dans la poudre par LC-HRMS, IR ou RMN. La spectroscopie RMN 1H unidimensionnelle et la spectroscopie RMN 1H-13C et 1-1H bidimensionnelle ont permis d'identifier sans ambiguïté les résonances RMN correspondant aux 16 atomes de carbone de la MXPr ainsi que les 12 signaux de protons. En outre, l'analyse RMN 1H a révélé une teneur en MXPr de ~95 % dans la poudre (les ~5 % restants étaient des composés secondaires) (146). Cette teneur a ensuite été prise en compte pour l'incubation des microsomes hépatiques (HLMs). Cette identification a confirmé les informations inscrites sur le pochon de poudre. Il faut savoir que de nombreuses publications montrent des discordances entre le produit supposé et le produit réellement identifié principalement dans le cas des NPS (45,147,148).

Concernant l'analyse des urines, le premier criblage toxicologique par LC-HRMS avait permis d'identifier de l'oxazépam, du nordiazépam, de la norbuprénorphine et de la N-éthylhexédrone. Un pic important ne correspondait à aucune substance présente dans les grandes bases de données disponibles. L'ajout du composé MXPr identifié par les spectroscopies RMN et IR, ainsi que l'analyse rétrospective de l'urine par LC-HRMS ont permis d'identifier le pic inconnu comme étant de la MXPr.

L'étude du métabolisme *in vitro* a permis d'identifier trois métabolites principaux sur la base de l'intensité des pics chromatographiques : N-despropyl(nor)MXPr, O-desméthyl MXPr, et dihydroMXPr. Ces métabolites étaient conformes à la voie métabolique prédite, à savoir la N-dépropylation en norMXPr, la réduction en dihydroMXPr et la O-déméthylation en

métabolites O-desméthylMXPr. Pour l'ensemble de ces molécules, la précision en masse des métabolites était conforme à la masse exacte théorique attendue, à moins de 3 ppm près. Aucun autre métabolite de phase I ou II n'a été identifié. La norMXPr était le métabolite le plus abondant. Ces résultats sont cohérents avec ceux obtenus récemment *in vitro* par Menzies *et al.* (149). Nous avons ensuite pu comparer les métabolites générés par les microsomes *in vitro* avec ceux trouvés dans l'urine du patient. Le NorMXPr (via la N-dépropylation) était le métabolite prédominant *in vitro* et *in vivo* d'après l'intensité de son signal MS, l'indiquant comme le principal métabolite de la MXPr, comme c'est le cas pour la kétamine (150). Hijazi *et al.* (151) ont démontré que le CYP3A4 est la principale enzyme responsable de la N-désalkylation de la kétamine avec le CYP2B6 et le CYP2C9. Le CYP3A4 pourrait donc être responsable du métabolisme de la MXPr en son principal métabolite, identifié ici comme étant la norMXPr. Comme la norkétamine est connue pour être un métabolite actif, nous pouvons faire la même hypothèse pour la norMXPr. Par conséquent, il serait intéressant d'évaluer la toxicité de ce métabolite.

Si on se base sur l'intensité du signal, les autres métabolites principaux étaient le O-desméthyl et le dihydro MXPr. Ils étaient à la fois retrouvés dans les incubats microsomaux et les échantillons de patients (urine et cheveux). Aucun métabolite de phase I n'a été détecté ni *in vitro* ni *in vivo*. Cela est contraire à ce qui a été décrit pour la MXE. En effet, Horsley *et al.* (152) ont identifiés trois metabolites glucuronides conjugués avec un groupe fonctionnel amine primaire et sept avec un groupe amine secondaire. La N-glucuronidation des amines primaires et secondaires aliphatiques pourrait jouer un rôle majeur chez d'autres espèces, mais il est peu probable qu'il s'agisse d'une voie importante pour le métabolisme de la MXPr, si elle existe chez l'homme, et il est raisonnable de prévoir une conjugaison à un groupe hydroxyle facilement accessible.

Cette étude souligne la difficulté d'identifier certains NPS lorsqu'ils sont absents des bibliothèques et que le standard n'est pas disponible. Nous montrons ici que la combinaison de plusieurs méthodes analytiques avec l'HRMS est une méthodologie puissante pour la caractérisation structurale des NPS. L'identification des métabolites de la MXPr va permettre d'agrémenter les bibliothèques de criblage toxicologique et élargir la fenêtre de détection de la MXPr dans les matrices biologiques. Grâce à ces résultats, nous suggérons d'ajouter le N-despropyl(nor) MXPr, le O-desméthyl MXPr et le dihydroMXPr aux bibliothèques de criblage toxicologique afin de réduire le risque de faux-négatifs dans les cas d'intoxication impliquant la MXPr. D'autres études *in vivo* seront nécessaires afin de confirmer ces données.

Publication n° 2

Methoxpropamine (MXPr) in powder, urine and hair samples: Analytical characterization and metabolite identification of a new threat

Ruben Goncalves, Nadège Castaing, Camille Richeval, Dominique Ducint, Karine Titier, Estelle Morvan, Axelle Grélard, Antoine Loquet, Mathieu Molimard

Publié en Avril 2022 dans *Forensic Science International*

Volume 333, 111215

https://doi.org/10.1016/j.forsciint.2022.111215

Forensic Science International 333 (2022) 111215

Contents lists available at ScienceDirect

Forensic Science International

journal homepage: www.elsevier.com/locate/forsciint

Methoxpropamine (MXPr) in powder, urine and hair samples: Analytical characterization and metabolite identification of a new threat

R. Goncalves[a,b,*], N. Castaing[a], C. Richeval[c,d], D. Ducint[a], K. Titier[a], E. Morvan[e], A. Grélard[f], A. Loquet[f], M. Molimard[a,b]

[a] CHU de Bordeaux, Laboratoire de Pharmacologie et Toxicologie, F-33000 Bordeaux, France
[b] Univ. Bordeaux, INSERM, BPH, U1219, F-33000 Bordeaux, France
[c] CHU de Lille, Unité Fonctionnelle de Toxicologie, F-59000 Lille, France
[d] Univ. Lille, ULR 4483 – IMPECS – IMPact de l'Environnement Chimique sur la Santé humaine, F-59000 Lille, France
[e] Univ. Bordeaux, UMS3033 US001, CNRS, INSERM, Institut Européen de Chimie et Biologie, F-33600 Pessac, France
[f] Univ. Bordeaux, CBMN (UMR5248), CNRS, IPB, Institut Européen de Chimie et Biologie, F-33600 Pessac, France

ARTICLE INFO

Article history:
Received 7 December 2021
Received in revised form 29 January 2022
Accepted 5 February 2022
Available online 8 February 2022

Keywords:
Methoxpropamine
MXPr
High-resolution mass spectrometry
LC-QTOF
NMR
IR spectroscopy
In vitro metabolism
Hair analysis

ABSTRACT

Methoxpropamine (MXPr) is an arylcyclohexylamine dissociative drug with structural similarities with 3-MeO-PCE, ketamine and deschloroketamine. MXPr was identified for the first time in Europe in October 2019 in Denmark and is considered a new psychoactive substance. We undertook the molecular identification and characterization of MXPr in urine, hair and powder samples. We used a combination of several analytical methods: liquid-state nuclear magnetic resonance (NMR), infra-red spectroscopy (IR) and liquid chromatography high-resolution mass spectrometry (LC-HRMS). The second objective was to explore the metabolism of MXPr in silico and in vitro. To detect characteristic metabolites that prove MXPr consumption by urine analysis, pooled human liver microsome (pHLM) assays were performed and evaluated using liquid chromatography quadrupole time-of-flight mass spectrometry (LC-QToF-MS). A software algorithm (Unifi®) was used to predict in silico biotransformations of MXPr. Three metabolites were identified in the in vitro studies including N-despropyl(nor)MXPr, O-desmethyl MXPr and dihydroMXPr. Most of these phase II metabolites were confirmed to be present in urine and hair samples collected from an MXPr consumer. This is the first report of the identification of MXPr in France with analytical findings. This study highlights the challenge of identifying new psychoactive substances (NPS) when they are missing from compound libraries and if a standard is not available. The use of various complementary analytical methods combined with HRMS offers a promising approach for the molecular characterization of NPS.

© 2022 Elsevier B.V. All rights reserved.

1. Introduction

Methoxpropamine (MXPr or 3-MeO-2′-oxo-PCPr) is an arylcyclohexylamine dissociative drug and a higher homologue of methoxetamine (MXE) that possesses structural similarities with 3-MeO-PCE, ketamine and deschloroketamine [1]. MXPr differs from MXE due to the replacement of ethylamino with propylamino. MXPr contains a chiral center; so two possible enantiomers of the substance may exist. It was originally sold online as a designer drug and it was first identified in Europe in October 2019 in Denmark. It was then reported in 2020 as a new psychoactive substance (NPS) by the European Monitoring Center for Drugs and Drug Addiction (EMCDDA) [2]. Little is known about the pharmacology, metabolism and toxicity of MXPr. Like its analogs ketamine and MXE, MXPr acts as a potent antagonist of N-methyl-D-aspartate (NMDA) receptors [3]. MXPr blocked NMDA receptor in a dose-dependent manner [4]. The IC50 of MXPr and MXE decreased in the following order: MXPr (1.647 μM) > MXE (0.841 μM). Because MXPr has structural similarity with other arylcyclohexylamines and has a potential antagonistic effect against the NMDA receptors, similar dissociative reactions and adverse effects would be induced when MXPr is abused. Deaths due to MXE abuse have been reported and it is banned in many countries [5–7]. The recreational use of MXE may induce tachycardia, confusion, agitation, ataxia, nystagmus and a dissociative state [7,8]. According to trip reports available on consumer forums, MXPr is mainly consumed by nasal route but the oral

* Correspondence to: Laboratoire de Pharmacologie et Toxicologie Médicale, CHU de Bordeaux - PTMP – 2ème étage, Place Amélie Raba Léon, 33076 Bordeaux Cedex, France.
E-mail address: ruben.goncalves@u-bordeaux.fr (R. Goncalves).

https://doi.org/10.1016/j.forsciint.2022.111215
0379-0738/© 2022 Elsevier B.V. All rights reserved.

and rectal routes (plug) are also used. Users describe feelings of euphoria, narrowing of the visual field and hallucinations, myosis, and a euphoric cottonwool-head sensation which can go as far as the "M hole" that led one user to be hospitalized (Psychoactif.org). Like MXE, abuse of MXPr is harmful to humans by blocking the NMDA receptors, so it has become a serious concern.

We report the first identification of MXPr in France with analytical findings. The primary aim was to identify and characterize MXPr in urine, hair and powder samples by combining several analytical methods: liquid state nuclear magnetic resonance (NMR), infrared (IR) spectroscopy and liquid chromatography high-resolution mass spectrometry (LC-HRMS). The second objective was to explore the metabolism of MXPr in silico and in vitro.

2. Case history

A 26-year-old man known as a polydrug user, especially of new psychoactive substances (NPS), was admitted to the emergency department. He was brought in by his mother after she found bags of white powder in his room. He was known for his psychiatric disturbances and polydrug addiction. He was asymptomatic. Only urine immunochemistry screening for classic psychoactive substances was requested and was negative. The patient was monitored for 6 h in the emergency room and then discharged. The clinicians did not call the analytical toxicologist to discuss these results. LC-HRMS screening identified oxazepam, nordiazepam, norbuprenorphine, N-ethylhexedrone and an unknown important peak not found in any of the large libraries. After discussion with the clinicians, the mother brought in a bag of white powder labeled "MXPr - Not for human Use". The powder was sent to the laboratory for analysis. A strand of brown hair was collected four months after hospitalization.

3. Material and methods

3.1. Chemicals and reagents

According to Richeval et al. [9], β-OH-ethyltheophyllin and methyl-clonazepam (internal standards), β-glucuronidase (Helix pomatia), alamethicin (Trichoderma viride), uridine diphosphate glucuronic acid (UDPGA), glucose-6-phosphate dehydrogenase (G6PD), glucose-6-phosphate (G6P), and 5-sulfosalicylic acid were all purchased from Sigma-Aldrich (Saint-Quentin-Fallavier, France). Tetrasodium salt of a reduced form of nicotinamide adenine dinucleotide phosphate (NADPH) was purchased from Roche (Mannheim, Germany). A pool of human liver microsomes (HLMs) at a concentration of 20 mg of microsomal protein per milliliter was purchased from Biopredic (La Bretèche, Saint Grégoire, France) and kept at – 80 °C until use. All other chemicals were of analytical grade and obtained from common commercial sources.

3.2. Nuclear magnetic resonance analysis

5.08 mg of “MXPr” powder were completely dissolved in 500 μL of deuterated methanol (Eurisotop, Saint Aubin, France). 1D and 2D experiments were conducted on a Bruker Avance III HD Nanobay spectrometer (Bruker, Wissembourg, France) operating at 400 MHz for proton and at 100 MHz for carbon by means of a 5 mm diameter smart probe. All NMR experiments were recorded at 25 °C. For 1D 1 H and 13 C experiments, acquisition parameters used were the following: 10 μs for 1 H and 13 C hard pulses, 24 and 2048 scans, relaxation delay of 2 s and 5 s and spectral windows of 10 and 24 kHz. 2D homonuclear experiments (1–1 H COSY, 1–1 H TOCSY and 1–1 H NOESY) were acquired with a spectral window of 3.6 kHz, scan number between 8 and 24, a relaxation delay of 1.3 s or 1.8 s, an acquisition time of 285 ms in direct dimension and 36 ms in indirect dimension. The same mixing time of 300 ms was used for TOCSY and NOESY experiments. To correlate 1 H and 13 C resonances, 2D heteronuclear experiments (1 H–13 C HSQC, 1 H-13 C HMBC) were performed with scan number between 16 and 24 scans, a proton spectral window of 3.6 kHz, a carbon spectral window of 17 kHz and 22 kHz, a relaxation delay of 1.3 s and 1.8 s, an acquisition time of 285 ms in direct dimension and 8 ms or 6 ms in indirect dimension. For 2D HSQC, delays were optimized for a 145 Hz coupling constant ($^1J_{CH}$) and for 2D HMBC, a coupling constant of 8 Hz was used for long distance correlation. All NMR spectra were processed by using the TopSpin software (Bruker, Wissembourg). A Lorentzian noise filtering respectively 3 Hz and 0.3 Hz was applied prior to Fourier transform for 1D carbon and proton spectra. 1 H and 13 C chemical shifts were calibrated against the CH3OH signals (3.31 ppm for residual 1 H and 49.0 ppm for 13 C).

3.3. FTIR spectroscopy analysis

Structural identification was confirmed by IR spectroscopy. Briefly, no sample preparation was necessary for Fourier Transform Infrared Spectroscopy (FTIR) measurements. The composition was examined by an FTIR (Nicolet Summit (Thermo Fisher, MA, USA)) spectrometer operating in Attenuated Total Reflectance (ATR) mode through an integrating sphere by the Nicolet 6700 FTIR device. The signals were recorded between 4000 and 500 cm^{-1} with a resolution of 4 cm^{-1}. Spectrometer diagnostics and acquisition of spectroscopic data were performed by OMNIC Paradigm software (Thermo Fisher, MA, USA).

3.4. Toxicological screening test for product, urine and hair samples

3.4.1. Sample preparation

As previously published [10], liquid-liquid extraction was performed for urine sample. Briefly, a basic extraction was performed in a sodium carbonate buffer at pH 9.7 and an acid extraction was performed in hydrochloric acid 0.1 N. Prazepam (5 μg/mL) was used as an internal standard. Product sample was prepared in methanol. The hair sample (4 cm) was cut as close as possible from the vertex posterior of the head corresponding to a time frame analysis of 4 months after hospitalization. Segmental hair analysis was performed. The strand of brown hair was divided into four 1-cm segments each weighing 20 mg. The hair sample was decontaminated in a bath of dichloromethane for 2 min, followed by a 2-min bath in deionized water. The dried hair sample was ground into a fine, homogeneous powder using a ball mill (Precellys 24, Bertin Technologies, Montigny-le-Bretonneux, France). Hydrolysis was performed using 1 mL of methanol with 1 mL of 0.1% formic acid and 50 μL of internal standard (prazepam, 5 μg/mL) at 45 °C for 16 h. Extracts were evaporated to dryness under a gentle stream of nitrogen and reconstituted with 100 μL of water/acetonitrile (50:50, v/v).

3.4.2. Liquid chromatography-high-resolution mass spectrometry parameters

As previously published [10], the UPLC-HRMS system consisted of an Acquity I class system (waters, USA) coupled to a Xevo XS G2 QTOF analyzer (Waters, USA) using positive and negative mode electrospray ionization and controlled with Unifi 1.9 software. Chromatographic separation was performed on an ACQUITY HSS C_{18} column (150 ×2 mm, 1.8 μm, Waters). The mobile phase was composed of solvents A (ammonium formate buffer 5 mM, pH 3) and B (acetonitrile in 0.1% v/v formic acid). The column was maintained at 50 °C and a gradient elution was used at a flow rate of 0.4 mL/min: 13% of B held until 0.5 min, increasing to 50% at 10 min, then to 95% at 10.25 min, and finally held until 12.25 min, then return to initial condition in 0.25 min until 17 min. The injection volume was 2 μL. Data were analyzed with HighResNPS® and Waters® databases for

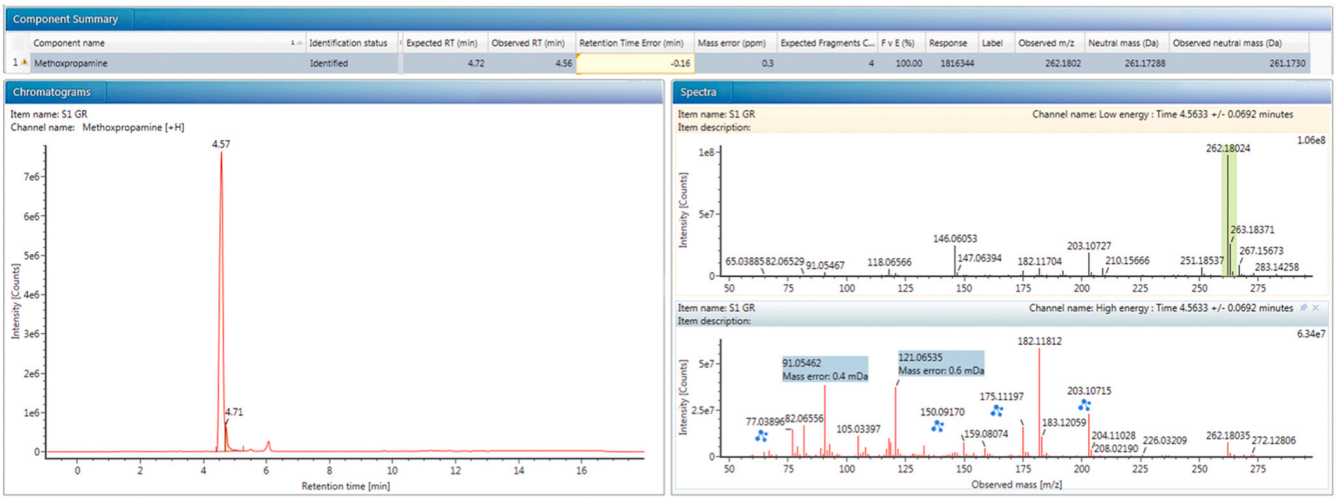


Fig. 1. Chromatogram of MXPr using LC-QTOF in powder.

over 3500 toxicologically relevant drugs, NPS and metabolites that included protonated molecules and fragmentation and chromatographic retention time data. Criteria used to identify compounds included 5 ppm as exact mass error of the precursor ion as well as product ions and + /- 0.5 min as tolerance in retention time for the precursor ion.

3.5. MXPr metabolism exploration

3.5.1. In silico metabolite identification [11]

Data was analyzed with Unifi software (Waters, Manchester, UK). A software algorithm (MetaboLynx) was used to predict in silico biotransformations. Using the available data on the metabolism of

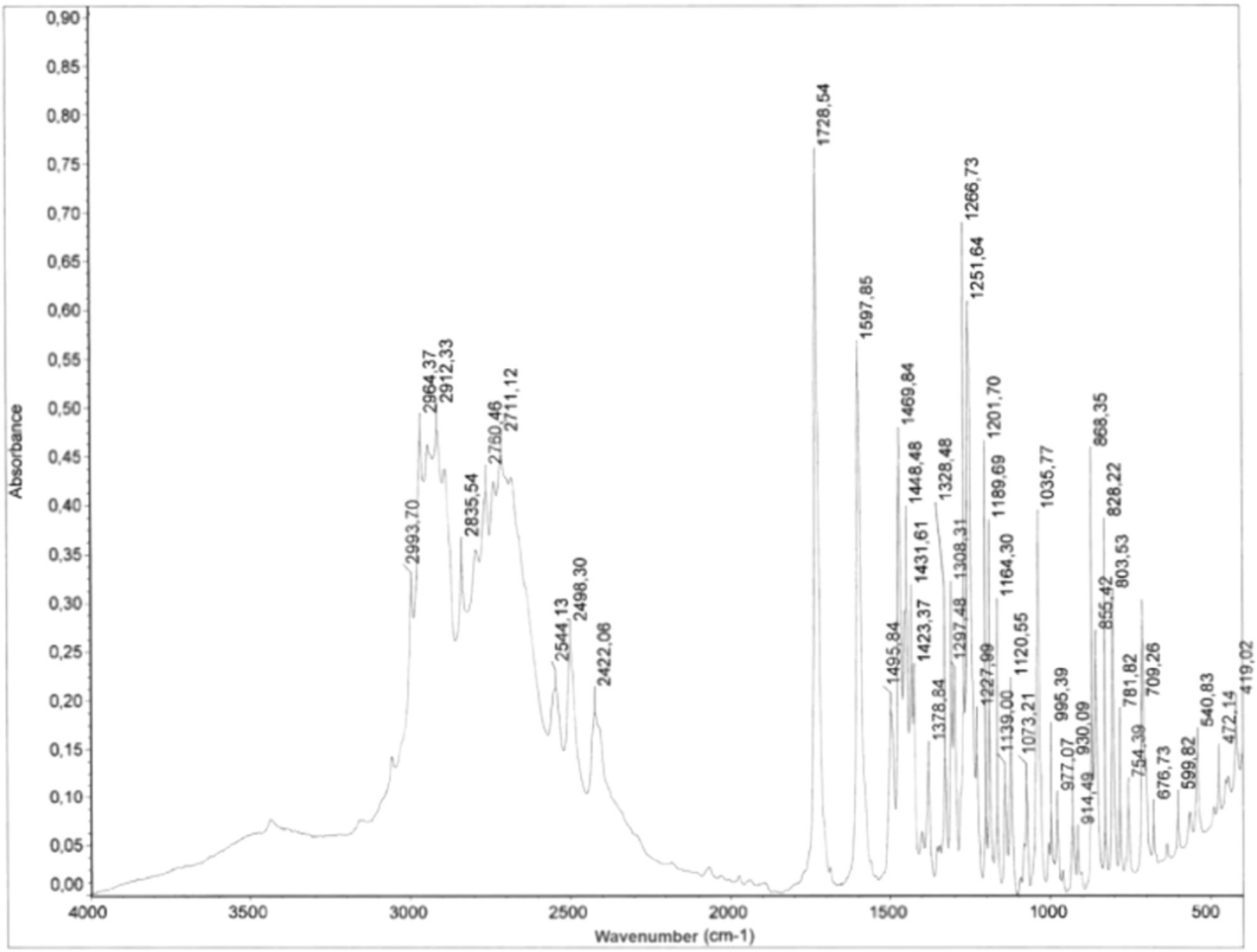


Fig. 2. FTIR analysis of MXPr powder.

related substances, sets of theoretically possible biotransformations (including possible hydroxylation, N-dealkylation, and glucuronidation) were created based on the structure of the drug. The automated metabolite profiling process was completed using the fragmentation interpretation software tool MassFragmentTM 4.1 (Waters) to enable software-driven assignment of metabolite structures from fragmentation patterns. The automated approach is driven from structural knowledge of the parent molecule using chemically intelligent algorithms which perform cleavage of the molecule in silico and identify possible functional groups. Criteria used to identify targeted compounds included 5 ppm as exact mass error of the precursor ion as well as product ions and + /- 0.5 min as tolerance in retention time for the precursor ion.

3.5.2. In vitro identification of metabolism [12]

Metabolites of MXPr were investigated in vitro by analyzing HLM incubates by liquid chromatography with high-resolution mass spectrometry detection (LC-HRMS) and then identified retrospectively in urine and hair samples [13–15]. Once thawed, HLMs (2 mg protein/mL for a final volume of 100 μL) were pre-activated by alamethicin (at 0.1 mmol/L) on ice in an intermediate volume of 50 μL in 0.1 M Tris-HCl- $MgCl_2$ (10 mM MgCl2 and 100 mM Tris-HCl solution) at pH 7.4. This mixture was added to a 50 μL dried residue of two different concentrations (100 μM or 500 μM) of MXPr methanolic solution. Fifty microliters of a cofactor mixture (5 mM UDPGA, 1.3 mM NADPH, 3.3 mM G6P, and 0.5 U/mL G6PD) in 0.1 M Tris-HCl were then added. The enzymatic reaction was performed at 37 °C for 60 min (incubation time resulting from experience gathered in similar metabolism studies of other NSOs [25,26]) and stopped by the addition of 100 μL of methanol. Cell culture supernatants were removed and frozen at − 20 °C until analysis (for 2 weeks at the most). HLM incubate samples were analyzed using the LC-HRMS method described above. The LC-HRMS-acquired data of HLM incubates were subsequently cross-checked with in silico predicted biotransformations. Criteria for identification of the targeted compounds included 5 ppm as exact mass error of the precursor ion, as well as product ions, and ± 0.25 min as tolerance in retention time for the precursor ion.

4. Results and discussion

4.1. Identification of MXPr powder

LC-HRMS (Fig. 1), IR (Fig. 2) and NMR (Fig. 3, 4, 5 and 6) were applied to identify and characterize the unknown powder sample collected from the patient. MXPr was detected with this combination of technical analyses. No significant organic impurity was detected in the powder using LC-HRMS, IR or NMR. One-dimensional 1H NMR and two-dimensional 1H-13C and 1–1H NMR spectroscopy enabled the unambiguous identification of NMR resonances corresponding to the 16 carbon atoms of MXPr as well as the 12 proton signals. Chemical shifts of MXPr are reported in Fig. 5 and could be used as a reference for further MXPr analysis by NMR spectroscopy. In addition, 1H NMR analysis revealed an MXPr content of ~95% in the powder (the remaining ~5% putatively consisting in secondary compounds) [16]. This content was subsequently considered for

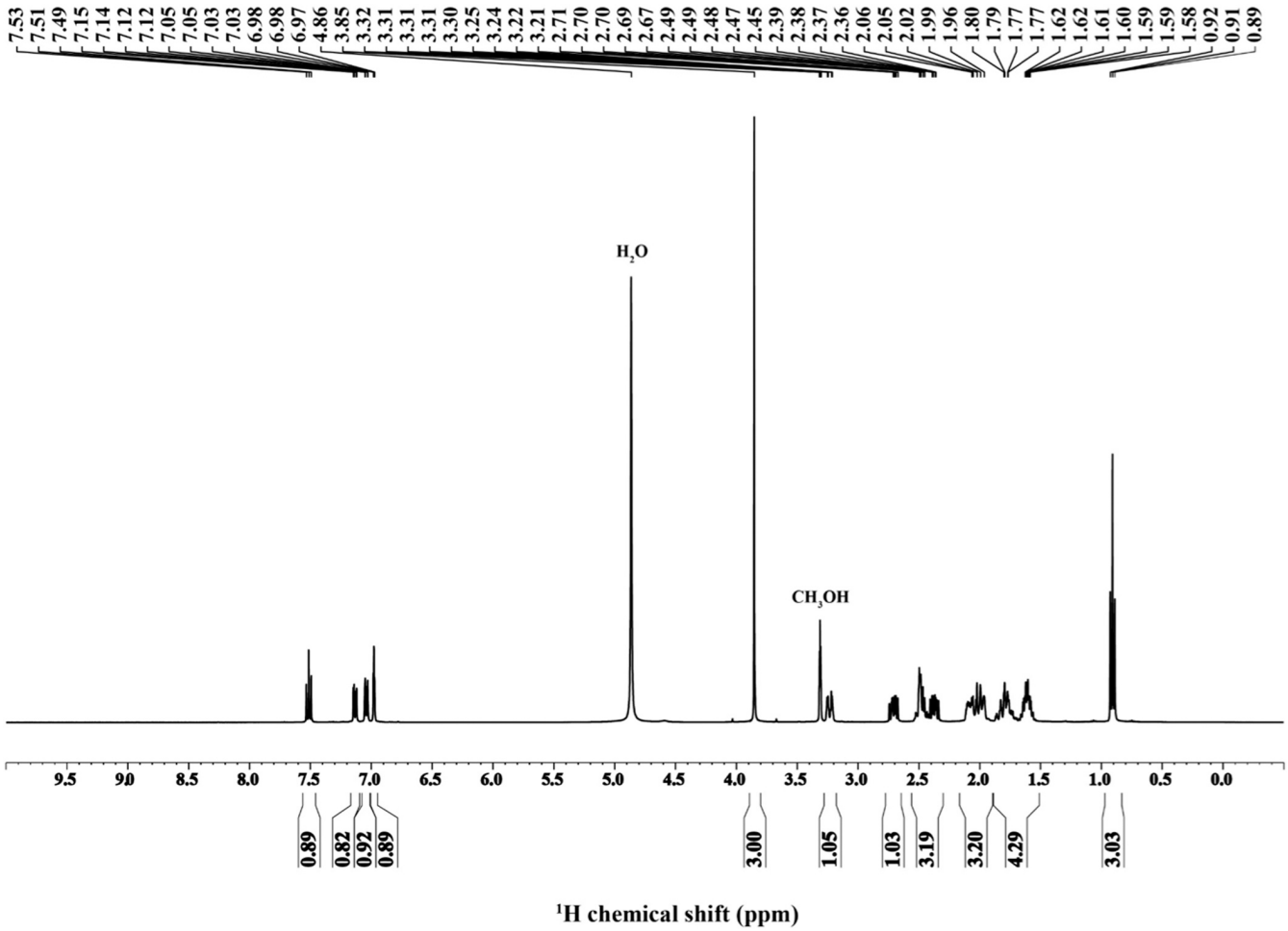


Fig. 3. NMR spectrum of MXPr powder solubilized in deuterated methanol at 400 MHz.

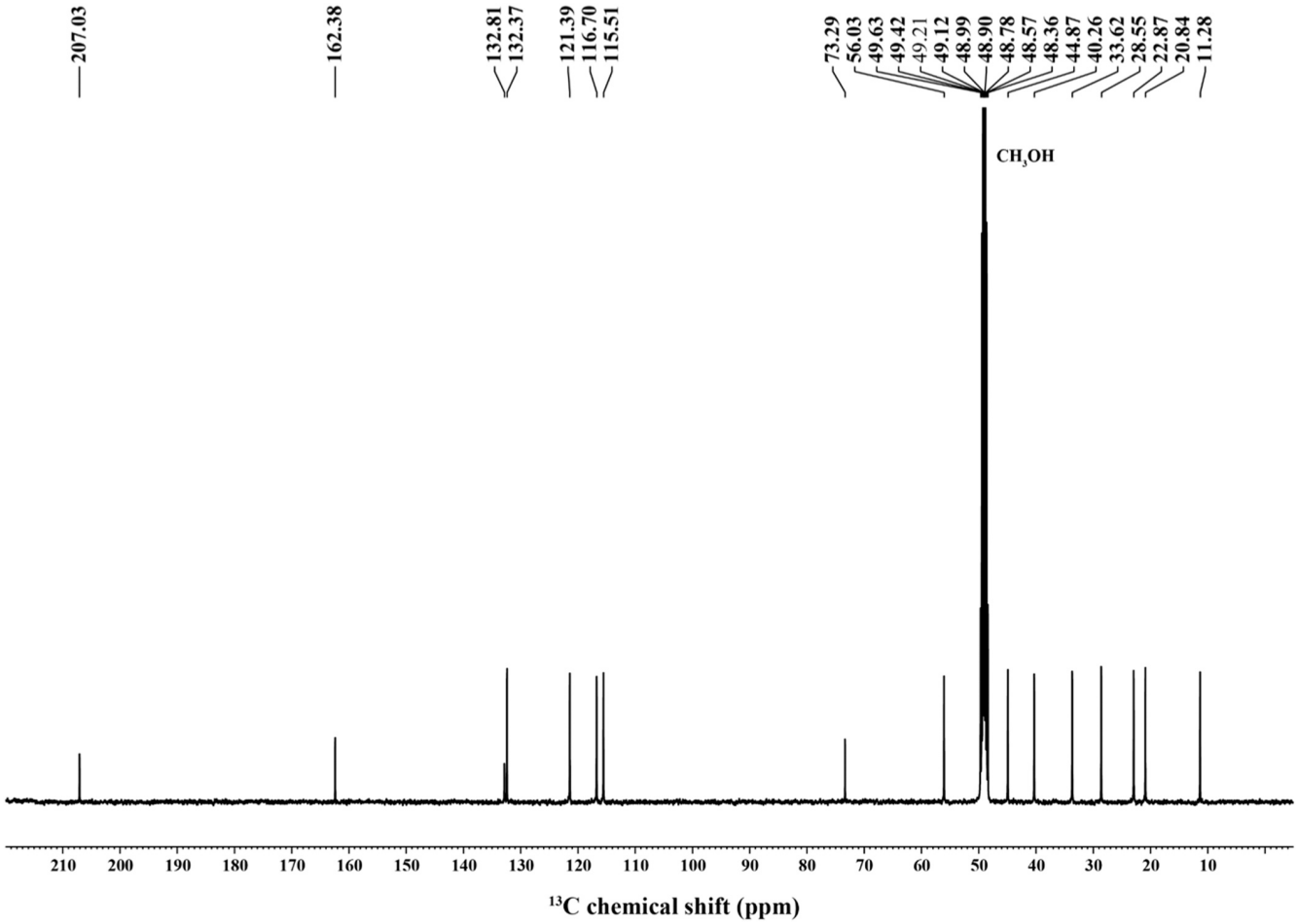
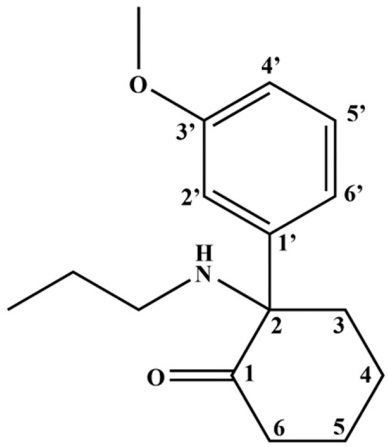


Fig. 4. ^{13}C NMR spectrum of MXPr powder solubilized in deuterated methanol at 100 MHz.

A



B

Assignment	1H chemical shift (ppm)	13C chemical shift (ppm)
O-$\underline{CH_3}$	3.85 (s)	56.03
1'		132.81
2'	6.98 (t, $^4J_{H-H}$: 2.14Hz)	115.51
3'		162.38
4'	7.13	116.70
5'	7.51 (t, $^3J_{H-H}$: 8.1Hz)	132.37
6'	7.04	121.39
N-$\underline{CH_2}$	2.37 (m) / 2.71 (m)	44.87
N-CH_2-$\underline{CH_2}$	1.61 (m)	20.84
N-CH_2-CH_2-$\underline{CH_3}$	0.91 (t, $^3J_{H-H}$: 7.4Hz)	11.28
1		207.03
2		73.29
3	2.02 (m) / 3.23 (m)	33.62
4	1.80 (m) / 1.98 (m)	22.87
5	1.77 (m) / 2.09 (m)	28.55
6	2.48 (m)	40.26

Fig. 5. chemical structure of MXPr (A) and table with ^{1}H and ^{13}C chemical shifts (B).

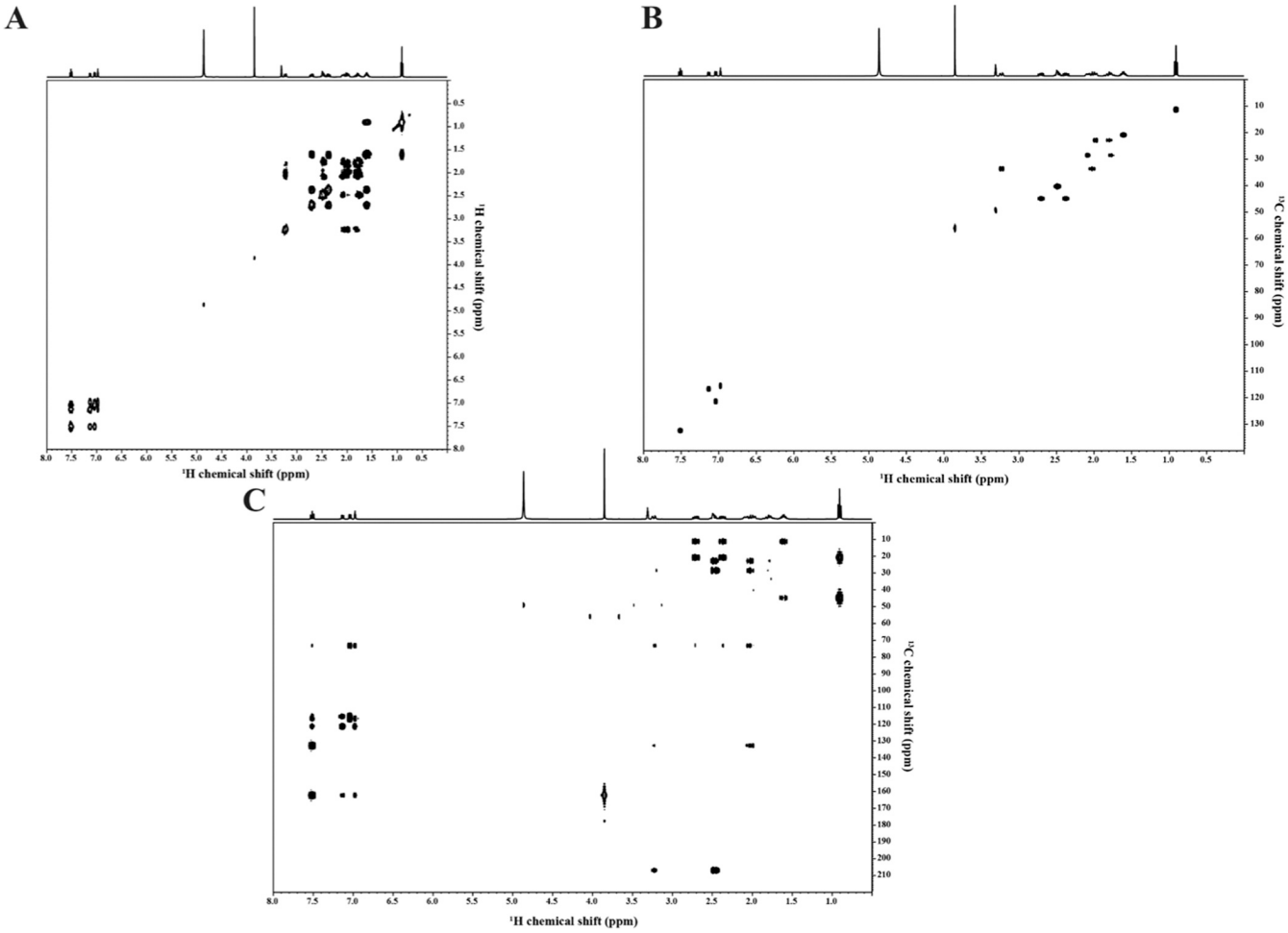


Fig. 6. ^{2}D NMR spectra of MXPr powder solubilized in deuterated methanol ^{1}H–^{1}H COSY(A) ^{1}H–^{13}C HSQC (B) and ^{1}H–^{13}C HMBC (C).

HLM cell incubation. This identification confirmed the information written on the bag. MXPr would seem to be a particular case, since many publications have demonstrated that the purported product and the product actually identified are not the same, especially in the case of NPS [17–19].

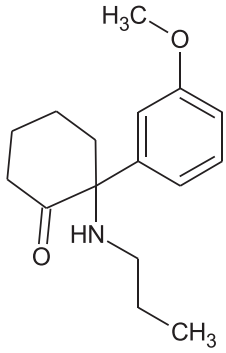
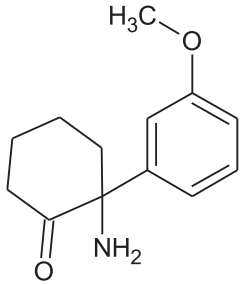
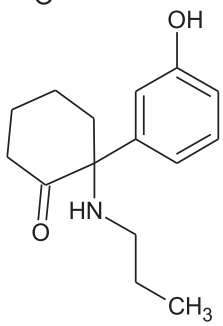
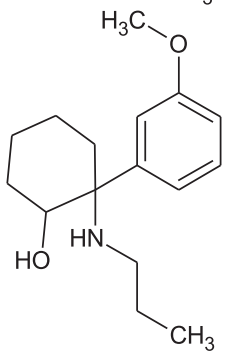
4.2. Toxicological analysis

Initial toxicological screening tests by LC-HRMS identified oxazepam, nordiazepam, norbuprenorphine, and N-ethylhexedrone. One large peak did not match with any substance present in the large available databases. The addition of the MXPr compound identified by NMR and IR spectroscopies and the retrospective urine analysis by LC-HRMS identified the unknown peak as MXPr. Hair analysis (Table 1) provides a useful history of past drug use, but the interpretation of hair concentrations must be done with caution. Hair analysis determined the molecules to which the patient had been exposed during the four months after hospitalization. It showed the presence of various illicit psychoactive substances and confirmed that the patient was a polydrug user. The substances identified in the hair specimen were not identified in the dichloromethane and water washes, suggesting no external contamination [20]. Interpretation of hair analysis data is problematic

Table 1
Results of toxicological screening in hair by LC-HRMS.

	Segment 1 1 cm – 20 mg	Segment 2 1 cm- 20 mg	Segment 3 1 cm – 20 mg	Segment 4 1 cm – 20 mg
NPS Identified	MAPB, **MXPr and NorMXPr**, 3-Fluorophenmetrazine, FMA, N-ethylhexedrone, U47700, MeO-PCP	MAPB, 3-Fluorophenmetrazine, FMA, N-ethylhexedrone, U47700, MeO-PCP	3-Fluorophenmetrazine, FMA, N-ethylhexedrone, U47700 and N-desmethyl-U47700, MeO-PCP	MAPB, 3-Fluorophenmetrazine, FMA, N-ethylhexedrone, U47700, MeO-PCP
Others drugs of abuse and cut agents	Deschloroketamine, cocaïne and metabolites (benzoylecgonine, hydroxycocaïne, cocaethylene), phenacetine, lidocaine, levamisole, doxylamine, methylphenidate	Deschloroketamine, cocaïne and metabolites (benzoylecgonine, hydroxycocaïne, cocaethylene), phenacetine, lidocaine, levamisole, doxylamine, methylphenidate	Deschloroketamine, benzoylecgonine, hydroxycocaïne, cocaethylene, phenacetine, lidocaine, levamisole, doxylamine, methylphenidate	Deschloroketamine, cocaïne and metabolites (benzoylecgonine, hydroxycocaïne, cocaethylene), phenacetine, lidocaine, levamisole, doxylamine, methylphenidate
Pharmaceuticals	Bromazepam, nordiazepam, diazepam, buprenorphine and norbuprenorphine, quetiapine and norquetiapine, risperidone	Bromazepam, nordiazepam, diazepam, buprenorphine and norbuprenorphine, quetiapine and norquetiapine, risperidone	Bromazepam, nordiazepam, diazepam, buprenorphine and norbuprenorphine, quetiapine and norquetiapine, risperidone	Bromazepam, nordiazepam, diazepam, buprenorphine and norbuprenorphine, quetiapine and norquetiapine, risperidone

Table 2
Metabolites structures for MXPr based on accurate mass measurements.

Metabolite number	Proposed metabolite structure	Trivial Metabolite name	Elemental composition	Theortical Mass [M + H]	LC t_r (min)	Response relative to parent compound (%)	Measured *m/z* (ppm)	Urine sample	Hair sample
1		MXPr	$C_{16}H_{23}NO_2$	262.1729	4.9	100	262.1798 (−1.5)	+	+
2		N-despropyl (nor)MXPr	$C_{13}H_{17}NO_2$	220,1338	3.47	4%	220.1329 (−1.4)	+	+
3		O-desmethyl MXPr	$C_{15}H_{21}NO_2$	248.1651	3.26	12%	248.1640 (−2.0)	+	+
4		DihydroMXPr	$C_{16}H_{25}NO_2$	264,1964	5,51	9.6%	264.1962 (−1,5)	+	+

+ : positive; -: negative
Table X. Putative phase I and phase II structures for MXPr metabolites based on accurate mass measurements

for NPS. Little is known about the incorporation of substances into the keratin matrix after intake and the correlation between dosage, frequency of use and hair concentrations remains elusive. Owing to these actual limitations, the authors decided not to measure the concentration of MXPr in the hair sample. However, it is difficult to obtain and purchase the reference standards of all substances. Some are very expensive, while others are not even listed in the catalogue of referenced suppliers. In addition to the difficulties associated with molecule characterization such as identification, quantification, and purity, the routine analytical methods like LC-MS/MS are not always sufficient to firmly identify NPS, e.g. in the case of positional isomers. It is therefore necessary to use another method such as NMR spectroscopy to discriminate the species [14,21].

4.3. Characterization of metabolites

These in vitro experiments revealed three main metabolites based on the intensity of the chromatographic peak areas: N-despropyl(nor)MXPr, O-desmethyl MXPr, and dihydroMXPr (Table 2). These metabolites were in keeping with the predicted metabolic

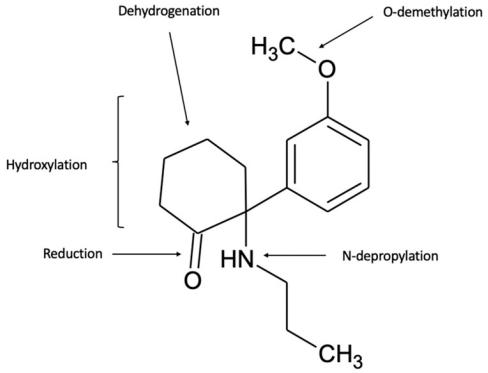


Fig. 7. Predicted metabolism of MXPr.

pathway (Figs. 7 and 8), namely N-depropylation to norMXPr, reduction metabolism to dihydroMXPr and O-demethylation to O-desmethylMXPr metabolites. In all cases, the mass accuracy of the metabolites was in keeping with the expected theoretical accurate mass by less than 3 ppm. No additional phase I or phase II metabolites were identified. NorMXPr was the most abundant metabolite. These results are coherent with those recently obtained in vitro by Menzies et al. [22].

To confirm whether the metabolites generated from phase I and phase II in vitro microsomal incubations were correlated with those found in the urine of the MXPr user, portions of microsomal incubations were analyzed along with urine sample. NorMXPr (via N-depropylation) was the predominant metabolite in vitro and in vivo based on its MS signal intensity, indicating that it is the main metabolite of MXPr, as is the case for ketamine [23]. Hijazi et al. [24] demonstrated CYP3A4 to be the major enzyme responsible for N-

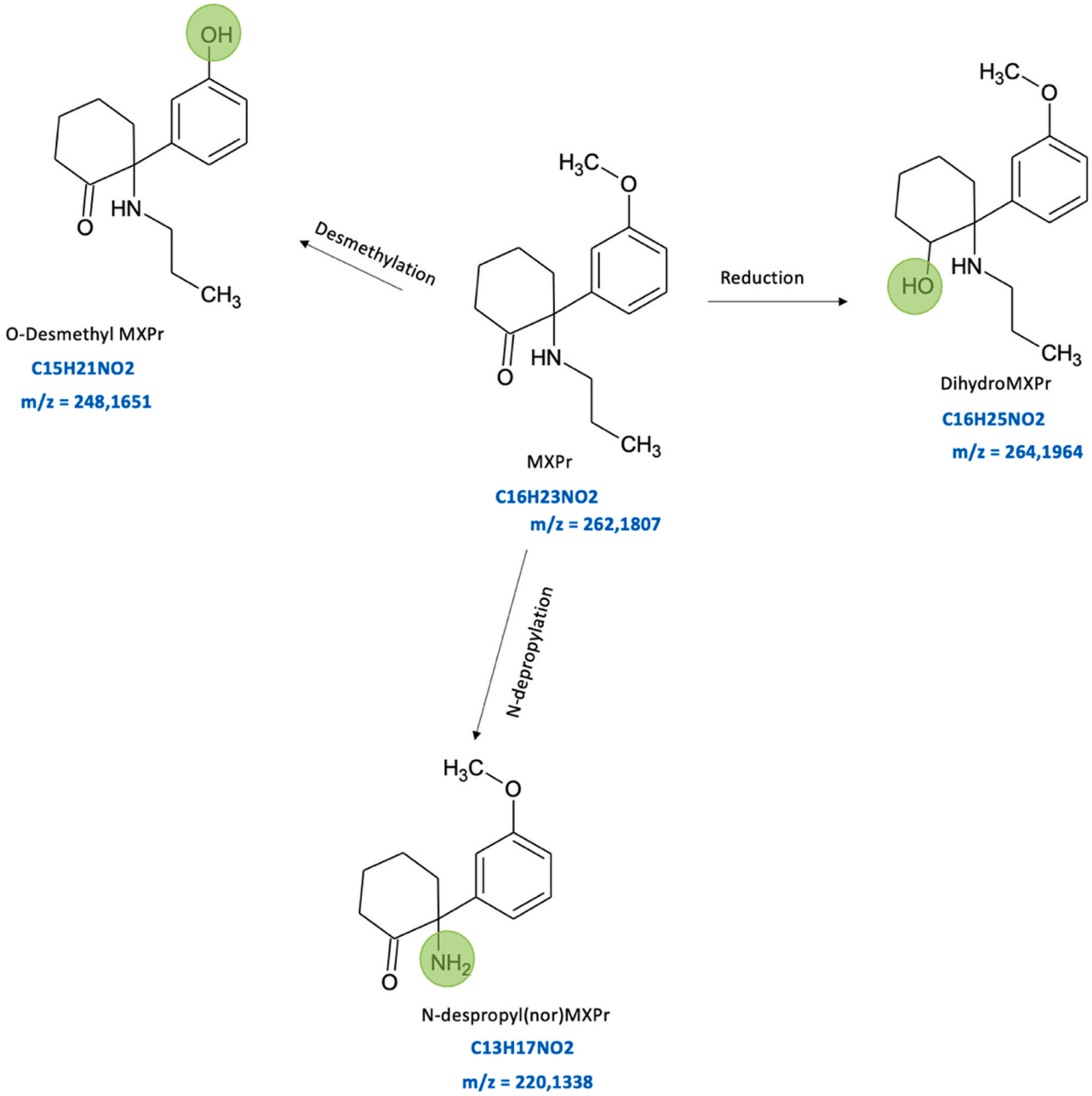


Fig. 8. Proposed metabolic pathway of MXPr in vitro analyses.

dealkylation of ketamine along with CYP2B6 and CYP2C9. CYP3A4 might therefore be responsible for the metabolism of MXPr to its main metabolite, identified here as norMXPr. As norketamine is known to be pharmacologically active, norMXPr might also be biologically active. Therefore, when investigating MXPr, norMXPr should also be considered as possibly contributing to impairment and/or toxicity.

The other major metabolites based on relative ion signal intensities were O-desmethyl and dihydro MXPr metabolites, the majority being matched for their presence in microsomal incubates and patient samples (urine and hair). No phase I metabolites were detected either in vitro or in vivo. This is contrary to what has been described for MXE. Indeed, Horsley et al. [25] identified three glucuronide conjugates of metabolites with a primary amine functional group and seven with a secondary amine group. N-glucuronidation of aliphatic primary and secondary amines might play a major role in other species, but this is unlikely to be a significant pathway for the metabolism of MXPr, if it exists at all in human, and it is reasonable to predict conjugation to an easily accessible hydroxyl group.

Metabolism studies play an important role in clinical and forensic toxicology. Metabolites should be sought when analyzing NPS, as detecting only the parent drug may lead to false-negative results when the delay between consumption and sampling is too long or when blood and urine concentrations are very low. From an analytical point of view, HRMS has shown the best performance in comparative studies of drug screening assays [26,27,28]. The in vitro (HLMs) and in silico approaches used in the present study may be considered complementary.

5. Conclusion

This study highlights the difficulty of identifying some NPS when they are missing from the libraries and the standard is not available. Here we show that the combination of several analytical methods with HRMS is powerful for the molecular characterization of NPS. The identification of MXPr metabolites will improve the efficiency of toxicological screening libraries and widen the window for detecting MXPr in biological matrices in cases of MXPr intake. In view of the results of the present study, we suggest adding N-despropyl(nor) MXPr, O-desmethyl MXPr and dihydroMXPr to toxicological screening libraries in order to reduce the risk of false-negative results in intoxication cases involving MXPr. Additional comparison with in vivo data from intoxication cases remains necessary to identify unambiguously the blood and urinary metabolites of MXPr.

CRediT authorship contribution statement

R. Goncalves: Writing – original draft. **N. Castaing:** Investigation, Validation. **C. Richeval:** Investigation. **D. Ducint:** Investigation, Validation. **K. Titier:** Investigation Writing – review & editing. **E. Morvan:** Resources, Investigation. **A. Grélard:** Resources, Investigation. **A. Loquet:** Resources, Investigation. **M. Molimard:** Project administration, Writing – review & editing.

Disclosure statement

No potential conflict of interest is reported by the author(s).

Acknowledgements

This work benefited from the facilities and expertise of the Biophysical and Structural Chemistry Platform (BPCS) at IECB, CNRS UMS3033, Inserm US001, Bordeaux University http://www.iecb.ubordeaux.fr/index.php/fr/plateformestechnologiques.

References

[1] C.J. Botanas, J.B. de la Peña, I.J. dela Peña, R. Tampus, R. Yoon, H.J. Kim, Y.S. Lee, C.G. Jang, J.H. Cheong, Methoxetamine, a ketamine derivative, produced conditioned place preference and was self-administered by rats: evidence of its abuse potential, Pharmacol. Biochem. Behav. 133 (2015) 31–36.
[2] EMCDDA, EU early warning system situation report. Situation report 1–June 2020 [Internet]. 2020 [cited on 16 avr 2021]. ⟨https://www.aekwien.at/documents/263869/449604/200629_EU+EARLY+WARNING+SYSTEM+SITUATION+REPORT+June.pdf⟩.
[3] T. Irie, D. Yamazaki, R. Kikura-Hanajiri, A potential of methoxpropamine to be a widespread recreational drug: it blocks NMDA receptors and inhibits NMDA receptor-mediated synaptic transmission in a brain preparation of mice, Forensic Toxicol. 39 (2) (2021) 474–480.
[4] B.L. Roth, S. Gibbons, W. Arunotayanun, X.-P. Huang, V. Setola, R. Treble, L. Iversen, The ketamine analogue methoxetamine and 3- and 4-methoxy analogues of phencyclidine are high affinity and selective ligands for the glutamate NMDA receptor, PLoS One 8 (3) (2013) e59334.
[5] D.M. Wood, S. Davies, M. Puchnarewicz, A. Johnston, P.I. Dargan, Acute toxicity associated with the recreational use of the ketamine derivative methoxetamine, Eur. J. Clin. Pharmacol. 68 (5) (2012) 853–856.
[6] S. Chiappini, H. Claridge, J.M. Corkery, C. Goodair, B. Loi, F. Schifano, Methoxetamine-related deaths in the UK: an overview, Hum. Psychopharmacol. Clin. Exp. 30 (4) (2015) 244–248.
[7] H. Morris, J. Wallach, From PCP to MXE: a comprehensive review of the non-medical use of dissociative drugs, Drug Test. Anal. 6 (7–8) (2014) 614–632.
[8] P. Adamowicz, D. Zuba, Fatal intoxication with methoxetamine, J. Forensic Sci. 60 (s1) (2015) S264–S268.
[9] C. Richeval, M. Baillieux, G. Pawlak, M. Phanithavong, J.ç Wiart, L. Humbert, A. Batisse, C. Lamoureux, G. Pfau, T. Nefau, D. Allorge, J.M. Gaulier, Benzoylfentanyl and parafluorobutyrfentanyl: some analytical and metabolism data, Toxicol. Anal. Clin. 31 (4) (2019) 258–267.
[10] R. Goncalves, N. Castaing, K. Titier, V. Dumestre-Toulet, Hair analysis of methoxphenidine in a forensic chemsex case, J. Anal. Toxicol. (2021) 1 bkab016.
[11] A. Ameline, C. Richeval, J.-M. Gaulier, J.-S. Raul, P. Kintz, Detection of the designer benzodiazepine flunitrazolam in urine and preliminary data on its metabolism, Drug Test Anal. 11 (2) (2019) 223–229.
[12] C. Richeval, J.-M. Gaulier, L. Romeuf, D. Allorge, Y. Gaillard, Case report: relevance of metabolite identification to detect new synthetic opioid intoxications illustrated by U-47700, Int J. Leg. Med. 133 (1) (2019) 133–142.
[13] C. Richeval, T. Gicquel, C. Hugbart, B. Le Dare, D. Allorge, I. Morel, J.M. Gaulier, In vitro characterization of NPS metabolites produced by human liver microsomes and the HepaRG cell line using liquid chromatographyhigh resolution mass spectrometry (LC-HRMS) analysis: application to Furanyl Fentanyl, Curr. Pharm. Biotechnol. 18 (10) (2017) 806–814.
[14] A. Ameline, D. Garnier, L. Gheddar, C. Richeval, J. Gaulier, J.é Raul, P. Kintz, Identification and analytical characterization of seven NPS, by combination of ^{1}H NMR spectroscopy, GC–MS and UPLC–MS/MS®, to resolve a complex toxicological fatal case, Forensic Sci. Int 298 (2019) 140–148.
[15] L. Jia, X. Liu, The conduct of drug metabolism studies considered good practice (II): in vitro experiments, Curr. Drug Metab. 8 (8) (2007) 822–829.
[16] S. Akoka, L. Barantin, M. Trierweiler, Concentration measurement by proton NMR using the ERETIC method, Anal. Chem. 71 (13) (1999) 2554–2557.
[17] A. Peacock, R. Bruno, N. Gisev, L. Degenhardt, W. Hall, R. Sedefov, J. White, K.V. Thomas, M. Farrell, P. Griffiths, New psychoactive substances: challenges for drug surveillance, control, and public health responses, Lancet 394 (10209) (2019) 1668–1684.
[18] C. Guillou, F. Reniero, J.L. Vicente, M. Holland, K. Kolar, H. Chassaigne, S. Tirendi, H. Schepers, Collaboration of the Joint Research Centre and European Customs Laboratories for the identification of new psychoactive substances, Curr. Pharm. Biotechnol. 19 (2) (2018) 91–98.
[19] C.V. Giné, I.F. Espinosa, M.V. Vilamala, New psychoactive substances as adulterants of controlled drugs. A worrying phenomenon? Drug Test. Anal. 6 (7–8) (2014) 819–824.
[20] G.A.A. Cooper, R. Kronstrand, P. Kintz, Society of Hair Testing guidelines for drug testing in hair, Forensic Sci. Int. 218 (1) (2012) 20–24.
[21] A. Ameline, D. Garnier, J.-S. Raul, P. Kintz, Use of nuclear magnetic resonance (NMR) spectroscopy to identify unknown powders, Toxicol. Anal. Clin. 31 (4) (2019) 197–250.
[22] E.L. Menzies, S.C. Hudson, P.I. Dargan, M.C. Parkin, D.M. Wood, A.T. Kicman, Characterizing metabolites and potential metabolic pathways for the novel psychoactive substance methoxetamine, Drug Test. Anal. 6 (6) (2014) 506–515.
[23] C.-Y. Chen, M.-R. Lee, F.-C. Cheng, G.-J. Wu, Determination of ketamine and metabolites in urine by liquid chromatography-mass spectrometry, Talanta 72 (3) (2007) 1217–1222.
[24] Y. Hijazi, R. Boulieu, Contribution of CYP3A4, CYP2B6, and CYP2C9 isoforms to N-demethylation of ketamine in human liver microsomes, Drug Metab. Dispos. Biol. Fate Chem. 30 (7) (2002) 853–858.
[25] R.R. Horsley, E. Lhotkova, K. Hajkova, B. Jurasek, M. Kuchar, T. Palenicek, Detailed pharmacological evaluation of methoxetamine (MXE), a novel psychoactive ketamine analogue-behavioural, pharmacokinetic and metabolic studies in the Wistar rat, Brain Res. Bull. 126 (Pt 1) (2016) 102–110.
[26] D. Pasin, A. Cawley, S. Bidny, S. Fu, Current applications of high-resolution mass spectrometry for the analysis of new psychoactive substances: a critical review, Anal. Bioanal. Chem. 409 (25) (2017) 5821–5836.
[27] H.H. Maurer, M.R. Meyer, High-resolution mass spectrometry in toxicology: current status and future perspectives, Arch. Toxicol. 90 (9) (2016) 2161–2172.
[28] I. Ojanperä, M. Kolmonen, A. Pelander, Current use of high-resolution mass spectrometry in drug screening relevant to clinical and forensic toxicology and doping control, Anal. Bioanal. Chem. 403 (5) (2012) 1203–1220.

IV. Apport clinique de la spectrométrie de masse haute résolution en QTOF, *focus* sur l'exposition aux cannabinoïdes de synthèse à Mayotte (Outre-mer) : Protocole HOMACHI – Publication n° 3

A. Introduction et présentation

À Mayotte, la consommation de substances psychoactives était principalement représentée par le tabac et les feuilles de cannabis (appelées « bangué »), et dans une moindre mesure par l'alcool. Depuis une dizaine d'années, est apparu l'usage d'une drogue inconnue, appelée « chimique ». Une étude rétrospective s'est intéressée au profil médico-social des patients ayant consulté le centre d'addictologie de Mayotte en 2015 pour une consommation de « chimique ». Elle a décrit la diffusion rapide de cette consommation, en particulier chez les adolescents et les jeunes hommes en situation de précarité, qui sont des cibles faciles pour les dealers (153). Ces cigarettes imprégnées de substances étaient longtemps passées inaperçues sur cette île où on consomme régulièrement du tabac. Un lien a finalement été établi entre la consommation de « chimique » et l'augmentation des admissions aux urgences. Selon les études, elle est actuellement la troisième cause de consultation aux urgences de Mayotte (154,155), et concerne principalement les mineurs et les jeunes adultes. Le CEIP-A [4] d'Aquitaine et des DOM (Centre d'évaluation et d'information sur la pharmacodépendance et l'addictovigilance, référent de l'Aquitaine et des départements d'Outre-mer) a alerté sur cette problématique de consommation de « chimique » (154).

À ce jour, la composition exacte de la « chimique » est encore mal documentée. Roussel *et al.* ont réalisé une étude à partir des différentes saisies qui ont pu être effectuées sur l'île au moment des pics d'hospitalisation, indiquant qu'elle serait principalement composée de cannabinoïdes de synthèse (CS) (156), mais aucun lien n'a pu être établi entre cette augmentation des saisies de CS et les substances effectivement consommées via la « chimique ». De plus, il existe peu de données sur la toxicité à moyen et long terme de la « chimique » ou sur son potentiel de dépendance.

L'objectif de cette étude était d'identifier quelles substances parmi celles consommées sous le nom de « chimique », conduisent à des admissions hospitalières, en les associant aux tableaux cliniques identifiés (Annexe 2).

B. Discussion

Il s'agit de la première étude menée dans l'Océan Indien pour tenter d'identifier les composants de la « chimique » et les tableaux cliniques associés. Elle a également permis la mise en place d'un circuit d'analyse pour les composés non détectables par les analyses toxicologiques de routine. Entre le 1er mars et le 30 juin 2019, tous les patients, âgés de plus de 14 ans, hospitalisés aux urgences du centre hospitalier de Mayotte après consommation de « chimique » et pour lesquels le médecin a demandé une analyse toxicologique, ont été inclus. Des prélèvements sanguins et des données cliniques ont été recueillies pour chaque patient. Les analyses toxicologiques ont été réalisées par spectrométrie de masse à haute résolution (UPLC-MS/QTOF). Douze patients ont été inclus : 11 hommes et 1 femme, dont 2 mineurs. L'âge moyen était de 26 ans (âge médian : 22). Les présentations cliniques observées étaient variées, principalement des troubles psychiatriques et neurologiques. Aucun décès n'a été signalé. L'analyse toxicologique a permis d'identifier des substances psychoactives telles que le THC et/ou ses métabolites (n=3), et le MDMB-4en-PINCA (n=2). Les autres substances identifiées faisaient principalement partie du traitement des patients.

En dehors de trois patients âgés de plus de 37 ans, les sujets étaient jeunes (moins de 31 ans), dont deux mineurs. Cela est cohérent avec ce qui a été rapporté sur les usagers de « chimique » à Mayotte. Ce nombre peut paraître faible, mais le Centre Hospitalier de Mayotte (CHM) est le seul hôpital de l'île. Situé à Mamoudzou, il assure des consultations spécialisées, des hospitalisations, des interventions chirurgicales et plus de la moitié des accouchements. Le CHM est soutenu par des dispensaires répartis sur toute l'île, qui assurent les soins primaires de proximité et les actions de prévention (154). Il est tout à fait possible que les usagers de « chimique » se soient présentés dans les dispensaires où les médecins, commençant à être familiarisés avec les présentations cliniques liées à la « chimique », auraient pu les garder sous surveillance sans les envoyer au CHM.

Alors que tous les patients ont développé des effets suffisamment graves pour être admis au service des urgences du CHM, un CS, le MDMB-4en-PINACA (Methyl 3,3-dimethyl-2-(1-(pent-4-en-1-yl)-1H-indazole-3-carboxamido)butanoate), n'a été identifié que chez deux patients. Trois autres patients étaient positifs pour le Δ9-tétrahydrocannabinol (THC) et/ou ses métabolites. Cependant, pour tous les patients, le tableau clinique observé était cohérent avec la prise de THC ou d'un CS (157,158).

