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Mónica Gisel Arellano Sánchez

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Normandie Université

THESIS

Submitted in partial fulfillment of the requirements for the degree of
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Specialty: Chemistry

For the work performed at the: National Institute of Applied Sciences (INSA) of Rouen,
Normandy, France.

**Chromium speciation in leather by ion chromatography coupled
with molecular and elemental mass spectrometry**

Presented and defended by
Mónica Gisel Arellano Sánchez

Thesis publicly defended on October 17, 2022
in front of the examining board composed of

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Thesis supervised by Carlos AFONSO and Mélanie MIGNOT, COBRA Laboratory, UMR 6014



Normande de Chimie



GOBIERNO DE
MÉXICO





Normandie Université

THESE

Pour obtenir le diplôme de doctorat

Spécialité Chimie

Préparée au sein de l'Institut National des Sciences Appliquées Rouen Normandie

Spéciation du chrome dans le cuir par chromatographie ionique couplée à la spectrométrie de masse moléculaire et élémentaire

Présentée et soutenue par
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Thèse soutenue publiquement le 17 octobre 2022
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Publications and presentations at congresses and meetings

Throughout this PhD, several articles were published, both as first author, as well as in collaboration with other colleagues. Likewise, my thesis work was presented as a poster and/or oral communication in national congresses and in an international congress that took place in Belgium. As an additional work, I did an internship in the Forensic Services in Mexico and I participated as a reviewer for an international peer-reviewed journal.

Peer-reviewed articles:

- 1) **Arellano-Sánchez MG**, Devouge-Boyer C, Hubert-Roux M, Afonso C, Mignot M. Quantitative extraction of chromium VI and III from tanned leather: a comparative study of pretreatment methods. *J Leather Sci Eng.* 2021;3:30. **Impact Factor: under calculation.**
- 2) **Arellano-Sánchez MG**, Devouge-Boyer C, Hubert-Roux M, Afonso C, Mignot M. Chromium determination in leather and other matrices: a review. *Crit Rev Anal Chem.* 2021;1-20. **Impact Factor: 5.686.**
- 3) Chapter 4 will be submitted as an article.

Collaboration articles:

- 1) Vievard J, Amoikon TLS, Coulibaly NA, Devouge-Boyer C, **Arellano-Sánchez MG**, Aké MFD, Djeni NT, Mignot M. Extraction and quantification of pesticides and metals in palm wines by HS-SPME/GC-MS and ICP-AES/MS. Food Chem. 2022;393:133352. **Impact Factor: 9.231.**

Congresses and meetings:

- 1) "Chromium Determination in Leather: Challenges of Extraction and Speciation." 17th International Symposium on Hyphenated Techniques in Chromatography and Separation Technology. Poster. Ghent, Belgium. May 18-20, 2022.
- 2) "Chromium Determination in Leather: Challenges of Extraction and Speciation." Journée thématique AFSEP : Prélèvement et traitement de l'échantillon : quelles conséquences sur l'analyse ? Oral presentation. Online. December 9, 2021.
- 3) « Détermination du chrome dans le cuir: défis de l'extraction et de la spéciation. » 14ème Congrès Francophone sur les Sciences Séparatives et les Couplages de l'AFSEP. Poster. Paris, France. October 5-7, 2021.
- 4) « Détermination du chrome dans le cuir: défis de l'extraction et de la spéciation. » Journées Françaises de Spectrométrie de Masse. Oral presentation. Online. June 14-24, 2021.

Additional participations:

- 1) Reviewer for "Journal of Environmental Science and Pollution Research". 2022.

- 2) Three weeks stay at the Forensic Services Department of the Nuevo Leon State Attorney General's Office. "Development of an analytical method for the detection of carbon monoxide in blood through Gas Chromatography - Thermal Conductivity Detector". December 2019 - January 2020.

- 3) Supervisor of practical works in analytical chemistry: gas chromatography, Fourier transform infrared spectroscopy, flame atomic absorption.

Résumé

L'industrie du cuir joue un rôle essentiel dans l'économie mondiale. C'est l'une des plus anciennes industries traditionnelles qui permet la création d'une large gamme de produits tels que des chaussures, des vêtements, des meubles, des équipements sportifs, etc. Cependant, elle a également un impact négatif sur l'environnement en raison de la production de quantités importantes de déchets, dont certains sont classés comme produits chimiques dangereux tels que le chrome, les tanins synthétiques, les huiles, les résines, les biocides et les détergents^[1].

En moyenne, 3,5 millions de tonnes de produits chimiques différents sont utilisés chaque année pour le traitement du cuir. Parmi ces derniers figurent la chaux, le sulfure de sodium, les sels de chrome, les colorants et les acides (Figure 1). Une grande partie de ces produits est rejetée dans les effluents. Environ 50 m³ de déchets liquides sont déversés par tonne de cuir brut traité^[2].

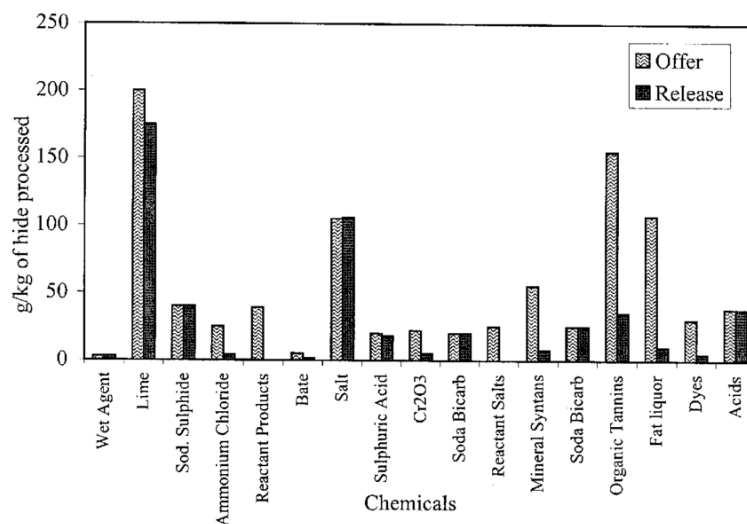


Figure 1. Principaux produits chimiques polluants utilisés dans le traitement du cuir^[2].

Il existe plusieurs méthodes de tannage pour transformer les peaux brutes en cuir (tannage végétal, tannage aux aldéhydes, tannage synthétique, etc.). Cependant, environ 90% des peaux sont traitées avec des sels de chrome trivalent, ce que l'on appelle le tannage au chrome^[3].

La structure électronique du chrome ($[\text{Ar}]3d^54s^1$) en fait un agent tannant parfait en raison de ses orbitales 3d incomplètes qui peuvent accueillir des électrons supplémentaires pour former des complexes de coordination, ce qui est l'une des raisons pour lesquelles le tannage au chrome est le système de tannage le plus courant^[4, 5]. Le tannage au chrome assure une excellente qualité du produit final en termes de résistance mécanique et hydrothermique, attribuable au taux élevé d'infiltration des sels de Cr(III) dans les espaces interfibrillaires de la peau^[3]. La réticulation avec le collagène des peaux permet d'obtenir un cuir avec des propriétés très intéressantes^[6]. Au cours de ce processus, les peaux décapées sont traitées avec une solution aqueuse de sulfate de chrome basique. Le pH du bain est ajusté de 2,8-3,0 à 3,8-4,0 avec une solution alcaline (bicarbonate de sodium). La durée du tannage au chrome est d'environ 6 heures. Après ce processus, le cuir est appelé « cuir bleu »^[7]. Il existe également le tannage végétal qui est une technologie plus écologique. Cependant, la réaction de tannage au chrome est plus rapide, confère une grande stabilité hydrothermique, ne modifie que faiblement la structure du collagène, rend ce dernier plus hydrophobe (donc résistant à l'eau) et permet la rétention des colorants puisque le Cr(III) peut agir comme un agent fixateur de ceux-ci^[8].

Le chrome trivalent est donc introduit dans le cuir sous forme d'agents tannants, mais aussi sous forme de colorants (colorants pré-métallisés au chrome). Néanmoins, différentes conditions (par exemple l'exposition aux rayons UV, une température élevée, ou une atmosphère sèche^[9]) peuvent provoquer la conversion du chrome trivalent (Cr(III)) en chrome hexavalent (Cr(VI)). Par conséquent, le Cr(VI) peut se retrouver dans les produits en cuir et provoquer des dermatites allergiques^[10, 11] ou déclencher des maladies plus graves. Il est donc

aujourd'hui important de limiter autant que possible l'utilisation des sels de chrome comme agents tannants. En effet, bien que le chrome trivalent soit essentiel pour la régulation du glucose, le chrome hexavalent est toxique. Il semble important de pouvoir quantifier les deux espèces séparément, et il n'existe actuellement aucune méthode standard pour l'extraction et la quantification des deux espèces dans les produits en cuir. Dans les systèmes aqueux, l'état d'oxydation du chrome est déterminé par le potentiel redox et le pH, ce qui pose une difficulté dans la spéciation du chrome, car les protocoles de préparation des échantillons et/ou les conditions analytiques peuvent conduire à des interconversions entre le Cr(III) et le Cr(VI), biaisant ainsi les résultats.

Le premier objectif de cette thèse était de développer une méthodologie pour extraire quantitativement les espèces de chrome du cuir tanné. Le second et ultime objectif de ce travail de thèse était de séparer les chromes trivalent et hexavalent et de les quantifier séparément, tout en conservant leur spéciation. En d'autres termes, l'objectif était d'extraire et d'analyser entièrement les espèces de chrome, avec la même technique (chromatographie ionique couplée à la spectrométrie de masse à plasma à couplage inductif (IC-ICP-MS)), tout en évitant la réduction du Cr(VI) en Cr(III), ainsi que l'oxydation du Cr(III) en Cr(VI).

Cette thèse est divisée en quatre chapitres :

Le chapitre 1 est basé sur un article de synthèse^[12] qui a été écrit pendant la première année du programme de doctorat et publié dans *Critical Reviews of Analytical Chemistry* en 2021 (Facteur d'impact : 5.686). Cet article présente l'état de l'art concernant la détermination du chrome dans le cuir et d'autres matrices. Il aborde l'importance du cuir dans l'économie mondiale et l'impact environnemental de ses processus de production. Après avoir décrit le tannage au chrome et le risque d'interconversions entre le chrome trivalent et le chrome hexavalent au cours du cycle de vie d'un produit en cuir, une recherche bibliographique exhaustive a été menée et, en conséquence, un tableau compile et résume les différentes méthodes de prétraitement et de détermination des

espèces de chrome dans le cuir et d'autres matrices (aliments, produits cosmétiques, échantillons environnementaux et pharmaceutiques). Enfin, les perspectives d'avenir concernant la production de cuir et la spéciation du chrome y ont été présentées. Cet article a permis de tirer les conclusions suivantes :

- En ce qui concerne la détermination du chrome, il n'existe pas de norme actuelle décrivant une technique permettant d'extraire et de quantifier simultanément le Cr(III) et le Cr(VI) dans les produits en cuir issus du tannage au chrome. Il est donc particulièrement important de développer un protocole qui prenne en compte les processus d'interconversion possibles, éventuellement par l'utilisation de la dilution isotopique. La production de matériaux de référence certifiés (MRC) pour la spéciation du chrome dans le cuir serait également intéressant pour le développement méthodologique.
- L'examen des méthodes analytiques existantes d'extraction et de quantification du chrome a montré que la plupart des études a été réalisée sur des aliments et des échantillons environnementaux. Toutefois, il convient d'accorder plus d'attention aux articles en cuir afin de mettre au point des protocoles analytiques plus fiables qui permettent de contrôler les interconversions éventuelles et de révéler le risque réel pour la santé auquel les consommateurs sont exposés.
- Les réglementations futures doivent également tenir compte de l'évolution du chrome hexavalent dans le temps. Compte tenu du fait que tout au long du cycle de vie du cuir la spéciation du chrome a un comportement dynamique influencé par un grand nombre de paramètres, l'analyse du Cr(VI) réalisée indépendamment de celle du Cr(III) peut être considérée comme peu pertinente. De plus, aucune relation n'a été trouvée entre le Cr(III) libéré et le Cr(VI)^[13].

Le chapitre 2 est consacré à la méthodologie suivie tout au long de la partie expérimentale du projet. Il décrit l'instrumentation, les matériaux, les procédures

générales, ainsi que les bases théoriques et les concepts des différentes techniques qui ont été utilisées au cours de la thèse tels que les différentes extractions, la minéralisation, la chromatographie ionique (IC), la spectrométrie de masse à plasma à couplage inductif (ICP-MS), l'IC-ICP-MS et la spectrométrie de masse à résonance cyclonique ionique et à transformée de Fourier (FTICR-MS).

Le chapitre 3 présente un article^[14] qui a été rédigé au cours de la deuxième année du programme de doctorat et publié dans le Journal of Leather Science and Engineering en 2021 (Facteur d'impact : en cours de calcul). L'objectif de ce travail était d'évaluer la capacité d'extraction du chrome d'échantillons de cuir par sept méthodes différentes de prétraitement.

Concernant le chrome hexavalent, depuis 2015, l'Union européenne limite sa teneur à 3 mg/kg (du poids sec total) dans les articles en cuir entrant en contact direct avec la peau^[15]. Cependant, l'Agence européenne des produits chimiques (ECHA) a récemment adopté un avis visant à diminuer la limite de restriction du chrome hexavalent de 3 mg/kg à 1 mg/kg dans le cuir, les fourrures et les peaux. Cette mesure prendra effet 5 ans après l'entrée en vigueur de la restriction^[16]. Actuellement, la norme 17075 de l'Organisation internationale de normalisation (ISO) spécifie une procédure d'extraction sélective du chrome hexavalent dans le cuir en utilisant du $K_2HPO_4 \cdot 3H_2O$ à pH 8,0. L'analyse ultérieure de l'extrait est effectuée par analyse colorimétrique^[17] ou chromatographique^[18]. L'analyse colorimétrique consiste en une réaction entre le Cr(VI) et la 1,5-diphénylcarbazine (DPC), le complexe résultant est détecté par spectroscopie UV Visible (UV-Vis) à 540 nm. L'analyse chromatographique peut être effectuée par détection directe du pic de chromate à 372 nm, ou par réaction post-colonne avec la DPC en mesurant le pic d'absorption à 540 nm. La limite de quantification (LOQ) de cette procédure standard est de 3 mg/kg pour les deux techniques. Néanmoins, lorsqu'on travaille avec des matrices de cuir, la sélectivité de la détection UV peut être compromise car d'autres composés peuvent absorber à la même longueur d'onde que le complexe cible ; il est important de noter que le cuir est

fréquemment traité avec une variété de produits chimiques (colorants azoïques, pigments, fongicides, bactéricides, liants, tensioactifs cationiques/anioniques...).

D'après la littérature, il a été possible de remarquer que les méthodes de prétraitement et d'analyse qui ont été utilisées pour la détermination du chrome hexavalent dans les échantillons de cuir reposent sur une extraction du Cr(VI) avec un tampon phosphate^[17-20] ou avec du NaOH^[21], puis sur la complexation du chrome hexavalent avec de la DPC pour une détection UV-Vis. Peu d'études ont été menés concernant l'extraction et la quantification du chrome dans le cuir. Cependant, la spéciation du chrome dans d'autres matrices est plus documentée : aliments, produits cosmétiques, échantillons environnementaux et pharmaceutiques.

Fabregat-Cabello *et al.*^[22] ont rapporté une extraction assistée par micro-ondes du Cr(VI) dans plusieurs MRC (sol contenant des résidus de traitement de minerai de chromite, poussière de soudure chargée sur un filtre, et sol argileux sableux) en utilisant de l'acide éthylènediaminetétraacétique (EDTA) 50 mmol/L à pH 10. D'après leurs résultats, ils ont pu extraire et mesurer le Cr(VI) et le complexe Cr(III)-EDTA⁻, simultanément. La séparation et l'analyse des espèces ont été effectuées par IC-ICP-MS. L'acide diéthylènetriaminepentaacétique (DTPA) a également été testé comme agent chélateur du chrome trivalent mais il a montré une stabilité cinétique inférieure à celle de l'EDTA^[23]. Pour extraire le Cr(VI) des matrices solides, l'extraction alcaline avec l'hydroxyde d'ammonium a été employée pour les produits laitiers, la farine, le chocolat, les légumes, les fruits, la viande, le poisson, les œufs et les boissons. La chromatographie en phase liquide à haute performance couplée à la spectrométrie de masse à plasma à couplage inductif (HPLC-ICP-MS) a été utilisée pour quantifier le Cr(VI) après ultrafiltration de l'extrait^[24]. Gürleyük et Wallschläger^[25] ont étudié la détermination du Cr(III) et du Cr(VI) dans l'eau par IC-ICP-MS en utilisant l'EDTA pour dériver le Cr(III). Cependant, le potentiel de l'EDTA pour extraire les espèces de chrome dans les échantillons de cuir n'a pas encore été testé. Pour les eaux^[25, 26], les sédiments de

fond^[26] et les médicaments traditionnels chinois^[27], la séparation des espèces de chrome a été effectuée par des méthodes chromatographiques utilisant le nitrate d'ammonium comme phase mobile après dérivatisation à l'EDTA ou extraction au tampon phosphate.

Sur la base de ces études, l'objectif de ce travail était d'évaluer sept méthodes de prétraitement du cuir tanné quant à leur capacité à extraire le chrome : minéralisation acide (qui permet la décomposition de la matière organique, permettant la quantification du chrome total par spectrométrie d'émission atomique à plasma à couplage inductif (ICP-AES)), extraction à l'EDTA, extraction au DTPA, extraction à l'hydroxyde d'ammonium (NH₄OH), extraction au nitrate d'ammonium (NH₄NO₃), extraction à l'eau (pour les composés de chrome hydrosolubles) et extraction au tampon phosphate (ISO 17075^[17, 18]). Les efforts de recherche sur le cuir sont limités et aucun de ces systèmes d'extraction n'avait été essayé sur des échantillons de cuir.

Pour tester les différentes méthodes d'extraction, la première étape a consisté à préparer les échantillons et à les découper en petits morceaux (3 à 5 mm par côté) conformément à la norme ISO 4044:2017, 6.3^[28]. Ils ont été coupés au laboratoire et immédiatement après, ils ont été stockés dans des sacs en plastique refermables. Pour chaque échantillon, une minéralisation directe a été effectuée, et l'échantillon digéré a été analysé par ICP-AES pour connaître la teneur en chrome total et l'avoir comme référence. Ensuite, les différentes extractions ont été testées et, après chaque extraction, l'extrait a également été minéralisé et le chrome a été déterminé par ICP-AES. Un schéma de la méthodologie peut être observé sur la Figure 2. Le taux d'extraction consistait en un rapport entre les milligrammes de chrome extrait et les milligrammes de chrome total obtenus par la minéralisation directe de l'échantillon. Enfin, le taux d'extraction le plus élevé a été obtenu avec l'extraction par EDTA, comme on peut l'observer sur la Figure 3.

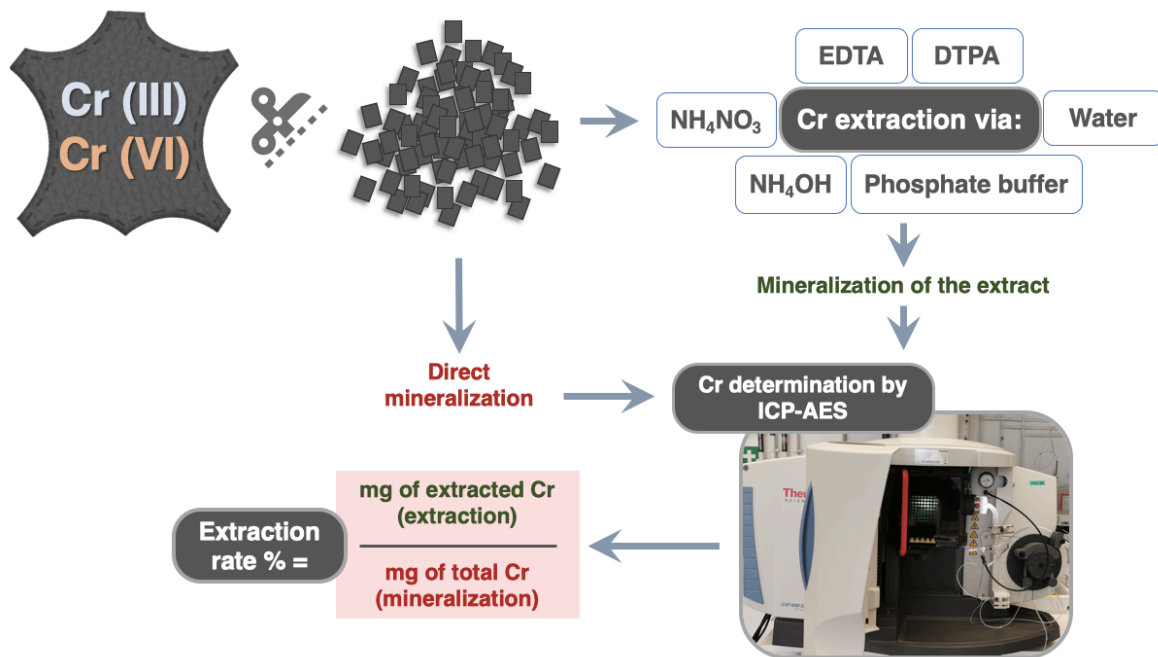


Figure 2. Schéma de la méthodologie utilisée pour tester les différentes conditions d'extraction^[14].

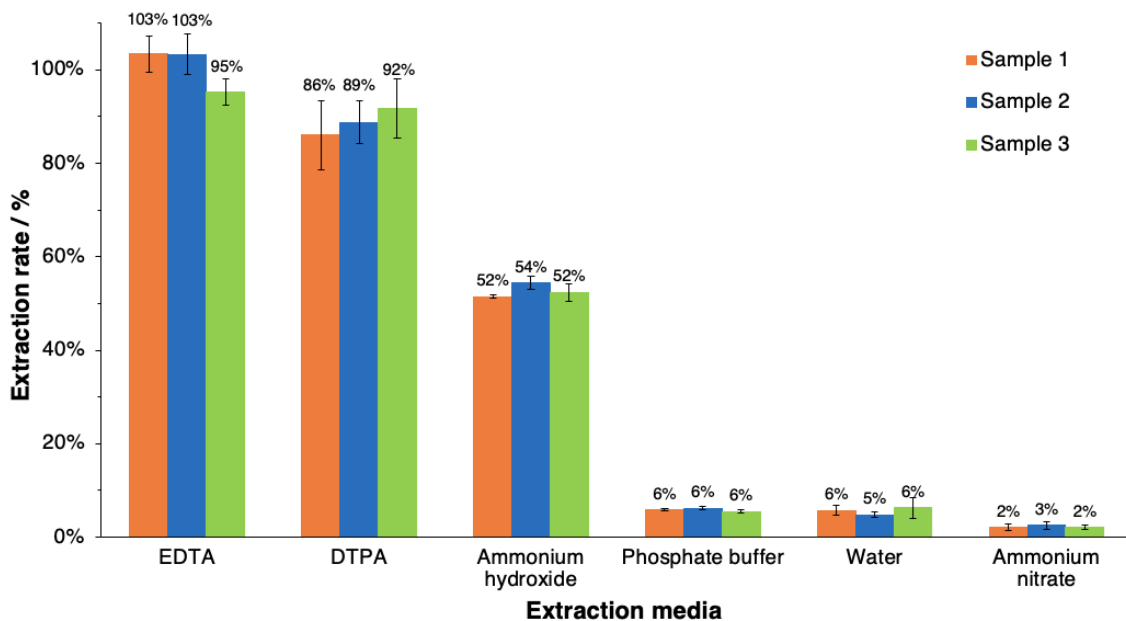


Figure 3. Résumé des taux d'extraction de six méthodes de prétraitement différentes (3 échantillons par méthode)^[14].

Par la suite, cette méthode d'extraction à l'EDTA a été validée en termes de linéarité, de limite de détection (LOD), LOQ, fidélité intermédiaire et taux de récupération. Le coefficient de détermination (R^2) était supérieur à 0,9998 pour

toutes les analyses ICP-AES, la limite de détection et de quantification était de 3,4 et 11,2 ppb, respectivement, la fidélité intermédiaire a été évaluée sur trois jours et pour trois échantillons différents et le coefficient de variation était inférieur à 4,2 %. Concernant le pourcentage de récupération, il a été évalué à trois niveaux de concentration différents pour trois échantillons différents et il variait entre 91 et 108,6 %. En ce qui concerne la séparation et l'interconversion des espèces de chrome, dans une première approche, des standards isotopique de chrome ($^{50}\text{Cr(VI)}$ et $^{53}\text{Cr(III)}$) ont suivi les conditions d'extraction de la méthode sélectionnée (l'extraction EDTA), et la séparation des espèces de chrome a été suivie par IC-ICP-MS en utilisant une colonne de garde NG1, une colonne de garde AG7, une colonne échangeuse d'ions AS7, et 75 mmol/L de NH_4NO_3 à pH 3 comme éluant chromatographique.

Lorsque le $^{50}\text{Cr(VI)}$ a été injecté, un pic correspondant au $^{50}\text{Cr(VI)}$ a été observé (temps de rétention : 920 secondes) (Figure 4 a)). Lorsque le $^{53}\text{Cr(III)}$ a été injecté en présence d'EDTA, un pic correspondant au $^{53}\text{Cr(III)-EDTA}^-$ a été identifié (temps de rétention : 230 secondes), aucune oxydation en $^{53}\text{Cr(VI)}$ n'a été observée (Figure 4 b)). Par contre, lorsque le $^{50}\text{Cr(VI)}$ a été injecté en présence d'EDTA, il ne s'est pas complexé avec l'EDTA, il est resté sous forme de $^{50}\text{Cr(VI)}$ (temps de rétention : 920 secondes) mais une petite partie a été réduite en $^{50}\text{Cr(III)}$ qui s'est complexée avec l'EDTA (temps de rétention : 230 secondes) (Figure 4 c)). Néanmoins, cela ne représentait que 1,5% du pic principal. Par conséquent, la méthode utilisant de l'EDTA s'est avérée être la plus efficace pour l'extraction quantitative du Cr(III) sous forme d'un complexe Cr(III)-EDTA^- du Cr(VI). Seulement une quantité limitée de Cr(VI) a été convertie en Cr(III) lors de l'analyse des standards isotopiques de chrome qui avaient suivi les conditions d'extraction à l'EDTA.

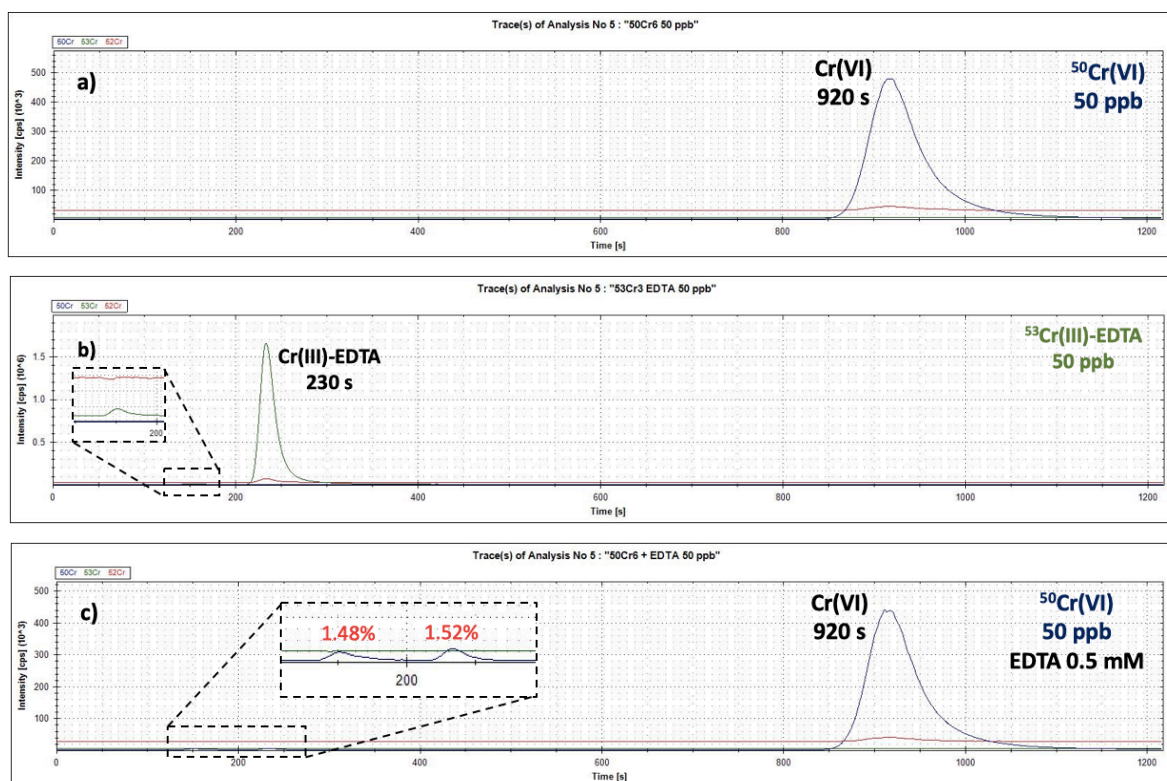


Figure 4. Chromatogrammes IC-ICP-MS de : a) une solution contenant 50 ppb de $^{50}\text{Cr(VI)}$, b) une solution contenant 50 ppb de $^{53}\text{Cr(III)}$ complexé avec EDTA 0,5 mmol/L, et c) une solution contenant 50 ppb de $^{50}\text{Cr(VI)}$ et EDTA 0,5 mmol/L^[14].

Le chapitre 4 est rédigé dans un format de publication car il est destiné à être publié dans une revue à comité de lecture telle que Journal of Chromatography, A ou Journal of Leather Science and Engineering. Ce chapitre traite du défi que représente la préservation de la spéciation du chrome tout au long des étapes de prétraitement et d'analyse des échantillons de cuir. Une étude comparative des méthodes d'analyse est présentée dans ce chapitre. Plusieurs conditions ont été testées telles que : deux colonnes différentes (AS7 et CS5A), différentes phases mobiles (NH_4NO_3 et HNO_3), différents pH des échantillons et différents agents de complexation (DPC, DTPA et EDTA). Des données supplémentaires concernant les tests préliminaires sont intégrées dans ce chapitre (qui n'apparaîtra pas dans la publication finale) pour refléter l'effort que cette étude a nécessité.

Après avoir testé plusieurs conditions lors des essais préliminaires et considérant que le complexe Cr(III)-EDTA^- élué très près du temps mort, les conditions retenues ont été les suivantes :

- HNO_3 1,9% au lieu de 1,7% (v/v) comme phase mobile car la diminution de sa concentration n'a aucun effet sur la rétention du Cr(VI) et augmente significativement la rétention du Cr(III) .

- Un débit de 0,4 mL/min au lieu de 0,6 mL/min car il est plus proche du débit optimal de la colonne.

- Compartiment de la colonne à température ambiante puisque la chaleur diminue légèrement le temps de rétention du Cr(VI) et augmente le temps de rétention du Cr(III) .

- Pas d'ajout d'acétonitrile car il diminue les temps de rétention des deux espèces de chrome.

- Une colonne analytique AS7 au lieu d'une colonne de garde AG7.

- Les échantillons ont été injectés avec une concentration de HNO_3 de 0,1% (v/v) afin de préserver la stabilité du complexe.

Ceci a permis de séparer le Cr(III) et le Cr(VI) . Le Cr(VI) a été élué à 190 secondes, tandis que le Cr(III) a été élué à 1300 secondes. Néanmoins, le Cr(III)-EDTA^- a été élué à 75 secondes, toujours très proche du temps mort qui était de 70 secondes. Ceci peut être observé dans les chromatogrammes présentés en Figure 5.

En effet, la colonne échangeuse d'ions AS7 est une colonne pelliculaire à base de latex. La bille centrale polymérique est sulfonatée (échangeur de cations) tandis que le latex porte des groupes d'ammonium quaternaire (échangeur d'anions). Par conséquent, cette colonne contient une capacité d'échange d'ions à la fois anionique et cationique^[29]. Le latex est aggloméré de façon électrostatique à la bille centrale. La particularité de cette structure offre une possible pénétration des cations métalliques (dont la sphère d'hydratation est réduite) vers les sulfonates de la bille centrale, et donc, leur rétention. En analyse

classique (éluant de force ionique plus faible et pH basique) il y aurait une rétention plus importante des espèces anioniques (espèces Cr(VI)) et le cation métallique (Cr(III)) resterait coincé au cœur de la phase stationnaire. L'utilisation d'un acide permet l'élution du Cr(III) puisque la force ionique du nitrate à pH acide a pour but de minimiser la rétention des anions.

Le Cr(III)-EDTA⁻ étant une grosse structure moléculaire par rapport à la taille des pores de la phase stationnaire, il entre peu dans les pores et s'écoule librement. Ceci explique pourquoi le complexe élué en premier (temps de rétention = 75 s) et très proche du temps mort, suivi du Cr(VI) qui élué 115 secondes plus tard (temps de rétention = 190 s). Le Cr(III) élué enfin, lorsque l'acide nitrique réussit à éluer le cation qui interagit avec le cœur de la bille centrale (temps de rétention = 1300 s).

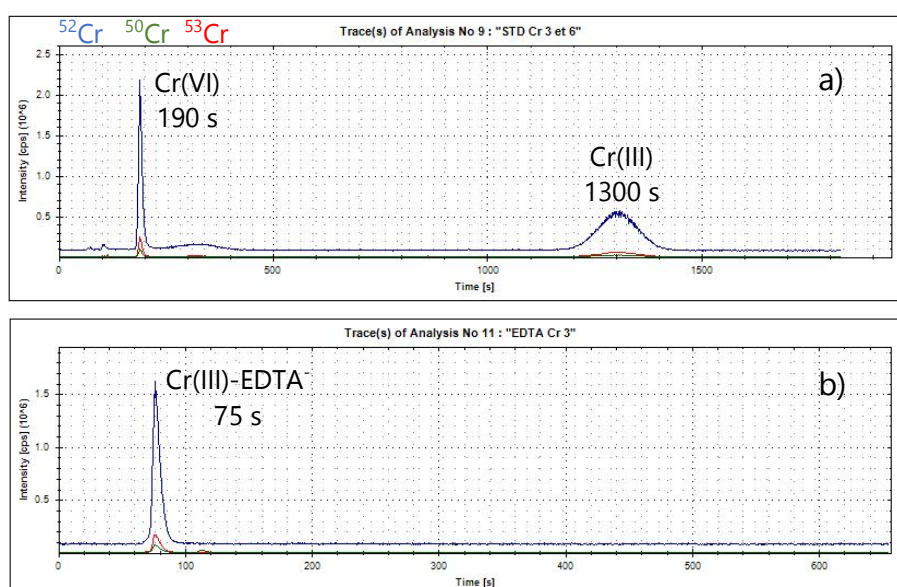


Figure 5. Chromatogrammes IC-ICP-MS de : a) Cr(VI) et Cr(III), 50 ppb, HNO₃ 0,1% (v/v). b) 50 ppb de Cr(III) en présence d'EDTA (après avoir suivi les conditions d'extraction/complexation de l'EDTA), HNO₃ 0,1% (v/v). Colonne de garde NG1 + colonne AS7, phase mobile : HNO₃ 1,9% (v/v), débit : 0,4 mL/min, mode STD, temps mort : 70 s.

Par la suite, une colonne de garde NG1, une colonne de garde CG5A, une colonne échangeuse d'ions CS5A et acide nitrique comme phase mobile (A :

HNO₃ 1,1 M, B : H₂O) en mode gradient ont été testés. Des solutions standards (⁵³Cr(III) et ⁵⁰Cr(VI)) ainsi que le complexe Cr(III)-EDTA⁻ ont été dilués et injectés et, comme on peut le voir sur la Figure 6 a), la séparation des trois espèces a été réussie (temps mort = 120 s). Le Cr(III)-EDTA⁻ a été élué en premier (temps de rétention = 178 s), il a été suivi par le Cr(VI) (temps de rétention = 243 s), et le Cr(III) a été élué en dernier (temps de rétention = 542 s).

En fait, la colonne CS5A possède deux groupes échangeurs d'ions : l'acide sulfonique (groupe échangeur de cations) et l'ammonium quaternaire (groupe échangeur d'anions)^[30]. Cela peut expliquer l'ordre des temps de rétention. En raison de la taille du complexe Cr(III)-EDTA⁻, il n'est pas fortement retenu dans la colonne et il élue dans des conditions douces (32% de phase mobile A). Les espèces anioniques de Cr(VI) éluent lorsque les conditions de la phase mobile sont plus acides (100 % de la phase mobile A) et sont donc capables de rompre les interactions entre les anions de l'analyte et les groupes d'ammonium quaternaire de la phase stationnaire. Le cation Cr(III) élue à la fin, en raison des fortes interactions avec les groupes d'acide sulfonique.

Par la suite, une extraction EDTA d'un échantillon de cuir a été réalisée. L'extrait a été dilué dans l'eau (dilution 5000X) et injecté. Ensuite, l'échantillon a été dopé avec 50 ppb de ⁵⁰Cr(VI) et injecté à nouveau. La Figure 6 b) correspond à l'échantillon dopé et on peut remarquer qu'il y a un pic à 178 secondes (la ligne rouge correspondant à l'isotope naturel ⁵²Cr) qui correspond au complexe Cr(III)-EDTA⁻, et un pic à 243 secondes (la ligne bleue correspondant à l'isotope ⁵⁰Cr) qui correspond au ⁵⁰Cr(VI) dopé. La partie zoomée de la Figure 6 b) montre l'échantillon avant le dopage, et un petit pic au temps de rétention de 243 secondes peut être observé. On peut conclure de cette partie que le Cr(VI) est effectivement présent dans l'extrait d'EDTA mais que le pic du Cr(III)-EDTA⁻ couvre le pic du Cr(VI), donc la détermination n'est pas possible pour les échantillons où il y a une grande différence de concentration entre le Cr(III) (Cr(III)-EDTA⁻ une fois complexé) et le Cr(VI). Dans le but de vérifier que la complexation du Cr(III) avec

l'EDTA est complète, le complexe $^{53}\text{Cr(III)-EDTA}^-$ a été dilué et injecté seul. On a pu constater que, lorsque le $^{53}\text{Cr(III)}$ est injecté en présence d'EDTA après avoir suivi le protocole d'extraction, il est possible d'observer le pic principal du complexe de chrome trivalent à 178 secondes mais aucun pic supplémentaire n'est observé en dehors du pic correspondant au complexe Cr(III)-EDTA^- (pas de Cr(III) libre). Donc, ces conditions ont permis de confirmer que la complexation du Cr(III) avec l'EDTA est complète.

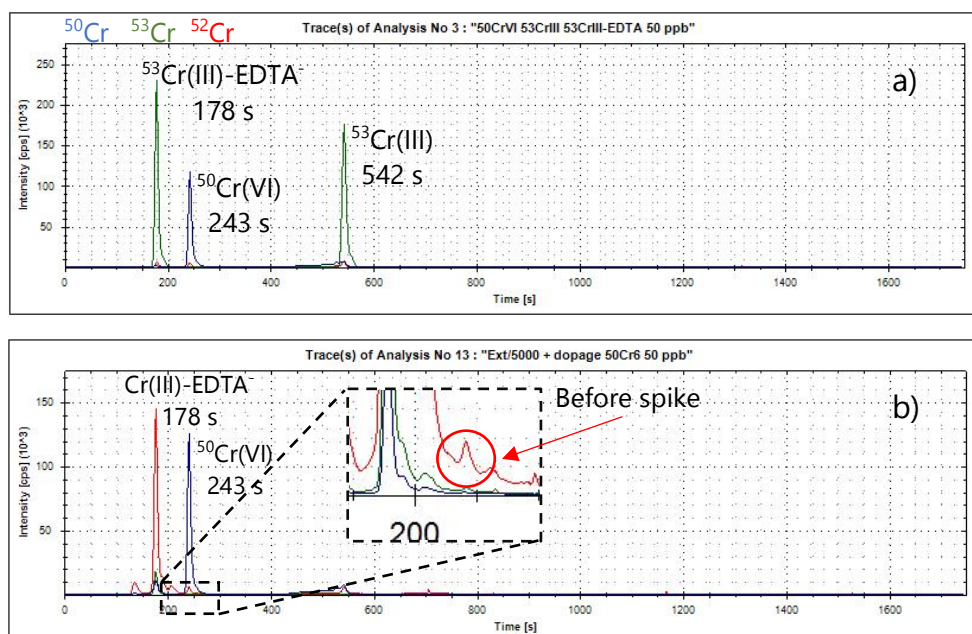


Figure 6. Chromatogrammes IC-ICP-MS de : a) 50 ppb de $^{53}\text{Cr(III)}$, 50 ppb de $^{50}\text{Cr(VI)}$, et 50 ppb de $^{53}\text{Cr(III)}$ en présence d'EDTA (après avoir suivi les conditions d'extraction/complexation à l'EDTA), HNO_3 0,1% (v/v). b) Extraction à l'EDTA d'un échantillon de cuir dilué 5000X dans l'eau, dopé avec 50 ppb de $^{50}\text{Cr(VI)}$. Colonne de garde NG1 + colonne de garde CG5A + colonne CS5A, phase mobile : A : HNO_3 1,1 mol/L, B : eau ultrapure, débit : 0,275 mL/min, mode KED, temps mort : 120 s.

Après cela, les conditions de séparation utilisées lors de la première approche de spéciation au chapitre 3 (une colonne de garde NG1, une colonne de garde AG7, une colonne échangeuse d'ions AS7 et 75 mmol/L de NH_4NO_3 à pH 3 comme éluant chromatographique) ont été utilisées mais, contrairement au chapitre 3, dans le chapitre 4 des échantillons de cuir ont été analysés et

différentes conditions de pH des échantillons ont été testées. Après l'injection des différents échantillons, les conclusions suivantes ont pu être tirées :

- À pH 3,00, la complexation n'a pas eu lieu (Figure 7 a)). Cependant, la complexation des standards a eu lieu à pH 9,70 (Figure 7 b)). Ceci peut être expliqué par le fait qu'à faible pH (conditions acides), la forme protonée de l'EDTA est prédominante et le H^+ entre en compétition avec le Cr^{3+} . Dans des conditions basiques, la forme déprotonée de l'EDTA est prédominante et la complexation entre Cr^{3+} et EDTA se produit rapidement et quantitativement.
- Une fois que le $^{50}Cr(VI)$ et le $^{53}Cr(III)$ ont suivi les conditions d'extraction de l'EDTA à pH 4,14, le $^{50}Cr(VI)$ s'est transformé en $^{50}Cr(III)$ qui s'est complexé avec l'EDTA (Figure 7 c)). Ceci est possible car le chauffage accélère la cinétique de complexation du chrome avec l'EDTA. En effet, une fois complexé, le $Cr(III)-EDTA^-$ est stable pour analyse dans une gamme de pH assez large (inférieure à 10), mais la complexation doit être réalisée à pH 10 afin de préserver l'état d'oxydation du $Cr(VI)$, car le chauffage à pH acide favorise sa réduction en $Cr(III)$.
- Lorsque l'extrait de cuir non dilué a été dopé avec du $^{50}Cr(VI)$, le pic de $^{50}Cr(VI)$ est apparu à 420 secondes (Figure 7 d)). Cependant, lorsque l'extrait de cuir dilué a été dopé avec du $^{50}Cr(VI)$, le pic de $^{50}Cr(VI)$ est apparu à 970 secondes (Figure 7 e)). Ainsi, lorsque le $Cr(VI)$ est présent dans un extrait de cuir EDTA non dilué, il est probablement lié à un constituant présent dans la matrice du cuir. D'autre part, lorsque le $Cr(VI)$ est présent dans un échantillon dilué, les conditions sont plus proches de celles où le $Cr(VI)$ est seul dans une solution aqueuse, donc le temps de rétention reste le même. Une autre hypothèse est que le $Cr(VI)$ est présent sous différentes formes ioniques lorsque l'échantillon est dilué ou non, d'où l'élution du $Cr(VI)$ à deux temps de rétention différents.

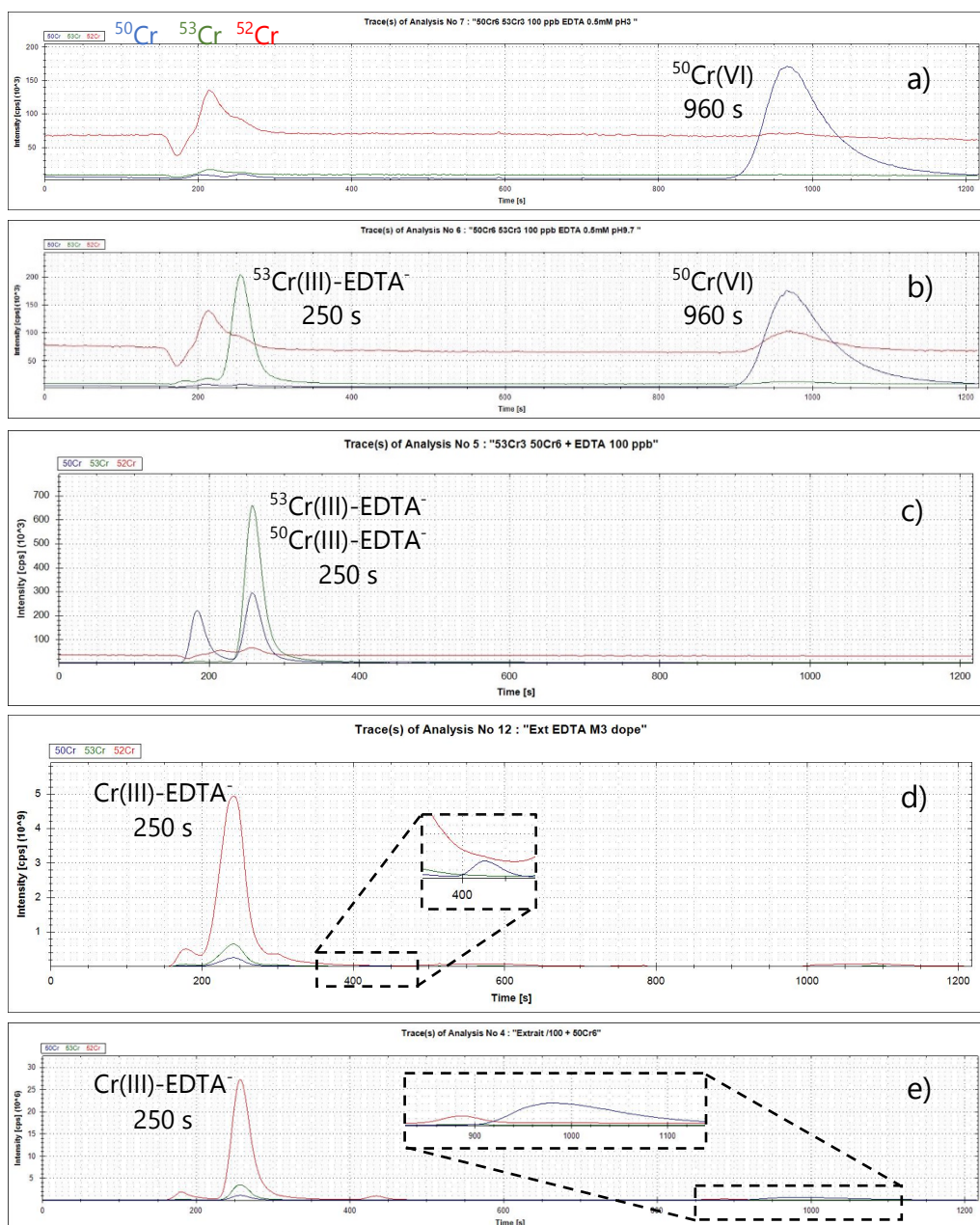


Figure 7. Chromatogrammes IC-ICP-MS de : a) $^{53}\text{Cr(III)}$ et $^{50}\text{Cr(VI)}$, 100 ppb, EDTA 0,5 mM, pH 3,00. b) $^{53}\text{Cr(III)}$ et $^{50}\text{Cr(VI)}$, 100 ppb, EDTA 0,5 mM, pH 9,70. c) 100 ppb de $^{53}\text{Cr(III)}$ et $^{50}\text{Cr(VI)}$ en présence d'EDTA 0,5 mM après avoir suivi les conditions d'extraction/complexation à l'EDTA à pH 4,14. d) Extrait non dilué dopé au $^{50}\text{Cr(VI)}$. e) Extrait de cuir dilué (100X) dopé au $^{50}\text{Cr(VI)}$. Colonne de garde NG1 + colonne de garde AG7 + colonne AS7, phase mobile : 75 mmol/L NH_4NO_3 pH 3, débit : 0,25 mL/min, mode KED, temps mort : 170 s.

En outre, après avoir observé que le temps de rétention du Cr(VI) se déplaçait en fonction du facteur de dilution de l'extrait de cuir EDTA, une solution de DPC a été utilisée pour essayer de complexer le Cr(VI) et éviter ce problème.

Cependant, cela n'a pas été possible en raison de la précipitation de la matière organique lors de l'ajout de la solution de DPC à l'extrait. Le résultat était une solution trouble même après filtration à travers des filtres seringues en nylon de 0,45 μm . De plus, l'extraction à l'EDTA est réalisée avec une solution d'EDTA à pH 10, et la solution de DPC est très acide, donc les deux solutions ne sont pas compatibles.

Enfin, étant donné que l'extraction au DTPA était la deuxième méthode d'extraction la plus performante comme rapporté dans une étude précédente^[14], l'injection de $^{53}\text{Cr(III)}$ après avoir suivi les conditions d'extraction au DTPA a également été testée. Cependant, un large pic a été observé juste après le temps mort qui était de 170 secondes. Cela peut s'expliquer par une grande quantité de composés non retenus pendant le temps mort. Par conséquent, cette méthode n'était pas optimale pour extraire les espèces de chrome en utilisant ces conditions analytiques pour leur détermination.

Après toutes les expériences réalisées, il a été possible de confirmer que le complexe Cr(III)-EDTA^- est stable dans une large gamme de pH, sans déplacement du pic en fonction du facteur de dilution. Par contre, le pic du Cr(VI) peut se déplacer en fonction du facteur de dilution de l'extrait de cuir (extraction EDTA) et, vu que la complexation du Cr(VI) avec la DPC n'a pas été possible, l'étape suivante a été d'essayer l'extraction du Cr(VI) des échantillons de cuir par le protocole ISO 17075^[17, 18] avec une analyse par IC-ICP-MS. Pour ce faire, des standards isotopiques de chrome, ainsi qu'un échantillon de cuir, ont suivi l'ensemble du protocole ISO 17075^[17, 18] dans le but de suivre les éventuelles interconversions. Comme on peut le voir sur la Figure 8 a), lorsqu'on injecte du $^{53}\text{Cr(III)}$, il ne se transforme pas en $^{53}\text{Cr(VI)}$. De plus, lors de l'injection du $^{50}\text{Cr(VI)}$, un seul pic est apparu à 925 secondes (Figure 8 b)). Par conséquent, la méthode standard ISO^[17, 18] peut être utilisée pour extraire le Cr(VI) des échantillons de cuir et l'extrait peut être analysé à la suite par IC-ICP-MS dans les conditions d'analyse optimisées et aucune interconversion ne se produit. Lors de l'injection d'un extrait

de cuir, un petit pic a été observé à 925 secondes, ce qui correspond au temps de rétention du Cr(VI) (Figure 8 c)).

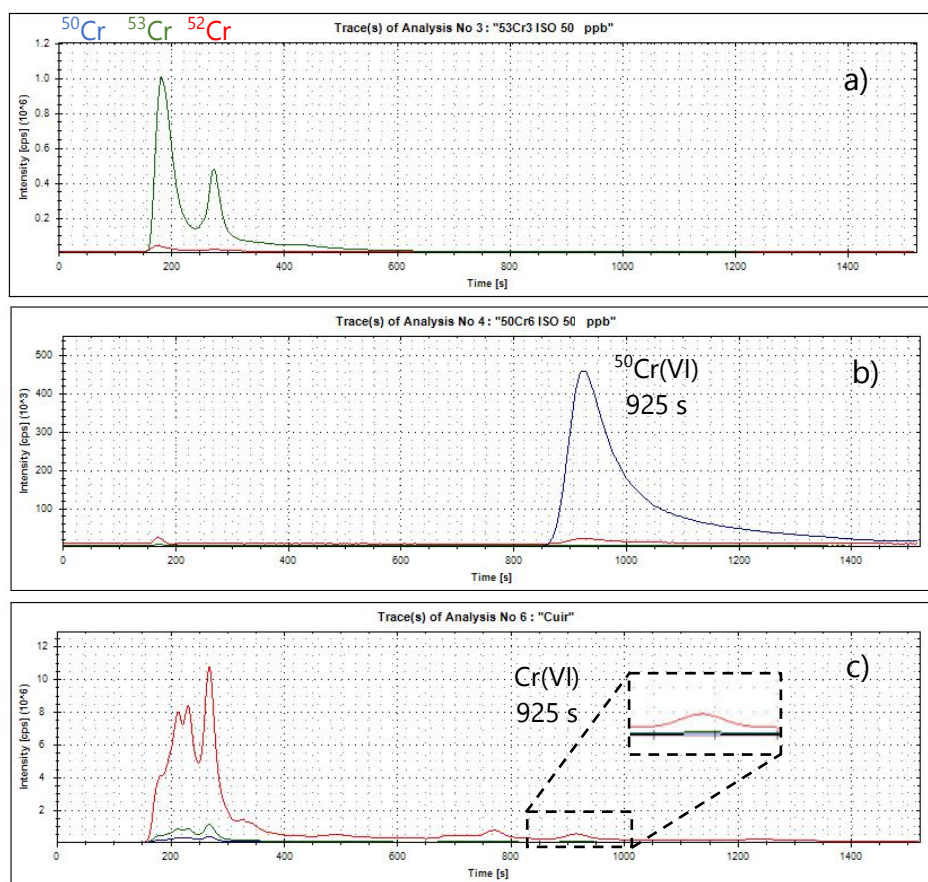


Figure 8. Chromatogrammes IC-ICP-MS de : a) 50 ppb de $^{53}\text{Cr(III)}$ après avoir subi l'extraction ISO 17075. b) 50 ppb de $^{50}\text{Cr(VI)}$ après avoir subi l'extraction ISO 17075. c) Extrait de cuir non dilué (extraction ISO 17075). Colonne de garde NG1 + colonne de garde AG7 + colonne AS7, phase mobile : 75 mmol/L NH_4NO_3 pH 3, débit : 0,25 mL/min, mode KED, temps mort : 170 s.

Un dernier test a été effectué avec du nitrate d'ammonium comme phase mobile. Une colonne de garde NG1, une colonne de garde CG5A, une colonne échangeuse d'ions CS5A et 75 mmol/L NH_4NO_3 à pH 3 comme phase mobile ont été testées. Un échantillon contenant $^{53}\text{Cr(III)}$, $^{50}\text{Cr(VI)}$ et $^{53}\text{Cr(III)-EDTA}^-$ a été injecté avec une concentration acide de HNO_3 0,1% (v/v). A partir de la Figure 9 a), on peut observer que le $^{53}\text{Cr(III)-EDTA}^-$ élué juste après le temps mort (175 secondes) avec un temps de rétention de 195 secondes, suivi par le $^{50}\text{Cr(VI)}$ qui

élue à 360 secondes. Aucune interconversion n'a été détectée dans ces conditions mais il n'y a pas eu de pic pour le Cr(III) avant 3600 secondes, c'est à dire que le Cr(III) est resté collé à la phase stationnaire. Ensuite, un échantillon de cuir a été extrait par la méthode d'extraction EDTA. L'extrait concentré a été injecté postérieurement et un pic a été observé à 195 secondes qui correspond au Cr(III)-EDTA⁻. De plus, un petit pic a été observé à 360 secondes qui correspond au Cr(VI). Les deux pics ne sont pas co-élus pour les standards mais en raison de la forte différence de concentration entre Cr(III) et Cr(VI) dans l'échantillon réel de cuir, les deux pics sont co-élus, empêchant la quantification des deux espèces, comme on peut le comprendre dans la partie zoomée de la Figure 9 b).

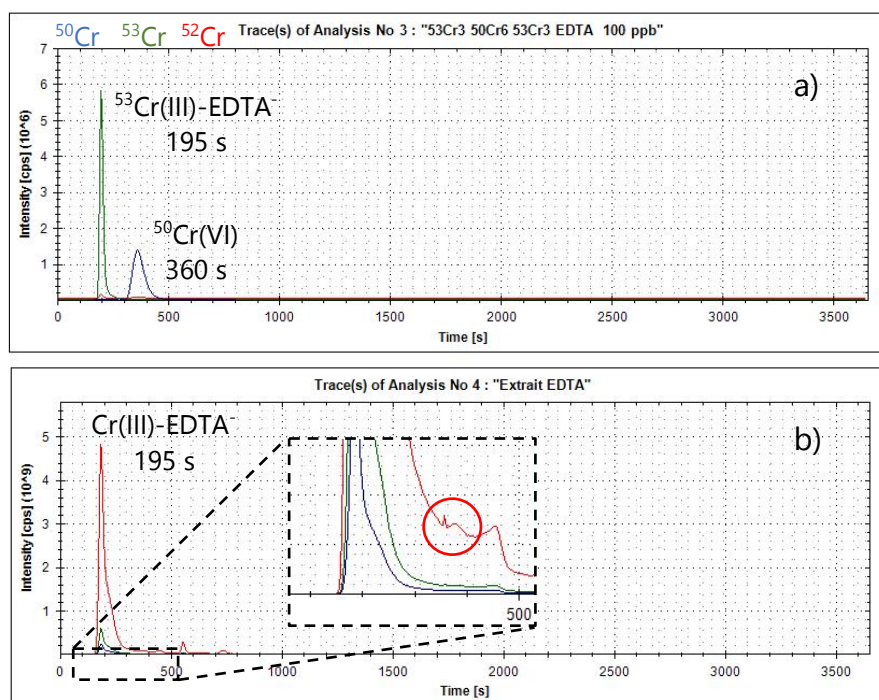


Figure 9. Chromatogrammes IC-ICP-MS de : a) 100 ppb de $^{53}\text{Cr(III)}$, 100 ppb de $^{50}\text{Cr(VI)}$ et 100 ppb de $^{53}\text{Cr(III)}$ en présence d'EDTA (après avoir suivi les conditions d'extraction/complexation de l'EDTA), HNO_3 0,1% (v/v). b) Extraction EDTA d'un échantillon de cuir, pas de dilution. Colonne de garde NG1 + colonne de garde CG5A + colonne CS5A, phase mobile : 75 mmol/L NH_4NO_3 pH 3, débit : 0,25 mL/min, mode STD, temps mort : 175 s.