Les CS sont conçus pour stimuler le système endocannabinoïde et imiter les effets du Δ9-THC, le principal constituant psychoactif du cannabis, avec une puissance 2 à 200 fois

supérieure (158,159). Les CS ont démontré un effet inhibiteur sur la neurotransmission de l'acide gamma aminobutyrique (GABA) dans le cerveau via plusieurs voies, plus efficacement que le THC (160). Ainsi, les CS peuvent provoquer une anxiété intense, une agitation et des crises d'épilepsie (51,158,161). Les CS peuvent également avoir des effets secondaires cardiovasculaires, notamment des arythmies cardiaques et même des arrêts cardiaques (162). Il existe une grande variabilité inter- et intra-individuelle, de sorte que leurs effets cliniques sont imprévisibles (158,163). Compte tenu de leur grande affinité pour leurs sites récepteurs et de leur forte puissance, la dose efficace est faible, la quantité de métabolites excrétés est également faible, ce qui rend difficile la détection de ces composés (158,164,165), *a fortiori* sur des prélèvements sanguins, comme dans la présente étude (166). Les meilleurs marqueurs de l'ingestion sont les métabolites urinaires, et non la molécule mère, qui est généralement largement métabolisée avec des demi-vies courtes. Par conséquent, l'urine est une matrice de choix pour augmenter la fenêtre de détection des CS. Un prélèvement précoce permet également une meilleure identification. En outre, les dépistages actuels par immunochimie ne sont pas conçus pour détecter les CS en raison de leur structure chimique qui est très variable entre les familles et les générations. Ils ne peuvent être détectés que par des méthodes analytiques hautement spécifiques et sensibles, telle la spectrométrie de masse haute résolution (167).

Depuis octobre 2020, plusieurs alertes sur la présence de CS dans des échantillons de cannabis ont été émises en France : plusieurs centres d'addictovigilance ont été informés de la circulation de matériel végétal vendu comme du cannabis contenant, en plus du THC, un CS puissant, identifié par la suite comme le MDMB-4en-PINACA. Des consommateurs réguliers et non réguliers de cannabis ont décrit des symptômes inhabituels pour le THC (fièvre, vertiges, tremblements, sueurs, nausées et vomissements, hypotension et tachycardie, troubles psychiatriques, sensation de mort imminente), suggérant la présence de produits plus puissants (158). Le même CS a été retrouvé dans notre étude chez deux patients.

Il s'agit de la première étude menée dans l'Océan Indien confirmant la présence de CS dans la « chimique ». Pendant un certain temps, la consommation de CS en France a semblé avoir une importance limitée. Cependant, leur utilisation est devenue importante dans l'Océan Indien depuis la diffusion de la « chimique » à Mayotte. Elle continue de se répandre notamment à La Réunion depuis 2017 sous le nom de « chamane ».

Publication n° 3

Minors and young adult's hospitalizations after "chimique" consumption in Mayotte Island : which substances are involved?

Ruben Goncalves, Alexandre Peyré, Nadège Castaing, Thomas Beeken, Patrice Combe, Ghada Miremont-Salamé, Karine Titier, Mathieu Molimard, Amélie Daveluy

Publié en 2022 dans *Therapies*

https://doi.org/doi:10.1016/j.therap.2022.07.007

Journal Pre-proof

THERAPIES
ADDICTOVIGILANCE

Minors and young adult's hospitalizations after "chimique" consumption in Mayotte Island: which substances are involved?

"Chimique" consumption in Mayotte Island

Ruben Goncalves[a,b], Alexandre Peyré[c], Nadège Castaing[a], Thomas Beeken[d], Sophie Olivier[e], Patrice Combe[e], Ghada Miremont-Salamé[b,c], Karine Titier[a], Mathieu Molimard[a,b], Amélie Daveluy[b,c,*]

[a] *CHU de Bordeaux, laboratoire de pharmacologie et toxicologie, service de pharmacologie médicale, 33000 Bordeaux, France*
[b] *Univ. Bordeaux, INSERM, BPH, U1219, 33000 Bordeaux, France*
[c] *CHU de Bordeaux, centre d'addictovigilance de Bordeaux et Départements d'Outre-Mer, service de pharmacologie médicale, 33000 Bordeaux, France*
[d] *CH de Mayotte, service des urgences, 97600 Mamoudzou, France*
[e] *CH de Mayotte, laboratoire de biologie médicale, 97600 Mamoudzou, France*

Received 25 February 2022; accepted 11 July 2022

***Corresponding author.** Centre d'addictovigilance de Bordeaux, service de pharmacologie médicale, CHU de Bordeaux, Bât. 1A Zone Nord, 33076 Bordeaux cedex, France
E-mail address: amelie.daveluy@u-bordeaux.fr (A. Daveluy)

Summary

Journal Pre-proof

Objectives.- Over the last 10 years, the use of an unknown drug called "*chimique*" has emerged, among adolescents and young adults in precarious situations in Mayotte Island. To date, the exact composition of "*chimique*" is still poorly documented, but seizures made on the Island at the same time indicated that it would be mainly composed of synthetic cannabinoids receptor agonists (SCRAs). The objective of this study was to identify which substances, among those consumed under the name of "*chimique*", leading to hospital admissions. *Methods*.- Between 1st march and 30th June 2019, all patients, over 14 years old, hospitalized in the emergency department of Mayotte hospital after use of "*chimique*" for which the physician required toxicological analysis were included. Blood samples and clinical data were recorded for each patient. Toxicological analyses were performed using high resolution mass spectrometry (UPLC-MS/QTOF). *Results*.- Twelve patients were included: 11 males and 1 female. The mean age was 26 years (median age: 22). There were 2 minors. Clinical presentations varied, mainly psychiatric and neurologic disorders were observed. No death was reported. Toxicological analysis identified psychoactive substances such as THC and/or its metabolites (n = 3) and MDMB-4en-PINCA (n = 2). The other substances identified were mainly part of the patients' treatment. *Conclusion*.- This is the first study conducted in the Indian Ocean confirming the presence of SCRAs in the "*chimique*". For a while, the consumption of SCRAs in France seemed to be of limited importance. However, their use has become important in the Indian Ocean since the spread of "*chimique*" in Mayotte. It continues to spread especially in Reunion Island since 2017 under the name of "*chamane*".

KEYWORDS

Chimique; Synthetic cannabinoids; Mayotte Island; High resolution mass spectrometry; LC-MS/QTOF; Addictovigilance; Substance abuse detection; MDMB-4en-PINACA

Journal Pre-proof

Journal Pre-proof

Abbreviations

CEIP: Centre for Evaluation and Information on Drug Dependence and Addictovigilance

CHM: Mayotte Hospital (centre hospitalier de Mayotte)

DOM: French Overseas Departments (départements d'Outre-Mer)

EU: European Union

EWS: early warning system

GABA: gamma aminobutyric acid

MDMA: 3,4methylenedioxymethamphetamine

MDMB-4en-PINACA: Methyl 3,3-dimethyl-2-(1-(pent-4-en-1-yl)-1H-indazole-3-carboxamido)butanoate

QTOF: quadrupole time of flight

SCRA: synthetic cannabinoids receptor agonists

THC: tetrahydrocannabinol

THC-COOH: 11-nor-delta 9-tetrahydrocannabinol-9-carboxylic acid

UPLC-HRMS: ultraperformance liquid chromatography-high resolution mass spectrometry

Journal Pre-proof

Journal Pre-proof

Introduction

Until the 2010s, in Mayotte, the consumption of psychoactive substances was mainly represented by tobacco and cannabis leaves (called *bangué*) and to a lesser extent by alcohol [1]. Over the last 10 years, the use of an unknown drug called "*chimique*" has emerged. A retrospective study investigated the medico-social profile of patients who consulted the Mayotte addictology centre in 2015 for use of "*chimique*". It described the rapid spread of this consumption in particular among teenagers and young men in precarious situations who are easy targets for dealers [2]. These substance-impregnated cigarettes went unnoticed for a long time on this island where tobacco was widely consumed. A link has finally been established between "*chimique*" consumption and the increase in emergency room admissions. According to studies, it is currently the 3rd cause of consultation in the emergency department of Mayotte [1,3], and concerns mainly minors and young adults. The Centre for Evaluation and Information on Drug Dependence and Addictovigilance, referent of Aquitaine and the Overseas Departments (CEIP-A [4] of Aquitaine and DOM has alerted on this problem of "*chimique*" consumption [1]. To date, the exact composition of "*chimique*" is still poorly documented. Roussel *et al*. conducted a study based on various seizures that may have been made on the island at the time of hospitalization peaks, indicating that it would be mainly composed of synthetic cannabinoids receptor agonists (SCRAs) [5], but no confirmation could be made between this increase in seizures of SCRAs and the substances effectively consumed *via* the "*chimique*". Moreover, there is little data on the mid-and long-term toxicity of the "*chimique*" or on its potential for addiction.

The objective of this study was to identify which substances, among those consumed under the name of “chimique”, leading to hospital admission, through toxicological analysis and clinical presentation.

Journal Pre-proof

Material and Methods

Patients

Between 1st march and 30th June 2019, all patients, over 14 years old, hospitalized in the emergency department of Mayotte hospital (CHM) after use of "*chimique*" and for which the physician required toxicological blood test were included. Blood samples were collected in heparinized tubes and stored at -20°C. Samples, patients consents and anonymized clinical data were sent to the pharmaco-toxicology laboratory and to the addictovigilance centre of Bordeaux.

Toxicological analysis

Blood samples were submitted to toxicological examination. Cannabis identification was performed according to Jamey et al [6].

Toxicological screening

Sample preparation

Precipitation extraction was performed on blood samples. Briefly, 100 µL of sample were deproteinized by addition of 25 µL of ZnSO4 0,1N in ammonium acetate 5M and 300 µL of internal standards (THC-COOH-d3, estazolam-d5, cocaine-d3, morphine-d3, methadone-d3, MDMA-d5) in solvents (acetone/methanol/formic acid; 80:20:0.1 ; v/v/v). After vortex-mixing and centrifugation, 25 µL of the supernatant was added to 25 µL of formic acid 0.1%. Five µL were injected in the LC-MS/QTOF system.

Liquid chromatography-high-resolution mass spectrometry

The UPLC-HRMS system consisted of an Acquity I class system (waters, USA) coupled to a Xevo XS G2 QTOF analyzer (Waters, USA) using positive and negative mode electrospray ionization

Journal Pre-proof

and controlled with Unifi 1.9 software. Chromatographic separation was performed on an ACQUITY HSS C18 column (150 x 2 mm, 1.8 µm, Waters). The mobile phase was composed of solvents A (ammonium formate buffer 5 mM, pH 3) and B (acetonitrile in 0.1 % v/v formic acid). The column was maintained at 50°C and a gradient elution was used at a flow rate of 0.4 mL/min: 13% of B held until 0.5 min, increased to 50% at 10 min, then to 95% at 10.25 min, and finally held until 12.25 min, return to initial condition in 0.25 min until 17 min. The injection volume was 5 µL. Data were analyzed with HighResNPS® and Waters® databases for over 3500 toxicologically relevant drugs, NPS and metabolites that included protonated molecules and fragmentation and chromatographic retention time data. Criteria used to identify compounds included 5 ppm as exact mass error of the precursor ion as well as product ions and +/- 0.5 minutes as tolerance in retention time for the precursor ion.

Results

Results are summarized in Table 1. Between March and June 2019, 12 patients were included: 11 males and 1 female. The mean age was 26 years (median age: 22; extremes: 14 - 51). There were 2 minors. In our study, "*chimique*" drug was smoked in cigarette form. The clinical presentations varied. Some patients presented behavioural problems: agitation (n = 5), incoherent speech (n = 2), aggressive (n = 2), logorrheic (n = 2). Conversely, some patients were calm (n = 3), drowsy (n = 2), and mute (n = 2). Some had consciousness disorders (n = 3). Other symptoms could be highlighted: tachycardia (n = 3), red eyes (n = 1), dizziness (n = 1) spasticity (n = 1). No seizure or death were recorded. Among all included patients, toxicological analysis by UPLC-HRMS identified psychoactive substances such as THC and/or its metabolites in 3 patients. An SCRA, the Methyl 3,3-dimethyl-2-(1-(pent-4-en-1-yl)-1H-indazole-3-carboxamido)butanoate (MDMB-4en-PINACA) was identified in 2 other patients. The other substances identified were mainly part of the patients' treatments, mainly diazepam (n = 5) and loxapine (n = 5). No substances were detected in 2 patients.

Discussion

Journal Pre-proof

This is the first study conducted in the Indian Ocean to attempt to identify the components of the "*chimique*" and the associated clinical presentations. It also allowed the setting up of a circuit allowing the analysis of these substances which are not detectable by routine toxicological analysis.

Apart from 3 patients aged over 37 years, the subjects were young (under 31 years), including 2 minors. This is consistent with what has been reported about “chimique” users in Mayotte. This number may seem low, but the CHM is the only hospital on the island. Located in Mamoudzou, it provides specialized consultations, hospitalization, surgical procedures and more than half of all deliveries. The CHM is supported by dispensaries across the island which provide local primary care and preventive actions [1]. It is quite possible that "*chimique*" users could have attended the dispensaries where physicians, beginning to be familiar with "*chimique*"-related clinical presentations, could have kept them under surveillance without referral to the CHM.

While all patients developed symptoms severe enough to be admitted to the emergency department of the CHM, a SCRA, the MDMB-4en-PINACA (Methyl 3,3-dimethyl-2-(1-(pent-4-en-1-yl)-1H-indazole-3-carboxamido)butanoate), was only identified in 2 patients. Three others patients were positive for Δ9-tetrahydrocannabinol (THC) and/or its metabolites. However, for all patients, the clinical presentation observed was consistent with the intake of THC or a SCRA [7,8].

SCRAs represent the largest group of so-called new psychoactive substances. Since 2008, 209 new SCRAs have been detected in Europe, of which 11 were identified for the first time in 2020 [9]. Although originally developed as research chemicals for exploring the endocannabinoid system, the use of SCRAs in a recreative context has become popular since 2008 [7,8]. SCRAs are designed to stimulate the endocannabinoid system and mimic the effects of Δ9-THC, the main psychoactive constituent of cannabis, with 2 to 200 times its potency [8,10]. SCRAs have demonstrated an inhibitory effect on gamma aminobutyric acid (GABA) neurotransmission in the brain *via* several pathways more effectively than THC [11]. Thus, SCRAs may cause intense anxiety, agitation and induce seizures [8,12,13]. SCRAs may also have cardiovascular side effects including cardiac arrhythmias and even cardiac arrest [14]. There is a large inter- and intra-individual variability so that their clinical effects are unpredictable [8,15]. Given their high affinity for their receptor sites and high potency, the effective dose is small, the amount of excreted metabolites is low, making the detection of these compounds difficult [8,16,17], *a fortiori* on blood samples, as in the present study [18]. The best markers of intake are urinary metabolites, not the parent molecule, which is usually extensively metabolized with short half-lives. Therefore, urine is a matrix of choice to increase the detection window for SCRAs. Early collection also allows for better identification. This may explain why we have some negative results. However, urine

Journal Pre-proof

sampling remains difficult in cases of severe behavioural changes. We chose to take blood samples in order to facilitate the collection of samples by health care workers given the symptoms of patients hospitalised after taking "*chimique*" drug. In addition, current immunoassays screenings are not designed to detect SCRAs due to their chemical structure that is highly variable between families and generations. They can be detected only by highly specific, sensitive analytical methods such as high-resolution mass spectrometry [19].

For a while, the consumption of SCRAs in France seemed to be of limited importance. However, their use has become important in the Indian Ocean, with a spread to Reunion Island from 2017, where it is becoming a real public health problem [20], but also in continental France. Indeed, since 2013, the French addictovigilance network has reported the presence of synthetic cannabinoids in the form of e-liquids sold on the Internet, particularly in the north of France, although this practice seems to have spread throughout the country [21]. In particular, in 2018/2019, neurological and psychiatric but also digestive and cardiovascular effects were reported in adolescents and young adults after vaping e-liquids supposed to contain CBD; in 5 cases, the analysis of the e-liquid had found the presence of four different SCRAs: 5F-MDMB-PINACA, 5F-CUMYL-PINACA, XLR-11, 5F-MDMB-PICA [22]. In addition, since October 2020, several alerts on the presence of SCRAs in cannabis samples have been issued in France: several addictovigilance centres were informed about the circulation of herbal material sold as cannabis containing, in addition to THC, a powerful SCRA, later identified as MDMB-4en-PINACA. Regular and non-regular cannabis users described unusual symptoms for THC (fever, dizziness, tremors, sweating, nausea and vomiting, hypotension and tachycardia, psychiatric disorders, sensation of imminent death), suggesting the presence of more potent products [8]. The same SCRA was found in our study, in 2 patients. MDMB- 4en-PINACA is a SCRA that was first identified in powders seized by the German police in 2017 [23]. It was officially notified to the European Union (EU) early warning system (EWS) in August 2018 [24]. A total of 4 deaths with confirmed exposure to MDMB-4en-PINACA were reported in Europe, between 2019 and 2020 [24]. MDMB-4en-PINACA has been identified in five seizures in the Indian Ocean, since June 2020. The five seizures amounted to a total of 2.073 g, which were en route, *via* express freight, from China [24]. In France, MDMB-4en-PINACA is controlled under drug control legislation and is under assessment within the United Nations system [25]. MDMB-4en-PINACA could continue to rapidly spread in Europe. It is possible that MDMB-4en-PINACA has been a replacement for two recently internationally controlled SCRAs (4F-MDMB-BINACA and 5F-MDMB-PICA) [24]. This illustrates the turnover of available SCRAs and the difficulty of identifying them. The fact that these products are available *via* the Internet makes it

Journal Pre-proof

relatively easy for the general population to obtain them. In Mayotte, it affects mainly teenagers and young adults in precarious situations. Addiction seems to set in very quickly. Adolescents use "*chimique*" every day, in groups, after leaving school (around which the "*chimique*" dealers are settled). Secondary school teachers are very aware of the problem, but the information and prevention activities do not prevent the use of "*chimique*" [1].

Conclusion

This study confirmed the presence of SCRAs in two "*chimique*" consumers, the others presenting symptoms that could be consistent with the intake of such a substance and validated the toxicological analysis circuit between the two hospitals. This identification is important because it confirms that "*chimique*" may contain harmful substances, and thus, keeps the health authorities informed of the risks of this major public health problem in the Indian Ocean.

Funding

This research has been conducted with the support of the MILDECA (*Mission interministérielle de lutte contre les drogues et les conduites addictives*) / ARS (*Agence régionale de santé*) Mayotte.

Disclosure of interests

The authors declare that they have no competing interest

References

[1] Daveluy A, Haramburu F. Psychoactive substances use in Reunion Island and Mayotte, French departments in Indian Ocean. Therapie 2018;73:419-27.

[2] Fleury A, Daculsi P. Profil médico-social des patients ayant consulté au Centre d'addictologie de Mayotte en 2015 pour usage de nouveaux produits de synthèse, une étude rétrospective. Therapie 2017;72:151-2.

Journal Pre-proof

[3] Vilain P, Bah Assoumani S, Youssouf A, Filleul F. Recrudescence de passages liés à la consommation de cannabinoïdes de synthèse « la chimique » dans le service d'urgences du centre hospitalier de Mayotte, 2012—2016. Bulletin de veille sanitaire océan Indien 2017 http://invs.santepubliquefrance.fr/content/download/136947/492231/version/37/file/bvs_rm_33_2017.pdf. [Accessed 11 July 2022 (14 pp.)].

[4] Micallef J, Jouanjus E, Mallaret M, Lapeyre Mestre M. Safety signal detection by the French Addictovigilance Network: Innovative methods of investigation, examples and usefulness for public health. Therapie 2019;74:579-90.

[5] Roussel O, Carlin M, Bouvot X, Tensorer L. The emergence of synthetic cannabinoids in Mayotte. Toxicol Anal Clin 2015;27:18-22 .

[6] Jamey C, Szwarc E, Tracqui A, Ludes B. Determination of cannabinoids in whole blood by UPLC-MS-MS. J Anal Toxicol 2008;32:349-54.

[7] Le Boisselier R, Alexandre J, Lelong-Boulouard V, Debruyne D. Focus on cannabinoids and synthetic cannabinoids. Clin Pharmacol Ther 2017;101:220-9.

[8] Goncalves R, Labadie M, Chouraqui S, Peyre A, Castaing N, Daveluy A, *et al.* Involuntary MDMB-4en-PINACA intoxications following cannabis consumption: clinical and analytical findings. Clin Toxicol (Phila) 2021;1:1-6.

[9] EMCDDA. European Drug Report 2021: Trends and Developments. 2021 https://www.emcdda.europa.eu/system/files/publications/13838/TDAT21001ENN.pdf. [Accessed 11 July 2022 (60 pp.)].

[10] Mills B, Yepes A, Nugent K. Synthetic cannabinoids. Am J Med Sci 2015;350:59-62.

[11] Banister SD, Longworth M, Kevin R, Sachdev S, Santiago M, Stuart J, et al. Pharmacology of Valinate and tert-Leucinate Synthetic Cannabinoids 5F-AMBICA, 5F-AMB, 5F-ADB, AMB-FUBINACA, MDMB-FUBINACA, MDMB-CHMICA, and Their Analogues. ACS Chem Neurosci 2016;7:1241-54.

[12] Gurney SM, Scott KS, Kacinko SL, Presley BC, Logan BK. Pharmacology, toxicology, and adverse effects of synthetic cannabinoid drugs. Forensic Sci Rev 2014;26:53-78.

[13] Hess C, Schoeder CT, Pillaiyar T, Madea B, Muller CE. Pharmacological evaluation of synthetic cannabinoids identified as constituents of spice. Forensic Toxicol 2016;34:329-43.

[14] Ahmed T, Khan A, See VY, Robinson S. Cardiac arrest associated with synthetic cannabinoid use and acquired prolonged QTc interval: A case report and review of literature. HeartRhythm Case Rep 2020;6:283-6.

Journal Pre-proof

[15] Castaneto MS, Gorelick DA, Desrosiers NA, Hartman RL, Pirard S, Huestis MA. Synthetic cannabinoids: epidemiology, pharmacodynamics, and clinical implications. Drug Alcohol Depend 2014;144:12-41.

[16] Smith JP, Sutcliffe OB, Banks CE. An overview of recent developments in the analytical detection of new psychoactive substances (NPSs). Analyst 2015;140:4932-48.

[17] Pasin D, Cawley A, Bidny S, Fu S. Current applications of high-resolution mass spectrometry for the analysis of new psychoactive substances: a critical review. Anal Bioanal Chem 2017;409:5821-36.

[18] Cannaert A, Storme J, Franz F, Auwarter V, Stove CP. Detection and activity profiling of synthetic cannabinoids and their metabolites with a newly developed bioassay. Anal Chem 2016;88:11476-85.

[19] Goncalves R, Pelletier R, Couette A, Gicquel T, Le Daré B. Suitability of high-resolution massspectrometry in analytical toxicology: Focuson drugs of abuse. Toxicologie Analytique & Clinique 2022;34:29-41.

[20] Goncalves R, Mété D, Maillot A, Peyré A, Bastard S, Youssouf A, et al. Importance of the consumption of a new drug called chimique in the French departments of Indian Ocean (Mayotte and Reunion Island). Fund Clin Pharmacol 2021;35:61-2

[21] Batisse A, Le Boisselier R, Tournebize J, Chaouachi L, Djezzar S. Consommation de substances psychoactives illicites par vapotage : les données d'addictovigilance. Therapies 2021;76:168.

[22] Tournebize J, Gibaja V, Grossenbacher F, Plenier Y, Medhioub Y, Batisse A, et al. Le mésusage de la cigarette électronique : une centaine de jeunes hospitalisés après avoir vapoté dans le nord-est de la France. Therapies 2021;76:161.

[23] European Monitoring Centre for Drugs and Drug Addiction. EMCDDA initial report on the new psychoactive substance methyl 3,3-dimethyl-2-(1-(pent-4-en-1-yl)-1H-indazole-3-carboxamido)butanoate (MDMB-4en-PINACA), Initial reports, Publications Office of the European Union, Luxembourg, 2020. https://www.emcdda.europa.eu/system/files/publications/13363/emcdda-initial-report-MDMB-4en-PINACA.pdf. [Accessed 11 July 2022 31 pp.].

[24] EMCDDA. EMCDDA initial report on the new psychoactive substance MDMB-4en-PINACA. 2021 https://www.emcdda.europa.eu/publications/initial-reports/mdmb-4en-pinaca_sl. [Accessed 11 July 2022]

Journal Pre-proof

[25] WHO. Critical Review Report, MDMB-4en-PINACA 2021 https://www.who.int/docs/default-source/controlled-substances/43rd-ecdd/mdmb-4en-pinaca-review-2020.pdf?sfvrsn=5cd6e97e_4. [Accessed 11 July 2022].

Journal Pre-proof

Journal Pre-proof

Table 1. Description of clinical presentation and toxicology results.

Patient	Sex	Age	Clinical presentation	Blood toxicology results	
				Drugs of abuse	**Other drugs**
1	M	22	Conscious, oriented, logorrhea, bilateral reactive and symmetrical mydriasis, mild obnubilation, tachycardia, hypertension,	-	Cetirizine, diazepam, nordiazepam, hydroxyzine, loxapine, nicardipine, sertraline
2	M	22	Conscious, coherent, agitated, red eyes, dizzy, slight dermabrasion	THC, 11-OH-THC, THC-COOH	-
3	M	30	Coma quiet, disturbed consciousness, mute, elevated CPK (1,641 IU/L), abdominal pain	-	-
4	M	51	Incoherent, spastic, stereotyped movements, extrapyramidal syndrome, closed eyes, reactive intermediate pupils, tachycardia, disorder of consciousness	**MDMB-4en-PINACA**	Ketamine
5	M	37	Behavioral disturbance, conscious, auditory hallucinations, logorrheic, quiet, verbally aggressive, temporal disorientation, incoherent	THC, 11-OH-THC, THC-COOH	Loxapine
6	M	15	NA	-	Lidocaine, paracetamol
7	F	30	Delirious agitation, sinus tachycardia, microcytic anemia	THC-COOH	Diazepam, loxapine, paracetamol
8	M	39	Aggression, agitation, superficial	**MDMB-4en-PINACA**	Diazepam, nordiazepam,

Journal Pre-proof

			dermabrasion hemiface		Loxapine
9	M	14	Drowsy, calm, aware, oriented	-	-
10	M	18	Agitation, respiratory depression	-	Diazepam, nordiazepam loxapine, amoxapine
11	M	18	Alternating agitation/drowsiness, alertness disorder, hyperthermia	-	Diazepam, paracetamol lidocaine midazolam
12	M	20	Conscious, calm, mute and apyretic	-	Disulfiram

-: negative ; 11-OH-THC: 11-hydroxy-delta 9-tetrahydrocannabinol; MDMB-4en-PINACA: Methyl 3,3-dimethyl-2-(1-(pent-4-en-1-yl)-1H-indazole-3-carboxamido)butanoate; NA: not available; THC: tetrahydrocannabinol; THC-COOH ; 11-nor-delta 9-*tetrahydrocannabinol*-9-*carboxylic* acid

Journal Pre-proof

V. Application en pratique clinique, *focus* sur l'exposition aux cannabinoïdes de synthèse en France métropolitaine – Publication n° 4

A. Introduction et présentation

Depuis octobre 2020, plusieurs alertes sur la présence de cannabinoïdes de synthèse (CS) dans des échantillons de cannabis ont été émises en Suisse (168) et en France (39,169). Plusieurs pays européens ont été alertés après la découverte d'échantillons de cannabis à faible teneur en THC aient été signalés comme étant adultérés par le CS MDMB-4en-PINACA (5-CL-ADB-A, 3,3-di- méthyl-2-[1-(pent-4-en-1-yl)-1H-indazole-3-carboxamido] butanoate de méthyle). Ce NPS est un CS identifié pour la première fois dans des poudres saisies par la police allemande en 2017 (170) et officiellement notifié au système d'alerte précoce (SAP) de l'Union européenne (UE) en août 2018 (171). Hormis les commentaires sur les forums d'usagers de drogues en ligne, les informations sur ce NPS, y compris sa pharmacologie et sa toxicité, sont inconnues. Cependant, d'après l'étude de ses analogues structurels, ce CS est susceptible d'être un agoniste très puissant des récepteurs cannabinoïdes CB1 et CB2 (172,173). L'identification du MDMB-4en-PINACA dans les enquêtes médico-légales sur les décès toxiques suggère que ce nouveau CS a des effets toxiques importants pouvant causer le décès des patients ou y contribuer (171,172,174). Bien que le MDMB-4en-PINACA soit potentiellement très toxique, peu d'études ont abordé les conséquences cliniques de sa consommation et aucune dans un contexte de consommation de cannabis. Cette étude présente l'identification du MDMB-4en-PINACA chez des consommateurs de cannabis, associée à des données cliniques et analytiques.

B. Discussion

Entre novembre 2020 et mars 2021, 13 patients ont été inclus : 10 hommes et 3 femmes (âge médian : 22 ; [16-40]). Les patients ont été hospitalisés au service des urgences (n=9) et en service d'addictologie ambulatoire (n=4). Dans ce dernier, sont dispensées des consultations pour le traitement des dépendances incluant des programmes d'aide au sevrage. La plupart des patients (75 %) étaient des consommateurs réguliers de cannabis sous forme d'herbe. Tous pensaient consommer de l'herbe de cannabis fumée sous forme de joints. Chez tous les patients, les symptômes ont commencé après une seule bouffée de joint. Ils ont arrêté la

consommation immédiatement après le début des symptômes inhabituels. Les présentations cliniques étaient variées. Les patients présentaient des symptômes neurologiques, notamment des maux de tête (n=3), des hallucinations (n=3), une mydriase (n=3), une amnésie (n=2) et des convulsions (n=5), qui ont nécessité un transfert aux urgences. Les symptômes psychiatriques se sont manifestés par une paranoïa (n=6) et de l'anxiété (n=2). Les symptômes digestifs étaient des nausées (n=2) et des vomissements (n=6). Aucun symptôme respiratoire n'a été observé. Tous les patients présentaient généralement une altération de la conscience (n=13), et presque tous étaient revenus à un état normal ou quasi-normal après environ 11 heures d'observation (médiane : 14 ; [2-16]). Aucun décès n'a été enregistré. Le *Poison Severity Score* (PSS) a été évalué entre modéré (n=8) et sévère (n=5). Parmi tous les patients inclus, la première analyse toxicologique par immunochimie n'a identifié que du THC-COOH dans les échantillons de sang ou d'urine. Pour tous ces patients, les analyses par LC-QTOF ont montré la présence de MDMB-4en-PINACA et de son principal métabolite (acide MDMB-4en-PINACA 3,3-diméthylbutanoïque) dans les échantillons de sang et/ou d'urine. Aucune autre substance n'a été détectée. Cinq échantillons d'herbe ont été également analysés. Ils se présentaient tous sous la forme d'herbe de cannabis conventionnelle. Pour tous ces échantillons, les analyses toxicologiques en LC-QTOF ont identifié la présence de MDMB-4en-PINACA, une faible teneur en THC (moins de 1 %) et du cannabidiol (CBD). Si ce profil peut correspondre au chanvre industriel, les effets indésirables rapportés par les utilisateurs sont très éloignés des effets attendus. Dans le contexte de la pandémie de COVID-19, le réseau français d'addictovigilance a montré que certains patients rapportaient des symptômes de sevrage en raison de difficultés dans la chaîne d'approvisionnement en cannabis (23). La question est de savoir si les dealers contournent maintenant le manque de cannabis naturel sur le marché en ajoutant des CS aux produits de cannabis à faible teneur en THC, afin d'augmenter la psychoactivité. Si le phénomène est récent avec ce CS spécifiquement, il a déjà été décrit avec d'autres CS, notamment avec l'AB-FUBINACA (175) ou/et l'ADB-FUBINACA (6), ou le 4-cyano CUMYL-BUTINACA (4-CCB) (176), qui sont vendus comme du cannabis ou du CBD.

Publication n° 4

Involuntary MDMB-4en-PINACA intoxications following cannabis consumption: clinical and analytical findings

Ruben Goncalves, Magali Labadie, Simon Chouraqui, Alexandre Peyré, Nadège Castaing, Amélie Daveluy, Mathieu Molimard

Publié en 2021 dans *Clinical Toxicology*

1 déc. 2021;1-6

https://doi.org/10.1080/15563650.2021.1994144

CLINICAL TOXICOLOGY
https://doi.org/10.1080/15563650.2021.1994144

Taylor & Francis
Taylor & Francis Group

CLINICAL RESEARCH

Check for updates

Involuntary MDMB-4en-PINACA intoxications following cannabis consumption: clinical and analytical findings

Ruben Goncalves[a,b], Magali Labadie[c], Simon Chouraqui[d], Alexandre Peyré[e], Nadège Castaing[a], Amélie Daveluy[b,e] and Mathieu Molimard[a,b]

[a]CHU de Bordeaux, Laboratoire de Pharmacologie et Toxicologie, Bordeaux Cedex, France; [b]Univ. Bordeaux, INSERM U1219, BPH, Bordeaux Cedex, France; [c]CHU de Bordeaux, Centre-Antipoison et Toxicovigilance de Nouvelle Aquitaine, Bordeaux Cedex, France; [d]CHU de Bordeaux, Urgences adultes, Place Amélie Raba Léon, Bordeaux Cedex, France; [e]CHU de Bordeaux, Centre d'addictovigilance, Place Amélie Raba Léon, Bordeaux Cedex, France

ABSTRACT

Background and aims: MDMB-4en-PINACA is a synthetic cannabinoid receptor agonist (SCRA) that has recently emerged. Data regarding clinical presentations in the event of intoxication is scarce. This study presents MDMB-4en-PINACA identification in cannabis consumers with clinical and analytical descriptions.

Methods: Between November 2020 and March 2021, all patients with unexpected or unusually severe effects and Poisoning Severity Score (PSS) greater than or equal to 2 after cannabis consumption were included. Blood and/or urine samples were collected for toxicological analysis. When available, drug material samples were also collected for analysis.

Results: Between November 2020 and March 2021, 13 patients were included. All cases typically presented with altered mental status ($n = 13$), and nearly all had returned to a normal or quasi-normal state after around 11 h of observation. Neurological symptoms included headaches ($n = 3$), hallucinations (3), mydriasis (3), amnesia (2) and seizures (5). Psychiatric symptoms were paranoia (6) and anxiety (2). Digestive symptoms were nausea (2) and vomiting (6). No deaths were recorded. All patients were positive for the SCRA MDMB-4en-PINACA in urine, blood and/or drug material sample. Results from toxicology testing paired with case history showed the potential for MDMB-4en-PINACA to cause or contribute to different clinical disorders.

Conclusions: This study highlights the risk of intoxication by SCRAs when taking low-THC cannabis products. Forensic scientists, public health and public safety officials, law enforcement personnel and clinicians should be aware of the impact that these emergent SCRAs may have in their work, especially MDMB-4en-PINACA.

ARTICLE HISTORY
Received 23 June 2021
Revised 23 September 2021
Accepted 11 October 2021

KEYWORDS
MDMB-4en-PINACA; synthetic cannabinoid receptor agonist; LC-QTOF; cannabis; intoxication; cannabidiol

Introduction

Synthetic cannabinoids receptor agonists (SCRAs) are designed to stimulate the endocannabinoid system and mimic the effects of delta-9-tetrahydrocannabinol, the main psychoactive constituent of cannabis [1]. Although originally developed as research chemicals for exploring the endocannabinoid system, the use of SCRAs in a recreative context has become popular since 2008 [2]. One of the reasons for the use of these emerging SCRAs is that they and/or their metabolites are not detectable by routine toxicological analysis, especially due to their modified chemical structure and thermal lability. Current immunoassays screenings are not designed to detect SCRAs due to their chemical structure that is highly variable between families and generations. They can be detected only by highly specific, sensitive analytical methods such as high-resolution mass spectrometry [3]. Most SCRAs initially appeared on the Europe market as powders, after which they are added into smoking preparations or e-liquids.

Cannabis is the most widely used illicit drug worldwide [4] and an increase use was reported during the first lockdown period of the COVID-19 pandemic in some countries [5,6]. Since October 2020, several alerts on the presence of SCRAs in cannabis samples have been issued in Switzerland [7] and in France since October 2020 [8,9]. Several European countries have been alerted after low-THC cannabis products were reported to be adulterated with the SCRA MDMB-4en-PINACA (5-CL-ADB-A, methyl 3,3-di- methyl-2-[1-(pent-4-en-1-yl)-1H-indazole-3-carboxamido] butanoate). This New Psychoactive Substance (NPS) is a SCRA that was first identified in powders seized by the German police in 2017 [10]. It was officially notified to the European Union (EU) Early Warning System (EWS) in August 2018 [11]. Information on this NPS including its pharmacology and toxicity is largely unknown, apart from comments on online drug forums. However, based on the study of its structural analogs, the

CONTACT Ruben Goncalves ruben.goncalves@chu-bordeaux.fr Laboratoire de Pharmacologie et Toxicologie, CHU de Bordeaux, Place Amélie Raba Leon, Bordeaux Cedex 33076, France

© 2021 Informa UK Limited, trading as Taylor & Francis Group

drug is likely to be a highly potent and efficacious agonist on the CB1 and CB2 cannabinoid receptors [12,13]. The detection of MDMB-4en-PINACA in medico-legal death investigation casework coupled with limited case history suggest that these new SCRAs can have significant physiological effects and may cause or contribute to death [11,12,14]. While MDMB-4en-PINACA is potentially highly toxic, only a few studies have discussed its clinical presentation, none has yet investigated it in the context of cannabis consumption. This study presents the identification of MDMB-4en-PINACA in cannabis consumers with clinical and analytical descriptions.

Material and methods

Reported cases

Cases presented in this study are the result of collaboration between the Addictovigilance Center [15], the Poison Control Center and the Laboratory of Pharmacology and Toxicology in Bordeaux. Between November 2020 and March 2021, as part of their care, all patients with unexpected or unusually severe effects and Poisoning Severity Score (PSS) greater than or equal to 2 after cannabis consumption were included. Clinical information and drug material description was collected by poison control centre toxicologists during interview with physicians. The reporting physician also filled in an information sheet including data on clinical presentation as a part of addictovigilance reporting. Regular use was defined as greater than or equal to twice a week. Occasional use was defined as less than twice a week. Poisoning Severity Score (PSS) was evaluated for each patient [16]. Blood and/or urine samples were collected for toxicological analysis. When available, drug material samples were also collected for analysis during the period of inclusion.

Toxicological analysis

Blood, urine and drug material samples were submitted to toxicological examination.

Sample preparation

Liquid-liquid extraction was performed on blood and urine samples. Briefly, 500 µL of sample were alkalized by addition of 500 µL of sodium carbonate buffer at pH 9.7 and 500 µL of sample were acidified by addition of 500 µL hydrochloric acid 0.1 N. In each solution, 50 µL of internal standard (prazepam, 5 µg/mL) and a mixture of 6 mL of organic solvents (dichloro-methane/ether/hexane/isoamyl alcohol; 30:50:20:0.5 v/v/v) were added. After vortex-mixing and centrifugation, the two extracts where then mixed, evaporated to dryness (nitrogen gas at 45 °C) and finally redissolved in 100 µL of water/acetonitrile (50:50, v/v). For drug samples, 100 µL of working solutions of each sample were prepared at 100 ng/mL in a water-acetonitrile mixture (50:50, v/v) with 5 µL of internal standard (prazepam, 5 µg/mL).

Liquid chromatography-high-resolution mass spectrometry

The UPLC-HRMS system consisted of an Acquity I class system (waters, USA) coupled to a Xevo XS G2 QTOF analyzer (Waters, USA) using positive and negative mode electrospray ionization and controlled with Unifi 1.9 software. Chromatographic separation was performed on an ACQUITY HSS C18 column (150 × 2 mm, 1,8 µm, Waters). The mobile phase was composed of solvents A (ammonium formate buffer 5 mM, pH 3) and B (acetonitrile in 0.1% v/v formic acid). The column was maintained at 50 °C and a gradient elution was used at a flow rate of 0.4 ml/min: 13% of B held until 0.5 min, increased to 50% at 10 min, then to 95% at 10.25 min, and finally held until 12.25 min, return to initial condition in 0.25 min until 17 min. The injection volume was 2 µL. Data were analyzed with HighResNPS® and Waters® databases for over 3500 toxicologically relevant drugs, NPS and metabolites that included protonated molecules and fragmentation and chromatographic retention time data. Criteria used to identify compounds included 5 ppm as exact mass error of the precursor ion as well as product ions and ±0.5 min as tolerance in retention time for the precursor ion.

Metabolite identification

Data was analyzed using Unifi software (Waters, Manchester, UK). A software algorithm was used to predict biotransformations *in silico*. Briefly, after examining available data on the metabolism of related substances, sets of theoretically possible biotransformations were created based on the structure of the drug, including putative hydroxylation, N-dealkylation, ester hydrolysis and glucuronidation. The automated metabolite profiling process was completed by using the fragmentation interpretation software tool MassFragmentTM 4.1 (Waters) to enable the software-driven assignment of metabolite structures from fragmentation patterns. The automated approach is driven by structural knowledge of the parent molecule using chemically intelligent algorithms which perform a silicocleavage of the molecule and identify possible functional groups. Criteria used to identify targeted compounds included 5 ppm as exact mass error of the precursor ion as well as product ions and ± 0.5 min as tolerance in retention time for the precursor ion.

Results

Results are summarized in Table 1. Between November 2020 and March 2021, 13 patients were included: 10 males and 3 females (median age: 22; [16–40]). Patients were hospitalized at emergency department ($n = 9$) and outpatient addictology service ($n = 4$). In this service are provided consultations for the treatment of addictions including withdrawal programs. Most patients (75%) were regular users of herbal cannabis. In all cases, they thought they were using herbal cannabis smoked in joints. Symptoms in all patients started after only one hit of the joint. They stopped consumption immediately after the unusual symptoms started. Clinical presentation was varied. Patients had neurological symptoms including headaches ($n = 3$), hallucinations ($n = 3$), mydriasis ($n = 3$),

Table 1. Description of positive cases for MDMB-4en-PINACA.

Patient	Drug material form	Phytocannabinoids (THC, CBD) THC (<1%)	CBD	Sex	Age	Type of cannabis consumer	Sample type	Toxicology Results	Duration of hospitalization (h)	Clinical presentation
Emergency department										
1	Herbal	+	+	M	16	Occasional	Urine	THC-COOH, MDMB-4en-PINACA 3,3-dimethylbutanoic acid	4,5	Nausea, repeated vomiting dizziness, mydriasis
2	Herbal	+	+	M	17	Occasional	Urine	THC-COOH, MDMB-4en-PINACA 3,3-dimethylbutanoic acid	3	Nausea, repeated vomiting, mydriasis, anxiety, paranoia, headaches, dizziness, amnesia,
3	Herbal	+	+	M	16	Occasional	Urine	THC-COOH, MDMB-4en-PINACA ; MDMB-4en-PINACA 3,3-dimethylbutanoic acid	2	Multiple vomiting, mydriasis, palpitations and headaches, altered consciousness without loss of consciousness
4	Herbal	+	+	M	20	Regular	Plasma	THC-COOH, MDMB-4en-PINACA	16	Feeling of discomfort, hot flushes, ringing in ears, headaches before loss of consciousness, delirium after regaining consciousness, seizure, amnesia
							Urine	THC-COOH, MDMB-4en-PINACA ; MDMB-4en-PINACA 3,3-dimethylbutanoic acid		
5	Herbal	+	+	F	20	Regular	Plasma	THC-COOH, MDMB-4en-PINACA	13	Vomiting, asthenia, loss of consciousness with fall or head trauma, seizure
6	Herbal	+	+	M	23	Regular	Plasma	THC-COOH, MDMB-4en-PINACA 3,3-dimethylbutanoic acid	15	Intense psychomotor agitation, dysphoria, paranoia, hallucination, convulsions, aggressiveness, persistent tiredness
							Urine	THC-COOH, MDMB-4en-PINACA 3,3-dimethylbutanoic acid		
7	Herbal	+	+	M	22	Regular	Plasma	THC-COOH	16	Intense psychomotor agitation, dysphoria, paranoia, hallucination, convulsions, aggressiveness, persistent tiredness
							Urine	THC-COOH, MDMB-4en-PINACA 3,3-dimethylbutanoic acid		
8	Herbal	+	+	M	22	Regular	Plasma	THC-COOH	15	Intense psychomotor agitation, dysphoria, paranoia, hallucination, convulsions, aggressiveness, persistent tiredness
							Urine	THC-COOH, MDMB-4en-PINACA 3,3-dimethylbutanoic acid		
9	Herbal	+	+	M	30	Regular	N/A		13	Vomiting, malaise, depersonalization, anxiety, sweating, tachycardia and panic attack.
Addictology department										
10	Herbal	+	+	F	20	Regular	N/A			Vomiting, tachycardia, intense psychotropic effects, dry mouth, visual distortions
11	Herbal	+	+	F	24	Regular	N/A			Tachycardia, xerostomia, intense psychotropic effects
12	Herbal	+	+	M	30	Regular	N/A			Hot flushes, paranoid attack
13	Herbal	+	+	M	40	Regular	N/A			Hot flushes, paranoid attack

THC: tetrahydrocannabinol; CBD: cannabidiol; +: positive; grouped cases using the same cannabis preparation: (1,2,3) - (6,7,8) - (10,11) - (12,13).

amnesia ($n = 2$), and seizures ($n = 5$) that required ambulance transfer to the emergency department. Psychiatric symptoms were paranoia ($n = 6$) and anxiety ($n = 2$). Digestive symptoms were nausea ($n = 2$) and vomiting ($n = 6$). No respiratory symptoms were observed. All patients typically presented with altered mental status ($n = 13$), and nearly all had returned to a normal or quasi-normal state after around 11 h of observation (median: 14; [2–16]). No deaths were recorded. PSS was evaluated between moderate ($n = 8$) and severe ($n = 5$). Among all patients included, first toxicological analysis by immunoassay only identified THC-COOH in blood or urine samples. For all these patients, analyses by LC-QTOF showed the presence of MDMB-4en-PINACA and its main metabolite (MDMB-4en-PINACA 3,3-dimethylbutanoic acid) in blood and/or urine samples. No other substances were detected. Five drug material samples recorded. They were all in the form of conventional cannabis herb. For all drug material samples, toxicological analyses using LC-QTOF showed the presence MDMB-4en-PINACA in low-THC products which contained cannabidiol (CBD) and less than 1% THC.

Discussion

To our knowledge, these are the first descriptions of complications after the intake of the SCRA MDMB-4-en PINACA in cannabis consumers in France. Thirteen consumers had unknowingly consumed it and presented the unexpected effects described in Table 1. Such effects are rarely observed with cannabis, but the fact that they occurred in all members of the same group of naive young patients (cases 1 to 3) or in regular users who had not changed dealer (other cases) led us to search for substances other than THC.

Understanding of the pharmacokinetics and toxicology of SCRAs is required so that clinical and forensic toxicology can keep abreast, and for the purpose of interpreting drug screens. Evidence suggests that SCRAs are more potent than cannabis and could have longer half-lives, potentially leading to prolonged toxicological effects [17,18]. In addition, there is considerable inter- and intra-batch variability in terms of substances and their quantities, so their clinical effects are unpredictable [19]. Given their high affinity for their receptor sites and high potency, their effective dose is small, so the amount of excreted metabolites is low [20]. This makes the detection of these compounds difficult [3,21]. Krotulski et al. previously described the detection of butanoic acid metabolites as the result of the instability of parent SCRAs, especially with methyl ester species. The metabolic profiles of methyl ester SCRAs, including MDMB-4en-PINACA, have been well documented, and this species was reported as a primary metabolite of ester hydrolysis (MDMB- 4en-PINACA 3,3-dimethylbutanoic acid) and feasible biomarker for monitoring the use of MDMB-4en-PINACA [12]. In our case, due to early sampling, the toxicological screening only identified MDMB-4en-PINACA and its metabolite in blood. No others substances were identified thus patients included were not polydrug users. We can therefore link the clinical presentations to the substance identified. Patients were under the influence of the product during his hospitalization. Drug history was not taken directly from the patients therefore some details may be omitted. Indeed, THC-COOH blood concentration remains at a significant concentration 6 h later after starting to smoke and becomes very low and often undetectable 24 to 48 h later in the case of occasional cannabis users. However, the presence of THC-COOH in the blood usually lasts longer than 48 h in the regular user. In our cases, we can't exclude that THC-COOH identification may be from an earlier episode of drug use. However, as our aim was to link the identification of the substance to the clinical presentation, we did not perform a quantification of MDMB-4en-PINACA. Since pharmacological knowledge of MDMB-4en-PINACA is far from complete, further research is needed to establish a toxic level in blood and gain a much clearer understanding of their physical and psychological impact.

The 5 drug material samples in which this SCRA has been detected since September 2020 have all the same profile regarding the natural cannabinoids of the carrier plant: a low THC level, below 1% or even undetectable, and a CBD level normal or high. While these profiles may correspond to industrial hemp, the adverse effects reported by users (Table 1) are far removed from their expected effects. In the context of the COVID-19 pandemic, the French addictovigilance network has shown some patient reporting withdrawal symptoms because of difficulties in the cannabis supply chain [22]. The question is whether dealers are now circumventing the lack of natural cannabis on the market by adding SCRAs to low-THC cannabis product to increase psychoactivity. While the phenomenon is recent with this specific SCRA, it has already been described with other SCRAs, especially with AB-FUBINACA [23] or/and ADB-FUBINACA [24], or 4-cyano CUMYL-BUTINACA (4-CCB) [25], which are sold as cannabis or CBD. This phenomenon is occurring in a context in which THC levels in seized herb and resin are gradually increasing (from 5% to 25% for resin and from 5% to around 11% for herb between 2000 and 2019) [26]. Moreover, these results must be interpreted in the light of the levels reached in recent years, which may also promote the perception of unexpected or undesirable effects. In this situation, only toxicological analysis can determine the nature of the substance consumed. Since these compounds are much more potent than those from previous generations and are found at lower concentrations, toxicology testing for emerging SCRAs requires the use of highly sensitive platforms (e.g. LC-QTOF)

Adverse reactions to MDMB-4en-PINACA have not been widely reported [27]. Taylor M reported bizarre and impulsive behavior following consumption of heroin that had been cut with MDMB-4en-PINACA [28]. In general, numerous complications have been observed in SCRA users. Owing to their pharmacological properties, SCRAs may cause more severe adverse effects than those observed with cannabis. Delta-9-THC has demonstrated an inhibitory effect on gamma aminobutyric acid (GABA) neurotransmission in the brain *via* several pathways [29]. Thus, agents that have full agonist activity such as MDMB-4en-PINACA may cause intense anxiety and agitation, and may induce seizures and convulsions by inhibiting GABA neurotransmission more effectively

than THC [30,31]. These clinical effects of potent cannabinoid receptor agonists used excessively may have serious consequences. The effects are mainly neurological, psychiatric, cardiovascular, cerebrovascular, and gastrointestinal, with different degrees of seriousness [32–36]. In this context, recognition of the signs and symptoms of patients with SCRA intoxication can help physicians to be more efficiency and prepared to diagnose and manage adolescents in case of intoxication.

Conclusion

This study described in a short period of time a large number of intoxications with low-THC cannabis products adulterated with the SCRA MDMB-4en-PINACA. Clinical and forensic toxicologists should be aware of the possibility of cannabis preparations being fortified with MDMB-4en-PINACA or other NPS, resulting in unexpected toxicity.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Funding

The author(s) reported there is no funding associated with the work featured in this article.

ORCID

Ruben Goncalves http://orcid.org/0000-0002-9389-6226
Magali Labadie http://orcid.org/0000-0002-6546-6886
Mathieu Molimard http://orcid.org/0000-0002-4346-8346

References

[1] Mills B, Yepes A, Nugent K. Synthetic cannabinoids. Am J Med Sci. 2015;350(1):59–62.
[2] Le Boisselier R, Alexandre J, Lelong-Boulouard V, et al. Focus on cannabinoids and synthetic cannabinoids. Clin Pharmacol Ther. 2017;101(2):220–229.
[3] Smith JP Sutcliffe OB, Banks CE. An overview of recent developments in the analytical detection of new psychoactive substances (NPSs). Analyst. 2015;140(15):4932–4948.
[4] UNODC World Drug Report 2020 [Internet]. United Nations: World Drug Report 2020. 2020. [cited 12 August 2021]. Available at: wdr.unodc.org/wdr2020/en/index2020.html
[5] Manthey J, Kilian C, Carr S, et al. Use of alcohol, tobacco, cannabis, and other substances during the first wave of the SARS-CoV-2 pandemic in Europe: a survey on 36,000 european substance users. Subst Abuse Treat Prev Policy. 2021;16(1):36.
[6] van Laar MW, Oomen PE, van Miltenburg CJA, et al. Cannabis and COVID-19: Reasons for concern. Front Psychiatry. 2020;11: 601653.
[7] Infodrog. Alertes actuelles – Centrale nationale de coordination des addictions [Internet]. 2021. [cited 3 June 2021]. Available at: https://www.infodrog.ch/fr/aide/alertes-actuelles.html
[8] CEIP-A. Information – alerte relative aux cannabinoïdes de synthèse et risques [Internet]. 2020. [cited 3 June 2021]. Available at: http://www.dicadd13.fr/images/boiteaoutils/alerteCEIPcannabis.pdf
[9] OFDT. Circulation d'herbe de cannabis adultérée avec des cannabinoïdes de synthèse [Internet]. 2021. [cited 3 June 2021]. Available at: https://www.ofdt.fr/BDD/sintes/ir_19102020_Cannabis.pdf
[10] Morais JD, Brandt S, Jorge R, et al. EMCDDA technical report on the new psychoactive substance methyl 3,3-dimethyl-2-{[1-(pent-4-en-1-yl)-1H-indazole-3-carbonyl]amino}butanoate (MDMB-4en-PINACA).
[11] EMCDDA initial report on the new psychoactive substance MDMB-4en-PINACA | www.emcdda.europa.eu. [Internet]. 2021. [cited 3 June 2021]. Available at: https://www.emcdda.europa.eu/publications/initial-reports/mdmb-4en-pinaca_sl
[12] Krotulski AJ, Cannaert A, Stove C, et al. The next generation of synthetic cannabinoids: Detection, activity, and potential toxicity of pent-4en and but-3en analogues including MDMB-4en-PINACA. Drug Test Anal. 2021;13(2):427–438.
[13] Potts AJ, Cano C, Thomas SHL, et al. Synthetic cannabinoid receptor agonists: classification and nomenclature. Clin Toxicol (Phila). 2020;58(2):82–98.
[14] Logan BK, Mohr ALA, Friscia M, et al. Reports of adverse events associated with use of novel psychoactive substances, 2013-2016: a review. J Anal Toxicol. 2017;41(7):573–610.
[15] Jouanjus E, Gibaja V, Kahn J-P, et al. Signal identification in addictovigilance: the functioning of the french system. Therapie. avr. 2015;70(2):113–131.
[16] Persson HE, Sjöberg GK, Haines JA, et al. Poisoning severity score. Grading of acute poisoning. J Toxicol Clin Toxicol. 1998;36(3): 205–213.
[17] ElSohly MA, Gul W, Wanas AS, et al. Synthetic cannabinoids: Analysis and metabolites. Life Sci. 2014;97(1):78–90.
[18] Pike E, Grafinger KE, Cannaert A, et al. Systematic evaluation of a panel of 30 synthetic cannabinoid receptor agonists structurally related to MMB-4en-PICA, MDMB-4en-PINACA, ADB-4en-PINACA, and MMB-4CN-BUTINACA using a combination of binding and different CB1 receptor activation assays: Part I-Synthesis, analytical characterization, and binding affinity for human CB1 receptors. Drug Test Anal. 2021;13(7):1383–1401.
[19] Castaneto MS, Gorelick DA, Desrosiers NA, et al. Synthetic cannabinoids: Epidemiology, pharmacodynamics, and clinical implications. Drug Alcohol Depend. 2014;144:12–41.
[20] Cannaert A, Storme J, Franz F, et al. Detection and activity profiling of synthetic cannabinoids and their metabolites with a newly developed bioassay. Anal Chem. 2016;88(23): 11476–11485.
[21] Pasin D, Cawley A, Bidny S, et al. Current applications of high-resolution mass spectrometry for the analysis of new psychoactive substances: a critical review. Anal Bioanal Chem. 2017; 409(25):5821–5836.
[22] Lapeyre-Mestre M, Boucher A, Daveluy A, et al.; French Addictovigilance Network. Addictovigilance contribution during COVID-19 epidemic and lockdown in France. Therapies. juill. 2020;75(4):343–354.
[23] Rianprakaisang T, Gerona R, Hendrickson RG. Commercial cannabidiol oil contaminated with the synthetic cannabinoid AB-FUBINACA given to a pediatric patient. Clin Toxicol. 2020;58(3): 215–216.
[24] Lam RPK, Tang MHY, Leung SC, et al. Supraventricular tachycardia and acute confusion following ingestion of e-cigarette fluid containing AB-FUBINACA and ADB-FUBINACA: a case report with quantitative analysis of serum drug concentrations. Clin Toxicol (Phila)). 2017;55(7):662–667.
[25] Horth RZ. Acute poisonings from a synthetic cannabinoid sold as cannabidiol — Utah, 2017–2018. MMWR Morb Mortal Wkly Rep [Internet]. 2018. [cited 3 June 2021]; 67. Available at: https://www.cdc.gov/mmwr/volumes/67/wr/mm6720a5.htm
[26] EMCDDA. European Drug Report. 2020.
[27] WHO, Critical Review Report, MDMB-4en-PINACA [Internet]. 2021. [cited 3 June 2021]. Available at: https://www.who.int/docs/default-source/controlled-substances/43rd-ecdd/mdmb-4en-pinaca-review-2020.pdf?sfvrsn=5cd6e97e_4.
[28] Taylor M. Police Warn of Heroin Cut with Research-only Cannabinoid [Internet]. 2021. [cited 3 June 2021]. Available at: http://www.forensicmag.com/561307-Police-Warn-of-Heroin-Cut-with-Research-only-Cannabinoid/.

[29] Banister SD, Longworth M, Kevin R, et al. Pharmacology of valinate and tert-Leucinate synthetic cannabinoids 5F-AMBICA, 5F-AMB, 5F-ADB, AMB-FUBINACA, MDMB-FUBINACA, MDMB-CHMICA, and their analogues. ACS Chem Neurosci. 2016;7(9):1241–1254.
[30] Gurney SMR, Scott KS, Kacinko SL, et al. Pharmacology, toxicology, and adverse effects of synthetic cannabinoid drugs. Forensic Sci Rev. janv. 2014;26(1):53–78.
[31] Hess C, Schoeder CT, Pillaiyar T, et al. Pharmacological evaluation of synthetic cannabinoids identified as constituents of spice. Forensic Toxicol. 2016;34(2):329–343.
[32] Pacher P, Steffens S, Haskó G, et al. Cardiovascular effects of marijuana and synthetic cannabinoids: the good, the bad, and the ugly. Nat Rev Cardiol. 2018;15(3):151–166.
[33] Robson H, Braund R, Glass M, et al. Synthetic cannabis: adverse events reported to the New Zealand pharmacovigilance Centre. Clin Toxicol (Phila). 2021;59(6):472–479.
[34] Choi NG, Marti CN, DiNitto DM, et al. Cannabis and synthetic cannabinoid poison control center cases among adults aged 50+, 2009-2019. Clin Toxicol (Phila). 2021;59(4):334–342.
[35] Darke S, Duflou J, Farrell M, et al. Characteristics and circumstances of synthetic cannabinoid-related death. Clin Toxicol (Phila). 2020;58(5):368–374.
[36] Arens AM, Olives TD, Simpson NS, et al. An outbreak of synthetic cannabinoid exposures reported to a regional poison center: "K2" identified as 5F-ADB. Clin Toxicol (Phila). 2019; 57(1):69–71.

DISCUSSION GÉNÉRALE ET PERSPECTIVES

Ces dernières années, nous avons assisté à l'émergence de centaines de NPS, ainsi qu'à une augmentation de la pureté des substances généralement utilisées dans les milieux festifs, comme la MDMA et la cocaïne (177–179). Le manque de connaissance concrète sur la composition et les dosages en principes actifs de ces produits, les rend particulièrement dangereux et pose un risque sanitaire important (180). L'analyse de SPA représente ainsi une réponse directe à la nécessité de réduire les risques sanitaires liés à l'utilisation de drogues illicites.

L'objectif principal de ces travaux était d'étudier l'apport analytique de la spectrométrie de masse haute résolution en QTOF pour l'identification et la prise en charge de l'exposition aux substances psychoactives. Pour cela, nous avons étudié la mise en place du *drug checking* par spectrométrie infrarouge dans un CAARUD. Ce travail nous a permis d'évaluer l'apport du QTOF par comparaison avec une méthode infrarouge. Ensuite, nous avons évalué les avantages pour l'identification des NPS et la mise en évidence de biomarqueurs d'exposition. Cette étude a reposé sur la combinaison des approches *in vivo*, *in silico* et *in vitro*, ainsi que sur celle avec d'autres méthodes d'identification, telles que la RMN et l'IR. Enfin, nous avons évalué l'apport en pratique clinique pour l'identification des cannabinoïdes de synthèse dans les départements d'Outre-mer et en France métropolitaine. Ce travail a mis en évidence l'apport du QTOF pour l'amélioration de la prise en charge des patients et de façon plus globale une amélioration des connaissances sur ces substances et de leurs dangers, afin d'établir des bases de réflexion pour les autorités sanitaires.

La première partie de ce travail nous a permis d'évaluer la technique de spectroscopie infrarouge comme outil de dépistage des SPA *in situ* dans un CAARUD en la comparant à la technique de référence (LC-QTOF). La spectroscopie IR présente un certain nombre d'avantages. Elle permet une détection rapide et fiable de la substance majoritaire dans un échantillon, avec un minimum de formation de l'opérateur. Elle ne nécessite pas de solvants ni de préparation d'échantillon ; il s'agit donc d'un appareil facile à utiliser et dont le coût d'utilisation est faible. Il utilise un processus non destructif qui permet une analyse ultérieure avec le même échantillon. Il s'agit d'un appareil hautement portable, ce qui permet de l'utiliser lors de festivals et d'autres événements festifs. Ces avantages permettent aux personnels de la réduction des risques de mieux diffuser leur message en proposant un outil réactif et bien adapté à une population urbaine qui achète en petites quantités et consomme rapidement. Cependant, la spectroscopie IR présente des limites importantes pour

l'identification de divers types d'échantillons, notamment le cannabis, les buvards, les mélanges et les produits présents en faible concentration. Dans ces cas, ainsi que dans les situations où les usagers de drogues rapportent des effets indésirables connus ou inhabituels, des analyses supplémentaires par spectroscopie de masse à haute résolution sont nécessaires pour mieux évaluer la composition de la substance. Il s'agit de la première étude en France à évaluer une méthode de *drugchecking* par spectroscopie IR réalisée sur place dans un CAARUD, avec une comparaison directe avec des méthodes de laboratoire de pointe comme la LC-QTOF. Il s'agit d'un travail original, qui n'a pas fait l'objet d'études antérieures. Cette étude a montré les limites de la spectrométrie IR dans le cadre du *drug checking* en conditions réelles d'activité d'un CAARUD. Elle a fourni des données qui pourrait permettre aux structures de connaître les limites de leur instrument et de guider leur discours de RDR. Grâce à ces résultats, d'autres structures pourront mieux appréhender la mise en place d'un tel dispositif.

La seconde partie de ce travail consacrée à l'identification et l'étude du métabolisme des NPS a donné lieu à la mise en pratique d'un outil analytique performant permettant la recherche de nouveaux biomarqueurs d'exposition à ces produits. Depuis une dizaine d'années en France, les NPS sont un phénomène émergent et inquiétant du fait de leur association à des cas d'intoxications et de décès (3). De nouvelles familles ou sous-familles de NPS apparaissent constamment sur le marché, mais les données sur leur pharmacodynamie ou leur pharmacocinétique font défaut. Il s'agit d'un problème majeur pour les composés qui sont largement métabolisés, comme les cannabinoïdes de synthèse (181,182) et les NBOMe (183,184), où la molécule mère est difficilement identifiable. Afin d'augmenter les chances de détecter la consommation de ces NPS et d'accroître la fenêtre de détection dans les matrices biologiques, l'identification des métabolites semble inévitable et des études sur le métabolisme doivent être menées. Ces études sont particulièrement intéressantes en toxicologie clinique et médico-légale, en particulier avec des matrices connues pour stocker les métabolites comme la bile et l'urine. Dans cette étude, nous avons rapporté la première identification de MXPr en France accompagnée de données analytiques. Le premier objectif était d'identifier et de caractériser le MXPr dans des échantillons d'urine, de cheveux et de poudre en combinant plusieurs méthodes analytiques : résonance magnétique nucléaire (RMN) à l'état liquide, spectroscopie infrarouge (IR) et la chromatographie liquide couplée à la spectrométrie de masse haute résolution (LC-HRMS). Le deuxième objectif était d'explorer le métabolisme du MXPr in silico et in vitro. Cette étude de métabolisme a pu apporter de

nouvelles données scientifiques quant au métabolisme de certains NPS jamais documentés dans la littérature. Cependant, cet outil analytique qui combine des approches *in vivo*, *in vitro* et *in silico* doit continuer d'être alimenté pour demeurer performant. Au cours de la dernière décennie, la spectrométrie de masse haute résolution a suscité un intérêt croissant dans le domaine de la toxicologie analytique. Durant ces années, nous avons observé un remplacement progressif des spectromètres de masse à basse résolution (LRMS) par des spectromètres de masse à haute résolution (HRMS) dans un grand nombre de laboratoires de toxicologie. Grâce à leur sensibilité croissante, les spectromètres de masse à haute résolution actuels permettent de distinguer certains isomères à de très faibles concentrations dans des matrices biologiques complexes telles que l'urine, le sang, les échantillons de sang séché (*dried blood spot*) ou de cheveux. De plus, l'utilisation de la spectrométrie de masse en *full scan* permet de diminuer le bruit de fond provenant de la matrice, réduisant ainsi les étapes de préparation des échantillons sans perdre en spécificité. Ainsi, l'HRMS est de plus en plus utilisé pour des études de métabolisme dans l'urine ou pour déterminer les fenêtres de détection urinaire de nombreux composés. La détection de la molécule mère seule peut conduire à des résultats faussement négatifs en raison soit d'un délai trop long entre la consommation et le prélèvement, soit de la concentration très basse dans le sang ou l'urine, généralement observée *in vivo*. Les études sur le métabolisme jouent un rôle important en toxicologie médico-légale car elles sont essentielles dans le défi de l'identification des NPS. Nous avons montré ici que la combinaison de plusieurs méthodes analytiques avec l'HRMS est très importante pour la caractérisation moléculaire des NPS. L'identification des métabolites de la MXPr améliorera l'efficacité des bibliothèques de criblage toxicologique et élargira la fenêtre de détection de la MXPr dans les matrices biologiques en cas d'ingestion de MXPr. Au vu des résultats de la présente étude, nous suggérons d'ajouter le N-despropyl(nor) MXPr, le O-desméthyl MXPr et le dihydroMXPr aux bibliothèques de dépistage toxicologique afin de réduire le risque de résultats faux-négatifs dans les cas d'intoxication impliquant le MXPr. Des études supplémentaires avec des données *in vivo* obtenues dans des cas d'intoxication semblent nécessaires afin de confirmer nos résultats. Cette étude souligne la difficulté d'identifier certains NPS lorsqu'ils sont absents des bibliothèques et que le standard n'est pas disponible.

La confirmation de l'identification des NPS nécessite la mise au point d'une méthode non seulement spécifique et sensible mais également fiable et robuste. Les capacités analytiques des laboratoires de toxicologie sont limitées, ils doivent donc s'adapter au matériel disponible

et mettre en place des échanges et collaborations entre laboratoires. Les méthodes analytiques utilisées en routine ne sont pas toujours suffisantes pour identifier formellement les NPS, notamment dans le cas des poudres ou des e-liquides. Il est donc parfois nécessaire de combiner plusieurs méthodes analytiques.

Par exemple, l'analyse des produits de saisies dépend des moyens instrumentaux disponibles. Traditionnellement, cela inclut une combinaison de techniques analytiques telles que la GC-MS, la LC-MS, la spectroscopie infrarouge à transformée de Fourier (FTIR) et la spectroscopie de résonance magnétique nucléaire (RMN) pour la confirmation et l'élucidation de la structure (185). Cependant, lorsqu'elles sont utilisées seules, toutes ces méthodes présentent des limites pour l'identification sans équivoque d'une molécule inconnue (185). Par exemple, la LC, qui est la méthode de séparation la plus largement utilisée, peut ne pas distinguer les isomères de positions (tels que la chloro-N, N-diméthylcathinone (CDC) et la chloroéthcathinone (CEC) ou la 3-MMC et la méphédrone (4-MMC)) (186,187). En LC-MS/MS, leur temps de rétention peut être identique ainsi que leur profil de fragmentation. Il est donc nécessaire d'utiliser une autre méthode, comme la spectroscopie par résonance magnétique nucléaire (RMN), la GC-MS, la spectroscopie infrarouge ou la chromatographie sur fluide supercritique (SFC) (188) pour distinguer les structures. La disponibilité de bibliothèques de GC/MS aide grandement à l'identification de nouvelles substances. Pour des échantillons relativement purs, une analyse GC-MS associée à un spectre FTIR peut conduire à l'identification complète de la substance si les deux spectres correspondent aux données existantes. Malheureusement, dans la plupart des cas impliquant des NPS, les bibliothèques commerciales de GC-MS ne sont pas suffisantes. Dans ce cas, l'HRMS est souvent utilisé suivie d'une confirmation par RMN et/ou spectroscopie IR (189). La combinaison des résultats en HRMS et de la RMN avec les données obtenues par IR peut aider à élucider les structures de substances inconnues. L'utilisation systématique de l'approche multi-méthodes permet de définir la structure et d'identifier avec succès des échantillons inconnus grâce à de puissantes techniques analytiques complémentaires, malgré l'absence d'un standard de référence.

Grâce à ces développements analytiques, la troisième partie a permis de mettre en place plusieurs études observationnelles afin d'étudier la prévalence des NPS dans différentes populations d'usagers. Ces études montrent une prévalence importante de ces produits, qui restent souvent méconnus par les cliniciens et ne sont pas systématiquement recherchés dans la plupart des laboratoires. Cette tendance de consommation, similaire à celle des drogues

classiques (par exemple, cocaïne ou cannabis), incite à une réflexion générale sur le plan législatif et scientifique pour inclure leur recherche systématique dans les différentes expertises cliniques ou médico-judiciaires.

Le criblage (ou screening) toxicologique est une approche spécifique de la toxicologie analytique qui permet la détection et l'identification simultanées d'un grand nombre de composés (190). Il est adapté aux applications médico-légales et cliniques. Dans le cadre des urgences hospitalières, il est indispensable de fournir des résultats rapidement car un diagnostic précoce de l'intoxication peut améliorer le pronostic du patient. Dans ce contexte, il est important de disposer de méthodes de dépistage sensibles et spécifiques telles que la LC-HRMS pour identifier des molécules nouvelles ou rares, ou des métabolites susceptibles d'éclairer les cliniciens ou les magistrats dans les cas d'enquêtes judiciaires. Les procédures de dépistage par HRMS sont principalement qualitatives et peuvent être appliquées à la plupart des matrices, par exemple l'urine, le sérum, les cheveux ou les échantillons de sang total. La principale différence entre la plupart des méthodes HRMS et MS/MS est que l'HRMS permet un dépistage non ciblé. Aujourd'hui, le criblage à grande échelle par HRMS est l'un des outils analytiques les plus puissants et les plus efficaces dans le domaine des NPS. L'HRMS établit avec précision la masse des analytes et de leurs fragments, permettant ainsi l'élucidation de leurs formules moléculaires. La liste des candidats possibles peut être réduite à un ou un nombre limité de composés, qui peuvent être étudiés plus en détail avec les bases de données disponibles. En d'autres termes, l'analyse peut être effectuée initialement sans standards de référence, car l'information globale fournie par l'HRMS (acquisition du spectre complet, masse précise de la molécule (dé)protonée et des ions fragments pertinents, et profil isotopique) est pertinente et permet l'identification des composés détectés (124). La possibilité de faire un screening sans *a priori* est très utile, car les laboratoires n'ont pas besoin d'acheter tous les standards de référence qui engendrent des problèmes de coût et de gestion. Les standards de référence sont nécessaires pour la confirmation finale, mais seulement en dernière étape, lorsqu'il existe des preuves solides et bien fondées que le composé a été identifié dans l'échantillon. L'ajout de nouveaux NPS dans les bases de données est simple et ne nécessite que la disponibilité de standards de référence. Grâce à des bibliothèques partagées, les données spectrales d'un instrument peuvent être utilisées comme données de référence sur un autre appareil ayant des paramètres comparables (191). Ces sources de données partagées comprennent des bibliothèques telles que le NPS Data Center (192), la base de données RESPONSE (193) ou des bases de données HRMS telles que Massbank

(109), MzCloud (194) ou HighResNPS (56). Chaque utilisateur ajoute des informations sur la configuration analytique qu'il utilise pour générer ses données. Si certains utilisateurs ont des installations analytiques identiques, ils peuvent également ajouter les temps de rétention avec les données MS, ce qui augmente la robustesse de l'identification. En outre, High-ResNPS.com a été utilisé avec succès pour identifier des NPS (186), des échantillons environnementaux (195) et pour l'analyse rétrospective d'échantillons biologiques post-mortem (123). Dans le cadre de la configuration actuelle, la qualité des données dans HighResNPS augmente lorsque plusieurs utilisateurs ajoutent des données sur le même composé.

Un autre avantage de l'HRMS est l'acquisition du spectre complet donnant la possibilité d'effectuer une analyse rétrospective. Ainsi, des composés qui n'auraient pas été identifiés lors d'une première analyse car inconnus à ce moment-là pourraient être identifiés ultérieurement sans qu'il soit nécessaire de procéder à une nouvelle analyse C'est principalement le cas pour les NPS et leurs métabolites, qui en sont souvent absents des bibliothèques avant leur première identification (123). Notre étude sur la MXPr illustre parfaitement ce cas.

En conclusion, ce travail a permis d'étudier les nouveaux produits de synthèse sous différents aspects liés à leur consommation, leur détection et leur métabolisme.

L'objectif principal à venir est la poursuite d'autres études mises en place au cours de cette thèse. Tout d'abord, l'étude DRUGETOF (Annexe 3) qui vise à décrire les consommations réelles de SPA objectivées par l'analyse des échantillons biologiques en LC-MS/QTOF, et à identifier les déterminants associés à cette consommation dans la population étudiante de la cohorte i-Share. En parallèle et afin de compléter notre première étude dans l'Océan Indien, il s'agira de mener à bien l'étude RuNPS (Annexe 4) dont le but principal est de faire une étude descriptive des passages aux urgences pour prise de NPS à l'île de La Réunion durant une année.

CONCLUSION GÉNÉRALE

L’ensemble de ces travaux a reposé sur de nombreuses collaborations avec des services cliniques (urgences, addictologie), le Centre Antipoison et Toxicovigilance (CAPTV) de Nouvelle Aquitaine, le CEIP-A de Bordeaux et la plateforme de biophysico-chimie structurale de l’Institut Européen de Chimie et de Biologie.

Ces travaux ont fait appel à différentes techniques analytiques telles que l’IR, la RMN, la LC-MS/MS et la LC-QTOF. Il a permis de mieux préciser les limites de l’IR qui a tout son intérêt dans le *drug checking*, et l’apport indispensable d’un QTOF à un laboratoire moderne pour, entre autres, permettre l’identification des nouveaux produits de synthèse en pratique.

En effet, la spectrométrie de masse en haute résolution repose sur des technologies de pointe, tel que le QTOF, permettant de réaliser des analyses plus rapides et avec une résolution accrue. C’est une technique très sensible et surtout spécifique pour l'identification des SPA, il s'agit clairement du *gold standard* pour l'analyse d'échantillons pouvant contenir des NPS, grâce à sa capacité à acquérir des données MS en mode *full scan*, qui peuvent ensuite être étudiées rétrospectivement. Grâce à une grande précision de masse, cette technologie permet une identification sans ambiguïté et l'élucidation structurelle de composés inconnus. Ces avancées sont particulièrement intéressantes dans le domaine de la toxicologie pour l'identification des métabolites, des produits de saisie, des nouveaux produits de synthèse, mais aussi des agents dopants. Seule ou en association avec d'autres techniques, l’HRMS est aujourd'hui une méthode de choix pour la recherche et les analyses de routine en toxicologie.

RÉFÉRENCES

1. EMCDDA 2022. Rapport européen sur les drogues: Tendances et évolutions. 2022. :60.

2. EMCDDA 2022. New psychoactive substances: 25 years of early warning and response in Europe. :42.

3. Darke S, Duflou J, Farrell M, Peacock A, Lappin J. Characteristics and circumstances of synthetic cannabinoid-related death. Clin Toxicol Phila Pa. mai 2020;58(5):368-74.

4. Chiappini S, Claridge H, Corkery JM, Goodair C, Loi B, Schifano F. Methoxetamine-related deaths in the UK: an overview. Hum Psychopharmacol Clin Exp. 2015;30(4):244-8.

5. Barceló B, Pichini S, López-Corominas V, Gomila I, Yates C, Busardò FP, et al. Acute intoxication caused by synthetic cannabinoids 5F-ADB and MMB-2201: A case series. Forensic Sci Int. avr 2017;273:e10-4.

6. Lam RPK, Tang MHY, Leung SC, Chong YK, Tsui MSH, Mak TWL. Supraventricular tachycardia and acute confusion following ingestion of e-cigarette fluid containing AB-FUBINACA and ADB-FUBINACA: a case report with quantitative analysis of serum drug concentrations. Clin Toxicol. 9 août 2017;55(7):662-7.

7. Kronstrand R, Guerrieri D, Vikingsson S, Wohlfarth A, Gréen H. Fatal Poisonings Associated with New Psychoactive Substances. Handb Exp Pharmacol. 2018;252:495-541.

8. Binks S, Hoskins R, Salmon D, Benger J. Prevalence and healthcare burden of illegal drug use among emergency department patients. Emerg Med J EMJ. déc 2005;22(12):872-3.

9. Magali Martinez ACT, Thomas Néfau. Nouveaux produits de synthèse - Dix ans de recul sur la situation française [Internet]. 2018 [cité 7 sept 2019]. Disponible sur: https://www.ofdt.fr/BDD/publications/docs/eftxmmya.pdf

10. Cadet-Tairou A. Les nouveaux produits de synthèse (NPS) : développement du phénomène. Eur Psychiatry. 1 nov 2015;30(8):S59-60.

11. Martinez M. Nouveaux produits de synthèse (OFDT). 2018;4.

12. Dresen S, Kneisel S, Weinmann W, Zimmermann R, Auwärter V. Development and validation of a liquid chromatography-tandem mass spectrometry method for the quantitation of synthetic cannabinoids of the aminoalkylindole type and methanandamide in serum and its application to forensic samples. J Mass Spectrom JMS. févr 2011;46(2):163-71.

13. Maurer HH. Liquid chromatography–mass spectrometry in forensic and clinical toxicology1Dedicated to Prof. Dr. Gottfried Blaschke, Münster, Germany, on the occasion of his 60th birthday.1. J Chromatogr B Biomed Sci App. 21 août 1998;713(1):3-25.

14. Maurer HH, Meyer MR. High-resolution mass spectrometry in toxicology: current status and future perspectives. Arch Toxicol. 1 sept 2016;90(9):2161-72.

15. Kaufmann A, Butcher P, Maden K, Walker S, Widmer M. Comprehensive comparison of liquid chromatography selectivity as provided by two types of liquid chromatography detectors (high resolution mass spectrometry and tandem mass spectrometry): “Where is the

crossover point?" Anal Chim Acta. 12 juill 2010;673(1):60-72.

16. Song L, Wang J, Gao Q, Ma X, Wang Y, Zhang Y, et al. Simultaneous determination of five azadirachtins in the seed and leaf extracts of Azadirachta indica by automated online solid-phase extraction coupled with LC–Q-TOF–MS. Chem Cent J. déc 2018;12(1):85.

17. Cannabis : usages actuels en population adulte - Tendances n° 119 - juin 2017 - OFDT [Internet]. [cité 3 juill 2019]. Disponible sur: https://www.ofdt.fr/publications/collections/periodiques/lettre-tendances/cannabis-usages-actuels-en-population-adulte-tendances-n-119-juin-2017/

18. 2018 European Drug Report | www.emcdda.europa.eu [Internet]. [cité 30 janv 2019]. Disponible sur: http://www.emcdda.europa.eu/edr2018_en

19. Observatoire français des drogues et des toxicomanies. Drogues, Chiffres clés 2015 [Internet]. 2015 [cité 7 mai 2019]. Disponible sur: https://www.ofdt.fr/publications/collections/periodiques/drogues-chiffres-cles/6eme-edition-2015/

20. OFDT (2021). Les niveaux d'usage de cannabis en France en 2020.

21. Richard N., Arditti J., Pépin G., Mallaret M., Castot A C. Drames (Décès en relation avec l'abus de médicaments et de substances) : outil d'identification des décès liés à la drogue en France. BEH 40-41. 26 oct 2010;16.

22. Néfau T, Janssen É. Stimulants de synthèse : MDMA/ecstasy, amphétamines et cathinones (OFDT). 2018;5.

23. Lapeyre-Mestre M, Boucher A, Daveluy A, Gibaja V, Jouanjus E, Mallaret M, et al. Addictovigilance contribution during COVID-19 epidemic and lockdown in France. Therapies. juill 2020;75(4):343-54.

24. Cadet-Taïrou A, Néfau T, Janssen É. Héroïne et autres opioïdes (OFDT). 2019;5.

25. Cadet-Taïrou A. Profils d'usage et d'usagers de drogues (OFDT). 2019;4.

26. Zvosec DL, Smith SW. γ-Hydroxybutyrate addiction and withdrawal: From the γ-Hydroxybutyrate addiction study. Ann Emerg Med. oct 2004;44(4):S91.

27. Garfein RS, Vlahov D, Galai N, Doherty MC, Nelson KE. Viral infections in short-term injection drug users: the prevalence of the hepatitis C, hepatitis B, human immunodeficiency, and human T-lymphotropic viruses. Am J Public Health. 1 mai 1996;86(5):655-61.

28. Selwyn PA, Alcabes P, Hartel D, Buono D, Schoenbaum EE, Klein RS, et al. Clinical Manifestations and Predictors of Disease Progression in Drug Users with Human Immunodeficiency Virus Infection. N Engl J Med. 10 déc 1992;327(24):1697-703.

29. Stein MD, Sobota M. Injection drug users: hospital care and charges. Drug Alcohol Depend. 1 sept 2001;64(1):117-20.

30. Del Giudice P. Cutaneous complications of intravenous drug abuse. Br J Dermatol. janv 2004;150(1):1-10.

31. Palepu A, Tyndall MW, Leon H, Muller J, O'Shaughnessy MV, Schechter MT, et al. Hospital utilization and costs in a cohort of injection drug users. :6.

32. Armenian P, Vo KT, Barr-Walker J, Lynch KL. Fentanyl, fentanyl analogs and novel synthetic opioids: A comprehensive review. Neuropharmacology. mai 2018;134:121-32.

33. Moody MT, Diaz S, Shah P, Papsun D, Logan BK. Analysis of fentanyl analogs and novel synthetic opioids in blood, serum/plasma, and urine in forensic casework. Drug Test Anal. 2018;10(9):1358-67.

34. Lewer D, Brothers TD, Van Hest N, Hickman M, Holland A, Padmanathan P, et al. Causes of death among people who used illicit opioids in England, 2001–18: a matched cohort study. Lancet Public Health. 1 févr 2022;7(2):e126-35.

35. Outils de surveillance et d'évaluation - Résultats d'enquêtes DRAMES - Résultats de l'enquête 2019 [Internet]. [cité 24 juill 2022]. Disponible sur: https://addictovigilance.fr/wp-content/uploads/2021/08/Plaquette-DRAMES-2019.pdf

36. MILD&CA. Brochure Nouveaux Produits de Synthèse - Nouvelles Substances Psychoactives [Internet]. [cité 25 août 2019]. Disponible sur: https://www.drogues.gouv.fr/sites/drogues.gouv.fr/files/atoms/files/brochure_elsa_nsp.pdf

37. Pochard L, Frauger E, Giocanti A, Micallef J. Consommation de nouveaux produits de synthèse en France : données issues de l'enquête OPPIDUM entre 2000 et 2016. Toxicol Anal Clin. 1 juin 2018;30(2, Supplement):S45.

38. Nouveaux produits de synthèse - Synthèse des connaissances - OFDT [Internet]. [cité 30 janv 2019]. Disponible sur: https://www.ofdt.fr/produits-et-addictions/de-z/nouveaux-produits-de-synthese/

39. OFDT (2021). Circulation d'herbe de cannabis adultérée avec des cannabinoïdes de synthèse [Internet]. [cité 3 juin 2021]. Disponible sur: https://www.ofdt.fr/BDD/sintes/ir_19102020_Cannabis.pdf

40. Choi NG, Marti CN, DiNitto DM, Baker SD. Cannabis and synthetic cannabinoid poison control center cases among adults aged 50+, 2009-2019. Clin Toxicol Phila Pa. avr 2021;59(4):334-42.

41. Deville M, Dubois N, Cieckiewicz E, De Tullio P, Lemaire E, Charlier C. Death following consumption of MDAI and 5-EAPB. Forensic Sci Int. juin 2019;299:89-94.

42. Center for Disease Control. Overdose Deaths Involving Synthetic Opioids [Internet]. 2018 [cité 26 juill 2019]. Disponible sur: https://www.cdc.gov/drugoverdose/data/fentanyl.html

43. Schneir A, Metushi IG, Sloane C, Benaron DJ, Fitzgerald RL. Near death from a novel synthetic opioid labeled U-47700: emergence of a new opioid class. Clin Toxicol. 2 janv 2017;55(1):51-4.

44. Helander A, Beck O, Bäckberg M. Intoxications by the dissociative new psychoactive substances diphenidine and methoxphenidine. Clin Toxicol Phila Pa. juin 2015;53(5):446-53.

45. Peacock A, Bruno R, Gisev N, Degenhardt L, Hall W, Sedefov R, et al. New psychoactive substances: challenges for drug surveillance, control, and public health responses. The Lancet. 2 nov 2019;394(10209):1668-84.

46. Synthetic Cannabinoid–Associated Coagulopathy. N Engl J Med. 3 janv 2019;380(1):100-2.

47. German CL, Fleckenstein AE, Hanson GR. Bath salts and synthetic cathinones: An emerging designer drug phenomenon. Life Sci. 27 févr 2014;97(1):2-8.

48. Prosser JM, Nelson LS. The Toxicology of Bath Salts: A Review of Synthetic Cathinones. J Med Toxicol. mars 2012;8(1):33-42.

49. Yonemitsu K, Sasao A, Mishima S, Ohtsu Y, Nishitani Y. A fatal poisoning case by intravenous injection of “bath salts” containing acetyl fentanyl and 4-methoxy PV8. Forensic Sci Int. oct 2016;267:e6-9.

50. Arens AM, Olives TD, Simpson NS, Laes JR, Anderson DL, Bangh SA, et al. An outbreak of synthetic cannabinoid exposures reported to a regional poison center: « K2 » identified as 5F-ADB. Clin Toxicol Phila Pa. janv 2019;57(1):69-71.

51. Gurney SMR, Scott KS, Kacinko SL, Presley BC, Logan BK. Pharmacology, Toxicology, and Adverse Effects of Synthetic Cannabinoid Drugs. Forensic Sci Rev. janv 2014;26(1):53-78.

52. Concheiro M, Chesser R, Pardi J, Cooper G. Postmortem Toxicology of New Synthetic Opioids. Front Pharmacol. 2018;9:1210.

53. Karila L, Marillier M, Chaumette B, Billieux J, Franchitto N, Benyamina A. New synthetic opioids: Part of a new addiction landscape. Neurosci Biobehav Rev. 12 sept 2018;

54. Salle S, Bodeau S, Dhersin A, Ferdonnet M, Goncalves R, Lenski M, et al. Novel synthetic opioids: A review of the literature. Toxicol Anal Clin. déc 2019;31(4):298-316.

55. Ameline A, Richeval C, Gaulier JM, Raul JS, Kintz P. Detection of the designer benzodiazepine flunitrazolam in urine and preliminary data on its metabolism. Drug Test Anal. févr 2019;11(2):223-9.

56. Mardal M, Andreasen MF, Mollerup CB, Stockham P, Telving R, Thomaidis NS, et al. HighResNPS.com: An Online Crowd-Sourced HR-MS Database for Suspect and Non-targeted Screening of New Psychoactive Substances. J Anal Toxicol. 23 août 2019;43(7):520-7.

57. Makarov A. Electrostatic Axially Harmonic Orbital Trapping: A High-Performance Technique of Mass Analysis. Anal Chem. 1 mars 2000;72(6):1156-62.

58. Balogh MP. Debating resolution and mass accuracy. LC-GC N Am. 1 févr

2004;22(2):118-26.

59. Canterbury JD, Merrihew GE, MacCoss MJ, Goodlett DR, Shaffer SA. Comparison of Data Acquisition Strategies on Quadrupole Ion Trap Instrumentation for Shotgun Proteomics. J Am Soc Mass Spectrom. 1 déc 2014;25(12):2048-59.

60. Rochat B. From targeted quantification to untargeted metabolomics: Why LC-high-resolution-MS will become a key instrument in clinical labs. TrAC Trends Anal Chem. 1 nov 2016;84:151-64.

61. Aydoğan C. Recent advances and applications in LC-HRMS for food and plant natural products: a critical review. Anal Bioanal Chem. 1 avr 2020;412(9):1973-91.

62. Andra SS, Austin C, Patel D, Dolios G, Awawda M, Arora M. Trends in the application of high-resolution mass spectrometry for human biomonitoring: An analytical primer to studying the environmental chemical space of the human exposome. Environ Int. 1 mars 2017;100:32-61.

63. Schymanski EL, Singer HP, Slobodnik J, Ipolyi IM, Oswald P, Krauss M, et al. Non-target screening with high-resolution mass spectrometry: critical review using a collaborative trial on water analysis. Anal Bioanal Chem. août 2015;407(21):6237-55.

64. Kaufmann A. The current role of high-resolution mass spectrometry in food analysis. Anal Bioanal Chem. mai 2012;403(5):1233-49.

65. Arrebola-Liébanas FJ, Romero-González R, Garrido Frenich A. Chapter 1 - HRMS: Fundamentals and Basic Concepts. In: Romero-González R, Frenich AG, éditeurs. Applications in High Resolution Mass Spectrometry [Internet]. Elsevier; 2017 [cité 24 juill 2022]. p. 1-14.

66. Kingdon KH. A Method for the Neutralization of Electron Space Charge by Positive Ionization at Very Low Gas Pressures. Phys Rev. 1 avr 1923;21(4):408-18.

67. Hecht ES, Scigelova M, Eliuk S, Makarov A. Fundamentals and Advances of Orbitrap Mass Spectrometry. In: Meyers RA, éditeur. Encyclopedia of Analytical Chemistry [Internet]. 1re éd. Wiley; 2019 [cité 24 juill 2022]. p. 1-40. Disponible sur: https://onlinelibrary.wiley.com/doi/10.1002/9780470027318.a9309.pub2

68. Carlier J, Diao X, Scheidweiler KB, Huestis MA. Distinguishing Intake of New Synthetic Cannabinoids ADB-PINACA and 5F-ADB-PINACA with Human Hepatocyte Metabolites and High-Resolution Mass Spectrometry. Clin Chem. 1 mai 2017;63(5):1008-21.

69. Helfer AG, Turcant A, Boels D, Ferec S, Lelièvre B, Welter J, et al. Elucidation of the metabolites of the novel psychoactive substance 4-methyl- *N* -ethyl-cathinone (4-MEC) in human urine and pooled liver microsomes by GC-MS and LC-HR-MS/MS techniques and of its detectability by GC-MS or LC-MS ^{n} standard screening approaches: Metabolism of 4-MEC. Drug Test Anal. mai 2015;7(5):368-75.

70. Wagmann L, Manier SK, Felske C, Gampfer TM, Richter MJ, Eckstein N, et al. Flubromazolam-Derived Designer Benzodiazepines: Toxicokinetics and Analytical Toxicology of Clobromazolam and Bromazolam. J Anal Toxicol. 9 nov 2021;45(9):1014-27.

71. Anizan S, Concheiro M, Lehner KR, Bukhari MO, Suzuki M, Rice KC, et al. Linear pharmacokinetics of 3,4-methylenedioxypyrovalerone (MDPV) and its metabolites in the rat: relationship to pharmacodynamic effects: MDPV PK and PD in Rats. Addict Biol. mars 2016;21(2):339-47.

72. Crews BO, Pesce AJ, West R, Nguyen H, Fitzgerald RL. Evaluation of High-Resolution Mass Spectrometry for Urine Toxicology Screening in a Pain Management Setting. J Anal Toxicol. 1 nov 2012;36(9):601-7.

73. Fabresse N, Larabi IA, Stratton T, Mistrik R, Pfau G, Lorin de la Grandmaison G, et al. Development of a sensitive untargeted liquid chromatography–high resolution mass spectrometry screening devoted to hair analysis through a shared MS2 spectra database: A step toward early detection of new psychoactive substances. Drug Test Anal. mai 2019;11(5):697-708.

74. Mueller DM, Rentsch KM. Online extraction toxicological MS(n) screening system for serum and heparinized plasma and comparison of screening results between plasma and urine in the context of clinical data. J Chromatogr B. 1 févr 2012;883-884:189-97.

75. Roche L, Pinguet J, Herviou P, Libert F, Chenaf C, Eschalier A, et al. Fully automated semi-quantitative toxicological screening in three biological matrices using turbulent flow chromatography/high resolution mass spectrometry. Clin Chim Acta. 1 avr 2016;455:46-54.

76. Bambauer TP, Wagmann L, Weber AA, Meyer MR. Analysis of α- and β-amanitin in Human Plasma at Subnanogram per Milliliter Levels by Reversed Phase Ultra-High Performance Liquid Chromatography Coupled to Orbitrap Mass Spectrometry. Toxins. nov 2020;12(11):671.

77. Concheiro M, Lee D, Lendoiro E, Huestis MA. Simultaneous quantification of Δ9-tetrahydrocannabinol, 11-nor-9-carboxy-tetrahydrocannabinol, cannabidiol and cannabinol in oral fluid by microflow-liquid chromatography–high resolution mass spectrometry. J Chromatogr A. 5 juill 2013;1297:123-30.

78. Jacobs CM, Wagmann L, Meyer MR. Development, validation, and application of a quantitative volumetric absorptive microsampling–based method in finger prick blood by means of LC-HRMS/MS applicable for adherence monitoring of antipsychotics. Anal Bioanal Chem. 1 mars 2021;413(6):1729-37.

79. Labat L, Goncalves A, Marques AR, Duretz B, Granger B, Declèves X. Liquid chromatography high resolution mass spectrometry for the determination of baclofen and its metabolites in plasma: Application to therapeutic drug monitoring. Biomed Chromatogr. 2017;31(8):e3936.

80. Meyer MR, Helfer AG, Maurer HH. Current position of high-resolution MS for drug quantification in clinical & forensic toxicology. Bioanalysis. août 2014;6(17):2275-84.

81. Pascual-Caro S, Fontanals N, Borrull F, Aguilar C, Calull M. Solid-phase extraction based on cation-exchange sorbents followed by liquid chromatography high-resolution mass spectrometry to determine synthetic cathinones in urine. Forensic Toxicol. 1 janv 2020;38(1):185-94.

82. Kaufmann A. Analytical performance of the various acquisition modes in Orbitrap MS and MS/MS. J Mass Spectrom. août 2018;53(8):725-38.

83. Zarrouk E, Lenski M, Bruno C, Thibert V, Contreras P, Privat K, et al. High-resolution mass spectrometry: Theoretical and technological aspects. Toxicol Anal Clin. janv 2022;34(1):3-18.

84. Grund B, Marvin L, Rochat B. Quantitative performance of a quadrupole-orbitrap-MS in targeted LC–MS determinations of small molecules. J Pharm Biomed Anal. 30 mai 2016;124:48-56.

85. De Vijlder T, Valkenborg D, Lemière F, Romijn EP, Laukens K, Cuyckens F. A tutorial in small molecule identification via electrospray ionization-mass spectrometry: The practical art of structural elucidation. Mass Spectrom Rev. sept 2018;37(5):607-29.

86. Rivier L. Criteria for the identification of compounds by liquid chromatography–mass spectrometry and liquid chromatography–multiple mass spectrometry in forensic toxicology and doping analysis. Anal Chim Acta. 15 sept 2003;492(1):69-82.

87. Maurer HH, Meyer MR, Helfer A, Weber AA. Maurer / Meyer / Helfer / Weber LC-HR-MS/MS Library of Drugs, Poisons, and Their Metabolites. :3.

88. Helfer AG, Michely JA, Weber AA, Meyer MR, Maurer HH. Orbitrap technology for comprehensive metabolite-based liquid chromatographic–high resolution-tandem mass spectrometric urine drug screening – Exemplified for cardiovascular drugs. Anal Chim Acta. 3 sept 2015;891:221-33.

89. Data-independent acquisition-based SWATH-MS for quantitative proteomics: a tutorial. Mol Syst Biol. août 2018;14(8):e8126.

90. Noble C, Weihe Dalsgaard P, Stybe Johansen S, Linnet K. Application of a screening method for fentanyl and its analogues using UHPLC-QTOF-MS with data-independent acquisition (DIA) in MS^{E} mode and retrospective analysis of authentic forensic blood samples. Drug Test Anal. avr 2018;10(4):651-62.

91. Leung DKK, Wong ASY, Zhou QL, Wan TSM, Ho ENM. Application of a non-target variable data independent workflow (vDIA) for the screening of prohibited substances in doping control testing. Drug Test Anal. mai 2021;13(5):1008-33.

92. Rajski Ł, Beraza I, Gómez Ramos MJ, Ferrer C, Fernández-Alba AR. Evaluation of segmented non-target data acquisition (SWATH/vDIA) in a QToF and QOrbitrap for pesticide residue analysis. Anal Methods. 2020;12(15):2027-38.

93. Elmiger MP, Poetzsch M, Steuer AE, Kraemer T. Assessment of simpler calibration models in the development and validation of a fast postmortem multi-analyte LC-QTOF quantitation method in whole blood with simultaneous screening capabilities using SWATH acquisition. Anal Bioanal Chem. nov 2017;409(27):6495-508.

94. Dresen S, Ferreirós N, Gnann H, Zimmermann R, Weinmann W. Detection and identification of 700 drugs by multi-target screening with a 3200 Q TRAP® LC-MS/MS

system and library searching. Anal Bioanal Chem. avr 2010;396(7):2425-34.

95. Maurer HH. What is the future of (ultra) high performance liquid chromatography coupled to low and high resolution mass spectrometry for toxicological drug screening? J Chromatogr A. 31 mai 2013;1292:19-24.

96. Arnhard K, Gottschall A, Pitterl F, Oberacher H. Applying 'Sequential Windowed Acquisition of All Theoretical Fragment Ion Mass Spectra' (SWATH) for systematic toxicological analysis with liquid chromatography-high-resolution tandem mass spectrometry. Anal Bioanal Chem. janv 2015;407(2):405-14.

97. Roemmelt AT, Steuer AE, Kraemer T. Liquid Chromatography, In Combination with a Quadrupole Time-of-Flight Instrument, with Sequential Window Acquisition of All Theoretical Fragment-Ion Spectra Acquisition: Validated Quantification of 39 Antidepressants in Whole Blood As Part of a Simultaneous Screening and Quantification Procedure. Anal Chem. 15 sept 2015;87(18):9294-301.

98. Scheibner O. Variable Data-Independent Acquisition (vDIA) Delivers High Selectivity and Sensitivity in Combined Targeted and Untargeted Analyses for Small Molecules. :5.

99. Rosano TG, Na S, Ihenetu K, Swift TA, Wood M. Multi-drug and Metabolite Quantification in Postmortem Blood by Liquid Chromatography–High-Resolution Mass Spectrometry: Comparison with Nominal Mass Technology. J Anal Toxicol. oct 2014;38(8):495-506.

100. Bidny S, Gago K, Chung P, Albertyn D, Pasin D. Simultaneous Screening and Quantification of Basic, Neutral and Acidic Drugs in Blood Using UPLC-QTOF-MS. J Anal Toxicol. avr 2017;41(3):181-95.

101. Whitman JD, Lynch KL. Optimization and Comparison of Information-Dependent Acquisition (IDA) to Sequential Window Acquisition of All Theoretical Fragment Ion Spectra (SWATH) for High-Resolution Mass Spectrometry in Clinical Toxicology. Clin Chem. 1 juill 2019;65(7):862-70.

102. Rauniyar N. Parallel Reaction Monitoring: A Targeted Experiment Performed Using High Resolution and High Mass Accuracy Mass Spectrometry. Int J Mol Sci. déc 2015;16(12):28566-81.

103. Danaceau JP, Wood M, Ehlers M, Rosano TG. Analysis of 17 fentanyls in plasma and blood by UPLC-MS/MS with interpretation of findings in surgical and postmortem casework. Clin Mass Spectrom. 1 nov 2020;18:38-47.

104. Gergov M, Boucher B, Ojanperä I, Vuori E. Toxicological screening of urine for drugs by liquid chromatography/time-of-flight mass spectrometry with automated target library search based on elemental formulas: LC/TOFMS screening for drugs in urine. Rapid Commun Mass Spectrom. 30 avr 2001;15(8):521-6.

105. Ojanperä S, Pelander A, Pelzing M, Krebs I, Vuori E, Ojanperä I. Isotopic pattern and accurate mass determination in urine drug screening by liquid chromatography/time-of-flight mass spectrometry. Rapid Commun Mass Spectrom. 15 avr 2006;20(7):1161-7.

106. Sumner LW, Amberg A, Barrett D, Beale MH, Beger R, Daykin CA, et al. Proposed minimum reporting standards for chemical analysis: Chemical Analysis Working Group (CAWG) Metabolomics Standards Initiative (MSI). Metabolomics. 19 sept 2007;3(3):211-21.

107. Wang Y, Xiao J, Suzek TO, Zhang J, Wang J, Bryant SH. PubChem: a public information system for analyzing bioactivities of small molecules. Nucleic Acids Res. 1 juill 2009;37(Web Server):W623-33.

108. Williams AJ. A perspective of publicly accessible/open-access chemistry databases. Drug Discov Today. 1 juin 2008;13(11):495-501.

109. Horai H, Arita M, Kanaya S, Nihei Y, Ikeda T, Suwa K, et al. MassBank: a public repository for sharing mass spectral data for life sciences. J Mass Spectrom. 7 juill 2010;45(7):703-14.

110. Ivanisevic J, Want EJ. From Samples to Insights into Metabolism: Uncovering Biologically Relevant Information in LC-HRMS Metabolomics Data. Metabolites. déc 2019;9(12):308.

111. Laks S, Pelander A, Vuori E, Ali-Tolppa E, Sippola E, Ojanperä I. Analysis of Street Drugs in Seized Material without Primary Reference Standards. Anal Chem. 1 déc 2004;76(24):7375-9.

112. Ellefsen KN, Wohlfarth A, Swortwood MJ, Diao X, Concheiro M, Huestis MA. 4-Methoxy-α-PVP: in silico prediction, metabolic stability, and metabolite identification by human hepatocyte incubation and high-resolution mass spectrometry. Forensic Toxicol. janv 2016;34(1):61-75.

113. Witting M, Böcker S. Current status of retention time prediction in metabolite identification. J Sep Sci. mai 2020;43(9-10):1746-54.

114. Ross DH, Xu L. Determination of drugs and drug metabolites by ion mobility-mass spectrometry: A review. Anal Chim Acta. 15 avr 2021;1154:338270.

115. Elyashberg M. Identification and structure elucidation by NMR spectroscopy. TrAC Trends Anal Chem. juin 2015;69:88-97.

116. Blaženović I, Kind T, Ji J, Fiehn O. Software Tools and Approaches for Compound Identification of LC-MS/MS Data in Metabolomics. Metabolites. juin 2018;8(2):31.

117. Wang M, Carver JJ, Phelan VV, Sanchez LM, Garg N, Peng Y, et al. Sharing and community curation of mass spectrometry data with Global Natural Products Social Molecular Networking. Nat Biotechnol. août 2016;34(8):828-37.

118. Quinn RA, Nothias LF, Vining O, Meehan M, Esquenazi E, Dorrestein PC. Molecular Networking As a Drug Discovery, Drug Metabolism, and Precision Medicine Strategy. Trends Pharmacol Sci. 1 févr 2017;38(2):143-54.

119. Yang JY, Sanchez LM, Rath CM, Liu X, Boudreau PD, Bruns N, et al. Molecular Networking as a Dereplication Strategy. J Nat Prod. 27 sept 2013;76(9):1686-99.

120. Knolhoff AM, Callahan JH, Croley TR. Mass Accuracy and Isotopic Abundance Measurements for HR-MS Instrumentation: Capabilities for Non-Targeted Analyses. J Am Soc Mass Spectrom. 1 juill 2014;25(7):1285-94.

121. Mohamed MK, Takyi-Williams J, Baudot B, Grobler A. Development and validation of a LC-HRMS method for the quantification of cannabinoids and their metabolites in human plasma. Eur J Pharm Sci. 1 avr 2021;159:105705.

122. Kronstrand R, Brinkhagen L, Birath-Karlsson C, Roman M, Josefsson M. LC-QTOF-MS as a superior strategy to immunoassay for the comprehensive analysis of synthetic cannabinoids in urine. Anal Bioanal Chem. juin 2014;406(15):3599-609.

123. Gundersen POM, Broecker S, Slørdal L, Spigset O, Josefsson M. Retrospective screening of synthetic cannabinoids, synthetic opioids and designer benzodiazepines in data files from forensic post mortem samples analysed by UHPLC-QTOF-MS from 2014 to 2018. Forensic Sci Int. juin 2020;311:110274.

124. Favretto D, Pascali JP, Tagliaro F. New challenges and innovation in forensic toxicology: Focus on the "New Psychoactive Substances". J Chromatogr A. 26 avr 2013;1287:84-95.

125. Pelletier R, Le Daré B, Grandin L, Couette A, Ferron PJ, Morel I, et al. New psychoactive substance cocktail in an intensive care intoxication case elucidated by molecular networking. Clin Toxicol. 4 juin 2021;0(0):1-4.

126. MILDECA. plan_mildeca_2018-2022 [Internet]. [cité 25 juill 2019]. Disponible sur: https://www.drogues.gouv.fr/sites/drogues.gouv.fr/files/atoms/files/plan_mildeca_2018-2022_def_190212_web.pdf

127. Wilkinson GR. Drug Metabolism and Variability among Patients in Drug Response. N Engl J Med. 26 mai 2005;352(21):2211-21.

128. Zhang Z, Tang W. Drug metabolism in drug discovery and development. Acta Pharm Sin B. sept 2018;8(5):721-32.

129. Almazroo OA, Miah MK, Venkataramanan R. Drug Metabolism in the Liver. Clin Liver Dis. févr 2017;21(1):1-20.

130. Richeval C, Gicquel T, Hugbart C, Le Dare B, Allorge D, Morel I, et al. In vitro Characterization of NPS Metabolites Produced by Human Liver Microsomes and the HepaRG Cell Line Using Liquid Chromatographyhigh Resolution Mass Spectrometry (LC-HRMS) Analysis: Application to Furanyl Fentanyl. Curr Pharm Biotechnol. 2017;18(10):806-14.

131. Jia L, Liu X. The conduct of drug metabolism studies considered good practice (II): in vitro experiments. Curr Drug Metab. déc 2007;8(8):822-9.

132. Zuber R, Anzenbacherová E, Anzenbacher P. Cytochromes P450 and experimental models of drug metabolism. J Cell Mol Med. avr 2002;6(2):189-98.

133. Harper L, Powell J, Pijl EM. An overview of forensic drug testing methods and their

suitability for harm reduction point-of-care services. Harm Reduct J. 31 2017;14(1):52.

134. Green TC, Park JN, Gilbert M, McKenzie M, Struth E, Lucas R, et al. An assessment of the limits of detection, sensitivity and specificity of three devices for public health-based drug checking of fentanyl in street-acquired samples. Int J Drug Policy. 1 mars 2020;77:102661.

135. Grobério TS, Zacca JJ, Botelho ÉD, Talhavini M, Braga JWB. Discrimination and quantification of cocaine and adulterants in seized drug samples by infrared spectroscopy and PLSR. Forensic Sci Int. déc 2015;257:297-306.

136. Ti L, Tobias S, Lysyshyn M, Laing R, Nosova E, Choi J, et al. Detecting fentanyl using point-of-care drug checking technologies: A validation study. Drug Alcohol Depend. 1 juill 2020;212:108006.

137. Botanas CJ, de la Peña JB, dela Peña IJ, Tampus R, Yoon R, Kim HJ, et al. Methoxetamine, a ketamine derivative, produced conditioned place preference and was self-administered by rats: Evidence of its abuse potential. Pharmacol Biochem Behav. 1 juin 2015;133:31-6.

138. EMCDDA. EU early warning system situation report. Situation report 1–June 2020 [Internet]. 2020 [cité 16 avr 2021]. Disponible sur: https://www.aekwien.at/documents/263869/449604/200629_EU+EARLY+WARNING+SYSTEM+SITUATION+REPORT+June.pdf

139. Irie T, Yamazaki D, Kikura-Hanajiri R. A potential of methoxpropamine to be a widespread recreational drug: it blocks NMDA receptors and inhibits NMDA receptor-mediated synaptic transmission in a brain preparation of mice. Forensic Toxicol. 18 mars 2021 [

140. Roth BL, Gibbons S, Arunotayanun W, Huang XP, Setola V, Treble R, et al. The ketamine analogue methoxetamine and 3- and 4-methoxy analogues of phencyclidine are high affinity and selective ligands for the glutamate NMDA receptor. PloS One. 2013;8(3):e59334.

141. Wood DM, Davies S, Puchnarewicz M, Johnston A, Dargan PI. Acute toxicity associated with the recreational use of the ketamine derivative methoxetamine. Eur J Clin Pharmacol. 1 mai 2012;68(5):853-6.

142. Morris H, Wallach J. From PCP to MXE: a comprehensive review of the non-medical use of dissociative drugs. Drug Test Anal. 2014;6(7-8):614-32.

143. Adamowicz P, Zuba D. Fatal Intoxication with Methoxetamine. J Forensic Sci. 2015;60(s1):S264-8.

144. Ameline A, Garnier D, Gheddar L, Richeval C, Gaulier J michel, Raul J sébastien, et al. Identification and analytical characterization of seven NPS, by combination of 1H NMR spectroscopy, GC–MS and UPLC–MS/MS®, to resolve a complex toxicological fatal case. Forensic Sci Int. 1 mai 2019;298:140-8.

145. Ameline A, Garnier D, Raul JS, Kintz P. Use of nuclear magnetic resonance (NMR) spectroscopy to identify unknown powders. Toxicol Anal Clin. 1 déc 2019;31(4):197-250.

146. Akoka S, Barantin L, Trierweiler M. Concentration Measurement by Proton NMR Using the ERETIC Method. Anal Chem. 1 juill 1999;71(13):2554-7.

147. Guillou C, Reniero F, Vicente JL, Holland M, Kolar K, Chassaigne H, et al. Collaboration of the Joint Research Centre and European Customs Laboratories for the Identification of New Psychoactive Substances. Curr Pharm Biotechnol. 1 févr 2018;19(2):91-8.

148. Giné CV, Espinosa IF, Vilamala MV. New psychoactive substances as adulterants of controlled drugs. A worrying phenomenon? Drug Test Anal. 2014;6(7-8):819-24.

149. Menzies EL, Hudson SC, Dargan PI, Parkin MC, Wood DM, Kicman AT. Characterizing metabolites and potential metabolic pathways for the novel psychoactive substance methoxetamine. Drug Test Anal. juin 2014;6(6):506-15.

150. Chen CY, Lee MR, Cheng FC, Wu GJ. Determination of ketamine and metabolites in urine by liquid chromatography-mass spectrometry. Talanta. 15 mai 2007;72(3):1217-22.

151. Hijazi Y, Boulieu R. Contribution of CYP3A4, CYP2B6, and CYP2C9 isoforms to N-demethylation of ketamine in human liver microsomes. Drug Metab Dispos Biol Fate Chem. juill 2002;30(7):853-8.

152. Horsley RR, Lhotkova E, Hajkova K, Jurasek B, Kuchar M, Palenicek T. Detailed pharmacological evaluation of methoxetamine (MXE), a novel psychoactive ketamine analogue-Behavioural, pharmacokinetic and metabolic studies in the Wistar rat. Brain Res Bull. sept 2016;126(Pt 1):102-10.

153. Daveluy A, Haramburu F. Consommation de substances psychoactives à la Réunion et à Mayotte, départements français de l'Océan Indien. Therapies. oct 2018;73(5):419-27.

154. Fleury A, Daculsi PH. Profil médico-social des patients ayant consulté au Centre d'addictologie de Mayotte en 2015 pour usage de nouveaux produits de synthèse, une étude rétrospective. Therapies. févr 2017;72(1):151-2.

155. Vilain P BAS, Youssouf A. Recrudescence de passages liés à la consommation de cannabinoïdes de synthèse « la chimique » dans le service d'urgences du centre hospitalier de Mayotte, 2012—2016. Bulletin de veille sanitaire océan Indien 2017. :14.

156. Roussel O, Carlin MG, Bouvot X, Tensorer L. The emergence of synthetic cannabinoids in Mayotte. Toxicol Anal Clin. mars 2015;27(1):18-22.

157. Jamey C, Szwarc E, Tracqui A, Ludes B. Determination of Cannabinoids in Whole Blood by UPLC-MS-MS. J Anal Toxicol. 1 juin 2008;32(5):349-54.

158. Le Boisselier R, Alexandre J, Lelong-Boulouard V, Debruyne D. Focus on cannabinoids and synthetic cannabinoids. Clin Pharmacol Ther. févr 2017;101(2):220-9.

159. EMCDDA (2021). European drug report: trends and developments. Luxembourg: Publications office of the European Union; 2021.

160. Mills B, Yepes A, Nugent K. Synthetic Cannabinoids. Am J Med Sci. 1 juill

2015;350(1):59-62.

161. Banister SD, Longworth M, Kevin R, Sachdev S, Santiago M, Stuart J, et al. Pharmacology of Valinate and tert-Leucinate Synthetic Cannabinoids 5F-AMBICA, 5F-AMB, 5F-ADB, AMB-FUBINACA, MDMB-FUBINACA, MDMB-CHMICA, and Their Analogues. ACS Chem Neurosci. 21 sept 2016;7(9):1241-54.

162. Hess C, Schoeder CT, Pillaiyar T, Madea B, Müller CE. Pharmacological evaluation of synthetic cannabinoids identified as constituents of spice. Forensic Toxicol. juill 2016;34(2):329-43.

163. Ahmed T, Khan A, See VY, Robinson S. Cardiac arrest associated with synthetic cannabinoid use and acquired prolonged QTc interval: A case report and review of literature. Hear Case Rep. mai 2020;6(5):283-6.

164. Castaneto MS, Gorelick DA, Desrosiers NA, Hartman RL, Pirard S, Huestis MA. Synthetic cannabinoids: Epidemiology, pharmacodynamics, and clinical implications. Drug Alcohol Depend. 1 nov 2014;144:12-41.

165. P. Smith J, B. Sutcliffe O, E. Banks C. An overview of recent developments in the analytical detection of new psychoactive substances (NPSs). Analyst. 2015;140(15):4932-48.

166. Pasin D, Cawley A, Bidny S, Fu S. Current applications of high-resolution mass spectrometry for the analysis of new psychoactive substances: a critical review. Anal Bioanal Chem. oct 2017;409(25):5821-36.

167. Cannaert A, Storme J, Franz F, Auwärter V, Stove CP. Detection and Activity Profiling of Synthetic Cannabinoids and Their Metabolites with a Newly Developed Bioassay. Anal Chem. 6 déc 2016;88(23):11476-85.

168. Infodrog. Alertes actuelles - Centrale nationale de coordination des addictions [Internet]. [cité 3 juin 2021]. Disponible sur: https://www.infodrog.ch/fr/aide/alertes-actuelles.html

169. CEIP-A (2020). Information - alerte relative aux cannabinoîdes de synthèse et risques [Internet]. [cité 3 juin 2021]. Disponible sur: http://www.dicadd13.fr/images/boiteaoutils/alerteCEIPcannabis.pdf

170. Morais JD, Brandt S, Jorge R, Christie R, Sedefov R, Evans-Brown M. EMCDDA technical report on the new psychoactive substance methyl 3,3-dimethyl-2-{[1-(pent-4-en-1-yl)-1H-indazole-3- carbonyl]amino}butanoate (MDMB-4en-PINACA). :53.

171. EMCDDA initial report on the new psychoactive substance MDMB-4en-PINACA | www.emcdda.europa.eu [Internet]. [cité 3 juin 2021]. Disponible sur: https://www.emcdda.europa.eu/publications/initial-reports/mdmb-4en-pinaca_sl

172. Krotulski AJ, Mohr ALA, Logan BK. Emerging Synthetic Cannabinoids: Development and Validation of a Novel Liquid Chromatography Quadrupole Time-of-Flight Mass Spectrometry Assay for Real-Time Detection. J Anal Toxicol. 30 avr 2020;44(3):207-17.

173. Potts AJ, Cano C, Thomas SHL, Hill SL. Synthetic cannabinoid receptor agonists: classification and nomenclature. Clin Toxicol Phila Pa. févr 2020;58(2):82-98.

174. Logan BK, Mohr ALA, Friscia M, Krotulski AJ, Papsun DM, Kacinko SL, et al. Reports of Adverse Events Associated with Use of Novel Psychoactive Substances, 2013-2016: A Review. J Anal Toxicol. 1 sept 2017;41(7):573-610.

175. Rianprakaisang T, Gerona R, Hendrickson RG. Commercial cannabidiol oil contaminated with the synthetic cannabinoid AB-FUBINACA given to a pediatric patient. Clin Toxicol. 3 mars 2020;58(3):215-6.

176. Horth RZ, Crouch B, Horowitz BZ, Prebish A, Slawson M, McNair J, et al. *Notes from the Field:* Acute Poisonings from a Synthetic Cannabinoid Sold as Cannabidiol — Utah, 2017–2018. MMWR Morb Mortal Wkly Rep. 25 mai 2018;67(20):587-8.

177. Day N, Criss J, Griffiths B, Gujral SK, John-Leader F, Johnston J, et al. Music festival attendees' illicit drug use, knowledge and practices regarding drug content and purity: a cross-sectional survey. Harm Reduct J. 5 janv 2018;15(1):1.

178. Evrard I, Legleye S, Cadet-Taïrou A. Composition, purity and perceived quality of street cocaine in France. Int J Drug Policy. 1 sept 2010;21(5):399-406.

179. Vidal Giné C, Ventura Vilamala M, Fornís Espinosa I, Gil Lladanosa C, Calzada Álvarez N, Fitó Fruitós A, et al. Crystals and tablets in the Spanish ecstasy market 2000–2014: Are they the same or different in terms of purity and adulteration? Forensic Sci Int. 1 juin 2016;263:164-8.

180. Dolan SB, Johnson MW. The drug purity discounting task: Ecstasy use likelihood is reduced by probabilistic impurity according to harmfulness of adulterants. Drug Alcohol Depend. 1 mars 2020;208:107772.

181. Watanabe S, Vikingsson S, Åstrand A, Gréen H, Kronstrand R. Biotransformation of the New Synthetic Cannabinoid with an Alkene, MDMB-4en-PINACA, by Human Hepatocytes, Human Liver Microsomes, and Human Urine and Blood. AAPS J. 17 déc 2019;22(1):13.

182. Diao X, Huestis MA. New Synthetic Cannabinoids Metabolism and Strategies to Best Identify Optimal Marker Metabolites. Front Chem [Internet]. 2019 [cité 14 juin 2019];7.

183. Grafinger KE, Stahl K, Wilke A, König S, Weinmann W. *In vitro* phase I metabolism of three phenethylamines 25D-NBOMe, 25E-NBOMe and 25N-NBOMe using microsomal and microbial models. Drug Test Anal. oct 2018;10(10):1607-26.

184. Halberstadt AL. Pharmacology and Toxicology of N-Benzylphenethylamine ("NBOMe") Hallucinogens. In: Baumann MH, Glennon RA, Wiley JL, éditeurs. Neuropharmacology of New Psychoactive Substances (NPS) [Internet]. Cham: Springer International Publishing; 2017 [cité 3 août 2022]. p. 283-311. (Current Topics in Behavioral Neurosciences; vol. 32). Disponible sur: https://link.springer.com/10.1007/7854_2016_64

185. Strano Rossi S, Odoardi S, Gregori A, Peluso G, Ripani L, Ortar G, et al. An analytical approach to the forensic identification of different classes of new psychoactive

substances (NPSs) in seized materials: Analytical approach for the identification of NPSs in seizures. Rapid Commun Mass Spectrom. 15 sept 2014;28(17):1904-16.

186. von Cüpper M, Dalsgaard PW, Linnet K. Identification of New Psychoactive Substances in Seized material Using UHPLC–QTOF-MS and An Online Mass Spectral Database. J Anal Toxicol. 1 nov 2020;44(9):1047-51.

187. Cheng WC, Wong WC. Forensic drug analysis of chloro-N,N-dimethylcathinone (CDC) and chloroethcathinone (CEC): Identification of 4-CDC and 4-CEC in drug seizures and differentiation from their ring-substituted positional isomers. Forensic Sci Int. 1 mai 2019;298:268-77.

188. Kolderová N, Jurásek B, Kuchař M, Lindner W, Kohout M. Gradient supercritical fluid chromatography coupled to mass spectrometry with a gradient flow of make-up solvent for enantioseparation of cathinones. J Chromatogr A. 16 août 2020;1625:461286.

189. Evans-Brown M, Gallegos A, Francis W, Christie R, Cunningham A, Sekula J, et al. New psychoactive substances in Europe: an update from the EU Early Warning System, March 2015. [Internet]. Luxembourg: Publications Office; 2015 [cité 28 juill 2019]. Disponible sur: http://dx.publications.europa.eu/10.2810/372415

190. Guitton J, Gaulier JM, Citterio-Quentin A, Abe E, Allibé N, Bartoli M, et al. Accreditation of the toxicological screening: recommendations of the SFBC-SFTA group. Ann Biol Clin (Paris). avr 2019;77(2):219-24.

191. Oberacher H, Pavlic M, Libiseller K, Schubert B, Sulyok M, Schuhmacher R, et al. On the inter-instrument and inter-laboratory transferability of a tandem mass spectral reference library: 1. Results of an Austrian multicenter study. J Mass Spectrom JMS. avr 2009;44(4):485-93.

192. Urbas A, Schoenberger T, Corbett C, Lippa K, Rudolphi F, Robien W. NPS Data Hub: A web-based community driven analytical data repository for new psychoactive substances. Forensic Chem. 1 juin 2018;9:76-81.

193. RESPONSE [Internet]. [cité 31 août 2021]. Disponible sur: https://www.policija.si/apps/nfl_response_web/seznam.php

194. mzCloud DataViewer [Internet]. [cité 31 août 2021]. Disponible sur: https://www.mzcloud.org/DataViewer#/Main/reference$4878/T8392%23Standard/Recalibrated/1461299

195. Diamanti K, Aalizadeh R, Alygizakis N, Galani A, Mardal M, Thomaidis NS. Wide-scope target and suspect screening methodologies to investigate the occurrence of new psychoactive substances in influent wastewater from Athens. Sci Total Environ. 1 oct 2019;685:1058-65.

ANNEXES

I. Annexe 1 - Autres travaux, publications et communications sur le thème de la toxicologie clinique, analytique et médico-légale

Publications

1. Salle S, Bodeau S, Dhersin A, Ferdonnet M, **Goncalves R**, Lenski M, Lima B, Martin M, Outreville J, Vaucel J, Fabresse N. « Novel synthetic opioids : A review of the literature ». *Toxicol Anal Clin.,* décembre 2019 ; 31(4):298-316.

2. Tjoncke J-A, **Goncalves R**, Castaing N, Molimard M, Tovagliaro F, Titier K. « Death related to nicotine replacement therapy: A case report ». *Forensic Sci Int.*, avril 2020 ; 309:110223.

3. Hertzog T, Cetran L, Labadie M, Braganca C, **Goncalves R**. « Delayed cardiotoxicity in venlafaxine and quetiapine overdose ». *Clin Toxicol.,* 19 avril 2021 ; 59:12, 1279-1280

4. **Goncalves R**, Castaing N, Titier K, Dumestre-Toulet V. « Hair Analysis of Methoxphenidine in a Forensic Chemsex Case ». *J Anal Toxicol.,* 1er février 2021 ; bkab016.

5. Beurton A, **Goncalves R**, Imbault J, Repusseau B, Ouattara A. « Refractory cardiogenic shock caused by methadone poisoning and treated with hybrid ECLS ». *Therapie,* mars 2022 ; S0040595722000439

6. Barbier O, Galdari A, Milpied B, Sanchez P, Kassab S, **Goncalves R**, Darrigade A-S. « Ecstasy-Induced Fixed Drug Eruption », *Contact Dermatitis*, sept 2022;87(3):280-1.

7. Daoud FC, **Goncalves R**, Moore N. « How Long Do Implanted Triclosan Sutures Inhibit Staphylococcus aureus in Surgical Conditions? A Pharmacological Model ». *Pharmaceutics*, 28 février 2022 ; 14(3):539.

Expertise pour un groupe de travail

1. **Goncalves R**, Peyré A, Daveluy A. « Expertise collective sur l'usage de cannabinoïdes de synthèse à La Réunion ». Réseau Régional d'Addictologie SAOME (Santé Addictions Outre-Mer), soumis et accepté en 2022

Communications orales dans des congrès nationaux

1. **Goncalves R**, Tjoncke J-A, Blanc-Brisset I, Daveluy A, Castaing N, Molimard M, Titier K, Tovagliaro F. « Death related to transdermal nicotine patches: a case report ». Congrès annuel de la Société Française de Toxicologie Analytique (SFTA), Lille (France), 22-24 mai 2019.

2. O. Barbier, A Galdari, B. Milpied, P. Sanchez, S. Kassab, **R. Goncalves**, A-S. Darrigade. « Érythème pigmenté fixe à l'ecstasy ». Congrès annuel du Groupe d'Études et de Recherche en Dermato-Allergologie (GERDA), Nantes (France), 23-24 septembre 2021.

Communications affichées dans des congrès nationaux

1. **R. Goncalves**, N. Castaing, K. Titier, V. Dumestre-Toulet. « Identification et quantification de la methoxphenidine dans les cheveux : à propos d'un cas de décès dans un contexte de chemsex ». Congrès annuel de la Société Française de Toxicologie Analytique (SFTA), Biarritz (France), 16-17 novembre 2020.

2. **R. Goncalves**, A. Beurton, J. Imbault, B. Repusseau, A. Ouattara. « Choc cardiogénique réfractaire et intoxication à la méthadone : à propos d'un cas. » 11es ateliers de la pharmacodépendance et de l'addictovigilance, Biarritz (France), 16-17 novembre 2020.

3. A. Peyré, N. Castaing, **R. Goncalves**, E. Mosnier, N. Garceran, F. Armanville, R. Perusseau-Lambert, C. Mathurin, G. Miremont-Salamé, A. Daveluy. Sukru, « Une nouvelle drogue en Guyane ? », 11es ateliers de la pharmacodépendance et de l'addictovigilance, Biarritz (France), 16-17 novembre 2020.

4. O. Barbier, A Galdari, B. Milpied, P. Sanchez, S. Kassab, **R. Goncalves**, A-S. Darrigade. « Érythème pigmenté fixe à l'ecstasy ». Journées dermatologiques de Paris (JDP), Paris (France), 30 novembre - 4 décembre 2021.

5. **Goncalves R**, Giraud F, Cordier L, Ely O, Labadie M. « Regional ileitis as an unspecific symptom of *Boletus sp* poisoning ». 38th congress EAPCCT Congress, Bucarest (Roumanie), 22-25 mai 2018.

6. **Goncalves R**, Dondia D, Es Salhi M, Labadie M, Courtois A, Capaldo L. « A misuse of snakebite antidote may be life-threatening for patients: a French case of European Vipera species envenomation treated with ViperaTAb® ». 38th congress EAPCCT Congress, Bucarest (Roumanie), 22-25 mai 2018.

7. **Goncalves R**, Tjoncke J-A, Blanc-Brisset I, Daveluy A, Castaing N, Molimard M, Titier K, Tovagliaro F. « Death related to transdermal nicotine patches: a case report ». Congrès annuel de la Société Française de Toxicologie Analytique (SFTA), Lille (France), 22-24 mai 2019.

Revue de la littérature n° 2

Novel synthetic opioids: A review of the literature

Sophie Salle, Sandra Bodeau, Alice Dhersin, Mathilde Ferdonnet, Ruben Goncalves, Marie Lenski, Benjamin Lima, Marie Martin, Jonathan Outreville, Jules Vaucel, Nicolas Fabresse

Publié en 2019 dans *Toxicologie Analytique et Clinique* (ToxAc)

Volume 31, Issue 4, Pages 298-316, ISSN 2352-0078

https://doi.org/10.1016/j.toxac.2019.10.001

Toxicologie Analytique & Clinique (2019) 31, 298—316

Disponible en ligne sur
ScienceDirect
www.sciencedirect.com

Elsevier Masson France
EM|consulte
www.em-consulte.com

REVIEW PAPER

Novel synthetic opioids: A review of the literature

Sophie Salle[a], Sandra Bodeau[b], Alice Dhersin[c], Mathilde Ferdonnet[d], Ruben Goncalves[e], Marie Lenski[f], Benjamin Lima[b], Marie Martin[g], Jonathan Outreville[h], Jules Vaucel[i], Nicolas Fabresse[g,*]

[a] *Pôle médico-légal, institut de recherche criminelle de la Gendarmerie nationale, 95000 Pontoise, France*
[b] *Laboratoire de pharmacologie et de toxicologie, centre hospitalo-universitaire d'Amiens, 80000 Amiens, France*
[c] *Laboratoire LTB, 11100 Narbonne, France*
[d] *Laboratoire LABEX, 60230 Chambly, France*
[e] *Laboratoire de pharmacologie et toxicologie médicale, centre hospitalo-universitaire de Bordeaux, 33000 Bordeaux, France*
[f] *EA 4483—IMPECS—IMPact de l'Environnement Chimique sur la Santé humaine, Institut Pasteur de Lille, université de Lille, centre hospitalo-universitaire de Lille, 59000 Lille, France*
[g] *Laboratoire de pharmacologie et de toxicologie, centre hospitalo-universitaire Raymond-Poincaré, AP—HP, 92380 Garches, France*
[h] *Laboratoire de biochimie-toxicologie, centre hospitalier du Taaone, Papeete, BP 1640, Tahiti, Polynésie française*
[i] *Centre antipoison Nouvelle Aquitaine, centre hospitalo-universitaire de Bordeaux, 33000 Bordeaux, France*

Received 1st August 2019; received in revised form 10 October 2019; accepted 10 October 2019
Available online 18 November 2019

KEYWORDS
Novel synthetic opioids;
New psychoactive substances;
Forensic toxicology;
Opioid overdose;

Summary Novel synthetic opioids (NSO) are an emerging class belonging to new psychoactive substances (NPS). They include fentanyl (e.g., acetylfentanyl, ocfentanil, carfentanil, furanylfentanyl, fluorofentanyl) and non-fentanyl analogs (e.g., U-47000, AH-7921, MT-45). NSO include potent μ-opioid receptor agonists with low dose required to produce the desired effects leading to a high risk of fatal overdose. The use of these molecules is spreading rapidly among opioid users, and is the main cause of opioid overdose in the United States. In recent years, we have seen an increase in cases of NSO poisoning in Europe, including fatal intoxications. The

* Corresponding author.
E-mail address: nicolas.fabresse@aphp.fr (N. Fabresse).

https://doi.org/10.1016/j.toxac.2019.10.001
2352-0078/© 2019 Société Française de Toxicologie Analytique. Published by Elsevier Masson SAS. All rights reserved.

© 2019 Elsevier Masson SAS.All rights reserved. - Document downloaded on 11/12/2019 by VAUCEL JULES (963264). It is forbidden and illegal to distribute this document.

Addiction;
Analysis

use of NSO among opioid users is dangerous and represents a major public health issue. These molecules have pharmacological properties different from those of conventional opiates. In case of overdose, respiratory depression appears quickly and could be life-threatening. As for conventional opioids, the management of intoxicated patients is based on naloxone. However, the doses and duration of the treatment vary according to the pharmacodynamic and pharmacokinetic characteristics of the molecules. NSO addiction management is based on the same molecules as conventional opioids: methadone and buprenorphine. Detection and quantification in biological matrices rely on liquid chromatography/tandem mass spectrometry and gas chromatography/tandem mass spectrometry, given the pharmacological potency of many NSO, the quantities administered are small and the concentrations found in biological matrices are weak, requiring the development of sensitive methods. Given the rapid diversification of this group of NPS, it is essential to continuously increment the libraries. Novel approaches were recently developed and are promising to detect unknown compounds such as activity based bioassays.

© 2019 Société Française de Toxicologie Analytique. Published by Elsevier Masson SAS. All rights reserved.

Introduction

Synthetic opioids (SO) refer to a class of molecules designed to relieve pain, mimicking natural opiates extracted from poppy, mainly represented by morphine and codeine. They include structurally related compounds (oxycodone, buprenorphine) and non-related molecules such as methadone, tramadol or fentanyl. These pharmaceuticals present several pharmacokinetic differences regarding the route of administration (buprenorphine/sublingual, tramadol/oral, fentanyl/transdermal), effect duration (tramadol 6 h, fentanyl 72 h) and metabolism (tramadol is metabolized by CYP2D6 through O-dealkylation while buprenorphine and fentanyl are metabolized through CYP3A4 through N-dealkylation). They also display pharmacological specificities regarding μ opioid receptor affinity, potency and activity: buprenorphine is a partial agonist μ, while methadone, fentanyl or tramadol are full agonists. These characteristics led to the positioning of these molecules in pain and addiction management, and explain the risks of poisoning encountered with these analogs, mainly due to central nervous system and respiratory depressant effects [1].