Parmi tous ces tests, les conditions optimisées pour l'analyse simultanée des espèces de chrome étaient les suivantes : une colonne de garde NG1, une colonne de garde AG7, une colonne échangeuse d'ions AS7 et 75 mmol/L NH_4NO_3 à pH 3 comme éluant chromatographique. La LOD et la LOQ pour le Cr(III) ont été déterminées à 0,016 et 0,054 $\mu\text{g/L}$ (0,3 et 1,1 $\mu\text{g/Kg}$), respectivement, et pour le Cr(VI) à 0,13 et 0,43 $\mu\text{g/L}$ (7 et 22 $\mu\text{g/Kg}$), respectivement. Même si les autres conditions testées ne convenaient pas aux échantillons de cuir, elles ont servi à prouver que l'extraction du Cr(III) par l'EDTA était complète et peuvent éventuellement servir pour d'autres échantillons pour lesquels la concentration entre Cr(III) et Cr(VI) sont du même ordre de grandeur. Les interconversions ont été suivies à l'aide des standards isotopiques de chrome ($^{53}\text{Cr(III)}$ et $^{50}\text{Cr(VI)}$). En ce qui concerne les méthodes d'extraction, la méthode utilisant de l'EDTA s'est avérée être la plus performante pour l'extraction du Cr(III), tandis que le protocole ISO 17075^[17, 18] était efficace pour l'extraction du Cr(VI). Aucune interconversion n'a été trouvée avec aucun de ces protocoles dans les conditions d'analyse optimisées. Les conditions optimales à respecter afin de préserver la spéciation aux étapes d'extraction et d'analyse sont décrites dans le diagramme de la Figure 10.

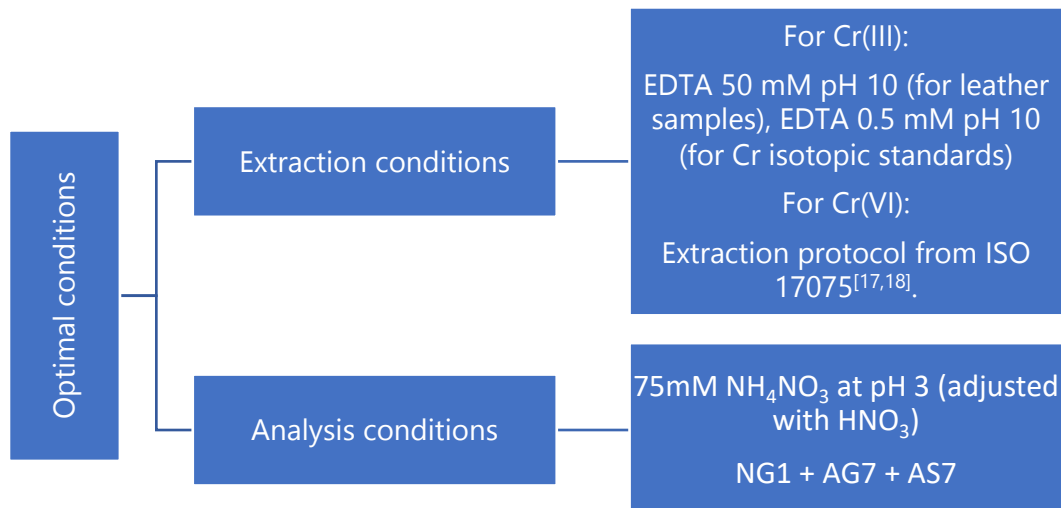


Figure 10. Conditions optimales d'extraction et d'analyse pour la colonne échangeuse d'ions AS7.

Dans le cadre de travaux futurs, il serait utile d'appliquer le protocole d'extraction et d'analyse à un cuir non tanné, un cuir tanné et un cuir tanné et coloré, afin d'avoir une idée des différences de concentrations de chrome en fonction des différents processus de fabrication auxquels les échantillons ont été soumis. En outre, la méthodologie développée pour la colonne CS5A en utilisant l'acide nitrique comme éluant chromatographique devrait également être explorée pour un autre type d'échantillons où la différence de concentration n'est pas si importante, et cela pourrait conduire à une publication supplémentaire.

Enfin, des analyses FTICR-MS ont été réalisées et une source ESI a été utilisée en mode d'ionisation positif et négatif. Le mode d'ionisation négatif a fourni la plus grande intensité pour le complexe Cr(III)-EDTA⁻, par rapport au mode d'ionisation positif. Le spectre de masse ESI FTICR en mode d'ionisation négatif de 10 ppm de Cr(III) en présence d'EDTA 0,5 mmol/L (après avoir suivi les conditions d'extraction/complexation à l'EDTA) (dilution 2X dans acétonitrile), ainsi que le spectre de masse ESI FTICR en mode d'ionisation négatif d'un extrait de cuir à l'EDTA (dilution 2X dans acétonitrile/H₂O, 50/50 v/v%), ont montré l'ion EDTA déprotoné, ([M-H⁺]⁻), à m/z 291, et l'ion du complexe métal-EDTA

déprotoné, $([M-4H^+ + Cr^{3+}]^-)$. De plus, la simulation du spectre du complexe Cr(III)-EDTA⁻ est cohérent avec le spectre obtenu expérimentalement, comme on peut le constater sur la Figure 11. En raison de la différence des abondances naturelles des isotopes du chrome (⁵⁰Cr : 4,345%, ⁵²Cr : 83,789%, ⁵³Cr : 9,501%, ⁵⁴Cr : 2,365%), les pics suivants sont observés (m/z arrondis à l'unité la plus proche, pour les valeurs exactes, voir le tableau 8, chapitre 4) :

- m/z 338 : $[EDTA-4H^+ + ^{50}Cr^{3+}]^-$
- m/z 340 : $[EDTA-4H^+ + ^{52}Cr^{3+}]^-$
- m/z 341 : $[EDTA-4H^+ + ^{53}Cr^{3+}]^-$
- m/z 342 : $[EDTA-4H^+ + ^{54}Cr^{3+}]^-$

La faible valeur de l'erreur obtenue pour la prédiction de la formule ionique pour les pics observés à m/z 291 et 340, en mode d'ionisation négatif, et les pics observés à m/z 293 et 342, en mode d'ionisation positif, a permis de confirmer la formule du complexe chrome-EDTA : $C_{10}H_{13}CrN_2O_8$ (Cr(III)-EDTA⁻).

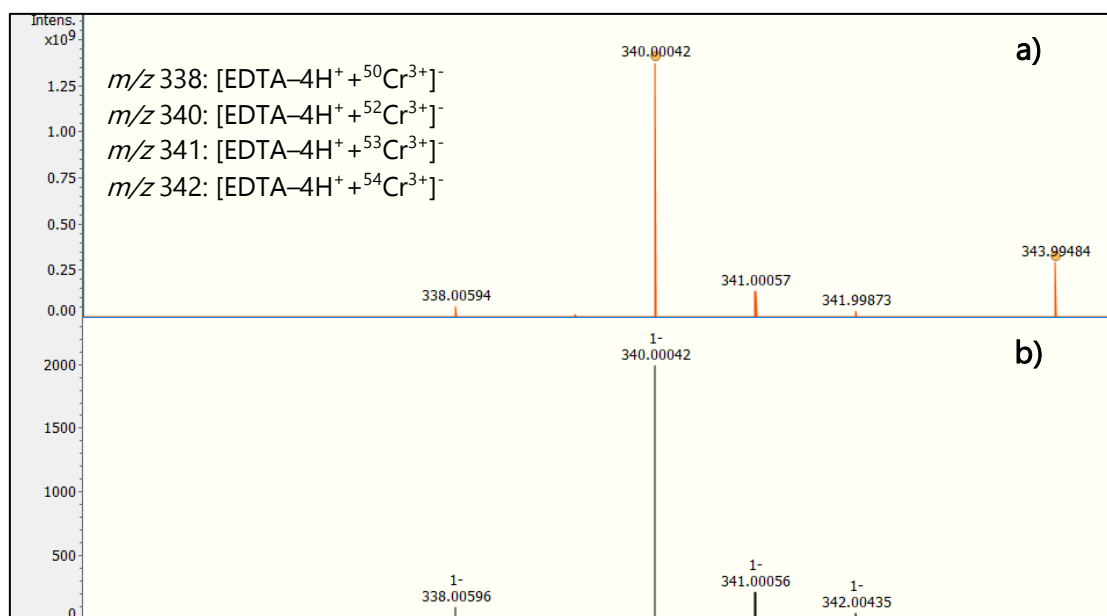


Figure 11. Spectres de masse ESI FTICR en mode d'ionisation négatif de : a) 10 ppm de Cr(III) en présence d'EDTA 0,5 mmol/L (après avoir suivi les conditions d'extraction/complexation de l'EDTA) (dilution 2X dans acétonitrile). b) Simulation du modèle isotopique de Cr(III)-EDTA⁻.

Pour terminer, une section est consacrée aux conclusions qui ont émergé de cette thèse, et la section « Annexes » présente quelques notions générales de chromatographie ainsi qu'un article qui a été réalisé en tant que travail collaboratif au cours de la dernière année de thèse. Cet article a été publié en collaboration avec Juliette Vievard (doctorante de mon laboratoire) *et al*^[31]. Il est intitulé « Extraction and quantification of pesticides and metals in palm wines by HS-SPME/GCMS and ICP-AES/MS » et a été publié dans Food Chemistry (Facteur d'impact : 9.231). Ces méthodes ont permis de quantifier 29 pesticides et 10 métaux dans 32 échantillons de vin de palme, et a été validée en termes de linéarité, répétabilité, LOD, LOQ et précision.

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Introduction

Introduction

The leather industry plays an essential role in global economy. It is one of the oldest traditional industries producing a wide range of goods such as footwear, clothing, furniture, sports equipment, and so on. However, it also has a negative impact on the environment due to the production of significant amounts of waste, some of which are classified as hazardous chemicals.

There are different tanning methods to transform raw hides into leather (vegetable tanning, aldehyde tanning, synthetic tanning, etc.). However, the vast majority of hides are treated with trivalent chromium salts; which is referred to as chrome tanning. Therefore, trivalent chromium is introduced into leather in the form of tanning agents, but also in the form of colorants (chromium premetallized dyes). Nevertheless, different conditions (i.e. exposure to UV light, high temperature, dry atmosphere) can provoke the conversion of trivalent chromium into hexavalent chromium. While trivalent chromium is essential for glucose regulation, hexavalent chromium is toxic; which highlights the importance of quantifying both species separately. It is worth noting that currently, there is no standard method for the extraction and quantification of both species in leather products. In aqueous systems, the oxidation state of chromium is determined by redox potential and pH, which poses a difficulty in chromium speciation, as sample preparation protocols and/or analytical conditions may lead to interconversions between Cr(III) and Cr(VI), thus biasing the results.

The first goal of this thesis was to develop a methodology to quantitatively extract chromium species from tanned leather. The second and ultimate goal of this PhD work was to separate and quantify trivalent and hexavalent chromium separately, while maintaining the speciation. In other words, the objective was to

entirely extract and analyze chromium species, with the same technique (Ion Chromatography - Inductively Coupled Plasma - Mass Spectrometry (IC-ICP-MS)), while avoiding the reduction of Cr(VI) into Cr(III), as well as the oxidation of Cr(III) into Cr(VI).

This thesis is divided into four chapters:

Chapter 1 is based on a review article which was written during the first year of the doctoral program and published in *Critical Reviews of Analytical Chemistry* in 2021. This article focuses on the state-of-the art on chromium determination in leather and other matrices. It discusses the importance of leather in global economy and the environmental impact of its production processes. After describing chrome tanning and the risk of interconversions between trivalent and hexavalent chromium during a leather product's life cycle, an exhaustive literature search was conducted and, as a result, a table compiles and summarizes the different pretreatments and determination methods for chromium species in leather and other matrices (foodstuffs, cosmetic products, environmental and pharmaceutical samples). Finally, the future outlooks regarding leather production and chromium speciation are presented.

Chapter 2 is dedicated to the methodology followed throughout the experimental part of the project. It describes the instrumentation, materials, general procedures, and the theoretical bases and concepts of the different techniques that were used throughout the PhD.

Chapter 3 presents an article that was written during the second year of the doctoral program and published in *Journal of Leather Science and Engineering* in 2021. Seven pretreatment methods are compared in terms of their capability to extract chromium from leather samples while maintaining the speciation. The method that showed the highest extraction rate was validated in terms of linearity, Method Detection Limit (MDL), Method Quantification Limit (MQL), interday precision and spike recovery. In a first approach, to pay attention to the possible interconversions, two chromium isotopic standards ($^{50}\text{Cr(VI)}$) and

$^{53}\text{Cr(III)}$) followed the extraction conditions of the selected method and the separation of chromium species was followed through IC-ICP-MS.

Chapter 4 is written in a publication format because it is intended to be published in a peer-reviewed journal. This chapter deals with the challenge of preserving chromium speciation throughout the pretreatment and analysis steps for real leather samples. A comparative study of analytical methods is presented in this chapter. Two different columns were tested, as well as different mobile phases, different pH of the samples and different complexing agents. The selected method (extraction/analysis) was validated in terms of linearity, Limit of Detection (LOD), Limit of Quantification (LOQ) and precision. Additional data concerning the preliminary tests are integrated in this chapter (which will not appear in the final publication) to reflect the effort that this study required.

Subsequently, there is a section devoted to the conclusions that emerged from this thesis. Finally, the appendix section presents some general concepts on chromatography and an article that was done as a collaborative work during the course of the last year of thesis.

Chapter 1

Chapter 1. State-of-the-art on chromium determination and leather production processes

This thesis is focused on the determination of chromium in leathers, which requires a first step of extraction to give access to chromium in solution for further analysis. Consequently, a deep state of the art was necessary on both sample pretreatment and analysis methods, with a particular attention on speciation. Few studies deal with chromium analysis in leather samples, so the research was broadened to chromium in leathers and other matrices. This article was written during the first year of the doctoral program and summarizes the literature research conducted at the beginning of the doctorate and during the lockdown due to the COVID-19 pandemic. It is entitled "Chromium determination in leather and other matrices: a review" and was published in *Critical Reviews in Analytical Chemistry* (Impact Factor: 5.686) in collaboration with Christine Devouge-Boyer, Marie Hubert-Roux, Carlos Afonso and Mélanie Mignot. It constitutes the first chapter of the thesis and focuses on the state-of-the-art on chromium determination and leather production processes.

Firstly, general aspects related to leather industry are discussed, such as the importance of leather industry in global economy and the environmental impact of its production processes. Next, leather manufacturing processes (preservation of hides and skins storage, beam house operations, tanning, post-tanning and finishing operations) are described. Later on, the article focuses on chrome-tanned leather and explains the evolution of chromium content during leather products' life-cycle, as well as the regulations on hexavalent chromium and the chemistry of chromium. After discussing the risk of interconversions between trivalent and hexavalent chromium because of pH and redox potential,

different complexing agents are presented as a useful way to achieve the separation of species.

An extensive part of this review corresponds to the description of different pretreatment and determination methods for chromium species in leather and other matrices (foodstuffs, cosmetic products, environmental and pharmaceutical samples). Determination methods are divided into five categories: UV-Visible detection, off-line analytical techniques, capillary electrophoresis, chromatographic methods coupled to mass spectrometry, and isotopic dilution techniques. A comprehensive table provides a complete summary of the reported literature on chromium speciation. It is arranged by analytical technique and includes an exhaustive revision of pretreatment and analytical methods.

To conclude, the future outlooks are presented. In this section, the importance of reducing wastes and developing alternatives to conventional chrome tanning are emphasized. In regard to chromium determination in chrome-tanned leather, developing a methodology to extract and quantify Cr(III) and Cr(VI) simultaneously is highly necessary. The production of Certified Reference Materials (CRMs) for chromium speciation in leather samples, in addition to the use of chromium isotopic standards could help dealing with the struggle of species interconversions.

Chromium Determination in Leather and Other Matrices: A Review

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Chromium Determination in Leather and Other Matrices: A Review

Abstract: Leather industry plays an essential role in the world's economy; however, it also has a negative environmental impact due to the generation of significant quantities of wastes, some of which are classified as hazardous chemicals. Chrome tanning, the most popular tanning process, employs chromium salts, acids, and some other chemicals. Some dyes can be also a source of chromium. As a result, hexavalent chromium, a known carcinogenic and mutagenic, can be found in leather products and cause allergic dermatitis or trigger other diseases. For this reason, it is important to quantify the total amount of chromium in final leather goods, as well as the oxidation state in which this element is found. This paper aims to summarize chromium contamination due to the leather production processes, and to review the analytical methods that have been used to determine chromium's most abundant species: Cr(III) and Cr(VI) in leather and other matrices (foodstuffs, cosmetic products, environmental, and pharmaceutical samples). The international and European regulations are presented as well as the last academic developments to extract and quantify chromium species. The future outlook of pre-treatment and quantification techniques are also discussed in this work, with a special focus on chromium interconversions.

Keywords: trivalent chromium; hexavalent chromium; chrome tanning; speciation analysis; isotope dilution

Introduction

Leather industry plays an essential role in world's economy. Leather and leather products are among the most extensively traded commodities globally. However, negative environmental impacts arise due to the generation of relevant quantities of wastes in the leather industry, and the use of hazardous chemicals in tanneries, such as chromium, synthetic tannins, oils, resins, biocides and detergents [1].

In average, 3.5 million tons of different chemicals are used for leather processing. Some of the chemicals used are lime, sodium sulfide, chromium salts, dyes, and acids (Figure 1). A huge fraction of these chemicals are discharged into the effluent. Approximately, around 50 m³ of liquid wastes are discharged per ton of raw hide treated [2].

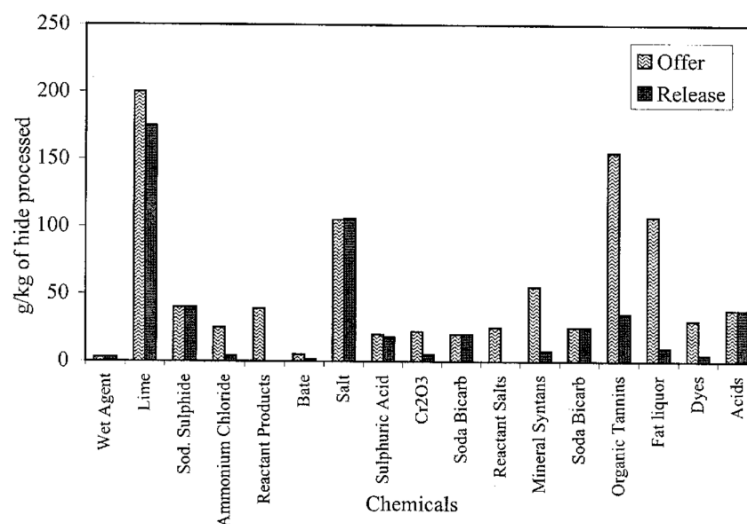


Figure 1. Major polluting chemicals used in leather processing. Obtained with permission from [2].

In the tanning process, trivalent chromium salts such as basic chromium sulfate ($\text{Cr}_2(\text{OH}(\text{SO}_4)_3)$) are widely used to transform raw skin into leather [3]. For chrome tanning, chromium waste emissions amount from 3.0 to 7.5 kg per ton of processed hide (Table 1) [2]. About 90% of all hides are treated with trivalent chromium salts [4].

Table 1. Waste emissions per leather manufacturing process. Modified from [2].

	<i>Soaking</i>	Liming	Deliming	Pickling	Chrome Tanning	Dyeing & Fatliquoring	Composite (incl. Washing)
BOD*	8.3-18.8	17.5-35.0	1.5-4.5	0.3-0.5	0.5-1.2	1.5-3.0	35.0-105.0
COD**	22.5-45.0	35.0-87.5	3.8-10.5	0.8-2.3	1.5-3.8	3.8-10.5	87.5-280.0
Total Solids	262.5-415.5	105.0-175.0	6.0-15.0	26.3-52.6	45.0-90.0	60-15.0	528.0-875.0
Solids suspended	22.5-52.5	21.0-70.0	2.3-6.0	0.8-2.3	1.5-3.8	0.9-1.5	70.0-140.0
Solids chlorides as Cl ⁻	112.5-225.0	14.0-28.0	1.5-3.0	1.5-3.8	23.0-38.0	0.8-1.5	210.0-332.5
Chromium (total) as Cr	-	-	-	-	3.0-7.5	-	3.5-8.8

(All values are expressed in kg/ton of hide processed).

* BOD: Biochemical Oxygen Demand. **COD: Chemical Oxygen Demand

Nevertheless, even if Cr(III) is not considered as toxic, a fraction of this chromium can be oxidized into Cr(VI), a hazardous species. This unintentional oxidation of Cr(III) into Cr(VI) can be attributed to a high temperature, a dry atmosphere, and exposure to UV light [5]. Consequently, Cr(VI) can still be found in leather products and cause allergic dermatitis or trigger more serious illnesses. As many leather products are directly in contact with the skin, it is important to limit as much as possible the use of chromium salts as tanning agents.

It is important to remark that even if chromium tanning effluents contribute to a high concentration of chromium in the soils, this represents a minimal risk to crops. To delve more deeply into this subject, both chromium species can be absorbed by plants, yet, Cr(VI) is preferred due to sulfate carriers. In the roots, Cr(VI) is reduced to Cr(III) by the action of Fe(III) reductase enzymes [6]. Therefore, even if crops are irrigated with contaminated effluents, Cr(VI) is not likely to be transferred to foodstuffs. Antioxidants also contribute to limit the presence of Cr(VI) species in foodstuffs, which is the case of tea leaves and infusions, since they have a high content of flavonoid compounds [7]. Additionally, in the acidic conditions of the stomach, hexavalent chromium is

likely to be reduced to trivalent chromium, representing no major risk to human health [8].

However, due to the dangerous effects of hexavalent chromium in leather, European and international agencies have adopted directives that limit its content in final products. Standard protocols have been developed in order to regulate the extraction and determination of Cr(VI). This review provides a summary of the pre-treatment techniques and the analytical methods that have been used for chromium speciation in leather, as well as in other matrices such as foodstuffs, cosmetic products, environmental, and pharmaceutical samples. The latest developments and the future outlooks on chromium speciation are also discussed herein. Abbreviations used in this article are given in Table 2.

Table 2. Full names and abbreviations.

Abbreviations	Full names
ATR-FTIR	Attenuated Total Reflectance – Fourier Transform Infrared Spectroscopy
CE	Circular Economy
CE-ICP-MS	Capillary Electrophoresis - Inductively Coupled Plasma - Mass Spectrometry
COD	Chemical Oxygen Demand
CPE	Cloud Point Extraction
CRMs	Certified Reference Materials
CrPic	Chromium Picolinate
CSTEE	Scientific Committee on Toxicity, Ecotoxicity and the Environment
DCTA	1,2-cyclohexanediaminetetraacetic acid
DPC	1,5-diphenylcarbazide
DPCO	1,5-diphenylcarbazone
DRS-FTIR	Diffuse Reflectance - Fourier Transform Infrared Spectroscopy
DTPA	Diethylenetriaminepentaacetic acid
EDTA	Ethylenediaminetetraacetic acid
EEAA	Egyptian Environmental Association Affair
EMSL	Environmental Monitoring Systems Laboratory
EPA	Environmental Protection Agency
ESI-MS	Electrospray Ionization - Mass Spectrometry
ETAAS	Electrothermal Atomic Absorption Spectrometry
FAAS	Flame Atomic Absorption Spectrometry
GFAAS	Graphite Furnace Atomic Absorption Spectrometry
HBIG	2-hydroxybenzaldiminoglycine

HPIC-ICP-MS	High-Performance Ionic Chromatography - Inductively Coupled Plasma - Mass Spectrometry
HPLC-ICP-MS	High-Performance Liquid Chromatography - Inductively Coupled Plasma - Mass Spectrometry
HR-CS AAS	High-Resolution Continuum Source Atomic Absorption Spectrometry
IARC	International Agency for Research on Cancer
IC-DAD	Ion Chromatography - Diode Array Detector
IC-ICP-MS	Ion Chromatography - Inductively Coupled Plasma - Mass Spectrometry
IC-MS/MS	Ion Chromatography Tandem Mass Spectrometry
IC-UV-Vis	Ion Chromatography - UV-Visible Detection
ICP-MS	Inductively Coupled Plasma - Mass Spectrometry
ICP-OES	Inductively Coupled Plasma - Optical Emission Spectrometry
IDL	Instrument Detection Limit
IDMS	Isotope Dilution Mass Spectrometry
ISO	International Organization for Standardization
IUPAC	International Union of Pure and Applied Chemistry
KED	Kinetic Energy Discrimination
LC-ICP-MS	Liquid Chromatography - Inductively Coupled Plasma - Mass Spectrometry
LOD	Limit of Detection
LOQ	Limit of Quantification
MDL	Method Detection Limit
PDCA	2,6-pyridinedicarboxylic acid
PQL	Practical Quantification Limit
SEC	Size-Exclusion Chromatography
SIDMS	Speciated Isotope Dilution Mass Spectrometry
SM&T	Standard, Measurement and Testing Programme
SPE	Solid Phase Extraction
TBAH	Tetrabutylammonium Hydroxide
TDS	Total Dissolved Solids
Tris	Tris(hydroxymethyl)aminomethane
UV-Vis	UV-Visible Spectroscopy

Leather production processes

The leather manufacturing process can be divided into four main steps: 1) preservation of hides and skins storage, 2) beam house operations, 3) tanning, and 4) post-tanning and finishing operations. A process diagram is shown in Figure 2.

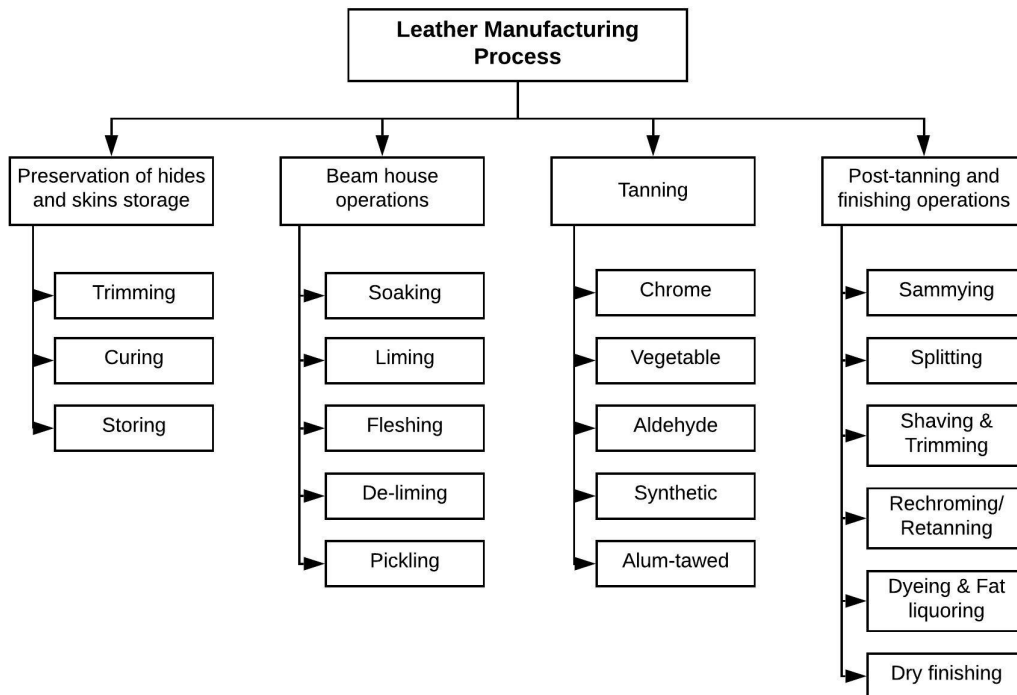


Figure 2. Leather manufacturing process.

Preservation of hides and skins storage

In this step, skins and hides are sorted and trimmed. A curing process is then implemented in order to prevent their decomposition, usually by salting and drying the raw material. This allows the skins and hides to be stored in ventilated or cooled areas for further processing [9].

Beam house operations

In the first instance, hides are soaked to get rid of salts and other solids, as well as to re-hydrate. Subsequently, a liming process (using lime liquors along with sodium sulfide) is performed to facilitate the removal of hair, flesh, fat, and inter-fibrillary protein. Hides are then fleshed to remove excess tissues, muscles and fat. In a de-liming procedure, pH is adjusted around 8.0 in order to convert some proteins to soluble forms. Pickling is required to correct the pH suitable to the tanning procedure (pH 2.8-3.0) with the use of a brine solution and sulfuric acid [1, 9, 10].

Tanning

The purpose of tanning is to transform raw hide into leather. There are several tanning methods, among which are chrome tanning, vegetable tanning, aldehyde tanning, synthetic tanning, and alum-tawing; being chrome and vegetable tanning the most widespread techniques [1].

The electronic structure of chromium ($[\text{Ar}]3d^54s^1$) makes it a perfect tanning agent due to its incomplete 3d orbitals that can host additional electrons to form coordination complexes, which is one of the reasons why chrome tanning is the most common tanning system [11, 12]. Chrome tanning is performed with trivalent chromium salts, which ensures an excellent quality of the final product in terms of mechanical and hydrothermal resistance, attributable to the high rate of infiltration of Cr(III) salts into the interfibrillar spaces of the skins [4].

Collagen, the most abundant protein in the skins, consists of three polypeptide chains in the form of a helix. Aspartic and glutamic acid, some of the principal amino acids of collagen, provide amine (R-NH_2) and carboxyl (R-COOH) groups that are essential for chrome tanning [11]. During this process, pickled skins are treated with basic chromium sulfate, $(\text{Cr}_2(\text{OH}(\text{SO}_4)_3)_3$, (6-8%) and water as the medium. The first step is carried out in acidic medium (part of the pickling bath) permitting carboxyl groups to protonate. The objective of this step is to allow the chromium salts to penetrate homogeneously in the skin. Subsequently, the pH of the bath is raised to 3.8-4.0 with 10% alkali solution. In this step, carboxylate ions (R-COO^-) are liberated from collagen, and chromium creates coordinate covalent bonds with the radicals containing the ionized carboxyl groups. After a self-polymerization reaction, this results in the formation of chromium-collagen complexes [10–12]. An example of a coordinate complex between collagen and chromium is shown in Figure 3.

Successful crosslinking of collagen in hides results in outstanding properties of the leather [13]. The duration of chrome tanning is around 6 hours. After this process, leather is called wet blue [10].

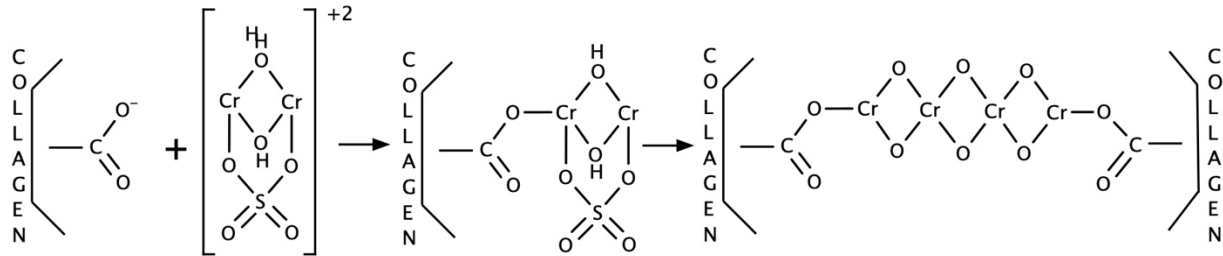


Figure 3. Interaction of chromium with collagen.

On the other hand, vegetable tanning, one of the most ancient tanning methods, works through the action of water infusions of tannins or tannic acid [14]. Vegetable tanning agents are leached from wood, bark, leaves, roots, etc.[15] They may be described as a mixture of polyphenols which bind to collagen in order to confer resistance to the hides. Vegetable tanning materials can be divided into two groups [14]:

- (1) Pyrogallol group. They provide a yellow to light brown deposit (ellagic acid) to the leather and improve wearing properties and water resistance. Examples: sumach, chestnut, myrabolams, divi-divi, oak-wood, oak galls valonia, oak-barks.
- (2) Catechol group. They provide a pink to red sediment (phlobaphenes) to the leather and the resultant leather is more solid. Examples: gambier, hemlock, quebracho, mimosa, mangrove, pine, oak-bark.

Although vegetable tanning is a greener technology, chrome tanning reaction is faster (6 hours instead of 3 weeks), confers high hydrothermal stability, alters the structure of collagen in only a small way, makes collagen more hydrophobic (hence, water-resistant) and allows the retention of colorants since Cr(III) can act as a dye fixing agent [16].

Post-tanning and finishing operations

Post-tanning operations include the removal of excess moisture (sammying), splitting the material into the desired thickness, mechanical shaving and trimming, and re-chroming or semi-chroming to improve the chromium content in leather. Subsequently, pH is neutralized and leather is dyed. Finally, the leather is fat-liquored and finished to obtain softness and fullness [9].

During the dyeing process, the leather is colored using: acid dyes, basic dyes, direct dyes, mordant dyes, premetallized dyes, reactive dyes or sulfur dyes. Azo dyes are organic colorants containing the azo group (N=N), usually bonded with chromophores. The azo group contributes to the delocalization of electrons in the chromophore, therefore, to the generation of color [16]. Orange II, shown in Figure 4, is an example of an azo dye widely used in the leather industry.

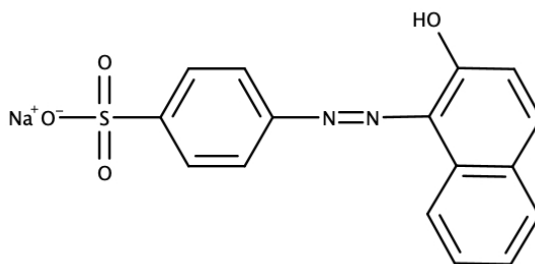


Figure 4. Molecular structure of Acid Orange 7 (Orange II).

These synthetic colorants are very popular in leather processing, though, its toxicity has been extensively investigated. Several azo dyes, specially benzidine-based colorants, have been reported to have a negative effect on human health, causing allergies or even triggering cancer [17, 18].

The European Union, after consulting the Scientific Committee on Toxicity, Ecotoxicity and the Environment (CSTEE) concerning the carcinogenicity of some azo dyes, provided a list of 22 prohibited aromatic amines. Azo dyes which may release these toxic aromatic amines in detectable concentrations, above 30 ppm, should not be used in textile and leather articles coming into contact with the human skin. This list includes the following aromatic amines: biphenyl-4-ylamine;

benzidine; 4-chloro-o-toluidine; 2-naphthylamine; o-aminoazotoluene; 5-nitro-o-toluidine; 4-chloroaniline; 4-methoxy-m-phenylenediamine; 4,4'-methylenedianiline; 3,3'-dichlorobenzidine; 3,3'-dimethoxybenzidine; 3,3'-dimethylbenzidine; 4,4'-methylenedi-o-toluidine; 6-methoxy-m-toluidine; 4,4'-methylene-bis-(2-chloroaniline); 4,4'-oxydianiline; 4,4'-thiodianiline; o-toluidine; 4-methyl-m-phenylenediamine; 2,4,5-trimethylaniline; o-anisidine; 4-aminoazobenzene [19].

Besides azo dyes, premetallized dyes are another class of colorants that are widely used in the leather industry. Its concept relies on avoiding the two-step mordanting-dyeing process. Consequently, premetallized dyes are beforehand prepared by complexing a metal atom, usually chromium, with the molecules of the dye. These premetallized dyes exhibit interesting properties such as: increased fastness, good penetration and levelness of coloring, high fixation, etc.[16] An example of a premetallized dye (Acid Blue 158) is shown in Figure 5. Evidently, the use of these type of colorants contributes to the total content of chromium, which could pose a problem in case of an unintentional oxidation of Cr(III) into Cr(VI).

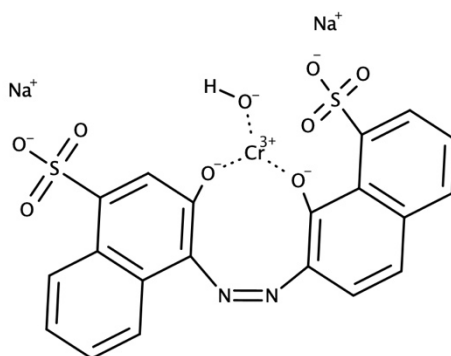


Figure 5. Molecular structure of a premetallized dye: Acid Blue 158.

Apart from the toxicity of some colorants, another inconvenient can emerge when hexavalent or trivalent chromium is determined through colorimetric methods. Some colorants (examples shown in Table 3) absorb in the

same wavelength as some complexes used for chromium determination. Hence, for chromatographic applications, the efficiency of the pre-column or guard column is of great importance.

Table 3. Wavelengths of maximum absorbance of different colorants used in leather industry. Modified from [11].

Description	Acid Red 97	Acid Red 131	Brown HH 469
CAS Number	10169-02-5	70210-37-6	94933-05-8
λ_{max}	530 nm	550 nm	540 nm
Structure			

Evolution of chromium content during leather products' life-cycle

As we mentioned before, chrome-tanned leather can represent a risk to human health due to the unintentional oxidation of Cr(III) into Cr(VI).

In 2014, Hedberg et al[20] characterized four differently tanned leather samples and exposed them to artificial sweat and phosphate buffer for chromium extraction, observing that co-released leather-specific species can reduce released hexavalent chromium. Therefore, no Cr(VI) was found in artificial sweat or phosphate buffer. However, leather samples with no reduction capacity and fewer aromatic groups, identified by Attenuated Total Reflectance – Fourier Transform Infrared Spectroscopy (ATR-FTIR), revealed Cr(VI) at the surface.

Some years later, in 2018, the International Organization for Standardization (ISO) published a thermal pre-ageing procedure that permits to

predict the evolution of hexavalent chromium content in tanned leather. The ISO 10195 standard requires the sample to be heated in an oven for a specific period of time (60° and relative humidity <20%, or 80° and relative humidity <10%, 24 hours). Nevertheless, this method does not simulate real conditions and only provides a tendency for the oxidation of Cr(III) into Cr(VI) [21].

In order to study the different storage conditions that could influence the evolution of hexavalent chromium content in leather, Fontaine et al.[5] studied natural and accelerated ageing in 4 bovine leather samples. A factorial design of experiment was performed with the following factors: temperature (40° or 80°), relative humidity (20% or 50%), presence or not of UV light, and exposure duration (24h or 48h). The experiments showed that temperature, relative humidity, and exposure to UV light had a great impact on Cr(VI) formation, having UV light a predominant role. In parallel, the same leathers were naturally aged for 12 months at room temperature and analyzed for hexavalent chromium content. They found out that the conditions that best simulate natural ageing are the following: 1) 40°C, 50% relative humidity, presence of UV light, 48h, and 2) 40°C, 20% relative humidity, presence of UV light, 24h. Finally, they analyzed the same leather samples after undergoing through ISO 10195 artificial ageing, concluding that this standard does not accurately reflect what happens during natural ageing. Therefore, UV light should be taken into consideration for artificial ageing tests.

Regulations on hexavalent chromium

Because of the dangerous effects of hexavalent chromium in human health, its content in leather products should be closely monitored.

As a result of an increment in chromium allergy, 16 228 patients with dermatitis patch tested between 1985 and 2007 at the Department of Dermato-Allergology, Getofte Hospital (Denmark), were studied to determinate the possible causes of this allergy. In particular, this study showed that around 55%

of 197 patients with chromium allergy tested between 1995 and 2007 had been exposed to leather products (shoes, gloves and other sources) [22] .

Further determinations of hexavalent chromium in leather goods performed in Germany and Denmark between 2007 and 2008 showed that more than 30% of these leather articles contained above 3 mg/kg of hexavalent chromium [23].

In 2012, the Danish government submitted a report in which they proposed to the European Chemicals Agency a maximum hexavalent chromium content of 3 ppm in leather products sold in the European Union [23, 24].

Consequently, in 2014 the European Union adopted the restriction proposed by the Danish government, limiting the content of hexavalent chromium to 3 mg/kg of the total dry weight in leather articles coming into contact with the skin; otherwise these products could not be commercialized [25].

Currently, the ISO 17075 standard is the only internationally recognized procedure to determine the content of hexavalent chromium in leather via a colorimetric (part 1) [26] and a chromatographic (part 2) analysis [27]. This standard requires a previous selective extraction of Cr(VI) with $K_2HPO_4 \cdot 3H_2O$ at pH 8.0, and it is suitable for concentrations above 3 mg/kg. The colorimetric analysis requires a reaction between Cr(VI) and DPC, and the detection of the resulting complex by UV-Vis spectroscopy at 540 nm. Concerning the chromatographic analysis, it can be performed by direct detection of the chromate peak at 372 nm, or through a post-column reaction with DPC. Post-column reaction offers more sensitivity and should be preferred in case the sample may contain reducing species that interfere with the analyte [28, 29]. Though, an inconvenience can surge when using post-column reaction due to the interference of colorants that may absorb at the same wavelength as the complex analyzed.

Chemistry of chromium

Chromium is a transition metal that, after being processed, occurs in several chemical species: metallic chromium, trivalent chromium and hexavalent chromium [30].

Metallic chromium, chromium 0, can be found in alloys and coatings. As a coating, chromium exhibits anti-wear and anticorrosion properties, and can also be used as decoration. It is obtained as a result of an electrochemical reduction of chromium salts [31, 32].

Trivalent chromium, Cr(III), exists as cationic species (Cr^{3+} , $\text{Cr}(\text{OH})^{2+}$, $\text{Cr}(\text{OH})_2^+$) [6] and has been considered as an essential nutrient involved in the regulation of glucose [33, 34], due to its participation in the activation of an insulin receptor [35, 36]. It can be frequently found in the form of chromium picolinate in dietary supplements. Nonetheless, some studies suggest no relevant benefits in certain populations [37]. Though, more studies on proper dosage and length of time of supplementation should be performed in order to accurately define the advantages of chromium picolinate supplementation [38].

By the other hand, hexavalent chromium, Cr(VI), exists as anionic species (HCrO_4^- , CrO_4^{2-} , HCr_2O_7^- , $\text{Cr}_2\text{O}_7^{2-}$), and it is a known mutagenic, classified by the International Agency for Research on Cancer (IARC) as carcinogenic to humans [39]. For this reason, it is of great importance the selective determination of Cr(VI).

As shown in the Eh-pH diagram from Figure 6, in aqueous systems, the oxidation state of chromium is determined by redox potential and pH [40].

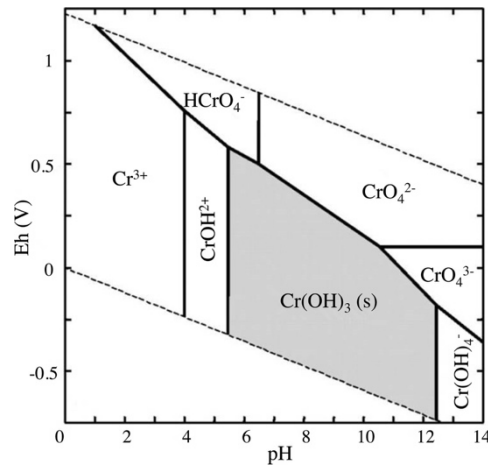
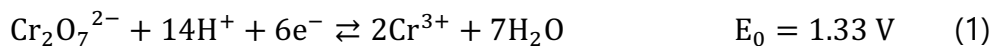
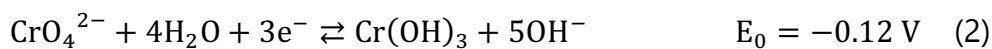


Figure 6. Eh-pH diagram of chromium. Reproduced of [40] with permission from Elsevier.

The reduction process in acidic solution can be expressed as [41]:



and in alkaline solution:



As we can see, interconversions can take place between the oxidation state of chromium. Cr(VI) can be reduced to Cr(III) under acidic conditions; by the other hand, Cr(III) can be oxidized to Cr(VI) in the presence of oxygen under alkaline conditions [3].

Dias da Silva et al.[4], after performing an extraction scheme that will be further described, noticed that in one of the steps (oxidizing conditions) about 48%, 5%, and 0.9% of chromium (percentages from three different samples) were converted into Cr(VI). Martone et al.[42], knowing the possibility of this type of transformations, employed isotopic dilution techniques to follow species interconversions during the analysis of Cr(VI) in dietary supplements. The results showed that 11 samples contained a highly reductive matrix that transformed all the spiked $^{53}\text{Cr(VI)}$ into $^{53}\text{Cr(III)}$. For 14 samples, the phosphate buffer extraction

(basic media) caused an oxidation of Cr(III) to Cr(VI) that ranged from 0.3 to 47.9%.

This poses a difficulty in the speciation of chromium, as the sample preparation protocols can lead to an interconversion between Cr(III) and Cr(VI), thus distorting the results [43, 44]. Therefore, the real challenge is to preserve, as much as possible, the initial distribution of both chromium species throughout the pre-treatment and analysis stages. Protocols should guarantee that no Cr(VI) is generated from the oxidation of Cr(III). In the same way, no Cr(VI) should be masked by its reduction to Cr(III) during its extraction and determination.

Complexing agents

The use of complexing agents can be a useful tool to achieve trivalent and hexavalent chromium separation. Some of the most popular chelating agents are 1,5-diphenylcarbazide (DPC), ethylenediaminetetraacetic acid (EDTA), diethylenetriaminepentaacetic acid (DTPA), 2,6-pyridinedicarboxylic acid (PDCA), 1,2-cyclohexanediaminetetraacetic acid (DCTA), and 2-hydroxybenzaldiminoglycine (HBIG). Some of them can form a complex with Cr(III), or with Cr(VI) after oxidation of the organic agent. The main examples are presented below.

DPC

In acidic medium, Cr(VI) oxidizes DPC to 1,5-diphenylcarbazone (DPCO), resulting in a violet Cr(III)-diphenylcarbazone complex that can be detected by UV-Vis spectroscopy at 540 nm [26, 45, 46]. The reaction is shown in Figure 7.

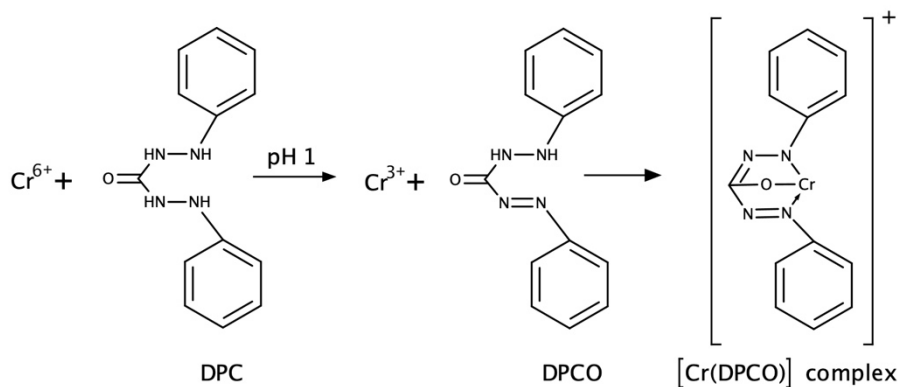


Figure 7. Complexation of Cr(VI) with DPC.

EDTA

EDTA can form colored complexes with some cations. In slightly acidic medium, EDTA can react with Cr(III) to form a violet $\text{Cr}(\text{III})\text{-EDTA}^-$ complex (anionic species) that absorbs strongly at 540 nm [45]. Due to the slow rate of complexation at room temperature, solutions are frequently heated to accelerate the reaction [47, 48].

DTPA

Similarly to EDTA, DTPA can react with trivalent chromium to produce the $[\text{Cr}(\text{DTPA})]^{2-}$ complex. Yet, poorer stability has been reported for $[\text{Cr}(\text{DTPA})]^{2-}$ [49].

PDCA

PDCA is also used to complex Cr(III) due to its high selectivity compared to other ligands, resulting in a $[\text{Cr}(\text{PDCA})_2]^-$ complex [50]. The reaction between Cr(III) and 2 molecules of PDCA is described in Figure 8. $[\text{Cr}(\text{PDCA})_2]^-$ can be quantified through direct UV detection at 335 nm and 535 nm [46].

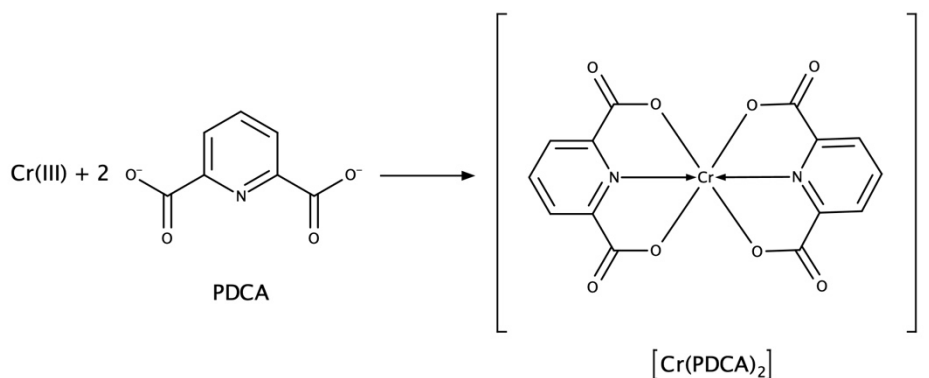


Figure 8. Complexation of Cr(III) with PDCA.

DCTA

In a slightly acidic medium (pH 6.5 - 7.0), Cr(III) can be chelated with DCTA to produce a single charged Cr-DCTA⁻ complex [36]. This reaction occurs slowly at room temperature but can be warmed up to 60 °C to speed up the rate of reaction. The Cr-DCTA⁻ complex can be measured through UV detection at 210 nm [51].

HBIG

HBIG has also showed potential to chelate Cr(III). Complexation occurs instantaneously at pH 7.5, and requires the use of a buffer solution: disodium hydrogen orthophosphate (0.01 M) and sodium hydrogen orthophosphate (0.01 M). The chromium(III)-HBIG complex has a maximum absorbance a 565 nm [52]. This method is selective and sensitive.

Nevertheless, UV detection is not a very selective method and some other compounds, especially when working on complexes matrices, can be extracted and absorb at the same wavelength than the complex formed between chromium and the ligand.

Pre-treatment and determination methods

Due to the nature of the samples, chromium speciation in leather requires a treatment before approaching its determination. Concerning leather products,

only few pre-treatment and analytical methods have been reported. Therefore, this section presents a review of pre-treatment and determination methods for chromium species in leather, but also includes chromium speciation studies in environmental and pharmaceutical samples, foodstuffs, and cosmetic products. In this way, we can see what is being done with other matrices, and what can be implemented when working with leather. Table 4 provides a summary of the reported literature of chromium speciation. It is arranged by analytical technique, and includes an exhaustive revision of pre-treatment and analytical methods. The way in which the different analytical figures of merit were calculated is specified in Table 5 (NA whenever the author did not specified the calculation).

Sample pre-treatment

Depending on the extraction conditions (i.e. pH, concentration, etc.), chromium can be encountered in different forms as specified in Table 6.

Table 6. Dominant Cr species in different aqueous conditions.

Cr oxidation state	Aqueous conditions	Dominant aqueous Cr species
Cr(III)	pH < 3.5	Cr^{3+}
	pH > 3.5	CrOH^{2+}
	Increased pH	$\text{Cr}(\text{OH})_2^+$
	Increased pH	$\text{Cr}(\text{OH})_3^{\circ} (\text{Aq})$
	Increased pH	$\text{Cr}(\text{OH})_4^-$
	Higher Cr concentrations	$\text{Cr}_2(\text{OH})_2^{4+}$
	Higher Cr concentrations	$\text{Cr}_3(\text{OH})_4^{5+}$
Cr(VI)	pH < 6.5 & conc. < 30 mM	HCrO_4^-
	pH < 6.5 & conc. > 30 mM	$\text{Cr}_2\text{O}_7^{2-}$
	pH > 6.5	CrO_4^{2-}

As previously described, ISO established a normalized method in which hexavalent chromium is leached from leather samples by means of a phosphate buffer ($\text{K}_2\text{HPO}_4 \cdot 3\text{H}_2\text{O}$) at pH 8.0 [26, 27]. This pre-treatment method was also implemented by Rezić and Zeiner[53] for Cr(VI) extraction from leather. Pastore and his colleagues[44] evaluated the normalized method and presented evidence of Cr(VI) formation, after the oxidation of Cr(III) by peroxides. As a consequence of the results obtained, they proposed a more suitable extraction solution

consisting of a 0.1M phosphate solution (0.1M NaH_2PO_4 and 0.1M H_3PO_4) at pH 4.4 in presence of 5% NaCl.

Costa et al.[54] tried an alkaline extraction of Cr(VI) in leather using a NaOH 0.1M solution. A different approach was studied by Dias da Silva et al[4] on which tanned leather shavings were pre-treated using the SM&T (Standard, Measurement and Testing Programme) sequential extractions scheme. Considering Cr concentrations, the extracted fractions can be ordered as follows: oxidizable > exchangeable > reducible > residual. The oxidizable fraction was tested to determine the concentration of Cr(VI). For this fraction, they observed that a part of the released chromium was oxidized to from Cr(III) to Cr(VI), which exposes the danger of the disposal of this type of waste under oxidative conditions.

For soils, sludges, sediments, and similar waste materials, the SW-846 Environmental Protection Agency (EPA) 3060A method defines an extraction protocol for hexavalent chromium using 0.28M Na_2CO_3 /0.5M NaOH, a phosphate buffer (0.5M K_2HPO_4 /0.5M KH_2PO_4) and MgCl_2 [55]. Mg^{2+} serves to suppress oxidation of Cr(III) into Cr(VI). Petrucci and Senofonte[56] adapted the EPA 3060A method for cosmetic products pre-treatment. This alkaline digestion has been employed for traditional Chinese medicines as well [57].

An alkaline extraction of hexavalent chromium with NH_4OH is frequently used when working with foodstuffs (milk, cheese, yoghurt, cereal products, chocolate, vegetables, fruits, meat, fish, eggs, and beverages) [58–60]. Some other selective alkaline extractions of Cr(VI) require the use of 0.1M Na_2CO_3 [61, 62] or 0.01M NaOH / 1M NH_4NO_3 [63].

Cloud Point Extraction (CPE) has been used for separation and preconcentration of metallic ions, among them chromium[64–66]. Kiran et al.[67] reported the determination of Cr(III) in industrial wastewater, natural water, and spiked water samples after its complexation with bis-[2-hydroxynaphthaldehyde] thiourea using CPE. For the determination of Cr(VI) in turmeric samples, Tiwari et

al.[68] complexed Cr(VI) with DPC and then used the non-ionic surfactant Triton X-100 for the determination of Cr(VI) by CPE. Likewise, Solid Phase Extraction (SPE) has also permitted the preconcentration of Cr(VI) on Amberlite XAD-1180 resin after its reaction with DPC, as reported by Narin et al.[69]

For total chromium determination in siliceous matrices, organic matrices, and other complex matrices, the SW-846 EPA 3052 method proposes a microwave digestion with HNO₃ and HF. HCl and H₂O₂ can be alternatively used [70].

Determination methods

UV-Visible detection

Regarding hexavalent chromium determination in leather, ISO proposes a colorimetric analysis by UV-Vis spectroscopy at 540 nm after the reaction between Cr(VI) and DPC, as well as a chromatographic analysis (using an anion exchange column and a pre-column) by direct detection of the chromate peak at 372 nm, or after a post-column reaction with DPC by measuring the absorption peak at 540 nm [26, 27]. Similarly, the SW-846 EPA 7196A method describes a UV-Visible determination of hexavalent chromium in groundwaters based on the same reaction[71]. In order to evaluate the ISO standardized protocol, Pastore et al.[44] performed Ion Chromatography and post-column reaction with DPC, and obtained a limit of quantification (LOQ) of 0.15 mg/kg after changing to a more suitable extraction solution to avoid Cr(III) oxidation into Cr(VI) (0.2 g of leather and 100 mL of 0.1M phosphate solution at pH 4.4, and 5% NaCl). For this study, they used an anion exchange Dionex IonPac AG7 guard column. To eliminate any interference of colorants, they employed SPE C18 cartridges that were activated before injection.

UV-Visible determination through Cr(VI) and DPC complexation has been extensively executed by several authors. Rezić and Zeiner[53] analyzed leather products from different chromium tanning processes and obtained a limit of detection (LOD) of 15 µg/L for Cr(VI). They also quantified total Cr after oxidation

of Cr(III). Costa et al.[54] evaluated leather samples using this complexation together with a colorimetric analysis of digital images by means of a mobile phone and PhotoMetrix App, achieving a LOD of 0.6 mg/kg. Dias da Silva et al.[4] determined extractable hexavalent chromium in leather samples, in the oxidizable fraction of the SM&T extractions scheme, by UV-Visible detection through the DPC reaction.

For drinking water, groundwater, and industrial wastewater effluents, Ion Chromatography (anion exchange Dionex IonPac AS7 column and NG1 guard column capable of removing strongly absorbing organics) with UV-Vis detection after post-column reaction with DPC has been reported in the SW-846 EPA 7199 method with a method detection limit (MDL) of 0.3-0.4 $\mu\text{g/L}$ [72] and by the Environmental Monitoring Systems Laboratory (EMSL) obtaining a MDL of 1 $\mu\text{g/L}$ [73]. For soil and wastewater samples, Phesatcha et al.[74] isolated Cr(III) by derivatization with PDCA on a pre-column and complexed Cr(VI) by post-column reaction with DPC. Both complexes were analyzed using an ion exchange Dionex IonPac CS5A column and a CG5A guard column. The detection was set at 335 nm from 0.0 to 4.7 min for Cr(III), and at 530 nm from 4.7 to 8.0 min for Cr(VI). Tiwari et al.[68] detected the same reddish-violet complex at 540 nm by Diffuse Reflectance - Fourier Transform Infrared Spectroscopy (DRS-FTIR) with a LOD of 1.22 $\mu\text{g/mL}$ for Cr(VI) in turmeric samples.

Off-line analytical techniques

Certain off-line techniques that have been used for chromium speciation in various matrices are Flame Atomic Absorption Spectrometry (FAAS) [67, 69], Electrothermal Atomic Absorption Spectroscopy (ETAAS) [63], Graphite Furnace Atomic Absorption Spectrometry (GFAAS) [62] and High-Resolution Continuum Source Atomic Absorption Spectrometer (HR-CS AAS) [61]. Nevertheless, these techniques do not allow the separation of species and require a selective extraction of either one of the two species. Generally, the other species is

calculated by subtraction of the extracted species from the total content of chromium.