Recently, potent synthetic analogs of opioids have emerged in the illicit drug market leading to the so called novel synthetic opioids (NSO). NSO have their own characteristics regarding pharmacodynamics and pharmacokinetics, with several very potent opioid analogs. The exposure to these compounds by mainstream population present a serious public health concern. NSO are consumed voluntarily as opioid substitutes or unwittingly when NSO are used as heroin adulterants.

In the United-States (US) and Canada, the current opioid epidemic is being driven by the use of NSO. In the US, opioids were involved in 33,091 deaths in 2015 and 70,200 in 2017 representing 68% of all overdoses drug deaths [2]. According to the Center for Disease Control, more than 28,000 deaths involving synthetic opioids (other than methadone) occurred in the United States in 2017, which is more deaths than from any other type of opioid [3]. In the European Union, there are 1.3 million high risks opioid users, among them 654,000 received a substitution treatment in 2017, and opioids were found in 85% of fatal overdoses [4].

Fifty NSO have been reported by the EU early warning on new psychoactive substances (NPS). According to the United Nations Office on Drugs and Crime (UNODC), the term NPS refers to ''substances of abuse, either in a pure form or a preparation, that are not controlled by the 1961 Single Convention on Narcotic Drugs or the 1971 Convention on Psychotropic Substances, but which may pose a public health threat''. NPS are not necessarily recently synthesized, but their marketing on the illicit drug market is recent (e.g. smart shops, internet, ''darknet''). They are labeled as ''legal highs'', ''bath salts'' and ''research chemicals'' [5]. Cost, availability, lack of detection in laboratory tests and the absence of legislation are the main reasons for the widespread use of these compounds [6].

Compared to morphine, NSO have a higher pharmacological potency, and requires lower doses for a similar effect. Consequently, biological levels in blood or urine are low and can only be detected with specific methods. Their quick metabolism is also an issue for parent drug detection in laboratories. It is essential to search the drug and its metabolites to lengthen the detection window.

The objective of this review is to summarize the current knowledge regarding epidemiology, pharmacology, clinical management and analytical aspects of NSO.

Presentation of synthetic opioids

Classification of opioids

Opioids are substances interacting with opioid receptors (agonist or antagonist) found in the body to produce

© 2019 Elsevier Masson SAS.All rights reserved. - Document downloaded on 11/12/2019 by VAUCEL JULES (963264). It is forbidden and illegal to distribute this document.

morphine-like effects, they can be distinguished according to their origin: natural opiates, semi-synthetics opioids and synthetics opioids [6].

Natural opiates refer to alkaloids extracted from opium, they include natural alkaloids contained in the latex of poppy Papaver somniferum L. (e.g., morphine, codeine, thebaine).

Semi-synthetics opioids are synthesized from natural opioid alkaloids and are structurally similar to morphine (e.g., oxycodone, hydrocodone, oxymorphone, and heroin).

Synthetic opioids present a chemical structure different from morphine. They are manufactured legally by pharmaceutical companies to provide pain relief and illegally in clandestine labs.

Chemical structures of fentanyl and the main NSO are presented in Fig. 1. The main class of SO is piperidine derivatives (fentanyl and its analogs). Fentanyl, a potent narcotic analgesic, was developed in 1960 by Janssen Pharmaceutica and used in human therapy for the management of severe pain and in anesthesia [6]. Sufentanil, remifentanil, and alfentanil only have applications in surgical anesthesia as a result of their potency, and have historically been less subject to diversion and abuse. There are also many non-pharmaceutical fentanyl analogs, not approved for human use, including acrylfentanyl, ocfentanil, methylfentanyl or furanylfentanyl. Carfentanil is indicated in veterinary medicine as tranquilizer for large animals such as elephants [7]. A new generation of SO, structurally different from fentanyl, has emerged on the drug market since 2010. Their chemical structures belong to benzamide (U-47700, U-48800 or AH-7921), acetamide (U-50488, U-51754), or piperazine (MT-45) [9]. The potency of the SO is often compared to morphine or fentanyl, rarely heroin. For example, carfentanil, the most powerful analog on the market, is 10,000 times more potent than morphine [7].

Legislation

NPS are defined by UNODC as ''substances of abuse, either in pure form or a preparation, that are not controlled by the 1961 Convention on Narcotic Drugs or the 1971 Convention on Psychotropic Substances, but which may pose a public health threat'' [9]. The main means of legislative control of NPS remains their inscription on the narcotics' list. In France, this list is fixed by a 1990 decree [10], established from the 1961 Convention on Narcotic Drugs and the 1971 Convention on Psychotropic Substances. This decree has been regularly completed since the emergence of NPS. The July 27, 2012 decree established for the first time in France a ban on the basis of a so-called ''generic'' classification, meaning being classified by a group of molecules instead of by molecules. The consolidated version of February 18, 2019 indicated thirty synthetic opioids [11].

Recently, Ireland and United Kingdom introduced a law to restrict production, sale and supply of NPS. The law passed in 2010 with the Criminal Justice (Psychoactive Substances) Act and 2016 with the Psychoactive Substance Act, respectively [12,13].

So far, the only regional response system to the spread of NPS is the European Early Warning System (EWS) of the European Union (EU). The EWS is operated by the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA). In collaboration with Europol, the EWS is designed to detect, assess, and respond to health and social threats caused by NPS. It was first set up in 1997, by the Joint Action 97/396/JHA, and then strengthened in 2005 by Council Decision 2005/387/JHA [14]. This decision provides for an assessment of the risks associated with NPS in order to permit the measures applicable in the EU Member States for control of narcotic and psychotropic substances to be applied also to NPS. By the end of 2017, the EMCDDA was monitoring more than 670 NPS identified in Europe [15]. NPS are also ranked by an international classification from the United Nations, between January 2013 and March 2018, 42 NPS were put under control, an unprecedented number since the first rankings in the 1970s [11]. Nevertheless, the change in the status of a molecule has little influence on users' motivation to obtain them. Illegality remains less important for users than the desire to experience new sensations or to find substitutes [11].

Epidemiology

The emergence in Europe of NSO associated with deaths and acute poisonings has led to the EMCDDA's assessment of an unprecedented number of substances in relation to their public health risks [11]. The development of these drugs poses significant problems for both health services and law enforcement agencies. These substances are easy to transport and conceal, small amounts often representing hundreds of thousands of doses that can potentially be sold as street doses. In the United States, fentanyl derivatives now contribute significantly to the current opioid crisis and, in a very short time, have become the first substances associated with overdose mortality [15]. Europe is not confronted with a problem of the same magnitude. According to the EMCDDA, however, many cases of fatal and non-fatal overdoses associated with SO highlights the need to continue to be vigilant. A total of 49 NSO have been detected in the Europe's drug market since 2009, 34 of them being fentanyl derivatives, and 6 of these opiates were reported for the first time in 2018 [15]. NSO have become the fourth largest group of substances monitored in 2017, after synthetic cannabinoids, cathinones, and phenethylamines. Still according to the EMCDDA, in Europe, fentanyl derivatives accounted for more than 70% of NSO police seizures in 2017. The prevalence of health problems related to opioids use among adults (aged 15 to 64-years-old) is estimated to 0.4% of the EU population, equivalent to 1.3 million users of opiates in 2016. Opioids use remains largely responsible for the morbidity and mortality associated with drug use. NSO pose a serious threat to individual health as well as to public health. In addition to the high risk of overdose, fentanyl derivatives also appear to have significant addictive effects, which could worsen public health and social problems commonly associated with opioid use [16]. NSO are found in powder, tablet or liquid forms. Users can swallow, snort, smoke or inject them. They can also use blotter papers. Rectal or sublingual routes of administration have also been reported [17]. They can also be vaporized with an electronic cigarette, smoked using burning powder or aluminum foil, or inhaled using a vaporizer [16]. In France, since 2013, 10 NSO have been identified out of 21 identified by the EMCDDA. Always seized in small

© 2019 Elsevier Masson SAS.All rights reserved. - Document downloaded on 11/12/2019 by VAUCEL JULES (963264). It is forbidden and illegal to distribute this document.

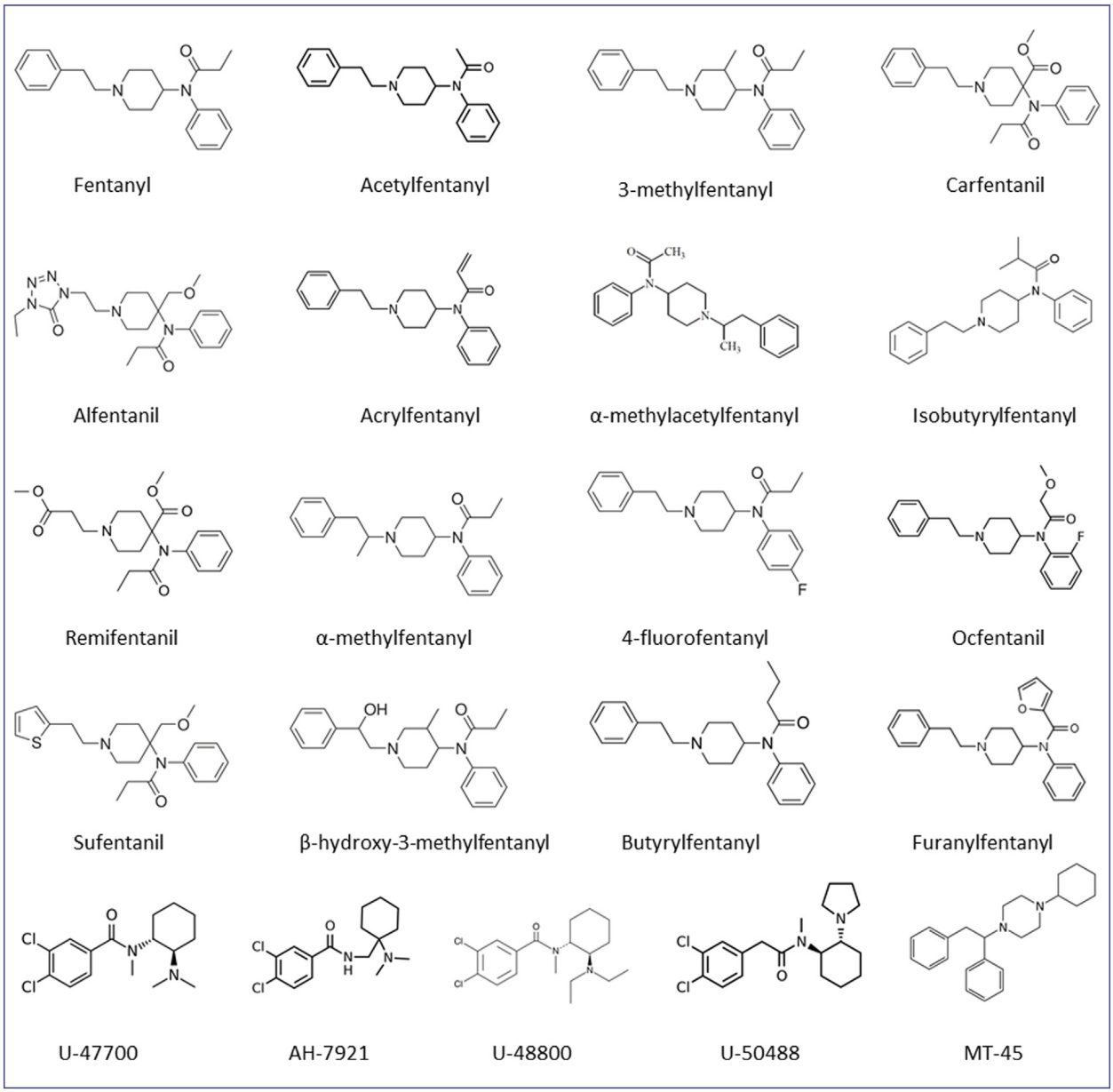


Figure 1. Chemical structures of fentanyl and some NSO.

quantities, they circulate in a very limited way. Between 2012 and 2017, the French addictovigilance network recorded several intoxications related to the consumption of non-medicated fentanyl ($N = 8$), ocfentanil (5 overdoses including 2 deaths), carfentanil ($N = 2$) and butyrylfentanyl ($N = 1$) [18]. These molecules have also been used as cutting products for heroin, sometimes without the user's knowledge, causing high risks of overdose [19]. Fentanyl analogs are a major cause of poisonings, fatal or not, in the United States and in Northern Europe. In contrast, their presence in France remains rare. In France, the availability and accessibility of opioid substitution (eg. buprenorphine and methadone) probably limit the consumption of NSO by opiate users. However, they remain a source of concern and are monitored by health authorities [11].

Pharmacology

Pharmacokinetics

Fentanyl and pharmaceutical analogs

Fentanyl can be administered through intravenous, oral, epidural, intranasal, intrathecal, and transdermal routes. As for fentanyl, NSO have several routes of administration: oral (powder, tablet, and solution), nasal (spray), smoked, intravenous or rectal. In recent years, fentanyl derivatives are sold ''ready to use'' in nasal spray or e-liquid for vaping. They are lipophilic with log P from 1.5 to 4.3 [20], pass through the membranes (including the blood brain barrier) and are widely distributed in tissues. Bioavailability depends on the route of administration. Low oral bioavailability is observed for fentanyl, as is the case for its analogs.

© 2019 Elsevier Masson SAS.All rights reserved. - Document downloaded on 11/12/2019 by VAUCEL JULES (963264). It is forbidden and illegal to distribute this document.

Intranasal and transmucosal fentanyl bioavailability are 89% and 50% respectively [21].

Fentanyl and its analogs are mainly metabolized (dealkylated) in the liver by cytochrome P450 3A4 and 3A5 isoenzymes in nor-compounds, 4-Aminophenyl-1-phenethylpiperidine (4-ANPP) is a minor metabolite of fentanyl. Thus, some interactions may occur with inhibitors of these enzymes, causing an increase of plasma levels and increasing the risk of toxicities. Furthermore, CYP3A4 activity varies from one individual to another that may influence metabolism of fentanyl and its derivatives. Fentanyl elimination half-life is 7 h for buccal and transmucosal routes, 3.7 h for intravenous, and 2.6 to 11.7 h for transdermal [22].

Alfentanil is bound to plasmatic proteins at 92%, volume of distribution is 0.75 L/kg and log *P* is 2.8. Alfentanil is mainly metabolized by CYP3A4 in noralfentanil and N-phenylpropionamide. Noralfentanil is also referred to as N-desalkylsufentanil, it is a common metabolite of alfentanil and sufentanil. Elimination half-life is 94 min and total body clearance is 0.48 L/h/kg [23].

Remifentanil is mainly administered intravenously. After administration of remifentanil, the effective elimination half-life ranges from 3 to 10 min. In healthy young adults, the mean clearance of remifentanil is 40 mL/min/kg, the central volume of distribution is 100 mL/kg and the steady-state volume of distribution is 350 mL/kg. Log *P* is 1.5. At the recommended doses, blood levels of remifentanil are proportional to the dose administered e.g. increase in the infusion rate of 0.1 μg/kg/min lead to a blood concentration increase of remifentanil by 2.5 ng/mL. Drug-protein binding is close to 70%. Remifentanil presents an ester bond rapidly hydrolyzed by plasma and non-tissue-specific esterases into a carboxylic acid. In healthy adults, metabolite half-life is 2 hours. In patients with normal renal function, 95% of the main metabolite of remifentanil is excreted in the urine in approximately 7 to 10 h [24]. Remifentanil have a common metabolite with carfentanil, which is norcarfentanil.

Sufentanil can be administered epidurally and intravenously. Following intravenous administration of sufentanil at doses ranging from 250 to 1500 μg, sufentanil has a triphasic kinetics whose half-lives associated with each phase ranged from 2.3 to 4.5 min and from 35 to 73 min for the distribution phases and from 10.9 h to 15.6 h (mean 13.1 h) for the terminal phase [23]. The volume of the central compartment is 14.2 L, the volume at equilibrium is 344 L. Log *P* is 3.6. Sufentanil pharmacokinetics is linear in the dose range studied. Sufentanil protein binding is 92.5%. Plasma protein binding rate in children is lower than adults and increases with age: 80.5% in newborns, 88.5% in infants and 91.9% in children. Sufentanil is mainly eliminated by metabolism, the two main metabolites are N-desalkylsufentanil and O-desmethylsufentanil. The liver and the small intestine are the main sites of biotransformation. In the liver, metabolism is mainly by CYP 3A4 [23]. Approximately 80% of the administered dose is eliminated within 24 h after administration, 2% are eliminated unchanged.

Illicit Fentanyl analogs

Acetylfentanyl can be smoked, snorted and intravenous injected. Half-life is between 90 and 111 min [22]. Metabolism generates 32 dealkylated, nor, hydroxyethyl and hydroxymethoxy compounds [25].

Acryloylfentanyl can be administered orally, intranasally (the most common route of administration), sublingually or transdermally, can be snorted, inhaled and injected [26]. Metabolism by cytochrome P450 (3A4, 3A5) generates 14 dealkylated, nor, hydroxyethyl and hydroxymethoxy compounds [27].

Alpha-methylfentanyl is lipophilic (log *P* = 4.2) and is metabolized by CYP 3A4, CYP 3A5, CYP 2D6, CYP 2C19, into N-dealkylated compounds before hydroxylation and excretion as glucuronide in urine [28,29].

Butyrylfentanyl can be used by rectal, nasal, intravenous, transdermal, sublingual routes. Butyrylfentanyl is a lipophilic drug (log *P* = 4.3), metabolized by CYP 3A4 and 2D6 into carboxybutyrfentanyl, hydroxybutyrfentanyl, norbutyrfentanyl and desbutyrfentanyl [30]. Butyrfentanyl seems to be rapidly eliminated, within 3 h after administration but elimination half-life is prolonged in case of intoxication [31].

Carfentanil is very rapidly absorbed and highly bound to plasmatic proteins [32]. Log *P* is 3.7. It is dealkylated and hydroxylated through several cytochromes (CYP 1A2, CYP 3A4/5, CYP 2C9, CYP 2C19, CYP 2D6) into 12 metabolites. In human liver microsomes, carfentanil half-life was 7.8 min [33].

Cyclopropylfentanyl can be used by the intranasal, oral, and intravenous routes. It seems to be metabolized in dealkylated (norcyclopropylfentanyl) and hydroxylated compounds but no controlled pharmacokinetic studies of cyclopropylfentanyl have been reported yet [34].

4-fluoroisobutyrylfentanyl (4F-iBF) is mainly metabolized by dealkylation into hydroxyethyl and hydroxymethoxy metabolites, 17 metabolites have been described [21].

Furanylfentanyl (Fu-F) can be used by oral, intramuscular injection, snorting, and inhalation routes, it crosses the blood-brain barrier and diffuses into fat and other tissues [35]. Fu-F is metabolized by microsomal oxidation in the liver. It could be predicted to undergo metabolism by hepatic CYP450 isoenzymes, including CYP 2C19, CYP 2D6, CYP 3A4, and CYP 3A5 [36]. Fu-F is mainly eliminated by urinary tract, several metabolites were found in urine: Fu-NF, 4-ANPP, ANPP-sulfate, Fu-F-dihydrodiol [37].

Methoxyacetylfentanyl can be administered intravenously, by nasal spray and orally. It crosses the blood-brain barrier and diffuses into fat and other tissues [38]. An important metabolic step appears to be O-demethylation leading to 2-hydroxy-N-phenyl-N-[1-(2-phenylethyl)piperidin-4-yl]acetamide. N-dealkylation, hydroxylation of the piperidine ring and the phenylethyl side chain, N-oxidation, and amide hydrolysis to 4-ANPP were also observed [39].

Methoxybutyrylfentanyl may be administered as a solution, orally as a powder, or by insufflation of a powder; it can also be administered sublingually or intranasally via a spray; administered by injection or inhaled by vaporizing [40]. No information was found regarding methoxybutyrylfentanyl in the current literature.

Ocfentanil is usually injected, snorted, or smoked [41]. The effects appear quickly (in a few minutes) and the duration of the effects is short (approximately 1 hour). In a study in human volunteers, the peak of analgesic effects of ocfentanil (up to 3 μg/kg) occurred at 6 min and disappeared beyond 1 h [42].

© 2019 Elsevier Masson SAS.All rights reserved. - Document downloaded on 11/12/2019 by VAUCEL JULES (963264). It is forbidden and illegal to distribute this document.

Tetrahydrofuranylfentanyl (THF-F) can be administered intravenously, orally and by nasal spray [43]. In a microsomal preparation of human liver, the predominant metabolic step of THF-F appears to be N-dealkylation, as in the case of fentanyl. Hydroxylation of the piperidine ring and the phenylethyl side chain, N-oxidation and amide hydrolysis to 4-ANPP were also observed [44].

Benzamide derivatives

AH-7921 belongs to the benzamide family. The administration can be nasal, intravenous, combined insufflation and oral, at doses ranging from 10 to 150 mg. Unconventional routes are associated with a higher risk of over-dose, such as sublingual and rectal administration [45,46]. Twelve different metabolites were described, metabolic steps include demethylation, hydroxylation, and combinations of different biotransformations. The two most abundant metabolites are desmethyl- and di-desmethyl-AH-7921 [45].

U47700 is an isomer of AH-7921. It can be used by oral, insufflation, intravenous, rectal and via an inhaler using a liquid solution (described as the main route of administration) [47], at doses ranging from 5 to 25 mg. Its time of action is 15 min and its effects last 5—7 h orally and respectively 1 min and 1—2 h intravenously [48,49]. It has two main metabolites: N-desmethyl-47700 and N,N-didesmethyl-47700 [48].

Piperazine derivatives

MT-45 can be administered orally, nasally, intravenously and rectally [45]. Oral doses of MT-45 ranged from 20 to 500 mg, although the majority of users reported limiting the dose to less than 100 mg. Regarding nasal insufflation doses ranged from 1 to 50 mg. MT-45 was also administered rectally by insertion of ''80 mg of MT-45 salt as the solution'' [50,51]. The psychoactive effects of MT-45 began within 15 min after nasal intake of 30 mg, or 60 min after oral intake of single doses in the range of 45—70 mg. At these dosages the effects of MT-45 subside after 2 h; by re-dosing, which is frequently reported, the effects may be extended for over 8 h [52].

Pharmacodynamics

All opioid substances (opiates, semi-synthetic opium alkaloids or their synthetic substitutes) may interact with mu (μ), but sometimes also kappa (κ) and delta (δ) opioid receptors. The activation of these receptors produces effects varying according to the subtype of stimulated receptor and the affinity of the agent [17,53,7]. With regard to synthetic opioids, and more particularly therapeutic and non-therapeutic derivatives of fentanyl, desired and toxic effects observed during acute intoxications result essentially from the stimulation of μ-type opioid receptors [54]. These effects are similar to those described for morphine secondary to the stimulation of μ-type opioid receptors [7,21].

SO have thus a stimulating action on the limbic system responsible for the euphoric effect sought by consumers. On the other hand, they have a depressant effect on the central nervous system (CNS) that induces analgesia and a state of stupor and alertness disorders (ranging from drowsiness to coma) observed during acute intoxications [8]. Central stimulation of μ-type opioid receptors leads to a dose-dependent decrease response of bulbar respiratory centers to hypoxemic and hypercapnic stimuli and to a depression of bulbar centers involved in respiratory rate regulation. This is responsible of respiratory depression comparable to that described for all opioids but which occurs more rapidly. Respiratory depression is the major component of opioid toxidrome, with the occurrence of bradypnea or even apnea that can lead to death in the absence of an effective management [55]. Fentanyl and its derivatives induce also a characteristic miosis secondary to central stimulation of the parasympathetic nucleus of the common ocular motor nerve (III) [21,56]. In the area postrema of the medulla, direct stimulation of the chemoreceptor trigger zone may also cause nausea and vomiting [53]. Concerning the peripheral effects of fentanyl and its derivatives, their direct depressant effects on the myocardium are weak. They have also cardiovascular effects secondary to histamine release, it reduces total peripheral vascular resistance and lead to hemodynamic instability [17,8]. On the contrary, as with all opioids, they can exert peripheral actions on the digestive and the urinary tracts leading to constipation and urine retention. These effects are related to a decrease in the tone and peristalsis of the longitudinal muscle fibers and an increase in the tone of the circular muscle fibers, which causes spasms of the sphincters (pyloric, Oddi, anal and vesical sphincter) [56].

NSOs toxicity comes in part from their high potency associated to a high affinity for the μ-opioid receptors [57,58]. Thus, fentanyl derivatives and NSOs have potency greater than that of morphine and comparable or greater to that of fentanyl. This results in the development of toxic effects at very low doses and the need to use higher doses of naloxone, a pure and complete μ-opioid receptor antagonist, when treating intoxications [21]. Literature data on the relative potencies of synthetic opioids related to fentanyl and used in clinical or veterinary practice, non-therapeutic fentanyl derivatives and NSOs compared to morphine and fentanyl are presented in Table 1. Most often, the data come from animal studies or case report studies [17,21,8]. Most of these molecules have been synthesized by the pharmaceutical industry and then abandoned because of their toxicity or addictive power, making the carrying out of clinical studies in humans impossible [59]. The high potency of some fentanyl derivatives, such as carfentanil having potency 10,000 times greater than morphine, raises the problem of the presence of these molecules even at very low doses as adulterants in powders of heroin or even cocaine [60] and poses a serious public health issue [21,57,61]. Another important issue concerning the toxicity of fentanyl derivatives and NSOs, stems from their presence in counterfeit drugs and leads to their consumption in naïve subjects, i.e. those who have not developed a state of tolerance for opioids [21,62,63]. Some unexpected toxic effects have also been described for some NSOs such as MT-45 or U-49900. Their consumption has been associated with an ototoxicity in several cases of intoxication for MT-45 [64,65] and loss of taste, smell and touch for U-49900 [7]. These ''off-target'' effects of NSOs, which have a chemical structure different from fentanyl and its derivatives, could be linked to their binding to other pharmacological targets than to μ-opioid receptors. During acute poisoning, it should also be emphasized that in combination with other CNS depressants, such as benzodiazepines or ethanol, it may be

© 2019 Elsevier Masson SAS. All rights reserved. - Document downloaded on 11/12/2019 by VAUCEL JULES (963264). It is forbidden and illegal to distribute this document.

Table 1 Relative potencies of fentanyl and its pharmaceuticals derivatives, non-pharmaceutical fentanyl derivatives and NSOs compared to morphine and fentanyl (NA: not available).

Compounds	Opioid receptor selectivity			Analgesic potency related to morphine	Analgesic potency related to fentanyl	References
	μ	κ	σ			
Fentanyl and pharmaceutical derivatives						
Fentanyl	+++			50–100	1	[53,8]
Sufentanil	+++	+	+	(500–) 1000 to 4000	5–10	[53,8,61,66]
Alfentanil	+++			72	0.1–0.2	[8,57,67,68]
Remifentanil	+++			220	2–20	[8,57,69]
Illicit fentanyl analogs						
Acetylfentanyl	+++			15.7	0.29	[8,70]
Acrylfentanyl	+++			170	NA	[57,71]
3-methyl-fentanyl	+++			48.5–569	(0.9–)10.5	[8,70,72]
β-hydroxy-3-methyl-fentanyl	+++			6300	28	[8,73]
α-methyl-fentanyl	+++			56.9	1.1	[8,70,72]
α-methyl-acetyl-fentanyl	+++			3.1	0.06	[8,70]
4-fluoro-fentanyl	+++			15.7	0.29	[8,70]
Butyr-fentanyl	+++			(1.5–) 7	0.03–0.13	[21,8,70,30]
Carfentanil	+++			10,000	100	[21,57,61,74]
Isobutyrylfentanyl	+++			NA	1.3–6.9	[57,70]
Ocfentanil	+++			90	1.7	[21,75]
Furanyl-fentanyl	+++			7	NA	[57,70]
Benzamide derivatives						
U-47700	+++			7.5	0.1	[7,21,57,72,76–78]
AH-7921	+++	+		1–1.7	NA	[17,7,57,79,80]
U-48800	+++	++		7.5	NA	[7]
U-50488	-	++		NA	NA	[7,81]
Piperazine derivatives						
MT-45	+++	+	+	≈1	NA	[7,57,74,81–83]

responsible for pharmacodynamics interactions increasing the risk of death from respiratory depression and should be taken into account in the management of the patient [57]. Finally, chronic consumption of fentanyl, its derivatives and NSOs leads to the rapid development of a tolerance state and to a psychological and physical dependence characterized by the appearance of a withdrawal syndrome when treatment is stopped [61].

Clinical management

Clinical picture

Although low doses of opiates induce analgesia, feelings of relaxation and euphoric effects, high doses lead to a classical opioid toxidrome characterized by a triad of neurovegetative troubles: reduced level of consciousness, respiratory depression and miosis. Death may occur by respiratory, circulatory or neurological failure [84]. Respiratory depression is the key symptom, and its absence should lead to reassessment of the diagnosis [85]. There is a significant inter individual dose/response variability leading to different toxic doses and clinical presentation [86]. Also, the great number of substances, forms (powders, pills, liquids, patches...) and administration routes (burning powder, inhalation via a vaporizer, and rectal and intravenous injection) lead to different delays in symptom onset and different duration of effects.

Neurological effects

The most common neurological symptom is a reduced level of consciousness. It can appear as drowsiness and/or disorientation, depression of central nervous system, lethargy, or calm coma leading to death. Side effects are miosis, allodynia, hyperalgesia, anesthesia, anxiety, dizziness depression, dissociation, memory loss, impairment of psychomotor performance and sleep disturbance [8,87,55,51,88–91]. Cerebral edema is observed in autopsies following fatal overdoses due to fentanyl [92], analogs [93–96] or other synthetic opioids [97–99]. In some case reports, specific effects were reported. Fentanyl or analogs overdoses cause immediate stiffening of the body, seizure-like activity or delayed leukoencephalopathy [100,101]. Cerebral hemorrhage was reported following acetylfentanyl intoxication [102]. MT-45 presents specific neurologic side effects like paresthesia, hand weakness, balance disturbance and ototoxicity [65,64]. U-49900 can induced loss of taste, smell and touch [7].

© 2019 Elsevier Masson SAS.All rights reserved. - Document downloaded on 11/12/2019 by VAUCEL JULES (963264). It is forbidden and illegal to distribute this document.

Respiratory effects

The most critical adverse effect is respiratory depression. Other symptoms are described such as cough suppression, hypoxia, cyanosis, pulmonary edema and pulmonary congestion [86,87,55,92,93,103—108,21,109—112]. Fentanyl and its analogs cause chest wall rigidity and apnea, this effect is not dose related and can appear at low dose [113]. Post mortem examination in a fentanyl user can reveal a hemorrhagic effect like alveolar hemorrhage and/or pleural petechiae [114—118].

Cardiovascular effects

Acute cardiac effects are induced by histamine release and parasympathetic system stimulation [88]. The clinical picture is orthostatic hypotension, hypotension, bradycardia, shock and chest pain mimicking acute coronary syndrome [87,21]. Non-specific T-wave changes are observed on the electrocardiogram [119]. In acute intake, acetyl fentanyl and 4-methoxybutyrfentanyl abuse induce a tachycardia contrary to other opioids [102]. Chronic users will develop cardiomegaly [92,109], cardiac fibrosis [103,115] and arteriosclerosis [104,109]. In autopsy, pericardial petechiae are observed appearing after asphyxia [110].

Gastrointestinal effects

Opioids induce constipation and bowel dysfunction [120—122]. The direct action is reinforced by inhibition of VIP (vasoactive intestinal peptide) secretion, decreased pancreatico-biliary secretion and gut absorption [123]. A lot of nonspecific symptoms are recorded with association of gastric, urinary and hepato-pancreatic symptoms. These symptoms appear in acute and chronic use. The gastric symptoms are spastic achalasia-like esophageal dysmotility, gastric hypomobility and paralytic ileus, generalized visceral congestion or cramp, nausea, vomiting, constipation, anorexia and a loss of weight. Nausea and vomiting were seen in 9% to 27% of patients [87,95,99,21,122—127]. The vesical symptoms are urinary urgency or retention [21]. The hepato-pancreatic effect is a dilatation of bile duct by sphincter of Oddi dysfunction. This induced biliary colic and epigastric discomfort [128]. Other chronic effects are reported such as hepatomegaly [96], fatty degenerative liver [104,129], chronic active hepatitis [114] or liver cirrhosis [104]. But these symptoms cannot be strictly associated with only opioid use. Some opioids have specific gastrointestinal effect, e.g. MT-45 can increase liver enzymes [91].

Other effects

Opioids can induce endocrinopathies by androgen deficiency [130—132]. The major symptom is sexual dysfunction for male, and oligomenorrhea or amenorrhea for female. For both, the opioid use evolves to hypogonadism and a libido decrease. The androgen deficiency promotes osteoporosis [88,89,133,134]. In chronic use, an insulin resistance can appear [133].

Miscellaneous other effects were observed such as asthenia, reduced body temperature [127] xerostomia [120], nasal burn or nasal drip after insufflation, bitter taste after oral ingestion [51,21]. Specific biological effects recorded were acidosis and immunosuppression [1]. Each opioid induces different inhibitions of cellular and humoral immune way [88,135]. Chronic use of opioid stimulates digestive and pulmonary cancer, organs affected are esophageal, gastric, laryngeal, lung, bladder and pancreas [136,137].

Overdose management

The respiratory failure appears in a few minutes after the use of new synthetic opioids. In case of acute intoxication, initial care is common to any emergency, with a focus on protecting the airway and maintaining vital parameters. Secondary, the quick use of naloxone is required to reverse opioid toxic effects [138—140]. The naloxone shortly reverses the respiratory depression with a competitive antagonist action on μ-opioid receptors [139]. The dose needs to be adapted according to clinical response, multiple doses of naloxone or continuous perfusion can be required [141—143]. Naloxone can be administered by several routes: intravenous, intramuscular, intranasal, subcutaneous, endotracheal, inhalational and sublingual [139]. In emergency cases, intravenous route is recommended due to a quick effect (30 sec) compared to intramuscular (5 min). Intranasal route is reserved for public or difficult situations, delay of action is close to the parenteral route. The dose required is higher due to a low bioavailability (50%), but intranasal route maintain blood levels above twice the intramuscular reference for the next 2 hours [144].

The naloxone dose needed to manage fentanyl and NSO poisonings is higher than other opioids. In heroin intoxication, a low dose (< 0,1 mg) is enough to reverse life threatening effects [21]. In observational NSO intoxications case series, the mean naloxone dose needed was far higher at 3.36 mg and the effect range was between 0.4 and 12 mg of naloxone. It was reported that only 15% of patients responded to 0.4 mg [145]. To counter the effect, the opioid involved must be known because the duration and the effect of naloxone is variable, depending on the type and the dose of NSO [139]. For example, the highest required dose of naloxone was described for a case of carfentanil poisoning, 18 mg of naloxone in multiple intravenous injections were required for management [146]. On the other hand, MT-45 and U-47700 respond well to naloxone, MT-45 intoxications responded to less than 2 mg of naloxone and U-47700 intoxications responded in range of 0.4 to 4 mg of naloxone [98,64,126,77,147,148,49].

Clinical effects and management differ for each NSO according to their potency, consequently, there is no consensus or global recommendations. Naloxone manufacturers recommend an initial dose of 2—4 mg intranasally or 0.4—2 mg intramuscularly/subcutaneously. These doses can be repeated after 3 min if respiratory rate is still under 10 movements per min [149]. The United Kingdom government recommends a first injection of 0.4 mg of naloxone, and, if no response appeared, a second dose of 0.4 mg is administered. If the two doses are ineffective a 2 mg dose can be provided [150]. In the US, the recommend intravenous starting dose of naloxone to withdraw respiratory depression is between 0.4 and 2 mg for an adult and 0.01 mg/kg weight in children [151]. A nasal spray kit of 2 mg naloxone is available for public user in 40 states, previously only 0.4 mg dose was available [21,152]. The world health organization (WHO) published novel guidelines on November 4, 2014 aiming to reduce the number of opioid-related deaths globally. The guidelines recommend countries expand naloxone access to

© 2019 Elsevier Masson SAS.All rights reserved. - Document downloaded on 11/12/2019 by VAUCEL JULES (963264). It is forbidden and illegal to distribute this document.

people likely to witness an overdose [153]. However, the naloxone nasal spray is currently not available in all countries. In 83% of 2 mg nasal spray use, the street user reports a second dose is needed to counter the opioid effects [101]. Canadian poison centers have different practices: they recommended an intravenous dose of 0.04 mg and additional dose every 3 min if the clinical response is unsatisfactory. This protocol has been chosen to avoid a withdrawal state [85].

Regarding the duration of supervision in emergencies, currently there is no recommendation for NSO. For heroin users, a study suggests to discharge patients after one hour of observation in the emergency department [63]. This can be applied to fentanyl, fentanyl derivatives and NSO because their elimination half-lives are greater than that of naloxone [119]. A recrudescence of symptoms can appear 2 hours after the last dose of naloxone. In these cases, second or multiple doses of naloxone can be administrated [17]. In the UK, a longer period of observation is advised for fentanyl patients than for heroin patients. For a carfentanil overdose, they recommend 24 h in a hospital setting [150]. Some US authors argue to increase naloxone first dose from 0.4 mg to 2 mg to avoid multiple injections and shorten the medical supervision [154]. The Canadian poison center recommends at least 2 hours of clinical supervision [85]. All these recommendations need to be adapted to each situation by considering the SO involved, the patient specificity and substances associated.

Co-abuse of other substances was frequently observed: cocaine (up to 65%), other opioids (up to 65%), benzodiazepines (up to 52.2%), cannabinoids (up to 50%), antidepressants (up to 48%), amphetamines (up to 40%), medicines like barbiturates (up to 27%) and ethanol (up to 22.9%) [1,84]. In multiple intoxications and during hospital care, physicians need to manage drug interactions. The major syndrome to advocate was the serotonin syndrome, appearing when serotoninergic drugs, tricyclic antidepressants, monoamine oxidase, triptans, 5-HT3 receptor agonists, tramadol, linezolid, intravenous methylene blue or lithium are associated to opiates or administered during hospital management. In alcoholic patients treated by baclofen or patients treated by other muscle relaxants, the risk of sedation and respiratory depression is enhanced. Similarly, in patient treated with benzodiazepine or central nervous system (CNS) depressant, the risk of CNS or cardiovascular effects is enhanced. In diabetic patients, metformin concentration is increased with a risk of lactic acidosis [86]. Adulterants can induce specific side effects, e.g. thallium poisoning-like symptoms: loss and depigmentation of hair, folliculitis and dermatitis, painful intertriginous dermatitis, mees' lines (leukonychia striata) on the fingernails and toenails [91].

Addiction management

Opioids induce an addictive behavior and opioid users present a suicide rate 14 times higher than that of the general population [155]. A long-term management needs to be performed to avoid risk of overdose or suicidal death. The two main medications available are buprenorphine and methadone. Both are oral medications, methadone can be presented as pills or syrup and buprenorphine as sublingual tablet or film. The oral or sublingual route induces a slow liberation to avoid the addiction peak which contributed to addiction [156]. Methadone is a μ- opioid receptors full agonist. Methadone efficiency is greater than buprenorphine [157]. Its use needs to be restrained to trained medical units to avoid overdose that leads to respiratory depression. Methadone therapy is essential for the most severely opioid-dependent patients, but not appropriate every for patient due to misuses [158,159]. Buprenorphine is a partial agonist of μ-opioid receptors having a very low dissociation rate [160,161]. This characteristic reinforces its inability to be displaced by other opioids. Buprenorphine seems more specific to respiratory center [162]. This decreases the number of severe respiratory depression [163]. To avoid misuses (an intravenous administration of the tablet) buprenorphine can be combined with naloxone. Naloxone has a poor oral bioavailability, but when injected it prevents adverse effects of buprenorphine [158]. Buprenorphine is recommended for ambulatory medicine patients who are not stabilized, patients with a high risk for misusing, patients with serious comorbidities, and pregnant women [164,165]. Opioid substitution treatments have side effects such as: decrease of cognitive performance and sexual dysfunctions in men were common. These effects can decrease the treatment compliance [166—168].

The classical therapy with a drug abuse therapist is the cornerstone of the abstinence management. The care must be adapted to each patient. A psychological approach can be proposed. Numerous other approaches are also available. The motivational interview is conversational person-centered to identify readiness, ability, and willingness to change. It helps to decrease misuse, depression and increase motivation to change [169]. The cognitive behavioral therapy is a type of psychotherapy associated with improved social functioning and quality of life in various populations [170]. The Cognitive Behavioral Therapy focuses on multiple tasks like avoiding stimuli or mood regulation [171]. The association of a psychological and pharmaceutical support is the best way to have a lasting remission in time.

Analytical aspects

Biological matrices

Different matrices of interest can be used depending on the clinical or forensic context. For decades, blood and urine samples are largely used for drug detection or quantification. However, these matrices are not always available and testing unconventional or alternative matrices like hairs, oral fluids, meconium and various forensic samples have to be considered.

Blood and urine

Blood concentrations give information about drug exposure for a few hours according to drug's half-life, and are more correlated with clinical symptoms than urine concentration. Very few ''normal'' or toxic concentrations are known [172]. Most of the time authors report lethal concentrations of fentanyl or fentanyl analogs from fatal poisonings [173,174].

Analysis in urine samples is interesting for long term detection of opiates, and has been used worldwide in different situations for decades: road prevention, addiction

19 Elsevier Masson SAS.All rights reserved. - Document downloaded on 11/12/2019 by VAUCEL JULES (963264). It is forbidden and illegal to distribute this document.

monitoring or control of additional opiate consumption for patient with opiate substitution treatment. Several publications describe the identification and quantification of NSO and their metabolites in urine, in order to improve the detection window in clinical and forensic intoxications [175].

Hairs

Hair is a useful matrix for drug detection and quantification after months or years. Drug stability is a main advantage with this matrix. After hair incorporation, in the absence of cosmetic treatment (discoloration, coloration, hair abrasion by smoothing, non-exhaustive list) NSO can persist in hairs. Identification and quantification of therapeutic or misused opioids in hair samples are more described [176] than NSO. Capillary analysis is of interest in situations related to unexplained intoxications. Conventional biological matrices can be inappropriate because of the significant delay between sampling and presumed intake. In addictology, the monitoring by hair analysis plays a major role in risk reduction. It makes possible to study long-term exposure profiles, consumption trends and a possible discrepancy in the actual composition of the absorbed substances, often unbeknownst to the consumer due to mislabelling. A study confirmed a historic NSO use of U-47700 with pubic hair again in a 30 years old man in an addiction monitoring context [177]. Larabi et al. highlighted the presence of 3-fluorofentanyl and furanylfentanyl in hair samples for a chronic opioid user [178]. Hairs are also used in forensic science. Two recent papers describe sensitive UPLC-MS/MS methods able to quantify in hair fentanyl, fentanyl analogs and a non-fentanyl compounds (U-47700) in ranges concentration of 1 to 50 pg/mg and 0.01 to 100 pg/mg [179,180]. With the same goal, pubic hair quantification was described in the context of U-47700 intoxication [181]. Interpretation of hair concentrations remains difficult, especially because correlation between hair and blood concentrations is few documented and uncertain. Indeed, all drugs are not incorporated in the hair at the same rate, depending on their physic-chemical properties. The speed of hair growth is about 1 cm/month (0.7—1.4 cm/month) and must be taken into account for the interpretation [182]. The repeated measure of hair concentration by segment (1-2 cm) could be useful in the case of chronic use of NSO. Hair levels found in literature are presented in Table 2.

Oral fluids

Collection of oral fluid is simple, non-invasive, and less subject to adulteration compared to urine samples. Driving under the influence of drugs (DUID) is a worldwide problem with potentially major judiciary and life-threatening consequences. Oral fluids are largely used in road prevention for control of illicit products consumption, especially frequent drugs of abuse (DOA). Currently, on-site oral fluid screening tests are limited to standard DOA classes (opiates, cannabis, cocaine, ecstasy and amphetamine) [185]. These tests do not cross react with NSO [186]. Results of a recent study highlighted this drawback, NPS were detected in 33 out of 391 ''negative'' on-site oral fluid samples. It shows a lack of on-site detection methods to screen NPS [187]. Moreover, the low doses administered given the pharmacological potential of the NSO make their detection hardest.

Forensic analysis

There is a wide diversity of matrices that could be used in forensic investigations, including whole blood (peripheral or cardiac), urine, vitreous humor [188], bile, gastric contain, liver, kidney, lung, hairs, other organs, dried blood spots [189] or seized materials [190—192]. The interpretation of measured concentrations is problematic due to post-mortem tissue redistribution. Most of the time, concentrations in blood and forensic matrices of fentanyl, fentanyl analogs and non-fentanyl compounds are reported from forensic cases [173,174,193]. Blood concentrations found in fatal cases are presented in Table 3.

Critical issues for the detection and the interpretation are due to low NSO concentrations and high pharmacological potencies [173]. Concheiro et al. also exposed in their study the ratio C/P (cardiac-to-peripheral blood) and L/P (liver-to-peripheral) from several forensic cases to predict NSO candidates of post-mortem redistribution [173]. However due to scarce amount of data available, no conclusion could be drawn. Nevertheless, it is still a controversial issue for classical opioids. Given the difficulties described above, the French Society of Analytical Toxicology (SFTA) proposed some guidelines for the achievement of toxicological analysis for deaths involving NPS [211].

NSO stability

Several studies have been conducted to evaluate the stability of fentanyl and some of its analogs in fortified biological samples, such as blood, plasma and urine. Furanylfentanyl showed no significant degradation in blood samples at 5 and 10 ng/mL for 48 h at room temperature, at +4 °C for 7 days [212] and up to 30 days [98]. Regarding NSO not related to fentanyl, U-47700 was stable in blood refrigerated up to 30 days [98], appeared stable in urine at room temperature (+21—27 °C) for 16 h, in the autosampler (+6 °C) for 72 h, with three freeze—thaw cycles, frozen (− 15 °C) for 28 days [213]. AH-7921 was found to be stable for at least 21 days in blood and plasma at room temperature [209]. MT-45, was determined to be stable for up to 30 days at room temperature, refrigerated and frozen [214]. Further studies are necessary to evaluate the stability of the different synthetic opioids and their metabolites, and in additional biological matrices of forensic interest, such as vitreous humor and tissues.

Analytical methods

Different analytical methods are appropriated for NSO identification, involving an important step of method validation for clinical toxicology laboratories. They can refer to the validation consensus of SFTA [215], or international recommendations for the validation of new analytical methods (EMA, SWGTOX) [216,217].

Immunoassay

Immunoassay panels are based on the reaction of antibodies that recognize epitopes of a drug or a class of drugs with similar structures. Standard opiate immunoassays, widely used for their cost-effectiveness and rapid reporting, do not permit the detection of NSO. Their structural differences lead to the absence of cross reaction. In this context, development of new immunoassay for NSO remains an

© 2019 Elsevier Masson SAS.All rights reserved. - Document downloaded on 11/12/2019 by VAUCEL JULES (963264). It is forbidden and illegal to distribute this document.

Table 2 Hair concentrations found in current literature.

Compounds	Hair concentrations (pg/mg)	References
Fentanyl and pharmaceutical derivatives		
4-ANPP	1–11.2	[179,180]
Fentanyl	2.3–2800	[178–180,183]
Norfentanyl	15.1, 149	[179,180]
Illicit fentanyl analogs		
3-fluoro-fentanyl	60–150	[178]
4-fluoro-fentanyl	60–150	[178]
Acetylfentanyl	2.1–3200	[180,183]
Carfentanil	84–166	[184]
Furanyl-fentanyl	0.7–44	[178,179,183]
Benzamide derivatives		
U-47700	1.4–4.5	[183]

Table 3 Blood concentrations found in fatal cases.

Compounds	Lethal blood concentrations found in fatal cases	References
Fentanyl and pharmaceutical derivatives		
Fentanyl	0.5–383 ng/mL	[1,114,194–196]
Sufentanil	27 ng/mL	[197]
Alfentanil	100–200 ng/mL	[198]
Illicit fentanyl analogs		
α-methyl-fentanyl	3.1 ng/mL	[199]
3-fluoro-fentanyl	2.4 ng/mL	[200]
3-methyl-fentanyl	0.3–1.9 ng/mL	[108,196,201]
4-fluoro-fentanyl	0.24 ± 0.21 ng/mL	[200]
4-methoxy-butyryl-fentanyl	79 ng/mL	[200]
Acetylfentanyl	153-260 ng/mL	[93,107,110,196,202]
Acrylfentanyl	1.86 ± 3.08 ng/mL	[200]
Butyryl-fentanyl	0.1–99 ng/mL	[94,200,203]
Carfentanil	0.1–4.9 ng/mL	[184,200,204]
Cyclopropylfentanyl	15.3 ± 11.9 ng/mL	[205]
Fluoro-butyryl-fentanyl	91–112 ng/mL	[206]
Furanyl-fentanyl	0.4-26 ng/mL	[95,207]
Methoxyacetylfentanyl	17.7 ± 11 ng/mL	[205]
Ocfentanil	5.3-15.3 ng/mL	[1,96,208]
Valerylfentanyl	0.44 ng/mL	[200]
Benzamide derivatives		
U-47700	13.8–490 ng/mL	[151,207]
AH-7921	31–6600 ng/mL	[1,151,82,209]
Piperazine derivatives		
MT-45	8.3–1989 ng/mL	[1,151,209,210].

important issue, largely due to the structure variability and unpredictable creativity of NSO drug designers. The strong demand of clinicians associated with the public health problem in the United States, led to the development of fentanyl immunoassays. Different fentanyl derivatives are expected to cross react according to the kit used [235,218]. Regarding benzamine derivatives (AH-7921, U-47700) and piperazine derivatives (MT-45), specific NSO immunoassays have been developed (Randox toxicology), but a lack of independent validation studies and their low laboratory deployment limit their use [230]. Anyway, a positive result in any of those tests has to be confirmed by a more specific technique. In one case report, U-47700 caused a false-positive result for the presence of benzodiazepines using immunoassay methods [219].

On the other hand, some alternative methods have to be encouraged. A newly developed cell-based activation bioassay uses the NanoLuc® Binary technology (Promega), and detects in vitro μ-opioid receptor activation by recruitment of β-arrestin 2 that produce a luminescent signal [220]. It was applied successful in forensic case of carfentanil intoxication [221].

LC-DAD, GC-MS and LC-MS

With their high specificity, gas chromatography coupled to mass spectrometry (GC-MS) or liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) allow a qualitative and quantitative analysis of NSO in different biological matrices. The combined approaches between a

© 2019 Elsevier Masson SAS.All rights reserved. - Document downloaded on 11/12/2019 by VAUCEL JULES (963264). It is forbidden and illegal to distribute this document.

separation process and mass spectrometry allow the identification of features depending on their mass to charge ratio, mass spectrum, and retention time. GC-MS has been historically an analyzer of choice in toxicology, and a gold standard for volatile drug detection and quantification [199]. This method presents several drawbacks as it is a cumbersome sample preparation that can require derivatization steps. It also presents a limited capacity of detection of non-volatile or polar molecules that can be of interest in the context of excretion of hydrophilic metabolites in urines for example. LC-MS/MS can overcome those limits, and permits the analysis of polar molecules, with limited sample preparation steps and lower limits of detection, and is wildly prioritized in NSO methods development. Several targeted LC-MS/MS methods were published for the different classes of NSO, including fentanyl derivatives [205,222], benzamine derivatives [98], or piperazine derivatives [214], and summarized by Marchei and al. in 2018 [223]. A recommendation report was also published in 2017 by United Nations Office on Drug and Crime (UNODC), about identification and analysis of fentanyl and its analogs in biological specimens [193]. A review on analytical methods developed for NSO detection has been recently published by Tabarra et al. [224]. Liquid-liquid or solid-phase extractions have been described for sample preparation with sample volumes ranging from 0,08 mL to 2 mL. GC-MS, GC-MS/MS, LC-MS/MS, LC-QTOF or LC-DAD instruments are used. They present limits of quantification ranging from 0.01 (LC-MS/MS) to 312 ng/mL (LC-DAD). Beside chromatographic considerations, the identification of NSO is based on a targeted approach consisting in the comparison of sample data with mass spectral libraries of compounds of interest. However, due to the constant emergence of new molecules, laboratory incrementation of reference libraries can be of interest. It can be achieved with analysis of standard samples when they are commercially available, or from a solution of the powder of interest after purity check. Beside NSO parent molecule incrementation, metabolites have to be referenced in order to extend the detection window of NSO, especially in case of urine screening [225]. Metabolites referencing remains an important challenge. Different methods were published, frequently using a combination of in vitro and in vivo individual metabolism studies [175,226]. Briefly, powder of pure NSO of interest is incubated with human hepatocytes, allowing an in vitro production of NSO metabolites by mimicking cytochrome P450 activity. Meanwhile, an in silico prediction of biotransformation of NSO is performed, allowing the comparison with in vitro biotransformation data. Finally, an in vivo confirmation of metabolite identification data on biological specimens is performed with real clinical cases of NSO exposure.

LC-HRMS

GC-MS and LC-MS/MS require reference libraries for targeted NSO of interest, so they can hardly face challenges of the continuous emergence of new NSO. Fortunately, recent advances in mass spectrometry have permitted the development of high resolution (LC-HRMS) methods, with both targeted and untargeted workflows for NSO identification [227,228]. Time-of-flight or Orbitrap analyzers acquire untargeted data on exact mass with high precision, and allow a gain in structural information on analytes of interest based on the fragmentation patterns. Thus, they permit to elucidate the molecular formula of compounds of interest, and its identification, without reference libraries. Since data have been acquired in full scan mode, NSO can also be retrospectively identified [228,229]. Possible combination of LC-HRMS with ion mobility spectrometry can provide an additional benefit on NSO and its metabolites identification. It is offering an additional dimension of separation with ion mobility data, completing accurate mass and fragmentation data already available, and increasing the specificity of the identification, including isomeric substances [229].

However, we are currently far from the routine use of high resolution techniques, and most of the clinical toxicology laboratories do not offer such equipment, requiring advanced expertise and financial investment.

Conclusion

The opioid epidemic currently observed in USA and Canada is driven by NSO use. This health issue is now emerging in Europe with fatal cases of NSO poisoning. Fifty NSO have been referenced by the EU early warning system on NPS. This class of opioids includes some potent agonists of μ-opioid receptors, up to 10,000 more potent than morphine for carfentanil, generating a high risk of fatal overdose. To fight against the spread of NPS including NSO, legislations have evolved, with laws based on the ban of groups of molecules instead of by molecules. As with others opioids, the clinical management of NSO overdose relies on naloxone. However, due to drug potency and half-life, higher doses of naloxone can be required for medical care. Addiction management is identical to classical opioids, relying on methadone and buprenorphine. Regarding NSO fatal overdoses, blood levels are highly variable; this is probably related to different levels of tolerance between individuals. Numerous analytical methods have been developed for NSO detection and quantification in blood, urine and hair. These methods rely on targeted approaches with a screening of known compounds and can miss newly marketed compounds. This point underlines the need to continuously increment homemade and shared libraries, and to use original methods like activity based immunoassay and combine it with HRMS in order to characterize these molecules.

Disclosure of interest

The authors declare that they have no competing interest.

References

[1] Frisoni P, Bacchio E, Bilel S, Talarico A, Gaudio RM, Barbieri M, et al. Novel Synthetic Opioids: The Pathologist's Point of View. Brain Sci 2018;8(9.).

[2] Seth P, Scholl L, Rudd RA, Bacon S. Overdose Deaths Involving Opioids, Cocaine, and Psychostimulants - United States, 2015-2016. Morb Mortal Wkly Rep 2018;67(12):349—58.

[3] Center for Disease Control. Overdose Deaths Involving Synthetic Opioids, 2018. Consulted on website https://www. cdc.gov/drugoverdose/data/fentanyl.html

© 2019 Elsevier Masson SAS. All rights reserved. - Document downloaded on 11/12/2019 by VAUCEL JULES (963264). It is forbidden and illegal to distribute this document.

on July 26 2019. Consulted on website https://www.cdc.gov/drugoverdose/data/fentanyl.html on July 26 2019.
[4] European Monitoring Centre for Drugs and Drug Addiction. European Drug Report 2018 - Trends and Developments, 2018. Consulted on website http://www.emcdda.europa.eu/system/files/publications.
[5] United Nations Office on Drugs and Crime. NPS (New psychoactive substances), 2014. Consulted on website https://www.unodc.org/documents/scientific/NPS_leaflet_2014_EN.pdf on July 26 2019.
[6] Seither J, Reidy L. Confirmation of Carfentanil. U-47700 and Other Synthetic Opioids in a Human Performance Case by LC-MS-MS. J Anal Toxicol 2017;41(6):493—7.
[7] Solimini R, Pichini S, Pacifici R, Busardò FP, Giorgetti R. Pharmacotoxicology of Non-fentanyl Derived New Synthetic Opioids. Front Pharmacol 2018;9:654.
[8] Suzuki J, El-Haddad S. A review: Fentanyl and non-pharmaceutical fentanyls. Drug Alcohol Depend 2017;171:107—16.
[9] United Nations Office on Drugs and Crime. What are NPS?, 2019. Consulted on website https://www.unodc.org/LSS/Page/NPS on June 17 2019.
[10] Code de la santé publique. Arrêté du 22 février 1990 fixant la liste des substances classées comme stupéfiants.
[11] Office Français des Drogues et des Toxicomanies. Nouveaux produits de synthèse - Dix ans de recul sur la situation française, 2018. Consulted on website https://www.ofdt.fr/publications/collections/periodiques/lettre-tendances/nouveaux-produits-de-synthese-dix-ans-de-recul-sur-la-situation-francaise-tendances-127-octobre-2018/ on July 26 2019.
[12] Government of Ireland. Electronic Irish Statute Book, 2010. Consulted on website http://www.irishstatutebook.ie/eli/2010/act/22/enacted/en/html on July 27, 2019.
[13] United Kingdom legislation. Psychoactive Substances Act, 2016. Consulted on website http://www.legislation.gov.uk/ukpga/2016/2/contents/enacted/data.htm on July 26 2019.
[14] Early Warning System on NPS. European monitoring centre for drugs and drug addiction, 2018. Consulted on website http://www.emcdda.europa.eu/publications/topic-overviews/eu-early-warning-system_en on July July 26 2019.
[15] European monitoring centre for drugs and drug addiction. European drug report, 2019. Consulted on website http://www.emcdda.europa.eu/system/files/publications/11364/20191724_TDAT19001ENN_PDF.pdf on July 26 2019.
[16] Karila L, Marillier M, Chaumette B, Billieux J, Franchitto N, Benyamina A. New synthetic opioids: Part of a new addiction landscape. Neurosci Biobehav Rev 2019;106:133—40.
[17] Prekupec MP, Mansky PA, Baumann MH. Misuse of Novel Synthetic Opioids: A Deadly New Trend. J Addict Med 2017;11(4):256—65.
[18] Agence Nationale de Sécurité du Médicament. Comité technique des centres d'Evaluation et d'Information sur la Pharmacodépendance - CT022017033, 2017. Consulted on website https://www.ansm. sante.fr/var/ansm_site/storage/original/application/0e7e5e89a7a37b615ae0fc5b86ecf2c1.pdf on July 26 2019.
[19] Ciccarone D, Ondocsin J, Mars S. Heroin uncertainties: exploring users' perceptions of fentanyl-adulterated and -substituted 'heroin'. Int J Drug Policy 2017;46:146—55.
[20] Gerace E, Salomone A, Vincenti M. Analytical Approaches in Fatal Intoxication Cases Involving New Synthetic Opioids. Curr Pharm Biotechnol 2018;19(2):113—23.
[21] Armenian P, Vo KT, Barr-Walker J, Lynch KL. Fentanyl, fentanyl analogs and novel synthetic opioids: A comprehensive review. Neuropharmacology 2018;134:121—32.
[22] Pérez-Mañá C, Papaseit E, Fonseca F, Farré A, Torrens M, Farré M. Drug interactions with new synthetic opioids. Front Pharmacol 2018;9:1145. Consulted on website https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6193107/on July 26 2019.
[23] Scholz J, Steinfath M, Schulz M. Clinical pharmacokinetics of alfentanil, fentanyl and sufentanil. An update. Clin Pharmacokinet 1996;31(4):275—92.
[24] Agence Nationale de Sécurité du Médicament. Résumé des caractéristiques du produit du remifentanil, 2012. Consulted on website http://agence-prd.ansm.sante.fr/php/ecodex/rcp/R0216301.htm on July 26 2019.
[25] Patton AL, Seely KA, Pulla S, Rusch NJ, Moran CL, Fantegrossi WE, et al. Quantitative measurement of acetyl fentanyl and acetyl norfentanyl in human urine by LC-MS/MS. Anal Chem 2014;86(3):1760—6.
[26] World Health Organization. Critical Review Report: Acryloylfentanyl, Expert Committee on Drug Dependence Thirty-ninth Meeting, Geneva, 2017. Consulted on website https://www.who.int/medicines/access/controlled-substances/CriticalReview_Acrylolyfentanyl.pdf?ua = 1 on July 26 2019.
[27] European Monitoring Centre for Drugs and Drug Addiction. EMCDDA—Europol Joint Report on a new psychoactive substance: N-(1-phenethylpiperidin-4-yl)-N-phenylacrylamide (acryloylfentanyl), Joint Reports, Publications Office of the European Union, Luxembourg, 2017. Consulted on website http://www.emcdda.europa.eu/system/files/publications/3873/TI_PUBPDF_TDAS17001ENN_PDFWEB_20170221105322.pdf on July 26 2019.
[28] Sato S, Suzuki S, Lee X-P, Sato K. Studies on 1-(2-phenethyl)-4-(N-propionylanilino)piperidine (fentanyl) and related compounds VII. Quantification of alpha-methylfentanyl metabolites excreted in rat urine. Forensic Sci Int 2010;195(1—3):68—72.
[29] Meyer MR, Dinger J, Schwaninger AE, Wissenbach DK, Zapp J, Fritschi G, et al. Qualitative studies on the metabolism and the toxicological detection of the fentanyl-derived designer drugs 3-methylfentanyl and isofentanyl in rats using liquid chromatography-linear ion trap-mass spectrometry (LC-MS(n)). Anal Bioanal Chem 2012;402(3):1249—55.
[30] Steuer AE, Williner E, Staeheli SN, Kraemer T. Studies on the metabolism of the fentanyl-derived designer drug butyrfentanyl in human in vitro liver preparations and authentic human samples using liquid chromatography-high resolution mass spectrometry (LC-HRMS). Drug Test Anal 2017;9(7):1085—92.
[31] Alburges ME, Hanson GR, Gibb JW, Sakashita CO, Rollins DE. Fentanyl receptor assay. II. Utilization of a radioreceptor assay for the analysis of fentanyl analogs in urine. J Anal Toxicol 1992;16(1):36—41.
[32] European Monitoring Centre for Drugs and Drug Addiction. EMCDDA—Europol Joint Report on a new psychoactive substance: methyl 1-(2-phenylethyl)-4-[phenyl(propanoyl)amino]piperidine-4-carboxylate (carfentanil), Joint Reports, Publications Office of the European Union, Luxembourg, 2017. Consulted on website http://www.emcdda.europa.eu/system/files/publications/6502/2017.6256_EN_04-WEB.pdf on July 26 2019.
[33] Feasel MG, Wohlfarth A, Nilles JM, Pang S, Kristovich RL, Huestis MA. Metabolism of Carfentanil, an Ultra-Potent Opioid, in Human Liver Microsomes and Human Hepatocytes by

© 2019 Elsevier Masson SAS. All rights reserved. - Document downloaded on 11/12/2019 by VAUCEL JULES (963264). It is forbidden and illegal to distribute this document.