Capillary electrophoresis

A different approach was reported by Chen et al.[36] via Capillary Electrophoresis - Inductively Coupled Plasma - Mass Spectrometry (CE-ICP-MS), achieving the separation of Cr(III), Cr(VI) and Chromium Picolinate (CrPic) in nutritional food samples. This was accomplished by chelating Cr(III) with DCTA, allowing the separation of the single-charged Cr-DCTA⁻ complex from the double-charged molecule CrO₄²⁻ (Cr(VI)), and CrPic (no charge).

Chromatographic methods coupled to Mass Spectrometry

Coupling chromatographic methods with Inductively Coupled Plasma - Mass Spectrometry (ICP-MS) has facilitated and increased speciation studies, allowing the separation and posterior quantification of trivalent and hexavalent chromium. Hernandez and her team[58] analyzed Cr(VI) in dairy (milk, cheese, yoghurt) and cereal products by High-Performance Ionic Chromatography – ICP-MS (HPIC-ICP-MS) with an ion exchange Dionex IonPac CS5A column and a CG5A guard column, attaining the optimization of a sensitive and specific on-line method where no pre-concentration was required. In a further study, they determined Cr(III) in dairy products by in-situ complexation of EDTA and the determination of the Cr(III)-EDTA⁻ complex by High-Performance Liquid Chromatography – ICP-MS (HPLC-ICP-MS) (using the same columns as in the previous study) [59]. Vacchina et al.[60] also worked with HPLC-ICP-MS (ion exchange Dionex IonPac CS5A column) to accomplish hexavalent chromium determination in dairy products, flour, chocolate, vegetables, fruits, meat, fish, eggs, and beverages. To study the interactions between Cr(VI) and the matrices, they employed size-exclusion HPLC-ICP-MS (gel filtration Superdex-200 HR 10/30 column). However, no Cr(VI) was found in any of the samples, attributing these results to the instability of Cr(VI) in food matrices.

HPLC-ICP-MS was also implemented for the analysis of Cr(III) and Cr(VI) in environmental samples. Jabłońska-Czapla et al.[75] accomplished chromium speciation in water and bottom sediments using an anion exchange Dionex IonPac AG7 guard column and 0.1M NH_4NO_3 / 0.8M HNO_3 gradient elution after buffer extraction. According to Séby et al.[76], who worked with an ion exchange Dionex IonPac CS5A column and a CG5A guard column, they were not able to quantify Cr(III) in natural water samples due to its possible precipitation in alkaline medium. To avoid this problem, Hu and his colleagues[77] stabilized Cr(III) with EDTA, and were able to separate it from hexavalent chromium and polymeric Cr(III) using a reversed phase Spheri-5 C18 column, obtaining a LOD of 0.9 $\mu\text{g/L}$ for both chromium species in wood leachates, surface waters, tap water, and wastewater. Li et al.[57] attained a good recovery of Cr(VI) in traditional Chinese medicines by HPLC-ICP-MS using a weak anion exchange Agilent Bio WAX column and NH_4NO_3 as mobile phase. Evaluating iron supplements, Araujo-Barbosa et al.[78] reported a fast chromatographic run (5 minutes) using 1.0mM tetrabutylammonium hydroxide (TBAH), 0.7mM EDTA and 5% methanol at pH 7.2. as mobile phase and a Phenomenex Kinetex reversed phase C18 column, allowing the separation of Cr(III) and Cr(VI).

Speciation in drinking water has been addressed by Ion Chromatography – ICP-MS (IC-ICP-MS) with minimal sample preparation using nitric acid as mobile phase and an anion exchange Dionex IonPac AG7 guard column for separation of both species, attaining low LOD (ng/g, pg/L) [79, 80]. Similar conditions were used by Kutscher et al.[81] for Cr(VI) quantification in toys and toy material with the aim of studying the migration of elements in these samples. IC-ICP-MS with an anion exchange Dionex IonPac AS7 column and an AG7 guard column was also employed by Petrucci and Senofonte[56], where they found that one of the 4 cosmetic products evaluated contained more than 5 mg/L of Cr(VI). Chen et al.[49] investigated wastewater by IC-ICP-MS using 20mM NH_4NO_3 and 10mM $\text{NH}_4\text{H}_2\text{PO}_4$ at pH 7.0 as mobile phase, an anion exchange Agilent G3154A/101

column, and a G3154A/102 guard column. Subsequently, they evaluated the stability of Cr(III) complexes with three aminopolycarboxylic acids (EDTA, DTPA and PDCA) by Electrospray Ionization - Mass Spectrometry (ESI-MS). They attained the separation of Cr(VI) and Cr(III) as Cr(III)-EDTA⁻, and confirmed the kinetical stability of the chromium-chelate complex. Direct injection of the samples was possible and no Cl⁻ interferences were found. Hagendorfer and Goessler[82] studied chromium speciation in cement and homeopathic drugs by IC-ICP-MS without derivatization. This was attained by taking into account the distribution of Cr species in water and working in the window of pH where Cr(VI) is not reduced and Cr(III) does not precipitate, which can be described as the gray shading in Figure 9. For this reason, they used a mobile phase of pH 3.5 (90 mM (NH₄)₂SO₄ and 10 mM NH₄NO₃) and a metal-free Rspak NN-814 PEEK column.

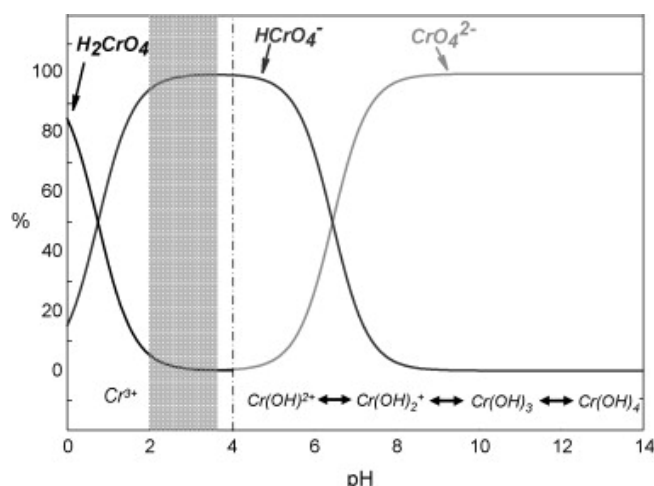


Figure 9. Distribution of Cr species in water. Reproduced of [82] with permission from Elsevier.

Gürleyük and Wallschläger[48] complexed Cr(III) with EDTA and achieved the separation of chromium species in CRMs (synthetic and estuarine water) by IC-ICP-MS using an anion exchange Dionex IonPac AS7 column, an AG7 guard column, a self-regenerating suppressor, and 50mM NH₄NO₃ (pH 8) as eluent. They obtained an LOD of 12 ng/L for Cr(VI) and 5 ng/L for Cr(III).

When working with ICP-MS for Cr determination, it is important to precise that polyatomic interferences can arise with the presence of carbon or chlorine. For $^{52}\text{Cr}^+$ the interferences could be $^{35}\text{Cl}^{16}\text{O}^{1}\text{H}^+$, $^{35}\text{Cl}^{17}\text{O}^+$ and $^{40}\text{Ar}^{12}\text{C}^+$; and for $^{53}\text{Cr}^+$ the interferences could be $^{37}\text{Cl}^{16}\text{O}^+$, $^{40}\text{Ar}^{13}\text{C}^+$ and $^{40}\text{Ar}^{12}\text{C}^{1}\text{H}^+$. These interferences can be reduced by using collision/reaction cell and kinetic energy discrimination (KED) with He as collision gas[58, 83].

Isotopic dilution techniques

To improve the accuracy of chromium speciation, several authors have implemented isotope dilution techniques. The two different approaches of isotope dilution are Isotope Dilution Mass Spectrometry (IDMS) and Speciated Isotope Dilution Mass Spectrometry (SIDMS). While IDMS serves for total element determination through the addition of a known quantity of an enriched isotope, SIDMS is capable of tracing species interconversions by labelling each species with enriched isotopes.

The SW-846 EPA 6800 method describes a procedure for the implementation of IDMS and SIDMS, for the speciation of different elements, among which chromium is part of the list, in a variety of samples such as waters, solids, blood, foods, extracts or digests [84]. Mädler et al. implemented SIDMS by adapting the EPA 6800 method for trace (MDL of $0.007\ \mu\text{g/L}$)[85] and ultra-trace level analysis (MDL of $2\ \text{ng/L}$)[86] of Cr(VI) in environmental water and sediment samples by Ion Chromatography Tandem Mass Spectrometry (IC-MS/MS) through an anion exchange Dionex IonPac AS20 column and an AG20 guard column. $^{50}\text{Cr(VI)}$ and $^{53}\text{Cr(III)}$ tracers were used by Drinčić et al.[87] to follow possible interconversions in the determination of hexavalent chromium by HPLC-ICP-MS using an anion exchange Mono Q HR 5/5 column. However, Cr(VI) was not detected in river sediments. SIDMS has also been reported for teas, bread samples [43], and dietary supplements [42].

IDMS was studied by Fabregat-Cabello et al.[88], who quantified Cr(VI) in several Certified Reference Materials (CRMs) after microwave assisted extraction

with 5mM EDTA (5 min at 90 °C and 5 min at 110 °C) by IC-ICP-MS using an anion exchange Dionex IonPac AS9-HC column. The CRMs analyzed in this work were: soil containing chromite ore processing residue, welding dust loaded on a filter, and sandy clay soil. In this work, only $^{50}\text{Cr(VI)}$ was added to the samples as Cr(III) was found to be stable in the extracting conditions. Guidotti et al.[89] modified these extraction conditions (only one cycle of 5 min at 80 °C) to minimize Cr(VI) reduction during the analysis of soil samples from a contaminated area. $^{53}\text{Cr(VI)}$ was traced to attain accurate quantification by Liquid Chromatography – ICP-MS (LC-ICP-MS) using the same column as Fabregat-Cabello et al.[88]. The application of both IDMS and SIDMS was reported by Wolle et al[90] in soil samples.

In the absence of CRMs for chromium speciation in leather samples, only spiking and isotope dilution techniques can help to improve the accuracy of the measurements. Therefore, these techniques that had been employed in other matrices should set the tone for what should be considered when working with leather.

Table 4. Pre-treatment and determination methods for chromium speciation in different matrices.

Matrix	Sample pre-treatment	Analytical technique	Analyte	Analytical Conditions	Analytical Figures of Merit	Important Remarks	Reference
Nutritional food samples	Extraction with 25% methanol solution under ultrasonic agitation.	CE-ICP-MS	Cr(III), Cr(VI), CrPic	CE voltage: 13 kV. 50 cm x 75 µm i.d. x 375 µm o.d. fused silica capillary	IDL: 0.10 ng/mL for Cr(VI), 0.18 ng/mL for Cr(III), 0.20 ng/mL for CrPic. MDL: 1.0 ng/g for Cr(VI), 1.8 ng/g for Cr(III), 2.0 ng/g for CrPic.	Cr(III) was chelated with DCTA to form a single charged Cr-DCTA complex.	Chen et al.[36]
Turmeric samples	The Cr(VI)-DPC complex was extracted of using Triton X-100 as non-ionic surfactant. CPE was used as preconcentration process.	DRS-FTIR	Cr(VI)	Wavelength: 540 nm.	LOD Cr(VI): 1.22 µg/mL. LOQ Cr(VI): 4.02 µg/mL.	Cr(VI) is complexed with DPC prior to extraction.	Tiwari et al.[68]
Bread samples	Acid digestion (total Cr) and selective alkaline extraction of Cr(VI) with 0.01M NaOH and 1M NH ₄ NO ₃ .	ETAAS	Total Cr, Cr(VI)	Ashing temperature: 1600 °C. Atomization temperature: 2500 °C	LOD: 1.5 µg/kg for total Cr and 1.7 µg/kg for Cr(VI). LOQ: 4.95 µg/kg for total Cr and 5.60 µg/kg for Cr(VI).	From 152 bread samples, about 10% of the total Cr in bread is in the Cr(VI) form.	Soares et al.[63]
Industrial wastewater, natural water, and spiked water samples	Cr(III) is complexed with bis-[2-hydroxynaphthaldehyde] thiourea and extracted with the non-ionic surfactant Triton X-100.	FAAS	Total Cr, Cr(III)		LOD~0.18 µg/L.	Total Cr: determined after reducing Cr(VI) to Cr(III). Cr(VI): calculated by subtracting Cr(III) from the total Cr.	Kiran et al.[67]
Tap water, bottled mineral water, and red lentil	Cr(VI) is complexed with DPC. Then the Cr(III)-diphenylcarbazone complex was analyzed on Amberlite XAD-1180 resin.	FAAS	Total Cr, Cr(VI)	Elution with 1M HNO ₃ in acetone.	LOD: 7.7 µg/L for Cr(VI) and 8.6 µg/L for total Cr.	Total Cr was determined after oxidizing Cr(III) to Cr(VI). Cr(III) was calculated by subtracting Cr(VI) from total Cr.	Narin et al.[69]
Tanned leather	Acid digestion (total Cr) and SM&T Sequential Extractions Scheme (oxidizable > exchangeable > reducible > residual).	FAAS, UV-Vis	Total Cr, Cr(VI)	Wavelength: 540 nm.		Cr was determined in each extracted fraction. Cr(VI) was determined in the oxidizable fraction by reaction with DPC.	Dias da Silva et al.[4]
Teas	Acid digestion (total Cr) and alkaline extraction of Cr(VI) with 0.1M Na ₂ CO ₃ .	GFAAS	Total Cr, Cr(VI)	Atomization temperature: 2500 °C.	LOD: 0.020 µg/g for Cr(VI). LOQ: 0.07 µg/g for Cr(VI) (tea samples).	Kinetic of Cr(VI) leaching from tea sample was also studied.	Mandiwana et al.[62]
Dairy (milk, cheese, yoghurt) and cereal products	Acid digestion (total Cr) and extraction of Cr(VI) with NH ₄ OH.	HPIC-ICP-MS	Total Cr, Cr(VI)	Column: Dionex IonPac CG5A, CS5A (4x250 mm). Mobile phase: 2M HNO ₃ , H ₂ O. Gradient mode.	LOQ: 0.8 µg/kg (cereals), 0.6 µg/kg (dairy products) for Cr(VI).	Cr(VI) was not found in dairy and cereal products.	Hernandez et al.[58]
Iron supplement samples	Acid digestion (total As, Cr) and extraction with ultrapure water and microwave assisted radiation (for speciation).	HPLC-ICP-MS	Total As/Cr, As(III)/As(V), Cr(III)/Cr(VI)	Column: Phenomenex Kinetex C18 (4.6x100 mm). Mobile phase: 1.0mM TBAH, 0.7mM EDTA and 5% methanol at pH 7.2.	LOD: 0.029 µg/g for Cr, 0.014 µg/g for Cr(III), 0.04 µg/g for Cr(VI). LOQ: 0.089 µg/g for Cr, 0.5 µg/g for Cr(III), 0.14 µg/g for Cr(VI).	Fast chromatographic run (5 min).	Araujo-Barbosa et al.[78]
Dairy products, flour, chocolate, vegetables, fruits, meat, fish, eggs, and beverages	Acid digestion (total Cr) and extraction of Cr(VI) with NH ₄ OH at pH 11.5.	HPLC-ICP-MS	Total Cr, Cr(VI)	SEC column: Superdex-200 HR 10/30 (10x300 mm). Ion exchange column: Dionex IonPac CS5A (4x250 mm). Mobile phase (SEC): 30mM tris buffer pH 7.5. Mobile phase (Ion exchange): 85mM HNO ₃ .	LOD Cr(VI): 1 µg/L and 10 µg/kg. LOQ Cr(VI): 3 µg/L and 30 µg/kg.	SEC HPLC-ICP-MS for Cr(VI)/matrix(milk) interactions. Ion-exchange HPLC-ICP-MS for Cr(VI) quantification.	Vacchina et al.[60]

Natural waters samples	Filtration prior to analysis.	HPLC-ICP-MS	Cr(III), Cr(VI)	Column: Dionex IonPac CG5A (4x50 mm), CS5A (4x250 mm). Mobile phase: HNO ₃ .	LOD: 0.19 µg/L for Cr(VI), 0.32 µg/L for Cr(III). LOQ: 0.6 µg/L for Cr(VI), 0.9 µg/L for Cr(III).	Recovery of Cr(III) was not completely quantitative due to its high reactivity in alkaline medium.	Séby et al.[76]
Sediment samples	Acid digestion (total Cr) and Cr(VI) extraction with 2% NaOH + 3% Na ₂ CO ₃ .	HPLC-ICP-MS	Total Cr, Cr(VI)	Column: Mono Q HR 5/5 (5x50 mm). Mobile phase: Linear gradient elution from 100% water to 100% 0.7M NaCl.	LOD: 1.25 µg/kg for Cr(VI). LOQ: 4.0 µg/kg for Cr(VI).	The addition of 0.4M MgCl ₂ prevented oxidation of Cr(III). SIDMS was implemented.	Drinčić et al.[87]
Teas and bread samples	Acid digestion (total Cr) and alkaline extraction of Cr(VI) with 0.1M Na ₂ CO ₃ or 0.01M NaOH/1M NH ₄ NO ₃ .	HPLC-ICP-MS	Total Cr, Cr(III), Cr(VI)	Column: Mono Q HR 5/5 (5x50 mm). Mobile phase: Linear gradient elution from 100% water to 100% 0.7M NaCl .	LOD: 0.5 µg/kg for total Cr, 0.03 µg/L for Cr(VI), 0.02 µg/L for Cr(III). LOQ: 1.7 µg/kg for total Cr, 0.11 µg/L for Cr(VI), 0.07 µg/L for Cr(III).	Cr(VI) cannot exist in tea and bread samples due to the presence of organic matter and antioxidants. SIDMS was implemented.	Novotnik et al.[43]
Traditional Chinese medicines	Extraction with 0.28M Na ₂ CO ₃ /0.5M NaOH, phosphate buffer (0.5M K ₂ HPO ₄ /0.5M KH ₂ PO ₄) and MgCl ₂ .	HPLC-ICP-MS	Cr(VI)	Column: Agilent Bio WAX (4.6x250 mm). Mobile phase: 75mM NH ₄ NO ₃ at pH 7.	LOD: 0.1 µg/L. LOQ: 0.3 µg/L.	Good recovery of Cr(VI) in the present of high amount of Cr(III).	Li et al.[57]
Water and bottom sediments	Acid digestion (total As, Cr, Sb), extraction with buffer (5mM Na ₂ HPO ₄ and 50mM KH ₂ PO ₄ pH 6.0), and extraction with deionized water.	HPLC-ICP-MS	Total As/Cr/Sb, As(III)/As(V), Cr(III)/Cr(VI), Sb(III)/Sb(V)	Column: Dionex IonPac AG7 (4x50 mm) for Cr; AS7 (4x250 mm) for As and Sb. Mobile phase: A: 0.1M NH ₄ NO ₃ pH 4.0, B: 0.8M HNO ₃ gradient mode for Cr; 1mM phthalic acid, 10mM EDTANa ₂ pH 4.5 for As and Sb.	LOD: 0.11 µg/L for Cr(III), 0.17 µg/L for Cr(VI).	A guard column was used for separation of Cr species.	Jabłońska-Czapla et al.[75]
Wood leachates, surface waters, tap water, and wastewater	Filtration prior to analysis.	HPLC-ICP-MS	Cr(III), polymeric Cr(III), Cr(VI)	Column: PerkinElmer Spheri-5 RP-18 (4.6x220 mm). Mobile phase: 5% (v/v) methanol/0.5mM EDTA + 1 mM TBAH (pH 7.2).	LOD: 0.9 µg/L for Cr(III) and Cr(VI).	EDTA was added to complex and stabilize monomeric Cr(III) during sample preparation and measurement.	Hu et al.[77]
Yoghurt and cheese samples	Acid digestion (total Cr) and extraction of Cr(VI) with NH ₄ OH.	HPLC-ICP-MS	Total Cr, Cr(III), Cr(VI)	Column: Dionex IonPac CG5A, CS5A (4x250 mm). Mobile phase: 2M HNO ₃ , H ₂ O. Gradient mode.	LOD: 12.6 µg/kg for Cr(III). LOQ: 38 µg/kg for Cr(III).	In-situ complexation of Cr(III) with EDTA. Good agreement between Cr(III) and total Cr.	Hernandez et al.[59]
Bread and cereals	Acid digestion (total Cr) and alkaline extraction of Cr(VI) with 0.1M Na ₂ CO ₃ .	HR-CS AAS	Total Cr, Cr(VI)	Pyrolysis temperature: 1200 °C. Atomization temperature: 2450 °C	LOD of Cr: 0.045 µg/g.	Cr levels in bread do not exceed the daily maximum acceptable concentration.	Mathebula et al.[61]
Tanned leather	Extraction of Cr(VI) with 0.1M phosphate solution (0.1M NaH ₂ PO ₄ and 0.1M H ₃ PO ₄) at pH 4.4 in presence of 5% NaCl.	IC-DAD	Cr(VI)	Column: Dionex IonPac AG7. Mobile phase: 1) H ₂ O, 2) 250mM (NH ₄) ₂ SO ₄ /100mM NH ₃ (pH 8-8.5), 3) 2.5M (NH ₄) ₂ SO ₄ /1M NH ₃ (pH 8-8.5). Gradient mode. Wavelength: 543 nm.	LOQ: 0.15 mg/kg.	Post-column reaction with DPC for Cr(VI) determination. Cr(III) was complexed with EDTA. Evidence of Cr(VI) formation (ISO 17075).	Pastore et al.[44]
Cement samples and homeopathic drugs	Acid digestion (total Cr) and Cr(VI) extraction with water or dissolution in 0.1M HCl for both species determination.	IC-ICP-MS	Total Cr, Cr(III), Cr(VI)	Column: Shodex Rspak NN-814 4DP (4x150 mm). Mobile phase: 90mM (NH ₄) ₂ SO ₄ and 10mM NH ₄ NO ₃ (pH 3.0-3.5).	LOQ~0.5 µg/L for both species.	Minimum sample preparation, no derivatization, multi-mode column.	Hagendorfer & Goessler[82]
Cosmetic products: face powder and eye shadow	Defatted samples (defatted with ethanol-hexane) are extracted with 0.28M Na ₂ CO ₃ /0.5M NaOH.	IC-ICP-MS	Cr(VI)	Column: Dionex IonPac AS7 (2x250 mm), AG7(4x50 mm). Mobile phase: 0.25M (NH ₄) ₂ SO ₄ + 0.1M NH ₄ OH.	LOQ: 0.11 mg/kg.	Cr(VI) was found in 1 out of 4 evaluated products.	Petrucchi & Senofonte[56]
CRMs: soil containing chromite ore processing residue, welding dust loaded	Cr(VI) is extracted with 50mM EDTA (Cr(III) complexation with EDTA).	IC-ICP-MS	Cr(VI)	Column: Dionex IonPac AS9-HC (4x250 mm). Mobile phase: EDTA 5mM (pH 10).		IDMS was implemented.	Fabregat-Cabello et al.[88]

on a filter, and sandy clay soil							
CRMs: synthetic and estuarine water	Cr(III) is complexed with EDTA 0.2mM.	IC-ICP-MS	Cr(III), Cr(VI)	Column: Dionex IonPac AS7 (4x250 mm), AG7 (4x50 mm). Mobile phase: 50mM NH ₄ NO ₃ (pH 8).	LOD: 12 ng/L for Cr(VI) and 5 ng/L for Cr(III).	Self-regenerating suppressor was used.	Gürleyük & Wallschläger[48]
Dietary supplements	Digestion through EPA 3052 (total Cr) or extraction through EPA 3060A (Cr(VI)).	IC-ICP-MS	Total Cr, Cr(III), Cr(VI)	Column: Hamilton PRP-X100 (4.6x150 mm). Mobile phase: A: 0.06M NO ₃ ⁻ (pH 9.3), B: 0.06M NO ₃ ⁻ (pH 1.2). Gradient mode.	LOD: 0.9 µg/L for Cr(III) and Cr(VI).	Total Cr determined by ICP-MS. Cr(VI) determined by IC-ICP-MS or EPA 7196A or 7199. Cr(III) determined by ICP-MS after Cr(VI) extraction. SIDMS was implemented.	Martone et al.[42]
Drinking water	No sample preparation.	IC-ICP-MS	Cr(III), Cr(VI)	Column: Dionex IonPac AG7 (2x50 mm). Mobile phase: 0.4M HNO ₃ .	LOD: 0.20 µg/g for Cr(VI) and 0.38 µg/g for Cr(III).	A guard column was used for separation of both species.	Kutscher et al.[79]
Drinking water	Samples are acidified with HNO ₃ .	IC-ICP-MS	Cr(III), Cr(VI)	Column: Dionex IonPac AG7 (2x50 mm). Mobile phase: 0.3M HNO ₃ .	LOD: 4.0 ng/L for Cr(VI) and 9.0 ng/L for Cr(III).	A guard column was used for separation of both species.	Schmelzel et al.[80]
Soil samples	Digestion through EPA 3052 (total Cr and insoluble Cr species), extraction through EPA 3060A (Cr(VI)), EDTA extraction (Cr(VI) and soluble Cr(III)).	IC-ICP-MS	Total Cr, soluble Cr(III), Cr(VI), insoluble Cr species.	Column: Metrosep A Supp 4 (Metrohm), (4x250 mm). Mobile phase: 2mM EDTA pH 10.		IDMS and SIDMS were implemented.	Wolle et al.[90]
Toys & toy material	Samples are subjected to migration process and diluted (1eq of 1M NH ₄ OH + 4eq of migration solution).	IC-ICP-MS	Total element quantification, Cr(VI)	Column: Dionex IonPac NG1 (2x50 mm), AG7 (2x50 mm). Mobile phase: 0.35M HNO ₃ .	IDL: 0.9 ng/L for Cr(VI).	Cr(VI) was stable for at least 24h. Cr(III) was prone to hydrolysis and precipitation.	Kutscher et al.[81]
Contaminated waters	Direct injection of the samples.	IC-ICP-MS, ESI-MS,	Cr(VI)	Column: Agilent guard (G3154A/102) and separation (G3154A/101) columns. Mobile phase: 20mM NH ₄ NO ₃ and 10mM NH ₄ H ₂ PO ₄ (pH 7.0).	LOD: 0.2 µg/L for Cr(III), 0.1 µg/L for Cr(VI).	Stability of Cr(III) complexes was studied by ESI. Separation was performed with IC.	Chen et al.[49]
Environmental water samples	Samples were preserved with NH ₄ OH or NH ₄ OH/(NH ₄) ₂ SO ₄ , and filtered prior to analysis.	IC-MS/MS	Cr(VI)	Column: Dionex IonPac AG20 (2x50 mm), AS20 (2X250 mm). Mobile phase: 1) 30mM KOH, 2) 60mM KOH, 3) 30mM KOH. Gradient mode.	MDL: 2 ng/L. PQL: 0.010 µg/L.	Ultra-trace level analysis. SIDMS was implemented.	Mädler et al.[86]
Sediment samples	Alkaline digestion (0.28M Na ₂ CO ₃ /0.5M NaOH) and EDTA extraction.	IC-MS/MS, UV-Vis	Cr(VI)	Column: Dionex IonPac AG20 (2x50 mm), AS20 (2X250 mm). Mobile phase: 1) 30mM KOH, 2) 60mM KOH, 3) 30mM KOH. Gradient mode. Wavelength: 540 nm (CRMs).	MDL: 0.007 µg/L.	Trace level analysis. CRMs were additionally analyzed by UV-Vis (reaction with DPC). SIDMS was implemented.	Mädler et al.[85]
Drinking water, groundwater, and industrial wastewater effluents	Sample preparation through appropriate SW-846 method.	IC-UV-Vis	Cr(VI)	Column: Dionex IonPac AS7, NG1 (or equivalent). Mobile phase: 250mM (NH ₄) ₂ SO ₄ + 100mM NH ₄ OH. Wavelength: 530 nm.	MDL: 0.3-0.4 µg/L.	Post-column reaction with DPC.	SW-846 EPA 7199[72]
Drinking water, groundwater, and industrial wastewater effluents	Not described.	IC-UV-Vis	Cr(VI)	Column: Dionex IonPac AS7, NG1. Mobile phase: 250mM (NH ₄) ₂ SO ₄ + 100mM NH ₄ OH. Wavelength: 530 nm.	MDL: 1 µg/L.	Post-column reaction with DPC.	EMSL[73]
Leather	Extraction of Cr(VI) with K ₂ HPO ₄ ·3H ₂ O at pH 8.0.	IC-UV-Vis	Cr(VI)	Annex B (direct detection of chromate peak). Column: Anion exchange column (4,6	LOQ: 3 mg/kg.	Direct detection of chromate peak at 372 nm (Annex B) or post-	ISO 17075-2[27]

				×75 mm), 1 mm pre-column. Mobile phase: 25mM (NH ₄) ₂ SO ₄ + 1mM NaOH. Annex C (detection after complexation with DPC). Column: Anion exchange column (4x250 mm), guard column (4x35 mm). Mobile phase: 250mM (NH ₄) ₂ SO ₄ + 116mM NH ₄ OH.		column reaction with DPC by measuring the absorption peak at 540 nm (Annex C).	
Soil and wastewater samples	Digestion with HNO ₃ and eluent stock (10x concentrate).	IC-UV-Vis	Cr(III), Cr(VI)	Column: Dionex IonPac CG5A (4x50 mm), CS5A (4x250 mm). Mobile phase: 2mM PDCA, 2mM Na ₂ HPO ₄ , 10mM NaI, 50mM NH ₄ CH ₃ CO ₂ and 2.8mM LiOH. Wavelength: 335 (0.0-4.7 min) and 530 nm (4.7-8.0 min).		Cr(III) is complexed with PDCA (pre-column derivatization) and Cr(VI) reacts with DPC (post-column reaction).	Phesatcha et al.[74]
Tanned leather	Acid digestion (total Cr) and alkaline extraction of Cr(VI) with 0.1M NaOH.	ICP-OES, Mobile Phone, PhotoMetrix App	Total Cr, Cr(VI)	Wavelength: 540 nm.	LOD: 0.6 mg/kg for Cr(VI). LOQ: 2 mg/kg for Cr(VI).	Reaction with DPC for Cr(VI) determination.	Costa et al.[54]
Waters, solids, blood, foods, extracts or digests.	Sample preparation through appropriate SW-846 method.	IDMS, SIDMS	Multielements			Applicable to the determination of total elements, elemental and molecular species at ppb, ppt and sub-ppt levels.	SW-846 EPA 6800[84]
Soil samples	Cr(VI) is extracted with 50mM EDTA (Cr(III) complexation with EDTA).	LC-ICP-MS	Cr(VI)	Column: Dionex IonPac AS9-HC (4x250 mm). Mobile phase: EDTA 5mM (pH 10).		IDMS was implemented.	Guidotti et al.[89]
Groundwaters	Sample preparation through appropriate SW-846 method.	UV-Vis	Cr(VI)	Wavelength: 540 nm.		Reaction with DPC.	SW-846 EPA 7196A[71]
Leather	Extraction of Cr(VI) with K ₂ HPO ₄ ·3H ₂ O at pH 8.0.	UV-Vis	Cr(VI)	Wavelength: 540 nm.	LOQ: 3 mg/kg.	Reaction with DPC.	ISO 17075-1[26]
Tanned leather	Extraction of Cr(VI) with K ₂ HPO ₄ at pH 8.0.	UV-Vis	Total Cr, Cr(VI)	Wavelength: 540 nm.	LOD: 15 µg/L for Cr(VI).	Reaction with DPC for Cr(VI) determination. Total Cr was measured after oxidation of Cr(III).	Rezić & Zeiner[53]
Siliceous matrices, organic matrices, and other complex matrices	Microwave digestion with HNO ₃ and HF.	Via appropriate SW-846 method.	Multielements			Alternative acid and reagent combinations (HCl and H ₂ O ₂)	SW-846 EPA 3052[70]
Soils, sludges, sediments, and similar waste materials	Digestion with 0.28M Na ₂ CO ₃ /0.5M NaOH, phosphate buffer (0.5M K ₂ HPO ₄ /0.5M KH ₂ PO ₄) and MgCl ₂ .	Via SW-846 EPA 7196A or 7199	Cr(VI)			Mg ²⁺ can suppress oxidation of Cr(III) to Cr(VI).	SW-846 EPA 3060A[55]

Table 5. Description of the calculation of LOD, LOQ, IDL, MDL, and/or PQL according to the different authors cited in Table 4.

Reference	LOD	LOQ	IDL	MDL	PQL
Chen et al.[36]			Concentration necessary to yield a net signal equal to 3 times the standard deviation of the background ($3\sigma/S$)	Calculated from IDL and mass ratio of final solution used for CE-ICP-MS	
Tiwari et al.[68]	$3SD/b$ for 3 times the standard deviation for 7 replicates and b the slope of the calibration curve	$10SD/b$			
Soares et al.[63]	$3s/m$ where s is the standard deviation of the measurements of the blank and m is the slope of the calibration curve	$10s/m$			
Kiran et al.[67]	5 times signal-to-noise ratio				
Narin et al.[69]	3 times the standard deviation of the blank				
Mandiwana et al.[62]	$3s/b$ for 3 times the standard deviation of 22 measurements of the blanks divided by the slope	$10s/b$			
Hernandez et al.[58]		Calculated through statistical method. Obtained from the intersection between acceptance and tolerance limits. Acceptance: set to $\pm 35\%$ (corresponding to an intermediate precision coefficient of variation estimated at 12% at $k=3$ ($p=0.99$)). Tolerance: calculated from the mean bias at each concentration level. Probability set to 90 or 85%.			
Araujo-Barbosa et al.[78]	$3\sigma/s$, where σ is the standard deviation of the measurements of the blank and s is the slope of the calibration curve	$10\sigma/s$			
Vacchina et al.[60]	3 times the standard deviation of 10 blanks	10 times the standard deviation of 10 blanks			
Séby et al.[76]	NA	NA			
Drinčić et al.[87]	Concentration that gives a signal equal to 3 times the standard deviation of 8 blanks	Concentration that gives a signal equal to 10 times the standard deviation of 8 blanks			
Novotnik et al.[43]	Concentration that gives a signal equal to 3 times the standard deviation of 8 blanks	Concentration that gives a signal equal to 10 times the standard deviation of 8 blanks			
Li et al.[57]	$3S/N$ ratio	$10S/N$ ratio			
Jabłońska-Czapla et al.[75]	$3s/b$, where s is the standard deviation and b the slope of the calibration curve				
Hu et al.[77]	NA				

Hernandez et al.[59]	3 times the standard deviation of 10 blanks	10 times the standard deviation of 10 blanks			
Mathebula et al.[61]	$x + 3s$, where x is the signal of the blank sample and s is the standard deviation of the blank				
Pastore et al.[44]		NA			
Hagendorfer & Goessler[82]		Calculated from the calibration method			
Petrucci & Senofonte[56]		10σ analyzing 10 blanks			
Gürleyük & Wallschläger[48]	3 times the standard deviation of the baseline noise				
Martone et al.[42]	NA				
Kutscher et al.[79]	Calculated from calibration curves				
Schmelzel et al.[80]	Calculated based on the standard deviation of the peak area of 15 injections of unspiked water				
Kutscher et al.[81]			3σ for the lowest concentration standard		
Chen et al.[49]	Signal/Noise = 3				
Mädler et al.[86]				Standard deviation of the analysis of multiple standard solutions multiplied by one-tailed student t-value ($\alpha=0.01$, $n=18$) of 2.567	Same approach as MDL
Mädler et al.[85]				Standard deviation of the analysis of multiple standard solutions multiplied by one-tailed student t-value ($\alpha=0.01$, $n=18$) of 2.567	
SW-846 EPA 7199[72]				NA	
EMSL[73]				NA	
ISO 17075-2[27]		NA			
Costa et al.[54]	Calculated according to IUPAC recommendations				
ISO 17075-1[26]		NA			
Rezić & Zeiner[53]	NA				

Future outlooks

As a result of the environmental concerns regarding leather industry, researchers have been continuously trying to find ways to reduce wastes, as well as alternatives to conventional chrome tanning.

Recently, Md.A. Moktadir et al.[91] analyzed the challenges of implementing Circular Economy (CE) practices in leather industry. CE is a strategy that aims to limit the input of virgin materials and the production of wastes, creating a closed-loop system. This work revealed that the major challenge is the lack of financial support from the authorities, followed by the lack of technological advancement. According to them, financial support could help implement CE policies for environmental protection. Meanwhile, technological advancement could improve tannery effluent treatment, which would reduce wastes and protect the environment. The analysis of wastewater, solid waste, sulfide, Chemical Oxygen Demand (COD), ammonium salt, chloride and chromium with CE practices is essential to accomplish sustainable development of leather industry [92].

According to Abdel-Shafy et al.[93], the liming/unhairing operation produces highly polluted effluents (high levels of COD, BOD, TDS and sulfides) that should be treated separately before its discharge. They were able to develop a wastewater treatment based on the Fenton technique for the oxidation of organic matter in water employing H_2O_2 and Fe^{2+} (Fenton reagent). This method was followed by membrane filtration and showed to be effective for the non-biodegradable portions of wastewater. The final effluent could meet the permissible limits of the unrestricted water reuse according to the Egyptian Environmental Association Affair (EEAA). El-Khateeb et al.[94] employed the electrocoagulation process assisted by chemical oxidation to treat chrome tanning effluents. The electrocoagulation treatment consists in forming an agglomeration (that can be subsequently separated by filtration) of the suspended matter by means of an electrical current. H_2O_2 was used to support

the oxidation process. This methodology permitted a 99.99% of chromium removal and 99.97% of sulfides removal. In further studies, El-Khateeb et al.[95] combined chemical coagulation process (using FeCl_3 and lime) and an aerobic reactor to treat effluents generated from beam house operations. The developed system efficiently reduced the COD, BOD and sulfides levels: 96.1%, 85.2% and 99.3% removal, respectively. The final treated effluent could be used for irrigation of trees or reused in the tanning industry.

Another option to minimize the environmental problems caused by chrome tanning is the extraction of chromium from leather scrap. Ferreira et al[96]. developed a method for the acidic extraction of chromium, using concentrated H_2SO_4 , recovering 30-60% of chromium and rendering the residue suitable for anaerobic biodegradation. Similarly, Popiolsky and her coworkers[97] achieved 98% of chromium removal from residual leather tannery via an EDTA ultrasound-assisted extraction. Additionally, they proposed EDTA's recovery by adding H_2SO_4 to precipitate EDTA- H_4 .

On the other hand, alternatives to conventional chrome tanning have also been explored. Some of these alternatives include: high exhaustion chrome tanning using exhaust aids or high exhaust tanning salts [10, 98], ultrasound assisted chrome tanning which improves chromium requirement and reduces shrinkage [99], microwave/ultrasound assisted chrome tanning which additionally decreases tanning time [100], salt-free pickling chrome tanning which diminishes the discharge of COD and Total Dissolved Solids (TDS) [101], and enzyme assisted vegetable tanning which enhances the diffusion of vegetable tannins into the leather fibers [15].

Still, chrome tanning remains the preferred alternative due to the versatility of the resulting leather. Ultrasound assisted [99] and salt-free pickling chrome tanning [101] seem to be promising alternatives as they maintain the good quality of the leather. On the other hand, high exhaustion and microwave/ultrasound assisted chrome tanning resulted in some properties that were inferior than the

conventional technique, such as thermal stability and non-uniform distribution of chromium [10, 100].

Eco-friendly chrome-free tanning is another alternative to conventional chrome tanning. This approach has been attempted with different tanning agents such as titanium salts[102], natural and unnatural amino acids[103–105], and an aluminum sulfate - composite material[106]. Leathers obtained by way of these tanning agents have shown good qualities, comparable or superior to the properties of chrome-tanned leather. The exploitation of these promising approaches could help reduce the environmental impacts of tanning industry.

Regarding chromium determination, there is no current standard describing a technique to extract and quantify Cr(III) and Cr(VI) simultaneously in leather products coming from chrome tanning. Hence, it is of particular importance to develop a protocol that takes into account the possible interconversion processes, eventually through the use of SIDMS. The production of CRMs for speciation of Cr in leather articles would also represent a huge step on the mitigation of these obstacles.

Conclusion

In the leather manufacturing process, chrome tanning is the most common tanning system due to the quality of the final products. However, the environmental impacts of this process have encouraged researchers to find cleaner alternatives to conventional chrome tanning. Even if only trivalent chromium salts are used during chrome tanning, hexavalent chromium can still be found in leather, which represents a risk to human health. Additionally, natural ageing of the leather could also favor its formation. The European regulation restricts Cr(VI) content to 3 mg/kg in leather articles coming into contact with the skin. As the oxidation state of chromium is determined by redox potential and pH, the current international standard for hexavalent chromium determination in leather goods should consider any possible interconversion between Cr(III) and

Cr(VI) during the extraction and analysis procedure. The introduction of isotope dilution techniques and the production of CRMs could help mitigate this struggle. The review of the analytical methods for chromium extraction and quantification showed that most of the studies have been performed in foodstuffs and environmental samples, which is comprehensible. However, more attention should be given to leather articles in order to develop more reliable analytical protocols that monitor possible interconversions and reveal the real health risk consumers are exposed to. Further regulations must also contemplate the evolution of hexavalent chromium over time.

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Conflict of interest

The authors declare no conflict of interest.

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Chapter 2

Chapter 2. Methodology

The second chapter of this thesis presents the methodology followed throughout the experimental part of the project.

The first section of this chapter describes the general procedure for the pretreatment of the samples, which includes sample collection and preparation procedure, as well the reagents and equipment used for the different extraction conditions.

Subsequently, in the second section, the methodology for the separation and quantification of the extracted species by Ion Chromatography - Inductively Coupled Plasma - Mass Spectrometry (IC-ICP-MS) is described, with a presentation of the substances and equipment used. Some theoretical considerations are discussed to understand the results obtained (Ion Chromatography (IC), Inductively Coupled Plasma - Mass Spectrometry (ICP-MS), speciation). Several conditions were tested in order to achieve the good separation of trivalent and hexavalent chromium species, once complexed or not. These conditions include two different mobile phases (various concentrations and pH) and two different chromatographic columns. The specifications and characteristics of both columns are also presented in this section.

Finally, the third section explains the basic principles of Fourier-Transform Ion Cyclotron Resonance Mass Spectrometry (FTICR), a technique that was useful for the elucidation of the structure of the complex formed during the ethylenediaminetetraacetic acid (EDTA) extraction. The specifications of the equipment and the conditions of the analyses are exposed as well.

Figure 1 shows a process flow diagram of the general outline of the methodology. On the same sample, a direct mineralization was first achieved to give access to the total amount of chromium in the leather sample. Then, various

conditions were tested with the aim of extracting as quantitatively as possible all chromium species, while maintaining the speciation. To evaluate the best extractions conditions (in terms of extraction rate), each protocol was applied, and measurements were done by Inductively Coupled Plasma - Atomic Emission Spectrometry (ICP-AES). Then, for the selected conditions, subsequent analysis of the extract was done by IC-ICP-MS to elucidate the speciation.

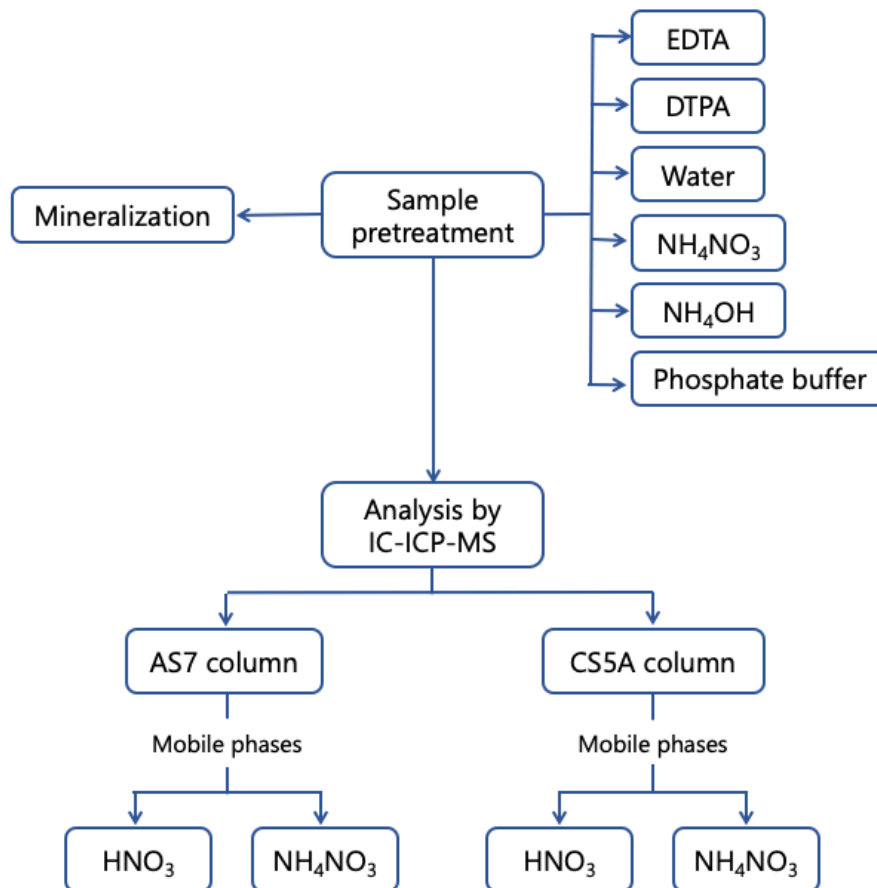


Figure 1. Process flow diagram of the general methodology of extraction and quantification of chromium species in leather samples.

2.1 Sample pretreatment

2.1.1 Instrumentation

Extraction and mineralization of the samples were performed with a Milestone ETHOS 1 microwave digestion system (shown in Figure 2) equipped with a temperature sensor. TFM reactors were used with a maximum pressure of 100 bar. ICP-AES measurements were performed on an iCAP 6300 series (Thermo Fisher Scientific) using the axial view. This instrument can be appreciated in Figure 3. The quantification of chromium was performed at 283.5 nm. As example, optimized conditions for the plasma and nebulizer for ICP-AES are given in Table 1.

Table 1. Optimized operating conditions for ICP-AES.

	iCAP 6300 ICP-AES
Radio frequency power (W)	1050
Plasma gas flow rate (L/min)	14
Auxiliary argon flow rate (L/min)	1.0
Nebulizer gas flow rate (L/min)	0.5
Purge gas (nitrogen) flow rate (L/min)	12
Peristatic pump rate (rpm)	50
Sample uptake delay (s)	30
Replicates	3
Rinse time (s)	30
Sampler	ASX520-CETAC
Chamber	Cyclonic
Nebulizer type	Concentric

The pH of the solutions was measured using a PHM240 pH/Ion Meter (MeterLab, Radiometer Analytical, Villeurbanne, France). Centrifugation of the extracts was performed with an Eppendorf Centrifuge 5702. Alkaline and ammonium nitrate extractions were performed using an Elmasonic S ultrasonic bath (Elma, Singen, Germany).

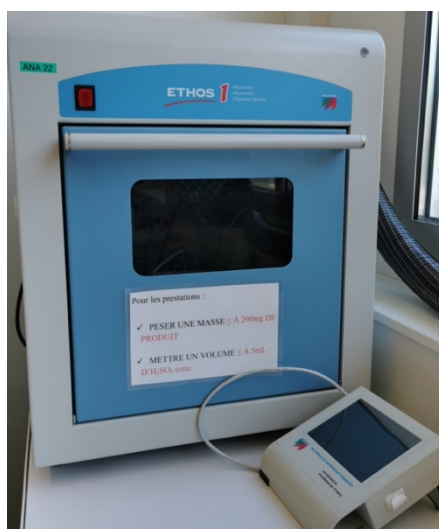


Figure 2. ETHOS 1 Microwave Digestion System from Milestone.

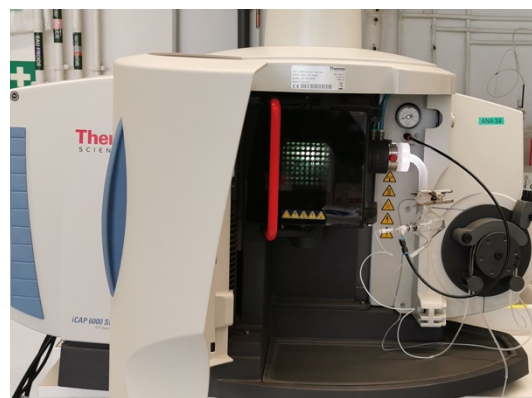


Figure 3. iCAP 6300 ICP-AES from Thermo Fisher Scientific.

2.1.2 Materials

Ultrapure water (resistivity 18.2 MW•cm), obtained from a Milli-Q system (Millipore, Bedford, MA, USA), was used for preparation of all solutions unless stated otherwise. Nitric acid 67-69% (Ultrapure Normatom, VWR Chemicals, Leuven, Belgium) was used for the mineralizations. For the extractions, EDTA (Analytical reagent, VWR Chemicals, Leuven, Belgium), diethylenetriaminepentaacetic acid (DTPA) (97%, Aldrich-Chemie, Germany), NH₄OH 20-22% NH₃ (Optima grade, Fisher Scientific, UK), NH₄NO₃ (Normapur guaranteed reagent, Prolabo, Paris, France), K₂HPO₄·3H₂O (AnalaR Normapur, VWR Chemicals, Leuven, Belgium) and phosphoric acid (85 wt. % in H₂O, Sigma-

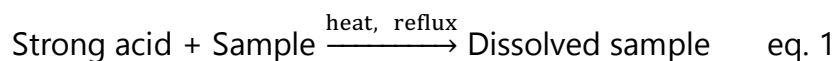
Aldrich, Germany) were employed. Filtration was performed with 0.45 μm nylon syringe filters (Fisher Scientific). Potassium dichromate (Normapur guaranteed reagent, Prolabo, Paris, France) was used for preparing calibration standards and spikes. Leather samples came from the luxury industry and were all chrome-tanned and colored.

2.1.3 Principle

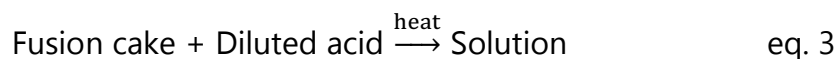
A digestion (or mineralization) is a sample preparation technique that permits the quantitative dissolution of the species to be analyzed, the reduction of interferences and the concentration of the sample. Some drawbacks might be an incomplete dissolution, the loss of a part of the analyte (if volatile), the presence of the analyte in the dilution solvent, and the presence of impurities (container, solvent, etc.). There are different types of mineralization^[1].

For mineral samples:

- Acidic digestion: It uses concentrated acids or mixtures thereof, such as HNO_3 at 65% (14 M), H_2SO_4 at 98% (18 M) or HCl at 32% (10M). In these cases, a counterion is introduced (NO_3^- , SO_4^{2-} , Cl^-).

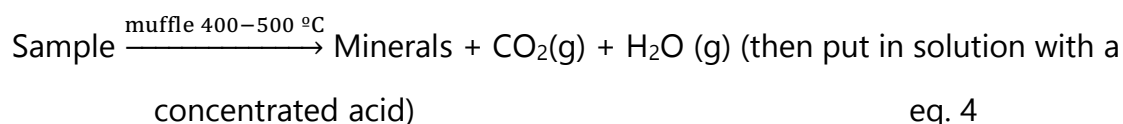


- Alkaline fusion: Specially used for silicate minerals and rocks. Examples of fluxes: pure NaOH , pure Na_2CO_3 and pure K_2CO_3 .

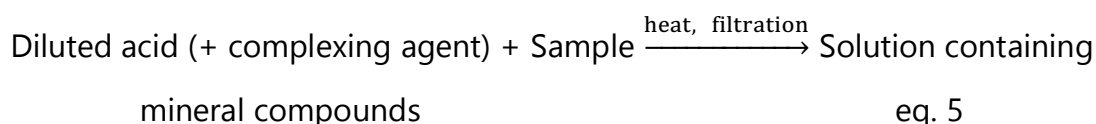


For organic samples:

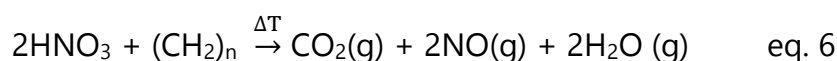
- Dry ashing: It permits to ignite and oxidize organic compounds by air at high temperatures and at atmospheric pressure. The advantage is the low volume of reagents (reduces contaminations by solvent) while the inconvenient is the loss by volatilization.



- Wet digestion: It involves the chemical degradation of sample matrices in solution. Microwave systems can be used, which allow rapid heating and limit the loss of the analyte by volatilization (closed reactor). A disadvantage could be the investment in the device.



In the case of nitric acid:



During this thesis, a microwave assisted acidic digestion was performed for each leather sample in order to decompose the organic matter and being able to determine the total chromium content by ICP-AES, and to have it as a reference for the calculation of the extraction rates of the different extraction conditions that were tested.

2.1.4 General procedure

2.1.4.a) Sample preparation

As previously mentioned, leather samples came from the luxury industry and were all chrome-tanned and colored. However, they have gone through different manufacturing processes. Therefore, different types of leather were used for the extractions, such as: chrome-tanned, colored, polished and satin leathers.

Dry leather samples were cut into small pieces (3 to 5 mm per side) in accordance with the International Organization for Standardization (ISO) 4044:2017, 6.3^[2] as depicted in Figure 4. They were cut in the laboratory and immediately after, they were stored in resealable plastic bags. This was done for each sample in the same way during the study.

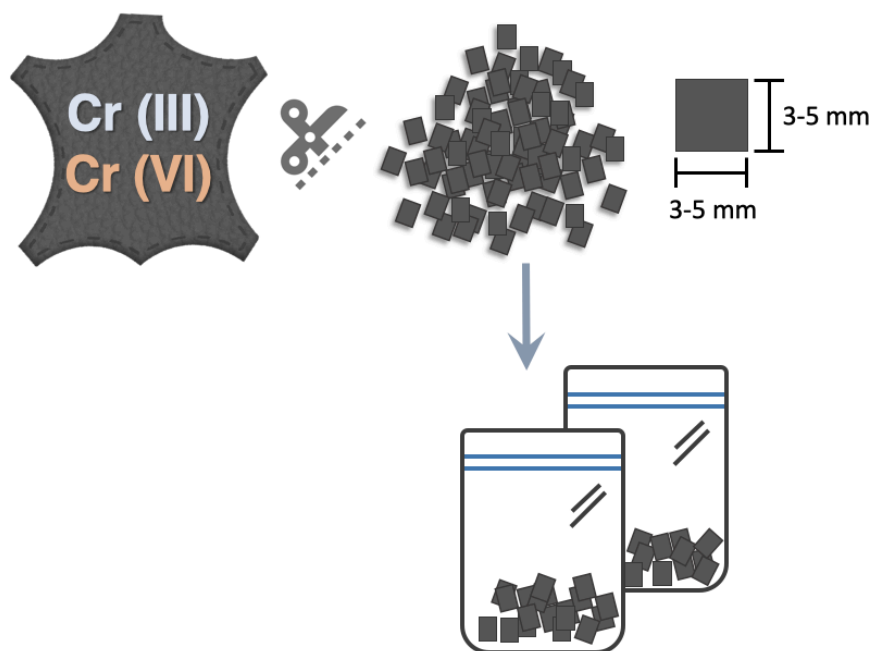


Figure 4. Depiction of sample preparation.

2.1.4.b) Extractions

Different extraction conditions were tested: EDTA, DTPA, NH_4OH , NH_4NO_3 , water and phosphate buffer. In order to calculate the extraction rate of each procedure, the extracts were mineralized and the amount of total chromium was quantified by ICP-AES. Therefore, the extraction rate consisted in the ratio between the milligrams of extracted chromium and the milligrams of total chromium obtained from the direct mineralization of the same sample. The different extraction conditions that were tested are subsequently described.

EDTA extraction

An amount of 200 mg of leather was extracted with 4 mL of EDTA 50 mmol/L (adjusted to pH 10 with NH_4OH) at 100 °C in a microwave system: 10 minutes from room temperature (21 °C) to 100 °C, 30 minutes at 100 °C (Figure 5). Once the extraction was over, the samples were collected when the temperature of the

microwave digestion system was below 50 °C. The extract was afterwards centrifuged for 15 min at 4000 rpm, the supernatant was collected and mineralized for subsequent total Cr quantification.

DTPA extraction

Similarly, for the DTPA extraction 200 mg of leather were extracted with 4 mL of DTPA 50 mmol/L (adjusted to pH 10 with NH₄OH) at 100 °C in a microwave system: 10 minutes from room temperature (21 °C) to 100 °C, 30 minutes at 100 °C (Figure 5). Once the extraction was over, the samples were collected when the temperature of the microwave digestion system was below 50 °C. The extract was afterwards centrifuged for 15 min at 4000 rpm, the supernatant was collected and mineralized for subsequent total Cr quantification.

Alkaline extraction

For the alkaline extraction, 500 mg of leather were extracted with 10 mL of NH₄OH at ultrasonic bath for 1 hour, as previously described by Vacchina et de la Calle^[3] for dry food samples. The extract was centrifuged for 15 min at 4000 rpm and filtered using a nylon 0.45 µm filter. Subsequent digestion of the extract was performed for total Cr quantification.

Ammonium nitrate extraction

An amount of 500 mg of leather was extracted with 10 mL of NH₄NO₃ 0.1 M at ultrasonic bath for 1 hour. After the ultrasonic assisted extraction, the solution was centrifuged for 15 min at 4000 rpm and filtered using a nylon 0.45 µm filter. The digestion of the extract was performed for total Cr quantification.

Water extraction

An amount of 200 mg of leather was extracted with 4 mL of ultrapure water at 100 °C in a microwave system: 10 minutes from room temperature (21 °C) to 100

°C, 30 minutes at 100 °C (Figure 5). Once the extraction was over, the samples were collected when the temperature of the microwave digestion system was below 50 °C. The extract was centrifuged for 15 min at 4000 rpm and the supernatant was collected. Then, the supernatant was mineralized for total Cr quantification.

Phosphate buffer extraction

In order to evaluate the ISO 17075^[4, 5] standardized method, 500 mg of leather were extracted with 25 mL of $K_2HPO_4 \cdot 3H_2O$ at pH 8.0 (adjusted with phosphoric acid) for three hours under magnetic stirring. The extracts were subsequently filtrated through nylon 0.45 μm filter and mineralized for total Cr determination.

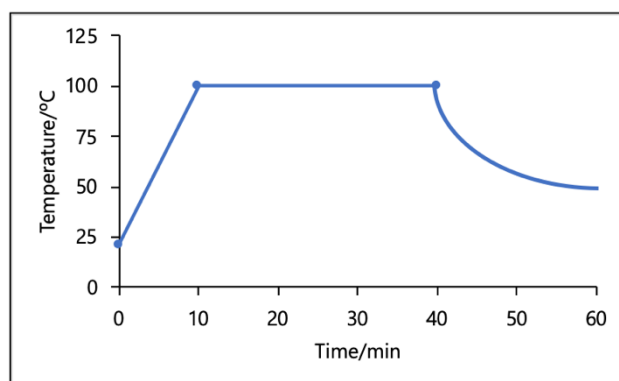


Figure 5. EDTA, DTPA and water extraction program.

2.1.4.c) Mineralization

Direct mineralization of leather

Acidic digestion (or mineralization) was performed using a microwave digestion system. It permits the decomposition of the organic matter allowing the total chromium quantification by ICP-AES.

The procedure was as follows:

- 1) An amount of 200 mg of leather was digested with 2 mL of nitric acid at 150 °C in a microwave system: 10 minutes from room temperature (21 °C) to 150 °C, 30 minutes at 150 °C. Once the mineralization was over, the samples were collected when the temperature of the microwave digestion system was below 50 °C (Figure 6).
- 2) After the mineralization, the digest was diluted up to 50 mL with milli-Q water.
- 3) For total Cr determination by ICP-AES, 1.25 mL of the last solution were diluted up to 25 mL with water. Calibration standards were freshly prepared with potassium dichromate and they were acidified at the same level as the samples. In this part, as total chromium is determined, no matter if Cr(VI) is transformed into Cr(III) under acidic conditions. The most important is to have the same nitric acid amount in the standards and the samples.

This was done for each sample in the same way in order to know its total chromium content and to be able to calculate the extraction rate for the different extraction conditions.

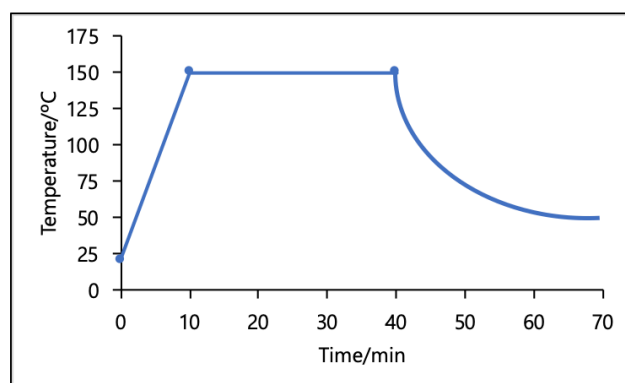


Figure 6. Mineralization program.

Mineralization of the extracts

After testing the different extraction conditions, the supernatants were mineralized to subsequently quantify by ICP-AES the total amount of chromium

extracted by the different media (EDTA, DTPA, NH_4OH , NH_4NO_3 , water, phosphate buffer).

The procedure was as follows:

- 1) Two milliliters of the extract (EDTA, DTPA, NH_4OH , NH_4NO_3 , water, or phosphate buffer extraction) were digested with 2 mL of nitric acid at 150 °C in a microwave system: 10 minutes from room temperature (21 °C) to 150 °C, 30 minutes at 150 °C (Figure 6). Once the mineralization was over, the samples were collected when the temperature of the microwave digestion system was below 50 °C.
- 2) After the mineralization, the digest was diluted up to 50 mL with water.
- 3) For total Cr determination by ICP-AES, 1.25 mL of the last solution were diluted up to 25 mL with water. Calibration standards were freshly prepared with potassium dichromate and they were acidified at the same level as the samples.

2.2 Separation and speciation

2.2.1 Instrumentation

Chromatographic separation was performed using a Dionex ICS-6000 HPIC System (Thermo Fisher Scientific) shown in Figure 7. A Dionex ADRS 600 Anion Dynamically Regenerated Suppressor (2 mm) (Thermo Fisher Scientific) was set before the entrance into the ICP-MS to avoid salt deposition and was operated in the external regeneration mode. A constant flow of 0.6 mL/min of ultrapure water was supplied by means of a Dionex AXP auxiliary pump (Thermo Fisher Scientific). Two guard columns were placed before the analytical column: a Dionex IonPac NG1 (2 × 50 mm, particle size: 10 µm) and a Dionex IonPac AG7 (2 × 50 mm, particle size: 10 µm). Ions were separated with a Dionex IonPac AS7 column (2 × 250 mm, particle size: 10 µm). The analytical column was coupled to the nebulizer

of the ICP-MS using a PEEK tubing (0.25 mm i.d., 150 cm long). Detection of chromium species was performed on an iCAP RQ ICP-MS instrument (Thermo Fisher Scientific) (Figure 8) with a collision/reaction cell. Two different measurement modes were used: standard mode (STD) and kinetic energy discrimination mode (KED). In the KED mode, He gas was injected at 10 mL/min in the collision/reaction cell in order to remove unwanted polyatomic interferences (possible interference in mass 52 (Ar + C) because of the organic matter in leather). In general, the STD mode provides a better sensitivity. However, because the KED mode is more specific, it was used for the analysis of real samples. Data processing was performed using ThermoFisher Scientific Qtegra software. As example, optimized conditions for the plasma and nebulizer for ICP-MS are given in Table 2. The pH of the solutions (mobile phase and samples) was adjusted using a PHM240 pH/Ion Meter (MeterLab, Radiometer Analytical, Villeurbanne, France).

Table 2. Optimized operating conditions for ICP-MS.

	iCAP RQ ICP-MS
Radio frequency power (W)	1550
Plasma gas flow rate (L/min)	14
Auxiliary argon flow rate (L/min)	0.8
Nebulizer gas flow rate (L/min)	1.0
Purge gas (nitrogen) flow rate (L/min)	-
Peristaltic pump rate (rpm)	15
Sample uptake delay (s)	30
Replicates	3
Rinse time (s)	30

Sampler	SC-2DX (with <i>FAST</i> valve system) or online coupling with IC system
Chamber	Cyclonic
Nebulizer type	Concentric

A second column was tested when trying to optimize the separation conditions. For these experiments, a Dionex IonPac CG5A guard column (2 × 50 mm, particle size: 9 μm) and a Dionex IonPac CS5A ion-exchange column (2 × 250 mm, particle size: 9 μm) were installed. The setup will be later described.



Figure 7. Dionex ICS-6000 HPIC System from Thermo Fisher Scientific.



Figure 8. iCAP RQ ICP-MS from Thermo Fisher Scientific.

2.2.2 Materials

Two mobile phases were tested. The first mobile phase, which corresponds to the optimized analysis conditions, was prepared with NH_4NO_3 75 mmol/L (Normapur

guaranteed reagent, Prolabo, Paris, France) and adjusted to pH 3.0 with HNO₃ 67-69% (Ultrapure Normatom, VWR Chemicals, Leuven, Belgium). HNO₃ 1.1 mol/L was used as the second mobile phase. Preliminary tests were performed with HNO₃ 1.9% as mobile phase. ⁵³Cr(III) (97.01%) and ⁵⁰Cr(VI) (97.36%) chromium isotopic standards (100 ppm, Certified Reference Material, ISC Science, Oviedo, Spain) were diluted and used to monitor possible chromium interconversions.

2.2.3 Principle

2.2.3.a) Ion Chromatography

This thesis is going to focus on ion chromatography, more specifically, ion-exchange chromatography. Ion chromatography can be subdivided into ion-exchange chromatography, ion pair chromatography and ion exclusion chromatography. However, ion-exchange chromatography is the predominant form of ion chromatography and it is based on ionic (electrostatic) interactions between ionic and polar analytes, eluent ions and ionic groups fixed to the stationary phase. Its mechanism is based on competitive ionic binding (attraction). In other words, it is based on the formation of ionic bonds between charged groups of the analyte and an oppositely charged ion-exchange support. Ion-exchange chromatography was first reported by Thompson in 1850^[6]. Nowadays, it is extensively used for the separation and determination of peptides, proteins, nucleic acids and related biopolymers^[6]. It is going to be called hereafter simply "ion chromatography". This technique has several advantages but also some limitations, some of them are listed in Table 3.

Table 3. Strengths and limitations of ion chromatography. Based on the information obtained from [7].

Strengths	Limitations
<ul style="list-style-type: none"> • High resolving power • Fast separations • High recoveries • Buffers are non-denaturing (in the case of proteins determination) • The process can be used as a preconcentration step 	<ul style="list-style-type: none"> • Samples must be injected under low ionic strength and controlled pH of the mobile phase, which may require an additional buffer exchange step • Chromatographic instrumentation should be resistant to salt-induced corrosion • Post-chromatographic concentration of dilute solutions may result in high salt concentration • Coupling with MS can be limited due to high salt concentration in the mobile phase

The mechanism of ion chromatography is the following: analyte ions and similarly charged eluent ions compete to bind to the oppositely charged ions (e.g. H^+ , OH^- , Na^+ , K^+ , Cl^- , Ca^{2+} , Mg^{2+} , SO_4^{2-} , PO_4^{3-} , NR_2H^+ , COO^- , etc.) on the surface of the stationary phase (see Figure 9). Cations are separated on cation-exchange columns, and anions are separated on anion-exchange columns.

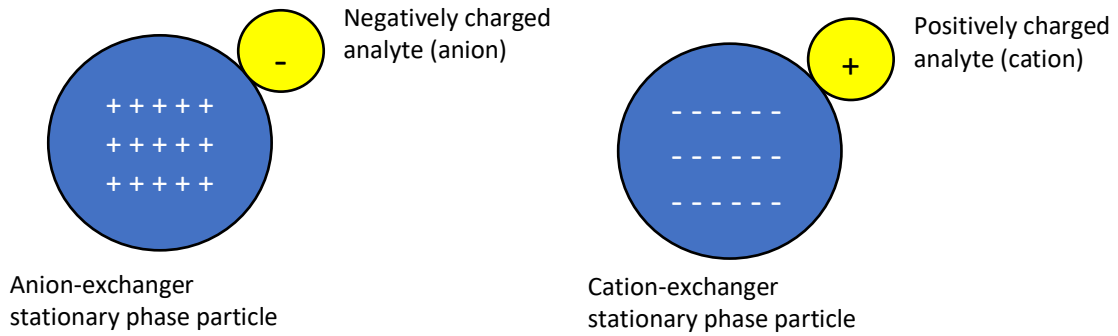
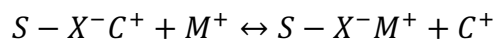


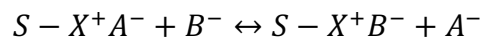
Figure 9. Anion and cation ion-exchangers.

When the exchanging ions (analyte and eluent ions) are cations, it is called cation chromatography and the competition is the following:



In this case, the cation of the eluent, M^+ , replaces the analyte cation, C^+ , bound to the anion, X^- , which is fixed to the chromatographic support, S .

In anion chromatography, the exchanging ions (analyte and eluent ions) are anions and the competition is the following:



In this case, the anion of the eluent, B^- , replaces the analyte anion, A^- , bound to the cation, X^+ , which is fixed to the chromatographic support, S ^[6].

There are five steps to the process of ion chromatography^[8]:

1. **Equilibration:** The stationary phase is washed with the initial eluent composition until the baseline is stabilized and the eluent pH remains constant.
2. **Loading:** The sample is dissolved in the eluent and injected into the column.
3. **Wash:** The column is washed again with the initial eluent to remove uncharged molecules and molecules with the same charge as the stationary phase.

4. **Elution:** A gradient or isocratic mode is used to elute the bound analytes as the eluent ions compete and replace the analytes.
5. **Column regeneration:** The column capacity is restored for the next injection by washing out any molecules bound to the stationary phase.

The more the analyte interacts with the functional groups of the stationary phase, the longer its retention time will be. This depends on^[9]:

- **The ionic charge:** As the charge of the analyte increases, its retention time increases as well.
- **The size of the ion:** Retention time increases with increasing ionic radius.
- **The polarization of ions:** Ions which can be polarized easily have higher retention time.
- **The ionic strength:** The larger the ionic strength, the weaker the interaction with the resin, and therefore the smaller the retention time.
- **The column temperature:** Increasing the column temperature reduces the viscosity of the mobile phase, and thus reduces the retention time (most of the time).
- **The pH of the mobile phase:** Specially for weak anion and cation exchangers.
- **Flow rate:** Ions elute faster with higher flow rates but separation efficiency decreases (once above the optimal flow rate).

The main components of an ion chromatography system are depicted in Figure 10 and are the following^[6]:

- A high-pressure pump that delivers the eluent
- An injector for introducing the sample into the eluent stream
- A separation column
- An oven (optional)
- A detector (conductivity detector, UV detector, ICP-MS) to measure the analyte peaks as they elute from the column

- A data system for data processing and analysis

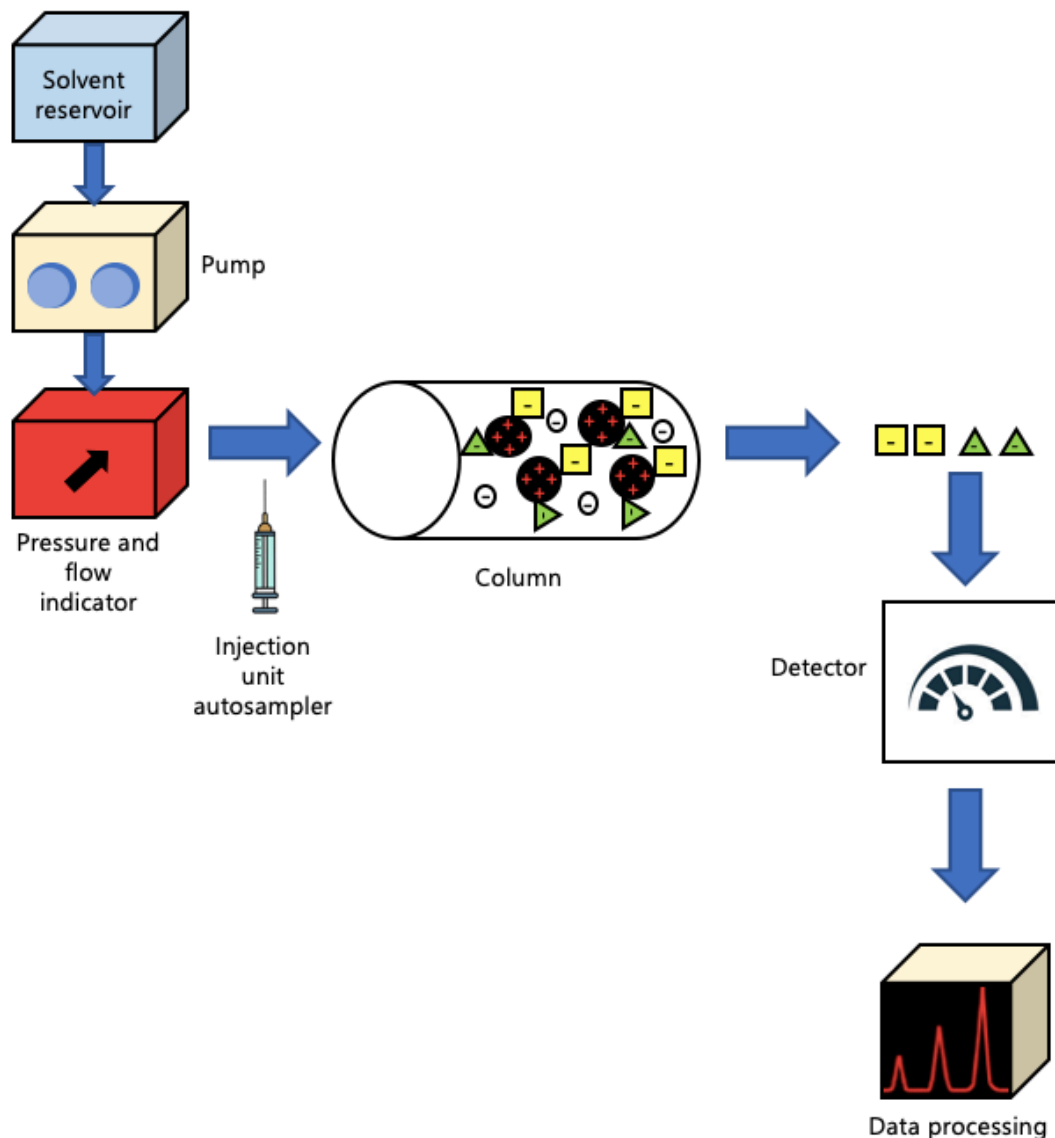


Figure 10. Diagram of an ion chromatography system. Modified from [6].

Columns

Regarding chromatographic columns, they can be classified as strong and weak anion and cation exchangers. Strong anion and cation exchangers maintain their ion exchange capacity over a broad pH range (they do not accept or lose protons with changes in the eluent pH), while weak anion and cation exchangers work over a limited pH range. Table 4 shows different ion-exchange groups. Moreover, ion-exchange columns are packed with porous, non-porous, inert, polymeric resin

or gel beads with ion-exchange groups bonded to them. The porosity and size of the polymeric resin impacts the resolution of the separation. Small particle size beads improve resolution but increase the system backpressure^[8]. The adequate selection of the packing material plays an important role in the ion chromatography process.

Table 4. Different types of ion-exchange columns. Modified from ^[8].

Column type	Column	Functional group	pH range
Strong anion exchanger (SAX)	Quaternary ammonium salts	$(\text{CH}_3)_3\text{N}^+\text{CH}_2\text{O}^-$	0-14
Weak anion exchanger (WAX)	Diethylamino ethyl	$(\text{C}_2\text{H}_5)_2\text{NCH}_2\text{CH}_2\text{O}^-$	2-9
Strong cation exchanger (SCX)	Sulfopropyl	$-\text{OCH}_2\text{CH}_2\text{CH}_2\text{SO}_3^-$	0-14
Weak cation exchanger (WCX)	Carboxymethyl	$-\text{OCH}_2\text{COO}^-$	2-9

A Dionex IonPac NG1 (2 × 50 mm, particle size: 10 μm) guard column was used before the AG7/AS7 columns (first setup) and before the CG5A/CS5A columns (second setup). The stationary phase of the NG1 guard column is divinylbenzene and the packing material is a macroporous ethylvinylbenzene polymer. It is a reverse-phase guard column resistant to solvents, acids and bases, which permits the use of eluent from pH 0 to 14^[10]. During this thesis project, this column was used to protect the analytical column by trapping the non-polar and hydrophobic co-extracted colorants from the leather samples.

The analytical column that was used for the optimized conditions was a Dionex IonPac AS7 column (2 × 250 mm, particle size: 10 μm). A Dionex IonPac AG7 (2 × 50 mm, particle size: 10 μm) guard column was placed between the NG1 guard column and the AS7 analytical column. Both the AG7 and the AS7 columns

are made up of a polymer packing with 5% divinylbenzene crosslinking, the ion-exchange group is alkyl quaternary ammonium, they have a medium-high hydrophobicity and a solvent compatibility from pH 0 to 14. These columns contain both anionic and cationic ion-exchange capacity and are ideal for separation of polyvalent anions and chelating agents in complex sample matrices^[11].

For the second setup that was tested, a Dionex IonPac CG5A guard column (2 × 50 mm, particle size: 9 μm) and a Dionex IonPac CS5A ion-exchange column (2 × 250 mm, particle size: 9 μm) were installed. These columns are made up of a polymer packing with 10% divinylbenzene crosslinking, they have two ion-exchange groups: sulfonic acid (cation-exchange group) and quaternary ammonium (anion-exchange group) and are compatible with typical HPLC solvents up to 50%. They have been designed for the determination of lanthanides and transition metals^[12].

Suppressors

The conductometric detector is the most popular detector in IC. In the mid-1980s, suppressors surged as a method to separate analyte ions against the background of eluent ions, as the eluent is also an electrolyte. In order to reduce the conductivity of the solution in the suppressor, the eluent and the determined ions are converted into compounds that differ significantly in electric conductivity. When anions are determined, the cation-exchanger in the H⁺ form is placed in the suppressor. When cations are determined, the anion-exchanger in the OH⁻ form is placed in the suppressor. The cation-exchanger exchanges the H⁺ ions for the eluent cations, the eluent is converted into a weakly dissociated acid and the determined anions are converted into strongly dissociated acids. Analogous reactions take place in the suppressor for cations determination.

The conductivity measured with a conductometric detector is defined as follows:

$$K_{measured} = c_{sample}^+ \cdot |\Lambda_{sample}^+ - \Lambda_{eluent}^+| + c_{sample}^- \cdot |\Lambda_{sample}^- - \Lambda_{eluent}^-| \quad \text{eq. 7}$$

Where:

c_{sample}^+ is the cation concentration in the sample,

$|\Lambda_{sample}^+ - \Lambda_{eluent}^+|$ is the difference in the mobility of the sample and eluent cations,

c_{sample}^- is the anion concentration in the sample, and

$|\Lambda_{sample}^- - \Lambda_{eluent}^-|$ is the difference in the mobility of the sample and eluent anions

When anions are determined with suppressed ion chromatography, the sample and eluent cations are exchanged for H^+ ions in the suppressor, and the measured conductivity is calculated as follows:

$$K_{measured} = c_{sample}^- \cdot |\Lambda_{sample}^- + \Lambda_{H^+}| \quad \text{eq. 8}$$

When cations are determined with suppressed ion chromatography, the sample and eluent anions are exchanged for OH^- ions in the suppressor, and the measured conductivity is calculated as follows:

$$K_{measured} = c_{sample}^+ \cdot |\Lambda_{sample}^+ + \Lambda_{OH^-}| \quad \text{eq. 9}^{[13]}$$

Suppressors have three main functions:

- 1) To remove conductive ions, thus reducing the background and increasing the sensitivity.
- 2) To remove counter ions in the sample.
- 3) To convert the determined ion into a more conductive form, thus increasing its sensitivity^[14].

Diagram in Figure 11 shows the difference between suppressed and non-suppressed conductivity in anion separation using KOH as eluent. Diagram in Figure 12 shows the setup of an ion chromatograph using suppressed conductivity detection, which reduces the conductivity of the eluent and increases the signal-to-noise ratio (S/N) of the analytes^[15].

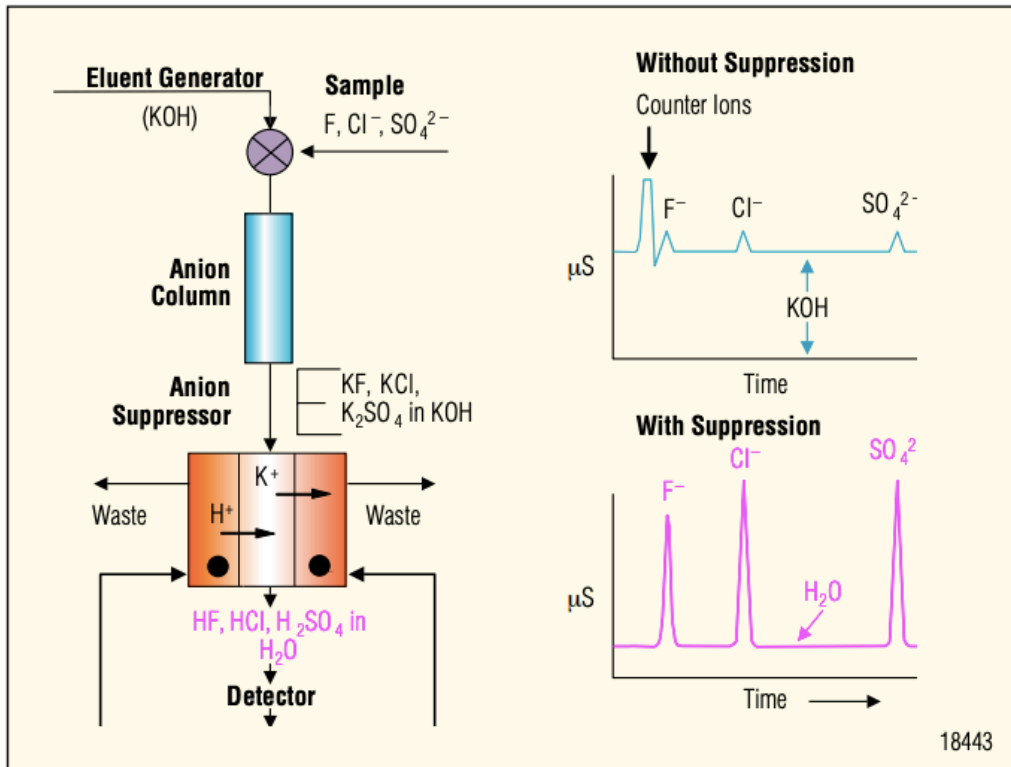


Figure 11. Difference between suppressed and non-suppressed anion chromatography. Obtained from [15].

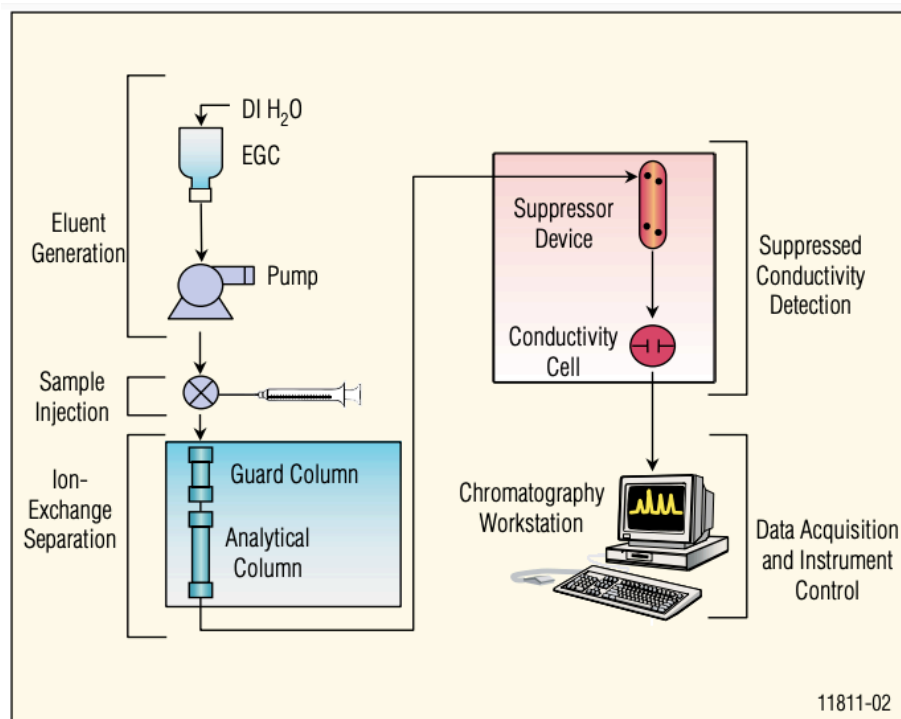


Figure 12. Setup for suppressed ion chromatography. Obtained from [15].

There are different types of suppressors. During this thesis project, we used a Dionex ADRS 600 Anion Dynamically Regenerated Suppressor (2 mm) from Thermo Fisher Scientific, which allows external water mode operation and enables analyses that require the hyphenation of IC with ICP-MS. Chromatographic separation was performed using a Dionex ICS-6000 HPIC System (Thermo Fisher Scientific) and the columns previously described. In our conditions, NH_4NO_3 (the eluent in the optimized conditions) was transformed into diluted HNO_3 as NH_4^+ was transformed into H^+ in the suppressor, allowing a good hyphenation with ICP-MS.

2.2.3.b) ICP-MS

Inductively Coupled Plasma - Mass Spectrometry is a type of mass spectrometry that uses a high temperature ionization source to determine metals and non-metals at very low concentration levels. ICP-MS is capable of simultaneously determining the majority of elements in the periodic table^[16]. It was developed in 1980 by Gray and Houk and first commercialized in 1983 by the Canadian company Sciex^[17]. Today, ICP-MS is a popular technique that has displaced several techniques such as atomic absorption or emission spectrometry (element-specific techniques).

An ICP-MS uses an argon plasma to convert samples into ions. Subsequently, ions are measured using a mass spectrometer, usually a quadrupole (other mass spectrometers are sector-based and time-of-flight (TOF) based instruments). In Figure 13 we can appreciate the main components of an ICP-MS instrument. ICP operates at atmospheric pressure, while the MS and detector operate in a vacuum chamber. Therefore, a vacuum pump, a vacuum interface and some "lenses" are required to focus the ions through the system^[18].

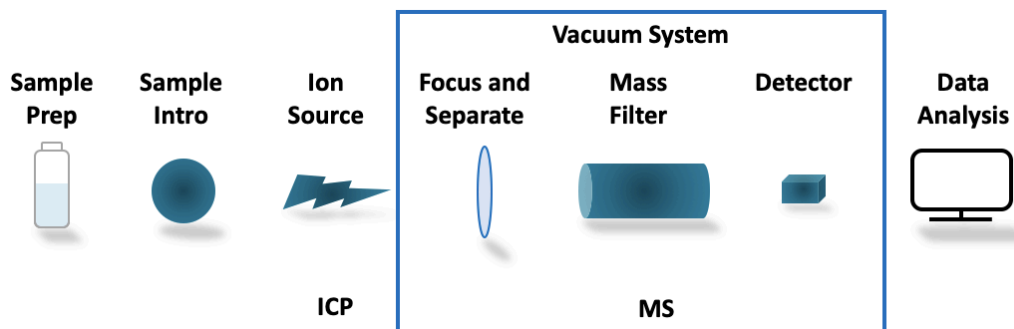


Figure 13. Main components of an ICP-MS instrument. Modified from ^[18].

The main components of a single quadrupole ICP-MS instrument are the following^[18] (Figure 14):

1. **Sample introduction system:** The sample solution is pumped into a nebulizer where it is transformed into a fine aerosol mist using a jet of argon gas. The aerosol mist passes through a spray chamber where larger droplets are removed. Fine droplets are carried to the ICP plasma torch by the argon gas flow.
2. **Plasma (ICP):** It is formed from argon gas that flows in a quartz tube where it is ionized by a radio frequency generator. Its objective is to dry, decompose, dissociate into individual atoms and ionize the aerosol droplets. The degree of ionization of an element in the plasma depends on the element's ionization potential and the plasma temperature. The amount of energy required to generate argon ions in this process is around 15.8 eV (first ionization potential). This energy is enough to ionize the majority of elements in the periodic table^[19].
3. **Vacuum interface:** Interface to extract the ions into the vacuum system (from the plasma to the mass spectrometer). It consists of a series of cooled metal cones (sampling cone and skimmer cone) with small holes in them to allow the ions to pass through.

4. ***Ion lens:*** It focuses the ions and separates them from background signals (photons and neutral particles extracted from the plasma).
5. ***Collision/reaction cell:*** To resolve the analyte ions from polyatomic interferences.
6. ***Mass spectrometer (MS):*** The quadrupole filters the analyte ions by mass to charge ratio (m/z) allowing only one m/z at a time to pass through the detector.
7. ***The electron multiplier detector:*** It uses a high voltage electrode or dynode so that ions that emerge from the quadrupole strike the dynode, and then electrons are released from the dynode surface. After several strikes with the multiple dynodes, a cascade of electrons is recorded as a count. The electron multiplier detector can detect individual ions, hence, very low concentrations.
8. ***Data processing:*** A data analysis software (ThermoFisher Scientific Qtegra software for this thesis project).

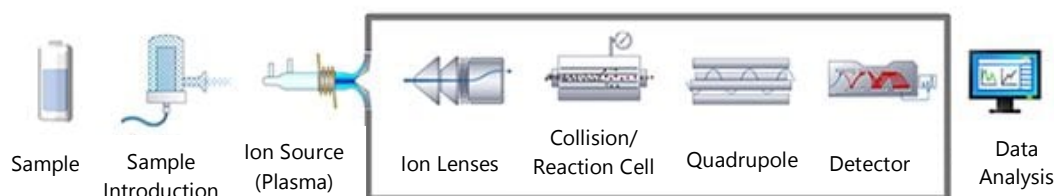


Figure 14. Main components of a single quadrupole ICP-MS instrument. Modified from ^[18].

Additionally, ICP-MS can analyze almost any sample type. The main applications of ICP-MS are^[18]:

- Liquid sample analysis
- Speciation analysis
- Nanoparticles and single cells
- Solid sample analysis
- Gas analysis

In ICP-MS two types of interferences exist: non-spectral interferences and spectral interferences. Non-spectral interferences can be subdivided into physical interferences and matrix effects. Physical interferences are related to the sample's viscosity and surface tension, and can affect the sample's transport through the autosampler probe, nebulizer and spray chamber. Additionally, the presence of sample matrix can suppress or enhance the analyte signal in comparison to standards without matrix. Samples with high total dissolved solids or organic matrices are examples of matrix interferences. By the other hand, spectral interferences are caused by ionic species with the same or very similar mass to that of the analyte. Spectral interferences can be isobaric or polyatomic. Isobaric interferences happen when naturally occurring isotopes exist for more than one element at the same mass (Ba^+ , Ce^+ , and La^+ at mass 138), or when a double-charged ion appears at the same mass as an analyte (i.e. Pb^{2+} on Rh^+ at mass 103). Polyatomic interferences surge when elements from the matrix combine each other or with plasma gases to form molecular ions with the same mass as the analyte^[20].

The principal disadvantage of ICP-MS is the formation of polyatomic interferences as a consequence of the interactions with the carrier gas, atmospheric gases and matrix ions. This obstacle can be minimized with the implementation of collision/reaction cells.

Collision/reaction cells are devices used in ICP-MS to remove interfering ions. They can be operated in two modes:

- *Reaction mode:* Which uses reaction gases to remove known interferences from each analyte isotope. It can remove some interferences very efficiently as the reactions occur quickly (few collisions are required)^[21]. The drawbacks of this mode are the following: 1) a reaction gas will only remove interferences that react with that gas, consequently some residual interferences may remain; 2) reaction gases will react with matrix, interference and analyte ions, so new interferences may appear; 3) the

reactive gas will react with some of the analyte ions, as well as the target polyatomic interferences, and this may cause signal loss for the analytes^[22].

- *Collision mode*: Which uses a non-reactive gas (e.g. helium) and a KED process to selectively reduce all polyatomic interferences based on their size. As a matter of fact, polyatomic ions are larger than analyte ions that share the same mass. Therefore, they collide with the cell gas more frequently, emerging with lower residual energy. Subsequently, these low energy ions are excluded from the ion beam by means of a bias voltage at the cell exit^[21]. In other words, at the cell entrance, the analyte and the polyatomic ion energies overlap. Energy spread of both group of ions is narrow. Energy loss from each collision with a helium atom is the same for the analyte and for the polyatomic ion, but the polyatomic ion is bigger so it collides more repeatedly. At the cell exit, ion energies no longer overlap, polyatomic interferences are rejected using bias voltage. Analyte ions have enough residual energy, polyatomic ions don't (energy discrimination) (Figure 15).

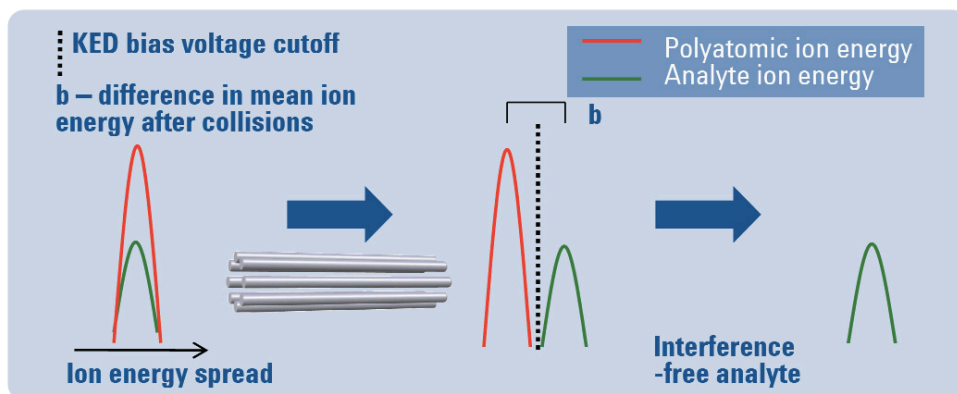


Figure 15. Principle for removing polyatomic interferences by KED mode. Obtained from ^[21].

KED mode needs multiple collisions before the difference in residual energy between the analyte ion and polyatomic ions is enough to separate them. The requirements for an effective collision are:

- A cell that operates with a light cell gas at high cell gas pressure (large number of collisions).
- An ion guide that maintains ion focusing across the mass range.
- Ions that enter the cell with a narrow spread of ion energy^[21].

When working with ICP-MS for chromium determination, polyatomic interferences can arise with the presence of carbon or chlorine. For $^{52}\text{Cr}^+$ the interferences could be $^{35}\text{Cl}^{16}\text{O}^{1}\text{H}^+$, $^{35}\text{Cl}^{17}\text{O}^+$ and $^{40}\text{Ar}^{12}\text{C}^+$; and for $^{53}\text{Cr}^+$ the interferences could be $^{37}\text{Cl}^{16}\text{O}^+$, $^{40}\text{Ar}^{13}\text{C}^+$ and $^{40}\text{Ar}^{12}\text{C}^{1}\text{H}^+$. These interferences can be reduced by using the ICP-MS in KED mode with He as collision gas^[23].

In this thesis project, detection of chromium species was performed on an iCAP RQ ICP-MS instrument (Thermo Fisher Scientific) fitted with a collision/reaction cell.

2.2.3.c) IC-ICP-MS

Separation and speciation were studied by IC-ICP-MS. While ion chromatography allows the separation of different ionic species, ICP-MS, on the other hand, allows the detection of specific isotopes. The IC-ICP-MS system allows to have information on the isotope and also in which ionic form the isotope is present.

Simply quantifying the total amount of an element does not provide complete information on the toxicity of the sample. To determine a sample's toxicity or safety, it is important to identify and quantify the different species of an element which may display different physicochemical properties.

Chemical speciation is defined by the International Union of Pure and Applied Chemistry (IUPAC) as follows:

- Chemical species: specific form of an element defined as to isotopic composition, electronic or oxidation state and/or complex or molecular structure.

- Speciation analysis: analytical activities of identifying and/or measuring the quantities of one or more individual chemical species in a sample.
- Speciation of an element: distribution of an element among defined chemical species in a system^[24].

In the case of chromium, speciation is necessary since trivalent chromium is considered as essential for glucose regulation while hexavalent chromium is highly toxic. The hyphenation of ion chromatography with ICP-MS is a powerful tool for the determination of different organic and inorganic compounds in a single run. By manipulating the conditions of the mobile phase, IC-ICP-MS can provide optimal separation for complex speciation^[25].

This hyphenation is suitable for:

- Speciation of metals, metalloids and non-metals
- Identification and quantification of heterorganic compounds with respect to the detectable heteroatoms (sulfur, phosphorous, silicon, bromine, iodine)^[13]

An IC-ICP-MS chromatogram for the detection of Gd complexes is shown in Figure 16. The advantages and disadvantages of ICP-MS as detector for ion chromatography are shown in Table 5.

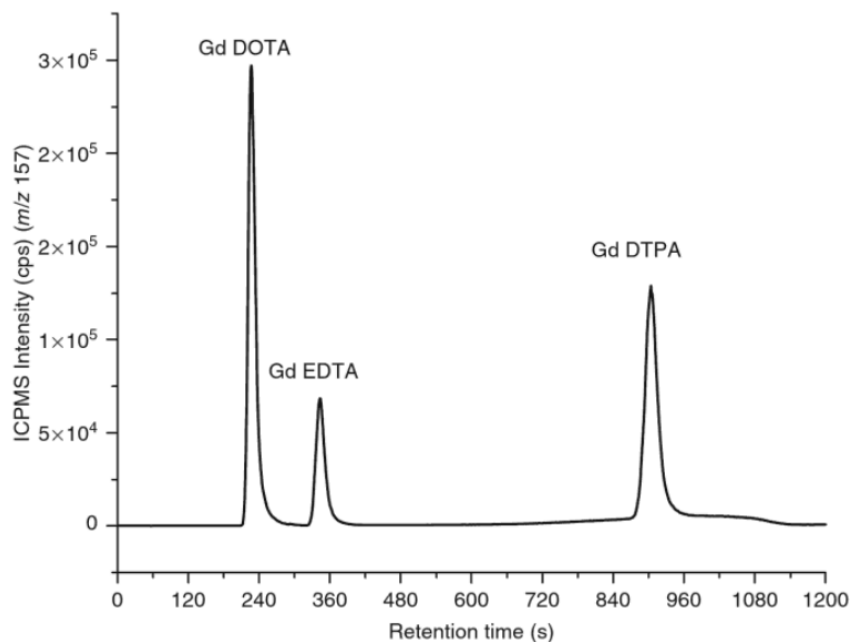


Figure 16. IC-ICP-MS chromatogram of Gd complexes. Detection: m/z 157 (Gd). Separation: anion-exchange column, ammonium acetate gradient, flow rate 1 mL/min. Obtained from ^[13].

However, identification of detected compounds is provided by only two parameters:

- 1) m/z , which identifies an element by its mass
- 2) retention time of the ionic species

Consequently, standard compounds (commercially available or synthesized) are required in order to precisely identify the analytes. Moreover, sensitivity depends highly on the composition of the mobile phase. High organic content in eluent (>20%) may cause an instable plasma. To prevent these problems, it is possible to add a gas mixture of argon/oxygen (80/20, v/v) to the nebulizer aerosol, to apply a plasma torch with reduced aerosol channel, to reduce the flow rate of the eluent, or to post-column split the eluent. Additionally, to protect the sample and skimmer cones of the ICP-MS, mobile phases should not contain sodium, potassium and their hydroxides because they form metal oxide deposits on the orifice of the cones. Mobile phases should preferably

contain volatile components such as ammonium salts of acetate, carbonate, nitrate or aliphatic carbonic acids ^[13].

Table 5. Advantages and drawbacks of IC-ICP-MS. Modified from ^[13].

Advantages	Disadvantages
<ul style="list-style-type: none"> • Suitability for multi-elemental analysis (e.g. heavy metal complexes) • High sensitivity (ng/L) • High selectivity (exact masses, collision/reaction cell modes) • Detection stability 	<ul style="list-style-type: none"> • Organic solvents can interfere • Non-volatile buffer unsuitable • Standard compounds are necessary for precise identification

Even if there are some inconveniences of using this hyphenated technique, IC-ICP-MS is still a popular and effective tool for separation and speciation in foodstuffs, cosmetic products, environmental and pharmaceutical samples. Some of these applications were discussed in Chapter 1. For this thesis project, IC-ICP-MS served to separate and quantify trivalent and hexavalent chromium species from leather samples coming from the luxury sector that had undergone different and multiple manufacturing processes. This is a complex matrix because leathers contain many organic compounds that can also be extracted during the extraction step, and possibly interfere during analysis. Besides, chromium can be present in various ionic forms, and a particular attention should be paid to ensure the conservation of the initial species (no interconversions). Moreover, leather samples are often treated with trivalent chromium salts, so the concentration of Cr(III) is expected to be much more important than the concentration of Cr(VI). For both analytical challenges, IC-ICP-MS appeared to be a promising technique.

2.2.4 General procedure

Different conditions were tested in order to optimize the separation of ionic species by IC-ICP-MS. These conditions were:

- different mobile phases (with different concentrations and pH)
- addition of organic solvent to the mobile phase
- two different chromatographic columns (with their guard columns)
- isocratic and gradient modes
- different pH of the samples

However, two main setups were used, one setup for each type of chromatographic column (AS7 or CS5A), even if the optimized conditions were found when using the AS7 ion-exchange column for the separation of ionic species.

Preliminary tests were conducted using a simple setup. In this setup, a NG1 guard column and an AS7 ion-exchange column were used. However, as the mobile phase was nitric acid, no suppressor was used, therefore, no AXP pump was used neither. The setup is described in Figure 17.



Figure 17. Setup for preliminary studies with the Dionex Ionpac AS7 ion-exchange column.

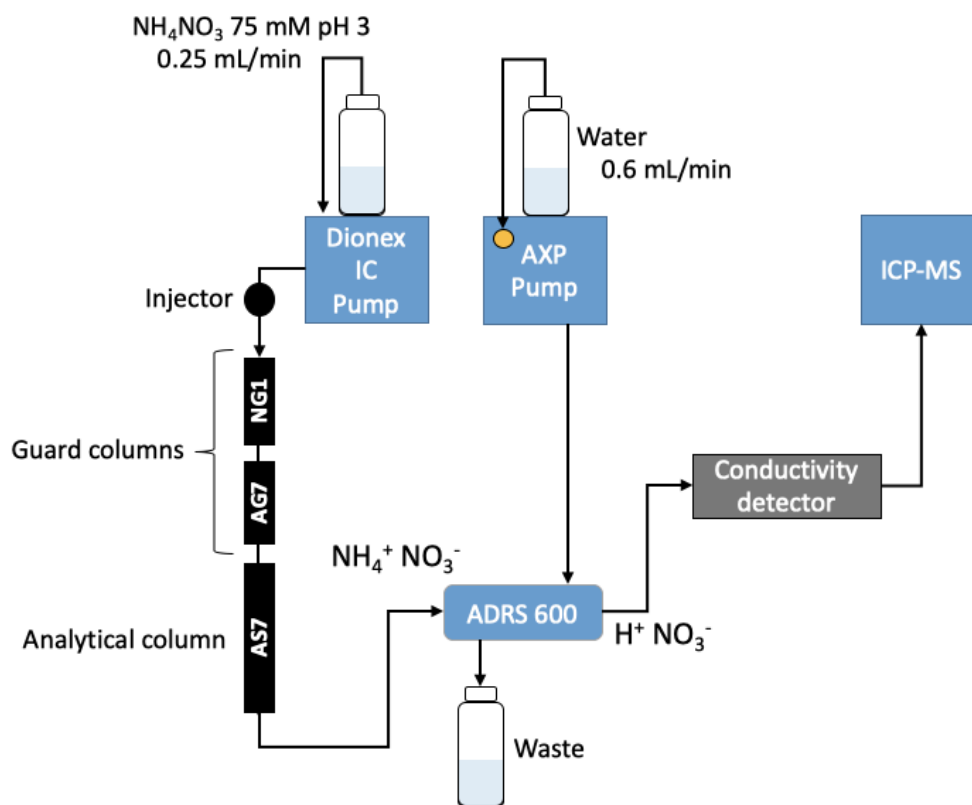


Figure 18. Setup for the Dionex Ionpac AS7 ion-exchange column. Suppressor was operated in external regeneration mode.

The first setup is described in Figure 18. As previously indicated, this setup was used for column AS7. In this setup, we used a NG1 guard column, an AG7 guard column and an AS7 ion-exchange column. Then, a Dionex IC pump delivered the mobile phase and the sample in isocratic mode at 0.25 mL/min. This solution traveled through the columns in the order previously mentioned. Ionic species were separated and eluted at different retention times. The mobile phase (NH_4NO_3 75 mmol/L at pH 3) together with the sample arrived at the suppressor, which permitted the exchange of ammonium ions for hydronium ions. Therefore, diluted nitric acid arrived at the ICP-MS, which served to avoid salt deposition. The suppressor was operated in external regeneration mode, so a constant flow of 0.6 mL/min of ultrapure water was supplied by means of an AXP auxiliary pump.

After the solution passed through the suppressor, it arrived at the conductivity detector and finally at the ICP-MS where detection was completed.

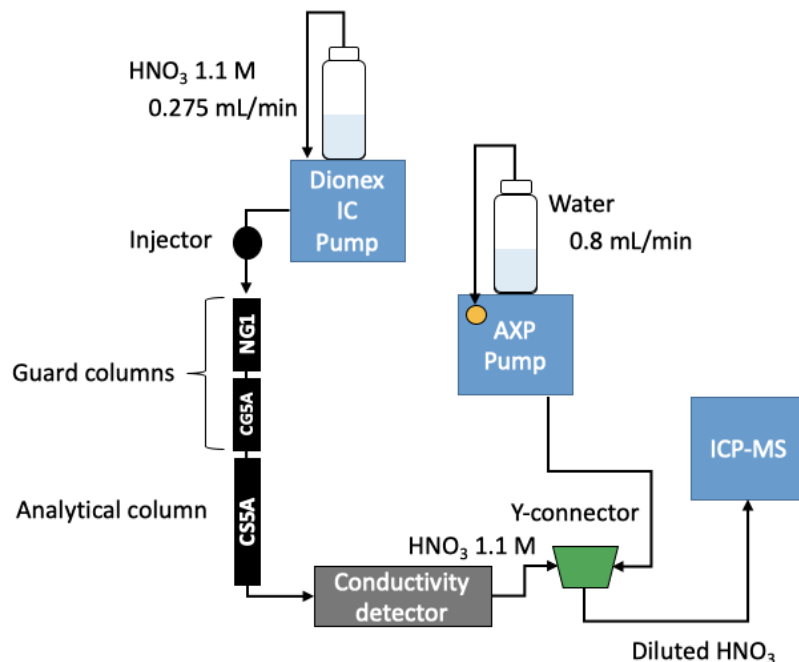


Figure 19. Setup for the Dionex Ionpac CS5A ion-exchange column.

The second setup is described in Figure 19. This setup was designed for column CS5A. Two guard columns were used: a NG1 and a CG5A guard column. The analytical column was placed right after the two guard columns. In the same way as the first setup, a Dionex IC pump delivered the mobile phase (HNO₃ 1.1 mol/L) and the sample, but in this case, in gradient mode. This gradient is shown in Figure 20. This solution traveled through the columns where species were separated and eluted at different retention times. After passing through the conductivity detector, a Y-connector was installed. This permitted the arrival of the sample and the mobile phase at 0.275 mL/min from one side, and the arrival of ultrapure water at 0.8 mL/min by means of an AXP auxiliary pump from the other side. The final solution (at the exit of the Y-connector) was diluted nitric acid. This solution finally arrived at the ICP-MS where detection was completed.

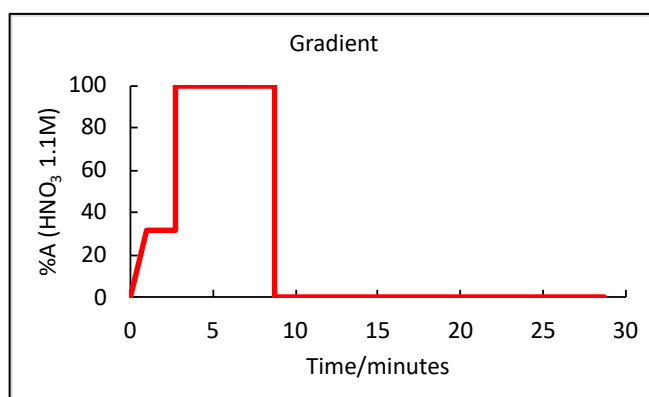


Figure 20. Gradient used for the second setup.
 $\%A = \%HNO_3 \text{ 1.1M}$, $\%B = (100\% - \%A)H_2O$.

Samples were diluted and their pH and redox potential were monitored. Finally, they were injected, and the resulting chromatograms were interpreted. (See Chapter 4 for results and interpretation).

2.3 FTICR

2.3.1 Instrumentation

FTICR MS analyses were achieved using a Solarix XR instrument (Bruker Daltonics, Bremen, Germany) equipped with a 12T superconducting magnet and a dynamically harmonized ICR cell (ParaCell®). An ESI source was used in positive and negative ion modes. The operating parameters for direct infusion analyses on ESI(+) and ESI(-) are reported in Table 6. The analogue image current was digitized with 4 million data points resulting in the recording of a 1.118 s time domain signal for ESI analyses. The time domain signals were transformed into the corresponding frequency domain by Fourier transform (after full-sine apodization and one zero fill). These settings resulted in a mass resolving power of 500,000 at m/z 200. Mass spectra were acquired using Bruker Compass FtmsControl 2.3, and processed with Bruker Compass DataAnalysis 5.1 (Bruker

Daltonics GmbH, Bremen, Germany). Molecular formulas were assigned to the peaks with a S/N greater than 6, with even and odd electron configuration.

Table 6. FTICR parameters for the analysis of the Cr(III)-EDTA⁻ complex.

		ESI(+)	ESI(-)
Source parameters	Flow ($\mu\text{L}\cdot\text{h}^{-1}$)	80	120
	Nebulizer gas pressure (bar)	2.0	2.0
	Drying flow ($\text{L}\cdot\text{min}^{-1}$)	4.0	4.0
	Drying temperature ($^{\circ}\text{C}$)	200	180
	Capillary voltage (kV)	4.2	4.1
	Ion transfer parameters	Quadrupole m/z	100
	Time of flight (ms)	1.0	1.0
Acquisition parameters	m/z range	98-1200	98-1200
	Scan number	16	100
	Accumulation time (s)	0.05	0.05
	Transient length (s)	1.118	1.118

2.3.2 Materials

Optima LC-MS grade water and acetonitrile (purchased from Fisher Chemical) were employed for the dilution of samples: 2X dilution in acetonitrile for Cr(III)-EDTA⁻ complexes (initial concentration of 10 ppm of Cr(III)); 2X dilution in acetonitrile/H₂O (50/50, v/v) for non-diluted leather extracts.

2.3.3 Principle

Electrospray ionization (ESI) is a technique used in mass spectrometry to produce ions from solutions. In this technique, the analyte is dissolved in a liquid (e.g. in

acetonitrile/H₂O 50/50, v/v for instance) and it goes through a stainless steel capillary tube maintained at a high voltage (typically between 1-4 kV). This process generates a plume of charged liquid droplets. A heated gas helps to evaporate and break up clusters of ions. Subsequently, analyte ions are transferred from the charged droplets to the gas phase by series of Coulomb explosions and are conducted into the mass spectrometer vacuum system^[26].

Fourier-Transform Ion Cyclotron Resonance Mass Spectrometry is a mass analyzer used to determine m/z of analyte ions based on their cyclotron frequency within a fixed high magnetic field^[27]. This method was introduced by Comisarow and Marshall in 1974^[28].

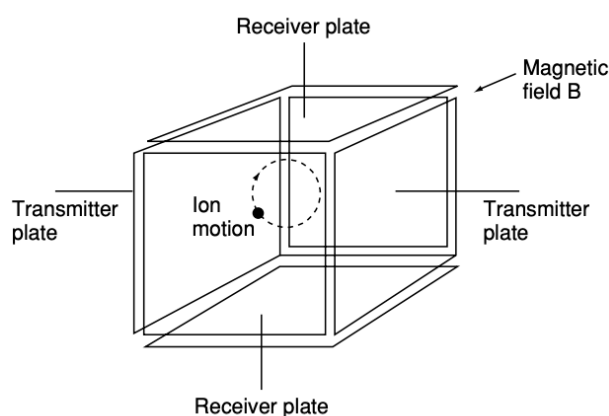


Figure 21. Schematic diagram of an FTICR. Obtained from ^[26].

FTICR uses a Penning ion trap in which ions are confined axially by an electric field and radially by a strong magnetic field, B . In the trap, ions move circularly with a characteristic cyclotron frequency, ω . The motion of the ions is defined by the eq. 10.

$$\omega = z \cdot \frac{B}{m} \quad \text{eq. 10}$$

For their detection, ions are excited in the trapping cell at their cyclotron frequency by applying a radiofrequency voltage in the excitation plates. This yields a higher cyclotron radius and a coherent motion. The orbiting ions induce

an alternating image current in the walls of the cell receiver plates. This complex signal (since each m/z has a characteristic frequency) is resolved by a Fourier transform, which yields to the frequency and consequently the m/z ratios of all ions in the spectrum^[26]. A schematic diagram of an FTICR can be appreciated in Figure 21.

This technique offers the highest mass accuracy (below 100 ppb) and resolving power (above 1,000,000) among all type off mass analyzers, making FTICR a reference for the analysis of highly complex mixtures. Although it is an expensive equipment and running costs are very high, it has been used in many fields such as proteomics, metabolomics, petroleomics and investigation of complex mixtures^[28].

During this thesis, an ESI-FTICR (SolariX XR, Bruker Daltonics) equipped with a 12 T superconducting magnet (Figure 22) was used for the elucidation of the structure of the chromium complex formed during the EDTA extraction.



Figure 22. FTICR SolariX XR 12T (Bruker Daltonics, Bremen, Germany) from Cobra Laboratory (Université de Rouen).

2.3.4 General procedure

Samples were diluted, injected and analyzed in the conditions previously indicated. Mass spectra were internally calibrated using confident ions signal over the whole mass range.

Abbreviations

$a_{1,2}$	Selectivity factor
A_s	Peak asymmetry factor
DTPA	Diethylenetriaminepentaacetic acid
EDTA	Ethylenediaminetetraacetic acid
ESI	Electrospray ionization
f	Flow rate
FTICR	Fourier-Transform Ion Cyclotron Resonance Mass Spectrometry
IC	Ion Chromatography
IC-ICP-MS	Ion Chromatography - Inductively Coupled Plasma - Mass Spectrometry
ICP-AES	Inductively Coupled Plasma - Atomic Emission Spectrometry
ICP-MS	Inductively Coupled Plasma - Mass Spectrometry
ISO	International Organization for Standardization
IUPAC	International Union of Pure and Applied Chemistry
k	Retention factor
KED	Kinetic Energy Discrimination mode
MS	Mass Spectrometry
N	Efficiency or number of theoretical plates
PDMS	Polydimethylsiloxane
PEG	Polyethylene glycol
R_s	Resolution

S/N	Signa-to-noise ratio
SAX	Strong anion exchanger
SCX	Strong cation exchanger
STD	Standard mode
T _f	Tailing factor
t ₀	Dead time
TOF	Time-of-flight
t _r	Retention time
V _m	Dead volume
V _r	Volume of retention
W	Peak width at the base
WAX	Weak anion exchanger
WCX	Weak cation exchanger

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Chapter 3

Chapter 3. Sample pretreatment

The third chapter of this thesis corresponds to the first experimental essays. It consists of the second peer-reviewed article which is entitled "Quantitative extraction of chromium VI and III from tanned leather: a comparative study of pretreatment methods". It was published in the Journal of Leather Science and Engineering (Impact Factor: under calculation) in collaboration with Christine Devouge-Boyer, Marie Hubert-Roux, Carlos Afonso and Mélanie Mignot, and its objective was to compare the chromium extraction rate of the following methods: acidic mineralization, ethylenediaminetetraacetic acid (EDTA) extraction, diethylenetriaminepentaacetic acid (DTPA) extraction, alkaline extraction (NH_4OH), ammonium nitrate extraction (NH_4NO_3), water extraction and phosphate buffer extraction.

Due to the importance of developing a method to extract and determine trivalent and hexavalent chromium simultaneously, several methods were tested to evaluate the extraction of total chromium from leather samples. The results were compared to the results obtained from a complete acidic digestion (acidic mineralization) which permitted the decomposition of the organic matter allowing the total chromium quantification, in order to calculate the chromium extraction rate.