High-Resolution Mass Spectrometry. Am Assoc Pharmac Sci J 2019;18:1489—99.
[34] European Monitoring Centre for Drugs and Drug Addiction. EMCDDA—Europol Joint Report on a new psychoactive substance: N-phenyl-N-[1-(2-phenylethyl)piperidin-4-yl] cyclopropanecarboxamide (cyclopropylfentanyl), Joint Reports, Publications Office of the European Union, Luxembourg, 2017. Consulted on website http://www.emcdda.europa.eu/system/files/publications/7926/20181014_TDAS18001ENN_PDF.pdf on July 26 2019.
[35] European Monitoring Centre for Drugs and Drug Addiction. EMCDDA—Europol Joint Report on a new psychoactive substance: N-(4-fluorophenyl)-N-(1-phenethylpiperidin-4-yl)isobutyramide (4-fluoroisobutyrylfentanyl; 4F-iBF), Joint Reports, Publications Office of the European Union, Luxembourg, 2017. Consulted on website http://www.emcdda.europa.eu/system/files/publications/5482/2017.4967_TDAS17007ENN_PDFWEB.pdf on June 17 2019.
[36] World Health Organization. Critical Review Report: Furanylfentanyl, Expert Committee on Drug Dependence Thirty-ninth Meeting, Geneva, 2017. Consulted on website https://www.who.int/medicines/access/controlled-substances/Critical_Review_FuranylFentanyl.pdf on June 17 2019.
[37] Goggin MM, Nguyen A, Janis GC. Identification of Unique Metabolites of the Designer Opioid Furanyl Fentanyl. J Anal Toxicol 2017;41(5):367—75.
[38] World Health Organization. Critical Review Report: Methoxyacetylfentanyl, Expert Committee on Drug Dependence Forty-first Meeting, Geneva, 2018. Consulted on website https://www.who.int/medicines/access/controlled-substances/Methoxyacetylfentanyl.pdf on June 17 2019.
[39] European Monitoring Centre for Drugs and Drug Addiction. EMCDDA—Europol Joint Report on a new psychoactive substance: 2-methoxy-N-phenyl-N-[1-(2-phenylethyl) piperidin-4-yl]acetamide (methoxyacetylfentanyl), Joint Reports, Publications Office of the European Union, Luxembourg, 2017. Consulted on website http://www.emcdda.europa.eu/system/files/publications/7925/20181015_TDAS18002ENN_PDF.pdf on June 17 2019.
[40] World Health Organization. Critical Review Report: p-Methoxy-butyrylfentanyl, Expert Committee on Drug Dependence Forty-first Meeting, Geneva, 2018. Consulted on website https://www.who.int/medicines/access/controlled-substances/Paramethoxybutyrfentanyl.pdf on June 17 2019.
[41] Quintana P, Ventura M, Grifell M, Palma A, Galindo L, Fornís I, et al. The hidden web and the fentanyl problem: Detection of ocfentanil as an adulterant in heroin. Int J Drug Policy 2017;40:78—83.
[42] World Health Organization. Critical Review Report: Ocfentanil, Expert Committee on Drug Dependence Thirty-ninth Meeting, Geneva, 2017. Consulted on website https://www.who.int/medicines/access/controlled-substances/CriticalReview_Ocfentanil.pdf on June 17 2019.
[43] World Health Organization. Critical Review Report: Tetrahydrofuranylfentanyl, Expert Committee on Drug Dependence Thirty-ninth Meeting, Geneva, 2017. Consulted on website https://www.who.int/medicines/access/controlled-substances/CriticalReview_THFF.pdf?ua=1 on June 17 2019.
[44] European Monitoring Centre for Drugs and Drug Addiction. EMCDDA—Europol Joint Report on a new psychoactive substance: N-phenyl-N-[1-(2-phenylethyl)piperidin-4-yl]tetrahydrofuran-2-carboxamide (tetrahydrofuranylfentanyl; THF-F), Joint Reports, Publications Office of the European Union, Luxembourg, 2017. Consulted on website http://www.emcdda.europa.eu/system/files/publications/5487/2017.4968_TDAS17008ENN_PDFWEB.pdf on June 17 2019.
[45] Ventura L, Carvalho F, Dinis-Oliveira RJ. Opioids in the Frame of New Psychoactive Substances Network: A Complex Pharmacological and Toxicological Issue. Curr Mol Pharmacol 2018;11(2):97—108.
[46] World Health Organization. Critical Review Report: Furanylfentanyl, Expert Committee on Drug Dependence Thirty-sixth Meeting, Geneva, 2014. Consulted on website https://www.who.int/medicines/areas/quality_safety/4_21_review.pdf on June 17 20199.
[47] Ruan X, Chiravuri S, Kaye AD. Comparing fatal cases involving U-47700. Forensic Sci Med Pathol 2016;12(3):369—71.
[48] World Health Organization. Critical Review Report: U-47700, Expert Committee on Drug Dependence Thirty-eighth Meeting, Geneva, 2016. Consulted on website https://www.who.int/medicines/access/controlled-substances/4.1_U-47700_CritReview.pdf on June 17 2019.
[49] Rambaran KA, Fleming SW, An J, Burkhart S, Furmaga J, Kleinschmidt KC, et al. U-47700: A Clinical Review of the Literature. J Emerg Med 2017;53(4):509—19.
[50] World Health Organization. Critical Review Report: MT-45, Expert Committee on Drug Dependence Thirty-seventh Meeting, Geneva, 2017. Consulted on website: https://www.who.int/medicines/access/controlled-substances/5.1_MT-45_CRev.pdf on June 17 2019.
[51] Siddiqi S, Verney C, Dargan P, Wood DM. Understanding the availability, prevalence of use, desired effects, acute toxicity and dependence potential of the novel opioid MT-45. Clin Toxicol 2015;53(1):54—9.
[52] European Monitoring Centre for Drugs and Drug Addiction. Report on the risk assessment of 1-cyclohexyl-4-(1,2-diphenylethyl)piperazine (MT-45) in the framework of the Council Decision on new psychoactive substances, Risk Assessments, Publications Office of the European Union, Luxembourg, 2014. Consulted on website http://www.emcdda.europa.eu/system/files/publications/1865/TDAK14006ENN.pdf on June 17 2019.
[53] Opioids, Analgesia, Pain, Management. Goodman and Gilman's The Pharmacological Basis of Therapeutics, Twelfth Edition Brunton Laurence L; 2011.
[54] Maguire P, Tsai N, Kamal J, Cometta-Morini C, Upton C, Loew G. Pharmacological profiles of fentanyl analogs at mu, delta and kappa opiate receptors. Eur J Pharmacol 1992;213(2):219—25.
[55] Pattinson KTS. Opioids and the control of respiration. Br J Anaesth 2008;100(6):747—58.
[56] Beaulieu P. Les opioïdes. In: La douleur Guide pharmacologique et thérapeutique. Maloine, Montréal; 2016 [DOI: 10.1016/j.phclin.2016.01.004].
[57] Concheiro M, Chesser R, Pardi J, Cooper G. Postmortem Toxicology of New Synthetic Opioids. Front Pharmacol 2018;26(9):1210.
[58] Leen JLS, Juurlink DN. Carfentanil: a narrative review of its pharmacology and public health concerns. Can J Anesth Can Anesth 2019;66(4):414—21.
[59] Meyer MR. New psychoactive substances: an overview on recent publications on their toxicodynamics and toxicokinetics. Arch Toxicol 2016;90(10):2421—44.
[60] Subramanian G, Paterlini MG, Portoghese PS, Ferguson DM. Molecular docking reveals a novel binding site model for fentanyl at the mu-opioid receptor. J Med Chem 2000;43(3):381—91.
[61] United Nations Office on Drugs and Crime. Global smart update. Fentanyl and its analogues - 50 years on, 2017.

© 2019 Elsevier Masson SAS.All rights reserved. - Document downloaded on 11/12/2019 by VAUCEL JULES (963264). It is forbidden and illegal to distribute this document.

Consulted on website https://www.unodc.org/documents/scientific/Global_SMART_Update_17_web.pdf on June 17 2019.
[62] Arens AM, van Wijk XMR, Vo KT, Lynch KL, Wu AHB, Smollin CG. Adverse Effects From Counterfeit Alprazolam Tablets. JAMA Intern Med 2016;176(10):1554—5.
[63] Sutter ME, Gerona RR, Davis MT, Roche BM, Colby DK, Chenoweth JA, et al. Fatal Fentanyl: One Pill Can Kill. Acad Emerg Med 2017;24(1):106—13.
[64] Helander A, Bäckberg M, Beck O. MT-45, a new psychoactive substance associated with hearing loss and unconsciousness. Clin Toxicol Phila Pa 2014;52(8):901—4.
[65] Lindeman E, Bäckberg M, Personne M, Helander A. MT-45-a dangerous and potentially ototoxic internet drug. Lakartidningen 2014;111(40):1712—5.
[66] Reynolds L, Rauck R, Webster L, DuPen S, Heinze E, Portenoy R, et al. Relative analgesic potency of fentanyl and sufentanil during intermediate-term infusions in patients after long-term opioid treatment for chronic pain. Pain 2004;110(1):182—8.
[67] Vardanyan RS, Hruby VJ. Fentanyl-related compounds and derivatives: current status and future prospects for pharmaceutical applications. Future Med Chem 2014;6(4):385—412.
[68] Halliburton JR. The pharmacokinetics of fentanyl, sufentanil and alfentanil: a comparative review. Am Assoc Nurse Anesth J 1988;56(3):229—33.
[69] Glass PS, Iselin-Chaves IA, Goodman D, Delong E, Hermann DJ. Determination of the potency of remifentanil compared with alfentanil using ventilatory depression as the measure of opioid effect. Anesthesiology 1999;90(6):1556—63.
[70] Higashikawa Y, Suzuki S. Studies on 1-(2-phenethyl)-4-(N-propionylanilino)piperidine (fentanyl) and its related compounds VI. Structure-analgesic activity relationship for fentanyl, methyl-substituted fentanyls and other analogues. Forensic Toxicol 2008;26(1):1—5.
[71] Ujváry I, Jorge R, Christie R, Le Ruez T, Danielsson HV, Kronstrand R, et al. Acryloylfentanyl, a recently emerged new psychoactive substance: a comprehensive review. Forensic Toxicol 2017;35(2):232—43.
[72] Jannetto PJ, Helander A, Garg U, Janis GC, Goldberger B, Ketha H. The Fentanyl Epidemic and Evolution of Fentanyl Analogs in the United States and the European Union. Clin Chem 2019;65(2):242—53.
[73] Jin WQ, Xu H, Zhu YC, Fang SN, Xia XL, Huang ZM, et al. Studies on synthesis and relationship between analgesic activity and receptor affinity for 3-methyl fentanyl derivatives. Sci Sin 1981;24(5):710—20.
[74] European Monitoring Centre for Drugs and Drug Addiction. Fentanils and synthetic cannabinoids: driving greater complexity into the drug situation, 2018. Consulted on website http://www.emcdda.europa.eu/publications/rapid-communications/fentanils-and-synthetic-cannabinoids-ews-update_en on June 17 2019.
[75] Fletcher JE, Sebel PS, Murphy MR, Mick SA, Fein S. Comparison of ocfentanil and fentanyl as supplements to general anesthesia. Anesth Analg 1991;73(5):622—6.
[76] Coopman V, Blanckaert P, Van Parys G, Van Calenbergh S, Cordonnier J. A case of acute intoxication due to combined use of fentanyl and 3,4-dichloro-N-[2-(dimethylamino)cyclohexyl]-N-methylbenzamide (U-47700). Forensic Sci Int 2016;266:68—72.
[77] Domanski K, Kleinschmidt KC, Schulte JM, Fleming S, Frazee C, Menendez A, et al. Two cases of intoxication with new synthetic opioid U-47700. Clin Toxicol 2017;55(1):46—50.
[78] Harper NJ, Veitch GB, Wibberley DG. 1-(3,4-Dichlorobenzamidomethyl)cyclohexyldimethylamine and related compounds as potential analgesics. J Med Chem 1974;17(11):1188—93.
[79] Hayes AnnG, Tyers MichaelB. Determination of receptors that mediate opiate side effects in the mouse. Br J Pharmacol 1983;79(3):731—6.
[80] Zawilska JB, Andrzejczak D. Next generation of novel psychoactive substances on the horizon—A complex problem to face. Drug Alcohol Depend 2015;157:1—17.
[81] Baumann MH, Majumdar S, Le Rouzic V, Hunkele A, Uprety R, Huang XP, et al. Pharmacological characterization of novel synthetic opioids (NSO) found in the recreational drug marketplace. Neuropharmacology 2018;134:101—7.
[82] Logan BK, Mohr ALA, Friscia M, Krotulski AJ, Papsun DM, Kacinko SL, et al. Reports of Adverse Events Associated with Use of Novel Psychoactive Substances, 2013-2016: A Review. J Anal Toxicol 2017;41(7):573—610.
[83] Papsun D, Krywanczyk A, Vose JC, Bundock EA, Logan BK. Analysis of MT-45, a Novel Synthetic Opioid, in Human Whole Blood by LC—MS-MS and Its Identification in a Drug-Related Death. J Anal Toxicol 2016;40(4):313—7.
[84] Kuczyńska K, Grzonkowski P, Kacprzak Ł, Zawilska JB. Abuse of fentanyl: An emerging problem to face. Forensic Sci 2018;289:207—14.
[85] Larocque A. Protocole de traitement de l'intoxication aigüe aux opioides. Bull D'information Toxicol 2012;28(4):34—43.
[86] Pérez-Mañá C, Papaseit E, Fonseca F, Farré A, Torrens M, Farré M. Drug Interactions With New Synthetic Opioids. Front Pharmacol 2018;9:1145.
[87] Kieffer BL, Opioids:. first lessons from knockout mice. Trends Pharmacol Sci 1999;20(1):19—26.
[88] Benyamin R, Trescot AM, Datta S, Buenaventura R, Adlaka R, Sehgal N, et al. Opioid complications and side effects. Pain Physician 2008;11:S105—20.
[89] Arthur J, Hui D. Safe Opioid Use: Management of Opioid-Related Adverse Effects and Aberrant Behaviors. Hematol Oncol Clin North Am 2018;32(3):387—403.
[90] Lemon TI. Homemade heroin substitute causing hallucinations. Afr J Psychiatry 2013;16(6).
[91] Helander A, Bradley M, Hasselblad A, Norlén L, Vassilaki I, Bäckberg M, et al. Acute skin and hair symptoms followed by severe, delayed eye complications in subjects using the synthetic opioid MT-45. Br J Dermatol 2017;176(4):1021—7.
[92] Denton JS, Donoghue ER, McReynolds J, Kalelkar MB. An epidemic of illicit fentanyl deaths in Cook County, Illinois: September 2005 through April 2007. J Forensic Sci 2008;53(2):452—4.
[93] Cunningham SM, Haikal NA, Kraner JC. Fatal Intoxication with Acetyl Fentanyl. J Forensic Sci 2016;61:S276—80.
[94] Staeheli SN, Baumgartner MR, Gauthier S, Gascho D, Jarmer J, Kraemer T, et al. Time-dependent postmortem redistribution of butyrfentanyl and its metabolites in blood and alternative matrices in a case of butyrfentanyl intoxication. Forensic Sci Int 2016;266(170):7.
[95] Guerrieri D, Rapp E, Roman M, Druid H, Kronstrand R. Postmortem and Toxicological Findings in a Series of Furanylfentanyl-Related Deaths. J Anal Toxicol 2017;41(3):242—9.
[96] Dussy FE, Hangartner S, Hamberg C, Berchtold C, Scherer U, Schlotterbeck G, et al. An Acute Ocfentanil Fatality: A Case Report with Postmortem Concentrations. J Anal Toxicol 2016;40(9):761—6.
[97] Vorce SP, Knittel JL, Holler JM, Magluilo J, Levine B, Berran P, et al. A fatality involving AH-7921. J Anal Toxicol 2014;38(4):226—30.
[98] Fleming SW, Cooley JC, Johnson L, Frazee CC, Domanski K, Kleinschmidt K, et al. Analysis of U-47700, a Novel Synthetic Opioid, in Human Urine by LC-MS-MS and LC-QToF. J Anal Toxicol 2017;41(3):173—80.

© 2019 Elsevier Masson SAS.All rights reserved. - Document downloaded on 11/12/2019 by VAUCEL JULES (963264). It is forbidden and illegal to distribute this document.

[99] Fels H, Krueger J, Sachs H, Musshoff F, Graw M, Roider G, et al. Two fatalities associated with synthetic opioids: AH-7921 and MT-45. Forensic Sci Int 2017;277:e30—5.
[100] Foy L, Seeyave DM, Bradin SA. Toxic leukoencephalopathy due to transdermal fentanyl overdose. Pediatr Emerg Care 2011;27(9):854—6.
[101] Somerville NJ, O'Donnell J, Gladden RM, Zibbell JE, Green TC, Younkin M, et al. Characteristics of Fentanyl Overdose — Massachusetts, 2014-2016. MMWR Morb Mortal Wkly Rep 2017;66(14):382—6.
[102] Helander A, Bäckberg M, Beck O. Intoxications involving the fentanyl analogs acetylfentanyl, 4-methoxybutyrfentanyl and furanylfentanyl: results from the Swedish STRIDA project. Clin Toxicol Phila Pa 2016;54(4):324—32.
[103] Takase I, Koizumi T, Fujimoto I, Yanai A, Fujimiya T. An autopsy case of acetyl fentanyl intoxication caused by insufflation of ''designer drugs''. Leg Med Tokyo Jpn 2016;21:38—44.
[104] Coopman V, Cordonnier J, De Leeuw M, Cirimele V. Ocfentanil overdose fatality in the recreational drug scene. Forensic Sci Int 2016;266:469—73.
[105] Coopman V, Cordonnier J, Pien K, Van Varenbergh D. LC-MS/MS analysis of fentanyl and norfentanyl in a fatality due to application of multiple Durogesic transdermal therapeutic systems. Forensic Sci Int 2007;169(2—3):223—7.
[106] Thomas S, Winecker R, Pestaner JP. Unusual fentanyl patch administration. Am J Forensic Med Pathol 2008;29(2):162—3.
[107] McIntyre IM, Trochta A, Gary RD, Wright J, Mena O. An Acute Butyr-Fentanyl Fatality: A Case Report with Postmortem Concentrations. J Anal Toxicol 2016;40(2):162—6.
[108] Ojanperä I, Gergov M, Liiv M, Riikoja A, Vuori E. An epidemic of fatal 3-methylfentanyl poisoning in Estonia. Int J Legal Med 2008;122(5):395—400.
[109] Poklis J, Poklis A, Wolf C, Hathaway C, Arbefeville E, Chrostowski L, et al. Two Fatal Intoxications Involving Butyryl Fentanyl. J Anal Toxicol 2016;40(8):703—8.
[110] Yonemitsu K, Sasao A, Mishima S, Ohtsu Y, Nishitani Y. A fatal poisoning case by intravenous injection of « bath salts » containing acetyl fentanyl and 4-methoxy PV8. Forensic Sci Int 2016;267:6—9.
[111] Biedrzycki OJ, Bevan D, Lucas S. Fatal overdose due to prescription fentanyl patches in a patient with sickle cell/beta-thalassemia and acute chest syndrome: A case report and review of the literature. Am J Forensic Med Pathol 2009;30(2):188—90.
[112] Chaturvedi AK, Rao NG, Baird JR. A death due to self-administered fentanyl. J Anal Toxicol 1990;14(6):385—7.
[113] Burns G, DeRienz RT, Baker DD, Casavant M, Spiller HA. Could chest wall rigidity be a factor in rapid death from illicit fentanyl abuse? Clin Toxicol Phila Pa 2016;54(5):420—3.
[114] Lilleng PK, Mehlum LI, Bachs L, Morild I. Deaths after intravenous misuse of transdermal fentanyl. J Forensic Sci 2004;49(6):1364—6.
[115] Kronstrand R, Druid H, Holmgren P, Rajs J. A cluster of fentanyl-related deaths among drug addicts in Sweden. Forensic Sci Int 1997;88(3):185—95.
[116] Ruzycki S, Yarema M, Dunham M, Sadrzadeh H, Tremblay A. Intranasal Fentanyl Intoxication Leading to Diffuse Alveolar Hemorrhage. J Med Toxicol 2016;12(2):185—8.
[117] King NB, Fraser V, Boikos C, Richardson R, Harper S. Determinants of increased opioid-related mortality in the United States and Canada, 1990—2013: a systematic review. Am J Public Health 2014;104(8):e32—42.
[118] Dart RC, Surratt HL, Cicero TJ, Parrino MW, Severtson SG, Bucher-Bartelson B, et al. Trends in opioid analgesic abuse and mortality in the United States. N Engl J Med 2015;372(3):241—8.
[119] Kucuk HO, Kucuk U, Kolcu Z, Balta S, Demirkol S. Misuse of fentanyl transdermal patch mixed with acute coronary syndrome. Hum Exp Toxicol 2016;35(1):51—2.
[120] Swegle JM, Logemann C. Management of common opioid-induced adverse effects. Am Fam Physician 2006;74(8):1347—54.
[121] Bell TJ, Panchal SJ, Miaskowski C, Bolge SC, Milanova T, Williamson R. The prevalence, severity, and impact of opioid-induced bowel dysfunction: results of a US and European Patient Survey (PROBE 1). Pain Med Malden Mass 2009;10(1):35—42.
[122] Brock C, Olesen SS, Olesen AE, Frøkjaer JB, Andresen T, Drewes AM. Opioid-induced bowel dysfunction: pathophysiology and management. Drugs 2012;72(14):1847—65.
[123] Wood JD, Galligan JJ. Function of opioids in the enteric nervous system. Neurogastroenterol Motil 2004;16:17—28.
[124] Tuteja AK, Biskupiak J, Stoddard GJ, Lipman AG. Opioid-induced bowel disorders and narcotic bowel syndrome in patients with chronic non-cancer pain. Neurogastroenterol Motil 2010;22(4):424—30 [e96].
[125] Moore RA, McQuay HJ. Prevalence of opioid adverse events in chronic non-malignant pain: systematic review of randomised trials of oral opioids. Arthritis Res Ther 2005;7(5):R1046—51.
[126] Jones MJ, Hernandez BS, Janis GC, Stellpflug SJ. A case of U-47700 overdose with laboratory confirmation and metabolite identification. Clin Toxicol Phila Pa 2017;55(1):55—9.
[127] Khademi H, Kamangar F, Brennan P, Malekzadeh R. Opioid Therapy and its Side Effects: A Review. Arch Iran Med 2016;19(12):870—6.
[128] Sharma SS. Sphincter of Oddi dysfunction in patients addicted to opium: an unrecognized entity. Gastrointest Endosc 2002;55(3):427—30.
[129] Sinicina I, Sachs H, Keil W. Post-mortem review of fentanyl-related overdose deaths among identified drug users in Southern Bavaria. Germany, 2005—2014. Drug Alcohol Depend 2017;180:286—91.
[130] Daniell HW. Hypogonadism in men consuming sustained-action oral opioids. J Pain 2002;3(5):377—84.
[131] Daniell HW. Opioid endocrinopathy in women consuming prescribed sustained-action opioids for control of nonmalignant pain. J Pain 2008;9(1):28—36.
[132] Daniell HW. DHEAS deficiency during consumption of sustained-action prescribed opioids: evidence for opioid-induced inhibition of adrenal androgen production. J Pain 2006;7(12):901—7.
[133] Vuong C, Van Uum SHM, O'Dell LE, Lutfy K, Friedman TC. The effects of opioids and opioid analogs on animal and human endocrine systems. Endocr Rev 2010;31(1):98—132.
[134] Lugoboni F, Zamboni L, Federico A, Tamburin S. Gruppo Inter-SERT di Collaborazione Scientifica (GICS) Erectile dysfunction and quality of life in men receiving methadone or buprenorphine maintenance treatment. A cross-sectional multicentre study. PloS One 2017;12(11):e0188994.
[135] Sacerdote P, Manfredi B, Mantegazza P, Panerai AE. Antinociceptive and immunosuppressive effects of opiate drugs: a structure-related activity study. Br J Pharmacol 1997;121(4):834—40.
[136] Kamangar F, Shakeri R, Malekzadeh R, Islami F. Opium use: an emerging risk factor for cancer? Lancet Oncol 2014;15(2):e69—77.
[137] Shakeri R, Kamangar F, Mohamadnejad M, Tabrizi R, Zamani F, Mohamadkhani A, et al. Opium use, cigarette smoking, and alcohol consumption in relation to pancreatic cancer. Medicine (Baltimore) 2016;95(28):e3922.
[138] Green TC, Gilbert M. Counterfeit Medications and Fentanyl. JAMA Intern Med 2016;176(10):1555—7.

© 2019 Elsevier Masson SAS.All rights reserved. - Document downloaded on 11/12/2019 by VAUCEL JULES (963264). It is forbidden and illegal to distribute this document.

[139] Kim HK, Nelson LS. Reducing the harm of opioid overdose with the safe use of naloxone: a pharmacologic review. Expert Opin Drug Saf 2015;14(7):1137—46.
[140] Levine JD, Gordon NC, Fields HL. Naloxone dose dependently produces analgesia and hyperalgesia in postoperative pain. Nature 1979;278(5706):740—1.
[141] Daniulaityte R, Juhascik MP, Strayer KE, Sizemore IE, Harshbarger KE, Antonides HM, et al. Overdose Deaths Related to Fentanyl and Its Analogs - Ohio January—February 2017. MMWR Morb Mortal Wkly Rep 2017;66(34):904—8.
[142] Helander A, Bäckberg M, Signell P, Beck O. Intoxications involving acrylfentanyl and other novel designer fentanyls—results from the Swedish STRIDA project. Clin Toxicol 2017;55(6):589—99.
[143] Rogers JS, Rehrer SJ, Hoot NR. Acetylfentanyl: An Emerging Drug of Abuse. J Emerg Med 2016;50:433—6.
[144] McDonald R, Lorch U, Woodward J, Bosse B, Dooner H, Mundin G, et al. Pharmacokinetics of concentrated naloxone nasal spray for opioid overdose reversal: Phase I healthy volunteer study. Addict Abingdon Engl 2018;113(3):484—93.
[145] Schumann H, Erickson T, Thompson TM, Zautcke JL, Denton JS. Fentanyl epidemic in Chicago Illinois and surrounding Cook County. Clin Toxicol Phila Pa 2008;46(6):501—6.
[146] Gussow L. Toxicology Rounds: Who Said the Opioid Crisis Couldn't Get Any Worse? Emerg Med News 2016;38(11):1.
[147] Armenian P, Olson A, Anaya A, Kurtz A, Ruegner R, Gerona RR. Fentanyl and a Novel Synthetic Opioid U-47700 Masquerading as Street « Norco » in Central California: A Case Report. Ann Emerg Med 2017;69(1):87—90.
[148] Schneir A, Metushi IG, Sloane C, Benaron DJ, Fitzgerald RL. Near death from a novel synthetic opioid labeled U-47700: emergence of a new opioid class. Clin Toxicol 2017;55(1):51—4.
[149] Novel Psychoactive Treatment UK Network. Guidance on the Clinical Management of Acute and Chronic Harms of Club Drugs and Novel Psychoactive Substances; 2015. Consulted on website http://neptune-clinical-guidance.co.uk/wp-content/uploads/2015/03/NEPTUNE-Guidance-March-2015.pdf on July 17 2019.
[150] Zawilska JB. An Expanding World of Novel Psychoactive Substances: Opioids. Front Psychiatry 2017;8:110.
[151] Gupta R, Shah ND, Ross JS. The Rising Price of Naloxone - Risks to Efforts to Stem Overdose Deaths. N Engl J Med 2016;375(23):2213—5.
[152] World Health Organization. Naloxone: A take-home antidote to drug overdose that saves lives, 2019. Consulted on website https://www.who.int/features/2014/naloxone/en/on July 17 2019.
[153] Willman MW, Liss DB, Schwarz ES, Mullins ME. Do heroin overdose patients require observation after receiving naloxone? Clin Toxicol 2017;55(2):81—7.
[154] Darke S, Ross J. Suicide among heroin users: rates, risk factors and methods. Addict Abingdon Engl 2002;97(11):1383—94.
[155] Noble F, Marie N. Management of Opioid Addiction With Opioid Substitution Treatments: Beyond Methadone and Buprenorphine. Front Psychiatry 2019;9:742.
[156] Mattick RP, Breen C, Kimber J, Davoli M. Buprenorphine maintenance versus placebo or methadone maintenance for opioid dependence. Cochrane Drugs and Alcohol Group, éditeur. Cochrane Database Syst Rev 2014;6(2):CD002207.
[157] Vormfelde S, Poser W. Death Attributed to Methadone. Pharmacopsychiatry 2001;34(06):217—22.
[158] Maremmani I, Gerra G. Buprenorphine-Based Regimens and Methadone for the Medical Management of Opioid Dependence: Selecting the Appropriate Drug for Treatment: Choosing Buprenorphine or Methadone for Therapy. Am J Addict 2010;19(6):557—68.
[159] Yassen A, Olofsen E, Romberg R, Sarton E, Teppema L, Danhof M, et al. Mechanism-based PK/PD Modeling of the Respiratory Depressant Effect of Buprenorphine and Fentanyl in Healthy Volunteers. Clin Pharmacol Ther 2007;81(1):50—8.
[160] Megarbane B, Marie N, Pirnay S, Borron S, Gueye P, Risede P, et al. Buprenorphine is protective against the depressive effects of norbuprenorphine on ventilation. Toxicol Appl Pharmacol 2006;212(3):256—67.
[161] Raffa RB, Haidery M, Huang H-M, Kalladeen K, Lockstein DE, Ono H, et al. The clinical analgesic efficacy of buprenorphine. J Clin Pharm Ther 2014;39(6):577—83.
[162] Dahan A, Yassen A, Romberg R, Sarton E, Teppema L, Olofsen E, et al. Buprenorphine induces ceiling in respiratory depression but not in analgesia. Br J Anaesth 2006;96(5):627—32.
[163] Mendelson J. Clinical and pharmacological evaluation of buprenorphine and naloxone combinations: why the 4:1 ratio for treatment? Drug Alcohol Depend 2003;70(2):S29—37.
[164] Comer SD, Walker EA, Collins ED. Buprenorphine/naloxone reduces the reinforcing and subjective effects of heroin in heroin-dependent volunteers. Psychopharmacology (Berl) 2018;81:306—32.
[165] Pujol CN, Paasche C, Laprevote V, Trojak B, Vidailhet P, Bacon E, et al. Cognitive effects of labeled addictolytic medications. Prog Neuropsychopharmacol Biol Psychiatry 2018;81:306—32.
[166] Gerra G, Manfredini M, Somaini L, Maremmani I, Leonardi C, Donnini C. Sexual Dysfunction in Men Receiving Methadone Maintenance Treatment: Clinical History and Psychobiological Correlates. Eur Addict Res 2016;22(3):163—75.
[167] Yee A, Loh HS, Hisham Hashim HMB, Ng CG. Clinical factors associated with sexual dysfunction among men in methadone maintenance treatment and buprenorphine maintenance treatment: a meta-analysis study. Int J Impot Res 2014;26(5):161—6.
[168] Chang Y-P, Compton P, Almeter P, Fox CH. The Effect of Motivational Interviewing on Prescription Opioid Adherence Among Older Adults With Chronic Pain: The Effect of Motivational Interviewing to Enhance Prescription Opioid Adherence Among Older Adults With Chronic Pain. Perspect Psychiatr Care 2015;51(3):211—9.
[169] Mumba MN, Findlay LJ, Snow DE. Treatment Options for Opioid Use Disorders: A Review of the Relevant Literature. J Addict Nurs 2018;29(3):221—5.
[170] Waldron HB, Kaminer Y. On the learning curve: the emerging evidence supporting cognitive—behavioral therapies for adolescent substance abuse. Addiction 2004;99:93—105.
[171] Schulz M, Iwersen-Bergmann S, Andresen H, Schmoldt A. Therapeutic and toxic blood concentrations of nearly 1,000 drugs and other xenobiotics. Crit Care 2012;16(4):R136.
[172] Concheiro M, Chesser R, Pardi J, Cooper G. Postmortem Toxicology of New Synthetic Opioids. Front Pharmacol 2018;9:1210.
[173] Frisoni P, Bacchio E, Bilel S, Talarico A, Gaudio R, Barbieri M, et al. Novel Synthetic Opioids: The Pathologist's Point of View. Brain Sci 2018;8(9):170.
[174] Watanabe S, Vikingsson S, Roman M, Green H, Kronstrand R, Wohlfarth A. In Vitro and In Vivo Metabolite Identification Studies for the New Synthetic Opioids Acetylfentanyl, Acrylfentanyl, Furanylfentanyl, and 4-Fluoro-Isobutyrylfentanyl. Am Assoc Pharmac Sci J 2017;19(4):1102—22.
[175] Kim J, Ji D, Kang S, Park M, Yang W, Kim E, et al. Simultaneous determination of 18 abused opioids and metabolites in human hair using LC—MS/MS and illegal opioids abuse proven by hair analysis. J Pharm Biomed Anal 2014;89:99—105.
[176] Vogliardi S, Stocchero G, Maietti S, Tucci M, Nalesso A, Snenghi R, et al. Non-fatal Overdose with U-47700: Identification in Biological Matrices. Curr Pharm Biotechnol 2018;19(2):180—7.

© 2019 Elsevier Masson SAS.All rights reserved. - Document downloaded on 11/12/2019 by VAUCEL JULES (963264). It is forbidden and illegal to distribute this document.

[177] Larabi IA, Martin M, Fabresse N, Etting I, Edel Y, Pfau G, et al. Hair testing for 3-fluorofentanyl, methoxyacetylfentanyl, carfentanil, acetylfentanyl and fentanyl by LC/MS/MS after unintentional overdose. Forensic Toxicol 2019, http://dx.doi.org/10.1007/s11419-019-00502-0.
[178] Salomone A, Palamar JJ, Bigiarini R, Gerace E, Corcia DD, Vincenti M. Detection of Fentanyl Analogs and Synthetic Opioids in Real Hair Samples. J Anal Toxicol 2018;43(4):259—65.
[179] Busardò FP, Carlier J, Giorgetti R, Tagliabracci A, Pacifici R, Gottardi M, et al. Ultra-High-Performance Liquid Chromatography-Tandem Mass Spectrometry Assay for Quantifying Fentanyl and 22 Analogs and Metabolites in Whole Blood. Urine, and Hair. Front Chem 2019;7:184.
[180] Gerace E, Salomone A, Luciano C, Di Corcia D, Vincenti M. First Case in Italy of Fatal Intoxication Involving the New Opioid U-47700. Front Pharmacol 2018;9:747.
[181] Pötsch L. A discourse on human hair fibers and reflections on the conservation of drug molecules. Int J Legal Med 1996;108(6):285—93.
[182] Palamar JJ, Salomone A, Bigiarini R, Vincenti M, Acosta P, Tofighi B. Testing hair for fentanyl exposure: a method to inform harm reduction behavior among individuals who use heroin. Am J Drug Alcohol Abuse 2019;45:1—7.
[183] Gaulier J-M, Richeval C, Phanithavong M, Brault S, Allorge D, Dumestre-Toulet V. A case report of carfentanil-related fatality in France. Tox Anal Clin 2019, http://dx.doi.org/10.1016/j.toxac.2019.01.002.
[184] Wille SMR, Samyn N, Ramírez-Fernández M, del M, De Boeck G. Evaluation of on-site oral fluid screening using Drugwipe-5+®, RapidSTAT® and Drug Test 5000® for the detection of drugs of abuse in drivers. Forensic Sci Int 2010;198(1—3):2—6.
[185] European Monitoring Centre for Drugs and Drug Addiction. European Drug Report 2018: Trends and Developments, Publications Office of the European Union, Luxembourg, 2018. Consulted on website http://www.emcdda.europa.eu/system/files/publications/8585/20181816_TDAT18001ENN_PDF.pdf on July 17 2019.
[186] Richeval C, Wille SMR, Nachon-Phanithavong M, Samyn N, Allorge D, Gaulier J. New psychoactive substances in oral fluid of French and Belgian drivers in 2016. Int J Drug Policy 2018;57:1—3.
[187] Sofalvi S, Schueler HE, Lavins ES, Kaspar CK, Brooker IT, Mazzola CD, et al. An LC—MS-MS Method for the Analysis of Carfentanil, 3-Methylfentanyl, 2-Furanyl Fentanyl, Acetyl Fentanyl, Fentanyl and Norfentanyl in Postmortem and Impaired-Driving Cases. J Anal Toxicol 2017;41(6):473—83.
[188] Seymour C, Shaner RL, Feyereisen MC, Wharton RE, Kaplan P, Hamelin EI, et al. Determination of Fentanyl Analog Exposure Using Dried Blood Spots with LC—MS-MS. J Anal Toxicol 2019;43:266—76.
[189] Allibe N, Richeval C, Phanithavong M, Faure A, Allorge D, Paysant F, et al. Fatality involving ocfentanil documented by identification of metabolites. Drug Test Anal 2018;10(6):995—1000.
[190] Breindahl T, Kimergård A, Andreasen MF, Pedersen DS. Identification of a new psychoactive substance in seized material: the synthetic opioid N-phenyl-N-[1-(2-phenethyl)piperidin-4-yl]prop-2-enamide (Acrylfentanyl). Drug Test Anal 2017;9(3):415—22.
[191] Rojkiewicz M, Majchrzak M, Celiński R, Kuś P, Sajewicz M. Identification and physicochemical characterization of 4-fluorobutyrfentanyl (1-((4-fluorophenyl)(1-phenethylpiperidin-4-yl)amino)butan-1-one, 4-FBF) in seized materials and post-mortem biological samples: 4-fluorobutyrfentanyl (4-FBF) identification and physicochemical characterization. Drug Test Anal 2017;9(3):405—14.
[192] United Nations Office on Drugs and Crime. Recommended Methods for the Identification and Analysis of Fentanyl and its Analogues in Biological Spécimens, 2017. Consulted on website https://www.unodc.org/documents/scientific/Recommended_methods_for_the_identification_and_analysis_of_Fentanyl.pdf on July 17 2019.
[193] Palamalai V, Olson KN, Kloss J, Middleton O, Mills K, Strobl AQ, et al. Superiority of postmortem liver fentanyl concentrations over peripheral blood influenced by postmortem interval for determination of fentanyl toxicity. Clin Biochem 2013;46(7—8):598—602.
[194] Giorgetti A, Centola C, Giorgetti R. Fentanyl novel derivative-related deaths. Hum Psychopharmacol 2017;32(3).
[195] Martin TL, Woodall KL, McLellan BA. Fentanyl-related deaths in Ontario, Canada: toxicological findings and circumstances of death in 112 cases (2002—2004). J Anal Toxicol 2006;30(8):603—10.
[196] Eckart K, Röhrich J, Breitmeier D, Ferner M, Laufenberg-Feldmann R, Urban R. Development of a new multi-analyte assay for the simultaneous detection of opioids in serum and other body fluids using liquid chromatography-tandem mass spectrometry. J Chromatogr B Analyt Technol Biomed Life Sci 2015;1001:1—8.
[197] Hayashi T, Buschmann C, Riesselmann B, Roscher S, Tsokos M. Circumstantial and toxicological features of deaths from self-administered intravenous anesthetic/narcotic agents. Forensic Sci Med Pathol 2013;9(2):138—44.
[198] Gillespie TJ, Gandolfi AJ, Davis TP, Morano RA. Identification and quantification of alpha-methylfentanyl in post mortem specimens. J Anal Toxicol 1982;6(3):139—42.
[199] Moody MT, Diaz S, Shah P, Papsun D, Logan BK. Analysis of fentanyl analogs and novel synthetic opioids in blood, serum/plasma, and urine in forensic casework. Drug Test Anal 2018;10(9):1358—67.
[200] Ojanperä I, Gergov M, Rasanen I, Lunetta P, Toivonen S, Tiainen E, et al. Blood levels of 3-methylfentanyl in 3 fatal poisoning cases. Am J Forensic Med Pathol 2006;27(4):328—31.
[201] Fort C, Curtis B, Nichols C, Niblo C. Acetyl Fentanyl Toxicity: Two Case Reports. J Anal Toxicol 2016;40(9):754—7.
[202] Carson HJ, Knight LD, Dudley MH, Garg U. A fatality involving an unusual route of fentanyl delivery: Chewing and aspirating the transdermal patch. Leg Med Tokyo Jpn 2010;12(3):157—9.
[203] Sofalvi S, Schueler HE, Lavins ES, Kaspar CK, Brooker IT, Mazzola CD, et al. An LC-MS-MS Method for the Analysis of Carfentanil, 3-Methylfentanyl, 2-Furanyl Fentanyl, Acetyl Fentanyl, Fentanyl and Norfentanyl in Postmortem and Impaired-Driving Cases. J Anal Toxicol 2017;41(6):473—83.
[204] Fogarty MF, Papsun DM, Logan BK. Analysis of Fentanyl and 18 Novel Fentanyl Analogs and Metabolites by LC—MS-MS, and report of Fatalities Associated with Methoxyacetylfentanyl and Cyclopropylfentanyl. J Anal Toxicol 2018;42(9):592—604.
[205] Rojkiewicz M, Majchrzak M, Celiński R, Kuś P, Sajewicz M. Identification and physicochemical characterization of 4-fluorobutyrfentanyl (1-((4-fluorophenyl)(1-phenethylpiperidin-4-yl)amino)butan-1-one, 4-FBF) in seized materials and post-mortem biological samples. Drug Test Anal 2017;9(3):405—14.
[206] Mohr ALA, Friscia M, Papsun D, Kacinko SL, Buzby D, Logan BK. Analysis of Novel Synthetic Opioids U-47700, U-50488 and Furanyl Fentanyl by LC-MS/MS in Postmortem Casework. J Anal Toxicol 2016;40(9):709—17.
[207] Allibe N, Billault F, Moreau C, Marchard A, Gaillard Y, Hoizey G, et al. Ocfentanil in France: Seven case reports (2016—2018). Tox Anal Clin 2019, http://dx.doi.org/10.1016/j.toxac.2018.12.003.

© 2019 Elsevier Masson SAS. All rights reserved. - Document downloaded on 11/12/2019 by VAUCEL JULES (963264). It is forbidden and illegal to distribute this document.

[208] Karinen R, Tuv SS, Rogde S, Peres MD, Johansen U, Frost J, et al. Lethal poisonings with AH-7921 in combination with other substances. Forensic Sci Int 2014;244:21—4.
[209] Papsun D, Krywanczyk A, Vose JC, Bundock EA, Logan BK. Analysis of MT-45, a Novel Synthetic Opioid, in Human Whole Blood by LC-MS-MS and its Identification in a Drug-Related Death. J Anal Toxicol 2016;40(4):313—7.
[210] Société Française de Toxicologie Analytique. SFTA guidelines for the achievement of toxicological analyzes for deaths involving NPS — 2019 version, 2019. Consulted on website http://www.sfta.org/articles/view/recommandations-de-la-sfta-pour-la-realisation-des-analyses-toxicologiques-dans-les-cas-de-deces-impliquant-des-nps-version-2019 on July 17 2019.
[211] Guerrieri D, Rapp E, Roman M, Druid H, Kronstrand R. Postmortem and Toxicological Findings in a Series of Furanylfentanyl-Related Deaths. J Anal Toxicol 2017;41:242—9.
[212] Mohr ALA, Friscia M, Papsun D, Kacinko SL, Buzby D, Logan BK. Analysis of Novel Synthetic Opioids U-47700, U-50488 and Furanyl Fentanyl by LC—MS/MS in Postmortem Casework. J Anal Toxicol 2016;40(9):709—17.
[213] Soh YNA, Elliott S. An investigation of the stability of emerging new psychoactive substances: An investigation of the stability of emerging new psychoactive substances. Drug Test Anal 2014;6(7—8):696—704.
[214] Dingeon B, Dreyfuss M-F, Gaillard Y, Le Bouil A, Lhermitte M, Mazzega S, et al. Aide à la validation des méthodes en Toxicologie et Suivi Thérapeutique Pharmacologique. Ann Toxicol Anal 2005;17(3):1—20.
[215] European Medicines Agency. Guideline on bioanalytical method validation, 2011. Consulted on website https://www.ema.europa.eu/en/documents/scientific-guideline/guideline-bioanalytical-method-validation_en.pdf on June 17 2019.
[216] Scientific Working Group for Forensic Toxicology. Scientific Working Group for Forensic Toxicology (SWGTOX) standard practices for method validation in forensic toxicology. J Anal Toxicol 2013;37(7):452—74.
[217] Helander A, Stojanovic K, Villén T, Beck O. Detectability of fentanyl and designer fentanyls in urine by 3 commercial fentanyl immunoassays. Drug Test Anal 2018;10(8):1297—304.
[218] Armenian P, Vo KT, Barr-Walker J, Lynch KL. Fentanyl, fentanyl analogs and novel synthetic opioids: a comprehensive review. Neuropharmacology 2018;134(Pt A):121—32.
[219] Cannaert A, Vasudevan L, Friscia M, Mohr ALA, Wille SMR, Stove CP. Activity-Based Concept to Screen Biological Matrices for Opiates and (Synthetic) Opioids. Clin Chem 2018;64(8):1221—9.
[220] Cannaert A, Ambach L, Blanckaert P, Stove CP. Activity-Based Detection and Bioanalytical Confirmation of a Fatal Carfentanil Intoxication. Front Pharmacol 2018;9:486.
[221] Maurer HH. Position of chromatographic techniques in screening for detection of drugs or poisons in clinical and forensic toxicology and/or doping control. Clin Chem Lab Med 2004;42(11):1310—24.
[222] Marchei E, Pacifici R, Mannocchi G, Marinelli E, Busardò FP, Pichini S. New synthetic opioids in biological and non-biological matrices: A review of current analytical methods. TrAC Trends Anal Chem 2018;102:1—15.
[223] Tabarra I, Soares S, Rosado T, Gonçalves J, Luís Â, Malaca S, et al. Novel synthetic opioids - toxicological aspects and analysis. Forensic Sci Res 2019;4(2):111—40.
[224] Richeval C, Gaulier J-M, Romeuf L, Allorge D, Gaillard Y. Case report: relevance of metabolite identification to detect new synthetic opioid intoxications illustrated by U-47700. Int J Legal Med 2019;133(1):133—42.
[225] McKenzie C, Sutcliffe OB, Read KD, Scullion P, Epemolu O, Fletcher D, et al. Chemical synthesis, characterisation and in vitro and in vivo metabolism of the synthetic opioid MT-45 and its newly identified fluorinated analogue 2F-MT-45 with metabolite confirmation in urine samples from known drug users. Forensic Toxicol 2018;36(2):359—74.
[226] Liu L, Wheeler SE, Venkataramanan R, Rymer JA, Pizon AF, Lynch MJ, et al. Newly Emerging Drugs of Abuse and Their Detection Methods. Am J Clin Pathol 2018;149(2):105—16.
[227] Fabresse N, Larabi IA, Stratton T, Mistrik R, Pfau G, Lorin de la Grandmaison G, et al. Development of a sensitive untargeted liquid chromatography-high resolution mass spectrometry screening devoted to hair analysis through a shared MS2 spectra database: A step toward early detection of new psychoactive substances. Drug Test Anal 2018;11:697—708.
[228] Richeval C, Gicquel T, Hugbart C, Le Dare B, Allorge D, Morel I, et al. In vitro Characterization of NPS Metabolites Produced by Human Liver Microsomes and the HepaRG Cell Line Using Liquid Chromatographyhigh Resolution Mass Spectrometry (LC-HRMS) Analysis: Application to Furanyl Fentanyl. Curr Pharm Biotechnol 2017;18(10):806—14.
[229] Hall AB, Coy SL, Nazarov E, Vouros P. Development of rapid methodologies for the isolation and quantitation of drug metabolites by differential mobility spectrometry - mass spectrometry. Int J Ion Mobil Spectrom 2012;15(3):151—6.
[230] Larabi IA, Fabresse N, Etting I, Abe E, Alvarez J-C. Rapid and simultaneous screening of new psychoactive substances and conventional drugs of abuse - The use of Biochip Array Technology in whole blood and urine: a comparative study. TIAFT 2019;42(2):16—22.

© 2019 Elsevier Masson SAS. All rights reserved. - Document downloaded on 11/12/2019 by VAUCEL JULES (963264). It is forbidden and illegal to distribute this document.

Publication n° 5

Death related to nicotine replacement therapy: A case report

Julie-Anne Tjoncke, Ruben Goncalves, Nadège Castaing, Mathieu Molimard, Florence Tovagliaro, Karine Titier

Publié en 2020 dans *Forensic Science International*

Volume 309, 110223, ISSN 0379-0738

https://doi.org/10.1016/j.forsciint.2020.110223

Forensic Science International 309 (2020) 110223

Contents lists available at ScienceDirect

Forensic Science International

journal homepage: www.elsevier.com/locate/forsciint

Case Report

Death related to nicotine replacement therapy: A case report

J-A. Tjoncke[a], R. Goncalves[b,*], N. Castaing[b], M. Molimard[b], F. Tovagliaro[a], K. Titier[b]

[a] Institut de Médecine Légale, CHU De Bordeaux, Place Amélie Raba Leon, 33076 Bordeaux Cedex, France
[b] Laboratoire de Pharmaco-Toxicologie, Service de Pharmacologie Médicale, CHU De Bordeaux, Place Amélie Raba Leon, 33076 Bordeaux Cedex, France

ARTICLE INFO

Article history:
Received 17 June 2019
Received in revised form 16 January 2020
Accepted 26 February 2020
Available online 29 February 2020

Keywords:
Suicide
Nicotine poisoning
Nicotine substitution therapy
Transdermal nicotine patch

ABSTRACT

Transdermal nicotine patches and nicotine tablets are widely used for substitution therapies after cessation of smoking. Toxic concentrations of nicotine and cotinine, its main metabolite, are rarely reported, either in cases of misuse or in a fatal context. We report here a rare fatal case due to massive exposure to nicotine replacement therapy.

A 41-year-old man was found dead by his cellmate with 7 nicotine patches on the body. There were 14 nicotine patches (21 mg) and 5 empty blisters of nicotine tablets (Nicopass® 1.5 mg) in the bin. External, internal, and histological examinations revealed asphyxia syndrome. Toxicological analyses indicated lethal concentrations of nicotine and cotinine in femoral (2239 and 1230 ng/mL) and cardiac blood (1344 and 1090 ng/mL). Screening for ethanol, drugs, and illicit drugs revealed therapeutic concentrations of cyamemazine, lormetazepam, nordiazepam, oxazepam, and buprenorphine and its metabolite. THC and its metabolites were also detected, reflecting use of cannabis. The findings highlight the risk of nicotine poisoning in persons using nicotine patches. This case emphasises the importance of carrying out complete toxicological analyses to prevent other instances of nicotine poisoning from being overlooked.
© 2020 Elsevier B.V. All rights reserved.

1. Introduction

Nicotine is the main alkaloid of tobacco. It is of medical interest due to its toxicity and the frequency of its consumption (cigarettes, e-cigarettes, and substitution therapy). Nicotine binds to nicotine-acetylcholine receptors and induces stimulant or depressant effects depending on the concentration. Typical symptoms of mild nicotine poisoning include nausea and vomiting, pale skin due to vasoconstriction, tremor, sweating, dizziness, tachycardia, and increased blood pressure. In cases of acute intoxication, confusion, convulsions, bradycardia, and hypotension are observed. In severe poisoning, muscle weakness can lead to respiratory muscle paralysis and death. A dose of 30–60 mg (0.8–1 mg/kg) is considered to be lethal for an adult [1].

After absorption, nicotine readily penetrates biological membranes. It undergoes rapid biotransformation, mainly in the liver (75%), into a number of inactive metabolites, including cotinine, which has a longer half-life (15–19 h) than nicotine (2–3 h). Transdermal nicotine patches (TNPs) are widely used in substitution therapy for smoking cessation. Nicotine absorption through the skin is slow, and there is no peak concentration in the brain as happens after inhalation. During continuous transdermal application, with the skin acting as a reservoir, the apparent half-life of nicotine is up to 12 h. Gums and tablets release nicotine slowly through the buccal mucosa without a peak. The maximal concentration is about 4.20 ng/mL after 50 min [2,3].

A few cases of fatal nicotine poisoning have been reported: by ingestion [4–6], intravenous injection [7] and/or dermal application [1,8–11]. There are also studies of suicide attempts or intentional misuse by transdermal application [12–15]. Toxic concentrations of nicotine and cotinine, its main metabolite, are rarely reported, either in cases of misuse or in a fatal context.

We report here a rare fatal case due to massive exposure to nicotine replacement therapy.

2. Case report

2.1. Case history

A 41-year-old man was found dead by his cellmate in the morning. He was lying on his right side in his bed. There were 7 patches on the body. He was seen alive the day before by the prison guards. No suicide note was found near the body.

The bin contained 14 nicotine patches (21 mg), 5 empty blisters of nicotine tablets (Nicopass® 1.5 mg), cyamemazine (14 blisters of Tercian® 25 mg and 2 of Tercian® 100 mg), clorazepate (7 blisters

* Corresponding author at: Laboratoire de Pharmacologie et Toxicologie, CHU de Bordeaux, Place Amélie Raba Leon, 33076 Bordeaux Cedex, France.
E-mail address: ruben.goncalves@chu-bordeaux.fr (R. Goncalves).

http://dx.doi.org/10.1016/j.forsciint.2020.110223
0379-0738/© 2020 Elsevier B.V. All rights reserved.

of Tranxene® 20 mg), and 2 of Lormetazepam® 2 mg. The bin is normally emptied daily.

2.2. Autopsy findings

An external examination showed generalised rigor mortis, predominant at the cervical level and easily broken at the upper and lower limbs, and significant petechial livor mortis on the right side of the body. Furthermore, it revealed congestion of the face, conjunctival injection, reddish mucus at the nasal and oral levels, and cyanosis of the lips. In each subclavicular region there was a skin patch with the indication PF30. There was no traumatic injury on the skin.

The internal examination revealed multi-organ congestion, considerable pulmonary oedema, a diffuse petechial haemorrhage on the myocardium and the deep surface of the scalp. There were also whitish fibrous areas on the myocardium and a blackish indurated formation on the aortic valve. The gastric content was food being digested; there was no medicinal residue visible. There was no traumatic injury to the organs that could explain the death.

2.3. Histological examination

There were some fibrous scars on the left ventricle. Coronary arteries were slightly atheromatous, with a decrease in the lumen of 20%. The lungs were oedematous and congestive with some rupture of the inter-alveolar septa, without haemorrhagic raptus. The liver was substantially normal, without parenchymal or mesenchymal involvement. The kidneys did not show any lesion. Histological analyses did not provide additional information on the physiopathological mechanism of death.

2.4. Toxicological analysis

Samples of cardiac blood, peripheral blood, urine, gastric contents, and tissues from several organs were subjected to toxicological examination. Post-mortem samples were subjected to toxicological screening for ethanol (gas chromatography with flame ionisation detection [GC–FID]), drugs, and illicit drugs (liquid chromatography with diode array detection [LC–DAD], gas chromatography-mass spectrometry [GC–MS], and liquid chromatography-tandem mass spectrometry LC–MS/MS).

Nicotine and cotinine analyses were performed on an Agilent Technologies (Santa Clara, CA, USA) 7890B system coupled to a 5977B GC–MS system after liquid-liquid extraction under alkaline conditions (NaOH 0.1 N) by a mixture of organic solvents (dichloromethane/ether/hexane/isoamyl alcohol; 30:50:20:0.5 v/v/v). Chromatographic separation was achieved using an Agilent VF-5 ms capillary column (30 m; 0.25 mm; 0.25 μm). Nicotine and cotinine were quantitatively analysed by GC–MS using nicotine D4 and cotinine D3 as the internal standards. Nicotine, cotinine, nicotine D4, and cotinine D3 were from Dr. Ehrenstorfer® (Augsburg, Germany), LGC Standards® (Wesel, Germany), Sigma-Aldrich® (St. Louis, MO, USA), and Cerilliant® (Round Rock, TX, USA), respectively. Other quantifications were performed on an Acquity class I ultra-high-performance liquid chromatography system coupled to a Waters® TQD MS/MS (Milford, MA, USA).

Besides nicotine and cotinine (Table 1), the toxicological analysis identified cyamemazine (183 ng/mL), nordiazepam (1350 ng/mL), oxazepam (36 ng/mL), lormetazepam (2 ng/mL), buprenorphine (2.2 ng/mL) and cannabinoids (THC 0.6 ng/mL and THCCOOH 4.4 ng/mL) in femoral blood. These drugs were also detected in urine. The concentrations of all other drugs were within the therapeutic ranges. The toxicological analysis did not detect any other lethal toxic agent.

Table 1
Nicotine and cotinine concentrations in analyses samples.

Sample	Nicotine	Cotinine
Cardiac blood	1344 ng/mL	1090 ng/mL
Femoral blood	2239 ng/mL	1230 ng/mL
Urine	Not detected	2508 ng/mL
Gastric content	936 ng/mL	Not detected
Vitreous humor	188 ng/mL	578 ng/mL
Liver	677 ng/g	3029 ng/g
Bile	265 ng/mL	2424 ng/mL
Brain	147 ng/g	491 ng/g
Kidney	394 ng/g	515 ng/g
Lung	474 ng/g	704 ng/g
Heart	156 ng/g	491 ng/g

2.5. Case synopsis

According to the autopsy and toxicological findings, the cause of death was acute nicotine poisoning. In conformity with the police investigation, the fatality was categorised as suicide.

3. Discussion

High doses of nicotine inhibit the central nervous system, inducing hypotension and bradycardia followed by coma and respiratory muscle paralysis, leading to death by asphyxia. The first evidences of police investigation and autopsy findings suggested nicotine poisoning. Indeed, the autopsy revealed signs of asphyxia syndrome (congestion of the face, conjunctival injection, reddish mucus in the nose and mouth, cyanosis of the lips, multi-organ congestion and considerable pulmonary oedema).

The above hypothesis was confirmed by the results of the toxicological analyses. The validation of the GC method for nicotine and cotinine quantification was assessed according to the EMEA guideline on bioanalytical method validation (reference number EMEA/CHMP/EWP/192217/2009 Rev. 1). A weighted regression 1/x with excluded origin was used. Calibration curves were linear in the range of 10–1000 ng/mL (r2 > 0.998) with lower than 15%. The method showed an intra-day and inter-day precision (CV) of less than 15% (n = 20). A bias of less than 15% was achieved. The selected limit of quantification (LOQ) was 10 ng/mL. The limit of detection (LOD) was 5 ng/mL.

The nicotine concentration in peripheral blood was markedly higher than that typical for current smokers [16] and during nicotine replacement therapy [17]. The serum nicotine concentration is 10.5–50 ng/mL and the cotinine concentration is 94–444 ng/mL in adult smokers (average of 33 cigarettes per day) [17]. The peak blood concentration 2–8 h after application of a single nicotine patch is 3.5–23 ng/mL, depending on the type of transdermal nicotine system [15]. In the present case, the nicotine concentration in femoral blood was compatible with the application of several TNPs.

Empty blisters of nicotine tablets (1.5 mg of nicotine) were found in the cell. So, we must consider the possibility of associated oral absorption. There were only 5 empty blisters in the bin, and while no drug residue was found in the stomach, the nicotine level of the stomach contents was 936 ng/mL. Without post-mortem redistribution, this is consistent with concomitant oral intake of nicotine tablets and may have contributed to the death.

In total, 14 TNPs and 5 empty blisters of 1.5 mg nicotine capsules were found in the bin. That means a hypothetic maximal nicotine exposure of 342.25 mg (4.6 mg/kg) if the bin was emptied the day before. The lethal dose of nicotine in adults is 0.8–1.0 mg/kg body weight, corresponding to a total dose of 30–60 mg [1]. In comparison with previous reports (Table 2), the concentration of nicotine in femoral blood (2239 ng/mL) was sufficient to cause death.

Table 2
Comparison with other studies.

Literature	Sample type	Nicotine concentration	Cotinine concentration	Details
Presented case	Peripheral blood	2239 ng/mL	1230 ng/mL	Application of many TNPs
	Urine	Not detected	2508 ng/mL	
	Gastric content	936 ng/mL	Not detected	
Kemp et al. [11]	Cardiac blood	1400 ng/mL	1300 ng/mL	Application of 18 TNPs and plastic bag over nose and mouth
	Peripheral blood	460 ng/mL	–	
	Urine	2900 ng/mL	7600 ng/mL	
	Gastric content	90 ng/mL	20 ng/mL	
	Vitreous	270 ng/mL	–	
	Liver	2000 ng/mL	3800 ng/mL	
	Brain	800 ng/mL	900 ng/mL	
Lardi et al. [8]	Cardiac serum	190 ng/mL	1900 ng/mL	Application of self-made patches and ingestion of a lethal dose of diphenhydramine
	Peripheral serum	310 ng/mL	1600 ng/mL	
	Urine	1300 ng/mL	3772 ng/mL	
	Gastric content	2400 ng/mL	Detected	
Sanchez et al. [10]	Serum 11 h after cardiac arrest	3700 ng/mL	–	Application of one TNP and continue smoking
	Serum 57 h after cardiac arrest	1200 ng/mL	–	
Solarino et al. [1]	Peripheral blood	600 ng/mL	2000 ng/mL	Poisoning by tramadol and application of 25 TNPs
	Urine		Detected	
Solarino et al. [21]	Peripheral blood	2200 ng/mL	2200 ng/mL	Ingestion of nicotine-containing solution
Takayasu et al. [4]	Blood	6300 ng/mL	–	Taking orally the eluate from tobacco
	Urine	1500 ng/mL	–	
	Contents of stomach	30000 ng/mL	–	
	Small intestine	71 μg/g	–	
Moriya et al. [6]	Blood	222 to 733 ng/mL	–	Ingestion of tobacco leaves
Corkery et al. [9]	*First case*			Ingestion of nicotine
	Blood	5500 ng/mL	2500 ng/mL	
	Urine	>80000 ng/mL	7900 ng/mL	
	Second case		–	
	Blood	1000 ng/mL		
Lavoie et al. [5]	Blood	13,600 ng/mL	–	Sample taken before death; Autopsy not performed
Chaturvedi et al. [22]	Blood	3 000 ng/mL	–	Poisoning by ingestion of caffeine, nicotine and malathion
	Urine	1000 ng/mL	–	
	Liver	17 mg/kg		
	Gastric content	71.3 mg/20g		

To evaluate post-mortem redistribution (PMR), we determined the nicotine and cotinine concentrations in a variety of samples (Table 1). The nicotine concentration in the cardiac blood (1344 ng/mL) was lower than that in femoral blood (2239 ng/mL). The cardiac blood (C) to peripheral blood (P) ratio is 0.6, and the liver (L) to peripheral blood ratio is 0.3. These ratios suggest an absence of PMR, which is defined as a C/P ratio of < 1 [18] and L/P ratio of < 5 [19,20]. Lardi *et al.* [8] also described no PMR, contrary to Kemp *et al.* [11]. The localisation of the patches may explain the differences [8–11]. In the case described by Kemp *et al.* [11] and in the present case, most of the patches were attached to the thoracic skin.

The cotinine concentrations were high in the liver and urine, which was expected considering the metabolism and excretion of nicotine. In most previous studies the cotinine concentration was higher than the nicotine concentration (Table 2). In this case the opposite was observed, which was likely to be due to incomplete metabolism of nicotine caused by the short delay between absorption and death.

The toxicological analyses also revealed therapeutic concentrations of cyamemazine, lormetazepam, buprenorphine and nordiazepam in peripheral blood. Consequently, the results of the toxicological analyses indicated that death was caused by acute nicotine poisoning.

In conclusion, this case emphasises the necessity of a complete toxicological analyses to determine the cause of the death. Moreover, the risk of nicotine poisoning due to nicotine replacement therapies warrants further investigation. Finally, it is important to supervise nicotine substitution therapy in incarcerated individuals, particularly those with depression.

Declaration of Competing Interest

None.

References

[1] B. Solarino, B. Rießelmann, C.T. Buschmann, M. Tsokos, Multidrug poisoning involving nicotine and tramadol, Forensic Sci. Int. 194 (January (1)) (2010) e17–9, doi:http://dx.doi.org/10.1016/j.forsciint.2009.09.019.

[2] N.L. Benowitz, Nicotine replacement therapy. What has been accomplished–can we do better? Drugs 45 (February (2)) (1993) 157–170, doi:http://dx.doi.org/10.2165/00003495-199345020-00001.

[3] Base de données publique des médicaments, Résumé Des Caractéristiques Du Produit - NICOPASS 1,5 Mg SANS SUCRE MENTHE FRAICHEUR, Pastille Édulcorée à l'aspartam Et à l'acésulfame Potassique, (2018) Available on: http://base-donnees-publique.medicaments.gouv.fr/affichageDoc.php?specid=68852892&typedoc=R#RcpPropPharmacocinetiques. (Accessed 5 September 2019).

[4] T. Takayasu, T. Ohshima, Z. Lin, J. Nishigami, T. Nakaya, H. Maeda, et al., An autopsy case of fatal nicotine poisoning, Nihon Hoigaku Zasshi 46 (October (5)) (1992) 327–332.

[5] F.W. Lavoie, T.M. Harris, Fatal nicotine ingestion, J. Emerg. Med. 9 (May (3)) (1991) 133–136, doi:http://dx.doi.org/10.1016/0736-4679(91)90318-A.

[6] F. Moriya, Y. Hashimoto, A fatal poisoning caused by methomyl and nicotine, Forensic Sci. Int. 149 (May (2–3)) (2005) 167–170, doi:http://dx.doi.org/10.1016/j.forsciint.2004.06.013.

[7] W. Krauland, V. Schneider, E. Klug, Fatal nicotine poisoning due to miscalculated nicotine test, Z. Rechtsmed. 72 (June (4)) (1973) 269–277.

[8] C. Lardi, S. Vogt, S. Pollak, A. Thierauf, Complex suicide with homemade nicotine patches, Forensic Sci. Int. 236 (March) (2014) e14–8, doi:http://dx.doi.org/10.1016/j.forsciint.2013.12.017.

[9] J.M. Corkery, J. Button, A.E. Vento, F. Schifano, Two UK suicides using nicotine extracted from tobacco employing instructions available on the Internet, Forensic Sci. Int. 199 (June (1)) (2010) e9–13, doi:http://dx.doi.org/10.1016/j.forsciint.2010.02.004.

[10] P. Sanchez, J.L. Ducassé, M. Lapeyre-Mestre, O. Martinet, P. Rougé, M.F. Jorda, et al., Nicotine poisoning as a cause of cardiac arrest? J. Toxicol. Clin. Toxicol. 34 (4) (1996) 475–476.

[11] P.M. Kemp, G.S. Sneed, C.E. George, R.F. Distefano, Postmortem distribution of nicotine and cotinine from a case involving the simultaneous administration of multiple nicotine transdermal systems, J. Anal. Toxicol. 21 (August (4)) (1997) 310–313.
[12] C.J. Engel, A.H. Parmentier, Suicide attempts and the nicotine patch, JAMA 270 (July (3)) (1993) 323–324, doi:http://dx.doi.org/10.1001/jama.1993.03510030046026.
[13] A. Labelle, L. Boulay, An attempted suicide using transdermal nicotine patches, Can. J. Psychiatry Rev. Can. Psychiatr. 44 (March (2)) (1999) 190.
[14] A. Woolf, K. Burkhart, T. Caraccio, T. Litovitz, Self-poisoning among adults using multiple transdermal nicotine patches, J. Toxicol. Clin. Toxicol. 34 (6) (1996) 691–698, doi:http://dx.doi.org/10.1016/j.legalmed.2004.04.005.
[15] N. Montaldo, C.C. Brackett, T. Sobol, Use of transdermal nicotine systems in a possible suicide attempt, JABFP 7 (5) (1994).
[16] F. Moriya, Y. Hashimoto, Nicotine and cotinine levels in blood and urine from forensic autopsy cases, Leg. Med. Tokyo (Tokyo) 6 (July (3)) (2004) 164–169, doi:http://dx.doi.org/10.1016/j.legalmed.2004.04.005.
[17] R.D. Hurt, L.C. Dale, K.P. Offord, G.G. Lauger, L.B. Baskin, G.M. Lawson, et al., Serum nicotine and cotinine levels during nicotine-patch therapy, Clin. Pharmacol. Ther. 54 (July (1)) (1993) 98–106, doi:http://dx.doi.org/10.1038/clpt.1993.117.
[18] M. Dalpe-Scott, M. Degouffe, D. Garbutt, M. Drost, A comparison of drug concentrations in postmortem cardiac and peripheral blood in 320 cases, Can. Soc. Forensic Sci. J. 28 (January (2)) (1995) 113–121, doi:http://dx.doi.org/10.1080/00085030.1995.10757474.
[19] I.M. McIntyre, Liver and peripheral blood concentration ratio (L/P) as a marker of postmortem drug redistribution: a literature review, Forensic Sci. Med. Pathol. 10 (March (1)) (2014) 91–96, doi:http://dx.doi.org/10.1007/s12024-013-9503-x.
[20] I.M. McIntyre, J. Sherrard, J. Lucas, Postmortem carisoprodol and meprobamate concentrations in blood and liver: lack of significant redistribution, J. Anal. Toxicol. 36 (April (3)) (2012) 177–181, doi:http://dx.doi.org/10.1093/jat/bks011.
[21] B. Solarino, F. Rosenbaum, B. Riesselmann, C.T. Buschmann, M. Tsokos, Death due to ingestion of nicotine-containing solution: case report and review of the literature, Forensic Sci. Int. 195 (February (1–3)) (2010) e19–22, doi:http://dx.doi.org/10.1016/j.forsciint.2009.11.003.
[22] A.K. Chaturvedi, N.G. Rao, F.E. McCoy, A multi-chemical death involving caffeine, nicotine and malathion, Forensic Sci. Int. 23 (December (2–3)) (1983) 265–275, doi:http://dx.doi.org/10.1016/0379-0738(83)90155-X.

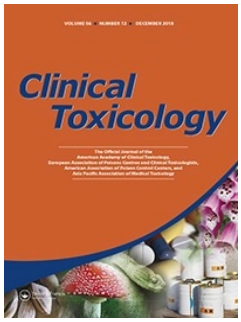
Publication n° 6

Delayed cardiotoxicity in venlafaxine and quetiapine overdose

Tatiana Hertzog, Laura Cetran, Magali Labadie, Coralie Bragança, Ruben Goncalves

Publié en 2021 dans *Clinical Toxicology*

https://doi.org/10.1080/15563650.2021.191434



Clinical Toxicology

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/ictx20

Delayed cardiotoxicity in venlafaxine and quetiapine overdose

Tatiana Hertzog, Laura Cetran, Magali Labadie, Coralie Braganca & Ruben Goncalves

To cite this article: Tatiana Hertzog, Laura Cetran, Magali Labadie, Coralie Braganca & Ruben Goncalves (2021): Delayed cardiotoxicity in venlafaxine and quetiapine overdose, Clinical Toxicology, DOI: 10.1080/15563650.2021.1914341

To link to this article: https://doi.org/10.1080/15563650.2021.1914341

Published online: 19 Apr 2021.

Submit your article to this journal

View related articles

View Crossmark data

Full Terms & Conditions of access and use can be found at
https://www.tandfonline.com/action/journalInformation?journalCode=ictx20

CLINICAL TOXICOLOGY
https://doi.org/10.1080/15563650.2021.1914341

Taylor & Francis
Taylor & Francis Group

LETTER TO THE EDITOR

Delayed cardiotoxicity in venlafaxine and quetiapine overdose

To the Editor,

Overdoses of drugs with QRS-prolonging activity have a mortality rate that can exceed 10% [1]. QRS prolongation and ventricular arrhythmias have been reported in large venlafaxine or quetiapine overdoses [2]. Flumazenil is a benzodiazepine reversant used as a diagnostic tool in comatose patients but also known to induce adverse effects like seizures [3]. We report a case of poly-intoxication with cardiotropic drugs complicated by severe and delayed cardiotoxicity.

A 38-year-old woman was suspected of ingesting 12 g of venlafaxine XR, 48 g of quetiapine XR, 105 mg of zopiclone, and an undefined quantity of bromazepam. Her medical history included anxiety depressive syndrome and a sleeve gastrectomy 4 years prior. Two hours after exposure, the Glasgow coma scale (GCS) was 12 with reactive intermediate pupils, blood pressure (BP) 109/56 mmHg, and normal blood glucose. Initial electrocardiogram (ECG) showed sinus tachycardia at 120 bpm, a narrow QRS (80 ms), and a corrected QT interval (QTc) of 450 ms (Fridericia's formula). Twelve hours after ingestion she developed increased central nervous system depression (GCS 3), mydriasis, and BP 80/60 mmHg. The ECG showed a sinus rhythm, with a prolonged QTc of 480 ms and QRS widening (182 ms) (Figure 1). Because of significant neurological depression supposed to be induced by benzodiazepines, flumazenil (0.3 mg) was administered, which was immediately followed by a tonic-clonic seizure. Immediately, intubation was performed due to neurological failure and noradrenaline was started due to mean BP at 50 mmHg. Administration of 250 mL of sodium bicarbonate (8.4%) was followed by a decrease in the QRS to 112 ms and then a decrease in the dose of noradrenaline with hemodynamic improvement. Twenty hours after ingestion, EEG showed seizure activity, therefore levetiracetam was started. Transthoracic echocardiography showed a 70% left ventricular ejection fraction under noradrenaline administration. At 29 h after ingestion, there was further hemodynamic

Figure 1. ECG 12 h after ingestion.

© 2021 Informa UK Limited, trading as Taylor & Francis Group

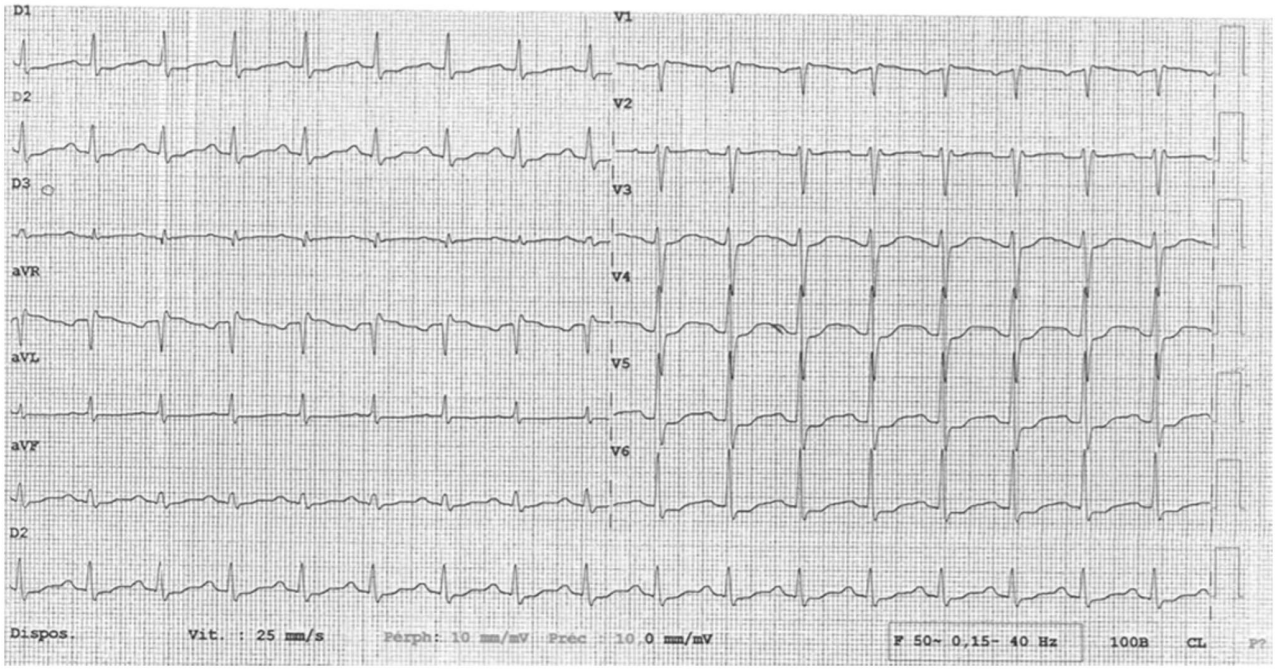


Figure 2. ECG 29 h after ingestion.

deterioration, with worsening of the ECG findings (Figure 2), which was interpreted as sinus rhythm with a prolonged QRS (160 ms) and QTc (455 ms). An additional 500 mL of sodium bicarbonate (8.4%) with 120 g of intralipid solution was administrated followed by a decrease in the QRS to 88 ms and the QTc to 375 ms and improved hemodynamic stability, allowing the weaning of noradrenaline. Toxicity resolved 4 days after admission without neurological sequelae.

Blood was analyzed 2 days after exposure using LC-HRMS with QTOF detection (Waters, Milford, MA, USA). The toxicological analysis identified: prazepam with metabolites (nordiazepam, oxazepam), venlafaxine (V) 6164 ng/mL with *O*-desmethylvenlafaxine (ODV) 2325 ng/mL [ref. (V + ODV) 100–400 ng/mL], zopiclone 32 ng/mL (ref. 10–50 ng/mL), quetiapine 945 ng/mL (ref. 100–500 ng/mL), norquetiapine, midazolam and levetiracetam.

In this case, venlafaxine could cause convulsive coma and slowdown in intra-myocardial conduction visualized by the QRS enlargement lie in the blockage of the sodium channels at 12 h. The second peak of toxicity occurred at 29 h which could be related to the extend-released form of the drugs; pharmacokinetics modifications induced by sleeve gastrectomy; or patient CYP2D6 genotype. Flumazenil must not be used on patients chronically taking benzodiazepines and also a seizure-genic co-ingestant.

Disclosure statement

No potential conflict of interest was reported by the author(s).

ORCID

Magali Labadie http://orcid.org/0000-0002-6546-6886
Ruben Goncalves http://orcid.org/0000-0002-9389-6226

References

[1] Henry JA, Cassidy SL. Membrane stabilising activity: a major cause of fatal poisoning. Lancet. 1986;327(8495):1414–1417.
[2] Bosse GM, Spiller HA, Collins AM. A fatal case of venlafaxine overdose. J Med Toxicol. 2008;4(1):18–20.
[3] Penninga EI, Graudal N, Ladekarl MB, et al. Adverse events associated with flumazenil treatment for the management of suspected benzodiazepine intoxication–a systematic review with meta-analyses of randomised trials. Basic Clin Pharmacol Toxicol. 2016; 118(1):37–44.

Tatiana Hertzog and Laura Cetran
Unité de Soins Intensifs de Cardiologie, CHU de Bordeaux, Bordeaux, France

Magali Labadie and Coralie Braganca
Centre-Antipoison et Toxicovigilance de Nouvelle Aquitaine, CHU de Bordeaux, Bordeaux, France

Ruben Goncalves
Laboratoire de Pharmacologie et Toxicologie, CHU de Bordeaux, Bordeaux, France;
INSERM, BPH, U1219, University of Bordeaux, Bordeaux, France
ruben.goncalves@chu-bordeaux.fr

Received 21 December 2020; revised 24 March 2021; Accepted 31 March 2021

Publication n° 7

Hair Analysis of Methoxphenidine in a Forensic Chemsex Case

Ruben Goncalves, Nadège Castaing, Karine Titier, Véronique Dumestre-Toulet

Publié en 2021 dans *Journal of Analytical Toxicology*

ISSN 0146-4760, 1945-2403

https://doi.org/10.1093/jat/bkab016

Journal of Analytical Toxicology, 2021;00:1–9
doi:10.1093/jat/bkab016
Advance Access Publication Date: 1 February 2021
Article

OXFORD

Article

Hair Analysis of Methoxphenidine in a Forensic Chemsex Case

Ruben Goncalves[1,2,*], Nadege Castaing[1], Karine Titier[1] and Véronique Dumestre-Toulet[3]

[1]Laboratoire de Pharmacologie et Toxicologie, CHU de Bordeaux, Amélie Raba Léon, 33076 Bordeaux, France; [2]Université de Bordeaux, INSERM U1219, BPH, 146 rue Léo Saignat, 33000 Bordeaux, France and [3]ToxGen Laboratoire, 11 rue du Commandant Cousteau, 33100 Bordeaux, France

*Author to whom correspondence should be addressed. Email: ruben.goncalves@chu-bordeaux.fr

Abstract

Methoxphenidine (MXP, 2-MeO-diphenidine) is a dissociative anesthetic drug of the diarylethylamine type, recently introduced for recreational purposes through the online-based sale of new psychoactive substances (NPSs). The concentration of MXP in hair has never been reported, either in cases of chemsex use or in fatal cases. A 55-year-old man was found dead at his home the morning after a chemsex party. Toxicological analyses indicated high concentrations of MXP in femoral blood (606 μg/L), cardiac blood (254 μg/L) and hair (13 ng/mg). We also identified 3-methylmethcathinone (3-MMC) in femoral blood (traces) and urine (238 μg/L). The concentrations of all other drugs were consistent with living subjects. This case highlights the risk of MXP poisoning in the context of chemsex and emphasizes the importance of including NPS in postmortem toxicology examinations.

Introduction

The term 'chemsex' is used to describe intentional sexual activity under the influence of psychoactive substances, mostly among men who have sex with men. The main substances used are crystal meth, mephedrone and gamma-hydroxybutyrate (GHB)/Gamma-butyrolactone (GBL) (1, 2). These drugs are often used in combination to facilitate sexual sessions lasting several hours or days with multiple sexual partners, sometimes with erectile dysfunction agents (sildenafil, tadalafil and vardenafil) (3, 4). Chemsex sessions do not necessarily include these drugs; many men engaging in chemsex also use other drugs, especially ecstasy, cocaine, other synthetic cathinones and ketamine (5–7). These substances have recently been accompanied by the use of illicit opioids and benzodiazepines (8). Rarely used in this context, methoxphenidine (methoxydiphenidine, 2-MeO-diphenidine, MXP) is a diarylethylamine sold online as a designer drug (9). Acting as an antagonist of the *N*-methyl-D-aspartate receptor, it is used for its dissociative effects. It is also used to replace other new psychoactive substances (NPSs) such as methoxetamine (MXE), a ketamine derivative (10). MXP enhances tactile sensations and has euphoric, empathogenic and stimulant characteristics that could help chemsex sessions (11). It was classified as a controlled substance on 3 October 2017 in France. The symptomatology of the dissociative state is complex and dose dependent and includes sensory hallucinations, tactile distortions, euphoria, derealization and depersonalization (12). Acute MXP intoxication has been reported to produce confusion, hypertension and tachycardia, which are responsive to treatment with intravenous lorazepam (13). MXP has also been associated with three published fatalities (14) and one case of impaired driving (15). To date, only a few cases of MXP toxicity have been reported, and no reports have been published on hair analysis in the context of chemsex.

We report here the first case of postmortem MXP quantification in hair in the context of chemsex with complete toxicological examination. We also present a short overview of fatal and non-fatal MXP intoxications.

Case History

A 55-year-old man who had sex with men was found dead at his home the morning after a chemsex party. He was known to be a regular participant in chemsex and especially SLAM (a term used for drug injection in the chemsex context). He had 32 stab wounds on his body. Two empty used syringes were found near the body.

© The Author(s) 2021. Published by Oxford University Press on behalf of Society of Forensic Toxicologists, Inc. All rights reserved.
For permissions, please e-mail: journals.permissions@oup.com

Downloaded from https://academic.oup.com/jat/advance-article/doi/10.1093/jat/bkab016/6125370 by TIAFT Member Access user on 21 June 2021

His medical history included cardiovascular disorders treated with amlodipine, valsartan and hydrochlorothiazide. He also occasionally took benzodiazepines. According to the autopsy findings, the cause of death was a stabbing homicide.

Materials and Methods

Toxicological screening

Autopsy samples of cardiac blood, peripheral blood, urine, hair and gastric contents and the two syringes were subjected to toxicological examination. Postmortem samples were subjected to toxicological screening for ethanol (headspace gas chromatography with flame ionization detection [HS-GC–FID]), drugs and illicit drugs (liquid chromatography–tandem mass spectrometry [LC–MS-MS] and high-resolution mass spectrometry [LC–HRMS] with QTOF detection).

The hair sample (2.5 cm) was cut as close as possible from the vertex posterior of the head corresponding to a time frame analysis of 2.5 months prior to death. Segmental hair analysis was not performed in our case because of a non-oriented sample. Briefly, the hair sample (2.5 cm, brown) was decontaminated using a bath of dichloromethane for 2 min, followed by a 2-min bath in deionized water. The dried hair sample was ground into a fine, homogeneous powder using a ball mill (Precellys 24, Bertin Technologies, Montigny-le-Bretonneux, France). Hydrolysis was performed using 1 mL of methanol with 1 mL of 0.1% formic acid and 50 µL of internal standard (IS; prazepam, 5 µg/mL) at 45°C for 16 h. Extracts were evaporated to dryness under a gentle stream of nitrogen and reconstituted with 100 µL of water/acetonitrile (50:50, v/v).

Ultra-performance liquid chromatography (UPLC)-HRMS system consisted of an Acquity I class system (Waters, USA) coupled to a Xevo G2-XS Quadrupole Time of flight (QTof) analyzer (Waters, USA) using positive- and negative-mode electrospray ionization and controlled with UNIFI 1.9 software. Chromatographic separation was performed on an ACQUITY HSS C18 column (150 × 2 mm, 1.8 µm, Waters). The mobile phase was a gradient composed of solvents A (ammonium formate buffer 5 mM, pH 3) and B (acetonitrile in 0.1% v/v formic acid). The column was maintained at 50°C and a gradient elution was used at a flow rate of 0.4 mL/min: 13% of B held until 0.5 min, increased to 50% at 10 min, then to 95% at 1,025 min, and finally held until 1,225 min, return to initial condition in 0.25 min until 17 min. The injection volume was 2 µL. Data were analyzed with an exact mass database from Waters® for over 1,500 toxicologically relevant drugs and metabolites that included protonated molecule, fragmentation and chromatographic retention time data. Criteria used for identification of targeted compounds included 5 ppm as exact mass error of the precursor ion as well as product ions and ±0.5 min as tolerance in retention time for precursor ion.

MXP quantification in hair

After identification by LC–QTOF screening, the concentration of MXP was quantified. The calibration curve for MXP was constructed by analysis of extracts from 10 mg of control hair samples to which a standard solution of MXP had been added (0.25–25 ng/mg of hair), together with MBDB-D5 (deuterium IS). The patient sample and calibration samples were incubated in 1 mL of methanol with 1 mL of 0.1% formic acid at 45°C for 16 h. After 5 min of centrifugation, extraction was performed with 1 mL of supernatant using solid phase extraction (PCX column 60 mg, Agilent). Extracts were evaporated to dryness under a gentle stream of nitrogen and reconstituted with 100 µL of 0.1% formic acid. The injection volume was 2 µL (analysis time: 15 min). Analyses were performed using an Acquity UPLC system (Waters) coupled with a TQD detector and associated with MassLynx software. The column was an Acquity UPLC HSS C18 (2.1 × 150 mm, 1.8 µm, Waters) maintained at 45°C. The mobile phase consisted of a combination of phase A (4 mM ammonium formate buffer, pH 3.2) and phase B (acetonitrile in 0.01% v/v formic acid). Gradient elution was performed at a flow rate of 0.4 mL/min: 1% of B held until 0.5 min; increased to 5% at 2 min, then to 15% at 9 min and finally to 50% at 14 min; and then returned to the initial condition in 0.1-min increments until 15 min.

Table I. Validation Parameters of Methoxphenidine in Hair

	Methoxphenidine
Linearity (ng/mg)	0.25–25
LOD (ng/mg)	0.0125
Correlation coefficient	>0.998
Recovery (%)	
0.25 ng/mg	121
15 ng/mg	102
Mean matrix effect (%)	
0.25 ng/mg	16
15 ng/mg	28
Process efficiency (%)	
0.25 ng/mg	94
15 ng/mg	96
QC 0.25 ng/mg	
CV intra-assay (%)	5
CV inter-assay (%)	10.2
Accuracy (%)	105
QC 0.75 ng/mg	
CV intra-assay (%)	5
CV inter-assay (%)	9.8
Accuracy (%)	102.5
QC 4 ng/mg	
CV intra-assay (%)	7
CV inter-assay (%)	13.2
Accuracy (%)	101.3
QC 15 ng/mg	
CV intra-assay (%)	8
CV inter-assay (%)	12.4
Accuracy (%)	102.6

QC: quality control

Validation of the LC–MS-MS method for MXP quantification in hair (Table I) was performed according to the European Medicines Agency (EMEA) guideline on bioanalytical method validation (reference number EMEA/CHMP/EWP/192217/2009 Rev. 1). A weighted regression $1/x$ with excluded origin was used. Calibration curves were linear in the range of 0.25–25 ng/mg of hair ($r^2 > 0.998$) with coefficient of variation (CV) and bias lower than 15% for each calibration curve point. The method showed an intra-day and inter-day precision (CV) of less than 15% ($n = 20$). A bias of less than 15% was achieved for 0.25, 0.75, 4 and 15 ng/mg of hair. The selected limit of quantification was 0.25 ng/mg of hair. The limit of detection (LOD) was 0.0125 ng/mg of hair. The mean matrix effect was 16% and 40% for 0.25 and 15 ng/mg of hair, respectively. However, for the IS, no matrix effect was observed. Chromatograms of MXP and 3-MMC in femoral blood, cardiac blood, urine and hair samples are provided in Figures 1–4 respectively.

Downloaded from https://academic.oup.com/jat/advance-article/doi/10.1093/jat/bkab016/6125370 by TIAFT Member Access user on 21 June 2021

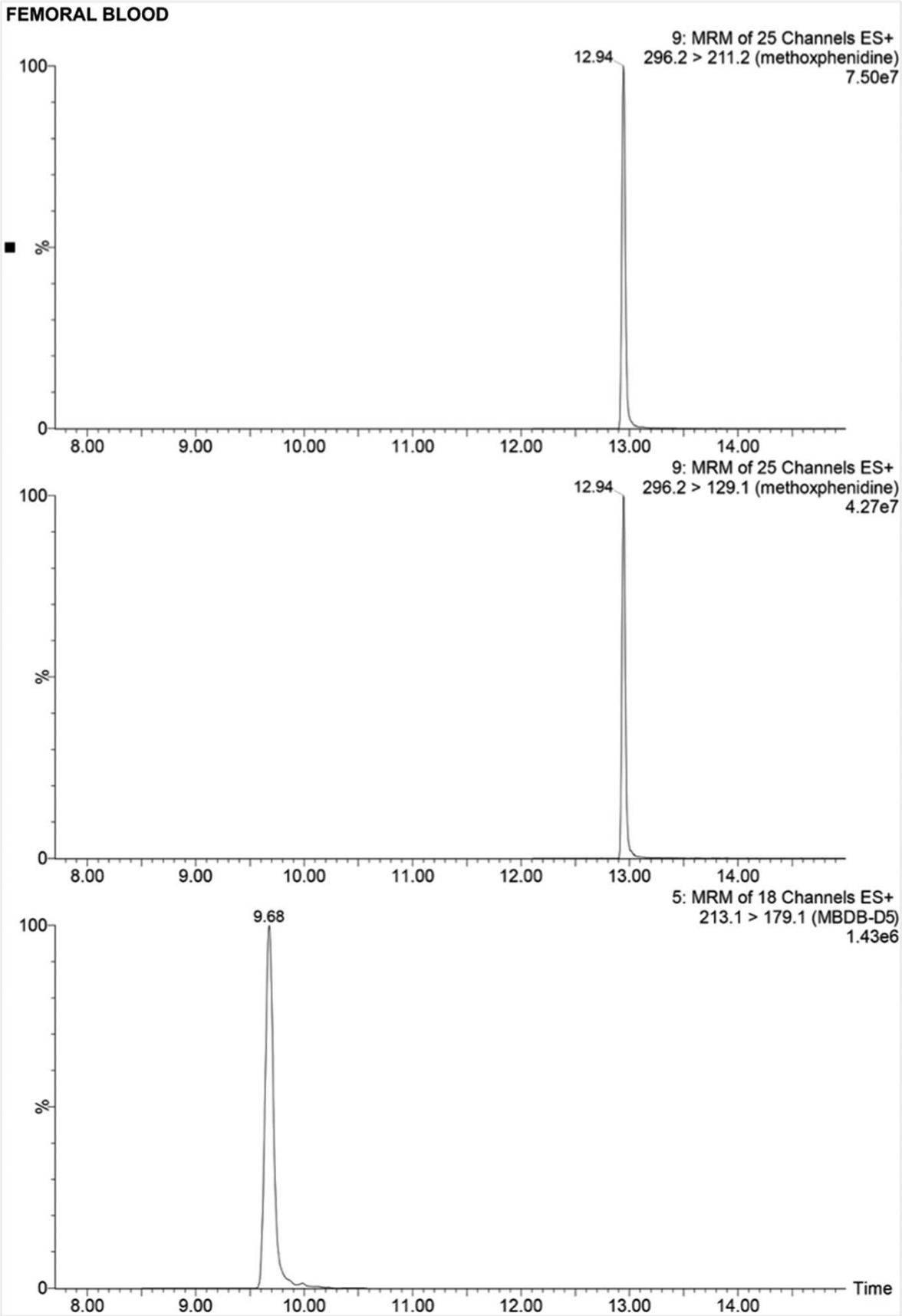
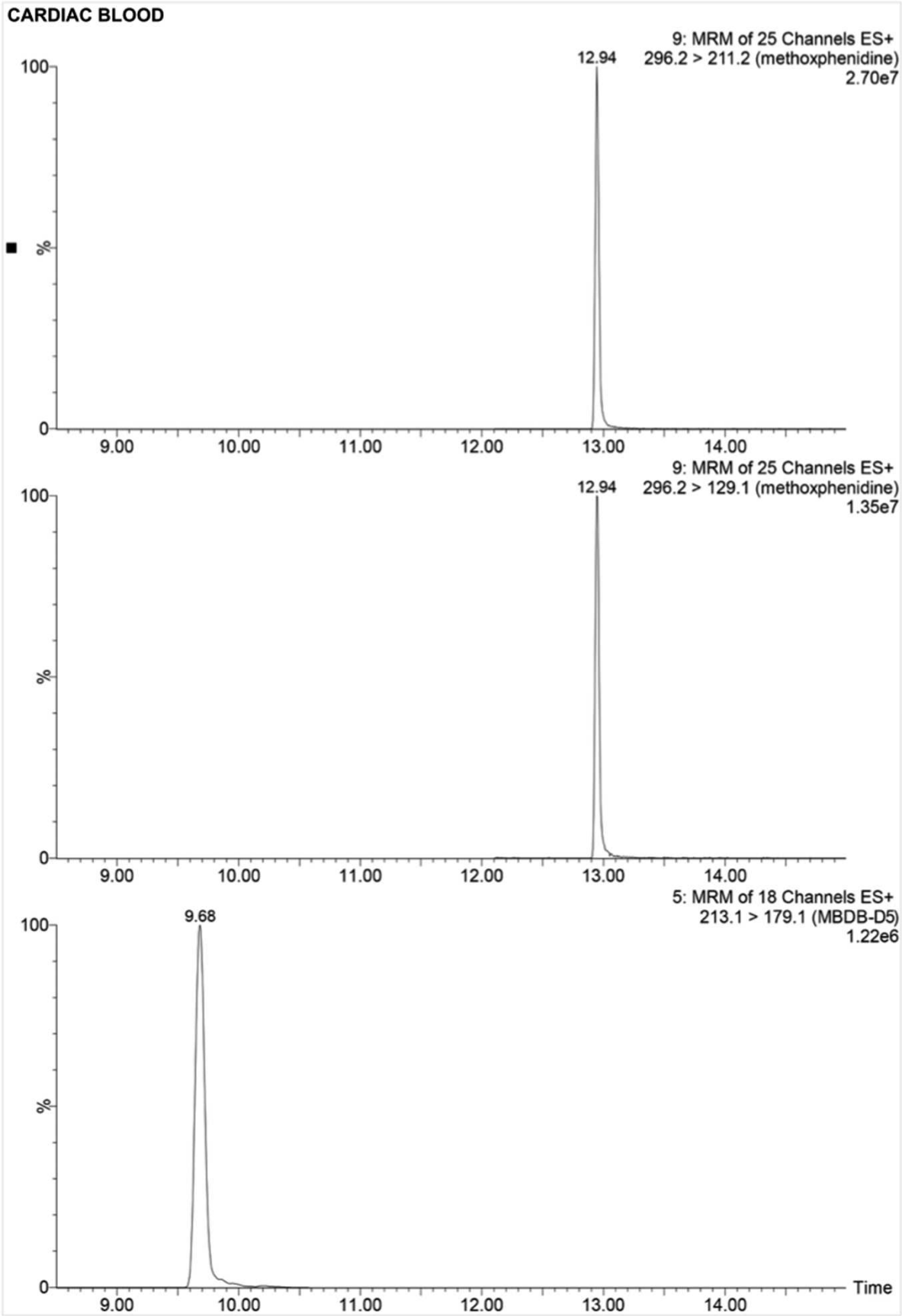


Figure 1. Chromatograms of methoxphenidine and MBDB-D5 used as IS in femoral blood.

Downloaded from https://academic.oup.com/jat/advance-article/doi/10.1093/jat/bkab016/6125370 by TIAFT Member Access user on 21 June 2021



Figure

Figure 2. Chromatograms of methoxphenidine and MBDB-D5 used as IS in cardiac blood.

Downloaded from https://academic.oup.com/jat/advance-article/doi/10.1093/jat/bkab016/6125370 by TIAFT Member Access user on 21 June 2021

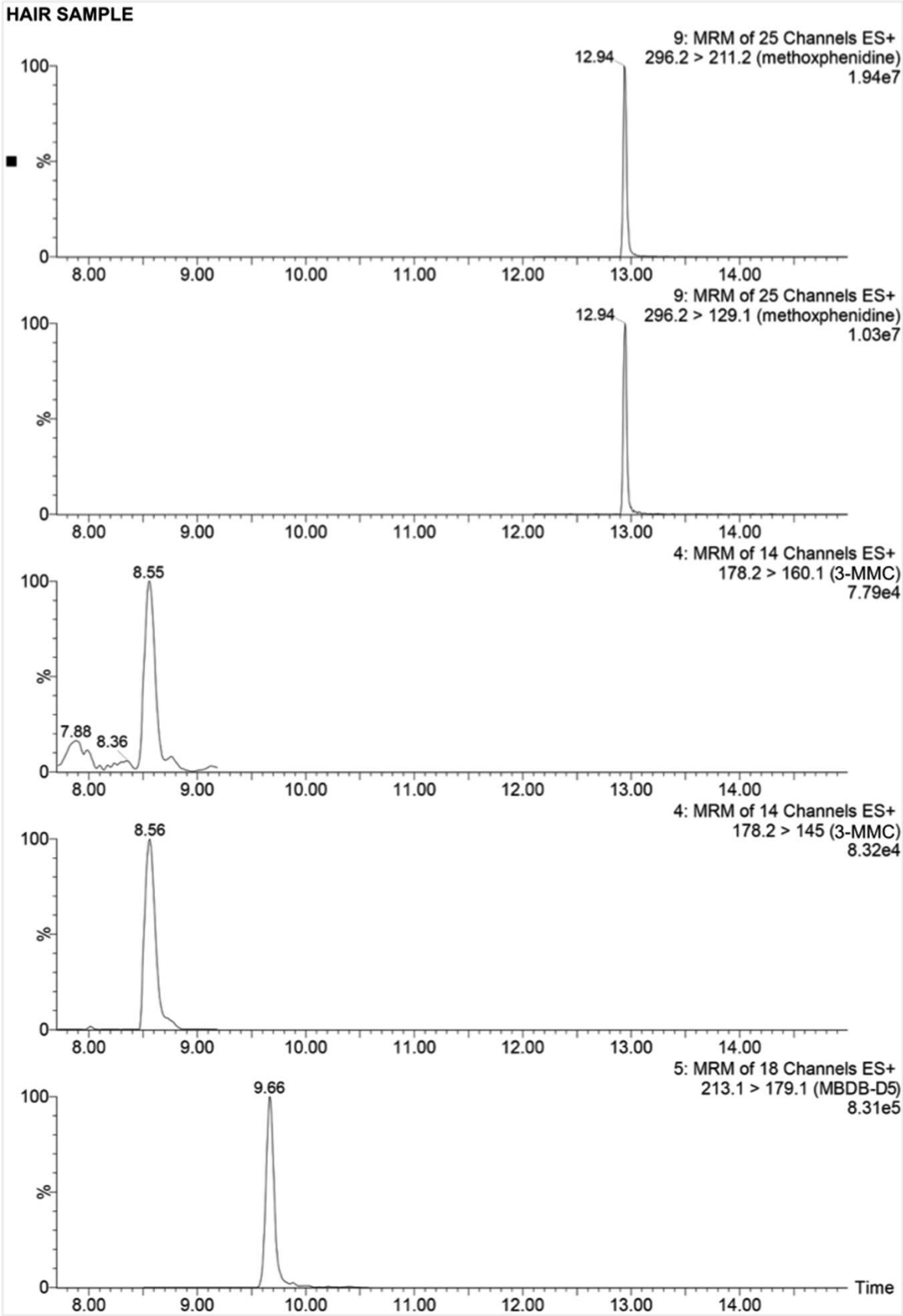


Figure 4. Chromatograms of methoxphenidine, 3-MMC and MBDB-D5 used as IS in hair sample.

Downloaded from https://academic.oup.com/jat/advance-article/doi/10.1093/jat/bkab016/6125370 by TIAFT Member Access user on 21 June 2021

Table II. Results of Toxicological Examination

Sample type	Toxicological screening identification (LC–QTOF)	MXP quantification (LC–MS-MS)	Other quantification (LC–MS-MS)
Femoral blood	MXP; lidocaine (traces)	MXP: 606 μg/L	Lidocaine (traces)
Cardiac blood	MXP	MXP: 254 μg/L	–
Urine	MXP; 3-MMC; oxazepam; valsartan; lidocaine	MXP: 1,066 μg/L;	MMC: 238 μg/L; oxazepam: 750 μg/L; valsartan (traces); lidocaine (traces)
Gastric content	Not detected	–	–
Hair	3-MMC; cocaine; benzoylecgonine; diphenidine; MXP; lidocaine; quetiapine; zopiclone	MXP: 13 ng/mg of hair;	3-MMC: <0.25 ng/mg of hair; diphenidine: <0.25 ng/mg of hair
Syringe 1	3-MMC; methoxphenidine	–	–
Syringe 2	3-MMC; methoxphenidine diphenidine	–	–

Results and Discussion

Besides MXP, the toxicological screening (Table II) identified lidocaine (traces) in femoral blood. These drugs were also identified in urine with a synthetic cathinone, 3-methylmethcathinone also called 3-MMC (238 μg/L), oxazepam (750 μg/L) and valsartan (traces). Except for MXP and 3-MMC, the concentrations of all other drugs were consistent with concentrations observed in human performance cases. The toxicological analysis did not detect any other lethal toxic agent.

Hair analysis (Table II) identified MXP (13 ng/mg), 3-MMC (<0.25 ng/mg), diphenidine (<0.25 ng/mg), cocaine, benzoylecgonine, lidocaine, quetiapine and zopiclone. All drugs identified in the hair sample were not detected (<0.25 μg) in dichloromethane and water washes.

According to the literature, MXP intoxication is rare. In Sweden, a national collaborative project reviewed the results of toxicological analyses in patients with acute poisoning following NPS consumption between January and December 2014. Among 1,580 consultations in the emergency/intensive care services with suspicion of NPS intoxication, 750 cases were analytically investigated and the involvement of MXP was demonstrated in three cases (0.4%) (10). More recently, in France, the French addictovigilance network showed an increase in the number of cases of MXP abuse from 2012 to 2016 (16). In the French annual drug-related death survey DRAMES, which collects and analyzes cases of deaths related to substance abuse (psychoactive drugs and illicit substances), MXP was involved in the death of a 42-year-old man in 2015, in association with other NPSs (synthetic cathinones and synthetic cannabinoids) and opioids (17, 18). In the chemsex context, co-exposure to other NPSs and/or classical drugs of abuse is common. In our case, MXP was associated with 3-MMC. These results are in accordance with the results of Helander et al. (10) because the three non-fatal cases identified in their study were all polydrug users.

Apart from a number of anecdotal reports, detailed information about the pharmacodynamic features of MXP is not available. It is tempting to consider the potential involvement of the NMDA receptor when making comparisons with what has been described for diphenidine, MXE and ketamine (12, 19). MXP effects after oral administration are said to begin after 60 min, with a duration of up to 18 h (13). Such effects may include drowsiness, dissociative and catatonic states, hallucinations, confusion and cardiovascular effects including hypertension and tachycardia. Moreover, rhabdomyolysis, acute kidney injury and ischemic cerebral disease were observed with MXP (20–22). In user forums, MXP is described as having a strong addictive potential that may lead to the development of a withdrawal syndrome (23). However, further research into the pharmacological properties of these particular diarylethylamines seems warranted to elucidate additional mechanisms of action that may be involved.

MXP is generally used orally (swallowed, parachuted or sprayed) in doses of 80–150 mg (in some reports up to 500 mg), and users report a steep dose–response curve (24). More exceptionally, it can be administered rectally, nasally or by inhalation. We presented a rare reported case of MXP injection in the context of chemsex (SLAM) confirmed by the absence of MXP in gastric contents and the presence of MXP and 3-MMC in the syringes tested. Since 3-MMC has not been identified in blood samples, we can assume that its half-life (0.8 h) is shorter than that of MXP (25).

Hair analysis provides a useful history of past drug use, but interpretation of hair concentration must be done with caution. Hair analysis results determine the molecules to which the victim had been exposed during the 2.5 months before death. Hair analysis showed the presence of different illicit psychoactive substances; these results were in accordance with those of a polydrug user. The hair results also highlight that this was not the first use of MXP and 3-MMC by the patient. MXP (logP: 4.53) concentration was higher than its metabolite diphenidine (logP: 4.62) concentration previously described (26). This can be explained because in most of the cases, drug metabolism leads to increased hydrophilicity. Polar metabolites enter the hair to a lesser extent than their lipophilic precursors (27). Lidocaine was probably present in cocaine powder as a cutting agent (28). Zopiclone identification is in accordance with the victim's prescription medications. On the other hand, we did not identify any antihypertensive treatment. We can hypothesize that the victim was not observant and that this may have increased the cardiovascular risk when taking the NPS. Quetiapine identification in hair did not correspond to any prescription at the time of death. Substances identified in the hair specimen were not identified in the dichloromethane and water washes, suggesting no external contamination (29–31).

In comparison with previous reports (Table III), the concentration of MXP in femoral blood (606 μg/L) is lower. Based on only these few cases, it is not possible to determine what concentrations may constitute excessive use, recreational use or otherwise. The variation in concentrations depends on a range of factors, such as routes of administration, dosage used, delay between consumption and death, and user pharmacokinetics specificities. The MXP concentration in cardiac blood (354 μg/L) was lower than that in femoral blood (606 μg/L). The cardiac blood (C) to peripheral blood (P) ratio was 0.6. This ratio suggests an absence of postmortem redistribution, which is defined as a C/P ratio of <1 (32). This confirms that the concentrations observed are those at the time of death.

Downloaded from https://academic.oup.com/jat/advance-article/doi/10.1093/jat/bkab016/6125370 by TIAFT Member Access user on 21 June 2021

Table III. Comparison with Other Studies

Literature	Sex/years	Sample type	MXP concentration	Other substances detected	Clinical details
Fatal stabbing					
Presented case	M/55	Femoral blood	606 μg/L	Lidocaine	Dead after MXP injection and 32 stab wounds
		Urine	1,066 μg/L	3-MMC; oxazepam; valsartan; lidocaine	
		Hair	13 ng/mg of hair	3-MMC; cocaine; benzoylecgonine; diphenidine; lidocaine; quetiapine; zopiclone	
Fatal stabbing					
Elliott et al. (14)	M/34	Femoral blood	24,000 μg/L	Mirtazapine, lamotrigine, citalopram	Dead at home after MXP use; hypertensive heart disease history
		Urine	Detected		
	M/34	Femoral blood	2,000 μg/L	Diazepam, quinine	Dead at home due to MXP toxicity
		Urine	Detected		
	M/38	Femoral blood	1,360 μg/L	Risperidone	Suicide by fatal car accident
		Urine	Detected		
Non-fatal cases					
Helander et al. (10)	M/25 [a]	Serum	187 μg/L	7-Aminonitrazepam; cannabis; lorazepam; methiopropamine; alpha-PBP; pentylone; pregabalin	Hypertension, anxiety
		Urine	610 μg/L (median between 1-2-3)		
	M/33 [b]	Serum	409 μg/L	3,4-CTMP; lorazepam; methiopropamine; thiothinone	Unconsciousness (GCS 8), dilated pupils on arrival. Flumazenil iv resulted in agitation and hallucinations
	F/48 [c]	Serum	Not detected	3-MeO-PCP; 4-MeOPCP, MT-45, oxazepam, temazepam	Unconsciousness (GCS 3), muscle rigidity, jaw clenching, hypertension on arrival followed by increasing level of consciousness, confusion, disorientation, horizontal and vertical nystagmus
	[a][b][c]	Urine	610 μg/L (median between [a], [b] and [c])	–	–
Stachel et al. (15)	M/33	Serum	57 μg/L	Amphetamine (111 g/L), MDA (28 μg/L), MDMA (3 μg/L)	Impaired driver

Conclusion

To the best of our knowledge, this is the first case of MXP identification and quantification in hair that confirms past use of MXP by the victim. The presented case illustrates that it is important to include several NPSs in postmortem toxicology screening especially in the chemsex context, including those from drug classes with relatively low prevalence of use.

Conflict of Interest

The authors declare that they have no conflict of interest.

References

1. Weatherburn, P., Hickson, F., Reid, D., Torres-Rueda, S., Bourne, A. (2017) Motivations and values associated with combining sex and illicit

Downloaded from https://academic.oup.com/jat/advance-article/doi/10.1093/jat/bkab016/6125370 by TIAFT Member Access user on 21 June 2021

drugs ('chemsex') among gay men in South London: findings from a qualitative study. *Sexually Transmitted Infections*, **93**, 203–206.
2. McCall, H., Adams, N., Mason, D., Willis, J. (2015) What is chemsex and why does it matter? *BMJ*, **351**, h5790.
3. Pichini, S., Marchei, E., Pacifici, R., Marinelli, E., Busardò, F.P. (2017) Chemsex intoxication involving sildenafil as an adulterant of GHB. *Drug Testing and Analysis*, **9**, 956–959.
4. Giorgetti, R., Tagliabracci, A., Schifano, F., Zaami, S., Marinelli, E., Busardò, F.P. (2017) When "chems" meet sex: a rising phenomenon called "chemsex". *Current Neuropharmacology*, **15**, 762–770.
5. Maxwell, S., Shahmanesh, M., Gafos, M. (2019) Chemsex behaviours among men who have sex with men: a systematic review of the literature. *International Journal of Drug Policy*, **63**, 74–89.
6. Batisse, A., Peyrière, H., Eiden, C., Courné, M.A., Djezzar, S. (2016) Réseau français des centres d'addictovigilance. [Use of psychostimulants in a sexual context: analysis of cases reported to the French Network of Addictovigilance Centers]. *Therapie*, **71**, 447–455.
7. Busardò, F.P., Gottardi, M., Pacifici, R., Varì, M.R., Tini, A., Volpe, A.R., et al. (2020) Nails analysis for drugs used in the context of chemsex: a pilot study. *Journal of Analytical Toxicology*, **44**, 69–74.
8. Pirani, F., Lo Faro, A., Tini, A. (2019) Is the issue of chemsex changing? *La Clinica Terapeutica*, **170**, 337–338.
9. McLaughlin, G., Morris, N., Kavanagh, P.V., Power, J.D., O'Brien, J., Talbot, B., et al. (2016) Test purchase, synthesis, and characterization of 2-methoxydiphenidine (MXP) and differentiation from its meta- and para-substituted isomers. *Drug Testing and Analysis*, **8**, 98–109.
10. Helander, A., Beck, O., Bäckberg, M. (2015) Intoxications by the dissociative new psychoactive substances diphenidine and methoxphenidine. *Clinical Toxicology (Philadelphia, PA)*, **53**, 446–453.
11. Van Hout, M.C., Hearne, E. (2015) "Word of mouse": indigenous harm reduction and online consumerism of the synthetic compound methoxphenidine. *Journal of Psychoactive Drugs*, **47**, 30–41.
12. Wallach, J., Kang, H., Colestock, T., Morris, H., Bortolotto, Z.A., Collingridge, G.L., et al. (2016) Pharmacological investigations of the dissociative 'legal highs' diphenidine, methoxphenidine and analogues. *PLoS One*, **11**, e0157021.
13. Luethi, D., Hoener, M.C., Liechti, M.E. (2018) Effects of the new psychoactive substances diclofensine, diphenidine, and methoxphenidine on monoaminergic systems. *European Journal of Pharmacology*, **819**, 242–247.
14. Elliott, S.P., Brandt, S.D., Wallach, J., Morris, H., Kavanagh, P.V. (2015) First reported fatalities associated with the 'research chemical' 2-methoxydiphenidine. *Journal of Analytical Toxicology*, **39**, 287–293.
15. Stachel, N., Jacobsen-Bauer, A., Skopp, G. (2016) A methoxydiphenidine-impaired driver. *International Journal of Legal Medicine*, **130**, 405–409.
16. Eiden, C., Leone-Burgos, S., Serre, A., Carton, L., Gerardin, M., Boisselier, R.L., et al. (2018) Ephenidine, diphenidine, and methoxphenidine complications reported to the French addictovigilance network. *Fundamental & Clinical Pharmacology*, **32**, 654–662.
17. Richard, N., Arditti, J., Pépin, G., Mallaret, M., Castot, A.C. (2010) Drames (Décès en relation avec l'abus de médicaments et de substances): outil d'identification des décès liés à la drogue en France. *Bulletin Épidémiologique Hebdomadaire*, **40–41**, 16.
18. ANSM. (2015) *Outils de surveillance et d'évaluation - Résultats d'enquêtes DRAMES - Résultats del'enquête 2015*. https://www.ansm.sante.fr/var/ansm_site/storage/original/application/ecb099a2a0e037284ce7f6ce0e8fc1cd.pdf (accessed Nov 17, 2020).
19. Morris, H., Wallach, J. (2014) From PCP to MXE: a comprehensive review of the non-medical use of dissociative drugs. *Drug Testing and Analysis*, **6**, 614–632.
20. Valli, A., Lonati, D., Locatelli, C.A., Buscaglia, E., Tuccio, M.D., Papa, P. (2017) Analytically diagnosed intoxication by 2-methoxphenidine and flubromazepam mimicking an ischemic cerebral disease. *Clinical Toxicology*, **55**, 611–612.
21. Lam, R.P.K., Yip, W.L., Tsui, M.S.H., Ng, S.W., Ching, C.K., Mak, T.W.L. (2016) Severe rhabdomyolysis and acute kidney injury associated with methoxphenidine. *Clinical Toxicology*, **54**, 464–565.
22. Chrétien, B., Bourgine, J., Hamel Sénécal, L., Bretaudeau-Deguigne, M., Boels, D., Lelong-Boulouard, V., et al. (2018) Severe serotonin syndrome in an autistic new psychoactive substance user after consumption of pills containing methoxphenidine and α-methyltryptamine. *Journal of Clinical Psychopharmacology*, **38**, 94–96.
23. Champeau, W., Eiden, C., Gambier, J., Peyriere, H. (2017) Methoxphenidine use disorder: first case notified to the French addictovigilance network. *Journal of Clinical Psychopharmacology*, **37**, 376–377.
24. Hofer, K.E., Degrandi, C., Müller, D.M., Zürrer-Härdi, U., Wahl, S., Rauber-Lüthy, C., et al. (2014) Acute toxicity associated with the recreational use of the novel dissociative psychoactive substance methoxphenidine. *Clinical Toxicology*, **52**, 1288–1291.
25. Shimshoni, J.A., Britzi, M., Sobol, E., Willenz, U., Nutt, D., Edery, N. (2015) 3-Methyl-methcathinone: pharmacokinetic profile evaluation in pigs in relation to pharmacodynamics. *Journal of Psychopharmacology (Oxford, England)*, **29**, 734–743.
26. Alvarez, J.C., Fabresse, N., Knapp, A., El Hajj Sleiman, I., Garnier, R., Langrand, J. (2017) Identification and quantification of diphenidine in hair by LC-MS/MS after single administration. *Toxicologie Analytique et Clinique*, **29**, 64–70.
27. Pragst, F., Balikova, M.A. (2006) State of the art in hair analysis for detection of drug and alcohol abuse. *Clinica Chimica Acta*, **370**, 17–49.
28. Cole, C., Jones, L., McVeigh, J., Kicman, A., Syed, Q., Bellis, M. (2011) Adulterants in illicit drugs: a review of empirical evidence. *Drug Testing and Analysis*, **3**, 89–96.
29. Cooper, G.A.A., Kronstrand, R., Kintz, P. (2012) Society of Hair Testing guidelines for drug testing in hair. *Forensic Science International*, **218**, 20–24.
30. Kintz, P. (2004) Value of hair analysis in postmortem toxicology. *Forensic Science International*, **142**, 127–134.
31. Kintz, P. (2012) Segmental hair analysis can demonstrate external contamination in postmortem cases. *Forensic Science International*, **215**, 73–76.
32. Dalpe-Scott, M., Degouffe, M., Garbutt, D., Drost, M. (1995) A comparison of drug concentrations in postmortem cardiac and peripheral blood in 320 cases. *Canadian Society of Forensic Science Journal*, **28**, 113–121.

Downloaded from https://academic.oup.com/jat/advance-article/doi/10.1093/jat/bkab016/6125370 by TIAFT Member Access user on 21 June 2021

Publication n° 8

Refractory cardiogenic shock caused by methadone poisoning and treated with hybrid ECLS

Antoine Beurton, Ruben Goncalves, Julien Imbault, Benjamin Repusseau, Alexandre Ouattara

Publié en 2022 dans *Therapies*

https://doi.org/doi:10.1016/j.therap.2022.02.004

Journal Pre-proof

Case report: Refractory cardiogenic shock caused by methadone poisoning and treated with hybrid ECLS*

Antoine Beurton Ruben Goncalves Julien Imbault Benjamin Repusseau Alexandre Ouattara

PII: S0040-5957(22)00043-9

DOI: https://doi.org/doi:10.1016/j.therap.2022.02.004

Reference: THERAP 670

To appear in: *Therapies*

Received Date: 3 September 2021

Please cite this article as: Beurton A, Goncalves R, Imbault J, Repusseau B, Ouattara A, Case report: Refractory cardiogenic shock caused by methadone poisoning and treated with hybrid ECLS*, *Therapies* (2022), doi: https://doi.org/10.1016/j.therap.2022.02.004

This is a PDF file of an article that has undergone enhancements after acceptance, such as the addition of a cover page and metadata, and formatting for readability, but it is not yet the definitive version of record. This version will undergo additional copyediting, typesetting and review before it is published in its final form, but we are providing this version to give early visibility of the article. Please note that, during the production process, errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

© 2020 Published by Elsevier.

Journal Pre-proof

THERAPIES

HEADING: Letter to editor

Case report: Refractory cardiogenic shock caused by methadone poisoning and treated with hybrid ECLS*

Antoine Beurton[a,b],* Ruben Goncalves[c], Julien Imbault[a,b], Benjamin Repusseau[a], Alexandre Ouattara[a,b]

[a] *CHU Bordeaux, Department of Anaesthesia and Critical Care, Magellan Medico-Surgical Centre, 33000 Bordeaux, France*

[b] *Univ. Bordeaux, INSERM, UMR 1034, Biology of Cardiovascular Diseases, 33600 Pessac, France*

[c] *CHU Bordeaux, Department of Toxicology, Pellegrin hospital, 33000 Bordeaux, France*

Received 3 September 2021; accepted 21 February 2022

***Corresponding author**. Department of Anaesthesia and Critical care, Magellan Medical and Surgical Center, Haut-Lévêque Hospital, Magellan Avenue, 33600 Pessac, France.
E-mail address: antoine.beurton@chu-bordeaux.fr (A. Beurton)

*This case has been declared at the addictovigilance unit of Bordeaux on the 06 june 2020 under the number N° BX20200606.

Journal Pre-proof

KEYWORDS
Methadone poisoning; Refractory cardiogenic shock; ECMO

Abbreviations

ALAT: alanine aminotransferase
ASAT: aspartate aminotransferase
CMV: cytomegalovirus
EBV: Epsein Barr virus
ECG: electrocardiogram
ECMO: extracorporeal membrane oxygenation
EDDP: 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine
HBV: hepatitis B virus
HCV: hepatitis C virus
HIV: human immunodeficiency virus
HHV6: human herpes virus 6
HSV: herpes simplex virus 1
IBW: ideal body weight
ICU: intensive care unit
LV: left ventricle
LVEF: LV ejection fraction
PCR: polymerase chain reaction
PEEP: positive end-expiratory pressure
RIJ: right internal jugular
SARS-CoV-2: severe acute respiratory syndrome coronavirus 2
TAPSE: tricuspid annular plane systolic excursion
VA: veino arterial
VTI: velocity time integra
VVA-ECMO: veno-veno-arterial extracorporeal membrane oxygenation

Methadone cardiotoxicity has been widely reported and includes principally QT interval prolongation leading potentially to torsade de pointes [1]. Hemodynamic instability, acute heart failure or more rarely cardiogenic shock have also been described following methadone overdose [2]. An excessive catecholamine release responsible for Takotsubo syndrome stress cardiomyopathy has been proposed to explain acute heart failure following methadone poisoning [2,3]. Here, we describe a patient with

Journal Pre-proof

multiple organ dysfunction syndrome complicating refractory cardiogenic shock caused by massive methadone intoxication without typical electrocardiogram (ECG) changes and/or echocardiography abnormalities and in whom a veno-veno-arterial extracorporeal membrane oxygenation (VVA-ECMO) was required.

Case history

A 39-year-old man with a history of drug addiction, but free from drug use for 7 years, was found comatose by his family. Paramedics found him to be hypotensive, hypopnoeic, hypoglycaemic (0.56 $g.L^{-1}$), hypothermic and cyanotic. He had miosis and empty methadone blisters were found nearby him (total dose of 1820 mg). He received 0.4 mg of Naloxone and his Glasgow score increased from 3 to 11 with acute agitation. After tracheal intubation, norepinephrine and epinephrine were given intravenously. Upon arrival in hospital, the blood gas showed pH 7.13, $PaCO_2$ 61 mmHg, PaO_2 63 mmHg, SaO_2 96%, lactates 6.0 $mmol.L^{-1}$. Chest X-ray revealed bilateral acute pulmonary oedema (Fig. 1A). Transthoracic echocardiography showed biventricular failure: dilated left ventricle (LV), LV ejection fraction (LVEF) 25-30%, LV outflow tract velocity time integral (VTI) 9 cm, absence of valvular disease, tricuspid annular plane systolic excursion (TAPSE) 6 cm. ECG revealed sinus tachycardia at 120 bpm with PR 160 ms, QRS 96 ms, QTc 369 ms, and upright and peaked P waves in leads II and III favouring a "P-pulmonale" wave. In the next few hours, the patient exhibited severe cardiogenic shock (refractory hypotension, hyperlactatemia 8.3 $mmol.L^{-1}$ and indexed cardiac output under 2 $L.min^{-1}.m^2$) while the dose of epinephrine was increased to 2.1 $\mu g.kg^{-1}.min^{-1}$. After a skin disinfection, a 19 Fr cannula (Maquet Getinge, Gothenburg, Sweden) was introduced in the left common femoral artery, a 21 Fr venous canula in the left femoral vein and a distal reperfusion for his leg in the superficial femoral artery. Because the patient suffered from severe hypoxemia, a 17 Fr cannula was introduced in his right internal jugular (RIJ) vein to ensure a precardiac oxygenation and limit the Harlequin syndrome (5). The extracorporeal membrane oxygenation (ECMO) blood flow was started at 5 $L.min^{-1}$ while the RIJ line was partially clamped to limit its flow to 1 $L.min^{-1}$. His theoretical cardiac output had been set at 3 $L.min^{-1}.m^{-2}$ for a patient of 166 cm, 75 kg (corporeal area 1.83 m^2). The blood flow and FiO2 to the RIJ cannula were set for an inlet saturation above 80% before the pulmonary circulation and PaO2 in right arterial blood above 95%. The arterial blood-gas obtained from the right radial artery under VVA-ECMO showed: pH 7.26, PaCO2 31.5 mmHg, PaO2 338 mmHg, lactates 12.8 $mmol.L^{-1}$ (maximal lactates). Mechanical ventilation was set at 6

Journal Pre-proof

ml.kg^{-1} of IBW x 10 c/min, positive end-expiratory pressure (PEEP) 8-10 cmH_2O, sweep gas on ECMO controlled for normal PaCO2. On ECMO day 1, epinephrine and norepinephrine infusions were reduced and an intravenous infusion of dobutamine was initiated at 5 µg.kg^{-1}.min^{-1}. The LVEF was 15%, VTI 1 cm with intraventricular sludge and an antero-lateral thrombus 1x1cm. An intra-aortic balloon pump was introduced for LV unloading. A resolution of pulmonary oedema could be rapidly observed (Fig. 1B). During the first days, the patient exhibited some organ failures including acute kidney injury (KDIGO 2) and hepatic failure (aspartate aminotransferase [ASAT] 4306 UI.L^{-1}, alanine aminotransferase [ALAT] 1162 UI.L^{-1}, PT failed at 45% with 22% factor V, all were normalised on day 4). Troponin-I (normal <30 ng.L^{-1}) was measured on the 1st day at 80 ng.L^{-1} and the peak was obtained at 5746 ng.L^{-1} on day 2. The serum creatinine peak was reached on the 1st day (192 µmol.L^{-1}) and was normal after the 3rd day without extrarenal epuration. Lactates were normalised on day 2. The jugular cannula (converting as a veino arterial [VA] ECMO) and the intra-aortic balloon pump were removed on the 3rd day. The VA-ECMO was removed on the 5th day without reintroduction of inotrope or pressor amines. He was extubated on day 6. He did not exhibit any neurological injury. Finally, the patient was discharged from the intensive care unit on the 10th day, his ECG was normal with the disappearance of his "p-wave pulmonary". Echocardiography gradually improved, with normalisation on day 10 (LV VTI 16cm, LVEF 55%, normal LV size, TAPSE 14 cm). There were no signs of either regional wall motion anomalies or genetic hypertrophic or obstructive cardiomyopathy.

Concerning the differential diagnosis, all our investigations were negative. Coronary angiography, full body CT-scan were normal. We also looked for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), influenza virus, Brucella, Chlamydia, Coxiella, Rickettsia, Syphilis, Zika, toxoplasma, human immunodeficiency virus (HIV), hepatitis B virus (HBV), hepatitis C virus (HCV), human herpesvirus 6 (HHV6), Epsein Barr virus (EBV), adenovirus, cytomegalovirus (CMV), B19, Chikungunya, herpes simplex virus 1 (HSV) 1 and 2 by polymerase chain reaction (PCR) [by bronchoalveolar lavage or blood sample] or serology. Dengue serology was compatible with a recent infection in the past 3 months (trip to Thailand 4 months previously). Auto-immune myocarditis test results were negative. Blood samples were subjected to toxicological screening using ultraperformance liquid chromatography–high-resolution time-of-flight mass spectroscopy. The analytical system consisted of an Acquity I class system (Waters, Milford, MA, USA) coupled to a Xevo XS G2 QTOF analyser (Waters). Data were analysed with an exact mass database from Waters for >1500 toxicologically relevant drugs and metabolites. The following drugs were identified: acetaminophen, methadone and its metabolite 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP), etomidate and laudanosine. Quantifications were performed for acetaminophen, methadone,

Journal Pre-proof

and EDDP. The respective plasma concentrations of methadone and EDDP upon arrival in the ICU (intensive care unit) were 1723 ng.mL^{-1} and 167 ng.mL^{-1}. These high levels decreased progressively over the following 10 days. One month later, a segmental toxicological hair analysis was performed (liquid chromatography–tandem mass spectrometry). Hair analysis was negative in all segments for illicit drugs and substitution therapy, corresponding to an absence of drug consumption before admission (0 to 6 cm of hair).

Discussion

Previous case reports described cardiogenic shock following methadone overdose, which was treated with inotropic drugs [2]. The use of VA-ECMO has been previously reported to treat opioid-induced pulmonary oedema [4]. The present case is the first to report multiple organ failure syndrome complicating refractory cardiogenic shock treated by VVA-ECMO. The severity of cardiogenic shock of our patient can be explained by the high plasma level of methadone (i.e. 1723 ng.mL^{-1} on intensive care unit [ICU] admission) which has been described as lethal [1]. Although the underlying mechanism of methadone cardiotoxicity remains unclear, QT prolongation and torsade de pointes have been described but did not apply in this case [1,5]. As previously reported, cardiac function was fully restored after 10 days [2]. The presumed cause of rapidly reversible cardiogenic shock was Takotsubo adrenergic cardiomyopathy which was previously described in patients with methadone poisoning [2]. It should be pointed out that our patient did not exhibit the typical echocardiographic pattern or ECG changes. Despite the absence of cardiac magnetic resonance imaging findings, the recent consensus regarding Takotsubo cardiomyopathy suggests that our patient exhibited adrenergic cardiomyopathy. The main supporting evidence is the ad-integrum reversibility after a few days of treatment [6]. An increase in circulating catecholamine levels has been observed after methadone injection in some species [7]. Excessive circulating catecholamines have been involved in the pathophysiology of this cardiomyopathy. Only a few case reports have described refractory cardiogenic shock after massive methadone poisoning and this concerned children [4]. Methadone has very good bio-availability (80%) with a long half-life (7-65 hours) and is mostly metabolised with cytochrome P450 3A4 and CYP2D6 [8]. Adrenergic cardiomyopathy has been described due to methadone withdrawal but he had not taken methadone in the months before intoxication, according to the hair analysis [3]. He had not taken any medication which could interact with CYP450. The elimination of methadone is, for the most part, assured by the kidney (15-40% during the first 24

hours) and he presented severe acute kidney injury. This could increase the toxicity of the drug [5]. Our patient had a methadone plasma level of 1723 $ng.mL^{-1}$ which is described as a lethal dose [9,10]. Concerning the final diagnosis, he did not have the typical pattern of Takotsubo cardiomyopathy but recent international expert consensus on Takotsubo syndrome proposed a new algorithm for the diagnosis [6]. This case demonstrates that we should be very aggressive during the treatment of refractory cardiogenic shock caused by poisoning, a fortiori, with a young healthy patient. Methadone or opioid poisoning can present many serious clinical symptoms. The patient is currently in good health and in the care of a cardiologist and an addictologist.

Conclusion

This case report describes multiple organ dysfunction syndrome complicating refractory cardiogenic shock following massive methadone intoxication. Neither ECG changes nor echocardiography abnormalities suggesting the diagnosis of Takotsubo syndrome could be observed on admission. It emphasises that such a diagnosis should be routinely suspected in patients with a history of drug abuse and access to methadone, even in the absence of typical ECG and/or echocardiography changes, and should be aggressively treated as it may be completely reversible. Also, direct methadone toxicity cannot be excluded. Toxicological analyses combining blood and hair testing should be performed to determine the cause of overdose and to exclude the use of other toxic substances.

Disclosure of interests

Authors have no competing interest to declare

Source and funding statement

None

Journal Pre-proof

Journal Pre-proof

References

[1] Alinejad S, Kazemi T, Zamani N, Hoffman RS, Mehrpour O. A systematic review of the cardiotoxicity of methadone. EXCLI J 2015;14:577–600.

[2] Dehghani K, Shojaie M, Pourdavood AH, Khajouei M. Stress cardiomyopathy (Takotsubo syndrome) following accidental methadone poisoning; report of two pediatric cases. Arch Acad Emerg Med 2019;7:e22.

[3] Faisal B. Saiful JL, Faisal B. Saiful JL. Takotsubo cardiomyopathy due to iatrogenic methadone withdrawal. Rev Cardiovasc Med 2011;12:164–7.

[4] Daugherty LE. Extracorporeal membrane oxygenation as rescue therapy for methadone-induced pulmonary edema. Pediatr Emerg Care 2011;27:633–4.

[5] Moore N, Berdaï D, Blin P, Droz C. Pharmacovigilance - The next chapter. Therapie 2019;74:557–67.

[6] Ghadri J-R, Wittstein IS, Prasad A, Sharkey S, Dote K, Akashi YJ, et al. International Expert Consensus Document on Takotsubo syndrome (Part I): clinical characteristics, diagnostic criteria, and pathophysiology. Eur Heart J 2018;39:2032–46.

[7] Garofalo NA, Teixeira Neto FJ, Pereira CDN, Pignaton W, Vicente F, Alvaides RK. Cardiorespiratory and neuroendocrine changes induced by methadone in conscious and in isoflurane anaesthetised dogs. Vet J Lond Engl 1997 2012;194:398–404.

[8] Fredheim Oms, Moksnes K, Borchgrevink Pc, Kaasa S, Dale O. Clinical pharmacology of methadone for pain. Acta Anaesthesiol Scand 2008;52:879–89.

[9] Lusetti M, Licata M, Silingardi E, Reggiani Bonetti L, Palmiere C. Therapeutic and recreational methadone cardiotoxicity. J Forensic Leg Med 2016;39:80–4.

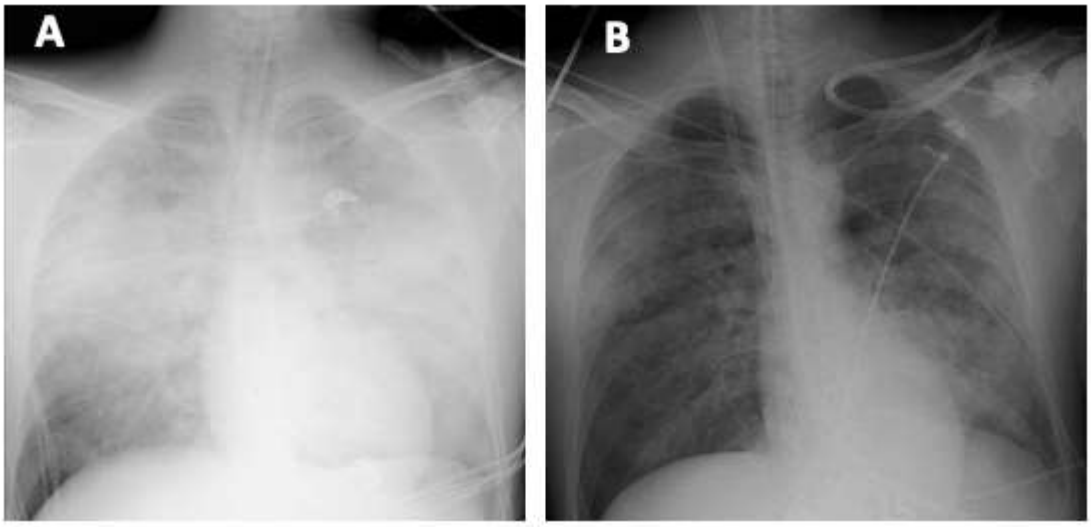
[10] Milroy CM, Forrest ARW. Methadone deaths: a toxicological analysis. J Clin Pathol 2000;53:277–81.

Journal Pre-proof

Journal Pre-proof

Legends

Figure 1. Posterior-anterior chest X-ray at the intensive care unit admission showing severe alveolar oedema (A) and under extracorporeal life support one day after the addition of intra-aortic balloon pump (B).