EDTA extraction was tested based on the work of Fabregat et al.^[1], who reported the quantification of Cr(VI) in several certified reference materials (CRMs) (soil containing chromite ore processing residue, welding dust loaded on a filter, and sandy clay soil) using EDTA 50 mmol/L at pH 10 as extractant. This was achieved through the application of a 10 minutes focused microwave assisted extraction (5 min at 90 °C plus 5 min at 110 °C). Using EDTA as extractant allowed to measure the Cr(III)-EDTA complex and Cr(VI). In our current work, we used an

EDTA solution (50 mmol/L at pH 10) to extract both chromium species. The extraction was performed in a microwave system: 10 minutes from room temperature (21 °C) to 100 °C, 30 minutes at 100 °C.

The DTPA extraction was performed in the same way as the EDTA extraction (same microwave program and same concentration of the extractant). In fact, DTPA has also been tested as chelating agent for trivalent chromium but it has shown lower kinetical stability than EDTA^[2].

The alkaline extraction with NH₄OH has been employed to extract hexavalent chromium from dairy products, flour, chocolate, vegetables, fruits, meat, fish, eggs, and beverages. Vacchina et al^[3]. reported the extraction from solid samples as follows: 500 mg of the sample were extracted by 10 mL of NH₄OH solution (pH 11.5) during 1 h in an ultrasonic bath. The supernatant was separated by centrifugation (14,000 rpm, 10 min) and filtration. Based on this work, our procedure was the following: 500 mg of leather were extracted with 10 mL of NH₄OH at ultrasonic bath during 1 hour. The extract was centrifuged for 15 min at 4000 rpm (maximum speed of our centrifuge) and filtered using a nylon 0.45 µm filter.

For waters^[4, 5], bottom sediments^[5], and traditional Chinese medicines^[6], chromium species separation has been performed by chromatographic methods using ammonium nitrate as mobile phase. The ammonium nitrate extraction was carried out in order to verify if the mobile phase that serves to elute both chromium species, can serve as extraction media as well. It was conducted similarly to the alkaline extraction (ultrasound assisted, same mass of leather and same volume of extractant). The ammonium nitrate solution had a concentration of 0.1 M, the same concentration of the mobile phase used in Jablonska's^[5] work.

The water extraction was tested in order to determine water soluble chromium compounds. This extraction was performed in the same way as the EDTA extraction (same microwave program and same volume of the extracting solution, water in this case).

Regarding the phosphate buffer extraction, it was done following the International Organization for Standardization (ISO) 17075^[7, 8] standardized method. Therefore, 500 mg of leather were extracted with 25 mL of $K_2HPO_4 \cdot 3H_2O$ at pH 8.0 (adjusted with phosphoric acid) for three hours under magnetic stirring. The extracts were subsequently filtrated through nylon 0.45 μm filter.

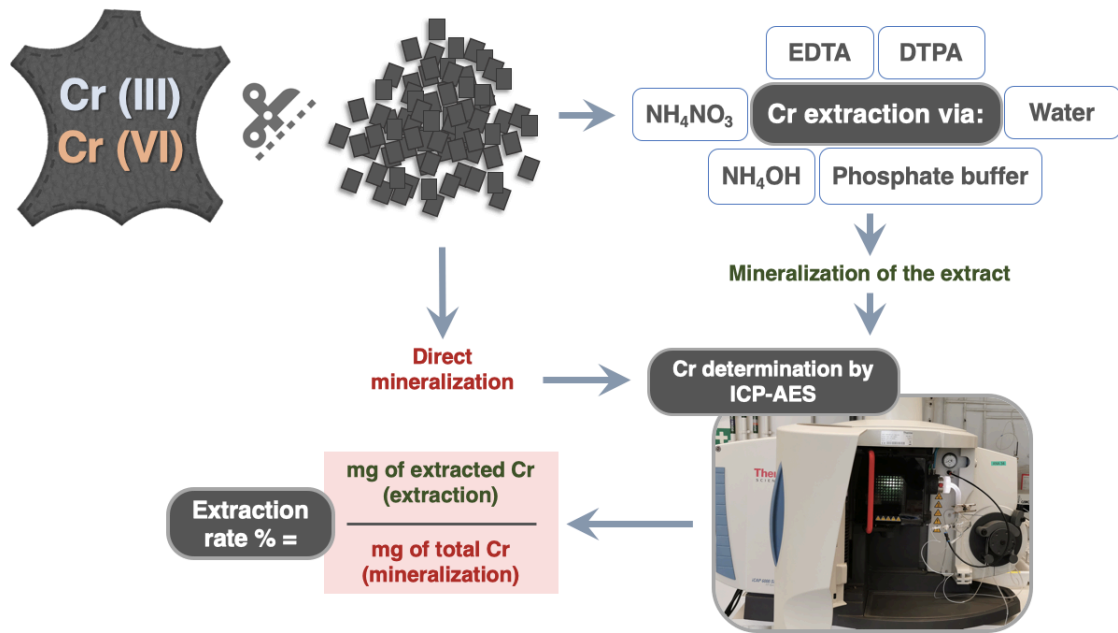
The most performant method was found to be the EDTA extraction which was subsequently validated in terms of linearity, Method Detection Limit (MDL), Method Quantification Limit (MQL), interday precision and spike recovery. Due to the non-existence of CRMs for chromium in leathers, accuracy was evaluated by spiking leather samples with an hexavalent chromium solution (Cr(VI) was spiked and not incubated) of a known concentration. The spike recovery of hexavalent chromium proved that EDTA was not only capable to extract trivalent chromium (found in larger quantities in leather samples), but hexavalent chromium as well.

The final part of this article shows an example of the separation of the extracted species through Ion Chromatography - Inductively Coupled Plasma - Mass Spectrometry (IC-ICP-MS). It provides an efficient methodology to quantitatively extract and subsequently separate and quantify chromium species (hexavalent and trivalent chromium) from leather samples while maintaining the speciation.

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Graphical Abstract.

RESEARCH

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Quantitative extraction of chromium VI and III from tanned leather: a comparative study of pretreatment methods

Mónica Gisel Arellano-Sánchez¹, Christine Devouge-Boyer¹, Marie Hubert-Roux², Carlos Afonso² and Mélanie Mignot^{1*}

Abstract

In this study, seven pretreatment methods for chromium speciation in tanned leather were evaluated: acidic mineralization, ethylenediaminetetraacetic acid (EDTA) extraction, diethylenetriaminepentaacetic acid (DTPA) extraction, alkaline extraction (NH₄OH), ammonium nitrate extraction (NH₄NO₃), water extraction, and phosphate buffer extraction. Acidic mineralization permitted the decomposition of the organic matter and ensured the complete digestion of leathers, giving access to the total content of chromium in each sample using inductively coupled plasma-atomic emission spectrometry (ICP-AES). From all the extractant media tested, EDTA proved to be the most efficient, allowing the extraction of Cr(VI) and Cr(III) as a Cr(III)-EDTA complex, quantitatively. Method validation is presented for EDTA extraction and direct mineralization. For the EDTA extraction, method detection limit (MDL) and method quantification limit (MQL) for total Cr in leather were 3.4 ppb and 11.2 ppb (µg of total Cr per L of extraction solution), respectively. Due to the lack of leather certified reference materials (CRMs) for Cr(VI), accuracy was evaluated by spiking leather samples with a Cr(VI) solution. The spike recovery of EDTA microwave assisted extraction ranged from 91.0 to 108.6%. Interday precision was also evaluated and all variation coefficients were below 5%, for both mineralization and EDTA extraction. This article provides an efficient procedure to extract quantitatively chromium from leather, while maintaining the speciation, which can be further followed by ion chromatography-inductively coupled plasma-mass spectrometry (IC-ICP-MS).

Keywords: Hexavalent chromium, Speciation, Tanned leather, Sample pretreatment, EDTA complexation, Inductively coupled plasma

1 Introduction

Leather industry occupies a very important role in the development of global economy. Leather is used every day and everywhere in a variety of articles such as footwear, furniture, book covers, gloves, handbags, etc. In order to transform raw hide into leather, a process called tanning is required [1]. Approximately 90% of all hides

are treated with trivalent chromium salts [2]; this process is called chrome-tanning.

The electronic structure of chromium allows it to form coordination complexes, which makes it a remarkable tanning agent (i.e. Cr-collagen complexes via carboxylate functions). Chrome-tanning reaction is fast (6 h instead of 3 weeks, compared with vegetable tanning), confers high hydrothermal stability, hardly alters the structure of collagen, makes collagen more hydrophobic (hence, water-resistant), and allows the retention of colorants since Cr(III) can act as a dye fixing agent [3]. Moreover, the cross-linking of these fibers makes the skin

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insensitive to decay or decomposition, and gives greater stability and resistance to mechanical action and heat.

Even if hides are not directly treated with hexavalent chromium, which is a known carcinogen and mutagen [4], it has been proved that Cr(VI) can be released from chrome-tanned leather. Indeed, different environmental conditions (i.e. pH, light, temperature ...) can induce trivalent chromium's oxidation into Cr(VI), as suggested by a recent publication from Fontaine et al. [5]. They studied the storage conditions that could influence the formation of Cr(VI) in four bovine leather samples, and found that a high temperature, a dry atmosphere, and presence of UV light had a great impact on Cr(VI) formation, with a predominant role of UV light. Consequently, these conditions should be considered when trying to predict the evolution of chromium in leather goods.

Furthermore, in direct contact with the skin, chrome-tanned leather products can cause chromium allergy and dermatitis [6, 7]. For most leathers, Cr(III) is the dominant form of chromium that is released [8–10]. In a recent study, 10 Cr-allergic subjects and 22 controls were patch tested with Cr(III), Cr(VI), and leather samples. The results suggested that repeated exposure to Cr-tanned leather with predominantly Cr(III) release is sufficient to elicit allergic contact dermatitis in Cr-allergic individuals. Even if the majority of Cr-allergic subjects reacted to Cr(III) and Cr(VI), a lower dose of Cr(VI) was required for a stronger reaction [11]. Taking into account that throughout the life-cycle of leather chromium speciation has a dynamic behavior influenced by a large number of parameters, the one-time testing of Cr(VI) may be considered irrelevant if trivalent chromium is not tested as well. Moreover, no relationship has been found between released Cr(III) and Cr(VI) [10]. Besides, an article by Feng et al. [12], reported that both trivalent and hexavalent chromium induce genetic mutations and DNA damage in yeast cells, the effect of Cr(III) being greater than that of Cr(VI). Consequently, it is important to quantitatively determine both chromium species separately.

Regarding hexavalent chromium in leather, since 2015 the European Union limits its content to 3 mg/kg (of the total dry weight) in leather goods coming into direct contact with the skin [13]. However, the European Chemicals Agency has recently adopted an opinion to decrease the restriction limit of hexavalent chromium from 3 to 1 mg/kg in leather, furs and hides. This shall come into effect 5 years after entry into force of the restriction [14]. Currently, the International Organization for Standardization (ISO) 17075 standard specifies a procedure for the selective extraction of hexavalent chromium in leather using $K_2HPO_4 \cdot 3H_2O$ at pH 8.0. Further analysis of the extract is performed via colorimetric [15] or chromatographic

analysis [16]. The colorimetric analysis consists of a reaction between Cr(VI) and 1,5-diphenylcarbazide (DPC), the resulting complex is detected by UV Visible (UV-Vis) spectroscopy at 540 nm. The chromatographic analysis can be performed by direct detection of the chromate peak at 372 nm, or through post-column reaction with DPC by measuring the absorption peak at 540 nm. The limit of quantification (LOQ) of this standard procedure is 3 mg/kg for both techniques. Table 1 shows a summary of the pretreatment and analytical methods that have been used for the determination of hexavalent chromium in leather samples. All of these techniques rely on a Cr(VI) extraction with phosphate buffer or NaOH, and then the complexation of hexavalent chromium with DPC for UV-Vis detection. Nevertheless, when working with leather matrices, the selectivity of the UV detection might be compromised as other compounds could absorb at the same wavelength as the target complex; it is important to remark that leather is frequently treated with a variety of chemicals (azo dyes, pigments, fungicides, bactericides, binders, cationic/anionic surfactants...).

However, it can be noticed that there is no current standard describing a method to extract and quantify Cr(III) and Cr(VI) simultaneously. A lot of studies have been conducted regarding chromium speciation in other matrices, for example foodstuffs, cosmetic products, environmental, and pharmaceutical samples [20], but as far as we know, the simultaneous extraction of both Cr species, while maintaining the speciation, has not been studied for leather samples. Fabregat-Cabello et al. [21] reported a focused microwave assisted extraction of Cr(VI) in several CRMs (soil containing chromite ore processing residue, welding dust loaded on a filter, and sandy clay soil) using EDTA 50 mmol/L at pH 10. According to their results, they were able to extract and measure Cr(VI) and Cr(III)-EDTA complex, simultaneously. The separation and analysis of the species was performed by IC-ICP-MS. DTPA has also been tested as chelating agent for trivalent chromium but it has shown lower kinetical stability than EDTA [22]. To extract Cr(VI) from solid matrices, alkaline extraction with NH_4OH has been employed for dairy products, flour, chocolate, vegetables, fruits, meat, fish, eggs, and beverages. High-performance liquid chromatography-inductively coupled plasma-mass spectrometry (HPLC-ICP-MS) was used to quantify Cr(VI) after ultrafiltration of the extract [23]. Gürleyük and Wallschläger [24] reported the successful determination of Cr(III) and Cr(VI) in water by IC-ICP-MS using EDTA to derivatize Cr(III). However, as far as we know, the potential of EDTA to extract Cr species in leather samples has not been tested. For waters [24, 25], bottom sediments [25], and traditional Chinese medicines [26], chromium species separation has been performed

Table 1 Pretreatment and determination methods for hexavalent chromium in leather samples

Sample pretreatment	Analytical technique	Analyte	Analytical figures of merit	Important remarks	References
Acid digestion (total Cr) and alkaline extraction of Cr(VI) with 0.1 M NaOH	ICP-AES, Mobile Phone, PhotoMetric App	Total Cr, Cr(VI)	Limit of detection (LOD): 0.6 mg/kg for Cr(VI); LOQ: 2 mg/kg for Cr(VI)	Reaction with DPC for Cr(VI) determination	Costa et al. [17]
Acid digestion (total Cr) and Standard, Measurement and Testing Programme (SM&T) Sequential Extractions Scheme (oxidizable > exchangeable > residual)	Flame Atomic Absorption Spectrometry (FAAS), UV-Vis	Total Cr, Cr(VI)		Cr was determined in each extracted fraction. Cr(VI) was determined in the oxidizable fraction by reaction with DPC	Dias da Silva et al. [2]
Extraction of Cr(VI) with $K_2HPO_4 \cdot 3H_2O$ at pH 8.0	UV-Vis	Cr(VI)	LOQ: 3 mg/kg	Colorimetric method: reaction with DPC	ISO 17075-1 [15]
Extraction of Cr(VI) with $K_2HPO_4 \cdot 3H_2O$ at pH 8.0	Ion Chromatography-UV-visible detection (IC-UV-Vis)	Cr(VI)	LOQ: 3 mg/kg	Chromatographic method: direct detection of chromate peak or post-column reaction with DPC	ISO 17075-2 [16]
Extraction of Cr(VI) with 0.1 M phosphate solution (0.1 M NaH_2PO_4 and 0.1 M H_3PO_4) at pH 4.4 in presence of 5% NaCl	Ion chromatography-diode array detector (IC-DAD)	Cr(VI)	LOQ: 0.15 mg/kg	Post-column reaction with DPC for Cr(VI) determination. Cr(III) was complexed with EDTA. Evidence of Cr(VI) formation (ISO 17075)	Pastore et al. [18]
Extraction of Cr(VI) with K_2HPO_4 at pH 8.0	UV-Vis	Total Cr, Cr(VI)	LOD: 15 µg/L for Cr(VI)	Reaction with DPC for Cr(VI) determination. Total Cr was measured after oxidation of Cr(III)	Rezić and Zeiner [19]

by chromatographic methods using ammonium nitrate as mobile phase after EDTA derivatization or phosphate buffer extraction. Therefore, there is a possibility that if the mobile phase serves to elute both chromium species, it can serve as extraction media as well.

Based on these studies, the objective of this work was to evaluate seven pretreatment methods in tanned leather: acidic mineralization (which permits the decomposition of the organic matter allowing the total Cr quantification by ICP-AES), EDTA extraction, DTPA extraction, NH_4OH extraction, ammonium nitrate extraction (NH_4NO_3), water extraction (for water-soluble chromium compounds), and phosphate buffer extraction (ISO 17075). The efforts in leather research are limited and, to the best of our knowledge, none of these extraction systems have been tried on leather samples. Method validation is presented for EDTA extraction and direct mineralization, and encompassed linearity, accuracy, interday precision, and method detection and quantification limits. IC-ICP-MS using Cr isotopic standards was performed in order to study the possible Cr interconversions resulting from the EDTA extraction conditions.

2 Experimental

2.1 Instrumentation

Extraction and mineralization of samples were performed with a Milestone ETHOS 1 microwave digestion system equipped with a temperature sensor. TFM reactors were used with a maximum pressure of 100 bar. ICP-AES measurements were performed on an iCAP 6300 series instrument (Thermo Fisher Scientific) using the axial view. The quantification of chromium was performed at 283.5 nm. Chromatographic separation was performed using a Dionex ICS-6000 Capillary

HPIC System (Thermo Fisher Scientific). A Dionex ADRS 600 Anion Dynamically Regenerated Suppressor (2 mm) (Thermo Fisher Scientific) was set before the entrance into ICP-MS to avoid salt deposition and was operated in the external regeneration mode. A constant flow of 0.6 mL/min of ultrapure water was supplied by means of a Dionex AXP auxiliary pump (Thermo Fisher Scientific). Two guard columns were placed before the analytical column: a Dionex IonPac NG1 (2×50 mm, particle size: 10 μm) and a Dionex IonPac AG7 (2×50 mm, particle size: 10 μm). Ions were separated with a Dionex IonPac AS7 column (2×250 mm, particle size: 10 μm). The analytical column was coupled to the nebulizer of the ICP-MS using a PEEK tubing (0.25 mm i.d., 150 cm long). Detection of chromium species was performed on an iCAP RQ ICP-MS instrument (Thermo Fisher Scientific) fitted with a collision cell. Two different measurement modes were used: standard mode (STD) and kinetic energy discrimination mode (KED). In the KED mode, He gas was injected in the collision cell and an energy discrimination barrier was applied in order to remove unwanted polyatomic interferences. Data processing was performed using ThermoFisher Scientific Qtegra software. As example, optimized conditions for the plasma and nebulizer for ICP-AES and ICP-MS are given in Table 2.

The pH of the solutions was measured using a PHM240 pH/Ion Meter (MeterLab, Radiometer Analytical, Villeurbanne, France). Centrifugation of the extracts was performed with an Eppendorf Centrifuge 5702. Alkaline and ammonium nitrate extractions were performed using an Elmasonic S ultrasonic bath (Elma, Singen, Germany).

Table 2 Optimized operating conditions for ICP-AES and ICP-MS

	iCAP 6300 ICP-AES	iCAP RQ ICP-MS
Radio frequency power (W)	1050	1550
Plasma gas flow rate (L/min)	14	14
Auxiliary argon flow rate (L/min)	1.0	0.8
Nebulizer gas flow rate (L/min)	0.5	1.0
Purge gas (nitrogen) flow rate (L/min)	12	–
Peristaltic pump rate (rpm)	50	15
Sample uptake delay (s)	30	30
Replicates	3	3
Rinse time (s)	30	30
Sampler	ASX520-CETAC	SC-2DX (with FAST valve system) or online coupling with IC system
Chamber	Cyclonic	Cyclonic
Nebulizer type	Concentric	Concentric

2.2 Materials

Ultrapure water (resistivity 18.2 MW cm), obtained from a Milli-Q system (Millipore, Bedford, MA, USA), was used for preparation of all solutions unless stated otherwise. Nitric acid 67–69% (Ultrapure Normatom, VWR Chemicals, Leuven, Belgium) was used for the mineralizations. For the extractions, EDTA (Analytical reagent, VWR Chemicals, Leuven, Belgium), DTPA (97%, Aldrich-Chemie, Germany), NH_4OH 20–22% NH_3 (Optima grade, Fisher Scientific, UK), NH_4NO_3 (Normapur guaranteed reagent, Prolabo, Paris, France), $\text{K}_2\text{HPO}_4 \cdot 3\text{H}_2\text{O}$ (AnalaR Normapur, VWR Chemicals, Leuven, Belgium), and phosphoric acid (85 wt. % in H_2O , Sigma-Aldrich, Germany) were employed. Filtration was performed with 0.45 μm nylon syringe filters (Fisher Scientific). Potassium dichromate (Normapur guaranteed reagent, Prolabo, Paris, France) was used for preparing calibration standards and spikes. Leather samples came from the luxury industry and were all chrome-tanned and colored. Depending on the treatment they had (chrome-tanned, colored, satin, or glossy leather), their chromium content was not necessarily the same. Consequently, for all the extractions done hereafter, a direct mineralization of the same sample was done to provide each time the reference amount of total chromium. Concerning chromium speciation by IC-ICP-MS, mobile phase was prepared with NH_4NO_3 and adjusted to pH with HNO_3 . $^{53}\text{Cr}(\text{III})$ (97.01%)

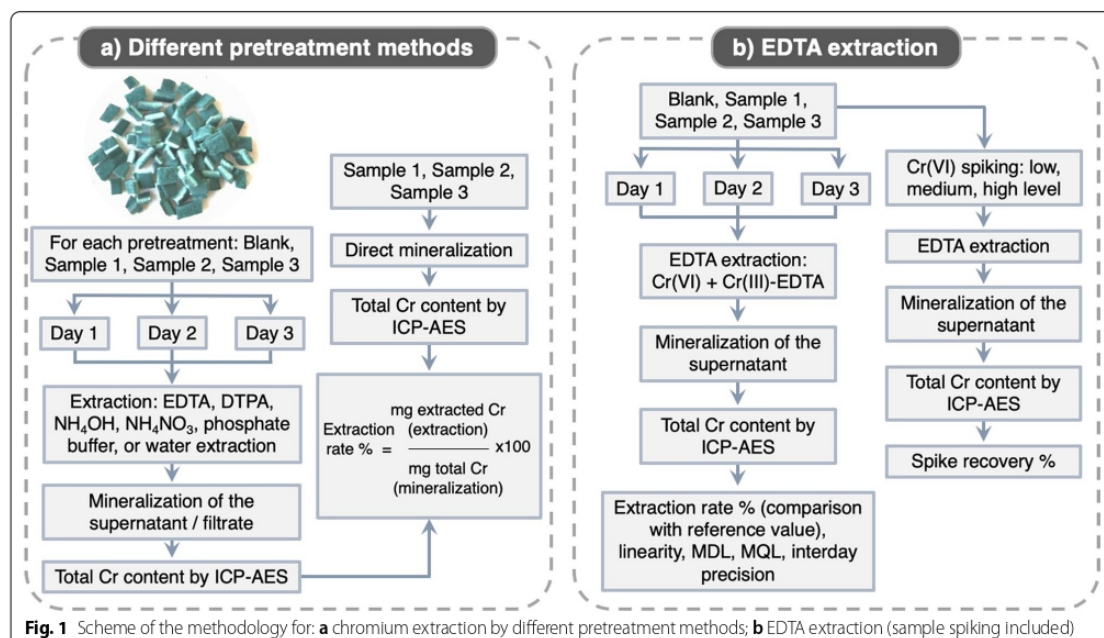
and $^{50}\text{Cr}(\text{VI})$ (97.36%) chromium isotopic standards (100 ppm, Certified Reference Material, ISC Science, Oviedo, Spain) were diluted and used to monitor possible chromium interconversions.

2.3 Total chromium determination

Leather was either directly mineralized or extracted prior to mineralization of the supernatant to further quantify by ICP-AES the amount of chromium extracted by the different media (EDTA, DTPA, NH_4OH , NH_4NO_3 , water, phosphate buffer). After the mineralization process, samples were diluted and total chromium was determined by ICP-AES. The calibration standards were freshly prepared with potassium dichromate and they were acidified as the same level as the samples. A scheme of the methodology is shown in Fig. 1a.

2.4 Sample preparation

Dry leather samples were cut into small pieces (3 to 5 mm per side) in accordance with ISO 4044:2017, 6.3 [27]. They were cut in the laboratory and immediately after this, they were stored in resealable plastic bags. This was done for each sample in the same way during the study. For each pretreatment method, three samples were treated in three different days.



2.5 Mineralization (or acidic digestion)

2.5.1 Direct mineralization of leather

An amount of 200 mg of leather was digested with 2 mL of nitric acid at 150 °C in a microwave system: 10 min from room temperature, 21 °C, to 150 °C, 30 min at 150 °C. After the mineralization, the digest was diluted up to 50 mL with water. For total Cr determination by ICP-AES, 1.25 mL of the last solution were diluted up to 25 mL with water. Calibration standards were acidified at the same level as the samples.

2.5.2 Mineralization of the extracts

Two milliliters of the extract (EDTA, DTPA, NH_4OH , NH_4NO_3 , water, or phosphate buffer extraction) were digested with 2 mL of nitric acid at 150 °C in a microwave system: 10 min from room temperature, 21 °C, to 150 °C, 30 min at 150 °C. After the mineralization, the digest was diluted up to 50 mL with water. For total Cr determination by ICP-AES, 1.25 mL of the last solution were diluted up to 25 mL with water. Calibration standards were acidified at the same level as the samples.

2.6 EDTA extraction

An amount of 200 mg of leather was extracted with 4 mL of EDTA 50 mmol/L (adjusted to pH 10 with NH_4OH) at 100 °C in a microwave system: 10 min from room temperature, 21 °C, to 100 °C, 30 min at 100 °C. The extract was afterwards centrifuged for 15 min at 4000 rpm, the supernatant was collected and mineralized for further total Cr quantification.

2.7 DTPA extraction

Similarly, for the DTPA extraction 200 mg of leather were extracted with 4 mL of DTPA 50 mmol/L (adjusted to pH 10 with NH_4OH) at 100 °C in a microwave system: 10 min from room temperature, 21 °C, to 100 °C, 30 min at 100 °C. The extract was afterwards centrifuged for 15 min at 4000 rpm, the supernatant was collected and mineralized for further total Cr quantification.

2.8 Alkaline extraction

For the alkaline extraction, 500 mg of leather were extracted with 10 mL of NH_4OH at ultrasonic bath during 1 h, as previously described by Vacchina et de la Calle [23] for dry food samples. The extract was centrifuged for 15 min at 4000 rpm and filtered using a nylon 0.45 μm filter. Further digestion of the extract was performed before total Cr quantification.

2.9 Ammonium nitrate extraction

An amount of 500 mg of leather was extracted with 10 mL of NH_4NO_3 0.1 M at ultrasonic bath for 1 h. After the ultrasonic assisted extraction, the solution was

centrifuged for 15 min at 4000 rpm and filtered using a nylon 0.45 μm filter. The digestion of the extract was performed as formerly described.

2.10 Water extraction

An amount of 200 mg of leather were extracted with 4 mL of ultrapure water at 100 °C in a microwave system: 10 min from room temperature, 21 °C, to 100 °C, 30 min at 100 °C. The extract was centrifuged for 15 min at 4000 rpm and the supernatant was collected. Then, the supernatant was mineralized for total Cr quantification.

2.11 Phosphate buffer extraction

In order to evaluate the ISO 17075 standardized method, 500 mg of leather were extracted with 25 mL of $\text{K}_2\text{HPO}_4 \cdot 3\text{H}_2\text{O}$ at pH 8.0 (adjusted with phosphoric acid) for three hours under magnetic stirring. The extracts were subsequently filtrated through nylon 0.45 μm filter and mineralized for total Cr determination.

2.12 Sample spiking

2.12.1 Sample spiking of mineralization

An amount of 200 mg of leather was spiked with 1, 3, and 5 mg of Cr(VI) coming from a concentrated solution of potassium dichromate freshly prepared. Subsequently, the samples were mineralized, diluted, and analyzed by ICP-AES for total Cr quantification.

2.12.2 Sample spiking of EDTA extraction

An amount of 200 mg of leather was spiked with 2, 6, and 10 mg of Cr(VI) with a concentrated solution of potassium dichromate freshly prepared. The samples were extracted with an EDTA solution (as specified in Sect. 2.6) and the supernatant was mineralized as previously specified. After proper dilution, the samples were analyzed by ICP-AES for total Cr quantification. This methodology is described in Fig. 1b.

2.13 Speciation by IC-ICP-MS

2.13.1 Complexation

In order to follow possible Cr interconversions during the separation and determination of species through IC-ICP-MS, 400 μL of a Cr isotopic standard ($^{53}\text{Cr}(\text{III})$ or $^{50}\text{Cr}(\text{VI})$) was complexed with 4 mL of EDTA 0.5 mmol/L (diluted from the EDTA extraction solution, specified at 2.6) at 100 °C in a microwave system: 10 min from room temperature, 21 °C, to 100 °C, 30 min at 100 °C. Solutions were subsequently diluted and analyzed by IC-ICP-MS.

2.13.2 Analysis of the samples

Separation was achieved using NH_4NO_3 75 mmol/L (adjusted to pH 3 with HNO_3) as mobile phase. Analysis were conducted on isocratic mode at a mobile phase flow

rate of 0.25 mL/min. The column temperature was set at 20 °C. Monitored signals were ^{50}Cr , ^{52}Cr , and ^{53}Cr .

3 Results and discussion

3.1 Evaluation of different pretreatment methods

Leather samples coming from the luxury industry were analyzed after going through seven different pretreatment procedures to evaluate their extraction rates. Samples used for these experiments had gone through different manufacturing processes, and were available in limited quantity, hence the content of chromium may vary from one sample to another (i.e. samples used for EDTA extraction were not the same as the samples used for NH_4OH extraction). Additionally, considering that leather is not manufactured, it is less homogeneous than other materials and chromium content may vary from one area to another. Consequently, for each sample (sample 1, sample 2, sample 3), a specific leather piece was selected, cut into small pieces, and used for the full procedure (day 1, day 2, day 3). For all the extractions done hereafter (EDTA, DTPA, NH_4OH , NH_4NO_3 , water, phosphate buffer extraction), a direct and complete mineralization of the same sample was done to provide each time the reference amount of total chromium, and the extraction rate was calculated with Eq. (1).

$$\text{Extraction rate\%} = \frac{\text{mg of extracted chromium(extraction)}}{\text{mg of total chromium(direct mineralization)}} \times 100 \quad (1)$$

The results are shown in Table 3 and are only valid for the conditions described on the experimental section. The highest extraction rate was achieved with the EDTA extraction, which ranged from 92.5 to 108.3% of total Cr (Table 3a). The advantage of the EDTA extraction is that it complexes trivalent chromium decreasing the probability of its conversion to Cr(VI), and allows the dissolution of insoluble forms of Cr(VI) [28]. Therefore, it permits the extraction of both Cr species. A slightly lower rate, from 78.7 to 96.1% of total Cr, as shown in Table 3b, was obtained with DTPA as extractant, as a consequence of a lower stability of the $[\text{Cr}(\text{DTPA})]^{2-}$ complex [22].

It is well-known that the behavior of chromium species in aqueous media is principally affected by pH and redox potential. The extraction of hexavalent chromium in environmental samples is generally performed in alkaline media. Under these conditions, redox potential decreases, which promotes the stabilization of Cr(VI) [29]. For leather samples, NH_4OH reached an extraction rate from 50.3 to 55.5% (Table 3c), half of the total chromium content, which means that a relevant part of Cr(III) was also extracted. At pH above 6, Cr(III) precipitates as $\text{Cr}(\text{OH})_3(\text{s})$. At higher pH, above 9, this precipitate

solubilizes as a $\text{Cr}(\text{OH})_4^-$ complex. Hence the possibility of extracting Cr(III) using NH_4OH as extractant.

Regarding the ISO 17075 standard, the phosphate buffer extraction exhibited an extraction rate from 5.1 to 6.6% of total Cr. As can be seen in Table 3f, total extracted chromium ranges from 700 to 800 mg/kg which is much higher than the restriction limit for hexavalent chromium. These values reveal that even if the protocol permits to extract hexavalent chromium, it also allows the extraction of a portion of trivalent chromium, present at levels much more important than the restriction limit of Cr(VI). The selectivity of this method comes from the selective reaction of DPC with Cr(VI), and the further detection of the complex.

The results for water extraction are shown in Table 3e. An extraction rate of 4.2 to 8.8% of total Cr (up to 1130 mg/kg of total Cr) was found when using only water, which implies that by submerging the sample in water and heating it to 100 °C, it is possible to extract some chromium species from leather. Considering that total extracted Cr was far above the restriction limit for Cr(VI), it can be affirmed that Cr(III) has been extracted although not in its entirety. Moreover, it is well-known that while Cr(III) compounds are barely soluble in water, Cr(VI) compounds are readily soluble in water [30].

Therefore, there is a possibility that Cr(VI) has been extracted as well.

According to the results presented in Table 3d, the lowest extraction rate was obtained when using ammonium nitrate as extractant (1.4 to 3.3% of total Cr). Hence, even if ammonium nitrate can be used for the successful elution of chromium species in some chromatographic methods [24–26], it is not an adequate extractant for chromium species in leather.

As mentioned before, a mineralization step succeeded each extraction. Mineralization permits the decomposition of the organic matter in the extract and makes possible the analysis and quantification of total Cr by ICP-AES. In Fig. 2 we can observe the summary of the results from Table 3. Error bars represent the standard deviation of the three results (three different days) obtained for each sample.

3.2 Validation of the method

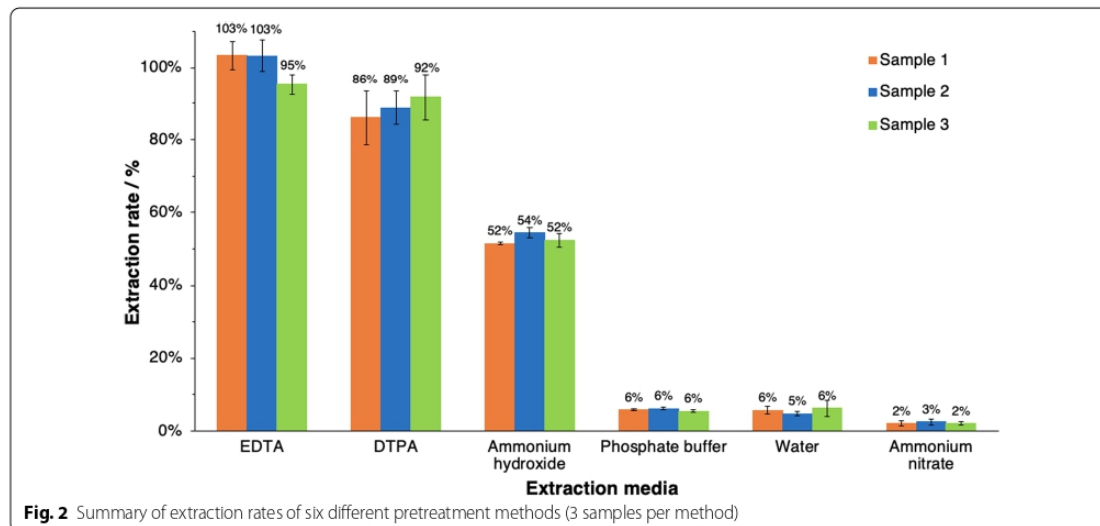
Due to the high extraction rate obtained with EDTA, and the possibility to preserve speciation from the extraction step until the chromatographic analysis by IC-ICP-MS for example, this method was validated together with

Table 3 Extraction rates of different pretreatment methods. Comparison between direct mineralization and: **a** EDTA extraction; **b** DTPA extraction; **c** NH₄OH extraction; **d** NH₄NO₃ extraction; **e** water extraction; **f** phosphate buffer extraction; 3 different samples were analyzed each day

(a) Day	EDTA mg of Cr/g of sample	Direct mineralization mg of Cr/g of sample	Extraction rate (%)
1	12.44	12.53	99.3
2	13.08	12.53	104.4
3	13.36	12.53	106.7
1	12.33	12.20	101.1
2	13.21	12.20	108.3
3	12.28	12.20	100.6
1	11.87	12.84	92.5
2	12.23	12.84	95.3
3	12.59	12.84	98.1
(b) Day	DTPA mg of Cr/g of sample	Direct mineralization mg of Cr/g of sample	Extraction rate (%)
1	13.91	16.12	86.3
2	15.07	16.12	93.5
3	12.68	16.12	78.7
1	15.13	16.57	91.3
2	15.18	16.57	91.6
3	13.86	16.57	83.6
1	15.47	16.33	94.7
2	15.70	16.33	96.1
3	13.82	16.33	84.6
(c) Day	NH ₄ OH mg of Cr/g of sample	Direct mineralization mg of Cr/g of sample	Extraction rate (%)
1	8.23	16.12	51.0
2	8.36	16.12	51.9
3	8.32	16.12	51.6
1	9.09	16.57	54.9
2	9.19	16.57	55.5
3	8.74	16.57	52.7
1	8.83	16.33	54.1
2	8.61	16.33	52.7
3	8.21	16.33	50.3
(d) Day	NH ₄ NO ₃ mg of Cr/g of sample	Direct mineralization mg of Cr/g of sample	Extraction rate (%)
1	0.48	16.12	3.0
2	0.31	16.12	1.9
3	0.23	16.12	1.4
1	0.54	16.57	3.3
2	0.42	16.57	2.6
3	0.27	16.57	1.6
1	0.42	16.33	2.5
2	0.32	16.33	2.0
3	0.28	16.33	1.7

Table 3 (continued)

(e) Day	Water mg of Cr/g of sample	Direct mineralization mg of Cr/g of sample	Extraction rate (%)
1	0.70	12.53	5.6
2	0.86	12.53	6.8
3	0.57	12.53	4.6
1	0.62	12.20	5.1
2	0.62	12.20	5.1
3	0.52	12.20	4.2
1	0.61	12.84	4.7
2	1.13	12.84	8.8
3	0.68	12.84	5.3
(f) Day	Phosphate buffer mg of Cr/g of sample	Direct mineralization mg of Cr/g of sample	Extraction rate (%)
1	0.74	12.55	5.9
2	0.70	12.55	5.5
3	0.78	12.55	6.2
1	0.71	11.84	6.0
2	0.71	11.84	6.0
3	0.78	11.84	6.6
1	0.75	13.61	5.5
2	0.70	13.61	5.1
3	0.80	13.61	5.9



the mineralization method. Linearity, Method Detection Limit (MDL), Method Quantification Limit (MQL), interday precision, and recovery are presented in the following subsections.

3.2.1 Linearity

Linearity was studied through the analysis of calibration solutions of Cr(VI) from 0 to 10 mg/L ($n=6$). Each time, the standards were prepared with the same amount

of nitric acid as the real samples and total Cr was determined in each solution. Calibration curve was done each day a sample was analyzed, and linearity was found to be 0.9998 for all ICP-AES analyses performed throughout this study.

3.2.2 Method detection limit and method quantification limit

MDL and MQL were estimated by the analysis of three blanks and three replicates per blank (9 measurements in total). Blanks consisted of the extraction solutions (nitric acid for the mineralization and EDTA 50 mmol/L pH 10 for the EDTA extraction) and had gone through all the steps of pretreatment and analysis, in the same way as leather samples. For the 9 measurements, a standard deviation was obtained. With this value, MDL was calculated as 3 times the standard deviation (3σ) and MQL was calculated as 10 times the standard deviation (10σ). For the mineralization method, MDL and MQL for total Cr in leather were 3.9 ppb and 13.1 ppb (μg of total Cr per L of mineralization solution), respectively. MDL and MQL for the EDTA extraction were 3.4 ppb and 11.2 ppb of total Cr (μg of total Cr per L of extraction solution), respectively.

3.2.3 Interday precision

Interday precision was determined by analyzing three different leather samples in three different days. It can be noticed in Table 4 that for each sample the interday precision provided a relative standard deviation (RSD) below 5%, for EDTA extraction and mineralization. Low RSD values show that both methods exhibit excellent interday precision.

3.2.4 Spike recovery

Due to the lack of leather certified reference materials for Cr(VI), accuracy was evaluated by spiking leather samples with a concentrated Cr(VI) solution prepared with potassium dichromate. Sample spiking was performed at three different levels of spike (low, medium, high), three different samples per level, the same day. For each level, total Cr was determined by ICP-AES after proper extraction, mineralization, and dilution. This allowed to measure the matrix effects. As observed in Table 5, the spike recovery for the mineralization ranged from 80.1 to 95.2%, so the recovery was good at all spike levels. Same tendency was found for EDTA extraction, as recovery ranged from 91.0 to 108.6% for all spike levels. Both techniques provided acceptable results, which proved that EDTA extraction was efficient to quantitatively extract

Table 4 Interday precision of: **a** direct mineralization; **b** EDTA extraction. 3 different samples were analyzed each day

(a)		Direct mineralization		
Day	mg of Cr / g of sample \pm SD			
1	17.01 \pm 0.10	Average	16.12	
2	15.51 \pm 0.02	SD	0.789	
3	15.83 \pm 0.09	RSD%	4.9%	
1	17.07 \pm 0.01	Average	16.57	
2	16.26 \pm 0.02	SD	0.431	
3	16.39 \pm 0.04	RSD%	2.6%	
1	16.73 \pm 0.03	Average	16.33	
2	16.25 \pm 0.03	SD	0.367	
3	16.00 \pm 0.03	RSD%	2.2%	
(b)		EDTA extraction		
Day	Extraction rate (%)	mg of Cr / g of sample \pm SD		
1	99.3	12.44 \pm 0.08	Average	12.96
2	104.4	13.08 \pm 0.06	SD	0.473
3	106.7	13.36 \pm 0.12	RSD%	3.6%
1	101.1	12.33 \pm 0.02	Average	12.61
2	108.4	13.21 \pm 0.00	SD	0.526
3	100.7	12.28 \pm 0.02	RSD%	4.2%
1	92.5	11.87 \pm 0.02	Average	12.23
2	95.3	12.23 \pm 0.02	SD	0.360
3	98.1	12.59 \pm 0.03	RSD%	2.9%

Table 5 Spike recovery for: **a** direct mineralization; **b** EDTA extraction

(a)		Direct mineralization	
Spiked Cr (mg)		Found (mg) ± SD	% Recovery
1.00		0.80 ± 0.02	80.1
1.00		0.93 ± 0.00	93.6
1.00		0.95 ± 0.01	95.2
2.99		2.67 ± 0.05	89.4
2.99		2.68 ± 0.02	89.6
2.99		2.68 ± 0.03	89.5
4.98		4.47 ± 0.01	89.7
4.98		4.64 ± 0.06	93.1
4.98		4.58 ± 0.03	91.8
(b)		EDTA extraction	
Spiked Cr (mg)		Found (mg) ± SD	% Recovery
2.00		2.17 ± 0.00	108.6
2.00		2.08 ± 0.01	103.9
2.00		1.96 ± 0.01	98.1
6.00		5.67 ± 0.02	94.4
6.00		5.46 ± 0.02	91.0
6.00		5.93 ± 0.01	98.9
10.00		9.67 ± 0.02	96.6
10.00		9.37 ± 0.02	93.7
10.00		9.55 ± 0.02	95.4

the hexavalent chromium that was used to spike the samples, as well as the total Cr content from leather.

3.3 Speciation by IC-ICP-MS

Regarding chromium speciation, IC-ICP-MS analyses were performed in order to verify if the EDTA extraction conditions permit to preserve chromium speciation. This was demonstrated by analyzing Cr isotopic standards. The analyses were carried out in the standard mode. KED mode was also performed but no differences were perceived. Figure 3a shows the chromatogram obtained from a $^{50}\text{Cr(VI)}$ solution. As can be seen, Cr(VI) elutes at 920 s and there is no other peak in the chromatogram. Figure 3b corresponds to a Chromium(III) isotopic reference standard enriched in ^{53}Cr , which was complexed with EDTA at the same conditions as the EDTA extraction used for leather. In this case, it is possible to observe that a $^{53}\text{Cr(III)-EDTA}$ complex was formed and it eluted at 230 s. An unidentified small peak with a retention time of 150 s was also noticed. Nevertheless, no peak was observed around 920 s. Thus, these conditions did not induce a conversion from Cr(III) to Cr(VI). Figure 3c corresponds to a Chromium(VI) isotopic reference standard enriched in ^{50}Cr . This solution was submitted to the same conditions as the EDTA extraction used for leather. As it was expected, no complex was formed

as EDTA complexes only with Cr(III). Anyhow, it is possible to see, when zooming in, two small peaks with a retention time of 150 and 230 s. Each of these peaks represent 1.5% of the principal peak. The peak with a retention time of 150 s has not been identified. On the other hand, the retention time of the second peak corresponds to that of the Cr(III)-EDTA complex. Consequently, in the reverse direction, there is a conversion from Cr(VI) to Cr(III) under these extraction conditions, but this conversion is minimal. From this study, we can conclude that the EDTA extraction conditions allowed quantitative chromium extraction while limiting chromium interconversions. An ongoing study focuses on different leathers samples (chrome-tanned, colored, satin, or glossy leather), extracted with EDTA as presented in this study, and analyzed by IC-ICP-MS. Different analysis conditions are tested to give access to the amount of both species (Cr(III) and Cr(VI)), with the same protocol (extraction and chromatographic method).

4 Conclusions

This work focused on the extraction of chromium from leather samples. On the one hand, the acidic digestion allowed the complete mineralization and gave access to the total chromium content in each sample, but such conditions do not preserve chromium speciation as

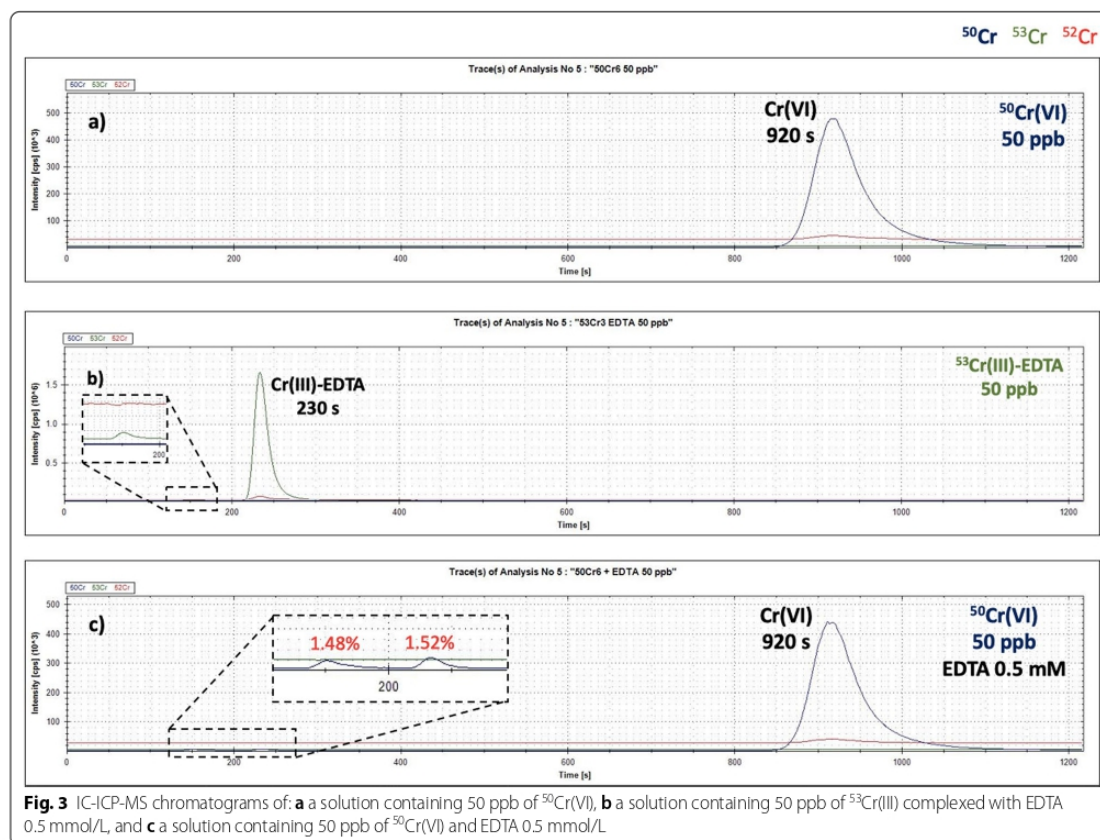


Fig. 3 IC-ICP-MS chromatograms of: **a** a solution containing 50 ppb of $^{50}\text{Cr(VI)}$, **b** a solution containing 50 ppb of $^{53}\text{Cr(III)}$ complexed with EDTA 0.5 mmol/L, and **c** a solution containing 50 ppb of $^{50}\text{Cr(VI)}$ and EDTA 0.5 mmol/L

Cr(VI) is reduced to Cr(III). Among the extraction conditions tested (i.e. EDTA, DTPA, NH_4OH , NH_4NO_3 , water, phosphate buffer), EDTA proved to be the most efficient and ensured quantitative extraction of Cr(VI) and Cr(III) as a Cr(III)-EDTA complex. The content of total chromium obtained by the EDTA extraction was similar to the content obtained by direct mineralization. The method was validated in terms of linearity, recovery, interday precision, method detection limit, and method quantification limit. The separation of the extracted species was studied through IC-ICP-MS using Cr isotopic standards, which proved that the EDTA extraction conditions permit a quantitative extraction while limiting the interconversions. No interconversion from Cr(III) to Cr(VI) was noticed, and a limited portion (1.5%) of Cr(VI) was transformed into Cr(III) under the extraction conditions used.

Abbreviations

CRMs: Certified reference materials; DPC: 1,5-Diphenylcarbazine; DTPA: Diethylenetriaminepentaacetic acid; EDTA: Ethylenediaminetetraacetic acid; FAAS:

Flame atomic absorption spectrometry; HPLC-ICP-MS: High-performance liquid chromatography-inductively coupled plasma-mass spectrometry; IC-DAD: Ion chromatography-diode array detector; IC-ICP-MS: Ion chromatography-inductively coupled plasma-mass spectrometry; IC-UV-Vis: Ion chromatography-UV-visible detection; ICP-AES: Inductively coupled plasma-atomic emission spectrometry; ISO: International Organization for Standardization; KED: Kinetic energy discrimination mode; LOD: Limit of detection; LOQ: Limit of quantification; MDL: Method detection limit; MQL: Method quantification limit; RSD: Relative standard deviation; SM&T: Standard, measurement and testing programme; STD: Standard mode; UV-Vis: UV visible spectroscopy.

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Authors' contributions

MGAS performed the extractions, analyzed, and interpreted the data. CDB and MHR were in charge of the instrumentation. CA was in charge of getting the funding and provision of equipment. MM corrected the manuscript and led scientific discussions. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Competing interests

The authors declare no competing interests.

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Chapter 4

Chapter 4. Separation and speciation

A comparative study of pretreatment methods was presented in Chapter 3. This last chapter focuses on the challenge of separation and speciation of chromium by ion chromatography – inductively coupled plasma – mass spectrometry (IC-ICP-MS). Various tests were necessary to find the best conditions that allow the quantitative extraction and analysis of trivalent and hexavalent chromium while maintaining speciation. The final method was validated and is presented hereafter. This chapter is intended to be submitted to a peer-reviewed journal. Consequently, results are presented in a publication format with additional data concerning the preliminary tests (which will not appear in the final publication) in order to present the whole context and trials, and as a way to reflect the huge effort that the experimental work required.

Quantitative analysis of chromium VI and III from tanned leather: a comparative study of analytical methods

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Keywords

Chromium speciation, Tanned leather, Interconversions, Ion chromatography, Inductively coupled plasma – mass spectrometry, Fourier-Transform Ion Cyclotron Resonance Mass Spectrometry.

Highlights

- Different analytical methods for chromium speciation in leather were evaluated.
- Interconversions were monitored through the use of chromium isotopic standards.
- Two different extractions were used to quantitatively extract Cr(III) and Cr(VI), separately, and without interconversions.
- The optimized analytical method, allowing to analyze both Cr(III) and Cr(VI) simultaneously, was validated in terms of linearity, LOD, LOQ and precision.

Abstract

In the present work, a comparative study of analytical methods for the quantitative determination of trivalent and hexavalent chromium is presented. For

the analysis by ion chromatography – inductively coupled plasma – mass spectrometry (IC-ICP-MS) two different columns were tested, as well as different mobile phases, different pH of the samples and different complexing agents. The optimized analytical method permitted the separation of Cr(III) and Cr(VI) using 75 mmol/L NH_4NO_3 pH 3 as chromatographic eluent. Limit of detection (LOD) and limit of quantification (LOQ) for Cr(III) were found to be 0.016 and 0.054 $\mu\text{g/L}$ (0.3 and 1.1 $\mu\text{g/Kg}$), respectively, and for Cr(VI) 0.13 and 0.43 $\mu\text{g/L}$ (7 and 22 $\mu\text{g/Kg}$), respectively. Possible species interconversions were monitored through the use of chromium isotopic standards, which confirmed that the optimized methodology preserves chromium speciation. Fourier-transform ion cyclotron resonance – mass spectrometry (FTICR-MS) permitted the structure elucidation of the complex formed during ethylenediaminetetraacetic acid (EDTA) extraction.

1. Introduction

Tanning is the process of transforming raw hide into leather. The main tanning methods are chrome tanning, vegetable tanning, aldehyde tanning, synthetic tanning and alum-tawing; being chrome and vegetable tanning the most common techniques^[1]. However, chrome tanning accounts for around 90% of global leather production^[2]. This process uses trivalent chromium salts such as basic chromium sulfate, $(\text{Cr}_2(\text{OH}(\text{SO}_4)_3))$ ^[3]. The chrome tanning reaction is fast (around 6 hours), confers high hydrothermal stability, makes collagen more hydrophobic and allows the retention of colorants^[4]. All of these advantages make of chrome tanning the most widespread technique.

Trivalent chromium exists as cationic species (Cr^{3+} , $\text{Cr}(\text{OH})^{2+}$, $\text{Cr}(\text{OH})_2^+$) and is not considered as toxic but it can be unintentionally oxidized into hexavalent chromium, a known mutagenic and carcinogenic that exists as anionic species (HCrO_4^- , CrO_4^{2-} , HCr_2O_7^- , $\text{Cr}_2\text{O}_7^{2-}$)^[5], as a consequence of different factors such as high temperature, dry atmosphere and presence of UV light^[6]. Additionally, in

direct contact with the skin, chrome-tanned leather products may elicit allergic contact dermatitis^[7, 8].

Recent studies have revealed that there is no relationship between released hexavalent and trivalent chromium^[8]. In other words, the one-time testing of hexavalent chromium does not correlate to the total allergic potential of a chrome-tanned leather item. Considering that chromium speciation has a dynamic behavior throughout the life-cycle of leather, it is important to quantify both chromium species separately in order to prevent the exposure to hazardous species^[9].

Regarding hexavalent chromium in leather, the European Union regulates its content to 3 mg/kg (of the total dry weight) in leather goods coming into direct contact with skin^[10]. However, the leather standard by Oeko-Tex[®] limits total chromium content to 2 mg/kg for leather products intended for babies, and to 200 mg/kg for leather products in direct contact with skin^[11].

Concerning hexavalent chromium determination, the International Organization for Standardization (ISO) 17075 standard describes a procedure for the selective extraction of Cr(VI) in tanned leather using a phosphate buffer: $K_2HPO_4 \cdot 3H_2O$ at pH 8.0. The analysis of the extract is performed via colorimetric^[12] or chromatographic analysis^[13]. Both methods rely on the complexation of hexavalent chromium with 1,5-diphenylcarbazide (DPC) for UV-Vis detection. An inconvenience may surge due to the interference of colorants that may absorb at the same wavelength (540 nm) as the complex formed between Cr(VI) and DPC. On the other side, trivalent chromium is generally measured after its oxidation to Cr(VI), or calculated by subtracting Cr(VI) from the total Cr content^[5]. This calculation is only reliable if no interconversions occur that interfere with Cr(VI) determination, which in turn decreases the validity of Cr(III) determination. In aqueous media the oxidation state of chromium is determined by the redox potential and pH. Therefore, the preservation of chromium speciation through the extraction and analysis steps, represents a huge challenge.

The aim of this work was to develop an analytical method to quantitatively determine hexavalent chromium and trivalent chromium as a Cr(III)-EDTA⁻ complex from leather samples, limiting the interconversions during extraction and analysis. Different conditions (columns, mobile phases, pH of the samples, complexing agents) were tested for the analysis through IC-ICP-MS. In order to follow possible chromium interconversions, chromium isotopic standards were used.

2. Experimental

2.1 Instrumentation

Extractions and complexations were performed with a Milestone ETHOS 1 microwave digestion system equipped with a temperature sensor. TFM reactors were used with a maximum pressure of 100 bar. The pH of the solutions was measured using a PHM240 pH/Ion Meter (MeterLab, Radiometer Analytical, Villeurbanne, France). Centrifugation of the extracts was performed with an Eppendorf Centrifuge 5702.

Chromatographic separation was performed using a Dionex ICS-6000 HPIC System (Thermo Fisher Scientific). The ion chromatography pump delivered the mobile phase and the sample at 0.25 mL/min. A Dionex ADRS 600 Anion Dynamically Regenerated Suppressor (2 mm) (Thermo Fisher Scientific) was set before the entrance into the Inductively Coupled Plasma - Mass Spectrometer (ICP-MS) to avoid salt deposition and was operated in the external regeneration mode. A constant flow of 0.6 mL/min of ultrapure water was supplied by means of a Dionex AXP auxiliary pump (Thermo Fisher Scientific). Two guard columns were placed before the analytical column: a Dionex IonPac NG1 (2 × 50 mm, particle size: 10 µm) and a Dionex IonPac AG7 (2 × 50 mm, particle size: 10 µm). Ions were separated with a Dionex IonPac AS7 column (2 × 250 mm, particle size: 10 µm). The analytical column was coupled to the nebulizer of the ICP-MS using a PEEK tubing (0.25 mm i.d., 150 cm long). Detection of chromium species was

performed on an iCAP RQ ICP-MS instrument (Thermo Fisher Scientific) fitted with a collision cell. Two different measurement modes were used: standard mode (STD) and kinetic energy discrimination mode (KED). In the KED mode, He gas was injected in the collision cell and an energy discrimination barrier was applied in order to remove unwanted polyatomic interferences. Data processing was performed using ThermoFisher Scientific Qtegra software. As example, optimized conditions for the plasma and nebulizer for ICP-MS are given in Table 1. Figure 1 shows a diagram of the setup used for the optimized conditions. For each setup (optimized conditions and tests), mobile phases were previously degassed using an Elmasonic S ultrasonic bath (Elma, Singen, Germany).