Journal

Publication n° 9

Ecstasy-induced fixed drug eruption

Ophélie Barbier, Alia Galdari, Brigitte Milpied, Paola Sanchez, Stéphanie Kassab, Ruben Goncalves, Anne-Sophie Darrigade

Publié en 2022 dans *Contact Dermatitis*

DOI: 10.1111/cod.14132

Check for updates

Received: 17 December 2021 | Revised: 16 February 2022 | Accepted: 17 February 2022
DOI: 10.1111/cod.14132

CONTACT POINT

CONTACT DERMATITIS WILEY

Ectasy-induced fixed drug eruption

Ophélie Barbier[1] | Alia Galadari[1] | Brigitte Milpied[1] | Paola Sanchez[2] | Stéphanie Kassab[1] | Ruben Goncalves[3] | Anne-Sophie Darrigade[1]

[1]Department of Dermatology, Hôpital Saint André, CHU de Bordeaux, Bordeaux, France

[2]Department of Pharmacoviligancy, Hôpital Pellegrin, CHU de Bordeaux, Bordeaux, France

[3]Department of Pharmacotoxicology, Hôpital Pellegrin, CHU de Bordeaux, Bordeaux, France

Correspondence
Anne-Sophie Darrigade, Department of Dermatology, Hôpital Saint-André, CHU Bordeaux, 1 rue Jean Burguet, 33000 Bordeaux, France.
Email: anne-sophie.darrigade@chu-bordeaux.fr

KEYWORDS: 3,4-methylenedioxy-*N*-methyl, amphetamine, case report, cocaine, cutaneous side effect, ecstasy, fixed drug eruption, illicit drugs, levamisole

CASE REPORT

Fixed drug eruption (FDE) is a drug hypersensitivity reaction mostly caused by antibiotics and anti-inflammatory non-steroidal drugs.[1,2] 3,4-Methylenedioxy-*N*-methylamphetamine (MDMA) is a party drug, available as a powder or a tablet, the latter often referred to as 'ecstasy'.[3] Skin reactions associated with illicit drug use have only rarely been reported.[4] We here report a first case of FDE induced by MDMA. A 25-year-old woman had developed several erythematous itchy macules on the arms (Figure 1A), back and face, in September 2019, 3 weeks following treatment with antibiotics (ceftriaxone, doxycycline, and metronidazole) for a salpingitis. Although at the time the antibiotics had been suspected as a possible cause of the skin eruption, history revealed that the skin lesions had since then recurred several times (with intervals of several months), on the exact same skin areas, and always within 12 h after the recreational intake of MDMA, a drug which she had also taken during the first episode of the skin eruption. Following an inflammatory phase, the lesions always healed with remarkable hyperpigmentation (Figure 1B). Besides MDMA, she also confessed regularly using cocaine and alcohol, none of which, however, seemed to induce any skin reactions. Physical examination revealed well-defined (~1-cm diameter), hyperpigmented lesions on the forearm and the back, as well as around the lips. Patch tests were performed with the European baseline series from Chemotechnique Diagnostics (Vellinge, Sweden) on the upper back. All patch test preparations were mounted on IQ Ultra Chemotechnique Diagnostics (Vellinge, Sweden) patch test chambers, and occluded for 2 days with Urgoderm non-woven stretch plaster URGO (Chenôve, France). Because an FDE was strongly suspected, we also patch tested an MDMA tablet, provided by the patient and diluted 50% pet., on both lesional and non-lesional skin; the previously suspected antibiotics, although considered a less likely cause at the time of patch testing, were tested in a similar way. Readings on day 2 (D2) and D4, performed according to ESCD guidelines, were positive to the MDMA tablet (50% pet.), but only on previously affected skin, whereas the same test remained entirely negative on previously uninvolved skin (Figure 2); all other patch tests were equally negative. The oral introduction of drugs (ceftriaxone, doxycycline, and metronidazole) caused no skin reaction. An MDMA tablet supplied by the patient was qualitatively analyzed by mass

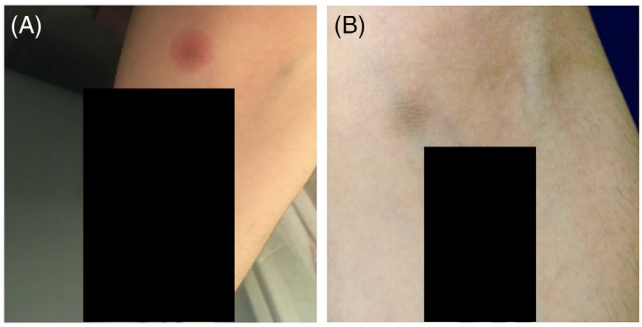


FIGURE 1 Photographs provided by the patient. (**A**) Erythematous and slightly papular well-defined macula on the left forearm. (**B**) Residual hyperpigmented lesions, lasting several months, on the same skin areas

This is an open access article under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.
© 2022 The Authors. *Contact Dermatitis* published by John Wiley & Sons Ltd.

FIGURE 2 Reading on day (D4) of patch tests containing the MDMA tablet (50% pet.), provided by the patient, on previously affected and non-affected skin

spectrometry, confirming the presence of MDMA, but also of cocaine and levamisole, the latter being a well-known cocaine adulterant. The concentration of cocaine and levamisole were above the detection limit but below the quantification limit (<5 ng/mL), so the vast majority of the tablet was therefore composed of MDMA. Additional patch tests with MDMA in methanol (1 mg/mL; 0.1%), cocaine in acetonitril (1 mg/mL; 0.1%), and levamisole powder diluted at 50% in pet., all remained negative on healthy and previously involved skin. Although further patch testing with MDMA in higher concentrations and alternative vehicles was proposed, the patient refused further skin tests. Follow-up, however, revealed that the accidental reintroduction of MDMA tablets led to severe recurrence of the skin lesions, whereas further and regular use of only cocaine did not.

DISCUSSION

In the 50s Nelson reported a case of FDE due to the inhalation of cocaine.[5] Only few cutaneous side effects have been attributed to MDMA, including guttate psoriasis occurring 4 days after the intake of ecstasy,[6] and an urticarial skin reaction developing 12 h after inhaling MDMA powder[7]; however, to the best of our knowledge, no cases of FDE due to ecstasy (MDMA tablets) have previously been reported. As skin tests may remain negative, the allergy work-up of an FDE often relies on a rechallenge with the suspected drug(s), although this evidently poses ethical questions when dealing with illicit drugs. Our patient had a positive patch test to the MDMA tablet (ecstasy) as such, whereas patch tests to its single components, including to pure MDMA, remained negative. We might not fully exclude that the actual culprit was an additive or impurity contained in the MDMA tablet, although the chemical analyses did not suggest the presence of these. It is likely that the negative patch test to (pure) MDMA was due to the low concentration used, and/or because an inappropriate vehicle was chosen (methanol instead of pet.).

To conclude, we present a case of FDE induced by an MDMA tablet (ecstasy), likely due to its MDMA content, based on the suggestive clinical history, characteristic skin lesions, positive patch test (only) on affected skin, as well as a positive oral reintroduction.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

AUTHOR CONTRIBUTIONS

Ophélie Barbier: Conceptualization (equal); investigation (equal); writing – original draft (lead); writing – review and editing (equal). **Alia Galadari:** Writing – original draft (equal). **Brigitte Milpied:** Supervision (equal). **Paola Sanchez:** Methodology (equal); validation (equal). **Stéphanie Kassab:** Data curation (equal). **Ruben Goncalves:** Data curation (equal); formal analysis (equal); writing – review and editing (supporting).

ORCID

Brigitte Milpied https://orcid.org/0000-0003-3679-6613
Anne-Sophie Darrigade https://orcid.org/0000-0003-4034-0395

REFERENCES

1. Phillips EJ, Bigliardi P, Bircher AJ, et al. Controversies in drug allergy: testing for delayed reactions. *J Allergy Clin Immunol*. 2019;143(1):66-73.
2. Patel S, John AM, Handler MZ, Schwartz RA. Fixed drug eruptions: an update, emphasizing the potentially lethal generalized bullous fixed drug eruption. *Am J Clin Dermatol*. 2020;21(3):393-399.
3. Edland-Gryt M, Sandberg S, Pedersen W. From ecstasy to MDMA: recreational drug use, symbolic boundaries, and drug trends. *Int J Drug Policy*. 2017;2017(50):1-8.
4. Hennings C, Miller J. Illicit drugs: what dermatologists need to know. *J Am Acad Dermatol*. 2013;69(1):135-142.
5. Nelson LM. Fixed drug eruptions—a report of two cases, one caused by niacin, the other by cocaine. *Calif Med*. 1955;82(2):127-128.
6. Tan B, Foley P. Guttate psoriasis following ecstasy ingestion. *Australas J Dermatol*. 2004;45(3):167-169.
7. Gómez-Fernández C, Feito Rodríguez M, Casado Verrier B, Herranz Pinto P, Beato-Merino M, Casado Jiménez M. Facial eruption related to snorting ecstasy. *Eur J Dermatol*. 2010;20(6):853-854.

How to cite this article: Barbier O, Galadari A, Milpied B, et al. Ectasy-induced fixed drug eruption. *Contact Dermatitis*. 2022; 1-2. doi:10.1111/cod.14132

Publication n° 10

How Long Do Implanted Triclosan Sutures Inhibit Staphylococcus aureus in Surgical Conditions? A Pharmacological Model

Frederic Christopher Daoud, Ruben Goncalves, Nicholas Moore

Publié en 2022 dans *Pharmaceutics*

https://doi.org/10.3390/ pharmaceutics14030539

pharmaceutics

MDPI

Article

How Long Do Implanted Triclosan Sutures Inhibit *Staphylococcus aureus* in Surgical Conditions? A Pharmacological Model

Frederic Christopher Daoud [1,*], Ruben Goncalves [1,2] and Nicholas Moore [1]

[1] INSERM U1219, Bordeaux Population Health, Bordeaux University, 146 rue Léo Saignat, CEDEX, F-33076 Bordeaux, France; ruben.goncalves@u-bordeaux.fr (R.G.); nicholas.moore@u-bordeaux.fr (N.M.)

[2] CHU de Bordeaux, Laboratoire de Pharmacologie et Toxicologie, Place Amélie Raba Léon, CEDEX, F-33076 Bordeaux, France

* Correspondence: frederic.daoud-pineau@u-bordeaux.fr or fcdaoud@gmail.com; Tel.: +33-(0)6-0300-6898

Abstract: (1) Background: Sutures with triclosan (TS) are used to reduce the risk of surgical site infections (SSI), but most clinical trials are inconclusive. The traceability of SSI risk to antimicrobial activity in operated tissues is needed. (2) Objectives: This study aimed to predict triclosan anti-staphylococcal activity in operated tissues. (3) Methods: Three TS were exposed to static water for 30 days, and triclosan release was recorded. Polyglactin TS explanted from sheep seven days after cardiac surgery according to 3Rs provided ex vivo acceleration benchmarks. TS immersion up to 7 days in ethanol-water cosolvency and stirring simulated tissue implantation. Controls were 30-day immersion in static water. The release rate over time was measured and fitted to a predictive function. Antistaphylococcal activity and duration were measured by time-kill analysis with pre-immersed polyglactin TS. (4) Fifteen to 60-fold accelerated in vitro conditions reproduced the benchmarks. The rate prediction with double-exponential decay was validated. The antistaphylococcal activity was bactericidal, with TS pre-immersed for less than 12 h before then *S. aureus* began to grow. The residual triclosan level was more than 95% and played no detectable role. (5) Conclusions: Polyglactin, poliglecaprone, and polydioxanone TS share similar triclosan release functions with parametric differences. Polyglactin TS is antistaphylococcal in surgical conditions for 4 to 12 h.

Keywords: sutures; triclosan; 3Rs; in vitro model; release kinetics

check for updates

Citation: Daoud, F.C.; Goncalves, R.; Moore, N. How Long Do Implanted Triclosan Sutures Inhibit *Staphylococcus aureus* in Surgical Conditions? A Pharmacological Model. *Pharmaceutics* **2022**, *14*, 539. https://doi.org/10.3390/pharmaceutics14030539

Academic Editor: Koyo Nishida

Received: 7 January 2022
Accepted: 22 February 2022
Published: 28 February 2022

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Copyright: © 2022 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https://creativecommons.org/licenses/by/4.0/).

1. Introduction

Triclosan (2,4-dichlorophenoxyphenol) is a broad-spectrum antimicrobial that inhibits bacterial fatty acid synthesis. The target receptor in various Gram-positive and negative species such as *Staphylococcus aureus* and *Escherichia coli* is NADH-dependent enoyl-[acyl carrier protein] reductase (FabI) [1–3].

Triclosan is an optional adjuvant in absorbable surgical sutures such as polyglactin 910 (Vicryl PLUS "V+"), polydioxanone (PDS PLUS "P+"), and poliglecaprone 25 (Monocryl PLUS "M+") [4–6]. The structural polymers provide different mechanical and absorption properties that drive suture selection given the surgical circumstances [7]. The intended effect from adding triclosan is to inhibit suture microbial colonisation.

In vitro and animal experiments have shown that triclosan sutures (TS) exhibit antimicrobial activity in microorganisms commonly associated with surgical site infections (SSI) such as *S. aureus*, *S. epidermidis* and *E. coli* [8–12].

The expected clinical efficacy is a reduced incidence of surgical site infections (SSIs). Comprehensive meta-analyses of clinical trials have consistently shown a significantly lower incidence of SSIs after wound closure with TS as compared to non-triclosan sutures (NTS) [13,14]. The meta-analysis with the most randomised controlled trials (RCTs) reported a pooled relative risk (RR) of 0.73 [0.65, 0.82], but individual study RRs ranged

210

Annexes

from 0.09 to 2.45, and only 12% of them were significant. Considering the moderate level of evidence, the WHO made a conditional recommendation to use TS *"for the purpose of reducing the risk of SSI, independent of the type of surgery"* [15].

Pooling can result in a statistical synergy of non-significant study RRs that are in the same direction, whether the cause bias or efficacy. Therefore, the tracing of individual study measures of efficacy to its cause, i.e., exposure to germs *versus* strength and duration of the antimicrobial activity in real-life conditions, is needed.

In vitro studies and experiments in rodents have shown TS antimicrobial activity lasting several days [8–12,16]. However, a literature review has shown that animal models of human surgery often translate poorly to clinical conditions [17]. Moreover, published studies do not relate the antimicrobial activity to triclosan bioavailability on TS.

A review of publication databases found articles on the range of triclosan load on a TS to meet antimicrobial efficacy and non-toxicity objectives, on TS surgical properties, microbiological assays and clinical trial outcomes [8–14,16,18]. No studies described triclosan bioavailability and pharmacodynamics in the three TS. Product labels indicate the maximum initial triclosan amount (472 µg/m in V+ and 2360 µg/m in P+ and M+), but the average initial amount and variability and the expected duration of bioavailability once implanted are not mentioned. No clinical claims are made [4–6].

One literature review reported the initial triclosan load on V+ to be 2.7 µg/cm, but the sources of that value and the methods used to determine it is published [19–21].

The use of translational models is unavoidable, given that absorbable sutures cannot be removed from patients for research purposes. Exploring the triclosan bioavailability of triclosan on sutures in animal studies would be scientifically acceptable only if the protocol were accurately specified with in vitro study results, had a significant benefit for patients, and was in compliance with animal ethics, compatible with 3Rs principles [22,23].

Therefore, this study developed an empirical in vitro model specified with ex vivo benchmarks using opportunistically explanted TS fragments from a valid large animal translational model of human surgery in the course of an independent preclinical study according to the *reduction* and *refinement alternatives* of the 3Rs and published approaches to predict drug dissolution in vitro [22–25].

The critical assumption of this study is that in vivo TS antimicrobial activity reduces the risk of SSI depending on activity duration, with two clinical period phases:

- Intraoperative: the objective is to avoid germ inoculation by the suture.
- Postoperative: the objective is to prevent suture colonisation while natural defences kill residual microorganisms.

2. Materials and Methods

2.1. Quantitative Methods

The marginal release rate $R(t)$ in µg/m/h was defined as the amount of triclosan released per unit of suture length and per unit of time (1):

$$R(t) = [triclosan]_{t+dt} \times \frac{V}{L \cdot dt} \quad (1)$$

All rates were derived from the triclosan concentration $[triclosan]_{t+dt}$ measured after immersing a segment of TS of length L in meters, in a volume of solvent V in litres for a duration dt in hours, starting at time t.

If the initial amount of triclosan per suture length unit was $A0$, the residual amount at time t since the beginning of the immersion was $A(t)$, calculated as the initial amount less the amount released between immersion start and t (2):

$$A(t) = A0 - \int_{t=0}^{t} R_{model}(t)dt \quad (2)$$

Triclosan is a solid at temperatures below 55 °C. It is moderately soluble in water (Cs = 0.001 g triclosan/100 g of water, i.e., 10 µg/mL) and highly soluble in ethanol (Cs > 100 g triclosan/100 g ethanol) [1,26,27]. Triclosan was assumed to dissolve in liquids according to the Noyes & Whitney relationship (3) [28,29]:

$$\frac{dM}{dt}(t) = \frac{S \cdot D}{d} \cdot (Cs - Ct) \quad (3)$$

$\frac{dM}{dt}(t)$ is the dissolution rate of the solute at a given time t, S is the dissolution surface (m^2), D is the diffusion coefficient ($m \cdot s^{-1}$), Cs is the solubility of the solute in the solvent ($Kg \cdot L^{-1}$ or $moles \cdot L^{-1}$), i.e., the maximum concentration of solute the solvent can hold, above which the solute precipitates. Ct ($Kg \cdot L^{-1}$ or $moles \cdot L^{-1}$) is the solute concentration in the bulk of the solvent at time t (s), and d (m) is the thickness of the solute's diffusion layer equal to the concentration gradient. Assuming the dissolution surface was constant for each suture during the assay, solubility and diffusion layer thickness were the two independent parameters to modify the dissolution rate.

2.2. Standardised Sample Preparation and Triclosan Concentration Determination

The studied sutures were V+, P+, and M+ calibre USP 2-0 (diameter: 0.35 to 0.4 mm), USP 0 (diameter: 0.4 to 0.5 mm) and USP 1 (diameter: 0.5 to 0.6 mm). All test sutures were unpackaged and cut as 35 cm long segments and immersed in 10 mL solvent. All immersions were in glass tubes and incubated at 37 °C in the dark to avoid photodegradation [30]. The tested solvents were pure water obtained by distillation or water plus absolute ethanol as a cosolvent. Immersions were either static or continuously stirred with a tube rotator/incubator (Roto-Therm™ model H2020, Benchmark Scientific, Sayreville, NJ, USA). An independent laboratory accredited for compliance with the EN ISO 17025 standard determined the triclosan concentrations [31,32]. The determinations were performed by liquid chromatography-mass spectrometry using direct sample injection into a high-performance liquid chromatography device (HPLC Shimadzu Nexera X2, Kyoto, Japan) with liquid chromatography-tandem mass spectrometry (LC-MS/MS Sciex 5500, AB Sciex Pte. Ltd., Singapour). The targeted quantification limit was 0.25 µg/L, but the validated limit was 0.01 µg/L.

The samples were prepared at Bordeaux University, Bordeaux, France, and concentrations were determined at Laboratoires des Pyrénées et des Landes (LPL), Mont De Marsan, France, who provided the test tubes and distilled water. Two copies of each in vitro sample were prepared in order to have a pair of determinations. Six copies of each ex vivo sample type and corresponding in vitro controls were prepared.

2.3. Opportunistic Explanted TS Fragments and Ex Vivo Triclosan Release Benchmarking

The government authorised the preclinical cardiac surgical trial involving three sheep with scheduled sacrifice on postoperative day-seven (DDPP Paris approval number: APAFIS 12534-2017121118586862 v3) and conducted at the IMMR laboratory, Paris, France. Bordeaux University's clinical pharmacology department solicited IMMR to piggyback that study. The plan consisted in replacing standard NTS used to close the surgical wounds of the animals by TS and to retrieve TS fragments immediately after animal sacrifice. The piggybacking was agreed because suture replacement did not cause bias to the cardiac surgery study, did not cause additional harm to the animals, and met the *reduction* and *refinement alternatives* of the 3Rs. Moreover, this cardiac surgery involved a validated translational model of human cardiac surgery [22,23,33].

The animals were operated on in conditions similar to human surgery. The surgeons closed the deep muscle layer with V+ 1 and the subcutaneous tissue with V+ 0. The explanted suture fragments were immediately packaged in biohazard bags, one per animal and tissue, labelled, deep-frozen, and forwarded to Bordeaux University, Bordeaux, France.

The explanted intramuscular V+ 1 fragments were long and allowed for the cutting of six 35 cm fragments to be immersed in 10 mL of water. The explants had lost much of their stiffness compared to new sutures.

The explanted subcutaneous V+ 0 fragments were much shorter and were grouped to form pairs of equal length: four groups reached 22 cm and were immersed in 6.3 mL of water, while the two others reached 17.25 cm and were immersed in 4.95 mL, thus ensuring the same length to volume ratio in all samples.

The immersion was static and lasted 12 h. Fragments were then removed, and the tubes with the solvent were forwarded for triclosan concentration determination.

2.4. In Vitro Triclosan Release Controls

Segments of V+ 2-0, P+ 2-0, and M+ 2-0 were unpackaged and immersed in static water. Early marginal release rates were calculated with triclosan concentrations determined after segment immersion in a single tube at 4, 8, and 12 h. Subsequent marginal release rates were estimated after segment pre-immersion in tubes during 1, 2, 3, 4, 5, 10, 15, 20, 25, and 30 days. After pre-immersion, each segment was transferred to a tube for the final 12-hour static water immersion used to calculate the marginal triclosan release rate. Segments V+ 0 and V+ 1 were pre-immersed for seven days, then transferred to the final tube for 12 h. Segments were removed from the final tubes before forwarding the tubes for triclosan concentration determination. After 30 days in water, sutures remained stiffer than sutures explanted on day-seven from animal muscle.

2.5. Benchmark-Driven Accelerated In Vitro Triclosan Release Models

In vitro conditions were modified targeting a 15-fold to a 60-fold acceleration in triclosan dissolution rate given the two parameters identified with the Noyes & Whitney formula [24]. It was assumed that minimising diffusion layer thickness (d) and increasing the difference between solubility and bulk concentration (C_s—C_t) would have multiplicative effects. The thickness was minimised by continuous rotation at 24 rounds-per-minute (rpm) and 37 °C incubation targeting a six-fold acceleration. The rotation speed was selected based on approaches described previously and was confirmed by checking that stirring dispersed triclosan flakes temporarily when triclosan concentration was equal to 60 µg/mL, i.e., six times triclosan solubility in water at 20 °C [26,27,34,35]. Triclosan solubility was increased by adding 1.2 g of absolute ethanol (EtOH) as a cosolvent to 9 g of pure water (i.e., a 13.3% w/w solvent). That ratio targeted a 10-fold increase in solubility because preparatory stepwise gravimetric titration showed that that ratio dissolved precipitated flakes of triclosan in a 100 µg/mL saturated solution. A parallel 2.5-fold increase in solubility was targeted with one-quarter of the amount of EtOH (0.3 g) in 9 g of water, i.e., a 3.3% w/w solvent. The choice of EtOH, among other organic solvents with high triclosan solubility such as acetone, was guided by published experience and by EtOH compatibility with the suture material [36,37]. Another effect of the organic solvent was stabilising triclosan flake dispersion by rotation.

V+ 0 and V+ 1 segments were immersed in parallel sequences of 11 tubes filled with 13.3% w/w solvent in the rotator/incubator. Immersions were from baseline to 4 h, then 4 to 12, 12 to 24, and then every 12 h until tube 11 from 168 to 192 h. Thus, the total immersion time was eight days. Parallel assays were conducted with similar segments in the 3.3% w/w solvent.

2.6. Estimation of the Initial Triclosan Load

The initial amount of triclosan on a suture segment was estimated with the assumption that it would completely dissolve in a 100% w/w EtOH/water solvent (1 g EtOH + 1 g water) at 37 °C with continuous stirring for six days. The released concentrations were determined with V+ 0, V+ 1, V+ 2-0, P+ 2-0, and M+ 2-0.

2.7. Release Kinetics—Pharmacodynamics Matching Tests

In vitro time-kill analyses were performed at Université de Bordeaux, Aquitaine Microbiologie, UMR 5234 CNRS, 33000 Bordeaux, France, according to CLSI standard M26-A [38]. Suture segments were pre-immersed in the accelerated conditions described previously to establish the relationship between the marginal triclosan release rate and the corresponding antimicrobial activity on triclosan-sensitive bacteria [39]. The tests were conducted at Bordeaux University's experimental microbiology laboratory. All tests were performed in a safety cabinet in sterile conditions. V+ 0 suture segments of the same batch as previously underwent accelerated triclosan release in 3.3% and 13.3% *w*/*w* solvents, respectively. Pre-immersion durations were 1, 4, 12, 24, and 48 h. At the end of the immersion period, segments were rinsed in distilled sterile water with vortex and then left to dry in a sterile environment. Each segment was then transferred into an Eppendorf tube filled with 10 mL of tryptic casein soy broth (TSB, Difco, BD Diagnostics, Sparks, MD, USA) for a suspension culture with a 0.5 McFarland microbial inoculum (i.e., 10^5 to 10^6 colony-forming units CFU/mL) of reference *S. aureus* ATCC 29213 incubated 24 h at 37 °C. Microbial concentration (CFU/mL) in the broth at 24 hours was compared with baseline. Culture tubes were vortexed with the segment inside; next, a 100 µL broth sample was drawn with an aliquot. The sample underwent five serial 1/10 *v*/*v* dilutions. The original broth and each dilution were each spread on an Agar plate with a Mueller Hinton (MH) culture medium and then incubated at 37 °C for 24 h. Viable colonies were counted on the plates at the end of the incubation. The plate selected for determining CFU concentration in the broth was the one where colonies were large enough to be identifiable and individualised. The microbial concentration (CFU/mL) in the culture at each time slot was calculated by multiplying the colony count on the plate by the dilution factor. Two copies of the time-kill assays were performed per solvent and pre-immersion duration.

2.8. Data Management and Statistical Analysis

The determined concentrations with sample identifiers and preparation details were recorded in a database. Each pair of copies provided a minimum, a maximum, and a mean. Groups of six copies provided 95% confidence intervals (CI). Statistical analysis computed the time plots of the marginal release rates for each type of suture and solvent. Marginal ex vivo release rates and their controls were computed with CI and tested against their respective controls with two-sided t-tests and a type-I error of 5%. Ex vivo benchmark release rates from subcutaneous and intramuscular explanted fragments were compared.

A mathematical model was fitted to in vitro marginal release rates measured in accelerated conditions and the ex vivo benchmark release rates and residual triclosan over time. The purpose of this fitting was to predict the marginal release rate at a given moment, such as when the antimicrobial activity stopped. Model fitting was performed by single and double exponential decay functions for each series of in vitro triclosan marginal release rates with V+ 0 and V+ 1 after immersion in 13.3% *w*/*w* and 3.3% *w*/*w* solutions, respectively. A similar fitting was performed on 30-day water immersed reference sutures to find out if the model type varied across sutures and triclosan dissolution conditions. The model parameters were solved for each suture/solvent combination. The model with the adjusted coefficient of determination ($R^2{}_{adjusted}$) closest to 1 was selected as the best fit (4) [40]:

$$R^2_{adjusted} = 1 - \left(1 - \frac{\sum_{i=1}^{n} (y_i - \hat{y}_i)^2}{\sum_{i=1}^{n} (y_i - \overline{y})^2}\right) \frac{n-1}{n-(k+1)} \quad (4)$$

The single-decay model estimated the release rate at time t since baseline $R(t)$ with $R0$ is the baseline release rate and L is the half-life (5):

$$R_{predicted}(t) = R0 \cdot \mathrm{e}^{\frac{-\mathrm{t.Ln2}}{L}} \quad (5)$$

The double-decay model decomposed the single decay baseline rate and half-life into an early decay with a partial baseline rate related to a short half-life overlapping with a later decay with another partial baseline related to a long half-life (6) [41]:

$$R_{predicted}(t) = R_1 \cdot e^{\frac{-t.Ln2}{L_1}} + R_2 \cdot e^{\frac{-t.Ln2}{L_2}} \quad (6)$$

The models that best predicted the animal ex vivo benchmarks were selected.

The observed residual amount of triclosan on the suture at time t was the extracted initial amount less the sum of the observed released amounts at each time slot since immersion started to time (7):

$$A_{observed}(t) = A0_{observed} - \sum_{t=0}^{t} A_{observed}(t) \quad (7)$$

The predicted residual amount at time t was the extracted initial amount less the integral of the fitted model from time 0 to t (8):

$$A_{predicted}(t) = A0_{observed} - \int_{t=0}^{t} R_{predicted}(t)dt \quad (8)$$

Data management and computations were performed in Stata 17, StataCorps LLC, College Station, TX, USA. Mathematical modelling was performed in Maple 2021.1, Maple Inc., Waterloo, ON, Canada.

3. Results

3.1. Marginal Release Rate in Water Control

The initial rate was high and declined during the first day. It was followed by a slow plateau up to 30 days in all suture types (Figures 1–3). The function with the best fit for all three sutures was a double exponential decay. The adjusted R-squared ranged from 0.88 to 0.99.

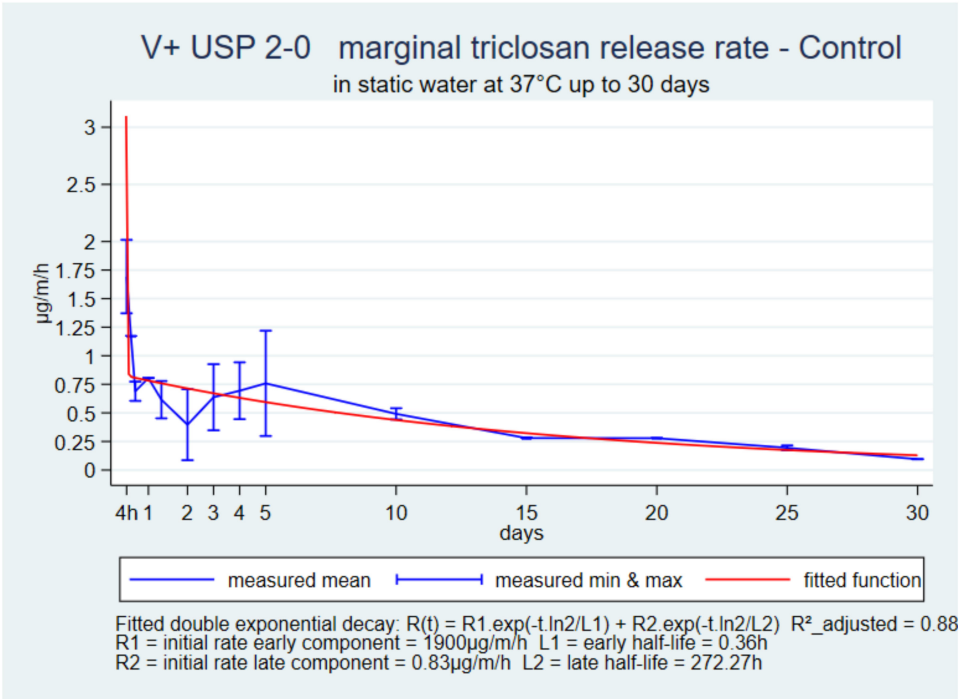


Figure 1. V+ USP 2-0, marginal release rate in static up to 30 days.

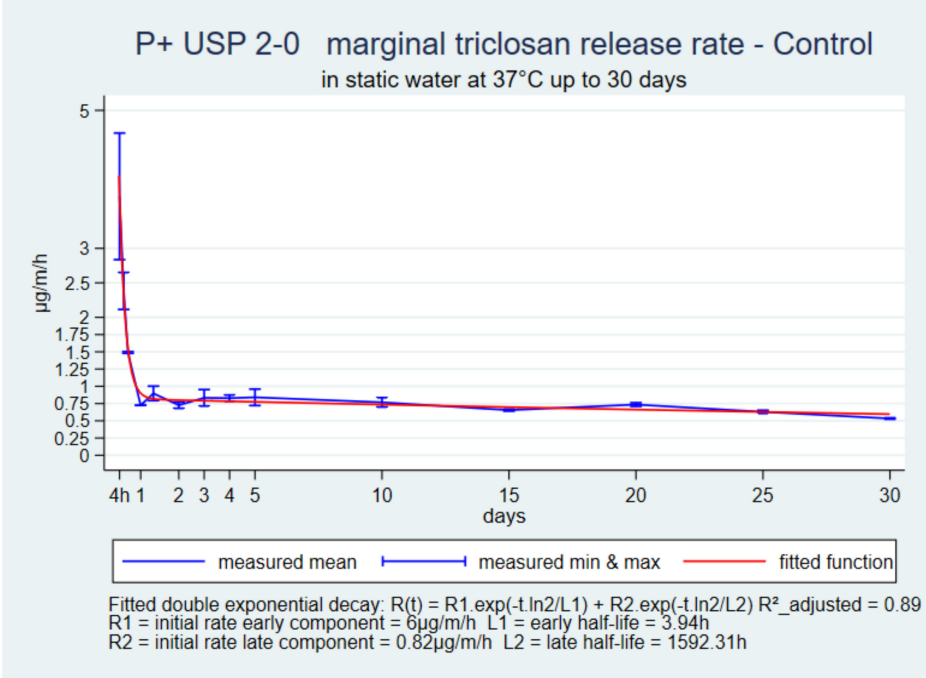


Figure 2. P+ USP 2-0, marginal release rate in static water up to 30 days.

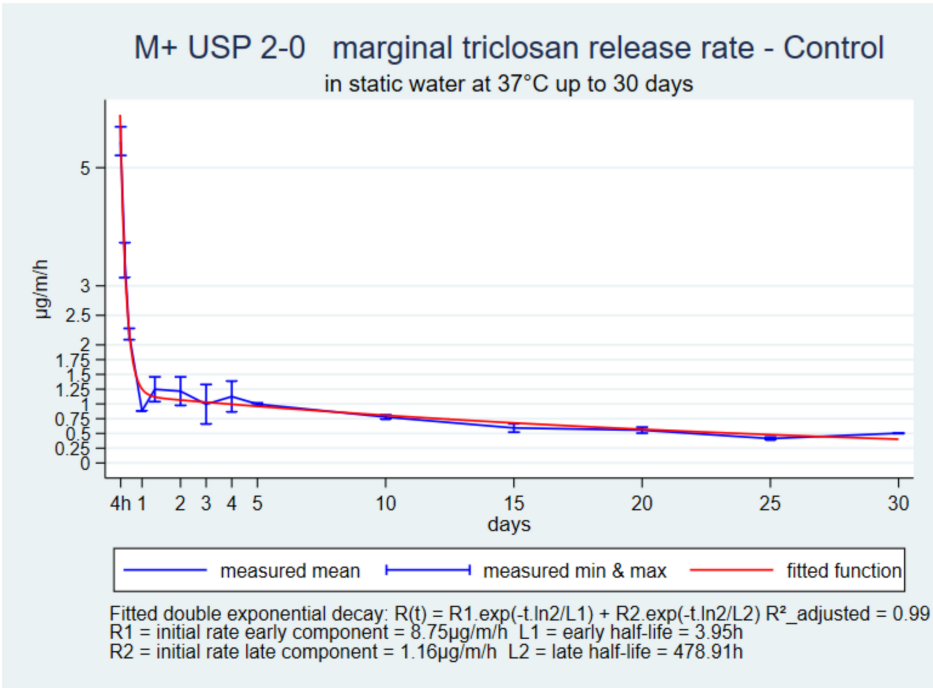


Figure 3. M+ USP 2-0, marginal release rate in static water up to 30 days.

3.2. Marginal Release Rate in Ex Vivo Animal Benchmarks

Ex vivo release rates with V+ on day seven were significantly lower than their controls (Figure 4). The ex vivo intramuscular V+ 1 rate was 0.005 µg/m/h [0.003, 0.006] *versus* 0.30 µg/m/h [0.26, 0.35] in control, thus a significant control/explant ratio of 64.2 was found [52.5, 75.9]. The ex vivo subcutaneous V+ 0 rate was 0.015 µg/m/h [0.009, 0.025] *versus* 0.203 µg/m/h [0.179, 0.220] in control, thus a significant control/explant ratio of 13.6 [8.8, 18.5] was realized. The subcutaneous V+ 0 over intramuscular V+ 1 ratio was also significant with 3.13 [1.92, 4.35].

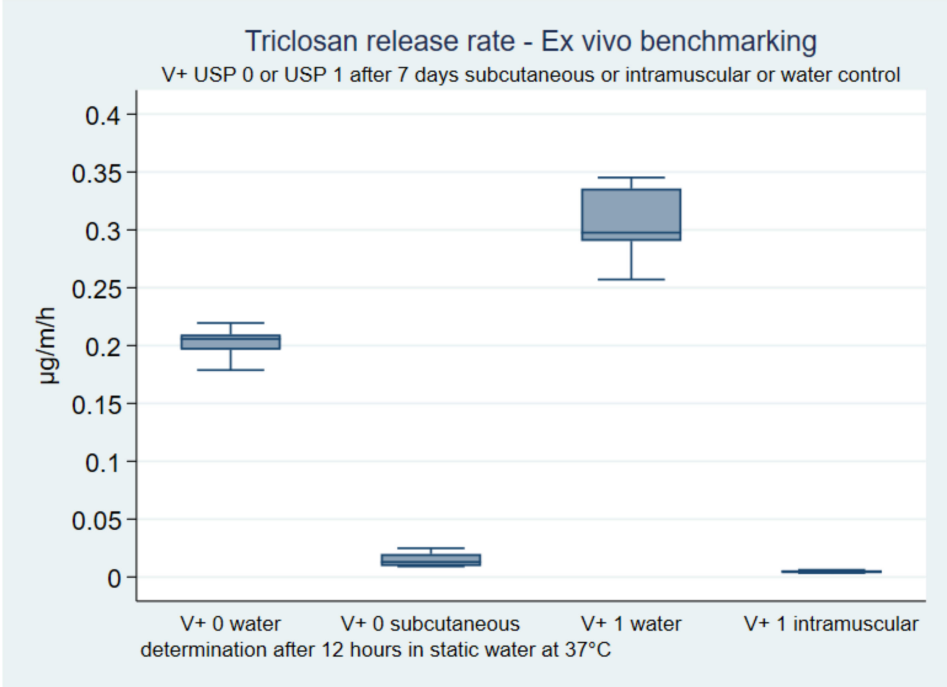


Figure 4. Ex vivo marginal release rate benchmarks *versus* in vitro controls.

3.3. Marginal Release Rate at Accelerated In Vitro Conditions

The shapes of the marginal release rate curves up to seven days of V+ USP 0 and USP 1, in 13.3% and 3.3% solutions, were similar to the shape in water with V+ USP 2-0 up to 30 days. The functions with the best fit were double-exponential decays as well (Figure 5). The initial rates with the 3.3% solution were about 50% lower than with the 13.3% solution, but the two rate functions converged during day-one, and the seven-day rates came close to the ex vivo benchmarks. (Figures for each other suture and solvent combination are in the Supplementary Materials).

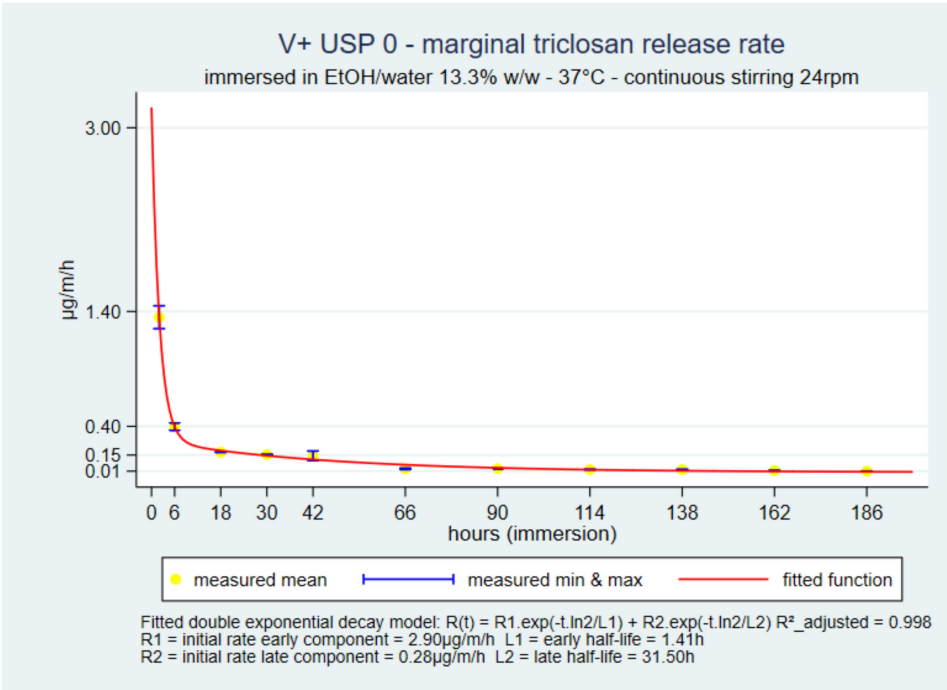


Figure 5. V+ 0 in 13.3% *w*/*w* marginal release rates up to 7 days.

3.4. Initial Amount of Triclosan on a Suture

The extraction with the three calibres of braided sutures (V+ 2-0, V+ 0, V+ 1) yielded between 10.1% and 11.1% of the 472 µg/m specified maximum amount. In the case of monofilament sutures with a common 2360 µg/m maximum amount, the extraction was 23% of the maximum with P+ 2-0, and 45% with M+ 2-0 (Table 1).

Table 1. Estimate of initial amount µg/m.

Release in µg/m	Suture	Days Immersed	N	Min	Mean	Max
Solvent: EtOH/water 100% *w*/*w*	V+ 2-0	6	2	42	47.6	53.1
	V+ 0	6	2	36.3	48.1	60
	V+ 1	6	2	40.3	52.3	64.3
	P+ 2-0	6	2	471.4	551.4	631.4
	M+ 2-0	6	2	971.4	1071.4	1171.4

3.5. Residual Amount of Triclosan on the Suture over Time

The residual triclosan amount in V+ 0 was close to 30 µg/m on day-7, i.e., 62% of the extracted initial amount. The residual amount was estimated by subtracting the cumulative release sum from the initial amount. The predicted cumulative release was also used as an alternate method (Figure 6). Overall, the residual amount on day-seven was approximately 93% of the maximum initial load. (Figures for each other suture and solvent combination are in the Supplementary Materials).

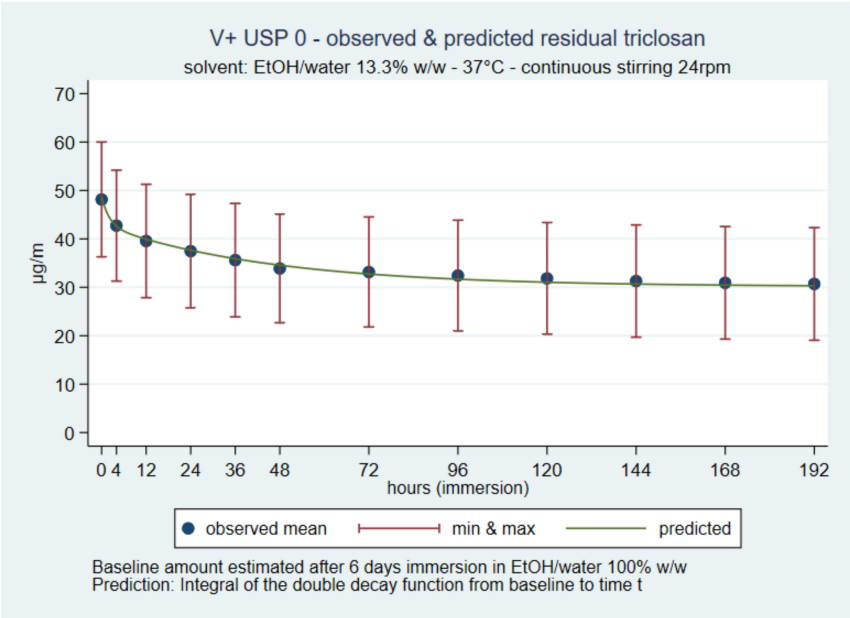


Figure 6. V+ 0 in 13.3% *w*/*w* predicted residual triclosan over time.

3.6. Release Kinetics—Pharmacodynamics Matching Results

S. aureus grew on the full surface of control plates after spreading samples of cultures without exposure to TS. No colonies grew on plates after spreading samples of cultures exposed to V+ 0 immersed in EtOH/water 13.3% *w*/*w* or 3.3% *w*/*w* during 1 h and 4 h, thus demonstrating the biocidal effect of the triclosan on the suture segment.

Viable colonies of *S. aureus* grew on plates after spreading samples of cultures exposed to suture segments immersed in 13.3% *w*/*w* or 3.3% *w*/*w* solvents between 12 h and 48 hours (Figure 7). These results demonstrated that microbial growth rate increased as the marginal triclosan release rate decreased, as predicted. Moreover, the growth rate was

faster in cultures exposed to segments pre-immersed in 13.3% *w*/*w* than in 3.3% *w*/*w*. That difference in rates was consistent with the steeper drop in triclosan marginal release rate with 13.3% *w*/*w* than 3.3% *w*/*w*, at equal immersion duration.

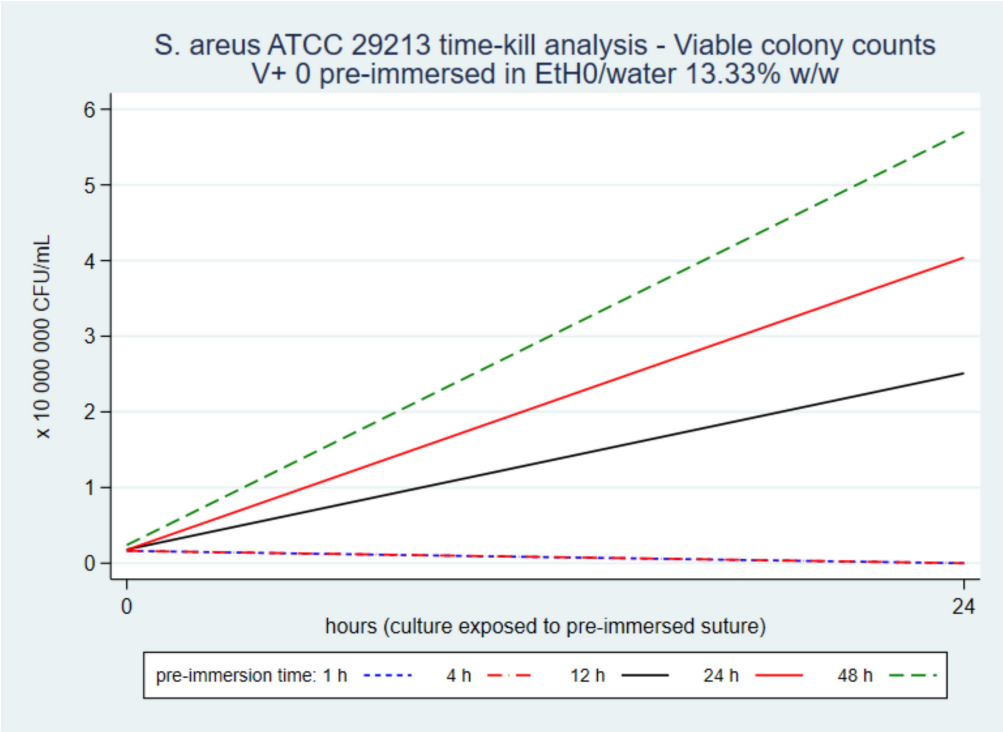


Figure 7. V+ 0 in 13.3% *w*/*w* 24-h time-kill analysis.

4. Discussion

This study achieved most of its goals. The in vitro dissolution model was defined according to approaches to predict drug dissolution in vitro, and they agree with the ex vivo benchmarks from TS [24]. Piggybacking of the planned preclinic study according to the 3Rs was achieved, and the opportunistic suture fragments were the source of the benchmarks as planned [22,23,33]. IMMR confirmed that piggybacking was rarely achieved, either because of confidentiality or incompatibilities between the protocol and the piggybacking objectives.

The common shapes and double-exponential decay functions of the 30-day references in static water with the three sutures V+, P+ and M+ USP 2-0 presented an excellent fit and showed that the release kinetics were very similar. The differences between suture types were in the four parameters of the prediction functions that described the initial high release rate, its rapid decline over one to two days and then a low-release rate plateau for up to 30 days. M+ and V+ had a higher initial rate than V+ and then a more horizontal plateau, which could be related to their higher initial load. The sutures were still reasonably stiff on day-30, and had a uniform surface and a marginal release rate still above 0.5 µg/m/h, which was incompatible with the known hydrolysis of the structural polymers once implanted. M+ loses 50 to 60% tensile strength during the first week, and P+ and V+ lose 20% to 25% within two weeks [4–6]. Accelerating triclosan release in vitro in proportions indicated by the ratio of the benchmarks and water controls was necessary to estimate the release kinetics as they occur in operated tissues.

The combination of continuous stirring and increased solubility with ethanol as an organic cosolvent were tested methods, and adapting those methods to the needs of this research was straightforward [24,26,27,34–37]. The expertise of LPL was instrumental in the success of this study because handling the considerable range of triclosan concentrations in the samples required proven procedures to ensure ad hoc dilution and conservation [31,32].

The similar shapes of the marginal release rate curves and the common double-exponential decay forms between V+ 2-0 in static water and V+ 0 and V+ 1 in EtOH/water solutions with stirring were an important interim internal validation test of the accelerated dissolution models.

One possible explanation for the shape of the triclosan release curves could be the rapid release of surface triclosan and the slow release of most of the triclosan embedded in the polymer and released with the progressive hydrolysis of the biomaterial [42,43].

This study also enables qualitative comparisons with prior studies. In vitro static culture medium models should have triclosan release-kinetics similar to the 30-day water reference in this study. In vivo studies performed with subcutaneous sutures in rodents should have release-kinetics with V+ closer to those or our accelerated models [8–12,16]. The main difficulty in interpreting those studies is translating their results to surgery because of the unavailability of triclosan release data. Planning an external validation of all those studies would have been possible if they had reported triclosan release data along with the observed TS antimicrobial activity.

The prediction functions suggested that the antistaphylococcal activity of V+ was lost when the marginal triclosan release rate was in a transition range between 0.175 μg/m/h and 0.287 μg/m/h. That change occurred when exposing cultures to sutures pre-immersed between 4 and 12 h, with 13.3% or 3.3% solutions. The end of the antistaphylococcal activity coincided with the end of the rapid decline in curves and the transition into the low-level plateau. The total triclosan released was between 1.7% and 5.3% of the extracted amount and between 0.2% and 0.4% of the maximum amount in V+ (472 μg/m) [5]. These results suggest that V+ has an estimated antistaphylococcal activity duration with bactericidal strength for 4 to 12 h postoperatively; covering the intraoperative and early postoperative risks. The residual amount of triclosan after that period is more than 95% of the actually extracted triclosan and more than 99% of the maximum authorised amount on V+.

According to the determinations with the explants from muscle and subcutaneous tissue, most triclosan remaining after that period is released progressively for at least seven days with either bacteriostatic activity or a non-lethal release.

The minimum inhibitory concentration (MIC) of triclosan in *S aureus* is close to the MIC in other common species found in SSIs, including *E. coli* and *S. epidermidis*. Therefore, the antistaphylococcal activity duration measured in the time-kill analyses should predict the antimicrobial activity duration in several other triclosan-sensitive microorganisms often isolated in SSIs with triclosan MIC < 1 μg/mL. Intrinsically, triclosan-resistant species such as *P. aeruginosa*, where the triclosan MIC ranges from 128 μg/mL to more than 1000 μg/mL, can colonise TS intraoperatively [8–12,18,39,44].

The intensive 4 to 12-h antistaphylococcal biocidal-level activity could explain significant differences in efficacy between trials. Differences could depend on the microbial load, microbial nesting at a distance from the suture (e.g., haematoma, devitalised tissue, implant), and the time the patient's natural defences required in order to kill residual microorganisms.

This study was executed according to the protocol, with some deviations. The main deviation was that ex vivo benchmarks were available only with V+ sutures. Regarding P+ and M+, the study yielded only water controls. In addition, the preparation of a few samples failed, resulting in outliers or missing concentrations. However, those failures were compensated by the backups (at least two copies of each determination) and the small repercussion of an error at a time slot on the next ones.

One potential bias concerning the ex vivo benchmarks was the risk of mechanical removal of triclosan during suture dissection. However, the visual examination of sutures when preparing them for immersion showed that most dissection damages concerned the subcutaneous fragments because of dense adhesion to tissue. Thus, subcutaneous dissection retrieved sutures incompletely and fragmented the available material into segments less than 5 cm long. The same type of suture in muscle had less adhesion, and several fragments were 35 cm long. Determinations showed that ex vivo triclosan marginal release rates in

subcutaneous explants were significantly higher than in intramuscular explants. Therefore, dissection in this study had little effect on the triclosan load compared with the differences between muscle and subcutaneous tissue over seven days [17].

The main limitations of this study were the incomplete extraction of the initial triclosan load, no benchmarks before seven days, no explanted fragments with polydioxanone and poliglecaprone 25 sutures, the uncertain of translating sheep benchmarks to humans, and not reproducing suture polymer erosion. The in vitro model focused on triclosan dissolution and did not release the bulk of triclosan that is assumed to be embedded in the polymer. If polymer hydrolysis had been reproduced, the time-kill analysis might have detected antistaphylococcal activity after 12-h of segment pre-immersion.

The difference between subcutaneous and intramuscular implantations show that different models are required to predict TS antimicrobial activity in various clinical situations.

Future studies should explore the amount of residual triclosan.

5. Conclusions

This study determined triclosan release rate kinetics and the antistaphylococcal activity duration of polyglactin 910 absorbable sutures. An in vitro dissolution model was developed and specified using ex vivo benchmarks. The dissolution model was continuous, stirring in 3.3% and 13.3% ethanol/water solvents at 37 °C. The benchmarks were the triclosan release rates measured in suture fragments opportunistically retrieved from sheep muscle and subcutaneous tissue after independently planned experimental cardiac surgery.

The exposure of similar sutures to the dissolution model showed an early high release rate that rapidly declined and transitioned to a long-lasting slow plateau after the second day.

A time-kill analysis showed antistaphylococcal activity with bactericidal intensity for 4 to 12 h, and staphylococcal growth with sutures exposed for more than 12 h, with both solvents. The end of the antistaphylococcal activity coincided with the end of the rapid decline in the marginal release rate. Therefore, the residual amount of triclosan was probably more than 99% of the maximum authorised amount on polyglactin 910 braided plus triclosan sutures.

This study showed that polyglactin 910, polydioxanone, and poliglecaprone 25 have similar triclosan release profiles. It also showed a similar profile between polyglactin 910 sutures in static water and an accelerated dissolution model. Differences between suture types and dissolution models seem to be mainly in the maximum initial release rate and the half-life of the decline of that rate.

Sheep are a valid translational model for human cardiac surgery. Polyglactin 910 sutures with triclosan exhibit an intense antistaphylococcal activity for a duration of four to twelve postoperative hours. This activity logically applies to microbial species with a minimal inhibitory concentration of less than 1 µg/mL.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/pharmaceutics14030539/s1. Table S1. In vitro water and ex vivo sample preparation schedule; Table S2. Modeling phase–Sample preparation–EtOH/water with stirring; Table S3. Estimate of marginal release rate µg/m/h—in static water; Table S4. Ex vivo marginal release rate benchmarks versus in vitro controls (µg/m/h); Table S5. Cumulative and marginal release rates calculated from raw data with EtOH/water solvent; Table S6. Residual amount of triclosan over time with in EtOH/water 13.3% *w*/*w*. Figure S1. V+ USP 0 in 3.3% *w*/*w* release rates and double exponential fitting; Figure S2. V+ USP 0 in 3.3% *w*/*w* predicted residual triclosan; Figure S3. V+ USP 1 in 13.3% *w*/*w* release rates and double exponential fitting; Figure S4. V+ USP 1 in 13.3% *w*/*w* predicted residual triclosan; Figure S5. V+ USP 1 in 3.3% *w*/*w* release rates and double exponential fitting; Figure S6. V+ USP 1 in 3.3% *w*/*w* predicted residual triclosan; Figure S7. V+ USP 0 in 3.3% *w*/*w* time-kill assay.

Author Contributions: F.C.D.: Designed and analysed the study, prepared the samples and participated in the article. R.G.: Reviewed the work and participated in the article. N.M.: Approved

and supervised the project and participated in the article. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by University of Bordeaux, Pharmacology department. INSERM U1219, Bordeaux, France. An unrestricted private research grant was provided to support this project by Medextens SARL, 75015 Paris FRANCE. The amount was 5500 Euros and covered the fees of Laboratoire des Pyrénées et des Landes.

Institutional Review Board Statement: This study was in vitro and used explants of animals that had participated in another preclinical cardiac surgical study and were sacrificed for reasons other than this study. That study had been conducted in Paris and was authorised by government DDPP Paris approval number: APAFIS#12534-2017121118586862 v3.

Informed Consent Statement: Not applicable.

Data Availability Statement: The complete datasets used in this study are available in the tables within the Supplementary Materials file: *TriclosanSutures_Pharmaceutics.SupplementaryMaterial.odt*.

Acknowledgments: The authors thank the following individual for their guidance and support: Nicolas Borenstein and Angélique Ente at IMMR, 75014 Paris, France, who provided ex vivo samples for benchmarking. M. Arnaud Zabala and Fatima M'Zali, Université de Bordeaux, Aquitaine Microbiologie, UMR 5234 CNRS, 33000 Bordeaux, France, who allowed the use of their laboratory to prepare the samples and storing the supplies. Nadège Pineau, Medextens, 75015 Paris, France, who helped prepare the samples, checked and entered the data.

Conflicts of Interest: The authors declare that they have no conflicts of interest. This study was carried in academic stings out of scientific interest only.

References

1. Scientific Committee on Consumer Safety (Sccs). Opinion on Triclosan 2010. European Commission-Directorate General for Health and Consumers. Available online: https://Op.Europa.Eu/En/Publication-Detail/-/Publication/3b684b59-27f7-4d0e-853c-4a9efe0fb4cb/Language-En (accessed on 3 December 2021).
2. Heath, R.J.; Li, J.; Roland, G.E.; Rock, C.O. Inhibition of the Staphylococcus Aureus Nadph-Dependent Enoyl-Acyl Carrier Protein Reductase by Triclosan and Hexachlorophene. *J. Biol. Chem.* **2000**, *275*, 4654–4659. [CrossRef]
3. Heath, R.J.; Rubin, J.R.; Holland, D.R.; Zhang, E.; Snow, M.E.; Rock, C.O. Mechanism of Triclosan Inhibition of Bacterial Fatty Acid Synthesis. *J. Biol. Chem.* **1999**, *274*, 11110–11114. [CrossRef] [PubMed]
4. *Pds Plus–389688.R02*; Ethicon Inc.: Raritan, NJ, USA, 2006.
5. *Vicryl Plus–389595.R04*; Ethicon Inc.: Raritan, NJ, USA, 2002.
6. *Monocryl Plus–389680.R02*; Ethicon Inc.: Raritan, NJ, USA, 2005.
7. Hochberg, J.; Meyer, K.M.; Marion, M.D. Suture Choice and Other Methods of Skin Closure. *Surg. Clin. N. Am.* **2009**, *89*, 627–641. [CrossRef] [PubMed]
8. Rothenburger, S.; Spangler, D.; Bhende, S.; Burkley, D. In Vitro Antimicrobial Evaluation of Coated Vicryl* Plus Antibacterial Suture (Coated Polyglactin 910 with Triclosan) Using Zone of Inhibition Assays. *Surg. Infect.* **2002**, *3* (Suppl. 1), S79–S87. [CrossRef] [PubMed]
9. Storch, M.L.; SRothenburger, J.; Jacinto, G. Experimental Efficacy Study of Coated Vicryl Plus Antibacterial Suture in Guinea Pigs Challenged with Staphylococcus Aureus. *Surg. Infect.* **2004**, *5*, 281–288. [CrossRef]
10. Ming, X.; Nichols, M.; Rothenburger, S. In Vivo Antibacterial Efficacy of Monocryl Plus Antibacterial Suture (Poliglecaprone 25 with Triclosan). *Surg. Infect.* **2007**, *8*, 209–214. [CrossRef]
11. Ming, X.; Rothenburger, S.; Yang, D. In Vitro Antibacterial Efficacy of Monocryl Plus Antibacterial Suture (Poliglecaprone 25 with Triclosan). *Surg. Infect.* **2007**, *8*, 201–208. [CrossRef]
12. Ming, X.; Rothenburger, S.; Nichols, M.M. In Vivo and In Vitro Antibacterial Efficacy of Pds Plus (Polidioxanone with Triclosan) Suture. *Surg. Infect.* **2008**, *9*, 451–457. [CrossRef]
13. Wu, X.; Kubilay, N.Z.; Ren, J.; Allegranzi, B.; Bischoff, P.; Zayed, B.; Pittet, D.; Li, J. Antimicrobial-Coated Sutures to Decrease Surgical Site Infections: A Systematic Review and Meta-Analysis. *Eur. J. Clin. Microbiol. Infect. Dis.* **2017**, *36*, 19–32. [CrossRef]
14. Ahmed, I.; Boulton, A.; Rizvi, S.; Carlos, W.; Dickenson, E.; Smith, N.; Reed, M. The Use of Triclosan-Coated Sutures to Prevent Surgical Site Infections: A Systematic Review and Meta-Analysis of the Literature. *BMJ Open* **2019**, *9*, e029727. [CrossRef]
15. WHO. *World Health Organization Global Guidelines for the Prevention of Surgical Site Infection*, 2nd ed.; Licence: Cc by-Nc-Sa 3.0 Igo. (2018); WHO: Geneva, Switzerland, 2018.
16. McCagherty, J.; Yool, D.A.; Paterson, G.K.; Mitchell, S.R.; Woods, S.; Marques, A.I.; Hall, J.L.; Mosley, J.R.; Nuttall, T.J. Investigation of the In Vitro Antimicrobial Activity of Triclosan-Coated Suture Material on Bacteria Commonly Isolated from Wounds in Dogs. *Am. J. Vet. Res.* **2020**, *81*, 84–90. [CrossRef]

17. Ruan, Y.; Robinson, N.B.; Khan, F.M.; Hameed, I.; Rahouma, M.; Naik, A.; Oakley, C.T.; Rong, L.; Girardi, L.N.; Gaudino, M. The Translation of Surgical Animal Models to Human Clinical Research: A Cross-Sectional Study. *Int. J. Surg.* **2020**, *77*, 25–29. [CrossRef]
18. Barbolt, T.A. Chemistry and Safety of Triclosan, and Its Use as an Antimicrobial Coating on Coated Vicryl* Plus Antibacterial Suture (Coated Polyglactin 910 Suture with Triclosan). *Surg. Infect.* **2002**, *3* (Suppl. 1), S45–S53. [CrossRef]
19. Leaper, D.; Assadian, O.; Hubner, N.O.; McBain, A.; Barbolt, T.; Rothenburger, S.; Wilson, P. Antimicrobial Sutures and Prevention of Surgical Site Infection: Assessment of the Safety of the Antiseptic Triclosan. *Int. Wound J.* **2011**, *8*, 556–566. [CrossRef]
20. Matl, F.D.; Obermeier, A.; Repmann, S.; Friess, W.; Stemberger, A.; Kuehn, K.D. New Anti-Infective Coatings of Medical Implants. *Antimicrob. Agents Chemother.* **2008**, *52*, 1957–1963. [CrossRef]
21. Obermeier, A.; Schneider, J.; Wehner, S.; Matl, F.D.; Schieker, M.; von Eisenhart-Rothe, R.; Stemberger, A.; Burgkart, R. Novel High Efficient Coatings for Anti-Microbial Surgical Sutures Using Chlorhexidine in Fatty Acid Slow-Release Carrier Systems. *PLoS ONE* **2014**, *9*, e101426. [CrossRef]
22. Tannenbaum, J.; Bennett, B.T. Russell and Burch's 3rs Then and Now: The Need for Clarity in Definition and Purpose. *J. Am. Assoc. Lab. Anim. Sci.* **2015**, *54*, 120–132.
23. Lewis, D.I. Animal Experimentation: Implementation and Application of the 3rs. *Emerg. Top. Life Sci.* **2019**, *3*, 675–679.
24. Zaborenko, N.; Shi, Z.; Corredor, C.C.; Smith-Goettler, B.M.; Zhang, L.; Hermans, A.; Neu, C.M.; Alam, M.A.; Cohen, M.J.; Lu, X.; et al. First-Principles and Empirical Approaches to Predicting In Vitro Dissolution for Pharmaceutical Formulation and Process Development and for Product Release Testing. *AAPS J.* **2019**, *21*, 32. [CrossRef]
25. Hanifeh, M.; Spillmann, T.; Huhtinen, M.; Sclivagnotis, Y.S.; Gronthal, T.; Hynonen, U. Ex-Vivo Adhesion of Enterococcus Faecalis and Enterococcus Faecium to the Intestinal Mucosa of Healthy Beagles. *Animals* **2021**, *11*, 3283. [CrossRef]
26. Karaffa, L.S. The Merck Index—An Encyclopedia of Chemicals, Drugs, and Biologicals, 15th ed.O'Neil, M.J., Maryadele, J., Eds.; The Royal Society of Chemistry (Great Britain): Cambridge, UK, 2013; p. 1789.
27. Yalkowsky, S.H.; He, Y.; Parijat, J. Triclosan. In *Handbook of Aqueous Solubility Data*; He, Y., Yalkowsky, S.H., Parijat, J., Eds.; CRC Press: Boca Raton, FL, USA, 2010; p. 844.
28. Smith, B.T. Solubility and Dissolution. In *Remington Education: Physical Pharmacy*; Pharmaceutical Press: London, UK, 2016; pp. 31–50.
29. Mircioiu, C.; Voicu, V.; Anuta, V.; Tudose, A.; Celia, C.; Paolino, D.; Fresta, M.; Sandulovici, R.; Mircioiu, I. Mathematical Modeling of Release Kinetics from Supramolecular Drug Delivery Systems. *Pharmaceutics* **2019**, *11*, 140. [CrossRef] [PubMed]
30. Tixier, C.; Singer, H.P.; Canonica, S.; Muller, S.R. Phototransfomation of Ticlosan in Surface Waters: A Relevant Elimination Process for This Widely Used Biocide—Laboratory Studies, Field Measurements, and Modeling. *Environ. Sci. Technol.* **2002**, *36*, 3482–3489. [CrossRef] [PubMed]
31. Liu, T.; Wu, D. High-performance liquid chromatographic determination of triclosan and triclocarban in cosmetic products. *Int. J. Cosmet Sci.* **2012**, *34*, 489–494. [CrossRef] [PubMed]
32. NF EN ISO/IEC 17025-2005. Exigences Générales Concernant la Compétence Des Laboratoires D'étalonnages et D'essais. Available online: https://www.boutique.afnor.org/fr-fr/norme/nf-en-iso-iec-17025/exigences-generales-concernant-la-competence-des-laboratoires-detalonnages-/fa120232/727 (accessed on 3 December 2021).
33. van der Velden, J.; Asselbergs, F.W.; Bakkers, J.; Batkai, S.; Bertrand, L.; Bezzina, C.R.; Bot, I.; Brundel, B.; Carrier, L.; Chamuleau, S.; et al. Animal Models and Animal-Free Innovations for Cardiovascular Research: Current Status and Routes to Be Explored. Consensus Document of the Esc Working Group on Myocardial Function and the Esc Working Group on Cellular Biology of the Heart. *Cardiovasc. Res.* **2022**, cvab370, Online ahead of print. [CrossRef] [PubMed]
34. Seeger, N.; Lange, S.; Klein, S. Impact of Vibration and Agitation Speed on Dissolution of Usp Prednisone Tablets Rs and Various Ir Tablet Formulations. *AAPS PharmSciTech* **2015**, *16*, 759–766. [CrossRef] [PubMed]
35. Qureshi, S.A. Choice of Rotation Speed (Rpm) for Bio-Relevant Drug Dissolution Testing Using a Crescent-Shaped Spindle. *Eur. J. Pharm. Sci.* **2004**, *23*, 271–275. [CrossRef] [PubMed]
36. Vandhana, S.; Deepa, P.R.; Aparna, G.; Jayanthi, U.; Krishnakumar, S. Evaluation of Suitable Solvents for Testing the Anti-Proliferative Activity of Triclosan—A Hydrophobic Drug in Cell Culture. *Indian J. Biochem. Biophys.* **2010**, *47*, 166–171.
37. Otto, D.P.; Otto, A.; de Villiers, M.M. Differences in Physicochemical Properties to Consider in the Design, Evaluation and Choice between Microparticles and Nanoparticles for Drug Delivery. *Expert Opin. Drug Deliv.* **2015**, *12*, 763–777. [CrossRef]
38. CLSI Clinical and Laboratory Standards Institute. *M26-a Methods for Determining Bactericidal Activity of Antimicrobial Agents*; Approved Guideline; 1999; Available online: https://clsi.org/standards/ (accessed on 3 December 2021).
39. Assadian, O.; Wehse, K.; Hubner, N.O.; Koburger, T.; Bagel, S.; Jethon, F.; Kramer, A. Minimum Inhibitory (Mic) and Minimum Microbicidal Concentration (Mmc) of Polihexanide and Triclosan against Antibiotic Sensitive and Resistant Staphylococcus Aureus and Escherichia Coli Strains. *GMS Krankenhhyg Interdiszip* **2011**, *6*, Doc06.
40. Chicco, D.; Warrens, M.J.; Jurman, G. The Coefficient of Determination R-Squared Is More Informative Than Smape, Mae, Mape, Mse and Rmse in Regression Analysis Evaluation. *PeerJ Comput. Sci.* **2021**, *7*, e623. [CrossRef]
41. Storm, N.; McKay, L.G.A.; Downs, S.N.; Johnson, R.I.; Birru, D.; de Samber, M.; Willaert, W.; Cennini, G.; Griffiths, A. Rapid and Complete Inactivation of SARS-CoV-2 by Ultraviolet-C Irradiation. *Sci. Rep.* **2020**, *10*, 22421. [CrossRef] [PubMed]
42. Lee, K.H.; Chu, C.C. The Role of Superoxide Ions in the Degradation of Synthetic Absorbable Sutures. *J. Biomed. Mater. Res.* **2020**, *49*, 25–35. [CrossRef]

43. Andrade, M.G.; Weissman, R.; Reis, S.R. Tissue Reaction and Surface Morphology of Absorbable Sutures after In Vivo Exposure. *J. Mater. Sci. Mater. Med.* **2006**, *17*, 949–961. [CrossRef] [PubMed]
44. Chuanchuen, R.; Karkhoff-Schweizer, R.R.; Schweizer, H.P. High-Level Triclosan Resistance in Pseudomonas Aeruginosa Is Solely a Result of Efflux. *Am. J. Infect. Control* **2003**, *31*, 124–127. [CrossRef] [PubMed]

Expertise collective sur l'usage des cannabinoïdes de synthèse à La Réunion

Présentation des cannabinoïdes de synthèse : approche pharmaco-toxicologique

Amélie Daveluy, Alexandre Peyré, Ruben Goncalves

En cours de publication par le Réseau Régional d'Addictologie SAOME (Santé Addictions Outre-Mer)

Présentation des cannabinoïdes de synthèse : Approche pharmaco-toxicologique

Amélie Daveluy[1,2], Alexandre Peyré[1], Ruben Goncalves[2,3]

[1] Centre d'addictovigilance, Bordeaux et DOM, CHU de Bordeaux,

[2] Univ. Bordeaux, INSERM U1219, BPH

[3] CHU de Bordeaux, Laboratoire de Pharmacologie et Toxicologie

1. Introduction

Les cannabinoïdes de synthèse (CS), principalement fabriqués en Chine, représentent le plus grand groupe de substances actuellement suivies par le système d'alerte rapide de l'Union Européenne. Depuis 2008, 224 nouveaux CS ont été détectés en Europe dont 15 ont été identifiés pour la première fois en 2021 [1].