Table 1. Optimized operating conditions for ICP-MS.

	iCAP RQ ICP-MS
Radio frequency power (W)	1550
Plasma gas flow rate (L/min)	14
Auxiliary argon flow rate (L/min)	0.8
Nebulizer gas flow rate (L/min)	1.0
Purge gas (nitrogen) flow rate (L/min)	-
Peristaltic pump rate (rpm)	15
Sample uptake delay (s)	30
Replicates	3
Rinse time (s)	30
Sampler	SC-2DX (with <i>FAST</i> valve system) or online coupling with IC system
Chamber	Cyclonic
Nebulizer type	Concentric

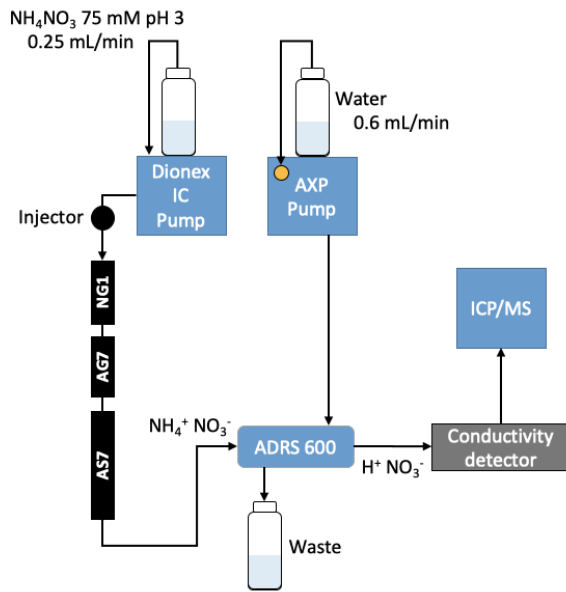


Figure 1. Chromatographic setup for the optimized separation conditions (AS7 column using NH_4NO_3 in isocratic mode).

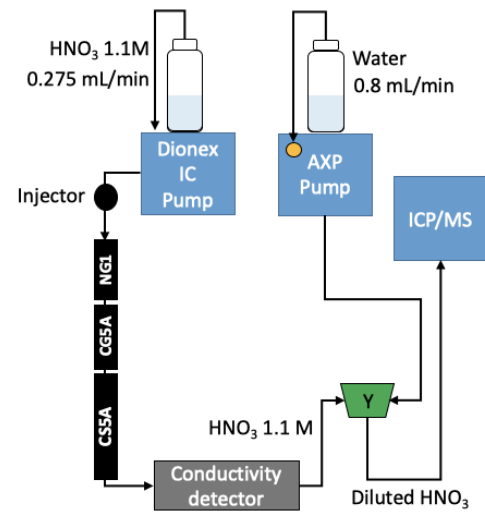


Figure 2. Chromatographic setup for the CS5A column using HNO_3 in gradient mode.

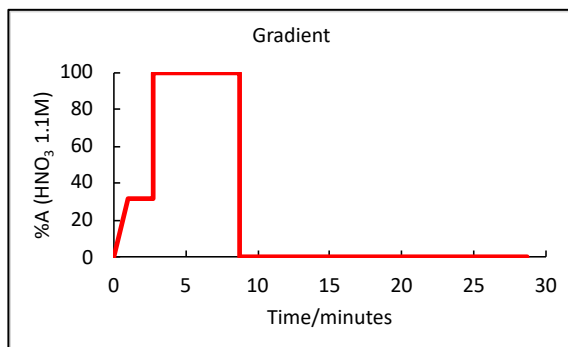


Figure 3. Gradient for the setup of the CS5A column. %A = $\%(\text{HNO}_3 \text{ 1.1M})$; %B = $(100\% - \%A)\text{H}_2\text{O}$.



Figure 4. Chromatographic setup used for preliminary studies.

A second setup was tested when trying to optimize the separation conditions (Figure 2). For these experiments, a Dionex IonPac NG1 guard column (2×50 mm, particle size: $10 \mu\text{m}$), a Dionex IonPac CG5A guard column (2×50 mm, particle size: $9 \mu\text{m}$) and a Dionex IonPac CS5A ion-exchange column (2×250 mm, particle size: $9 \mu\text{m}$) were installed. In the same way as the first setup, the ion chromatography pump delivered the mobile phase and the sample, but in

this case, in gradient mode (shown in Figure 3). This solution traveled through the columns where species were separated and eluted at different retention times. After passing through the conductivity detector, a Y-connector was installed. This permitted the arrival of the sample and the mobile phase at 0.275 mL/min from one side, and the arrival of ultrapure water at 0.8 mL/min by means of an AXP auxiliary pump (Thermo Fisher Scientific) from the other side. The final solution (at the exit of the Y-connector) was diluted nitric acid. This solution finally arrived to the ICP-MS where detection was completed.

Preliminary tests were conducted using a simple setup. In this setup, a NG1 guard column and an AS7 ion-exchange column were used. However, since the mobile phase was nitric acid, no suppressor was used, consequently, no AXP pump was used neither. The setup is described in Figure 4.

FTICR MS analyses were achieved using a SolariX XR instrument (Bruker Daltonics, Bremen, Germany) equipped with a 12T superconducting magnet and a dynamically harmonized ICR cell (ParaCell®). An ESI source was used in positive and negative ion modes. The operating parameters for direct infusion analyses on ESI(+) and ESI(−) are reported in Table 2. The analogue image current was digitized with 4 million data points resulting in the recording of a 1.118 s time domain signal for ESI analyses. The time domain signals were transformed into the corresponding frequency domain by Fourier transform (after full-sine apodization and one zero fill). These settings resulted in a mass resolving power of 500,000 at m/z 200. Mass spectra were acquired using Bruker Compass FtmsControl 2.3, and processed with Bruker Compass DataAnalysis 5.1 (Bruker Daltonics GmbH, Bremen, Germany). Molecular formulas were assigned to the peaks with a signal-to-noise (S/N) ratio greater than 6, with even and odd electron configuration.

Table 2. FTICR parameters for the analysis of the Cr(III)-EDTA⁻ complex.

		ESI(+)	ESI(-)
Source parameters	Flow ($\mu\text{L}\cdot\text{h}^{-1}$)	80	120
	Nebulizer gas pressure (bar)	2.0	2.0
	Drying flow ($\text{L}\cdot\text{min}^{-1}$)	4.0	4.0
	Drying temperature ($^{\circ}\text{C}$)	200	180
	Capillary voltage (kV)	4.2	4.1
	Ion transfer parameters	Quadrupole m/z	100
	Time of flight (ms)	1.0	1.0
Acquisition parameters	m/z range	98-1200	98-1200
	Scan number	16	100
	Accumulation time (s)	0.05	0.05
	Transient length (s)	1.118	1.118

2.2 Materials

Ultrapure water (resistivity 18.2 MW \cdot cm), obtained from a Milli-Q system (Millipore, Bedford, MA, USA), was used for preparation of all solutions unless stated otherwise. Nitric acid 67-69% (Ultrapure Normatom, VWR Chemicals, Leuven, Belgium) and NH_4NO_3 (Normapur guaranteed reagent, Prolabo, Paris, France) were used for the preparation of mobile phases. For preliminary studies, chromium standards were prepared with chromium(III) nitrate nonahydrate (99%, Thermo Scientific, Geel, Belgium) and potassium dichromate (R.P. Normapur, Prolabo, Paris, France). For the extractions and complexations, EDTA (Analytical reagent, VWR Chemicals, Leuven, Belgium), diethylenetriaminepentaacetic acid (DTPA) (97%, Aldrich-Chemie, Germany), 1,5-diphenylcarbazide (Sigma-Aldrich, Steinheim, Germany), $\text{K}_2\text{HPO}_4\cdot 3\text{H}_2\text{O}$ (AnalaR Normapur, VWR Chemicals, Leuven, Belgium), phosphoric acid (85 wt. % in H_2O , Sigma-Aldrich, Germany), NH_4OH 20-22% NH_3 (Optima grade, Fisher Scientific, UK), sulfuric acid (95%, Fisher Scientific,

Geel, Belgium) and methanol (LC-MS grade, Fisher Scientific, Geel, Belgium) were employed. Filtration was performed with 0.45 μm nylon syringe filters (Fisher Scientific). Leather samples came from the luxury industry and were all chrome-tanned and colored. Different types of leather were used for the extractions, such as: chrome-tanned, colored, polished and satin leather. $^{53}\text{Cr}(\text{III})$ (97.01%) and $^{50}\text{Cr}(\text{VI})$ (97.36%) chromium isotopic standards (100 ppm, Certified Reference Material, ISC Science, Oviedo, Spain) were diluted and used to monitor possible chromium interconversions. Chromium(III) nitrate nonahydrate was used for preparing the $\text{Cr}(\text{III})\text{-EDTA}^-$ complex analyzed by FTICR-MS. Optima LC-MS grade water and acetonitrile (purchased from Fisher Chemical) were employed for the dilution of samples analyzed by FTICR-MS.

2.3 Extractions and complexations

EDTA and DTPA extractions/complexations were carried out following the procedures reported in a previous study^[9]. Phosphate buffer extractions were performed with $\text{K}_2\text{HPO}_4 \cdot 3\text{H}_2\text{O}$ at pH 8.0 for three hours under magnetic stirring, and the extracts were subsequently filtrated through nylon 0.45 μm filter, as described in ISO 17075 protocol^[12]. In order to follow possible interconversions, chromium isotopic standards were used instead of the leather samples and followed the whole ISO 17075, EDTA, or DTPA extraction protocol, depending on the method to be tested. For complexations with DPC, extracts or standard solutions were left to react for 15 minutes with a DPC solution. This solution was prepared with DPC, methanol and sulfuric acid, in the same way as the post-column reagent from ISO 17075 chromatographic method^[13].

2.4 Speciation by IC-ICP-MS

Preliminary studies were performed with the setup previously described and depicted in Figure 4. For these studies, HNO_3 1.9% (v/v) was used as mobile phase at a flow rate of 0.4 mL/min. For the chromatographic setup used for the CS5A

column (Figure 2), HNO_3 1.1 M was used as mobile phase in gradient mode as described in Figure 3, %A = %(HNO_3 1.1M), %B = (100%-A) H_2O . The flow rate was 0.275 mL/min. Optimized conditions were found when using the AS7 ion-exchange column. For the optimized setup (Figure 1), 75 mmol/L NH_4NO_3 adjusted to pH 3 with nitric acid 1M was used as chromatographic eluent at a flow rate of 0.25 mL/min. An ADRS suppressor was operated in the external regeneration mode and an auxiliary pump supplied ultrapure water at a flow of 0.6 mL/min.

2.5 FTICR-MS Analyses

Samples were diluted (2X dilution in acetonitrile for Cr(III)-EDTA⁻ complexes (initial concentration of 10 ppm of Cr(III)); 2X dilution in acetonitrile/ H_2O (50/50, v/v) for non-diluted leather extracts), injected and analyzed in the conditions previously indicated. Mass spectra were internally calibrated using confident ions signal over the whole mass range.

3. Results and discussion

3.1 Preliminary studies

Preliminary speciation studies were performed with nitric acid as mobile phase as described on the application note by Thermo Scientific^[14] for the speciation analysis of Cr(III) and Cr(VI) in drinking waters (Cr(VI) eluted first, followed by Cr(III) 70 seconds later). However, the protocol required 0.4 mol/L (approximately 2.5 % v/v) nitric acid, and the ICP-MS should not be used at acidic concentrations beyond 2% (v/v). Therefore, HNO_3 1.9% (v/v) was used instead to prevent corrosion of the cones. Additionally, an AS7 ion-exchange column replaced the AG7 guard column proposed by the protocol.

One of the first parameters to optimize when starting to work with the IC-ICP-MS hyphenation was the dwell time of the mass spectrometer, which is the period of time that the instrument accumulates the signal or the time spent for

acquiring data. Dwell times tested were: 0.02, 0.05, 0.07, 0.2, 0.5, and 1 second. From all of these values, 0.05 seconds provided the best results in terms of chromatographic S/N ratio. Consequently, this was the value used for all the IC-ICP-MS analyses hereafter.

As previously mentioned, the setup for the preliminary tests consisted of a NG1 guard column and an AS7 ion-exchange analytical column. Once the dwell time was optimized, several conditions were tested with the aim of studying the retention of hexavalent and trivalent chromium species. A summary of the obtained results is reported in Table 3. Using the setup described in Figure 4, a mobile phase of HNO₃ 1.9% (v/v) was used at a flow rate of 0.4 and 0.6 mL/min. By varying the flow rate from 0.4 to 0.6 mL/min, the dead time decreased from 70 to 50 seconds, the retention time of Cr(III) decreased from 1300 to 940 seconds, and the retention time of Cr(VI) decreased from 190 to 130 seconds. A chromatogram of the injection of Cr(III) and Cr(VI) using HNO₃ 1.9% (v/v) at 0.4 mL/min can be found in Figure 5 (section 3.2.1).

Subsequently, the addition of organic solvent to the mobile phase was studied. The addition of 5% acetonitrile (ACN) at a flow rate of 0.6 mL/min decreased the retention time of Cr(III) from 940 to 860 seconds and the retention time of Cr(VI) from 130 to 125 seconds as compared to the condition without organic solvent (HNO₃ 1.9% (v/v), 0.6 mL/min). According to Rabin and Stillian^[15], acetonitrile causes a swelling of the latex containing the ion-exchange sites which provokes a less effective cross-linking of the latex and decreases the number of sites per unit area, hence decreasing the time that ions spend bound to the column.

Another test consisted on heating the column compartment at 35°C. When working at a flow rate of 0.6 mL/min, the retention time of Cr(III) increased from 940 to 970 seconds while the retention time of Cr(VI) decreased from 130 to 120 seconds as compared to the condition without heat (HNO₃ 1.9% (v/v), 0.6 mL/min, no heat).

Afterwards, two different concentrations of mobile phase were tested. Decreasing the concentration of the mobile phase from 1.9 to 1.7% (v/v) HNO₃, increased the retention of Cr(III) while the retention of Cr(VI) remained unchanged. For these last preliminary tests, an AG7 guard column replaced the AS7 analytical column in order to decrease run times and perform the experiments faster.

Table 3. Retention times of Cr(III) and Cr(VI) at different conditions (temperature, mobile phase, flow rate).

Dead time [s]	Retention time Cr(III) [s]	Retention time Cr(VI) [s]	Flow rate [mL/min]	Mobile Phase [% v/v]	Temperature [°C]	Columns
70	1300	190	0.4	HNO ₃ 1.9%	21.5	NG1 + AS7
50	940	130	0.6	HNO ₃ 1.9%	21.5	NG1 + AS7
50	860	125	0.6	HNO ₃ 1.9% / ACN 5%	21.5	NG1 + AS7
50	970	120	0.6	HNO ₃ 1.9%	35	NG1 + AS7
30	240	60	0.4	HNO ₃ 1.9%	21.5	NG1 + AG7
30	310	60	0.4	HNO ₃ 1.7%	21.5	NG1 + AG7

On a previous study^[9], EDTA proved to be the most performant method to extract Cr(VI) and Cr(III) as a Cr(III)-EDTA⁻ complex from leather samples, hence the importance of studying the retention of Cr(III)-EDTA⁻ as well.

Table 4 shows the retention times of the peaks observed after injecting three Cr(III)-EDTA⁻ samples at different pH using an NG1 guard column followed by an AG7 guard column for species separation. HNO₃ 1.9% (v/v) was used as mobile phase at a flow rate of 0.3 mL/min. The objective of this test was to study the stability of the complex at basic and acidic medium.

For the first injection, the sample had a pH of 11; this was achieved by adjusting the sample with a NaOH solution. As we can see from the Table 4, three peaks appeared: one peak at 55 seconds corresponding to the Cr(III)-EDTA⁻ complex, another peak at 90 seconds corresponding to Cr(VI), and a third peak at 330 seconds corresponding to Cr(III). The second sample was injected at a pH of

6; this was achieved by diluting the sample in water. For this sample, the chromatogram showed 2 peaks: one peak at 55 seconds corresponding to the Cr(III)-EDTA⁻ complex, and another peak at 90 seconds corresponding to Cr(VI). Finally, the third sample was injected at pH 2 which was achieved by acidifying the sample with nitric acid (final solution acidic concentration: HNO₃ 0.1% v/v). For this sample, only one peak appeared at 55 seconds. Then, acidifying the samples maintained the stability of Cr(III)-EDTA⁻ and no decomplexation was observed. However, if the solution is too acidic, Cr(VI) is transformed into Cr(III). In order to confirm the retention times of Cr(III) and Cr(VI) under these conditions, a standard solution was injected. This solution contained Cr(III) and Cr(VI), 50 ppb, HNO₃ 0.1% (v/v).

Nonetheless, it is important to mention that in those conditions Cr(III)-EDTA⁻ elutes very close to the dead time. Due to the size of the molecule, it has little entrance into the pores of the stationary phase and flows freely through the column.

Table 4. Retention times of peaks observed after injecting Cr(III)-EDTA⁻ at three different pH.

Dead time [s]	Retention time Cr(III) [s]	Retention time Cr(VI) [s]	Retention time Cr(III)-EDTA ⁻ [s]	Flow rate [mL/min]	Mobile Phase [% v/v]	Sample pH	Columns
50	330	90	55	0.3	HNO ₃ 1.9%	11	NG1 + AG7
50		90	55	0.3	HNO ₃ 1.9%	6	NG1 + AG7
50			55	0.3	HNO ₃ 1.9%	2	NG1 + AG7

3.2 Nitric acid as mobile phase

3.2.1 AS7 ion-exchange column

After testing several conditions during preliminary tests and considering that the Cr(III)-EDTA⁻ complex elutes very close to the dead time, the retained conditions were:

- HNO₃ 1.9% instead of 1.7% (v/v) as mobile phase since decreasing its concentration has no effect on the retention of Cr(VI) and increases significantly the retention of Cr(III).
- A flow rate of 0.4 mL/min instead of 0.6 mL/min because it is closer to the optimal flow rate of the column.
- Column compartment at room temperature since heat slightly decreases the retention time of Cr(VI) and increases the retention time of Cr(III). In ion chromatography the temperature increases or reduces the retention depending on whether the phenomenon is endothermic or exothermic (the interactions between the solute, the charged particle and the charged mobile phase). In this case, Cr(III) is cationic and Cr(VI) is anionic (they don't have the same charge). Thus, temperature can increase the retention of one species and decrease the retention of the other.
- No addition of acetonitrile since it decreases retention times of both chromium species.
- An AS7 analytical column instead of an AG7 guard column as depicted in Figure 4.
- Samples were injected with an HNO₃ concentration of 0.1% (v/v) in order to preserve the stability of the complex.

This permitted to separate Cr(III) and Cr(VI). Cr(VI) eluted at 190 seconds, while Cr(III) eluted at 1300 seconds. Nevertheless, Cr(III)-EDTA⁻ eluted at 75 seconds, still very close to the dead time which was 70 seconds. This can be appreciated in the chromatograms shown in Figure 5.

The AS7 ion-exchange column is a latex-based pellicular column. Its central polymeric packing is sulfonated (cation exchanger) while the latex carries quaternary ammonium groups (anion exchanger). Therefore, this column contains both anionic and cationic ion-exchange capacity^[16]. The latex is electrostatically agglomerated to the central backbone. The particularity of this structure induces a possible penetration of metallic cations (whose hydration sphere is reduced)

towards the sulfonates of the central bead, and thus, their retention. In classical analysis (eluent of lower ionic strength and basic pH) there would be a more important retention of anionic species (Cr(VI) species) and the metal cation (Cr(III)) would remain stuck to the core of the stationary phase. The use of acid allows the elution of Cr(III) since the ionic strength of the nitrate at acidic pH has the purpose of minimizing the retention of anions.

Being Cr(III)-EDTA⁻ a big molecular structure (Figure 6) compared to the size of the pores of the stationary phase, it has little entrance into the pores and flows freely. This explains why the complex elutes at first (retention time = 75 s) and very close to the dead time, followed by Cr(VI) which elutes 115 seconds later (retention time = 190 s). Cr(III) elutes at last, when nitric acid succeeds in removing the cation from the heart of the central bead.

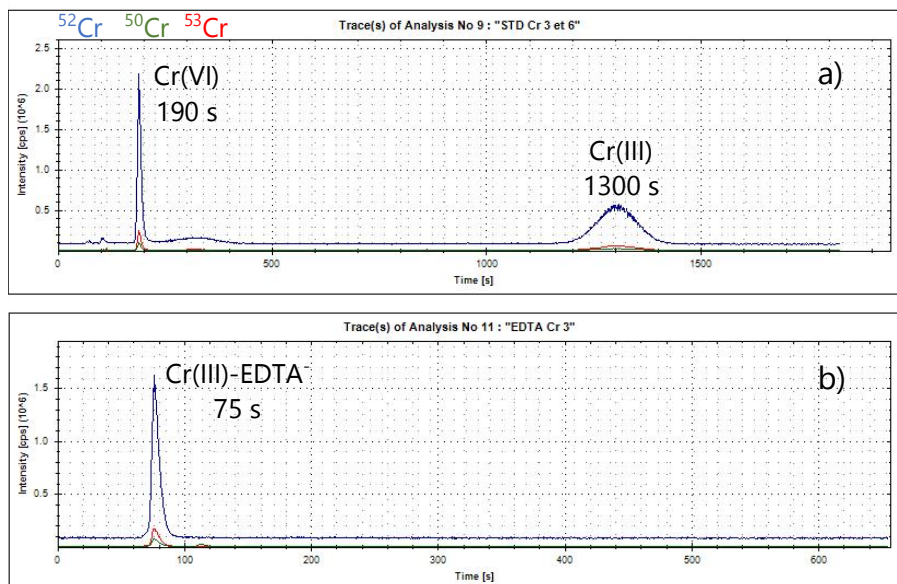
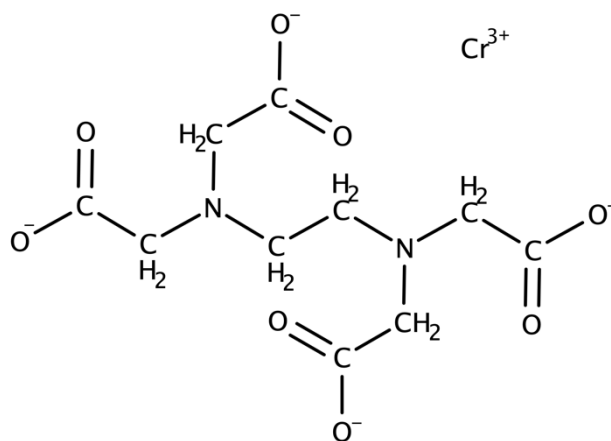


Figure 5. IC-ICP-MS chromatograms of: a) Cr(VI) and Cr(III), 50 ppb, HNO₃ 0.1% (v/v). b) 50 ppb of Cr(III) in presence of EDTA (after going through the EDTA extraction/complexation conditions), HNO₃ 0.1% (v/v). NG1 guard column + AS7 column, mobile phase: HNO₃ 1.9% (v/v), flow rate: 0.4 mL/min, STD mode, dead time: 70 s.

Figure 6. Molecular structure of Cr(III)-EDTA⁻.

3.2.2 CS5A ion-exchange column

Hernandez et al. reported a methodology for the determination of Cr(III) and Cr(VI) in dairy products through high performance liquid chromatography – inductively coupled plasma – mass spectrometry (HPLC-ICP-MS). They proposed two different gradient programs for the determination of Cr(III)^[17] and Cr(VI)^[18], separately, as well as two different extraction methods. Nonetheless, in order to adapt the gradient program to the current chromatographic conditions, the HPLC gradient method transfer calculator from University of Geneva was used^[19,20]. The method calculated was later optimized in order to be able to use one sole gradient program to elute both chromium species, Cr(III) and Cr(VI), together with Cr(III)-EDTA⁻. The final gradient is graphically described in Figure 3 and more explicitly described in Table 5. The setup used for these tests was the setup depicted in Figure 2.

Table 5. Chromatographic conditions of the study by Hernandez et al.^[17] versus conditions of the current study.

	Hernandez et al.		Current study
	Cr(VI)	Cr(III)	Cr(VI) and Cr(III)
Columns	IonPac CG5A (4 × 50 mm, particle size: 9 μm) + IonPac CS5A (4 × 250 mm, particle size: 9 μm)		IonPac NG1 (2 × 50 mm, particle size: 10 μm) + IonPac CG5A (2 × 50 mm, particle size: 9 μm) + IonPac CS5A (2 × 250 mm, particle size: 9 μm)
Column temperature	20°C		30°C
Mobile phase flow rate	1.1 mL/min		0.275 mL/min
Mobile phase A	Nitric acid 2 mol/L		Nitric acid 1.1 mol/L
Mobile phase B	Ultrapure water		Ultrapure water
Gradient program	17.5% A: 100 s 55% A: 360 s	0-17.5% A: 120 s 17.5% A: 102 s 55% A: 360 s	0-32% A: 60 s 32% A: 102 s 100% A: 360 s 0% A: 1200 s

The first chromatograms were obtained using the setup and conditions previously described but without guard columns, only the CS5A analytical column was used since only standards were used in this part. The EDTA extraction/complexation method was performed in the following way: 10 ppm of ⁵³Cr(III) (concentration of the stock solution: 100 ppm of ⁵³Cr(III)) were complexed with 4 mL EDTA 0.5 mM at pH 10. Standard solutions (⁵³Cr(III) and ⁵⁰Cr(VI))

together with the Cr(III)-EDTA⁻ complex were diluted and injected and, as it can be seen in Figure 7 a), the separation of the three species (Cr(III)-EDTA⁻, Cr(III) and Cr(VI)) was achieved. The dead time was 90 seconds. Cr(III)-EDTA⁻ eluted at first and had a retention time of 135 seconds, it was followed by Cr(VI) with a retention time of 195 seconds, and Cr(III) eluted at last with a retention time of 488 seconds.

As it can be seen, the order of retention times was the same as that obtained using the AS7 ion-exchange column with nitric acid as mobile phase. In fact, the CS5A column has two ion-exchange groups: sulfonic acid (cation-exchange group) and quaternary ammonium (anion-exchange group)^[21]. This can explain the order of retention times. Due to the size of the Cr(III)-EDTA⁻ complex, it is not strongly retained in the column and it elutes at mild conditions (32% mobile phase A). Cr(VI) anionic species elute when the mobile phase conditions are more acidic (100% mobile phase A) and, therefore, capable of breaking the interactions between the analyte anions and the quaternary ammonium groups of the stationary phase. The Cr(III) cation elutes at the end, 293 seconds after Cr(VI), as a consequence of the strong interactions with the sulfonic acid groups.

Contrary to the elution on the AS7 column, on column CS5A the Cr(III)-EDTA⁻ complex was more retained and appeared 45 seconds after the dead time. Different standard solutions containing the three species at 1, 25, 50, 75 and 100 ppb (HNO₃ 0.1% (v/v)) were injected (Figure 7 b)). For Cr(III)-EDTA⁻, the coefficient of determination (R^2) of the plotted calibration curve was 0.9986, for Cr(VI) it was equal to 0.9914, and for Cr(III) it was equal to 0.9966. It could be concluded from this part that the three species showed a linear behavior.

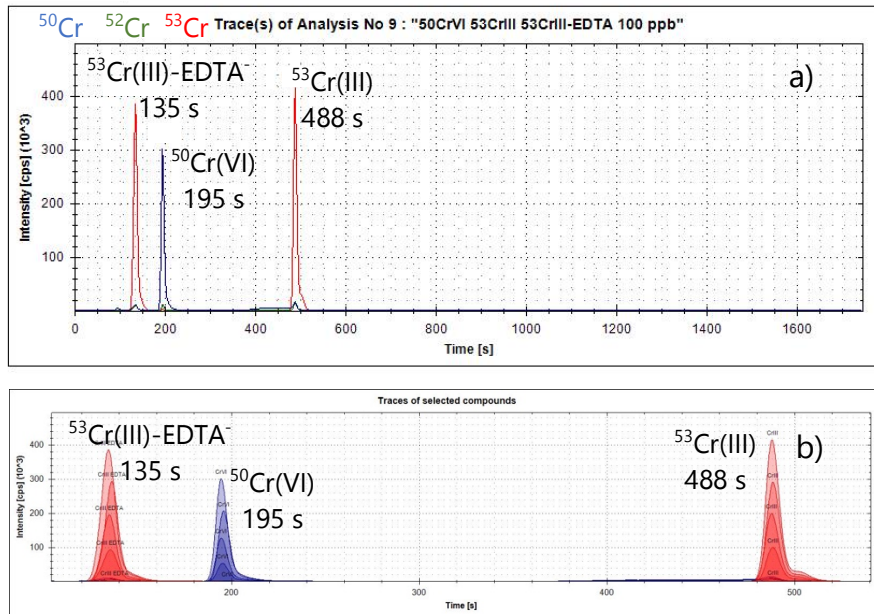


Figure 7. IC-ICP-MS chromatograms of: a) 100 ppb of $^{53}\text{Cr(III)}$, 100 ppb of $^{50}\text{Cr(VI)}$, and 100 ppb of $^{53}\text{Cr(III)}$ in presence of EDTA (after going through the EDTA extraction/complexation conditions), HNO_3 0.1% (v/v). b) Chromium standard solutions at 1, 25, 50, 75 and 100 ppb, HNO_3 0.1% (v/v). CS5A column, mobile phase: A: HNO_3 1.1 mol/L, B: ultrapure water, flow rate: 0.275 mL/min, KED mode, dead time: 90 s.

Figure 8 a) shows the chromatogram obtained from the injection of $^{53}\text{Cr(III)}$, $^{50}\text{Cr(VI)}$ and $^{53}\text{Cr(III)-EDTA}^-$, HNO_3 0.1% (v/v). For these experiments, two guard columns were added: a NG1 guard column and a CG5A guard column, since real samples were injected. It can be noticed that by adding two guard columns before the analytical column, the dead time shifted from 90 to 120 seconds, the retention time of Cr(III)-EDTA^- shifted from 135 to 178 seconds, the retention time of Cr(VI) shifted from 195 to 243 seconds, and the retention time of Cr(III) shifted from 488 to 542 seconds.

Afterwards, an EDTA extraction of a leather sample was performed. The extract was diluted in water (5000X dilution) and injected. Next, the sample was spiked with 50 ppb of $^{50}\text{Cr(VI)}$ and injected once again. Figure 8 b) corresponds to the spiked sample and it can be noticed that there is a peak at 178 seconds (the red line corresponding to the naturally occurring isotope) which corresponds

to the Cr(III)-EDTA^- complex, and a peak at 243 seconds (the blue line corresponding to the ^{50}Cr isotope) that corresponds to the spiked $^{50}\text{Cr(VI)}$. The inset from Figure 8 b) shows the sample before the $^{50}\text{Cr(VI)}$ spike. It can be concluded from this part that Cr(VI) is indeed present in the EDTA extract but the Cr(III)-EDTA^- peak covers the Cr(VI) peak, hence the determination is not possible for samples where there is a large discrepancy in concentrations between Cr(III) (Cr(III)-EDTA^- once complexed) and Cr(VI) .

In an effort to verify that the Cr(III) complexation with EDTA is complete, the $^{53}\text{Cr(III)-EDTA}^-$ complex was diluted and injected all alone. As it can be seen in Figure 9, when $^{53}\text{Cr(III)}$ is injected in the presence of EDTA after following the extraction protocol, it is possible to observe the main peak of the trivalent chromium complex at 178 seconds. This permitted to confirm that the Cr(III) complexation with EDTA is complete since no additional peak is observed other than the peak corresponding to the Cr(III)-EDTA^- complex.

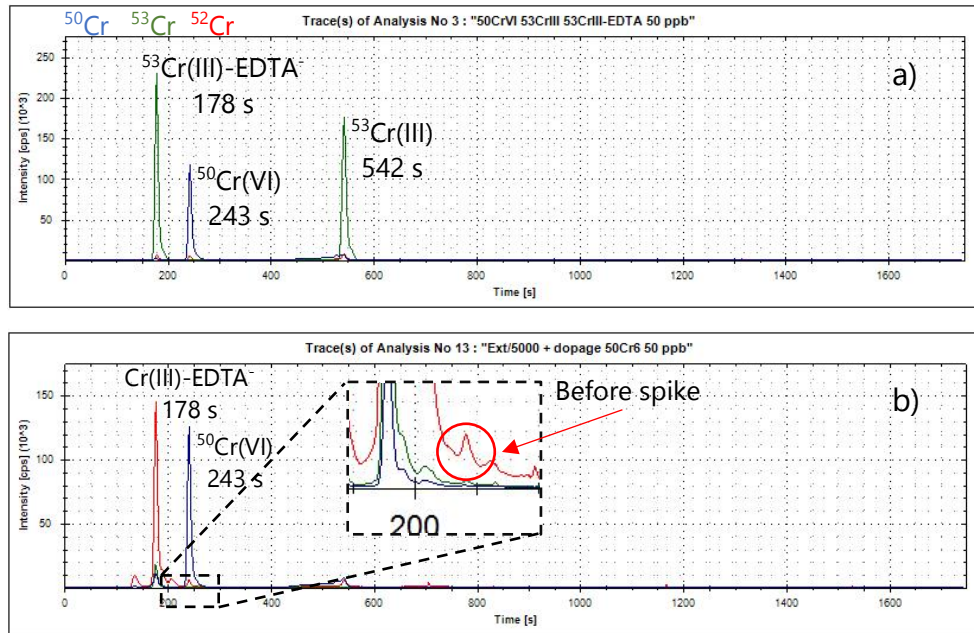


Figure 8. IC-ICP-MS chromatograms of: a) 50 ppb of $^{53}\text{Cr(III)}$, 50 ppb of $^{50}\text{Cr(VI)}$, and 50 ppb of $^{53}\text{Cr(III)}$ in presence of EDTA (after going through the EDTA extraction/complexation conditions), HNO_3 0.1% (v/v). b) EDTA extraction of a leather sample 5000X dilution in water, spiked with 50 ppb $^{50}\text{Cr(VI)}$. NG1 guard column + CG5A guard column + CS5A column, mobile phase: A: HNO_3 1.1 mol/L, B: ultrapure water, flow rate: 0.275 mL/min, KED mode, dead time: 120 s.

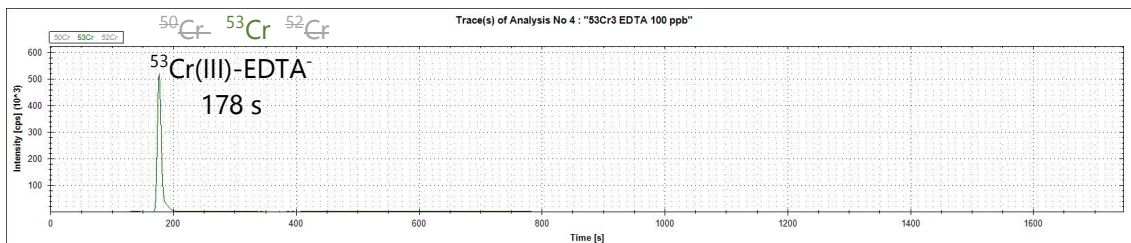


Figure 9. IC-ICP-MS chromatogram of 100 ppb of $^{53}\text{Cr(III)}$ in presence of EDTA (after going through the EDTA extraction/complexation conditions). NG1 guard column + CG5A guard column + CS5A column, mobile phase: A: HNO_3 1.1 mol/L, B: ultrapure water, flow rate: 0.275 mL/min, KED mode, dead time: 120 s.

3.3 Ammonium nitrate as mobile phase

3.3.1 AS7 ion-exchange column

Gürleyük and Wallschläger^[22] derivatized trivalent chromium with EDTA and achieved its separation from hexavalent chromium in certified reference materials (CRMs) (synthetic and estuarine water) by IC-ICP-MS using a self-regenerating

suppressor to avoid salt deposition, an ion-exchange AS7 column (4 × 250 mm, particle size: 10 μm), an AG7 guard column (4 × 50 mm, particle size: 10 μm), and 50mM NH₄NO₃ at pH 8 as chromatographic eluent. They obtained an LOD of 12 ng/L for Cr(VI) and 5 ng/L for Cr(III).

Encouraged by the reported results, the chromatographic setup from Figure 1 was used (NG1 and AG7 guard columns, and an AS7 analytical column). The first mobile phase tested was 50mM NH₄NO₃ at pH 8. The first experiments consisted in injecting Cr(III)-EDTA⁻ at different pH at a flow rate of 0.4 mL/min. Samples were thus injected at pH 2 (adjusted with HNO₃), at pH 6 (diluted in water) and at pH 11 (adjusted with NaOH). Dead time was 150 seconds, the retention time of Cr(III)-EDTA⁻ at pH 2 was 270 seconds, and the retention time of Cr(III)-EDTA⁻ at pH 6 and at pH 11 was 295 seconds. Nevertheless, at pH 11 a very small peak appeared around 650 seconds, which was the retention time of Cr(VI) as confirmed later by injecting a Cr(VI) standard solution. From this preliminary test, under these conditions, it was evident that a highly basic pH (pH equal to 11) of the samples needed to be avoided with the aim of preventing possible decomplexations/interconversions.

For the next experiments, Cr(III) and Cr(VI) standard solutions were injected together with Cr(III)-EDTA⁻, at a flow rate of 0.25 mL/min. As shown in Figure 10 a), when injecting Cr(III) no peak was observed before 2500 seconds. Indeed, there was only a small peak around 1200 seconds, though this small peak was also present in the blank. Later on, a Cr(VI) standard solution was injected (Figure 10 b)) and a peak appeared at 1200 seconds. Therefore, the peak present in the blank and after the injection of Cr(III) was a contamination of Cr(VI) in the column, as a result from previous injections of hexavalent chromium. The chromatogram that resulted from the injection of Cr(III)-EDTA⁻ is shown in Figure 10 c). A peak corresponding to the Cr(III)-EDTA⁻ complex appeared at 325 seconds, 155 seconds after dead time.

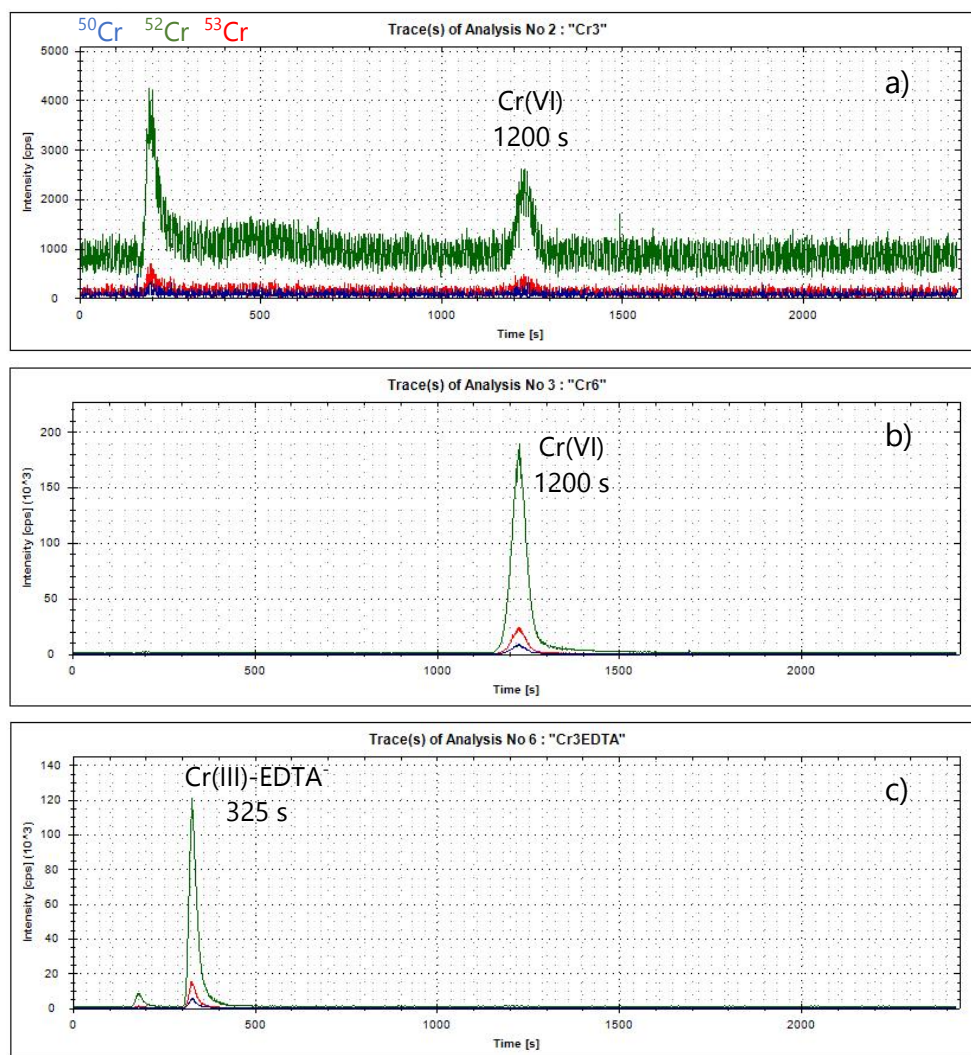


Figure 10. IC-ICP-MS chromatograms of: a) Cr(III) standard solution, 50 ppb. b) Cr(VI) standard solution, 50 ppb. c) 50 ppb of Cr(III) in presence of EDTA (after going through the EDTA extraction/complexation conditions). NG1 guard column + AG7 guard column + AS7 column, mobile phase: 50 mmol/L NH_4NO_3 pH 8, flow rate: 0.25 mL/min, KED mode, dead time: 170 s.

The concentration of NH_4NO_3 was increased to 75 mmol/L with the aim of reducing run times. Additionally, further research was done and pH was adjusted to 3 to prevent reduction of Cr(VI) as well as the precipitation of Cr(III). According to the diagram of species distribution from Figure 11, there is only a small pH window (shaded area) where Cr(VI) and Cr(III) species are present in ionic form. Above pH 4, Cr(III) forms insoluble hydroxo-aqua complexes; pH values below 2 may result in the formation of H_2CrO_4 , which is a strong oxidizing agent^[23].

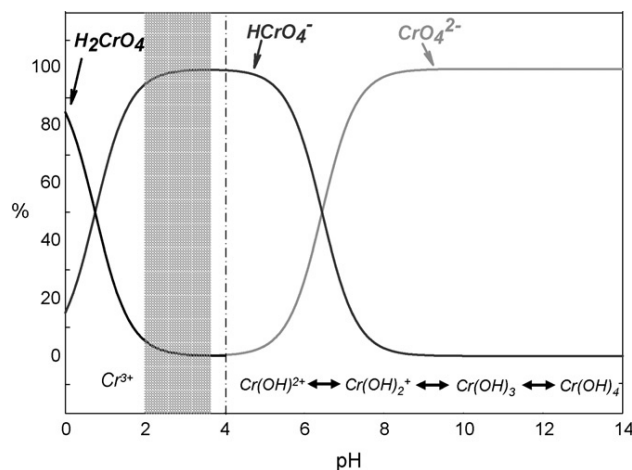


Figure 11. Distribution of chromium species in water. Obtained from [23].

Under these new conditions (75mM NH_4NO_3 at pH 3, adjusted with HNO_3 1M), interconversions were followed with the aid of chromium isotopic standards and the adapted EDTA extraction/complexation conditions (10 ppm of the Cr isotopic standard and EDTA 0.5 mM at pH 10). There was a slight shift in retention times, as well as in dead time (140 seconds instead of 170 seconds) due to some changes in terms of PEEK tubing because of a technical problem, so dead time is precise for each setup.

As it can be seen from Figure 12 a), when $^{50}\text{Cr(VI)}$ is injected only one peak is observed. This peak has a retention time of 920 seconds. When $^{53}\text{Cr(III)}$ is injected after going through the EDTA extraction conditions, a peak appears at 230 seconds. This peak corresponds to the $^{53}\text{Cr(III)-EDTA}^-$ complex (Figure 12 b)). On the other hand, when $^{50}\text{Cr(VI)}$ is injected after going through the EDTA extraction conditions (Figure 12 c)), it does not complex with EDTA, it remains as $^{50}\text{Cr(VI)}$ but a small part is transformed into $^{50}\text{Cr(III)}$ which complexes with EDTA (secondary peak), but this represents only 1.5% of the main peak. This is not a major problem because trivalent chromium is present in leather in much larger quantities than hexavalent chromium, so a minimal conversion of Cr(VI) to Cr(III) represents a negligible amount of the total amount of Cr(III). As previously mentioned, no peak was observed when injecting Cr(III), probably because it was too strongly linked to the sulfonic group of the core of the column.

Samples were injected at 1, 25, 50, 75 et 100 ppb to test for linearity. This was performed in STD and KED modes. Table 6 shows the retention time of the principal peak and the R^2 of its calibration curve for each sample injected. A secondary peak was observed when injecting $^{50}\text{Cr(VI)}$ in presence of EDTA (Figure 12 c)) and corresponded to $^{50}\text{Cr(III)-EDTA}^-$, as previously explained. Hence, the R^2 of the calibration curve of the secondary peak is also reported for this sample. As it can be perceived from Table 6, all peaks show a linear behavior.

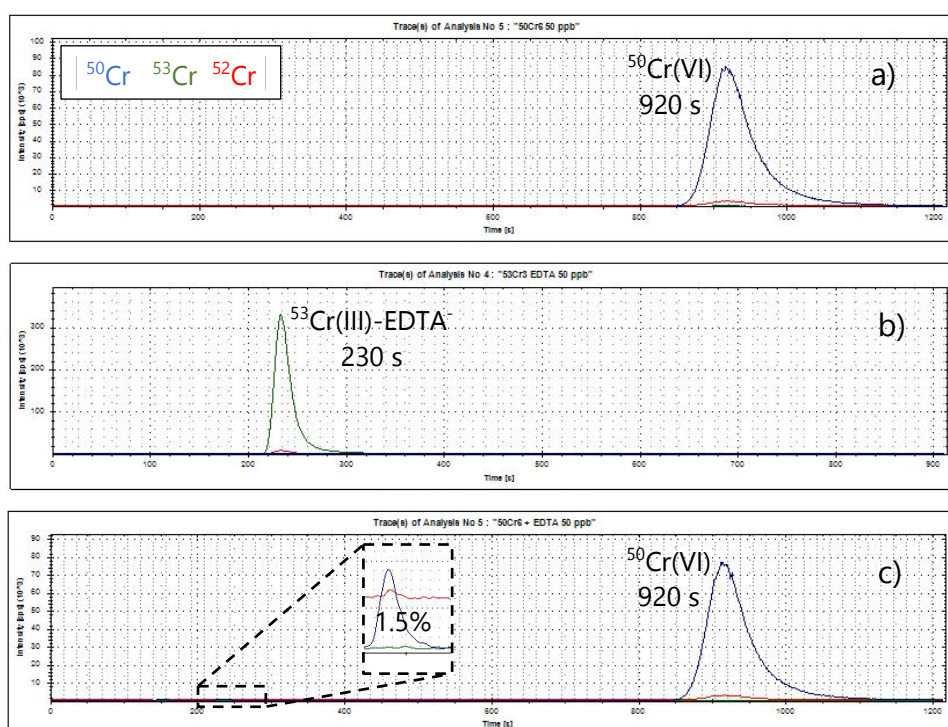


Figure 12. IC-ICP-MS chromatograms of: a) $^{50}\text{Cr(VI)}$, 50 ppb. b) 50 ppb of $^{53}\text{Cr(III)}$ in presence of EDTA (after going through the EDTA extraction/complexation conditions). c) 50 ppb of $^{50}\text{Cr(VI)}$ in presence of EDTA (after going through the EDTA extraction/complexation conditions). NG1 guard column + AG7 guard column + AS7 column, mobile phase: 75 mmol/L NH_4NO_3 pH 3, flow rate: 0.25 mL/min, KED mode, dead time: 140 s.

Table 6. Coefficient of determination (R^2) of the calibration curves of the peaks observed after the injection of $^{50}\text{Cr(VI)}$, $^{50}\text{Cr(VI)}$ in presence of EDTA, and $^{53}\text{Cr(III)-EDTA}^-$.

Sample	Mode	Retention time of main peak, [s]	Calibration curve of main peak, R^2	Retention time of secondary peak, [s]	Calibration curve of secondary peak, R^2
$^{50}\text{Cr(VI)}$	STD	920	0.9997		
$^{50}\text{Cr(VI)} + \text{EDTA}$	STD	920	0.9988	230	0.9956
$^{53}\text{Cr(III)-EDTA}^-$	STD	230	0.9949		
$^{50}\text{Cr(VI)}$	KED	920	0.9940		
$^{50}\text{Cr(VI)} + \text{EDTA}$	KED	920	0.9996	230	0.9975
$^{53}\text{Cr(III)-EDTA}^-$	KED	230	0.9995		

Back to the previous configuration in terms of PEEK tubing (before the technical problem), and under the same chromatographic conditions (75mM NH_4NO_3 at pH 3, flow rate equal to 0.25 mL/min), different samples were injected in order to evaluate the retention times of chromium peaks observed at different pH and redox potentials (dead time = 170 s). Table 7 summarizes the obtained results. It is important to highlight the following remarks:

1. At a sample pH of 3.00, complexation does not take place (Figure 13 a)). However, complexation of the standards was achieved at pH 9.57 (Figure 13 b)). This can be explained by the fact that at low pH (acidic conditions), the protonated form of EDTA is prevalent and H^+ competes with the metal ion, Cr^{3+} , for EDTA. At basic conditions, the deprotonated form of EDTA is prevalent and complexation between Cr^{3+} and EDTA occurs rapidly.
2. After $^{50}\text{Cr(VI)}$ and $^{53}\text{Cr(III)}$ went through the EDTA extraction conditions at pH 4.14, $^{50}\text{Cr(VI)}$ is transformed into $^{50}\text{Cr(III)}$ which complexed with EDTA (Figure 13 c)). This is possible because heating accelerates the kinetics of chromium's complexation with EDTA. In fact, once complexed, Cr(III)-EDTA^- is stable in a wide range of pH (below 10), but complexation must

- be performed at pH 10 in order to preserve the oxidation state of Cr(VI), because heating at acidic pH promotes its reduction to Cr(III).
- When the undiluted leather extract was spiked with $^{50}\text{Cr(VI)}$ and then injected, the $^{50}\text{Cr(VI)}$ peak appeared at 420 seconds, as it can be appreciated in the inset from Figure 13 d). However, when the diluted leather extract was spiked with $^{50}\text{Cr(VI)}$ and subsequently injected, the $^{50}\text{Cr(VI)}$ peak appeared at 970 seconds, as shown in the inset from Figure 13 e). Thus, when Cr(VI) is present in an undiluted EDTA leather extract, it is probably bonded to something that can be found in the leather matrix. On the other hand, when Cr(VI) is present in a diluted sample, the conditions are closer to when Cr(VI) is alone in an aqueous solution, therefore the retention time remains the same. Another hypothesis is that Cr(VI) is present in different ionic forms when the sample is diluted or not diluted, hence, Cr(VI) elutes at two different retention times.
 - It is important to mention that an EDTA solution was also injected after the injection of real samples and standards, and no peaks were observed. This permitted to confirm that there was a total elution of the compounds.

Table 7. Retention times of Cr species observed at different sample conditions (pH and redox potential).

Injected sample	Retention time of:		Sample:		Remarks
	Cr(VI), [s]	Cr(III)-EDTA, [s]	pH	Redox potential, [mV]	
$^{50}\text{Cr(VI)}$ + $^{53}\text{Cr(III)}$, 100 ppb, HNO_3 0.1% (v/v)	960		nd	nd	No Cr(III) peak was observed, as expected.
$^{50}\text{Cr(VI)}$ + $^{53}\text{Cr(III)}$, 100 ppb, EDTA 0.5 mM	960		3.00	nd	No heat; complexation did not take place.

$^{53}\text{Cr(III)}$ + EDTA, 100 ppb, EDTA 0.5 mM		260	3.99	175	Sample was diluted in EDTA 0.5 mM after going through the EDTA extraction/complexation conditions.
$^{50}\text{Cr(VI)}$ + $^{53}\text{Cr(III)}$, 100 ppb, EDTA 0.5 mM		250	4.14	nd	Sample was diluted in water after going through the EDTA extraction/complexation conditions at pH 4.14. No Cr(VI) peak was observed, $^{50}\text{Cr(VI)}$ was reduced into $^{50}\text{Cr(III)}$.
$^{50}\text{Cr(VI)}$ + EDTA, EDTA 0.5 mM	970		7.53	-37	Sample was diluted in EDTA 0.5 mM after going through the EDTA extraction/complexation conditions. At this pH, $^{50}\text{Cr(VI)}$ is preserved.
Diluted (x100) leather extract spiked with $^{50}\text{Cr(VI)}$	970	250	9.39	-151	$^{50}\text{Cr(VI)}$ peak appeared at 970 s.
Undiluted leather extract spiked with $^{50}\text{Cr(VI)}$	420	250	9.86	-178	$^{50}\text{Cr(VI)}$ peak appeared at 420 s.
$^{50}\text{Cr(VI)}$ + $^{53}\text{Cr(III)}$, 100 ppb, EDTA 0.5 mM	960	250	9.57	-160	No heat was required for complexation of standards in basic conditions.

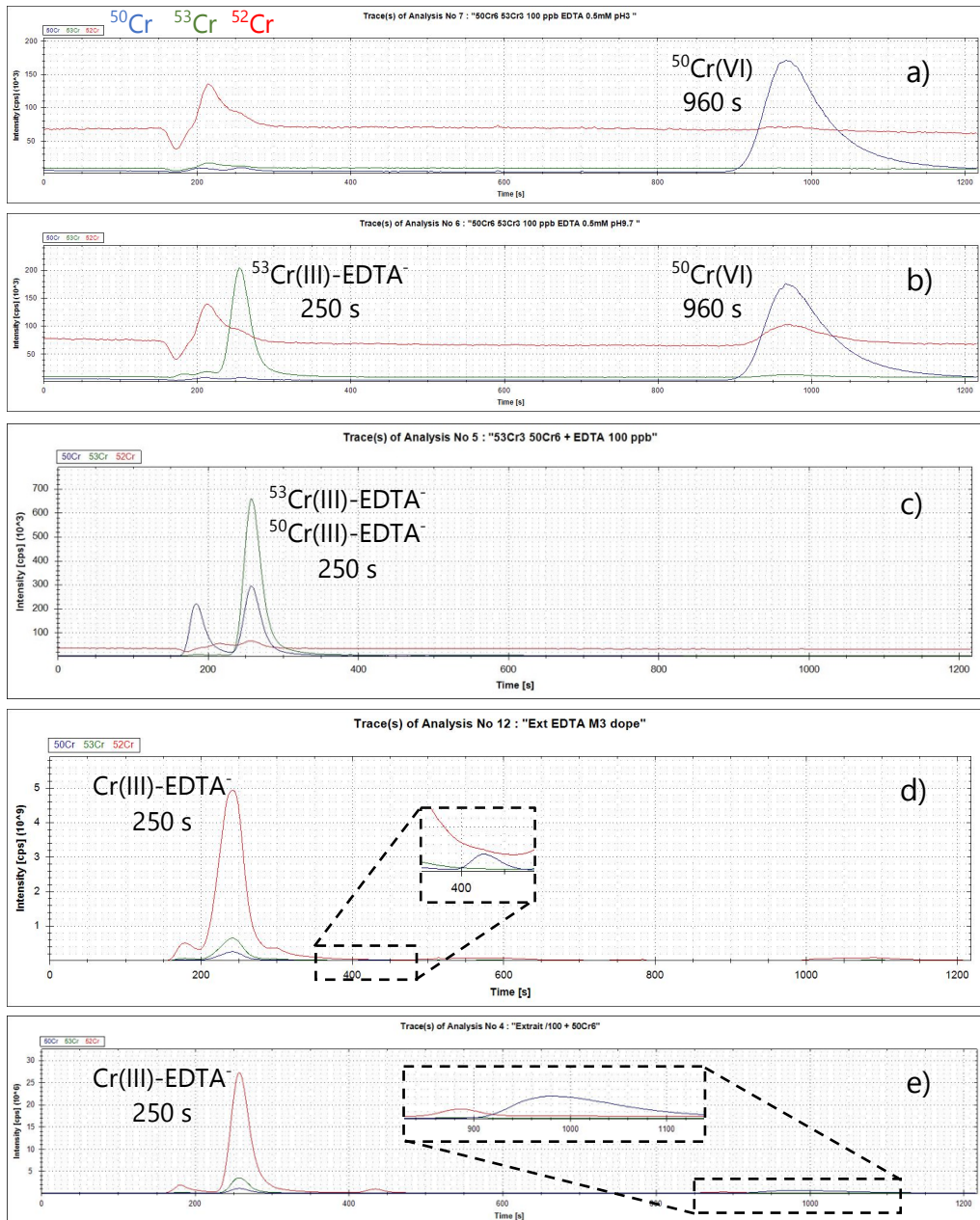


Figure 13. IC-ICP-MS chromatograms of: a) $^{53}\text{Cr(III)}$ and $^{50}\text{Cr(VI)}$, 100 ppb, EDTA 0.5 mM, pH 3.00. b) $^{53}\text{Cr(III)}$ and $^{50}\text{Cr(VI)}$, 100 ppb, EDTA 0.5 mM, pH 9.70. c) 100 ppb of $^{53}\text{Cr(III)}$ and $^{50}\text{Cr(VI)}$ in presence of EDTA 0.5 mM after going through the EDTA extraction/complexation conditions at pH 4.14. d) Undiluted extract spiked with $^{50}\text{Cr(VI)}$. e) Diluted (x100) leather extract spiked with $^{50}\text{Cr(VI)}$. NG1 guard column + AG7 guard column + AS7 column, mobile phase: 75 mmol/L NH_4NO_3 pH 3, flow rate: 0.25 mL/min, KED mode, dead time: 170 s.

Being DTPA the second most performant extraction method as reported on a previous study^[9], the injection of $^{53}\text{Cr(III)}$ after going through the DTPA extraction conditions (10 ppm of $^{53}\text{Cr(III)}$ were complexed with 4 mL DTPA 0.5 mM at pH 10, and subsequently diluted in water) was also tested. However, a large peak was observed just after the dead time which was 170 seconds (Figure 14). This can be explained by a large quantity of unretained material because in the dead time there are several things that elute. Consequently, this method was not optimal for extracting chromium species using these analytical conditions for their determination.