2. Classification des CS

Le nom des plus anciens CS commence par les initiales du chimiste les ayant synthétisés pour la première fois, suivi par un numéro (ex. **JWH** pour **J**ohn **W**. **H**uffman, JW-018). D'autres, les plus récents, peuvent avoir des noms issus de leur nom chimique (APINACA pour N-(1-**A**damantyl)-1-**P**entyl-1H-**IN**d**A**zole-3- **CA**rboxamide) également appelé AKB48).

Les CS ont exceptionnellement une structure chimique apparentée à celle du THC. La plupart proviennent de classes chimiques variées et différentes. Toutefois, des similarités structurelles sont observées avec d'une part, une queue (*tail*) reliée à un noyau (structure principale, *core*) composé d'un cycle insaturé incorporant au moins un atome d'azote (pyrrole), fusionné avec un autre cycle aromatique à 6 carbones. D'autre part, ils sont formés d'un groupe lié (*linked group*) ; le noyau et le groupé lié étant reliés entre eux par une chaine alkyl linéaire (section de liaison, *linker*) (cf. Figure). A noter que tous les CS n'ont pas cette structure schématique.

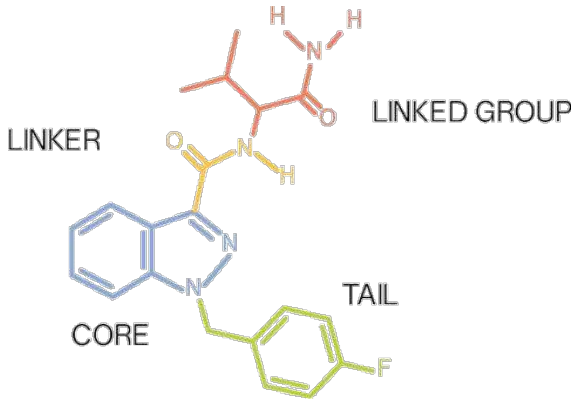


Figure. Structure des cannabinoïdes de synthèse (EMCDDA) [2]

Plusieurs classes chimiques se distinguent. On dénombre 7 groupes structuraux principaux (naphthoylindoles (ex. JWH-018), naphthylméthylindoles, naphthoylpyrrole, naphthylméthylindènes, phénylacétylindoles (ex. JWH-250), cyclohexylphénols (ex. CP 47,497), cannabinoïdes classiques (ex HU-210). Les CS dits de « 3ème génération » appartiennent le plus souvent à de nouveaux composés

dérivés du Noyau (ex. indole, indazole, benzimidazole, etc...), avec des substituts variés, reliés entre eux par des groupements chimiques de type carboxylate ou carboxamide (séries des CHMINACA, FUBINACA,...).

3. Législation des CS

Les CS sont le plus souvent retrouvés sous forme d'herbe ou de poudre pour être fumés mais on les retrouve également dans des e-liquides pour cigarette électronique.

Actuellement, la liste des substances contrôlées de l'annexe I de la CSA (Controlled Substances Act) comprend 221 substances, dont 43 sont des CS (United States Drug Enforcement Administration (DEA). Substances contrôlées - Ordre alphabétique. 2019).

En France, grâce aux travaux du réseau des centres d'addictovigilance, l'ANSM (Agence nationale de sécurité du médicament et des produits de santé), certains CS ont été inscrits sur la liste des stupéfiants dès 2009 (JWH-018, HU-210, CP 47,497 et analogues C6, C8, et C9). Puis en 2013, un classement dit « générique » de 7 classes chimiques de cannabinoïdes de synthèse a été décidé par arrêté du 27 mai 2015. Cet arrêté a été élargi à d'autres classes chimiques, dont les CS dits de 3ème génération par arrêté du 31 mars 2017.

Malgré les efforts législatifs déployés contre le problème des nouveaux produits de synthèse (NPS), de nouveaux CS continuent d'apparaître sur le marché des drogues. Entre 2016 et 2020, le nombre de nouveaux CS apparaissant sur le marché est resté stable, autour de 10 par an, alors qu'il était en moyenne de 27 par an entre 2011 et 2015. Une augmentation du nombre de nouveaux CS a été enregistrée en 2021, avec 15 substances signalées [1].

4. Pharmacologie

a. Pharmacocinétique

Les données sur la pharmacocinétique des CS sont peu nombreuses et/ou très spécifiques à chaque molécule. Les CS ont une bonne biodisponibilité par voie orale et inhalée. La voie inhalée est la principale voie d'administration permettant d'atteindre en quelques minutes le pic de concentration sanguine [3]. En cas de consommation par voie orale, l'absorption ainsi que le début de l'action peuvent être retardés par la prise de nourriture, la digestion et l'effet de premier passage hépatique. Les volumes de distribution sont souvent élevés pour ces composés lipophiles. En cas de consommation chronique, une accumulation dans les graisses est très souvent observée et peut entrainer des relargages tardifs. Les CS sont principalement et fortement métabolisés par voie hépatique. La voie d'élimination majoritaire est la voie urinaire sous forme de métabolites.

b. Pharmacodynamie

Les CS sont des molécules développées depuis les années 70 à des fins de recherche fondamentale. Ces substances n'ont pas une structure similaire à celle du Δ9-tétrahydrocannabinol (THC), principale substance responsable des effets centraux du cannabis, mais sont des **agonistes complets des récepteurs cannabinoïdes, CB1 surtout et CB2,** avec une affinité plus importante que le THC, agoniste partiel des récepteurs cannabinoïdes.

De plus certains CS présentent une puissance significativement supérieure à celle du THC. Elle peut être deux fois supérieure (ex. le CP- 47) voire 200 fois supérieure (pour le HU-210). Ainsi les doses « usuelles » peuvent être très faibles et les risques de toxicité beaucoup plus importants et souvent imprévisibles. Selon le cannabinoïde, la durée des effets est plus ou moins longue que celle du THC. Ainsi indépendamment de l'affinité au récepteur CB1, certains cannabinoïdes peuvent avoir une demi-vie longue et provoquer un effet psychoactif prolongé très différent du cannabis.

L'utilisation des CS dans un cadre festif a été mise en évidence pour la première fois en 2008. Les effets sont très variables selon les individus mais aussi selon le CS. Les effets débutent entre 20 et 60 minutes après la prise et peuvent persister quelques heures. Les effets recherchés sont les mêmes que ceux recherchés avec le cannabis : ivresse, relaxation, bien-être, euphorie, modification de l'état de conscience, hallucinations, mais ils peuvent être plus intenses et plus longs que pour le THC.

Les effets aigüs comprennent des effets neuropsychiatriques (délire/paranoïa, anxiété, agitation, irritabilité, convulsion, perte de connaissance, troubles mnésiques, confusion, tentative de suicide, impression de mort imminente), des nausées/vomissements (syndrome d'hyperhémèse cannabinoïde[1]), des effets cardio-vasculaires (tachycardie/palpitation, douleur angineuse, infarctus), trouble de l'usage de substances.

Des cas de décès ont été rapportés, en particulier par arrêt cardio-respiratoire.

5. Aspects analytiques : importance de l'identification biologique

L'identification des CS dans les matrices biologiques est essentielle pour documenter la consommation réelle dans des contextes hospitaliers et médico-légaux. Cette identification est le seul moyen fiable de pouvoir relier avec certitude un usage à la toxicité des CS (en particulier si la substance consommée n'a pu être récupérée pour analyse). Dans ce contexte, les laboratoires de toxicologie doivent mettre en œuvre des méthodes analytiques fiables capables d'identifier les CS dans les échantillons biologiques.

L'identification des CS pose 2 problèmes majeurs : premièrement, les doses consommées étant faibles en raison de la forte puissance, les concentrations sanguines retrouvées sont souvent très faibles et par conséquent difficilement détectables. Par ailleurs, les fenêtres de détection dans le sang en cas de prise aiguë sont courtes [4].

Deuxièmement, les meilleurs marqueurs de consommation sont les métabolites urinaires et non la molécule mère qui est généralement très fortement métabolisée. L'urine est donc une matrice de choix afin d'augmenter la fenêtre de détection des CS. Un prélèvement précoce permet également une meilleure identification.

---

[1] Syndrome décrit pour la première fois en 2004 ; se caractérise par des nausées et des vomissements récurrents accompagnés de douleurs abdominales intenses. Ils surviennent chez des consommateurs chroniques de cannabis ou de cannabis de synthèse. Ce qui est caractéristique est d'une part que la symptomatologie est améliorée par les bains et les douches chaudes et d'autre part que les symptômes disparaissent à l'arrêt de la consommation (d'où l'importance de bien interroger les patients sur une éventuelle consommation chronique de cannabis devant cette symptomatologie afin de ne pas demander des examens complémentaires inutiles).

a. Techniques immunochimiques

Ces substances ne sont pas détectables par un dépistage urinaire classique par immunochimie à cause de leurs variabilités structurales. Ainsi une discordance entre un tableau clinique évoquant la prise de cannabinoïdes de synthèse et un dépistage urinaire négatif ou positif uniquement au THC n'exclut pas la présence associée d'un cannabinoïde de synthèse.

b. Techniques de spectrométrie de masse

Les standards de référence des CS et de leurs métabolites n'étant pas toujours commercialisés ou très couteux pour les laboratoires, il apparait indispensable d'utiliser des techniques analytiques spécifiques pouvant être en mesure d'identifier des molécules inconnues avec une grande spécificité. Dans ce contexte, il est recommandé aux laboratoires de biologie des établissements de santé de conserver les échantillons du produit en cause et/ou les prélèvements biologiques à disposition et de contacter le Centre d'Addictovigilance afin de réévaluer la situation et le cas échéant d'envisager une analyse toxicologique spécialisée plus spécifique par spectrométrie de masse haute résolution [5]. Cette analyse nécessite une étape de préparation de l'échantillon (extraction), une étape d'analyse à proprement parlé (environ 24h) et enfin une étape de retraitement des résultats. Les données générées par le système haute résolution étant nombreuses et complexes, cette dernière étape peut parfois nécessiter plusieurs jours selon les cas. Il est d'ailleurs primordial de transmettre au laboratoire les informations liées au contexte afin de faciliter l'interprétation. Un partenariat entre le laboratoire CHU de St Denis de La Réunion et le laboratoire de pharmaco-toxicologie du CHU de Bordeaux est déjà existant.

6. Situation en France, données d'addictovigilance

Pendant longtemps, la consommation des cannabinoïdes de synthèse en France semblait peu importante. Cependant, leur consommation a pris une ampleur considérable autour de 2015 dans l'Océan Indien, avec le phénomène de la chimique, mais également en métropole.

a. Océan Indien

La chimique est l'appellation donnée à Mayotte et à la Réunion (également désignée sous le nom de chamane à la Réunion) aux cannabinoïdes de synthèse, fumés dans des cigarettes artisanales. La consommation de chimique peut être à l'origine de troubles potentiellement sévères entrainant parfois l'hospitalisation, en particulier chez une population jeune voire très jeune. Les premiers cas ont été observés à Mayotte (le centre d'addictovigilance de Bordeaux et DOM a été alerté dès 2015) [6], et il y a eu progressivement une diffusion à La Réunion (premiers cas signalés au centre d'addictovigilance de Bordeaux et DOM en 2017).

Depuis 2018, 68 signalements en provenance de la Réunion ont été faits au Centre d'addictovigilance de Bordeaux. Il s'agit de 10 femmes et 57 hommes (sexe inconnu dans un cas), dont l'âge moyen est de 30 ans, l'âge médian de 26 ans, les extrêmes allant de 1 an à 77 ans. Parmi eux, 10 mineurs, dont une petite fille de 1 an ayant ingéré un mégot de chimique laissé à sa portée.

Grâce au partenariat avec le laboratoire de pharmacologie-toxicologie du CHU de Bordeaux, il a pu être identifié au moins 8 cannabinoïdes différents chez 28 patients. Les 2 derniers cannabinoïdes de synthèse qui ont été identifiés par le laboratoire étaient classés comme stupéfiants, dont le MDMB-4en-PINACA, qui a également largement diffusé en métropole, mais aussi en Europe.
L'enquête d'addictovigilance OPPIDUM, réalisée par le centre d'addictovigilance de Bordeaux et DOM chaque mois d'octobre auprès des structures de prise en charge en addictologie retrouve en octobre 2018 un cas de prise de chamane (prise occasionnelle, par voie fumée, obtenu par deal ou don, sans souffrance à l'arrêt, pour une "substitution du cannabis qui n'a plus le même effet").
Plusieurs alertes ont été remontées à l'ARS, ainsi qu'une note réalisée en 2020 par le centre d'addictovigilance de Bordeaux et DOM.

b. Métropole

- Quelques cas de consommation de chimique ou phénomène s'y approchant ont été signalés, mais dans une beaucoup moins grande ampleur que ce qui est observé dans l'Océan Indien :
    - cas d'un patient Mahorais, consommateur de chimique à Mayotte, venu en Gironde et hospitalisé en psychiatrie pour trouble psychotique (pas d'analyse toxicologique)
    - cas médiatisés à Roanne dans la région Auvergne Rhône Alpes : 6 cas d'intoxication par des cigarettes "trafiquées" avec prise en charge hospitalière dans 4 cas (malaise, somnolence, suivi d'une phase d'agitation voire agressivité). Le centre d'addictovigilance de Bordeaux et DOM a été contacté par un urgentiste Mahorais pour alerter sur la possibilité que ce soit de la chimique, une communauté Mahoraise étant présente dans la région : (https://www.leparisien.fr/faits-divers/cigarettes-trafiquees-a-l-herbe-chamanique-trois-interpellations-a-roanne-07-08-2019-8130225.php).
    - un échantillon de chimique, en provenance de Nouvelle-Aquitaine (communauté comorienne), a été analysé par le dispositif SINTES et a retrouvé un cannabinoïde de synthèse (4F-MDMB-BINACA).
- Depuis 2013, le réseau français d'addictovigilance a signalé la présence de cannabinoïdes de synthèse sous forme d'e-liquides vendus sur Internet, notamment dans le nord de la France, bien que cette pratique semble s'être répandue sur l'ensemble du territoire, Internet sous les noms « commerciaux » de « buddha-blues », « C-liquid » ou plus récemment sous le nom de « PTC » (« Pête ton crâne ») [7]. En particulier, en 2018/2019, des effets neurologiques et psychiatriques mais aussi digestifs et cardiovasculaires ont été rapportés chez des adolescents et jeunes adultes après avoir fumé des e-liquides censés contenir du CBD ; dans 5 cas, l'analyse de l'e-liquide avait révélé la présence de quatre CS différents : 5F-MDMB-PINACA, 5F-CUMYL-PINACA, XLR-11, 5F-MDMB-PICA [8].
- Depuis octobre 2020, plusieurs alertes sur la présence de CS dans des échantillons de cannabis ont été émises en France : plusieurs centres d'addictovigilance (dont celui de Bordeaux et DOM) ont été alertés par la circulation d'herbe vendue comme du cannabis contenant, en plus du THC, un cannabinoïde de synthèse, identifié par la suite comme le MDMB-4en-PINACA. Des consommateurs réguliers et non réguliers de cannabis ont décrit des tableaux cliniques tout à fait inhabituels pour le THC, suggérant la présence de produits plus puissants (fièvre, vertiges,

tremblements, sueurs, nausées et vomissements, hypotension et tachycardie, troubles psychiatriques, sensation de mort imminente) après la consommation de ce produit [9].

7. Signaler en addictovigilance

L'expertise d'addictovigilance repose majoritairement sur les signalements réalisés par les professionnels au contact des consommateurs. Nous tenons à remercier l'ensemble des acteurs qui participent à cette veille sanitaire.

Si vous avez connaissance d'une nouvelle substance et/ou d'un effet somatique et/ou psychiatrique grave ou inattendu, n'hésitez pas à transmettre les éléments anonymisés à votre centre d'addictovigilance, directement ou par le portail de signalement (*cf.* encadré). En cas d'effet grave, des analyses toxicologiques peuvent aussi être réalisées en lien avec le laboratoire du CHU de Bordeaux.

Le Centre d'Addictovigilance est disponible pour toute question sur une substance psycho-active (médicament ou non), ses effets, le détournement de la voie d'administration, *etc.* sur simple demande par mail ou téléphone. Un bulletin d'information des centres d'addictovigilance est publié régulièrement sur divers points d'actualités.

Pour signaler un cas d'addictovigilance	**Pour poser une question et/ou pour recevoir le bulletin d'information des centres d'addictovigilance**
Portail : https://signalement.social-sante.gouv.fr ou directement, *via* ceip.addictovigilance@u-bordeaux.fr / 05 57 57 15 61	ceip.addictovigilance@u-bordeaux.fr / 05 57 57 15 61

Références

1. EMCDDA. New psychoactive substances: 25 years of early warning and response in Europe. 2022 https://www.emcdda.europa.eu/system/files/publications/14637/20222218_PDF_TD0522113ENN_002.pdf, Consulté le

2. EMCDDA. Les cannabinoïdes de synthèse en Europe. 2017 https://www.emcdda.europa.eu/system/files/publications/2753/POD_Synthetic%20cannabinoids_0.pdf , Consulté le

3. Castaneto MS, Gorelick DA, Desrosiers NA, Hartman RL, Pirard S, Huestis MA. Synthetic cannabinoids: epidemiology, pharmacodynamics, and clinical implications. Drug Alcohol Depend 2014; 144: 12-41 10.1016/j.drugalcdep.2014.08.005

4. Teske J, Weller JP, Fieguth A, Rothamel T, Schulz Y, Troger HD. Sensitive and rapid quantification of the cannabinoid receptor agonist naphthalen-1-yl-(1-pentylindol-3-yl)methanone (JWH-018) in human serum by liquid chromatography-tandem mass spectrometry. J Chromatogr B Analyt Technol Biomed Life Sci 2010; 878: 2659-63 10.1016/j.jchromb.2010.03.016

5. Goncalves R, Pelletier R, Couette A, Gicquel T, Le Daré B. Suitability of high-resolution massspectrometry in analytical toxicology: Focuson drugs of abuse. Toxicologie Analytique & Clinique 2021:

6. Daveluy A, Haramburu F. [Psychoactive substances use in Reunion Island and Mayotte, French departments in Indian Ocean]. Therapie 2018; 73: 419-27 10.1016/j.therap.2018.01.007

7. Batisse A, Le Boisselier R, Tournebize J, Chaouachi L, Djezzar S. Consommation de substances psychoactives illicites par vapotage : les données d'addictovigilance. Therapies 2021; 76: 168

8. Tournebize J, Gibaja V, Grossenbacher F, Plenier Y, Medhioub Y, Batisse A*, et al.* Le mésusage de la cigarette électronique : une centaine de jeunes hospitalisés après avoir vapoté dans le nord-est de la France. Therapies 2021; 76: 161

9. Goncalves R, Labadie M, Chouraqui S, Peyre A, Castaing N, Daveluy A*, et al.* Involuntary MDMB-4en-PINACA intoxications following cannabis consumption: clinical and analytical findings. Clin Toxicol (Phila) 2021: 1-6 10.1080/15563650.2021.1994144

II. Annexe 2 – Protocole HOMACHI

1. RESUME DE LA RECHERCHE

GESTIONNAIRE	CHU de Bordeaux
TITRE	HOspitalisations de mineurs et de jeunes adultes à Mayotte après prise de CHImique : quelles substances sont incriminées ?
TITRE COURT / ACRONYME	**HOMACHI**
NOMBRE D'EQUIPES ASSOCIES A L'ETUDE, RECHERCHE OU EVALUATION	4
NOM, TITRE ET FONCTION DU RESPONSABLE DE L'EQUIPE COORDINATRICE	Dr Amélie DAVELUY Service de pharmacologie médicale CHU Pellegrin - Centre addictovigilance Place Amélie Raba Leon 33076 BORDEAUX cedex Tel: 05.56.96.40.80 Fax : 05.57.57.46.60 Courriel : amelie.daveluy@u-bordeaux.fr / amelie.daveluy@chu-bordeaux.fr
JUSTIFICATION / CONTEXTE	En 2015, le CEIP-A de Bordeaux (Centre d'Evaluation et d'Information sur la Pharmacodépendance et Addictovigilance, référent d'Aquitaine et des Départements d'Outre-Mer) a été missionné par la MILD&CA (Mission Interministérielle de Lutte contre les Drogues et les Conduites Addictives) pour renforcer les échanges et les enquêtes d'addictovigilance à la Réunion et à Mayotte et pour mieux évaluer la nature et les modalités de consommation de substances psychoactives dans ces deux départements de l'Océan Indien. A Mayotte, le CEIP-A a été alerté sur le problème de "la chimique", drogue à l'origine de très nombreuses hospitalisations, en particulier de mineurs et de jeunes adultes au cours du dernier semestre 2015. La composition de la chimique n'est pas connue, même si au même moment, des saisies douanières ont permis de poser l'hypothèse qu'il puisse s'agir de cannabinoïdes de synthèse. Plusieurs études ont été menées sur le sujet, mais à ce jour, il n'y a pas eu de confirmation analytique permettant d'établir un lien entre la prise de chimique et les tableaux cliniques à l'origine des hospitalisations.
OBJECTIFS PRINCIPAL ET SECONDAIRES	*Objectif principal :* identifier les substances psychoactives contenues dans la chimique dont l'usage entraîne des tableaux cliniques graves amenant à des hospitalisations *Objectifs secondaires :* - valider une possibilité d'une recherche toxicologique de Nouveaux Produits de Synthèse (NPS) sur tâche de sang séché sur papiers buvards préparés à Mayotte et transportés en conditions tropicales, en les comparant à des buvards préparés en laboratoire à Bordeaux à partir du reliquat de sang. - dresser les tableaux cliniques associés à chaque substance afin d'améliorer la prise en charge ultérieure devant des tableaux similaires. - sensibiliser les acteurs institutionnels et associatifs aux risques liés à la prise de ces substances potentiellement retrouvées dans la chimique et à la déclaration de cas graves au CEIP-A, par l'organisation d'une visio-conférence (6 mois après la

	fin de la phase de collecte) - apporter des informations aux autorités sanitaires (ARS Océan-Indien, ANSM, MILD&CA) sur les molécules circulant à Mayotte, à l'origine de complications sévères (à travers la rédaction d'un rapport, issu de la synthèse de l'ensemble des éléments, après l'action de sensibilisation).
JUSTIFICATION D'INTERET PUBLIC DE L'ETUDE	La majorité des patients hospitalisés après consommation de chimique à Mayotte sont des mineurs isolés (environ les deux tiers selon des acteurs locaux). Ils utilisent d'autres circuits d'approvisionnement en drogues que les adultes socialement insérés. La problématique des jeunes des rues à Mayotte est spécifique de ce département français, de même que la consommation de la chimique. Ces adolescents sont livrés à eux-mêmes et n'ont en général pas de tuteur légal sur l'île. Une meilleure connaissance des produits consommés et des tableaux cliniques associés permettra aux urgentistes d'améliorer la prise en charge de ses patients. De plus, une analyse du buvard leur permettra d'avoir des réponses plus rapides sur les molécules consommées.
TYPE D'ETUDE	Etude rétrospective, non-interventionnelle, multicentrique.
CRITERES D'INCLUSION	- Patients hospitalisés en service d'urgences du CH de Mayotte après prise de chimique ou d'une autre substance psychoactive pour laquelle le médecin requière une recherche sanguine de prise de substances - Age ≥ 14 ans, âge établi à partir de documents d'identité, du carnet de santé ou de soins antérieurs. - Pour les patients majeurs : non-opposition et autorisation exprimée de participer à l'étude et d'analyser les données (sauf dérogation des autorités si les patients ne sont pas en état de comprendre et d'exprimer leur non-opposition) - Pour les patients mineurs de 14 ans : non-opposition du patient **et** de l'autorité parentale - Pour les patients mineurs de **15 ans et plus** : non-opposition du patient et de l'autorité parentale **sauf exception de l'article 59 de la loi du 6 janvier 1978 modifiée** : → Opposition formelle du patient mineur à ce que le ou les titulaires de l'autorité parentale soient informés/consultés **ou** → Liens de famille rompus (exprimé par le patient ou connu du service d'urgence) **et** mineur bénéficiaire à titre personnel du remboursement des prestations de l'assurance maladie et de la couverture complémentaire
CRITERES DE NON INCLUSION	- Patients mineurs de 14 ans sans titulaire de l'autorité parentale - Patients mineurs de 15 ans et plus <u>ne rentrant pas</u> dans l'exception l'article 59 de la loi du 6 janvier 1978
TAILLE D'ETUDE	20 participants
ORIGINE DES DONNEES DE SANTE	Dossiers patients informatisés du service des urgences du CH de Mayotte. Résultats des analyses d'échantillons sanguins pour recherche de toxiques par le laboratoire de pharmaco-toxicologie du CHU de Bordeaux.
MODE DE RECUEIL DES DONNEES A CARACTERE PERSONNEL ET LIEU D'HEBERGEMENT DE LA BASE DE DONNEES	Les données cliniques recueillies lors de l'hospitalisation sont saisies dans le cadre du soin sur le logiciel médical local du CH de Mayotte. Ces données sont anonymisées par le laboratoire d'analyse médicale du CH de Mayotte avant envoi des échantillons au laboratoire de pharmaco-toxicologie du CHU de Bordeaux.

	La base de données (données cliniques et résultats des analyses biologiques) est sous format électronique Excel protégée par mot de passe et hébergée sur le serveur sécurisé du CHU de Bordeaux. Les résultats sont communiqués au CH de Mayotte via la plateforme sécurisée du CHU de Bordeaux (NextCloud). Les données de la recherche seront conservées pendant 15 ans.
CIRCUIT DES DONNEES A CARACTERE PERSONNEL ET MODALITES DE PROTECTION DE LEUR CONFIDENTIALITE	Chaque participant se verra attribuer un code confidentiel d'identification, composé d'un numéro (2 chiffres) et d'un code lettre (4 lettres aléatoires) anonyme. L'anonymisation est effectuée par l'équipe investigatrice locale (laboratoire d'analyse médicale du CH de Mayotte). La copie des données médicales ainsi que chaque échantillon (buvard et tube sanguin) seront anonymisés par le laboratoire d'analyse médicale du CH de Mayotte à l'aide de ce code avant l'envoi au laboratoire du CHU de Bordeaux. Les résultats sont communiqués au CH de Mayotte via la plateforme sécurisée du CHU de Bordeaux (NextCloud). A l'issue des analyses, les échantillons seront détruits par le CHU de Bordeaux.
DUREE DE LA RECHERCHE	- Durée de la période d'inclusion : 4 mois - Durée de suivi par participant : < 5 heures (temps du prélèvement sanguin et examen médical effectués dans le cadre du soin) - Durée totale de la recherche : 4 mois
CALENDRIER DE LA RECHERCHE ET ORGANISATION DE L'ETUDE	- juillet à octobre 2019 : inclusions et conservation des échantillons au CH de Mayotte - novembre 2019 à juin 2020 : analyses des échantillons (laboratoire de pharmaco-toxicologie du CHU de Bordeaux) et synthèse des résultats (CEIP-A du CHU de Bordeaux).
CRITERES D'EVALUATION	Critère de jugement principal : Différence entre les nombres de NPS identifiés par le laboratoire de pharmaco-toxicologie du CHU de Bordeaux avec analyse ciblée par chromatographie liquide couplée à la spectrométrie de masse haute résolution (LC-HRMS) versus avec analyse ciblée « stupéfiants-NPS » par chromatographie liquide couplée à la spectrométrie de masse en tandem (LC-MS/MS). Les critères de jugement secondaires sont : - nombre de principes actifs trouvés (dont NPS). - nombre d'associations entre tableaux cliniques et principes actifs.
ANALYSE STATISTIQUE DES DONNEES	Les variables qualitatives seront décrites en termes d'effectif, de pourcentage et d'intervalle de confiance à 95% selon la loi binomiale exacte. Les variables quantitatives seront décrites en termes d'effectif, moyenne, écart-type et intervalle de confiance à 95% de la moyenne, médiane, étendue et étendue interquartile. Une corrélation sera recherchée entre NPS et tableaux cliniques
RETOMBEES ATTENDUES	Validation d'un dispositif d'analyse sur buvards qui permet aux médecins d'obtenir très rapidement des résultats toxicologiques précis, avec envoi sous enveloppe classique. Formation des professionnels mahorais en vue d'actions de prévention, réduction des risques et prise en charge des soins adaptée.

III. Annexe 3 – Protocole DRUGETOF mis en place dans le cadre de la thèse et en cours d'étude

Synopsis – Fiche synthétique

Intitulé du projet de thèse : Étude de la consommation de substances psychoactives et ses déterminants chez les étudiants de la cohorte iShare : apport de la spectrométrie de masse haute résolution en QTOF.

Problème, objet de la recherche, originalité du travail : Le profil des consommateurs de substances psychoactives (SPA) s'est élargi ces dernières années incluant des personnes insérées tels que les étudiants comme l'a montré une enquête nationale au Canada [1]. Alors que la consommation de SPA est bien étudiée chez les jeunes (collégiens et lycéens), les données de consommation de ces substances chez les étudiants sont plus rares. Des stratégies de prévention adaptées à la population étudiante ont été élaborées : un programme de prévention sur Internet a été établi pour la MDMA en Australie [2] ou des applications mobiles ont été utilisées en Suisse [3] et en Hongrie [4]. A ce jour, en France, peu de données sont disponibles dans cette population et les études réalisées portent le plus souvent sur l'alcool, le tabac et le cannabis [5,6], plus récemment sur les benzodiazépines, la cocaïne et la MDMA [7,8]. Bien que la consommation de SPA soit multifactorielle, certains contextes peuvent favoriser le recours à ces substances. Au regard des facteurs décrits comme associés à la consommation de SPA, la population étudiante apparait particulièrement exposée et vulnérable. Les étudiants constituent un public enclin à côtoyer des milieux festifs, ce qui augmente la possibilité et la fréquence d'usages récréatifs de SPA. Le passage dans la vie étudiante est en général associé à une pression sur les performances académique dans un milieu moins soutenant que le lycée et une plus grande autonomie dans la vie quotidienne avec le plus souvent le départ du foyer familial [9,10]. Cette période peut être pour certains étudiants, génératrice d'un stress important, voire de dépression [11]. Ces états peuvent alors amener le sujet à consommer des SPA pour trouver un réconfort (soulagement du stress, détente) [12]. Ces consommations, qu'elles soient expérimentales ou plus régulières, restent toutefois indissociables d'une mise en danger du sujet à court ou à long terme. Dans ce contexte, l'évaluation de la consommation de SPA chez les étudiants apparait comme un enjeu important de santé publique. L'évaluation auto-rapportée de la consommation de SPA peut amener à une sous-évaluation du fait d'un biais de désirabilité sociale. Il est donc intéressant (i) de disposer d'une étude des SPA réellement consommées et (ii) d'estimer ce biais dans la cohorte i-Share, large cohorte d'étudiants francophones qui repose sur des auto-évaluations en ligne. Le grand intérêt de la spectrométrie de masse haute résolution (LC-HRMS) en QTOF est d'identifier toutes les SPA (médicamenteuses ou non) présentes dans le plasma sans présumer des consommations du sujet.

Objectifs, hypothèses : L'objectif principal de ce projet est de décrire les consommations réelles de SPA objectivées par analyse des échantillons biologique en LC-QToF et identifier les déterminants associés à cette consommation dans la population étudiante de la cohorte i-Share. Les objectifs secondaires sont :

- Comparer les consommations réelles aux consommations déclarées
- Calculer les prévalences de consommation

Ce projet sera découpé en 3 grandes étapes :

- Étape 1 : *Création des groupes - inclusion*
- Étape 2 : *Analyses des échantillons*

- Étape 3 : *Analyses des données questionnaires (statistiques descriptives et mutlivariées)*

Méthodes : Inclusion de 400 étudiants ayants participés à la biobanque et ayant rempli le questionnaire optionnel de santé mentale, forts consommateurs de SPA ou non consommateurs. Analyses biologiques des échantillons réalisées en spectrométrie de masse haute résolution (LC-HRMS) en QTOF. Analyses statistiques descriptives (consommations déclarées ; résultats des analyses) et multivariées (déterminants associés aux différents niveaux de consommation de SPA)

Résultats attendus : Cette étude permettra de caractériser la consommation de SPA chez les étudiants de la cohorte et d'identifier les déterminants associés à cette consommation. Les informations sur la nature et la fréquence des consommations de SPA dans la cohorte i-Share permettront de fournir un état des lieux relativement représentatif des consommations en France chez les étudiants, et constitueront un soutien scientifique pour l'élaboration des futures campagnes de prévention pour la santé publique.

Étapes opérationnelles et calendrier de la recherche : Le projet a été prévu pour une durée de 1 an.
Insertion du projet dans des collectifs de recherche et rôle propre du candidat : Toutes les étapes de ce projet seront réalisées par le candidat dans le cadre de sa thèse de science, sous la direction du Professeur Mathieu Molimard. Cette recherche s'inscrit dans les thématiques phares de l'équipe Médicament et Santé des Populations du centre de recherche U1219.

Références :
[1] Les drogues à 17 ans : analyse de l'enquête ESCAPAD 2017, Tendances N°123, février 2018 - OFDT - Saint-Denis. L'observatoire français des drogues et des toxicomanies (OFDT), [en ligne]. https://www.ofdt.fr/BDD/publications/docs/eftxssy2.pdf

[2] Champion KE, Newton NC, Stapinski LA, Teesson M. Effectiveness of a universal internet-based prevention program for ecstasy and new psychoactive substances: a cluster randomized controlled trial. Addiction 2016; 111: 1396-405.

[3] Haug S, Paz Castro R, Meyer C, Filler A, Kowatsch T, P Schaub M. A Mobile Phone- Based Life Skills Training Program for Substance Use Prevention Among Adolescents: Pre-Post Study on the Acceptance and Potential Effectiveness of the Program, Ready4life. JMIR Mhealth Uhealth. 2017; 5(10): e143.

[4] Kapitány-Fövény M, Vagdalt E, Ruttkay Z, Urbán R, J Richman M, Demetrovics Z. Potential of an Interactive Drug Prevention Mobile Phone App (Once Upon a High): Questionnaire Study Among Students. JMIR Serious Games. 2018; 6(4): e19.

[5] Morvan Y, Rouvier JP, Oliéab JP, Lôo H, Krebs O. Consommations de substances illicites chez les étudiants : une enquête en service de médecine préventive. Student's use of illicit drugs: a survey in a preventive health service. Encéphale 2009; 35: 202-208.

[6] La santé des étudiants en France, 4ème enquête nationale. La mutuelle des étudiants, [en ligne]. https://www.lmde.fr/documents/20184/1997318/ense-4.pdf/5affe802-08a2-429d-8b60-044c3302419e

[7] David Balayssac D, Pereira B, Darfeuille M, Cuq P, Vernhet L, Collin A et al. Use of Psychotropic Medications and Illegal Drugs, and Related Consequences Among French Pharmacy Students – SCEP Study: A Nationwide Cross-Sectional Study. Front. Pharmacol. 2018; 9: 725.

[8] Hamel-Sénécal L, Chrétien B, Cabé N, Ritz L, Mange J, Sénemeaud C et al. ADUC (alcool et drogues à l'université de Caen Normandie): a useful contribution for addictovigilance on students' population. Therapie 2018; 73: 576.

[9] Verger P, Combes JB, Kovess-Masfety V, Choquet M, Guagliardo V, Rouillon F et al. Psychological distress in first year university students: socioeconomic and academic stressors, mastery and social support in young men and women. Soc Psychiatry Psychiatr Epidemiol 2009 ; 44: 643–50.

[10] Verger P, Guagliardo V, Gilbert F, Rouillon F, Kovess-Masfety V. Psychiatric disorders in students in six French universities: 12-month prevalence, comorbidity, impairment and help-seeking. Soc Psychiatry Psychiatr Epidemiol 2010 ; 45 : 189–99.

[11] Vandentorren S, Verret C, Vignonde M, Maurice-Tison S. Besoins d'information en santé des étudiants au service interuniversitaire de médecine préventive de Bordeaux. Santé Publique 2005 ; 17 : 47.

[12] Herault J, Dano C. Consommation de substances psychoactives des internes en médecine. Exercer 2013;107 : 122-3.

i-Share.fr
TA SANTÉ EN QUESTIONS

FORMULAIRE DE SOUMISSION D'UNE ÉTUDE ANCILLAIRE
université de Bordeaux / Étude i-Share

Tableau des versions

Version	Date	Rédigée par	Justification du changement de version
1.0	01/10/2015	Clothilde Pollet *Chef de projet opérationnel*	
2.0	26/10/2017	Catherine Fagard *Ingénieur de recherche Inserm ;* *Expert recherche/qualité*	Mise à jour des documents qualité des études ancillaires

Ce formulaire de soumission d'une étude ancillaire doit être complété par l'investigateur de l'étude ancillaire après **acceptation de la lettre d'intention par l'équipe i-Share.**

Il est transmis au chef de projet opérationnel de la cohorte avec le dossier de soumission qui comprend :

- ☐ Le protocole de l'étude ancillaire
- ☐ La charte des études ancillaires de la cohorte i-Share signée
- ☐ Les questionnaires de recueil de données (si applicable)

Supprimer les indications en italiques qui sont une aide au remplissage du document

Étude ancillaire N°	**EA _ _ _**
Titre complet de l'étude ancillaire	Étude de la consommation de substances psychoactives et ses déterminants chez les étudiants de la cohorte iShare : apport de la spectrométrie de masse haute résolution en QTOF.
Titre abrégé de l'étude ancillaire	DRUGETOF (A redéfinir)
Investigateur de l'étude ancillaire Titre, Nom Institution Adresse	Dr Ruben GONCALVES Service de Pharmacologie Médicale – Laboratoire de Pharmaco-Toxicologie Groupe Hospitalier Pellegrin - CHU de Bordeaux Place Amélie Raba Léon 33076 Bordeaux Cedex
Co-investigateurs	Pr Mathieu Molimard
Promoteur	*Toute recherche interventionnelle, interventionnelle à risques minimes ou non interventionnelle doit disposer d'un promoteur selon la loi Jardé (sauf si la recherche ne concerne que des données déjà recueillies dans la cohorte i-Share (type ❶ voir Annexe 1 de la charte).*

Personnes impliquées dans l'étude ancillaire	*Dr Ruben Goncalves** *Pr Mathieu Molimard* *Pr Marie Tournier* **correspondant principal*

Objectif principal	Dans un premier temps, décrire les consommations réelles de substances psychoactives objectivées par analyse des échantillons biologique en LC-QToF. Dans un second temps, identifier les déterminants associés à cette consommation dans la population étudiante de la cohorte i-Share.
Objectifs secondaires	Comparer les consommations réelles aux consommations déclarées Calculer les prévalences de consommation
Population-cible de la cohorte (définition des critères d'inclusion)	*Participants de la cohorte i-Share :* *Groupe de consommateurs :* - *Ayant réalisé un prélèvement sanguin disponible dans la biobanque* - *Ayant répondu au questionnaire de santé mentale* - *Et : Forts Consommateurs de cannabis (plusieurs fois par mois/tous les jours)* **Q3.1. <u>Au cours des 12 derniers mois,</u> avez-vous consommé du cannabis ?** ○ Oui ○ Non ○ Ne souhaite pas répondre ► Si Oui **Q3.1.1. Pouvez-vous préciser le nombre de fois :** ○ Tous les jours ○ Plusieurs fois par semaine ○ Une fois par semaine ○ Plusieurs fois par mois ○ Une fois par mois ou moins ○ Une seule fois - Et/Ou : Ayant consommé au moins une fois une autre substance psychoactive dans les 12 derniers mois Réponses questionnaire consommation **Q3.2. (T3) <u>Au cours des 12 derniers mois,</u> avez-vous pris un des produits suivants ?** **Q3.2.1. Ecstasy, MD, MDMA :** ○ Non jamais ○ Une fois ○ Entre 2 et 5 fois ○ 6 fois et plus ○ Ne souhaite pas répondre **Q3.2.2. Amphétamines (Speed) :** ○ Non jamais ○ Une fois ○ Entre 2 et 5 fois ○ 6 fois et plus ○ Ne souhaite pas répondre

Q3.2.3. Cocaïne :

- Non jamais
- Une fois
- Entre 2 et 5 fois
- 6 fois et plus
- Ne souhaite pas répondre

Q3.2.4. Champignons hallucinogènes :

- Non jamais
- Une fois
- Entre 2 et 5 fois
- 6 fois et plus
- Ne souhaite pas répondre

Q3.3. Au cours des 12 derniers mois, avez-vous pris d'autres drogues ?

- Oui
- Non
- Ne souhaite pas répondre

► *Si Oui*

Q3.3.1. Merci de préciser la ou lesquelles : .....

Groupe contrôle = **Groupe de non-consommateurs :**

- *Ayant réalisé un prélèvement sanguin disponible dans la biobanque*
- *Ayant répondu au questionnaire de santé mentale*
- Ayant répondu non à toutes les questions des consommations de substance
  Réponse questionnaire consommation :

Q3.4. Au cours des 12 derniers mois, avez-vous consommé du cannabis ?

- Oui
- Non
- Ne souhaite pas répondre

► *Si Oui*

Q3.4.1. Pouvez-vous préciser le nombre de fois :

- Tous les jours
- Plusieurs fois par semaine
- Une fois par semaine
- Plusieurs fois par mois
- Une fois par mois ou moins
- Une seule fois

	Q3.5. (T3) Au cours des 12 derniers mois, avez-vous pris un des produits suivants ? **Q3.5.1. Ecstasy, MD, MDMA :** o Non jamais o Une fois o Entre 2 et 5 fois o 6 fois et plus o Ne souhaite pas répondre **Q3.5.2. Amphétamines (Speed) :** o Non jamais o Une fois o Entre 2 et 5 fois o 6 fois et plus o Ne souhaite pas répondre **Q3.5.3. Cocaïne :** o Non jamais o Une fois o Entre 2 et 5 fois o 6 fois et plus o Ne souhaite pas répondre **Q3.5.4. Champignons hallucinogènes :** o Non jamais o Une fois o Entre 2 et 5 fois o 6 fois et plus o Ne souhaite pas répondre **Q3.6. Au cours des 12 derniers mois, avez-vous pris d'autres drogues ?** o Oui o Non o Ne souhaite pas répondre ► *Si Oui* **Q3.6.1. Merci de préciser la ou lesquelles :** .....
Schéma de l'étude	Étude transversale chez les participants de la cohorte i-Share. Les informations ont été

	fournies par le questionnaire d'inclusion dans la cohorte et le questionnaire de santé mentale.
Type d'étude ancillaire	❹a
Cadre réglementaire	*Défini en collaboration avec le chef de projet opérationnel*
Calendrier / Durée prévisionnelle de l'étude	**Début de l'Étude : 2020** **Durée Étude : 6 mois** ***Analyse des données*** *: 6 mois* ***Rédaction de l'article et soumission*** *: Septembre 2021*
Déroulement de l'étude	*Lister les étapes principales de l'étude (inclusion, suivi…)* 1. *Création des groupes - inclusion* 2. *Analyses des échantillons* 3. *Analyses des données questionnaires (statistiques descriptives et mutlivariées)* 4. *Rédaction de l'article*
Données de la cohorte nécessaires à l'étude ancillaire	Données sociodémographiques, autres données… (par catégories) Données socio-démographiques Données de consommations de substances psychoactives déclarées Données du questionnaire d'inclusion Données du questionnaire de santé mentale
Données additionnelles recueillies	*Aucune*
Aspects statistiques (taille de l'étude, méthodes d'analyse)	**Taille de l'étude** *: N = 400 (prévisionnel)* ***Méthodes d'analyse*** *: Analyse descriptive, analyses multivariées classiques (modèles de régression logistique et linéaires…), …* *Analyse descriptive sur les consommations* *Analyses multivariées entre les consommations et les données des questionnaires* ***Logiciels*** *: SAS Studio® ; R studio®*
Collection d'échantillons biologiques	*Non applicable*
Accès aux échantillons biologiques	*Modalités de recueil des échantillons selon le paragraphe « population cible de la cohorte »*
Accès aux données biologiques	*Non applicable*
Accès aux données génétiques	*Non applicable*

Partenaires institutionnels	CHU de Bordeaux Université de Bordeaux – Inserm U1219

Partenaires privés (si applicable)	Non applicable
Sous-traitants pressentis	Non applicable
Budget prévisionnel	*Non applicable*
Financement prévisionnel	*Étude réalisée dans le cadre de la thèse de science de Ruben Goncalves* *Financement par le laboratoire de Pharmacologie et Toxicologie du CHU de Bordeaux (Service de Pharmacologie Médicale)*

Je soussigné, Ruben GONCALVES, investigateur de l'étude ancillaire,

- sollicite la participation de cette étude ancillaire à la cohorte i-Share,
- garantit l'exactitude et l'honnêteté des informations fournies,
- accepte les termes de la charte des études ancillaires de la cohorte i-Share.

A Bordeaux, le 12 août 2020

Signature :

Docteur R. GONCALVES
Service de Pharmacologie
Laboratoire de Pharmacologie-Toxicologie
Groupe Hospitalier Pellegrin
33076 Bordeaux Cedex

IV. Annexe 4 – Protocole RuNPS mis en place dans le cadre de la thèse et en cours d'étude

Recherche - CHU de La Réunion - AOI 2020

LETTRE D'INTENTION

Titre de l'étude envisagée : Etude descriptive des cas suspects d'intoxications aiguës par Nouveaux Produits de Synthèse (NPS) se présentant aux Urgences du CHU de La Réunion
Titre abrégé : RuNPS

Porteur du projet

Prénom, Nom : David METE
Fonction : Chef de service
Service : Addictologie
Etablissement : CHU Réunion - Site CHFG
Adresse électronique : david.mete@chu-reunion.fr
Téléphone : 02.62.90.56.01

Projets de Recherche Clinique à promotion CHU déjà financés ? NON
Liste, état d'avancement, difficultés rencontrées ?

Praticien Hospitalier associé (le cas échéant)

Prénom, Nom :
Fonction :
Service :
Etablissement : CHU Réunion - Site CHFG/GHSR
Adresse électronique :
Téléphone :

Le cas échéant, équipes associé(e)s au CHU de La Réunion dans le projet :

Nom, Prénom	Service/Equipe	Etablissement
Henrion, Guy	Urgences	CHU Félix Guyon
Guihard, Bertrand	Urgences	CHU Félix Guyon
Marteau, Adrien	Urgences	CHU Sud Réunion
Bastard, Sophie	Laboratoire Biologie	CHU Félix Guyon
Goncalves, Ruben	Laboratoire de Pharmacologie et Toxicologie	CHU de Bordeaux

Préciser le nom du (ou des) METHODOLOGISTE(S) : Adrien Maillot & Anne-Françoise Casimir

Nom de la structure : Toxicovigilance CHU de La Réunion & CIC CHU de La Réunion

Nom et Signature du responsable de la structure : Pr Catherine MARIMOUTOU

Montant du budget demandé : 35 000€

Existence d'un cofinancement : OUI ○ NON ●

Si oui : Identité du co-financeur : _____ Montant : _____

Cofinancement : obtenu ○ demandé ○

Dans le cas d'un cofinancement, le budget détaillé de celui-ci doit être joint au dossier.
NB : Le cofinancement devra être obtenu avant le dépôt du dossier complet (signature du cofinanceur)

Mots clés
Nouveaux produits de synthèse, cannabinoïdes de synthèse, chimique, chamane, addictologie, La Réunion
Discipline/spécialité: urgences, addictologie

Justification de l'étude (maximum 250 mots) :
Bref rappel (données de la littérature scientifique, pathologie, domaine d'étude, contexte actuel et problématique soulevée)

Les Nouveaux Produits de Synthèses (NPS) constituent un ensemble hétérogène de substances psychoactives qui imitent les effets psychoactifs des substances illicites classiques. En France les premiers NPS ont été identifiés entre 2007-2008. Les cannabinoïdes de synthèse (CS) représentent une part importante des NPS, suivis des cathinones de synthèse. Produits sous forme de poudre, les CS sont ajoutés à un solvant et généralement pulvérisés sur un support qui sera fumé. Les effets indésirables sont variés et imprévisibles, ils peuvent avoir des conséquences graves sur la santé des consommateurs (jusqu'à 200 fois plus puissants que le cannabis ordinaire).

Les signes cliniques habituellement associés à la consommation de NPS se résument à : nausées, tachycardie, état d'agitation. Chez les cas plus sévères : malaise, hyperémèse, crises d'épilepsie, symptômes psychotiques. Plus rarement, une néphrotoxicité, une cardiotoxicité, une insuffisance hépatique avec plusieurs dizaines de décès enregistrés dans le monde. Comparée au cannabis, la consommation de CS s'accompagne d'un risque multiplié par trente de passage aux urgences.

Les premières consommations de CS à la Réunion (appelé localement "*Chimique*" ou "*Chamane*") ont été décrites en 2016, suivies d'une intensification à partir de 2018. Ces substances sont indétectables par les tests de routine. Leur identification est coûteuse et nécessite des moyens lourds (spectrographie de masse). Les NPS sont considérés par les soignants comme un problème de Santé Publique mais réellement peu quantifié avec de rares informations aujourd'hui sur les molécules consommées et les signes cliniques associés à la Réunion, impactant la prise en charge des patients.

Question posée et hypothèse de la recherche :
Définir l'hypothèse qui justifie la mise en place de la recherche, stratégie/procédure à l'étude, population cible.

Questions :
Quelle est la prévalence des intoxications aux NPS aux urgences ? Quelles sont les molécules retrouvées ?
Quels sont les signes cliniques associés à la consommation de NPS ?
Quelle est la part des soumissions chimiques et des personnes vulnérables dans la consommation de NPS ?

Hypothèses :
Il y aurait un nombre plus important, que préalablement estimé, d'intoxications aiguës aux NPS aux urgences à La Réunion.
Il y aurait des signes spécifiques, encore mal caractérisés, associés à certains NPS chez les cas se présentant pour intoxication aiguë aux urgences à La Réunion.
Il y aurait une part de cas graves nécessitant une prise en charge en médecine intensive.

Stratégie :
- Identification des cas suspects d'intoxication aux NPS aux urgences par les urgentistes selon les protocoles en vigueur dans leur service.
- Prescriptions d'analyses spécifiques à la recherche de NPS dans les liquides biologiques.
- Patient inclus dans l'étude dès lors qu'une prescription d'analyse spécifique à la recherche de NPS est réalisée.
- Collecte des données sociodémographique, cliniques et biologiques disponibles dans le dossier médical du patient.
- Analyse descriptive des différents paramètres recueillis.

Population source : patients admis aux urgences du CHU de La Réunion (Site Nord et site Sud).
Population cible : patients admis aux urgences du CHU de La Réunion, étiquetés suspects d'intoxication aux NPS par les urgentistes pour lesquels une prescription d'analyse biologique à la recherche de NPS est souhaitée.

Retombées attendues (patients, soins, valorisation) :
En termes d'amélioration des connaissances sur la pathologie, bénéfice attendu pour le patient, modifications éventuelles des pratiques, capacité de valorisation par publication notamment.

- meilleure connaissance des molécules de NPS qui sont consommées à La Réunion
- meilleure connaissance de la clinique associée en fonction du NPS consommé mais aussi en cas de poly consommation selon les habitudes de consommation de stupéfiants à La Réunion
- amélioration de l'identification de la part des professionnels de santé des patients concernés par ces intoxications
- amélioration de la prise en charge de ces patients et des cas graves
- amélioration du parcours de soin addictologique au sein des hôpitaux de La Réunion
- enrichissement et adaptation des messages de préventions à destination de cette population

Citer les principaux articles (maxi 5 articles) de la littérature internationale justifiant l'intérêt de mener cette étude :

European Monitoring Centre for Drugs and Drug Addiction. Rapport européen sur les drogues 2019: tendances et évolutions. [Internet]. 2019 [cited 2020 Jan 30]. Available from: https://data.europa.eu/doi/10.2810/820836

Scocard A, Benyamina A, Coscas S, Karila L. Cannabinoïdes de synthèse : une nouvelle matrice des addictions. La Presse Médicale. 2017 Jan;46(1):11–22.

Trecki J, Gerona RR, Schwartz MD. Synthetic Cannabinoid–Related Illnesses and Deaths. N Engl J Med. 9 juill 2015;373(2):103‑7.

Corazza O, Roman-Urrestarazu A. Handbook of novel psychoactive substances: what clinicians should know about NPS. 2019.

Zaurova, M.; Hoffman, R. S.; Vlahov, D.; Manini, A. F. Clinical Effects of Synthetic Cannabinoid Receptor Agonists Compared with Marijuana in Emergency Department Patients with Acute Drug Overdose. J. Med. Toxicol. 2016, 12 (4), 335–340. https://doi.org/10.1007/s13181-016-0558-4.

Grandes lignes du projet

Objectif principal :
Décrire les cas suspects d'exposition aux NPS ayant subi une analyse biologique toxicologique à la recherche de NPS dans le cadre d'un passage aux urgences à La Réunion durant une année.

Objectifs secondaires :
Décrire les profils sociodémographiques et cliniques en fonction des molécules consommées et retrouvées dans les analyses biologiques toxicologiques.

Critère de jugement principal :
Nombre de cas avérés d'exposition aux NPS rapporté au nombre de cas suspects d'exposition aux NPS chez des patients admis aux urgences de La Réunion pour suspicion d'intoxication aigue au NPS durant une année.

Schéma d'étude :
Décrire les grandes caractéristiques de la recherche par des termes standards : essai thérapeutique comparatif, étude diagnostique/pronostique, étude d'observation (descriptive, à visée étiologique), - monocentrique/multicentrique - Description succincte des traitements/stratégies/ procédures à l'étude et du déroulement de la recherche.

Étude descriptive transversale mono centrique

Lors de leur admission aux urgences, les patients sont pris en charge selon les procédures courantes des services. Lorsqu'un patient est suspect d'intoxication à un ou des stupéfiants, l'orientation vers une suspicion d'intoxication à un NPS se fait à l'aide d'un arbre décisionnel se basant sur les déclarations du patient, voire de ses proches, les circonstances de sa consommation et l'examen clinique. Lorsque ce patient est classé suspect d'intoxication aux NPS par l'urgentiste, celui-ci prescrit une analyse toxicologique spécifique aux NPS. Cette analyse se fera sur des prélèvements biologiques (urine et/ou sang) pratiqués lors de sa prise en charge aux urgences. Les prélèvements sont acheminés puis analysés au laboratoire du CHU de Bordeaux.

Les critères d'inclusion :
- Tout patient suspect d'intoxication aux NPS dont la consommation n'est pas antérieur à plus de 24h (défini suivant les recommandations en vigueur)
OU
- Tout patient suspect d'une soumission chimique dont la consommation n'est pas antérieure à plus de 24h
ET
- Tout patient à qui est prescrite une analyse toxicologique à la recherche de NPS

S'en suit le recueil des données concernant l'étude de manière anonyme, saisi dans un formulaire électronique. Puis, analyse descriptive des données recueillies : socio-démographiques, cliniques, biologiques, toxicologiques, addictologiques, de consommation, d'exposition, parcours du patient dans le système de soin.

Nombre de sujets :
Suite à l'analyse des dossiers patients réalisée sur la période du 23 Juin 2019 au 1er Septembre 2019 par l'équipe du Dispositif Toxicovigilance Océan Indien, il a été estimé qu'au CHU de La Réunion 2 passages par

semaine en moyenne aux urgences pour suspicion d'intoxications incriminants un NPS avait lieu. Soit une estimation de 104 patients sur une année. L'inclusion sur au moins un an pourrait refléter l'éventuelle saisonnalité de la consommation des NPS à La Réunion : en fonction des périodes d'activité (scolaire, vacances, festivités) de la population et également de l'approvisionnement en produit pouvant fluctuer au cours du temps. Le nombre de sujets nécessaires est donc arbitraire, il est surtout dépendant du nombre de bilans toxicologiques pouvant être financés dans le cadre de cet appel d'offre.

Nombre de centres concernés :
- CHU de La Réunion Site Félix Guyon et Site Sud Réunion

Durée du projet (les crédits sont attribués pour un maximum de 3 ans) :
2 ans :
- 6 mois préparation de l'étude (création e-CRF, formation ARC investigateur, communication auprès des urgentistes)
- 1 an d'inclusion
- 6 mois (data management, analyse des données, publication)

V. Annexe 5 – Fiche de renseignements pour la recherche de nouveaux produits de synthèse, mise en place dans le cadre de la thèse

CENTRE HOSPITALIER UNIVERSITAIRE DE BORDEAUX
LABORATOIRE DE PHARMACOLOGIE CLINIQUE ET TOXICOLOGIE
Pr M. MOLIMARD
Centre Hospitalier Pellegrin – Tripode
Plateau Technique 2e étage – 33076 BORDEAUX CEDEX
Tel : +33 (0)5 56 79 59 91 – Fax : + 33 (0)5 56 79 47 95

V1 29/05/2019

Fiche de renseignements
RECHERCHE DE NOUVEAUX PRODUITS DE SYNTHESE
Joindre obligatoirement le bon de demande de dosage de Pharmacologie

Identité du patient	**Coordonnées du service clinique**
Etiquette avec numéro identifiant **ou** Nom : …… Nom de jeune fille : …… Prénom : …… Sexe : …… Date de naissance : ……../……../……..	**Médecin prescripteur : ……** Service : …… Téléphone :……..……Fax :……

Code analyse : ☐ PPNPS (Plasma) – ☐ PUNPS (Urine) – ☐ PANPS (Autre)

Prélèvement : **Date** : ……../……../…….. **et** **Heure** : ….h….

Prélever sur **héparinate de lithium Vert foncé 7 mL** ou **tube non bornté** pour les urines (sauf mention spéciale). Acheminer à température ambiante jusqu'au laboratoire.

Pour les envois **hors CHU de Bordeaux**, veuillez congeler les prélèvements à **-20°C**.

Données sur la consommation : **Date** : ……../……../…….. **et** **Heure** : ….h….

Nom présumé de la (ou des) substance(s) : ……

Quantité consommée : ……

Produit(s) disponible(s) : ☐ Oui (joindre un échantillon) – ☐ Non

Présentation du produit	Comprimé	Poudre	Liquide	Autre (préciser) : ……..
Mode de conso.	Orale	Fumé	Injecté	Autre (préciser) : ……..
Fréquence de conso.	1ère conso.	Occasionnelle (<10x/an)	Répétée (au moins 10x/an)	Régulière (au moins 10x/mois)
Co-administration(s)	Alcool	Cannabis	Tabac	Autre(s) (préciser) : ……..
Contexte d'usage	Seul	Avec des amis	A son insu	Autre (préciser) : ……..
Provenance	Revendeur habituel	Revendeur occasionnel	Darknet	Autre (préciser) : ……..

Renseignements cliniques :

Evaluation neuropsychiatrique

Etat de conscience	Coma	Confus/réveil difficile	Conscient	Remarque : ……
Agitation psychomotrice	Minime	Modérée	Majeure	Remarque : ……
Autres	Anxiété	Hallucinations	Epilepsie	Autres (préciser) : ……
Autres complications				
Insuffisance rénale aigue	Modérée	Sévère	Non connue	Remarque : ……
Complications cardiaques	Préciser : ……			
Autres	Vomissements	Trismus	Autres (préciser) : ……..	

Titre: Apport analytique de la spectrométrie de masse haute résolution en QTOF pour l'identification et la prise en charge de l'exposition aux substances psychoactives

Résumé : Dans le monde, environ 271 millions d'adultes, soit 5,5 % des personnes âgées de 14 à 64 ans, auraient déjà consommé des drogues illicites au cours de leur vie (UNODC, 2019). Le nombre de consommateurs augmente avec des usages souvent problématiques. En parallèle, le développement permanent de nouvelles molécules rend le diagnostic d'exposition difficile. Le manque de connaissances concrètes sur la composition et les dosages en principes actifs de ces produits pose un risque sanitaire important, voire mortel. Au cours de la dernière décennie, la spectrométrie de masse à haute résolution a suscité un intérêt croissant dans le domaine de la toxicologie analytique. L'objectif principal de la présente étude a été d'évaluer l'apport analytique de la spectrométrie de masse haute résolution en QTOF pour l'identification et la gestion de l'exposition aux substances psychoactives. Pour cela, nous avons comparé une méthode de criblage toxicologique par QTOF avec une méthode par spectrométrie infrarouge en analysant des produits provenant directement de consommateurs de drogues. En combinant des approches *in vivo*, *in silico* et *in vitro*, ainsi que d'autres méthodes comme la RMN et l'IR, nous avons évalué l'apport du QTOF pour l'identification des nouveaux produits de synthèse et la mise en évidence de nouveaux biomarqueurs d'exposition. Enfin, nous avons évalué son intérêt en pratique clinique pour l'identification de cannabinoïdes de synthèse dans les départements d'Outre-mer et en France métropolitaine. Ces différents apports sont particulièrement intéressants dans le domaine de la toxicologie analytique et clinique, notamment pour l'identification de nouvelles substances psychoactives et de nouveaux métabolites.

Mots clés : Spectrométrie de masse haute résolution ; LC-QTOF ; Substances psychoactives ; Nouveaux produits de synthèse ; Étude de métabolisme

---

Title: Analytical contribution of high-resolution mass spectrometry by QTOF for the identification and management of psychoactive substances exposure

Abstract : Globally, an estimated 271 million adults, or 5.5% of people aged 14-64, have used illicit drugs in their lifetime (UNODC, 2019). The number of consumers is increasing and their use is often problematic. At the same time, the constant development of new molecules makes the diagnosis of exposure harder. The lack of concrete knowledge about the composition and dosage of active compounds in these drugs poses a significant, even lethal, health risk. Over the last decade, high-resolution mass spectrometry has attracted increasing interest in the field of analytical toxicology. The main objective of the present study was to evaluate the analytical contribution of high-resolution mass spectrometry by QTOF for the identification and management of psychoactive substances exposure. For this purpose, we compared a QTOF screening method with an infrared spectrometric screening method by analysing products directly from drug users. By combining *in vivo, in silico* and *in vitro* approaches, as well as other methods such as NMR and IR, we evaluated the contribution of QTOF to the identification of new psychoactive substances and the discovery of new exposure biomarkers. Finally, we evaluated its interest in clinical practice for the identification of synthetic cannabinoids in the French overseas departments and in mainland France. These various contributions are particularly interesting in the field of analytical and clinical toxicology, especially for the identification of new psychoactive substances and new metabolites.

Keywords: High resolution mass spectrometry; LC-QTOF; Drugs of abuse; New psychoactive substances ; metabolism study

---

Unité de recherche

Bordeaux Population Health Research Center INSERM U1219, Equipe « Pharmacoépidémiologie et impact des médicaments sur les populations ». Université de Bordeaux, Site de Carreire, Bâtiment Pharmacie, quatrième tranche, troisième étage. Case 121 146 rue Léo Saignat 33076 Bordeaux cedex, France