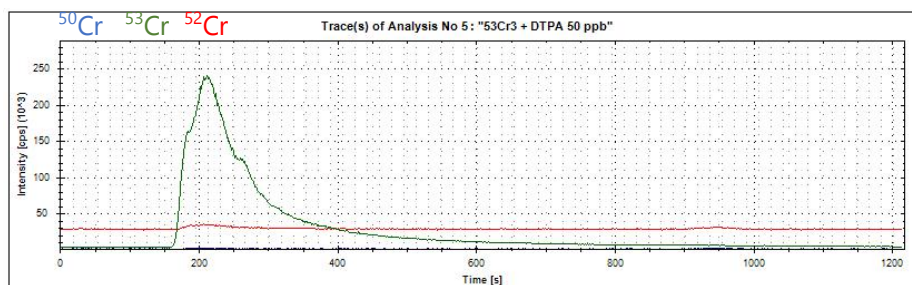


Figure 14. IC-ICP-MS chromatogram of 50 ppb of $^{53}\text{Cr(III)}$ in presence of DTPA (after going through the DTPA extraction/complexation conditions). NG1 guard column + AG7 guard column + AS7 column, mobile phase: 75 mmol/L NH_4NO_3 pH 3, flow rate: 0.25 mL/min, STD mode, dead time: 170 s.

Furthermore, after observing that the retention time of Cr(VI) shifted depending on the dilution factor of the EDTA leather extract, a DPC solution (see section 2.3) was used to try to complex Cr(VI) and to avoid this problem. However, this was not possible due to the precipitation of organic matter when adding the DPC solution to the extract. The result was a cloudy solution even after filtration through 0.45 μm nylon syringe filters. Moreover, EDTA extraction is performed with an EDTA solution at pH 10, and the DPC solution is highly acidic, therefore both solutions are not compatible.

After all the experiments performed, it has been possible to confirm that the Cr(III)-EDTA⁻ complex is stable under a large range of pH with no shifting of

the peak depending on the dilution factor. By the other hand, the Cr(VI) peak may shift depending on the dilution factor of the leather extract (EDTA extraction). Consequently, the next step was to try the extraction of Cr(VI) from leather samples through the ISO 17075^[13] protocol with subsequent analysis through IC-ICP-MS. In order to do this, chromium isotopic standards, as well as a leather sample, followed the whole ISO 17075 protocol with the aim of testing for possible interconversions.

As it can be seen from Figure 15 a), when injecting $^{53}\text{Cr(III)}$ after following the whole ISO 17075 protocol, it does not transform into $^{53}\text{Cr(VI)}$. Moreover, when injecting $^{50}\text{Cr(VI)}$, which had followed the whole ISO 17075 protocol, a single peak appeared at 925 s (Figure 15 b)). Therefore, the ISO standard method can be used to extract Cr(VI) from leather samples and the extract can be then analyzed through IC-ICP-MS under the optimized analysis conditions and no interconversions occur. When injecting an ISO leather extract, a small peak was observed at 925 s, which corresponds to the retention time of Cr(VI) (Figure 15 c)).

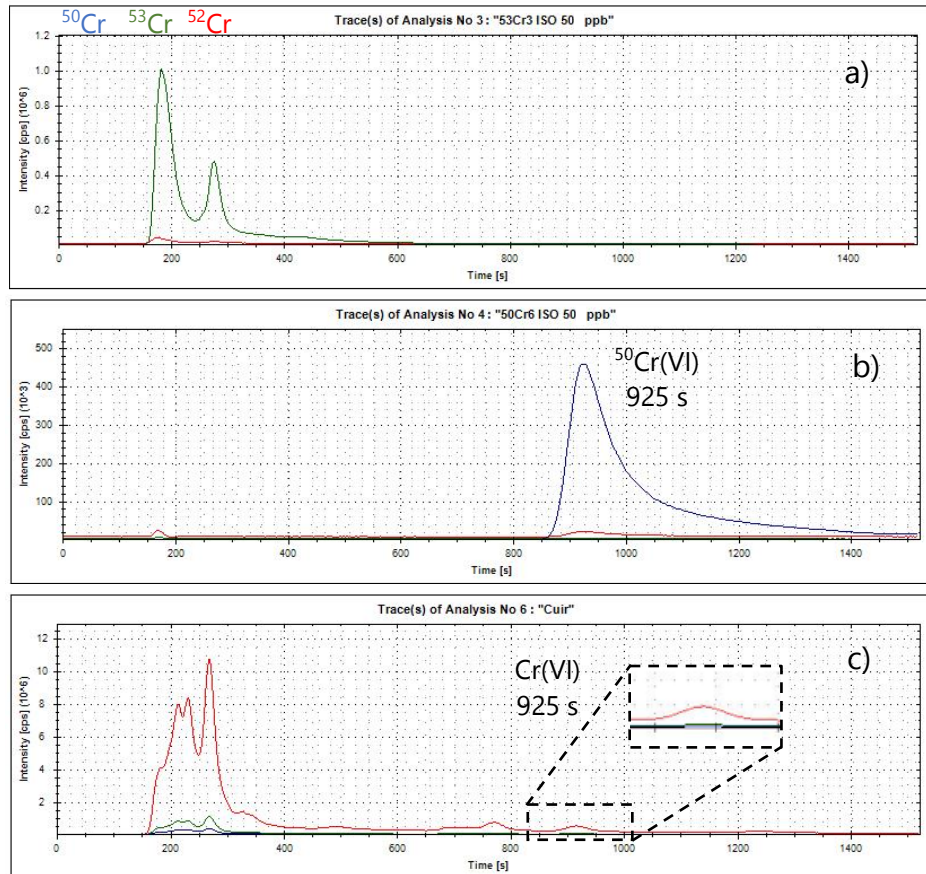


Figure 15. IC-ICP-MS chromatograms of: a) 50 ppb of $^{53}\text{Cr}(\text{III})$ after going through the ISO 17075 extraction. b) 50 ppb of $^{50}\text{Cr}(\text{VI})$ after going through the ISO 17075 extraction. c) Undiluted leather extract (through ISO 17075). NG1 guard column + AG7 guard column + AS7 column, mobile phase: 75 mmol/L NH_4NO_3 pH 3, flow rate: 0.25 mL/min, KED mode, dead time: 170 s.

Finally, the optimal conditions to be respected in order to preserve speciation at both extraction and analysis steps are described in diagram from Figure 16.

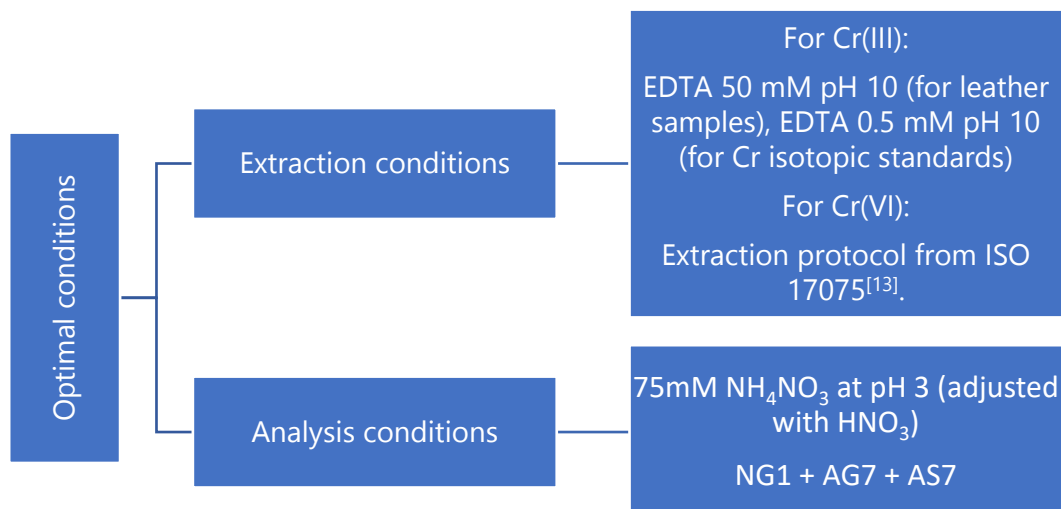


Figure 16. Optimal extraction and analysis conditions for the AS7 ion-exchange column.

3.3.2 CS5A ion-exchange column

One last test was done with ammonium nitrate as mobile phase. The chromatographic setup from Figure 1 was used but a CS5A column replaced the AS7 analytical column, and a CG5A guard column replaced the AG7 guard column. The mobile phase consisted of 75 mmol/L NH_4NO_3 adjusted to pH 3 at a flow rate of 0.25 mL/min, and the dead time was 175 seconds. A sample containing $^{53}\text{Cr(III)}$, $^{50}\text{Cr(VI)}$ and $^{53}\text{Cr(III)-EDTA}^-$ was injected with an acidic concentration of HNO_3 0.1% (v/v). From Figure 17 a), it can be observed that $^{53}\text{Cr(III)-EDTA}^-$ elutes just after the dead time with a retention time of 195 seconds, followed by $^{50}\text{Cr(VI)}$ which elutes at 360 seconds. No interconversions were appreciated under these conditions but there was no peak for Cr(III) before 3600 seconds. Even if the CS5A has two ion-exchange groups (sulfonic acid, cation-exchange group, and quaternary ammonium, anion-exchange group)^[21], the mobile phase was not strong enough to break the interactions between Cr(III) and the stationary phase. Consequently, Cr(III) remained stuck to the stationary phase and no peak was observed for this cation. Afterwards, a leather sample was extracted through the EDTA extraction method. The concentrated extract was

posteriorly injected and a peak was observed at 195 seconds which corresponds to Cr(III)-EDTA⁻. Additionally, a small peak was observed at 360 seconds which corresponds to Cr(VI). Nevertheless, coelution of both peaks impede the quantification of both species, as it can be appreciated from the inset on Figure 17 b).

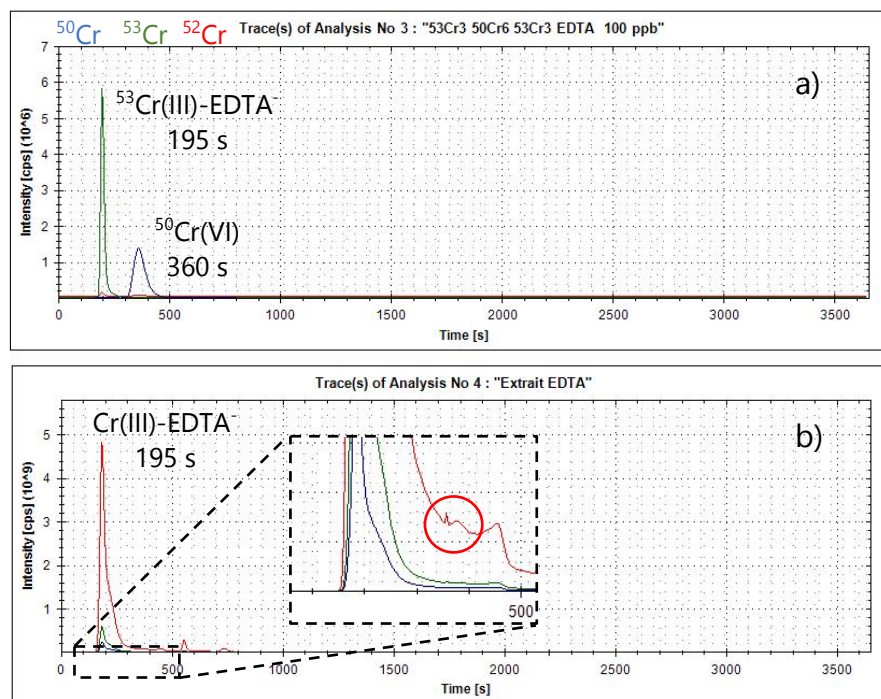


Figure 17. IC-ICP-MS chromatograms of: a) 100 ppb of ⁵³Cr(III), 100 ppb of ⁵⁰Cr(VI) and 100 ppb of ⁵³Cr(III) in presence of EDTA (after going through the EDTA extraction/complexation conditions), HNO₃ 0.1% (v/v). b) EDTA extraction of a leather sample, no dilution. NG1 guard column + CG5A guard column + CS5A column, mobile phase: 75 mmol/L NH₄NO₃ pH 3, flow rate: 0.25 mL/min, STD mode, dead time: 175 s.

3.4 FTICR MS

The negative ion mode ESI FTICR mass spectrum of 10 ppm of Cr(III) in presence of EDTA 0.5 mmol/L (after going through the EDTA extraction/complexation conditions) (2X dilution in ACN) is presented in Figure 18 a). This spectrum shows the deprotonated EDTA ion, [EDTA-H]⁻, at m/z 291.08339, as well as the deprotonated metal-EDTA complex ion, [EDTA-4H+Cr(III)]⁻, at m/z 340.00042,

and the peak that corresponds to the loss of formic acid from the EDTA molecule, $[\text{EDTA-H-HCOOH}]^-$, at m/z 245.07807.

Figure 18 b) shows the negative ion mode ESI FTICR mass spectrum of an EDTA leather extract (2X dilution in ACN/H₂O, 50/50 v/v%). Likewise, the deprotonated EDTA ion, $[\text{EDTA-H}]^-$, is present at m/z 291.08339, as well as the deprotonated metal-EDTA complex ion, $[\text{EDTA-4H+Cr(III)}]^-$, at m/z 340.00042, and the peak that corresponds to the loss of formic acid from the EDTA molecule, $[\text{EDTA-H-HCOOH}]^-$, at m/z 245.07782.

The positive ion mode ESI FTICR mass spectrum of 10 ppm of Cr(III) in presence of EDTA 0.5 mmol/L (after going through the EDTA extraction/complexation conditions) (2X dilution in ACN) is shown in Figure 19 a). Species were detected as protonated species with a single positive charge. The protonated EDTA ion, $[\text{EDTA+H}]^+$, is present at m/z 293.09794, as well as the protonated metal-EDTA complex ion, $[\text{EDTA-2H+Cr(III)}]^+$, at m/z 342.01497.

In Figure 19 b) we can observe the positive ion mode ESI FTICR mass spectrum of an EDTA leather extract (2X dilution in ACN/H₂O, 50/50 v/v%). Similarly, the protonated EDTA ion, $[\text{EDTA+H}]^+$, is present at m/z 293.09812, as well as the protonated metal-EDTA complex ion, $[\text{EDTA-2H+Cr(III)}]^+$, at m/z 342.01520.

As it can be seen from the spectra, negative ionization mode provided the highest intensity for the Cr(III)-EDTA⁻ complex, as compared to the positive ion mode. Additionally, it is important to mention that no peaks were found that suggest the formation of clusters of more than one metal ion per EDTA molecule, or vice versa, the formation of clusters of more than one EDTA molecule per metal ion.

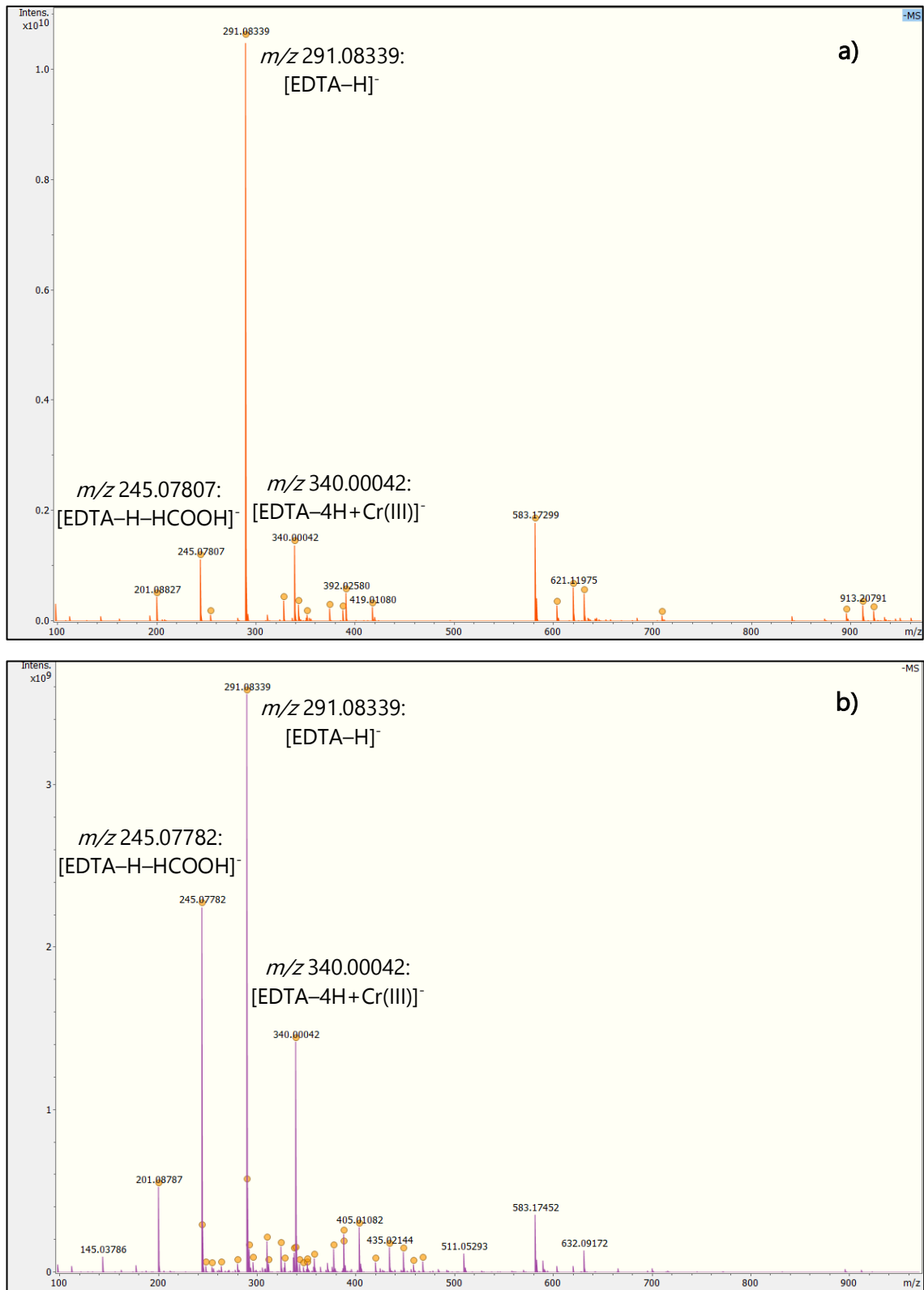


Figure 18. Negative ion mode ESI FTICR mass spectra of: a) 10 ppm of Cr(III) in presence of EDTA 0.5 mmol/L (after going through the EDTA extraction/complexation conditions) (2X dilution in ACN). b) EDTA leather extract (2X dilution in ACN/H₂O, 50/50 v/v%).

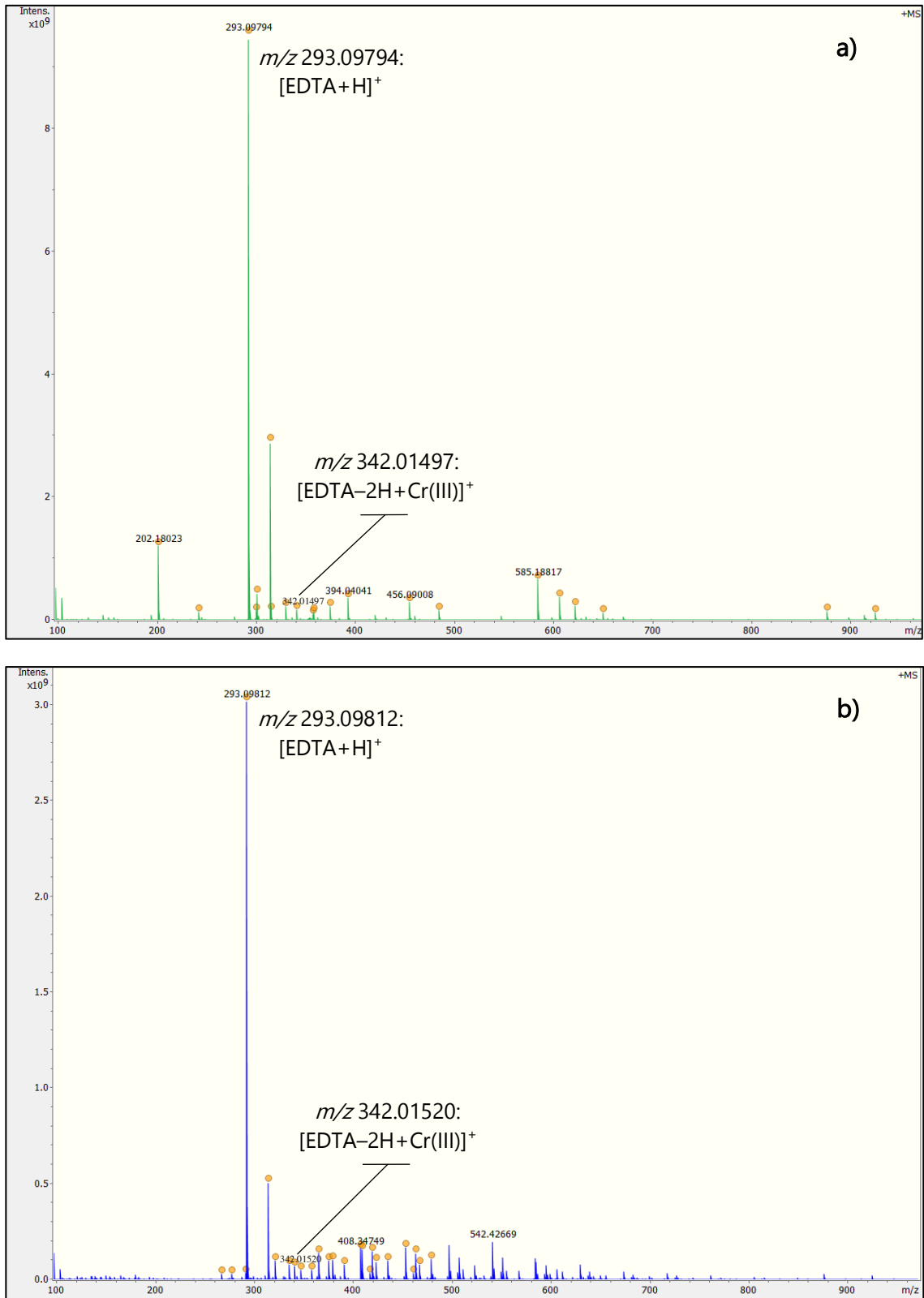


Figure 19. Positive ion mode ESI FTICR mass spectra of: a) 10 ppm of Cr(III) in presence of EDTA 0.5 mmol/L (after going through the EDTA extraction/complexation conditions) (2X dilution in ACN). b) EDTA leather extract (2X dilution in ACN/H₂O, 50/50 v/v%).

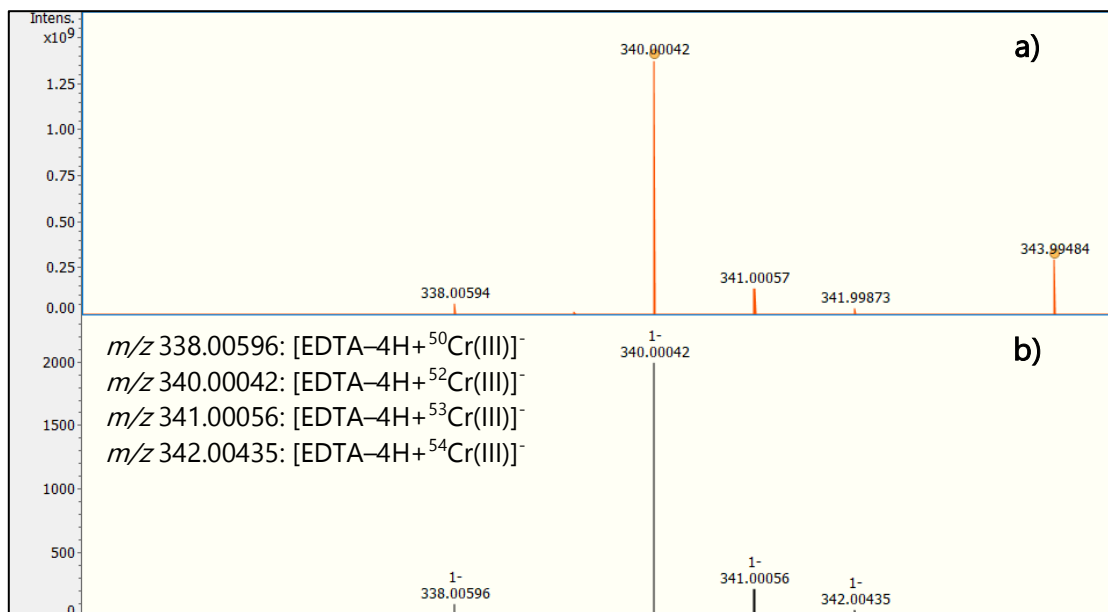


Figure 20. Negative ion mode ESI FTICR mass spectra of: a) 10 ppm of Cr(III) in presence of EDTA 0.5 mmol/L (after going through the EDTA extraction/complexation conditions) (2X dilution in ACN). b) Isotope pattern simulation of Cr(III)-EDTA⁻.

Figure 20 shows a spectrum of 10 ppm of Cr(III) in presence of EDTA 0.5 mmol/L (after going through the EDTA extraction/complexation conditions) (2X dilution in ACN), as well as a spectrum of the isotope pattern simulation of Cr(III)-EDTA⁻. As it can be seen, the pattern matches with the spectrum obtained experimentally. Due to the difference in natural abundances of chromium naturally occurring isotopes (⁵⁰Cr: 4.345%, ⁵²Cr: 83.789%, ⁵³Cr: 9.501%, ⁵⁴Cr: 2.365%), the following peaks are observed (m/z are rounded to the nearest unit. For exact values, see Table 8):

- m/z 338: [EDTA-4H+⁵⁰Cr(III)]⁻
- m/z 340: [EDTA-4H+⁵²Cr(III)]⁻
- m/z 341: [EDTA-4H+⁵³Cr(III)]⁻
- m/z 342: [EDTA-4H+⁵⁴Cr(III)]⁻

Table 8 shows the ion formula prediction for the peaks observed at m/z 291 and 340, at negative ion mode, and the peaks observed at m/z 293 and 342,

at positive ion mode. The low error value permitted to confirm the formula of the chromium-EDTA complex: $C_{10}H_{13}CrN_2O_8$ (Cr(III)-EDTA⁻).

Table 8. Ion formula prediction.

Sample	ESI ion mode	<i>m/z</i>	Ion formula	Err (ppm)
Cr(III)-EDTA ⁻ complex	-	291.083389	$C_{10}H_{15}N_2O_8$	0.0
		340.000420	$C_{10}H_{12}CrN_2O_8$	0.0
Leather sample	-	291.083389	$C_{10}H_{15}N_2O_8$	0.0
		340.000420	$C_{10}H_{12}CrN_2O_8$	0.0
Cr(III)-EDTA ⁻ complex	+	293.097942	$C_{10}H_{17}N_2O_8$	0.0
		342.014973	$C_{10}H_{14}CrN_2O_8$	0.0
Leather sample	+	293.098117	$C_{10}H_{17}N_2O_8$	-0.6
		342.015199	$C_{10}H_{14}CrN_2O_8$	-0.7

3.5 Validation

3.5.1 Trivalent chromium determination

As it has been proved by previous studies^[9], EDTA is the most performant method for the quantitative extraction of Cr(III) as a Cr(III)-EDTA⁻ complex. Furthermore, under the optimized analysis conditions (75 mmol/L NH_4NO_3 at pH 3), the Cr(III)-EDTA⁻ complex is stable under a large range of sample pH with no shifting of the peak depending on the dilution factor.

This method was validated in terms of linearity, LOD, LOQ and interday precision. Linearity was studied through the analysis of calibration solutions of Cr(III)-EDTA⁻ from 0 to 100 $\mu\text{g/L}$ of Cr(III) ($n=6$, 3 replicate injections per point). In order to do this, 400 μL of a Cr(III) solution were added to 4 mL of an EDTA solution (0.5 mM at pH 10). Once the microwave assisted complexation was completed, the solution containing the complex was diluted in water and each standard was analyzed through IC-ICP-MS, focusing on the peak with a retention time of 250 seconds, which corresponds to the Cr(III)-EDTA⁻ complex. Peak area

was plotted against concentration, and R^2 was found to be 0.9990. Calibration data and the calibration curve are shown in Table 9 and Figure 21.

Table 9. Calibration data for Cr(III) determination.

Concentration of Cr(III) / $\mu\text{g/L}$	Signal (Average of 3 replicates) / Counts
0	0
5	529086
25	3081015
50	5850666
75	9222595
100	12521735

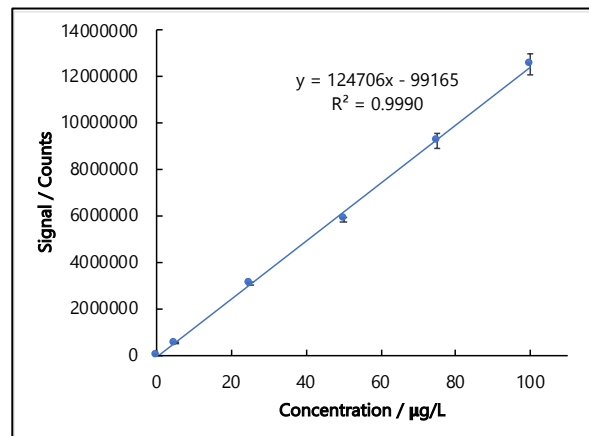


Figure 21. Calibration curve of trivalent chromium.

LOD and LOQ were calculated as the concentration necessary to give a S/N ratio equal to 3 and 10, respectively. LOD (S/N=3) was found to be 0.016 $\mu\text{g/L}$ or 0.3 $\mu\text{g/Kg}$ for Cr(III), and LOQ (S/N=10) was found to be 0.054 $\mu\text{g/L}$ or 1.1 $\mu\text{g/Kg}$ for Cr(III).

Interday precision was determined by analyzing three different leather samples (Sample 1: natural E2, Sample 2: after polish, Sample 3: UV-aging) from which trivalent chromium was extracted on three different days. As it can be seen

in Table 10, for each sample the interday precision provided a relative standard deviation (RSD) below 12% and a low standard deviation (SD). Low RSD values show that the method exhibits excellent interday precision.

Table 10. Interday precision for Cr(III) determination. 3 different samples were analyzed each day.

Sample	Day	mg of Cr(III) / kg of dry leather		
Sample 1: Natural E2	1	2445.01	Average	2659.06
	2	3007.27	SD	304.190
	3	2524.90	RSD%	11.4%
Sample 2: After polish	1	2922.23	Average	2717.18
	2	2765.36	SD	232.901
	3	2463.96	RSD%	8.6%
Sample 3: UV-aging	1	3836.42	Average	3494.66
	2	3456.85	SD	324.508
	3	3190.71	RSD%	9.3%

3.5.2 Hexavalent chromium determination

As mentioned before, the ISO 17075^[13] standard method was used to extract Cr(VI) from leather samples and the extract was then analyzed through IC-ICP-MS under the optimized analysis conditions and no interconversions were observed.

This method was validated in terms of linearity, LOD, LOQ and precision. Linearity was studied through the analysis of calibration solutions of Cr(VI) from 0 to 100 µg/L (n=6, 3 replicate injections per point). Each standard was analyzed through IC-ICP-MS, focusing on the peak with a retention time of 960 seconds, which corresponds to Cr(VI). Peak area was plotted against concentration, and R²

was found to be 0.9993. Calibration data and the calibration curve are shown in Table 11 and Figure 22.

Table 11. Calibration data for Cr(VI) determination.

Concentration of Cr(VI) / $\mu\text{g/L}$	Signal (Average of 3 replicates) / Counts
0	0
1	132371
25	3028055
50	5667130
75	8536624
100	11060999

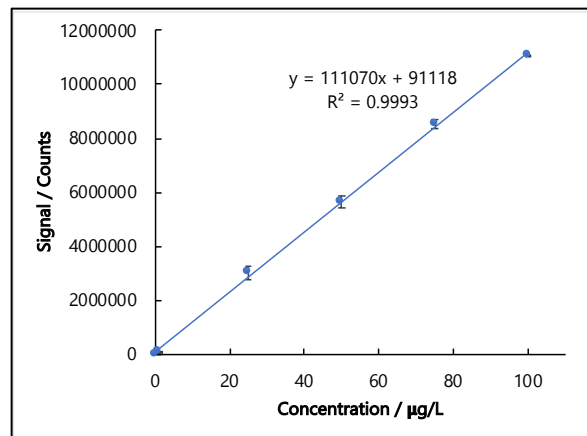


Figure 22. Calibration curve of hexavalent chromium.

LOD and LOQ were calculated as the concentration necessary to give a S/N ratio equal to 3 and 10, respectively. LOD (S/N=3) was found to be 0.13 $\mu\text{g/L}$ or 7 $\mu\text{g/Kg}$ for Cr(VI), and LOQ (S/N=10) was found to be 0.43 $\mu\text{g/L}$ or 22 $\mu\text{g/Kg}$ for Cr(VI).

Precision was determined by analyzing three different leather samples (Sample 1: UV-aging, Sample 2: mirage, Sample 3: after polish) from which hexavalent chromium was extracted three times the same day through the ISO

17075^[13] protocol. As it can be seen in Table 12, RSD was below 5.3%. Low RSD values show that the method exhibits excellent precision.

Table 12. Cr(VI) determination from a leather sample.

Sample	mg of Cr(VI) / kg of dry leather		
Sample 1: UV-aging	6.97	Average	6.98
	6.98	SD	0.00
	6.98	RSD%	0.1%
Sample 2: Mirage	3.00	Average	3.01
	3.00	SD	0.01
	3.02	RSD%	0.3%
Sample 3: After polish	8.13	Average	7.67
	7.52	SD	0.40
	7.37	RSD%	5.3%

4. Conclusions

This work focused on the analysis of trivalent and hexavalent chromium extracted from leather samples. Separation and speciation were studied by IC-ICP-MS. Several conditions were tested such as: two different columns, different mobile phases, different pH of the samples and different complexing agents. Among all these tests, the optimized conditions for the analysis of chromium species were: a NG1 guard column, an AG7 guard column, an AS7 ion-exchange column, and 75 mmol/L NH_4NO_3 pH 3 as chromatographic eluent at a flow rate of 0.25 mL/min. LOD and LOQ for Cr(III) were found to be 0.016 and 0.054 $\mu\text{g/L}$ (0.3 and 1.1 $\mu\text{g/Kg}$), respectively, and for Cr(VI) 0.13 and 0.43 $\mu\text{g/L}$ (7 and 22 $\mu\text{g/Kg}$), respectively. Even if the other tested conditions were not suitable for leather samples, they served to prove that the EDTA extraction of Cr(III) was complete, as long as the extraction is performed at basic conditions. Interconversions were monitored with the use

of chromium isotopic standards. Regarding the extraction methods, EDTA proved to be the most performant method for the extraction of Cr(III), while the ISO 17075 protocol was effective for the extraction of Cr(VI). No interconversions were found with none of these protocols under the optimized analysis conditions. Finally, FTICR-MS allowed the structure elucidation of the Cr(III)-EDTA⁻ complex formed during the EDTA extraction.

Abbreviations

ACN	Acetonitrile
CRMs	Certified Reference Materials
DPC	1,5-diphenylcarbazide
DTPA	Diethylenetriaminepentaacetic acid
EDTA	Ethylenediaminetetraacetic acid
FTICR	Fourier-Transform Ion Cyclotron Resonance Mass Spectrometry
HPLC-ICP-MS	High-Performance Liquid Chromatography - Inductively Coupled Plasma - Mass Spectrometry
IC-ICP-MS	Ion Chromatography - Inductively Coupled Plasma - Mass Spectrometry
ICP-MS	Inductively Coupled Plasma - Mass Spectrometry
ISO	International Organization for Standardization
KED	Kinetic Energy Discrimination mode
LOD	Limit of Detection
LOQ	Limit of Quantification
R ²	Coefficient of determination
RSD	Relative Standard Deviation
SD	Standard Deviation
STD	Standard mode
S/N	Signal-to-noise

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Conclusions

Conclusions

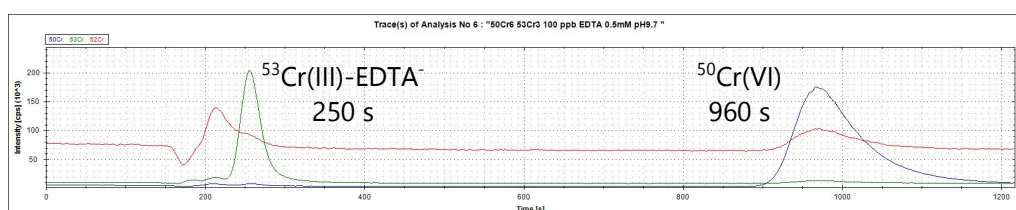
The goal of this thesis was to develop a methodology to quantitatively extract chromium species from tanned leather, and subsequently separate and quantify trivalent and hexavalent chromium, while maintaining the speciation. Taking into consideration that in aqueous systems the oxidation state of chromium is determined by redox potential and pH, chromium speciation represents a big challenge, as sample pretreatment and/or analytical methods may lead to interconversions between Cr(III) and Cr(VI), thus biasing the results. Dealing with the enormous difference of concentration between Cr(III) and Cr(VI) in real samples such as leather, it was also an important analytical challenge to quantify both species simultaneously.

After a deep bibliographic research leading to a review published in 2021 in *Critical Reviews in Analytical Chemistry*, the first part of the experimental work of this thesis consisted in comparing different pretreatment methods regarding their capacity to extract chromium from leather samples. To test the different extraction methods, the first step was to prepare the samples and to cut them into small pieces from 3 to 4 mm per side. For each sample, a direct mineralization was performed, and the digest was analyzed by Inductively Coupled Plasma - Atomic Emission Spectrometry (ICP-AES) to know the total chromium content and to have it as a reference. Then, different extractions were tested: ethylenediaminetetraacetic acid (EDTA) extraction, diethylenetriaminepentaacetic acid (DTPA) extraction, ammonium hydroxide extraction, phosphate buffer extraction, water extraction and ammonium nitrate extraction. Subsequently, after each extraction, the extract was also mineralized and chromium was determined using an ICP-AES equipment. Therefore, the extraction rate consisted in the ratio between the milligrams of extracted chromium and the milligrams of total

chromium obtained from the direct mineralization of the sample. Finally, the highest extraction rate was obtained with the EDTA extraction. Consequently, this method was validated in terms of linearity, limit of detection (LOD), limit of quantification (LOQ), interday precision and spike recovery. Regarding the separation and interconversion of chromium species, in a first approach, isotopic chromium standards ($^{50}\text{Cr(VI)}$ and $^{53}\text{Cr(III)}$) followed the extraction conditions of the selected method, and the separation of chromium species was followed through Ion Chromatography - Inductively Coupled Plasma - Mass Spectrometry (IC-ICP-MS) using a NG1 guard column, an AG7 guard column, an AS7 ion-exchange column, and 75 mmol/L of NH_4NO_3 pH 3 as chromatographic eluent. When $^{53}\text{Cr(III)}$ was injected in the presence of EDTA, a peak corresponding to $^{53}\text{Cr(III)-EDTA}^-$ (univocally confirmed by Fourier-transform ion cyclotron resonance – mass spectrometry (FTICR-MS)) was observed, no oxidation to $^{53}\text{Cr(VI)}$ was seen. On the other hand, when $^{50}\text{Cr(VI)}$ was injected in the presence of EDTA, it did not complex with EDTA, it remained as $^{50}\text{Cr(VI)}$ but a small part was reduced into $^{50}\text{Cr(III)}$ which complexed with EDTA. Nevertheless, this only represented 1.5% of the main peak. Therefore, EDTA proved to be the most efficient method for the quantitative extraction of Cr(VI) and Cr(III) as a Cr(III)-EDTA⁻ complex, and a limited amount of Cr(VI) was converted into Cr(III) when analyzing chromium isotopic standards that had followed the EDTA extraction conditions. This study led to the second publication in Journal of Leather and Science Engineering in 2021.

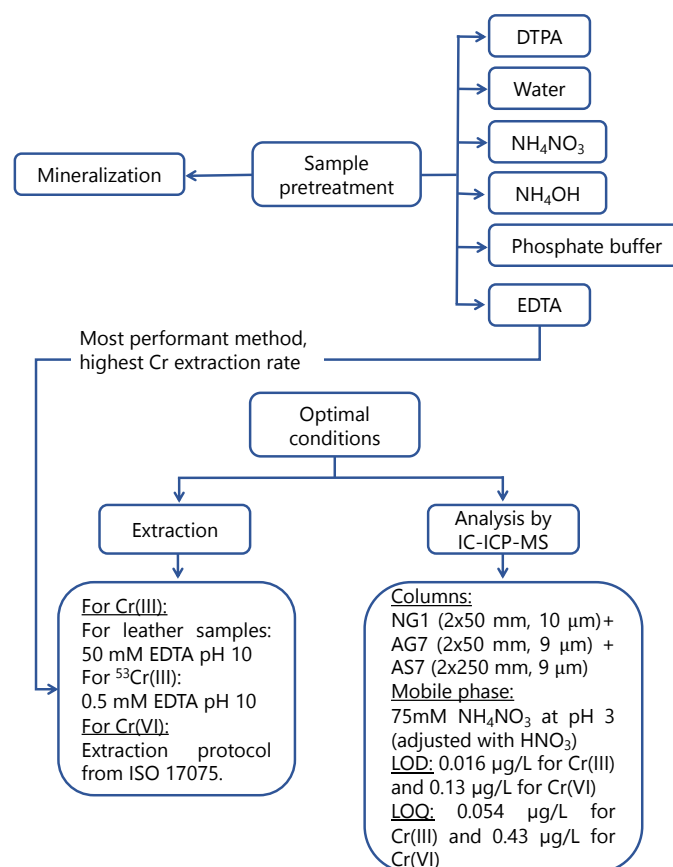
The second experimental part of this thesis consisted of a more in-depth study of separation and speciation of trivalent and hexavalent chromium from leather through IC-ICP-MS and is intended to be published in a peer-reviewed journal. An additional challenge when working with real samples such as leather is the enormous difference between Cr(III) (about 3000 ppm) and Cr(VI) (around 7 ppm for an aged leather sample). Several conditions were tested such as: two different columns (AS7 and CS5A), different mobile phases (NH_4NO_3 and HNO_3),

different pH of the samples and different complexing agents (1,5-diphenylcarbazide (DPC), DTPA and EDTA). Among all these tests, the optimized conditions for the analysis of chromium species were: a NG1 guard column, an AG7 guard column, an AS7 ion-exchange column, and 75 mmol/L NH_4NO_3 pH 3 as chromatographic eluent with external water regeneration. Those conditions allowed the unambiguous separation of Cr(III) and Cr(VI) regardless the stark difference in concentration levels. Extraction of Cr(III) was performed through the previously validated EDTA method, but the extraction of Cr(VI) was performed following the ISO 17075 protocol. The ISO protocol (phosphate buffer extraction: $\text{K}_2\text{HPO}_4 \cdot 3\text{H}_2\text{O}$ at pH 8.0) was preferred over the EDTA method because, even if the EDTA method allows the quantitative extraction of Cr(VI) as well, a small part (1.5%) was transformed into Cr(III). Moreover, when analyzing the extract, the retention time of Cr(VI) shifted depending on the dilution factor of the leather extract. The ISO 17075 protocol, in contrast, permitted the quantitative extraction of Cr(VI) and the eventual changes on the dilution factor did not affect the retention time of Cr(VI). Using the optimized extraction (EDTA extraction for Cr(III) and ISO 17075 for Cr(VI)) and analysis conditions, LOD and LOQ for Cr(III) were found to be 0.016 and 0.054 $\mu\text{g/L}$ (0.3 and 1.1 $\mu\text{g/Kg}$), respectively, and for Cr(VI) 0.13 and 0.43 $\mu\text{g/L}$ (7 and 22 $\mu\text{g/Kg}$), respectively. No interconversions were found with none of these protocols under the optimized analysis conditions. Additionally, even if the other tested conditions were not suitable for leather samples, they served to prove that the EDTA extraction of Cr(III) was complete, if the extraction is performed at basic conditions. Finally, FTICR-MS allowed the structure elucidation of the Cr(III)-EDTA^- complex formed during the EDTA extraction.



IC-ICP-MS chromatogram of 100 ppb of $^{53}\text{Cr(III)}$ and $^{50}\text{Cr(VI)}$ in presence of EDTA 0.5mM at basic conditions.

The following diagram summarizes the experimental work of this thesis, as well as the optimal extraction and analysis conditions that served to quantitatively extract and separate trivalent and hexavalent chromium from leather samples, while maintaining speciation and dealing with the enormous difference of concentration levels.



Summary of the experimental work done during the thesis.

As future work, it would be convenient to apply the extraction and analysis protocol to a non-tanned leather, a tanned leather and a tanned/colored leather, to have an idea of the differences in chromium concentrations depending on the different manufacturing processes that samples have gone through.

Concerning the extraction protocols, the best results have been obtained through microwave extraction (EDTA, DTPA). It could be interesting to perform

the other extractions through the use of microwave instead of ultrasound (NH_4OH , NH_4NO_3 , phosphate buffer) in order to compare under the same conditions.

Under the optimal analytical conditions, it is important to reduce the extra column volumes to obtain thinner peaks. Regarding the large mass of peaks obtained when analyzing leather samples after ISO 17075 extraction, it could be possible to recover fractions after separation by ion chromatography, then use solid-phase extraction to desalinate the solution and subsequently analyze it through FTICR for identification.

Additionally, the methodology developed for column CS5A using nitric acid as chromatographic eluent should also be explored for another type of samples where the difference in concentrations is not so great, and this could lead to an additional publication.

Regarding the interconversion rates for the EDTA extraction and the ISO 17075 protocol, it would be convenient to spike leather samples with a higher concentration of Cr(III) (to values similar to a real sample, around 20,000 ppm of Cr(III)) in order to see if the interconversion rates depend on chromium concentration, and to verify if speciation is preserved.

Concerning the results for FTICR-MS, it would be interesting to plot a Kendrick mass defect diagram applying a mass filtering in order to visualize elemental composition patterns in the mass spectra of our complex leather extracts.

Appendix A

Appendix A

General concepts of chromatography

Chromatography derives from Greek $\chi\rho\omega\mu\alpha$ *chroma*, which means "color", and $\gamma\rho\acute{\alpha}\phi\epsilon\iota\nu$ *graphein*, which means "to write or to express by written characters". The combination of these two terms was inherited from the invention of the method first used to separate a complex mixture of pigments^[1].

Chromatography is a physical method for separating components of a mixture by their distribution between two phases:

- A stationary phase: In the form of a porous bed, bulk liquid, layer or film. This phase is generally immobile.
- A mobile phase: In the form of a fluid that moves, permeates or infiltrates through or over the stationary phase. The solvent serves to move the sample through the stationary phase.

The separation takes place from repeated sorption/desorption events during the movement of the sample components along the stationary phase in the defined direction of the mobile phase migration. For optimal separations, the requirements are: 1) a difference in the strength of the physical interactions (differential affinities) for the sample components in the two phases and 2) a favorable contribution from system transport properties that control sample movement between phases^[2]. The principle may be defined as follows: differential affinities of the various components of the analyte towards the stationary and mobile phases result in the differential separation of the components^[3].

Chromatographic parameters include^[4, 5]:

Flow rate (*f*): The volume of mobile phase passing through the system per unit of time.

Retention time (t_r): The time that it takes for an analyte to pass through the system: from the injection to its exit from the system (see Figure 1).

Volume of retention (v_r): The required volume of mobile phase necessary to elute the solute. At constant flow rate: $v_r = t_r \times f$.

Dead time (t_0): The minimum time required for a non-retained compound to pass through the system and be analyzed.

Dead volume (V_m): Volume of mobile phase required to pass through the system; it depends on the geometry and porosity of the column. At constant flow rate: $v_m = t_0 \times f$.

Retention factor (k): The capacity of the system to retain a compound. It is independent from the geometry of the column. $k = (t_r - t_0)/t_0$.

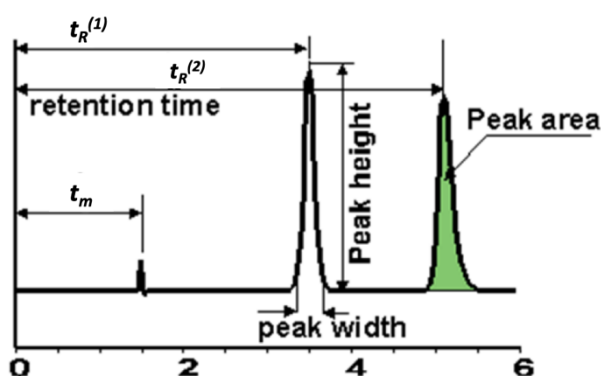


Figure 1. Chromatographic parameters: retention time, dead time, peak width, peak height and peak area. Modified from [4].

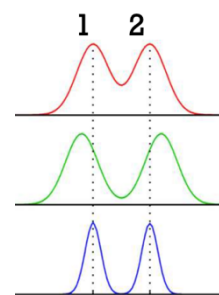


Figure 2. Chromatograms with increasing selectivity factor. Best selectivity: third chromatogram (blue line). Modified from [4].

Selectivity factor ($\alpha_{1,2}$): The capacity of the system to distinguish between two adjacent solutes. A separation is achieved if the selectivity factor is greater than 1 (see Figure 2). It is independent from the geometry of the column. $\alpha_{1,2} = (t_{r2} - t_0)/(t_{r1} - t_0)$.

Efficiency or number of theoretical plates (N): It is an index of the efficiency of the column (ability to provide thin peaks), but also an indirect measure of peak width. $N = 16(t_r/W)^2$, where W =peak width at the base.

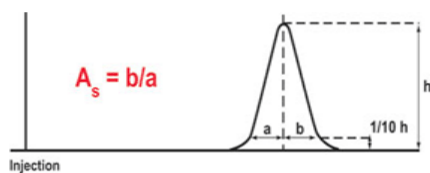


Figure 3. Asymmetry factor. Obtained from [5].

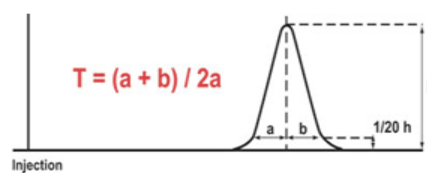


Figure 4. Tailing factor. Obtained from [5].

Peak asymmetry factor (A_s): $A_s = b/a$ where b is the distance from the peak midpoint to the trailing edge of the peak measured at $1/10$ of peak height, and a is the distance from the leading edge of the peak to the peak midpoint measured at $1/10$ of peak height. If $A_s > 1$: tailing, if $A_s < 1$: fronting. This factor is described in the chromatogram from Figure 3.

Tailing factor (T_f): $T_f = (a+b)/2a$ where a is the distance from the leading edge of the peak to the peak midpoint measured at $1/20$ of peak height, and b is the distance from the peak midpoint to the trailing edge of the peak measured at $1/20$ of peak height. This factor is described in the chromatogram from Figure 4.

Resolution (R_s): Global factor that determines the separation capacity between two peaks. $R_s = 2(t_{r2} - t_{r1}) / (W_1 + W_2)$. Purnell's relation: $R_s = \frac{1}{4} \sqrt{N_2} \frac{\alpha - 1}{\alpha} \times \frac{k_2}{1 + k_2}$, where N_2 is the plate number of the second peak, α is the separation factor between the two peaks, and k_2 is the retention factor of the second peak.

There are several types of chromatographic methods that have been developed to separate a variety of mixtures. The most common chromatographic methods are described in Table 1 and are the following^[3, 6]:

Gas chromatography: In this technique, the sample is vaporized and carried through the stationary phase. Gas chromatography columns are essentially silica-based capillary columns surrounded by polyimide, in which a film of stationary phase based on polydimethylsiloxane (PDMS) (for the most or moderately non-polar columns) or polyethylene glycol (PEG) (for the most polar columns) is deposited. The sample is carried along by a continuous flow of an inert gas or an

unreactive gas such as argon, helium, nitrogen or hydrogen. Separation of molecules is based principally upon differences of boiling point, vapor pressure and of polarity.

Liquid chromatography: In this technique, the sample is dissolved in a liquid and travels through a solid stationary phase, generally made up of silica. There are different types of liquid chromatography depending on the relative polarities of the phases (normal-phase versus reverse-phase) and whether the mobile phase is pressurized (high-performance) or not.

Thin-layer chromatography: In thin-layer chromatography, the stationary phase is a solid material, usually silica-based, which is supported by an inert backing. The sample is introduced on the deposition line of the plate. The eluent (mobile phase) diffuses along the support and drags the compounds according to their affinity for the stationary phase and the mobile phase (adsorption).

Ion chromatography or ion-exchange chromatography: This technique separates the components of a sample based on their charge, in addition to their size. Cations and anions are separated from a mixture using different stationary and mobile phases, and different pH mobile phases.

Size-exclusion chromatography: This technique separates the components of a sample based on their size and form.

Table 1. Basis of separation of the most commonly used chromatographic techniques.

Technique	Stationary phase	Mobile phase	Basis of separation	Important remarks
Gas chromatography	Liquid or solid support	An inert gas like argon or helium	Boiling point of the molecules	Samples are volatilized and separation of molecules is based principally upon

				differences of boiling point/ vapor pressure and of polarity.
Liquid chromatography	Solid (silica or alumina)	Liquid	Polarity of the molecules	Different varieties of liquid chromatography (normal-phase, reverse-phase, hydrophilic interaction chromatography).
Thin-layer chromatography	Solid (silica or alumina)	Liquid	Polarity of the molecules	An inert support (usually glass or aluminum) is coated with a thin layer of silica on which the mixture is spotted.
Ion chromatography or ion-exchange chromatography	Solid (cationic or anionic resin)	Liquid	Ionic charge of the molecules	Molecules with the same charge as the resin will flow through the column and elute out first. Molecules with an opposite charge as the resin will bind to the

				resin and elute out last.
Size-exclusion chromatography	Porous gel	Liquid	Size and form of the molecules	Large molecules are excluded and elute at first (dead time). Small and medium molecules are eluted next because they enter into the pores of the gel.

Chromatography can be classified (among other criteria) according to the interaction solute/mobile phase/stationary phase. This classification includes size-exclusion, ion-exchange, partition and adsorption chromatography. Partition chromatography is based on differential partitioning of the components of a sample between the stationary and the mobile phase. The stationary phase is a liquid film (immiscible with the mobile phase) impregnated on a rigid support (silica) or fixed by covalent bonds (grafted phases). In adsorption chromatography, by the other hand, chemicals are separated based on their absorption and desorption at the stationary phase^[4].

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Appendix B

Appendix B

This article was published in collaboration with Juliette Vievard (PhD student from my laboratory) *et al.* It is entitled "Extraction and quantification of pesticides and metals in palm wines by HS-SPME/GCMS and ICP-AES/MS" and was published in Food Chemistry (Impact Factor: 9.231). This method allowed to quantify 29 pesticides and 10 metals from 32 palm wine samples, and was validated in terms of linearity, repeatability, limit of detection (LOD), limit of quantification (LOQ) and accuracy.

Journal Pre-proofs

Extraction and quantification of pesticides and metals in palm wines by HS-SPME/GC-MS and ICP-AES/MS

Juliette Vievard, Tiemele Laurent Simon Amoikon, Aminata Nadjoua Coulibaly, Christine Devouge-Boyer, Monica Arellano Gisel Sanchez, Aké Francine, Theodore N'Dede Djéni, Mélanie Mignot

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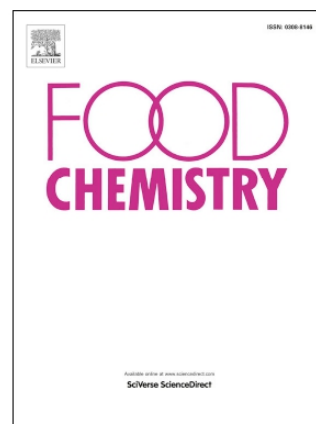
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Extraction and quantification of pesticides and metals in palm wines by HS-SPME/GC-MS and ICP-AES/MS

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ABSTRACT

In this study, a HS-SPME/GC-MS and ICP-AES/MS methods are presented to extract and quantify pesticides and metals in palm wines. Various parameters affecting the extraction were investigated: SPME fiber, equilibrium and extraction time, extraction temperature, salinity, and stirring, through an experimental design with 45 trials. The developed method allowed to identify 35 pesticides and quantify 29 of them, from different families of pesticides in 32 palm wine samples. Method performance was evaluated in terms of linearity, repeatability, LOD, LOQ, and accuracy. Among the 32 samples analyzed in 3 replicates, 7 pesticides were detected in 10 samples. Dichlorvos was the only pesticide detected at levels above the European maximal limits. Additionally, 10 of the 19 metals explored by ICP-AES and ICP-MS were found in all

samples. Six metals were detected in different samples at levels above the European or OIV maximal limits for drinking water or wine.

Keywords: palm wine, pesticides, metals, headspace solid-phase microextraction, gas chromatography, mass spectrometry, induced coupled plasma mass spectrometry

1. Introduction

Palms are believed to be among the oldest flowering plant in the world (Redhead, 1989). The oil palm (*Elaeis guineensis* Jacq.) is one of the major cultivated crops among the economically important palm species. Their growth is so vigorous that they are considered as a “weed” in neglected or abandoned farms. Different parts of the palm tree can be used for various purposes, but one important commercial activity associated with the tree is production of wine from the sap by the local populations (Yaméogo et al. 2008). Yet the oil palm in West Africa is highly valued for palm wine production, and could allow farmers to supplement and diversify their incomes. As an example, in western Ivory Coast, the resumption of wild oil palm in old cocoa farms played an important role in the 1990s, when farmers faced collapsing cocoa prices and inheritance conflicts, which resulted in the abandonment of many cocoa plantations where the density of oil palms trees grew rapidly. Thus, the felling of palm trees made it possible to produce palm wine for sale and for own consumption, thus generating income, reducing risks and creating jobs and new intergenerational and institutional arrangements (Ruf, 2014).

Palm wine is the collective name for a group of alcoholic beverages produced by the natural fermentation of the sap obtained from various tropical plants of the Palmae family (Okafor, 1978), such as *Elaeis guineensis*, *Raphia hookeri*, *Phoenix dactylifera*, *B. aethiopum* and *Cocos nucifera* (Mollet et al. 2000; Sambou et al. 2002; Stringini et al. 2009; Ziadi et al. 2011). It is mainly consumed by more than 10 million people in West Africa (Battcock et al., 1998) but it

is also available in Asia, India and Mexico (Chandrasekhar et al., 2012). A study by Fatoye and Morakinyo (2002) on alcoholic drinks (beer, locally fermented wine and locally distilled gins) in West Africa showed it as the most commonly used alcoholic beverages. It serves a variety of purposes, notably in traditional namings and marriage ceremonies, medicine (cure of low sperm count and poor sight), traditional incantation and also acts as catalyst for extraction of juice from herbs and roots for medical application.

Generally, the palm sap for palm wine production is obtained through a process known as tapping, operated in open-air, and involving a series of operations on live standing or felled or cut down tree, such as perforation of the trunk, insertion of a tube in the hole and collection of the sap in a container which can be a gourd, clay pot, plastic container, glass bottle or calabash as previously reported (Ouoba et al., 2012). The tapping process between 2 and 8 weeks and palm wine is collected twice a day (early in the morning and in the evening) and either immediately consumed on fields or stored for later sale.

Despite the enormous economic, medicinal and socio-cultural importance attached to the production of palm wine in rural African society, the consumption of this drink is becoming increasingly problematic because of two major facts. Indeed, it becomes fearful on the one hand, and this is a recurring observation, to frequent the palm wine from cabarets. This fear is generated by the fact that crooked sellers tend to sell an adulterated version of the drink, resulting from the adulteration of the original palm wine by using water (generally of low quality with a distinct possibility of contamination with undesirable elements such as metals) and artificial sweetness, which sometimes result in diarrhea, abdominal pains, and other stomach problems (Ukhun et al. 2005). In other hand, as palm trees mostly grow concomitantly in cocoa farms in rural areas, the second problem and hence the quieter comes from the potential contamination of this drink during tapping process by pesticides or their residues used in the agrochemical treatments of cocoa fields in which the sap extraction process takes place. Indeed,

in an attempt to reduce the incidence of insect pests and diseases on cocoa farms, a large number of pesticides are usually applied on farms. These pesticides are hazardous in very low concentrations and cause immense damage to the ecosystem. In most developing countries like Ivory Coast, consequences are often severe because approved pesticides are not used and recommended frequencies of pesticides application by government agencies for crops are not followed. Farmers however misuse, overuse and apply pesticides indiscriminately with disregard to safety measures and regulations on chemical use as reported by Konradsen (2007) and Sam et al. (2008). In consequence, the drink produced can be contaminated at multiple steps in the tapping process by plant material treated with pesticides, particularly leaves used to cover containers containing the drink in the palm wine processing environment, or insects when flying into bowls. According to the National Pesticide Information Center (2011), an increase in the use of agrochemicals has been observed over the past fifty years making humans more exposed to heavy metals, pesticides and insecticides. Heavy metals and pesticides have carcinogenic, mutagenic and teratogenic potential and exposure can cause irreversible damage to humans, including death, and are therefore considered a serious public health problem particularly in sub-Saharan countries. Although many studies have revealed that pesticides and heavy metals poses threats to the environment, and farmers themselves, their potential presence in palm wine and the risks associated have to be determined.

Several methods are available for extraction and quantification of pesticides and metals from complex matrices. For pesticides, liquid extraction with solvents, such as dichloromethane, acetone (Gambacorta et al., 2005), and ethyl acetate (Munawar and Waqas Hameed, 2013) has been used. Pesticide residues have also been extracted from apple juice using a solid phase extraction (SPE) method (Han et al. 2014; Velkoska-Markovska and Petanovska-Ilievska, 2013), from berry fruits using an Envi-Carb cartridge (Yang et al, 2011) and using an HS-SPME method, a fast, automatable, and inexpensive method, including in mangoes from Brazil (Filho,

Dos Santos, and De Paula Pereira 2010), in water samples from Argentina (Bonansea, Amé, and Wunderlin 2013) and in wines from Italy (Vitali et al. 1998). In general, the analysis of pesticides is done using gas (Han et al., 2014 and Yang et al., 2011) or liquid (Velkoska-Markovska and Petanovska-Ilievska, 2013) chromatography, depending on the polarity, volatility and decomposition risk of the compounds. For the detector, the electron capture detector is commonly used to quantify organochlorines, while the nitrogen-phosphorus detector is used for organophosphates. Mass spectrometry offers the advantage of being able to identify and quantify a wide range of molecules, without being limited to a single class of pesticides (Bonansea, Amé, and Wunderlin 2013). Metals are commonly analyzed by ICP-AES/MS in pharmaceutical compounds (Lewen et al., 2004), textile materials (Rezić and Steffan, 2007), or wines and alcoholic beverages (Plotka-Wasyłka et al., 2018), due to sensitivity, low limit of detection and speed of analysis. Prior to analysis, samples are mineralized to remove organic matter and ensure quantitative dissolution of metals in the case of solid matrices. Most of the time, acids or acid mixtures are used for this step (Lewen et al., 2004): nitric acid, hydrochloric acid, sulfuric acid, or mixtures, with aqua regia being the most famous. Due to the low detection levels required by regulatory agencies, efficient sample preparation and trace-level detection are important aspects in an analytical method.

This research was therefore conducted to determine the concentration of heavy metals and pesticides using optimized methods and then raise awareness of the possible health risk while consuming this beverage.

2. Materials and methods

2.1 Chemicals

Ultrapure water (resistivity 18.2 MW•cm), obtained from a Milli-Q system (Millipore, Bedford, MA, USA), sodium sulfate (GPR Rectapur, VWR Chemicals, Belgium), ethanol (99%, Fisher

Scientific, UK), (+) limonene (97%, Accros Organics, USA) decane (99%, Sigma-Aldrich, USA), L-ascorbic acid (99%, VWR Chemicals, Belgium), calcium chloride (98%, VWR Chemicals, Belgium), potassium chloride (99%, Carlo Erba, Italia), magnesium nitrate (98%, Sigma-Aldrich, USA), and sodium chloride (99.5%, Accros Organics, USA), were used for the preparation of the simulative palm wine. For the mineralizations, nitric acid (68%, trace analysis grade, Fisher Scientific, UK) and hydrogen peroxide (30%, VWR Chemicals, Belgium) were employed. Acetone (pesticides residue grade, Fisher Scientific, UK) and methanol (HPLC grade, Fisher Scientific, UK) were used for the preparation of the stock solutions of pesticides.

The pesticides standard consisted in six stock solutions. Two solutions were purchased from Restek (USA): an OrganoPhosphorus Pesticides (OPPs) solution in acetone containing acephate ($200 \mu\text{g mL}^{-1}$), azinphos methyl ($400 \mu\text{g mL}^{-1}$), chlorpyrifos ($100 \mu\text{g mL}^{-1}$), demeton-S-methyl ($200 \mu\text{g mL}^{-1}$), dichlorvos ($500 \mu\text{g mL}^{-1}$), dimethoate ($200 \mu\text{g mL}^{-1}$), ethion ($200 \mu\text{g mL}^{-1}$), malathion ($200 \mu\text{g mL}^{-1}$), methamidophos ($500 \mu\text{g mL}^{-1}$), methidathion ($200 \mu\text{g mL}^{-1}$), omethoate ($1,000 \mu\text{g mL}^{-1}$), pirimiphos methyl ($100 \mu\text{g mL}^{-1}$), profenofos ($200 \mu\text{g mL}^{-1}$), prothiofos ($200 \mu\text{g mL}^{-1}$), pyrazophos ($500 \mu\text{g mL}^{-1}$), and tolclofos-methyl ($100 \mu\text{g mL}^{-1}$), and an organohalide pesticides solution in methanol containing aldrin, alachlor, atrazine, γ -BHC (lindane), cis-chlordane, trans-chlordane, dieldrin, endrin, heptachlor, heptachlor epoxide (isomer B), hexachlorobenzene, hexachlorocyclopentadiene, methoxychlor, cis-nonachlor, trans-nonachlor, and simazine, at $200 \mu\text{g mL}^{-1}$ each. Two solutions were purchased from Fisher Scientific (France): an OrganoChloride Pesticides (OCPs) solution in acetone containing aldrin, α , β and δ -BHC, dieldrin, α and β -endosulfan, endosulfan sulfate, endrin, endrin aldehyde, endrin ketone, lindane, heptachlor, heptachlor epoxide (isomer B), methoxychlor, p,p'-DDD, p,p'-DDE, and p,p'-DDT at $200 \mu\text{g mL}^{-1}$ each, and a terbutylazine solution in acetone at $100 \mu\text{g mL}^{-1}$. A methyl parathion solution in acetone at $1000 \mu\text{g mL}^{-1}$, and a diazinon solution in methanol at $100 \mu\text{g mL}^{-1}$ were purchased from Merck (Germany).

The stock solution containing all pesticides was prepared in pure acetone and before each series of analyses. The pesticides standard solutions at different levels were prepared by spiking with the stock solution into 3 mL of the simulative palm wine contained in a 20 mL sealed glass vial. Calibration curves were prepared from dilutions of the stock solution. The concentration of pesticides ranged from 0.25 $\mu\text{g L}^{-1}$ for pirimiphos methyl and tolclofos-metyl to 1000 $\mu\text{g L}^{-1}$ for simazine.

A metal solution (VWR Chemicals, Leuven, Belgium) containing aluminum, silver, arsenic, boron, barium, calcium, cadmium, cerium, cobalt, chromium, copper, dysprosium, erbium, europium, iron, gadolinium, holmium, potassium, lanthanum, lithium, lutetium, magnesium, manganese, sodium, neodymium, nickel, phosphorus, lead, rubidium, selenium, samarium, strontium, thallium, thulium, vanadium, and zinc, at 10 mg L^{-1} each, was used. Three mono-element solutions containing bismuth at 10 mg L^{-1} (Spex CertiPrep, USA), molybdenum (Spex CertiPrep, USA) and tin at 1000 mg L^{-1} (Fisher Scientific, France) were used for preparing the stock solutions. Calibration curves were prepared from dilutions of the stock solution. The concentration of metals ranged from 0.05 $\mu\text{g L}^{-1}$ to 100 $\mu\text{g L}^{-1}$ for all metals except for rubidium which ranged from 0.05 to 2 mg L^{-1} and for phosphorus which ranged from 0.5 to 20 mg L^{-1} .

2.2 Samples

This study was carried out on different palm wines produced in four cities in Ivory Coast (Abidjan, Aboisso, Hiré and Toumodi). Four samples of each city were collected from local producers (P) and four from resellers (R), who could eventually modify the composition of the palm wines. Thirty two palm wines were analyzed (3 replicates) for determination of pesticides and metals. Samples were stored in a freezer at -18°C . Before analysis, samples were kept at 4°C during two hours in a fridge and thirty minutes at room temperature. The palm wines presented particles naturally present. To estimate the real concentrations to which the population is exposed, it was decided to let the samples as naturally present and consumed.

Consequently, no filtration nor centrifugation was done, and the samples were mixed before analysis in triplicate.

2.3 Instrumentation

2.3.1 GC-MS

Analyses of palm wine were performed using a GC-MS QP-2020 NX (Shimadzu, Germany) equipped with an auto-sampler AOC-6000 (automatic injection). Different GC conditions for analyzing pesticides were tested; to find a good compromise between the analysis time and the resolution. As final GC conditions, a ZB5MS+ (5% polysilarylene and 95% dimethylpolysiloxane, 30 m x 0.25 mm x 0.25 μ m, Phenomenex) column was used to separate pesticides; helium was used as carrier gas (1 mL/min); injection was splitless and at 260°C. The oven temperature was programmed as follows: initially maintained at 40°C (held for 5 min), then increased to 180°C (held for 2 min) at a rate of 10°C/min, a second increase was used to 280°C (held for 2 min) at a rate of 5°C/min. The optimal analysis time was 43 minutes.

The MS transfer line was kept at 250°C. The mass spectrometer, equipped with a quadrupole mass filter, was in the electron impact ionization mode (70 eV) with an ion source temperature of 200°C. Chromatograms were acquired in Single Ion Monitoring (SIM) scanning mode. The m/z associated with pesticides are specified in Table S1. All compounds could be separated excepted *cis* and *trans* chlordane. These isomers were identified on the same peak. Moreover, MS was not able to identify with certainty the three BHC (hexachlorocyclohexane), the three isomers were identified as BHC on chromatograms.

2.3.2 ICP-AES and ICP-MS

Extraction and mineralization of samples were performed with a Milestone ETHOS 1 microwave digestion system equipped with a temperature sensor. TFM reactors were used with a maximum pressure of 100 bar. ICP-AES measurements were performed on an iCAP 6300

series instrument (Thermo Fisher Scientific) using the axial view. The quantification of phosphorus and rubidium was performed at 178 nm and 780 nm, respectively.

Detection of lithium, boron, chromium, iron, manganese, cobalt, nickel, copper, zinc, strontium, molybdenum, silver, cadmium, tin, barium, lead, and bismuth was performed on an iCAP RQ ICP-MS instrument (Thermo Fisher Scientific) fitted with a collision cell. Two different measurement modes were used: standard mode (STD) and kinetic energy discrimination mode (KED). In the KED mode, Helium gas was injected in the collision cell and an energy discrimination barrier was applied in order to remove unwanted polyatomic interferences. Data processing was performed using ThermoFisher Scientific Qtegra software.

As example, optimized conditions for the plasma and nebulizer for ICP-AES and ICP-MS are given in Table S2.

2.4 Method optimization

2.4.1 The simulative palm wine

Because of the low volume of palm wine samples, a surrogate matrix (simulative palm wine) was developed to determine HS-SPME/GC-MS optimal conditions before application on real samples.

For this purpose, a palm wine sample was analyzed by GC-MS to determine the main constituents. It was found that palm wine contained around 88 % (m/m) of water, 2 % (m/m) of ethanol, and 10 % (m/m) of sucrose. Based on a previous study on palm wines (Simon Amoikon et al., 2020), the aromatic profile was recreated using 4 mg L⁻¹ of limonene and 10 mg L⁻¹ of decane, main compounds found in real palm wines. Calcium (5 mg L⁻¹), potassium (280 mg L⁻¹), magnesium (7 mg L⁻¹) and sodium (4 mg L⁻¹) concentrations were determined in the palm wine sample by ICP-AES. According to Cunningham and Wehmeyer (1988), vitamin

C (60 mg L^{-1}) is generally added. Finally, the simulative palm wine was doped to achieve the concentrations generally found in such samples.

2.4.2 Optimization of the HS-SPME procedure

Experimental parameters were evaluated to determine the optimal conditions for the analysis of pesticides in palm wines. Three fiber types were tested: $100 \mu\text{m}$ polydimethylsiloxane (PDMS), $85 \mu\text{m}$ polyacrylate (PA), and $50/30 \mu\text{m}$ DVB/Carbon WR/PDMS (Restek USA). Fibers were conditioned at 230°C during 30 min before each analysis, according to the manufacturer's recommendations. Three milliliters of the simulative palm wine were placed in a 20 mL sealed headspace glass vial (3 replicates). After extraction, the fiber was automatically retracted and transferred to the GC injector at 260°C for a desorption time of 3 min.

In order to optimize the extraction efficiency, an experimental design (Table S3) with 45 trials was implemented (Ellistat software, Chavanod, France) on a $100 \mu\text{m}$ PDMS fiber. Some parameters were tested, according to the literature for pesticides extraction by HS-SPME. Extraction temperature between 30 and 60°C (Cincotta et al., 2015), equilibrium time between 5 and 30 min (Bernardi et al. 2015, Pellati et al. 2013), and extraction time between 20 and 60 min (Pellati et al., 2013) were investigated. Moreover, stirring was studied between 250 and 500 rpm (Filho et al., 2010, Bernardi et al. 2015), and salinity (sodium sulfate) between 5 and 15 % (m/m) (Vitali et al., 1998, Zhu et al., 2009) of the sample. According to Zhu et al (2009), who worked on the determination of OPPs in textiles using SPME coupled with GC/MS, OPPs were more extracted with sodium sulfate than with sodium chloride.

Data processing was performed using the GC-Solution software.

2.5 Method validation

2.5.1 Analysis of palm wine by HS/SPME-GC/MS

For the study of pesticides in complex matrix, an HS-SPME extraction technique was used. As final conditions after optimization, a 20 mL vial was filled with 3 mL of each palm wine samples (3 replicates) and 7.5 % (m/m) of sodium sulfate. Extraction was done in the headspace vial kept at 52.5°C. A DVB/C-WR/PDMS fiber was used for all extractions because it allowed the determination of many pesticides with good repeatability (see results and discussions - fiber coating). The palm wine samples were equilibrated for 11.25 min and extracted for 50 min under continuous stirring (437.5 rpm). The fiber was automatically transferred to the GC injector at 260 °C for desorption time of 3 min. The method performance was evaluated in terms of linearity, limit of detection, limit of quantification, accuracy, and precision (repeatability).

The concentration of three stock solutions ranged from 0.5-2.5 $\mu\text{g L}^{-1}$ (for Tolclofos-methyl and Dichlorvos, respectively) to 5-25 $\mu\text{g L}^{-1}$ (for Tolclofos-methyl and Dichlorvos, respectively). These three stock solutions were each injected three times in order to study the repeatability (represented by a relative standard deviation (RSD)). To study the linearity, calibration curves with 3-5 points were established. LOD and LOQ were estimated as concentrations of pesticides which allowed a signal-to-noise ratio (S/N) of 3 and 10, respectively. Additionally, samples were spiked with stock solutions (to obtain three samples with concentrations from 0.5-2.5 $\mu\text{g L}^{-1}$ (for Tolclofos-methyl and Dichlorvos, respectively) to 5-25 $\mu\text{g L}^{-1}$ (for Tolclofos-methyl and Dichlorvos, respectively) three times and relative recoveries were determined.

2.5.2 Analysis of palm wine by ICP/AES and ICP/MS

In accordance to the French standard « NF EN ISO 17294-2 » for the determination of metals in drinking water, ICP-MS is the reference technique and is used after mineralization. No standard exists for palm wine, and it consists of 88% water, so the same methodology was followed and can be assimilated to drinking water.

A volume of 2 mL of palm wine was digested with 2 mL of nitric acid and 1 mL of hydrogen peroxide in a microwave system: from room temperature, 30 °C, to 180 °C, 20 minutes at 180

°C. After the mineralization, the digest was diluted up to 50 mL with ultrapure water. A blank was analyzed in the same conditions.

Samples were analyzed with ICP-AES for the determination of phosphorus and rubidium, and with ICP-MS for the determination of the other metals. To study the linearity, calibration curves with 5 points were established.

LOD and LOQ were obtained for the palm wine samples using equations 1 and 2, where BEC is the background equivalent concentration ($\mu\text{g L}^{-1}$), it represents the blank intensity divided by the angular coefficient of the analytical curve and RSD_{blank} is the relative standard deviation of the blank (n=10).

$$LOD = \frac{3 \times RSD_{\text{blank}} \times BEC}{100} \text{ (Equation 1)}$$

$$LOQ = \frac{10 \times RSD_{\text{blank}} \times BEC}{100} \text{ (Equation 2)}$$

3. Results and discussion

3.1 Determination of pesticides in palm wines

3.1.1 Determination of SPME conditions

Different parameters were tested in order to develop a HS/SPME-GC/MS method with the optimized extraction efficiency. Fiber coating, extraction temperature, equilibrium time, extraction time, stirring, and salinity were studied.

3.1.1.1 Extraction temperature, equilibrium and extraction time, stirring and salinity

Five parameters have been explored in this study on a 100 μm PDMS fiber: extraction temperature, equilibrium and extraction time, stirring and salinity. Among the 45 trials of the experimental design, one offered the highest peak area for 7/12 extracted pesticides of the OPPs solution (azinphos methyl, chlorpyrifos, ethion, methidathion, pirimiphos methyl, profenofos and tolclofos methyl). This method had a long extraction time and according to the exploitation

of the experimental design, the extraction time was the parameter that influences the most the extraction efficiency.

Finally, the extraction temperature was set at 52.5°C. Increasing the temperature allows reducing the extraction time, which increases the concentration of semi-volatiles in the vial headspace (Bianchin et al., 2014). Pesticides were mostly obtained with high peak areas for an extraction temperature of 52.5°C. Lower peak areas were obtained with a temperature of 37.5°C (comparison of trials 4 and 8 in Table S3, parameters were set at the optimized values, except for extraction temperature), especially for chlorpyrifos (peak areas of 889083 and 3829688 for temperatures of 37.5°C and 52.5°C, respectively), ethion (peak areas of 18863 and 2459896 for temperatures of 37.5°C and 52.5°C, respectively), and tolelofos methyl (peak areas of 6788178 and 21189553 for temperatures of 37.5°C and 52.5°C, respectively).

Equilibrium time was set at 11.25 min. Equilibrium time depends on the chemical nature and physico-chemical parameters of pesticides. The optimum equilibration time was selected as the time ensuring the highest peak area for the majority of pesticides (all other parameters being constant), with a reasonable time. Finally, 11.25 min was selected for equilibration time.

The highest extraction time tested with the experimental design was 60 min. A higher extraction efficiency was observed for a higher exposure time between the SPME fiber and the sample headspace (Ho and Hsieh, 2001), but a longer exposure could also damage the fiber and/or the pesticides. The extraction time of 50 min was a compromise between extraction efficiency and fiber lifetime and/or pesticides stability. This extraction time was compared with a lower time of 30 min (trials 6 and 8). All other parameters were constant at the optimized values. Pesticides were mostly obtained with high peak areas with an extraction time of 50 minutes. Ethion (peak areas of 744276 and 2459886 for times of 30 min and 50 min, respectively), and prothiofos (peak areas of 1725649 and 3690958 for times of 30 min and 50 min, respectively) were more extracted with the higher extraction time. However, malathion was more extracted with the

lower extraction time (peak areas of 502458 and 198595 for times of 30 min and 50 min, respectively).

Stirring and salinity of the sample allows the diffusion layer to reach the equilibrium faster (Ho and Hsieh, 2001). Stirring of 437.5 rpm and 7.5% (m/m) of Na_2SO_4 allowed to reduce the analysis time, by promotion of the pesticides from the liquid phase to the gas phase.

Finally, SPME optimized parameters for this study were: an extraction temperature of 52.5°C, an equilibrium time of 11.25 min, an extraction time of 50 min, stirring of 437.5 rpm, and salinity of 7.5% of Na_2SO_4 .

3.1.1.2 Fiber coating

Three SPME fiber coatings were used to extract the pesticides in the simulative palm wine solution: a 100 μm polydimethylsiloxane (PDMS), a 85 μm polyacrylate (PA), and a 50/30 μm DVB/Carbon WR/PDMS. Compounds were spiked between 2.5 to 12.5 $\mu\text{g L}^{-1}$ triplicate. SPME optimized parameters (see 3.1.1.1 Extraction temperature, equilibrium and extraction time, stirring and salinity) were used.

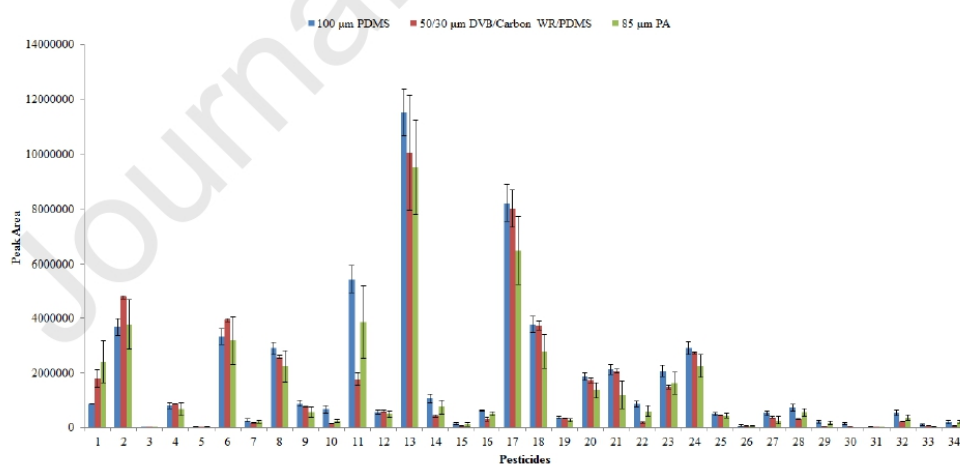


Fig.1. Comparison of three SPME fiber coatings for the extraction of 35 pesticides. Peaks: (1) dichlorvos, (2) BHC, (3) simazine, (4) BHC, (5) atrazine, (6) lindane, (7) terbuthylazine, (8) diazinon, (9) BHC, (10) parathion methyl, (11) tolclofos methyl, (12) alachlor, (13) heptachlor, (14) pirimiphos methyl, (15) malathion, (16) chlorpyrifos, (17) aldrin, (18) heptachlor epoxide,

(19) α -endosulfan, (20) chlordane, (21) trans-nonachlor, (22) prothiofos, (23) p,p'-DDE, (24) dieldrin, (25) endrin, (26) β -endosulfan, (27) cis-nonachlor, (28) p,p'-DDD, (29) ethion, (30) endrin aldehyde, (31) endosulfan sulfate, (32) p,p'-DDT, (33) endrin ketone, (34) methoxychlor. Hexachlorobenzene was not represented because its area was too important.

The three SPME fibers allowed the extraction of all pesticides as it can be observed from Table S4, except for the endrin aldehyde, which was not extracted by the PA fiber. The detector was saturated and no longer able to give an area for hexachlorobenzene using the DVB/Carbon WR/PDMS fiber. Some pesticides had low peak areas no matter the fiber, such as simazine, atrazine, malathion, β -endosulfan, endrin aldehyde, endosulfan sulfate or endrin ketone. Twenty-eight pesticides were extracted with higher area with the PDMS fiber, 5 with the DVB/Carbon WR/PDMS fiber and 2 with the PA fiber. A lot of pesticides were extracted with an important area with PDMS and DVB/Carbon WR/PDMS fibers, such as aldrin (peak area of 8228026 for the PDMS fiber and 8021739 for the DVB/Carbon WR/PDMS fiber), diazinon (peak area of 2886678 for the PDMS fiber and 2577749 for the DVB/Carbon WR/PDMS fiber), or heptachlor (peak area of 11522371 for the PDMS fiber and 10058902 for the DVB/Carbon WR/PDMS fiber).

However, as shown in Table S4, the DVB/Carbon WR/PDMS fiber exhibited the lowest RSDs for 25 pesticides (RSDs between 0.7 and 18%) and offered 22 RSDs under 10%. Only 14 and 1 RSDs were under 10% for the PDMS and PA fibers, respectively. The best repeatability was achieved with the DVB/Carbon WR/PDMS fiber. Therefore, this fiber was selected for the analysis of palm wines.

3.1.2 Analysis of palm wine samples

3.1.2.1 Method validation

3.1.2.1.1 Quadratic or linear behavior of calibration curves

Initially, a linear calibration model was developed for all pesticides. This type of calibration is the most used to obtain an analytical result from the measurement of the signal. According to Balsini and Parastar (2019), pesticides may have a quadratic behavior. In this study, 12 pesticides (aldrin, chlordane, α -endosulfan, endrin heptachlor, heptachlor epoxide, parathion methyl, pirimidiphos methyl, prothiofos, p,p-DDD, and simazine), followed a quadratic calibration curve and 23 of them had a linear behavior. The analytical results were listed in Table 1 (linear (L) or quadratic (Q) behavior, LODs, and LOQs).

Table 1

Quadratic or linear behavior, LODs, and LOQs of pesticides in this study and results from the literature

No.	Pesticides	Behavior ^a	Range ($\mu\text{g L}^{-1}$)	R ²	LOQs ($\mu\text{g L}^{-1}$)	LODs ($\mu\text{g L}^{-1}$)	LODs from the literature ($\mu\text{g L}^{-1}$)	LOQs from the literature ($\mu\text{g L}^{-1}$)	Fiber used from literature
1	Dichlorvos	L	1.25-250	0.9999	1.25	0.11	0.05		85 μm PA [27]
							3.80	11.4	65 μm PDMS/DVB [25]
2	BHC	L	1-100	0.9998	0.31	0.09			
3	Hexachlorobenzène	L	0.5-5	0.9971	0.10	0.03			
4	Simazine	Q	100-1000	1	92.1	27.6	0.10		85 μm PA [27]
5	BHC	L	0.5-100	0.9999	0.20	0.10			
6	Atrazine	L	10-1000	0.9993	10.0	3.00			
7	Lindane	L	2-200	0.9999	0.11	0.03			
8	Terbutylazine	L	1-100	0.9999	1.00	0.30			
9	Diazinon	L	5-100	0.9998	0.58	0.17	3.00		85 μm PA [33]
							0.01		85 μm PA [27]
10	BHC	L	0.5-100	0.9999	0.75	0.22			
11	Parathion methyl	Q	0.5-10	0.9991	0.02	0.01	50.0		85 μm PA [33]
12	Tolclofos-methyl	L	0.25-5	0.9993	0.13	0.04	0.01		85 μm PA [27]
13	Alachlor	L	1-100	0.9999	0.53	0.16			
14	Heptachlor	Q	1-200	0.9994	0.01	0.003			

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15	Pirimidiphos methyl	Q	0.25-50	0.9999	0.25	0.08				
16	Malathion	L	5-100	0.9998	1.20	0.36	45.0		85 μ m PA [33]	
							10.0		85 μ m PA [27]	
17	Chlorpyrifos	L	0.5-50	0.9997	0.03	0.01	80.0		85 μ m PA [27]	
							4.50	13.7	65 μ m PDMS/DVB [25]	
18	Aldrin	Q	1-200	0.9991	0.11	0.03				
19	Heptachlor epoxide	Q	1-200	0.9999	0.08	0.02				
20	α -Endosulfan	Q	0.5-100	0.9999	0.17	0.05				
21	Chlordane	Q	1-200	0.9997	0.16	0.05				
22	trans-Nonachlor	L	1-100	0.9999	0.01	0.002				
23	Prothiofos	Q	0.5-10	0.9999	0.22	0.07				
24	p,p'-DDE	L	0.5-10	0.9963	0.01	0.003				
25	Dieldrine	L	1-200	0.9996	0.09	0.03				
26	Endrin	Q	1-200	0.9999	1.00	0.03				
27	β -Endosulfan	L	0.5-100	0.9999	0.50	0.15				
28	cis-Nonachlor	L	1-100	0.9999	0.06	0.02				
29	p,p'-DDD	Q	0.5-100	0.9998	0.32	0.10				
30	Ethion	Q	1-100	0.9999	0.62	0.18	2.20	6.50	65 μ m PDMS/DVB [25]	
31	Endrin aldehyde	L	0.5-100	0.9999	0.50	0.15				
32	Endosulfan sulfate	L	1-100	0.9999	1.00	0.30				
33	p,p'-DDT	L	0.5-100	0.9999	0.07	0.02				
34	Endrin ketone	L	0.5-100	0.9999	0.50	0.15				
35	Methoxychlor	L	1-200	0.9997	1.00	0.30				

^aQ = quadratic calibration, L = linear calibration

All calibration curves had correlation coefficients between 0.9963 and 1, therefore, the quantification of pesticides in palm wines was acceptable.

3.1.2.1.2 LODs and LOQs

The obtained LODs ranged between 0.002 and 27.6 $\mu\text{g L}^{-1}$, for trans-nonachlor and simazine, respectively, and LOQs ranges between 0.01 and 92.05 for p,p'-DDE and simazine, respectively. For such compounds, some comparisons can be done with literature. For example, for the Dichlorvos, LOD with the optimized method was 0.11 $\mu\text{g L}^{-1}$, Sakamoto and Tsutsumi found a LOD of 0.05 $\mu\text{g L}^{-1}$ in water sample with a 85 μm PA fiber, and Rodrigues et al found a LOD of 3.8 $\mu\text{g L}^{-1}$ in cow milk sample with a 65 μm PDMS/DVB fiber. Tsoukali et al obtained higher LODs from whole blood and with a 85 μm PA fiber, as for the Malathion (0.36 $\mu\text{g L}^{-1}$ for palm wine sample and 45 $\mu\text{g L}^{-1}$ for whole blood sample) or Parathion methyl (0.01 $\mu\text{g L}^{-1}$ for palm wine sample and 50 $\mu\text{g L}^{-1}$ for whole blood sample). Rodrigues et al obtained higher LOQs with a LOQ of 6.50 $\mu\text{g L}^{-1}$ for Ethion (LOQ of 0.62 $\mu\text{g L}^{-1}$ for optimized method). The developed method offered better performances for those compounds.

3.1.2.1.3 Relative standard deviation

Table 2

Repeatabilities and recoveries of pesticides

No.	Pesticides	Replicate 1 (0.5-2.5 $\mu\text{g L}^{-1}$) ^a		Replicate 2 (2.5-12.5 $\mu\text{g L}^{-1}$) ^a		Replicate 3 (5-25 $\mu\text{g L}^{-1}$) ^a	
		RSD (%) (n = 3)	Relative recovery (%)	RSD (%) (n = 3)	Relative recovery (%)	RSD (%) (n = 3)	Relative recovery (%)
1	Dichlorvos	14.5	116	5.8	95	1	100
2	BHC	10.8	51	11.1	97	3.6	107
3	Hexachlorobenzene	7.9	114	1.4	100	2.5 ^b	75 ^b
4	Simazine	6.2 ^d	102 ^d	36.5 ^e	88 ^e	59.3 ^f	100 ^f
5	BHC	3.9	80	0.9	102	3.7	103
6	Atrazine	11.2 ^d	115 ^d	31.8 ^e	98 ^e	41.7 ^f	117 ^f
7	Lindane	22.1	73	12.2	100	2.7	103
8	Terbutylazine	18.7	94	0.7	98	1.1	102
9	Diazinon	14.7 ^d	95 ^d	11.6	115	6.1	85
10	BHC	22	96	6.9	96	3.1	92
11	Parathion methyl	7.2	110	3.8	99	2.7	102
12	Tolclofos-methyl	15.8	103	15.5	97	5.4	101

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13	Alachlor	4	100	4.2	99	6.2	100
14	Heptachlor	7.8	110	3.9	99	0.7	100
15	Pirimidiphos methyl	8.9	113	21.4	95	7.1	101
16	Malathion	4.4 ^d	100 ^d	5	82	1.4	109
17	Chlorpyrifos	21.9	44	22.1	93	6.6	110
18	Aldrin	7	109	4.3	99	0.3	100
19	Heptachlor epoxide	17.3	100	11.6	101	6.1	93
20	α -Endosulfan	14.1	102	10.3	105	2.9	99
21	Chlordane	19.1	107	12.9	100	18.2	100
22	trans-Nonachlor	40.4	116	37.6 ^d	90 ^d	13.2	98
23	Prothiofos	18.6	100	20.4	100	6.6	100
24	p,p'-DDE	20.7	113	19.3	92	6.5	102
25	Dieldrin	15	105	15.2	97	4.5	101
26	Endrin	13.5	115	14.2	100	6.5	100
27	β -Endosulfan	20.7	116	23.2	104	9.9	96
28	cis-Nonachlor	36.9	105	31.4 ^d	117 ^d	11.9	99
29	p,p'-DDD	17.9	110	15.1	95	9.3	102
30	Ethion	22	84	38	105	13.6	102
31	Endrin aldehyde	16.4	72	11.4	103	11.6 ^d	100 ^d
32	Endosulfan sulfate	10.6	108	11.9	98	11.3 ^d	100 ^d
33	p,p'-DDT	26.8	120	17.3	99	9.4 ^d	100 ^d
34	Endrin ketone	9.4	97	15.2	97	12.1 ^d	100 ^d
35	Methoxychlor	44 ^e	109 ^c	26.6	90	14.7	102

^a range of concentrations

^b at the concentration of 0.5 $\mu\text{g L}^{-1}$

^c at the concentration of 1 $\mu\text{g L}^{-1}$

^d at the concentration of 100 $\mu\text{g L}^{-1}$

^e at the concentration of 500 $\mu\text{g L}^{-1}$

^f at the concentration of 1000 $\mu\text{g L}^{-1}$

As the pesticides were not at the same concentration in the stock solution, spiking was at three levels and for each level the pesticides were not at the same concentration. For the first level, the concentrations were between 0.5 (for chlorpyrifos and pirimiphos methyl for example) and 2.5 $\mu\text{g L}^{-1}$ (for dichlorvos for example), between 2.5 and 12.5 $\mu\text{g L}^{-1}$ for the second level, and between 5 and 25 $\mu\text{g L}^{-1}$ for the final level. Some pesticides were not detected at these concentrations, such as simazine or atrazine, because its LOD were higher than the range of concentrations. Their RSDs and relative recoveries were calculated thanks to higher

concentrations (between 100 and 1000 $\mu\text{g L}^{-1}$). Some others were not detected at one concentration of the range tested, such as methoxychlor (replicate 1) or trans and cis-nonachlor (replicate 2), their RSDs and relative recoveries were calculated from another concentrations (at 1 $\mu\text{g L}^{-1}$ instead of 2 $\mu\text{g L}^{-1}$ and at 100 $\mu\text{g L}^{-1}$ instead of 5 $\mu\text{g L}^{-1}$, respectively for methoxychlor, and for trans-nonachlor, and cis-nonachlor).

Atrazine, cis-nonachlor, trans-nonachlor, ethion, methoxychlor, and simazine had RSDs higher than 20% for two replicates. Moreover, for these pesticides the calibration curve was only realized with 3 points, except for ethion (with 4 points), so they were identifiable but not quantifiable for this study.

The RSDs obtained, for the other pesticides, were between 3.9 and 26.8% for BHC and p,p'-DDT, respectively, at 1 $\mu\text{g L}^{-1}$ for the replicate 1, between 0.7 and 23.2% for terbuthylazine and β -endosulfan, respectively, at 5 $\mu\text{g L}^{-1}$ for the replicate 2, and between 0.3 and 18.2% for aldrin (at 20 $\mu\text{g L}^{-1}$) and chlordane (at 20 $\mu\text{g L}^{-1}$), respectively, for the replicate 3. The results are shown in Table 2. RSDs were mostly under 20% for replicates 1 and 2, respectively for 23/31 and 25/31 pesticides, and under 10% for replicate 3 for 25/31 pesticides. Little variation of peak areas used to calculate RSDs for low concentrations could have an important effect, so RSDs were lower for concentrations of the replicate 3. The first BHC (RSD of 3.9% for replicate 1, 0.9% for replicate 2, and 3.7% for replicate 3) and parathion methyl (RSD of 7.2% for replicate 1, 3.8% for replicate 2, and 2.7% for replicate 3) were pesticides with the lower RSDs for all replicates.

3.1.2.1.4 Relative recovery

Very good recoveries were obtained for all pesticides at level 3 (75% for hexachlorobenzene and 110% for chlorpyrifos) and level 2 (from 82% for malathion to 115% for diazinon). At level 1, the relative recoveries ranged from 72 to 120% (72% for endrin aldehyde and 120% for

p,p'-DDT at $1 \mu\text{g L}^{-1}$, respectively) except for BHC and chlorpyrifos, which showed the applicability of the developed method.

3.1.2.2 Pesticides in palm wine samples

Thirty-two palm wines were analyzed following the optimized HS-SPME-GC/MS method. Seven pesticides (dichlorvos, endrin ketone, parathion methyl, p,p-DDD, p,p-DDE, p,p-DDT, and pirimiphos methyl) were identified in 10 palm wine samples (AP1, AP2, AR3, BP4, HR4, TP1, TP2, TR1, TR2, and TR3) as presented in Table 3. Pesticides were found in palm wines from the 4 cities, in 3 samples from Abidjan, 1 from Aboisso, 1 from Hiré, and 5 from Toumodi, with concentrations from 0.26 to $164.7 \mu\text{g L}^{-1}$ for pirimiphos methyl in AP2 and dichlorvos in AR3, respectively. p,p'-DDT was identified in 5 palm wines, in 4 from Toumodi in low concentrations (from 0.503 for TP1 to $0.669 \mu\text{g L}^{-1}$). This study is among the first to detect these pesticides in alcoholic beverages.

In AP1, AP2, BP4, HR4, TP1, TP2, TR1, and TR3 palm wine samples, pesticides were quantified at concentrations close to LOQs. EML of pesticides in bunches of grapes are used because in France, the European Maximal Limits (EML) for wine does not exist. Parathion methyl was detected at $0.521 \mu\text{g L}^{-1}$ in AP1, which is a concentration below the EML, as well as a level below the concentration found in wines from different European countries, such as Spain or Portugal (Table 3). In AR3 and TR2, dichlorvos was detected at 164.7 and $38.4 \mu\text{g L}^{-1}$, respectively. As no study has detected this pesticide in alcoholic beverages, results were compared with the concentration of ethion and malathion (organophosphorus pesticides as dichlorvos) in wines from Brazil. Ethion was detected at concentrations between 0.29 and $0.32 \mu\text{g L}^{-1}$ and malathion at $4.4 \mu\text{g L}^{-1}$. Dichlorvos was found at a level above the EML and concentrations found in wines from Brazil (Table 3).

Table 3

Pesticides concentrations in palm wines, European Maximal Limits of pesticides in bunches of grapes and palm oil and comparison with other studies

Pesticides	European Maximal Limits (ug/kg)		Concentrations ($\mu\text{g L}^{-1}$)											Different countries (Brazil, Portugal, Argentina, Chile)	Spain	Brazil	
	Bunches of grapes ^a	Palm oil ^a	AP1	A2	AR3	BP4	HR4	TP1	TP2	TR1	TR2	TR3					
Dichlorvos	10	10			164.7							38.4					
Parathion methyl	10	20	0.521											2.80 ^b		3-4 ^c	
Pirimiphos methyl	10	10	0.26														
p,p'-DDE	50	50			0.645												
p,p'-DDD	50	50					0.609										
p,p'-DDT	50	50				0.575	0.503	0.669				0.671	0.669				
Endrin ketone	-	-									0.529						
Ethion	10	20															0.29-
Malathion	20	20															0.32 ^d
																	4.4 ^d

^a EU pesticides database

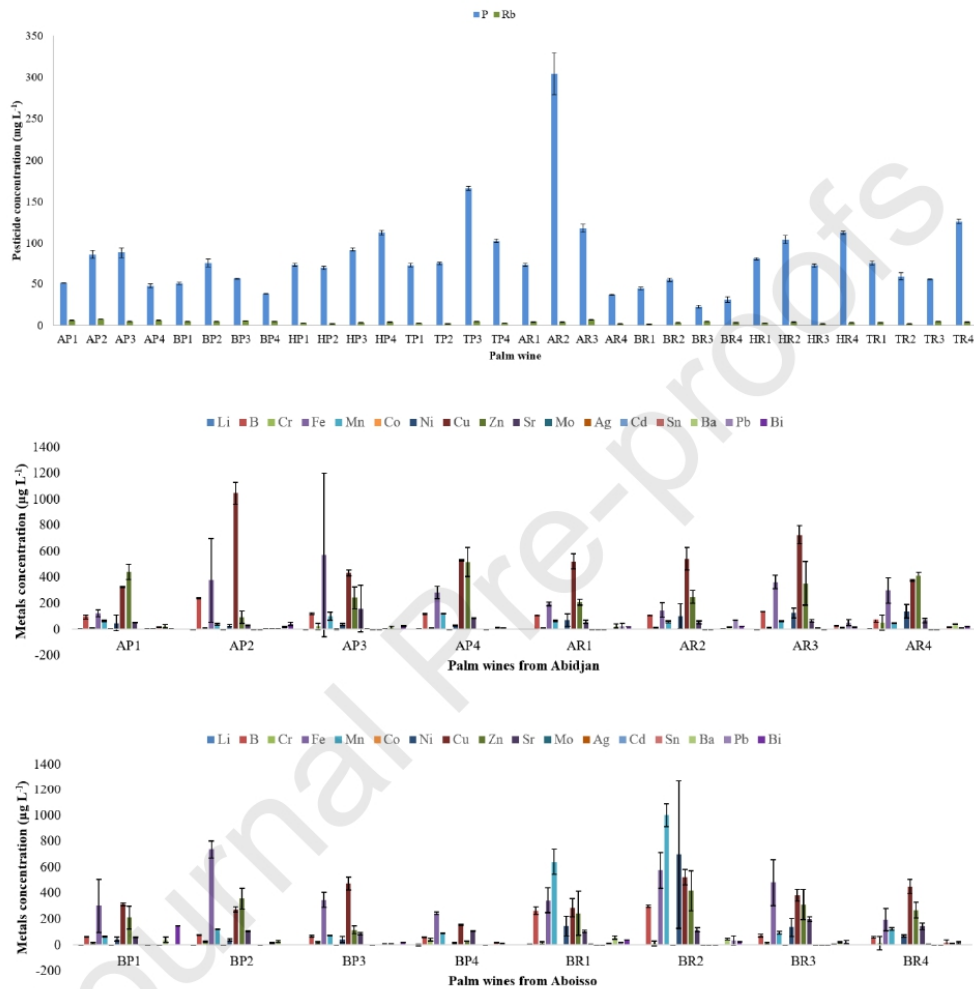
^b Santiago et al., 2021

^c Sala et al., 1997

^d dos Anjos & de Andrade, 2015

3.2 Determination of metals in palm wines

Thirty-two palm wine samples were mineralized and analyzed by ICP-AES and ICP-MS. The results are shown in Figure 2.



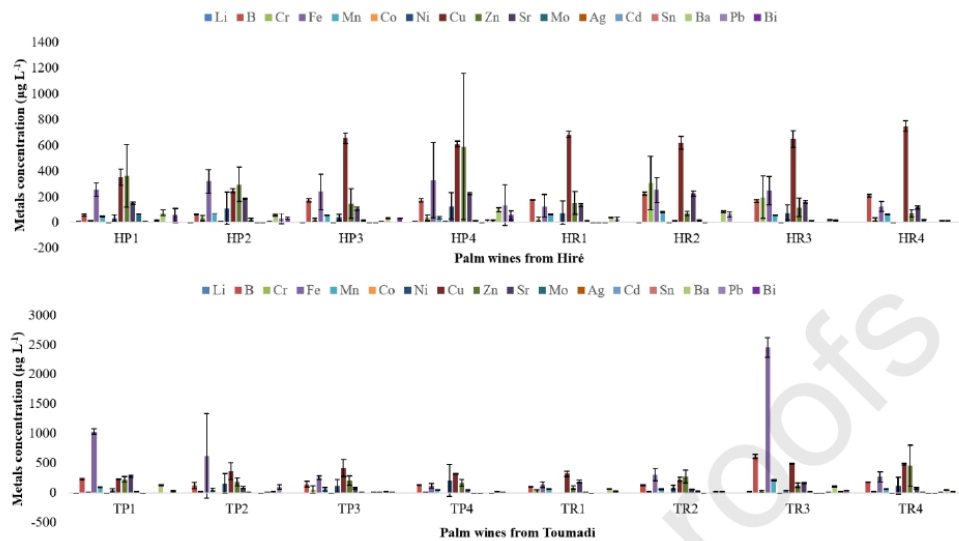


Fig. 2. Metals concentration in 32 palm wine samples, determined by ICP-AES and ICP-MS

The error bars on the graph can be important, because of the heterogeneity of the wine composition. Indeed, when an identical volume is taken, particles can be present, and those being certainly richer in metals, that can have an important role on the concentration of metals.

First, barium, boron, chromium, copper, iron, lead, manganese, phosphorus, rubidium, strontium, and zinc were found in all palm wine samples. The International Organization of Vine and Wine (OIV) recognized maximum limits for only a few elements in wine (Pb, Cd, Cu, Na, Zn, Ag, As, F, Br, F, and B). The levels of the other metals in palm wine, which is composed by 88% of water, can be compared with metal limits of drinking water. The higher concentrations for the most wine samples were found for phosphorus, rubidium, copper and iron, from 22.2 mg L⁻¹ (BR3) to 303.9 mg L⁻¹ (AR2) for phosphorus, from 1.5 mg L⁻¹ (BR1) to 7.6 mg L⁻¹ (AP2) for rubidium, from 155.6 (BP4) to 1046.9 µg L⁻¹ (AP2, this concentration was above the OIV maximal limit of 1000 µg L⁻¹) for copper and from 116.4 (TP4) to 2453.5 µg L⁻¹ (TR3) for iron. Manganese was mostly identified in samples from Aboisso, mainly in BR1

(641.6 $\mu\text{g L}^{-1}$) and BR2 (1001.3 $\mu\text{g L}^{-1}$) palm wines, at levels above the European maximal limits for drinking water.

Then, six metals (cadmium, chromium, copper, iron, manganese, and nickel) were detected in different samples at levels above the European maximal limits for drinking water or above the OIV maximal limits for wine. Cadmium was detected in TP3 (13.8 $\mu\text{g L}^{-1}$) at level above limits of 10 $\mu\text{g L}^{-1}$, and chromium in TP3 (51.4 $\mu\text{g L}^{-1}$), AR4 (52.2 $\mu\text{g L}^{-1}$), HR2 (313.4 $\mu\text{g L}^{-1}$), and HR3 (197.9 $\mu\text{g L}^{-1}$) at level above limits of 50 $\mu\text{g L}^{-1}$.

Iron was at level above limits of 200 $\mu\text{g L}^{-1}$ in 24 samples (AP2, AP3, AP4, AR1, AR2, AR3, AR4, BP1, BP2, BP3, BP4, BR1, BR2, BR3, BR4, HP1, HP2, HP3, HP4, HR1, HR2, HR3, HR4, TP1, TP2, TP3, TP4, TR1, TR2, TR3, and TR4), from 242.2 (HP3) to 2453.5 $\mu\text{g L}^{-1}$ (TR3), and nickel at level of 20 $\mu\text{g L}^{-1}$ in 28 samples too (AP1, AP2, AP3, AP4, AR1, AR2, AR3, AR4, BP1, BP2, BP3, BR1, BR2, BR3, BR4, HP1, HP2, HP3, HP4, HR1, HR2, HR3, HR4, TP1, TP2, TP3, TP4, TR1, TR2, TR3, and TR4), from 28.9 (AP4) to 699.8 $\mu\text{g L}^{-1}$ (BR2).

Zinc, which was detected at a range of concentrations between 28.6 and 589.9 $\mu\text{g L}^{-1}$, was also found in wines from different European countries, such as Italy or France (Table 4), at similar and higher levels (for example, between 289 and 967 $\mu\text{g L}^{-1}$ for Italian wines). As for Italian and French wines, zinc was not detected at levels above the OIV maximal limits for wine. Moreover, cadmium was detected in one sample (TP3) at concentration above the OIV maximal limit of 10 $\mu\text{g L}^{-1}$. However, its concentration in Italian and French wines was not at levels above the limits.

Metals, like copper, iron, and zinc, are essential for human at low concentrations. However, at important concentrations, they can pose a real danger. For example, drinking water contaminated by cadmium and lead can cause renal failure or chromium and nickel can induce hair loss (Singh Sankhla et al., 2016).

Finally, the palm wine TP3 from Toumodi was the most polluted sample with metals at levels above European and OIV maximal limits (cadmium, chromium, iron, manganese and nickel), given Table 4.

Table 4

KED, or STD mode, LODs and LOQs of metals in palm wines, European and OIV Maximal Limits of metal in drinking water and wine, and comparison with other studies

Metals	Mode	LOQ ($\mu\text{g L}^{-1}$)	LOD ($\mu\text{g L}^{-1}$)	European Maximal Limits in drinking water ($\mu\text{g L}^{-1}$)	OIV Maximal Limits ($\mu\text{g L}^{-1}$)	Concentrations in French wines (mg L^{-1}) ^a	Concentrations in Italian wines (mg L^{-1}) ^a	Concentrations in Italian wines ($\mu\text{g L}^{-1}$) ^b	This study ($\mu\text{g L}^{-1}$) or (mg L^{-1}) ^c
Barium	KED	1.5	0.45	-	-				3.65-132.4
Bismuth	STD	17	5.1	-	-				18.5-146.3
Boron	STD	4	1.2	1500	8000			1995-9489	55.7-614.4
Cadmium	STD	0.75	0.23	5	10	ND-0.0002	0.0012-0.0016	0.17-3.7	0.36-13.8
Chromium	KED	5	1.5	50	-	0.006-0.09	0.02-0.05		5.86-313.4
Cobalt	STD	0.5	0.15	-	-	0.004-0.005	0.003-0.006		0.52-7.35
Copper	KED	3	0.9	2000	1000	ND-0.48	0.001-1.34	14-456	155.6-1046.9
Iron	KED	16.5	4.95	200	-	0.81-2.51	1.35-27.8		116.4-2453.5
Lead	KED	8.5	2.5	10	150	0.006-0.023	0.01-0.35	3-59	0.38-135.4
Lithium	STD	0.25	0.08	-	-				0.33-18.0
Manganese	KED	1	0.3	50	-	0.63-0.96	0.67-2.5		33.2-1001.3
Molybdenum	KED	1.3	0.38	-	-				1.36-69.0
Nickel	KED	9.3	2.9	20	-	ND-0.052	0.015-0.21		12.9-699.8
Phosphorus	-	450	140	-	-				22.2-303.8 ^c

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Rubidium	-	80	20	-	-					1.52-7.62 ^c
Silver	KE	0.5	0.15	-	-					0.51-2.74
Strontium	D	2	0.6	-	-					33.2-281.7
Tin	KE	1.3	0.38	-	-					2.18-24.8
Zinc	D	12	3.6	-	5000	0.44-0.74	0.135-4.8	289-967		28.6-589.9

^a Plotka-Wasyłka et al., 2018

^b Caridi et al., 2019

4. Conclusion

In this study, the concentration of pesticides and metals in palm wines from Ivory Coast using HS-SPME/GC-MS and ICP-AES/MS were determined. SPME conditions (equilibrium time, extraction time and temperature, stirring, and salinity) were optimized thanks to an experimental design with 45 trials. The developed method used a DVB/C-WR/PDMS fiber and the palm wine samples were equilibrated for 11.25 min and extracted for 50 min under continuous stirring (437.5 rpm) at 52.5°C. The method was validated in terms of linearity, repeatability, LOD, LOQ, and accuracy, and allowed the identification of 35 pesticides and the quantification of 29 in 32 palm wine samples. Additionally, 7 pesticides were identified but dichlorvos was the only one identified in 2 samples at levels above the European maximal limits.

In a second step, ICP-AES and ICP-MS were used to study the presence of 19 metals in palm wine samples. Barium, boron, chromium, copper, iron, lead, manganese, rubidium, strontium, and zinc explored were identified in all samples of palm wine. Moreover, 6 metals (cadmium, chromium, copper, iron, manganese, and nickel) were detected in different samples at levels above the European maximal limits for drinking water or above the OIV maximal limits for wine.

Finally, pesticides (dichlorvos, endrin ketone, parathion methyl, p,p-DDD, p,p-DDE, p,p-DDT, and pirimidiphos methyl) were found in palm wines from the 4 cities, in 3 samples from Abidjan,

1 from Aboisso, 1 from Hiré, and 5 from Toumodi. In addition, one of the palm wine samples from Toumodi was the most polluted sample with metals (cadmium, chromium, iron, manganese and nickel) at levels above European or OIV maximal limits.

Maximal limits for metals have been only defined for boron, cadmium, copper, lead and zinc. As shown in this article, samples may contain pesticides and metals in high concentrations. This beverage is largely consumed by local population. It would be important to develop more studies on the composition of palm wine and maximal limits especially for pesticides in wines, in order to be aware of the potential danger of its consumption.

List of abbreviations

PA: polyacrylate

BHC: Hexachlorocyclohexane

Carbon WR: Carbon Wide Range

DVB: Divinylbenzene

EML: European Maximal Limits

GC: Gas Chromatography

GC-MS: Gas Chromatography coupled with Mass Spectrometry

HS-SPME: Headspace Solid-Phase Microextraction

ICP-AES: Inductively Coupled Plasma - Atomic Emission Spectrometry

ICP-MS: Inductively Coupled Plasma - Mass Spectrometry

KED: Kinetic Energy Discrimination mode

LOD: limit of detection

Log Kow: octanol-water partition coefficient

LOQ: limit of quantification

MS: mass spectrometry

OCPs: OrganoChloride Pesticides

OIV: International Organization of Vine and Wine

OPPs: OrganoPhosphorus Pesticide

PDMS: Polydimethylsiloxane

p,p'-DDD: Dichlorodiphenyldichloroethane

p,p'-DDE: Dichlorodiphenyldichloroethylene

p,p'-DDT Dichlorodiphenyltrichloroethane

RSD: relative standard deviation

SIM: Single Ion Monitoring

STD: Standard mode

SPME: Solid-Phase Microextraction

Authors' contributions

Juliette Vievard wrote the paper, performed the analysis, collected the data

Theodore N'Dede Djeni wrote the paper, gave samples

Christine Devouge-Boyer contributed to data and analysis

Monica Arellano Gisel Sanchez wrote the paper

Aké Francine collected the samples

Tiemele Laurent Simon Amoikon wrote the paper, gave samples

Aminata Nadjoua Coulibaly, wrote the paper, collected the samples

Mélanie Mignot oversight the study, reviewed the paper

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Authors' contributions

Juliette Vievard wrote the paper, performed the analysis, collected the data

Theodore N'Dede Djéni wrote the paper, gave samples

Christine Devouge-Boyer contributed to data and analysis

Monica Arellano Gisel Sanchez wrote the paper

Aké Francine collected the samples

Tiemele Laurent Simon Amoikon wrote the paper, gave samples

Aminata Nadjoua Coulibaly, wrote the paper, collected the samples

Mélanie Mignot oversight the study, reviewed the paper

Highlights

- A HS-SPME/GC-MS method was performed to analyse 35 pesticides from various families
- 19 metals were mineralized and analyzed by ICP/AES and ICP/MS in palm wines
- One pesticide and 6 metals were identified in samples at levels above maximal limits

Abstract

Leather industry plays an essential role in global economy but it may have a negative impact on the environment and health. Indeed, the vast majority of hides are treated with trivalent chromium (Cr(III)) salts and, under some conditions, it can be converted into hexavalent chromium (Cr(VI)). While Cr(III) is essential for glucose regulation, Cr(VI) is toxic, hence the importance of quantifying both species separately. In aqueous systems, the oxidation state of chromium is determined by redox potential and pH, which poses a difficulty since sample preparation and analysis protocols may lead to interconversions between Cr(III) and Cr(VI), thus biasing the results. The goal of this thesis was to establish a robust protocol for extraction and quantification of Cr(III) and Cr(VI) in leather samples while avoiding interconversions.

Résumé

L'industrie du cuir joue un rôle essentiel dans l'économie mondiale mais elle peut avoir un impact négatif sur l'environnement et la santé. En effet, la grande majorité des peaux sont traitées avec des sels de chrome trivalent (Cr(III)) et sous certaines conditions, celui-ci peut se convertir en chrome hexavalent (Cr(VI)). Alors que le Cr(III) est essentiel à la régulation du glucose, le Cr(VI) est toxique, d'où l'importance de quantifier les deux espèces séparément. Dans les systèmes aqueux, l'état d'oxydation du Cr est déterminé par le potentiel redox et le pH, ce qui pose une difficulté puisque la préparation des échantillons et les protocoles d'analyse peuvent conduire à des interconversions entre le Cr(III) et le Cr(VI), biaisant ainsi les résultats. L'objectif de cette thèse a été de mettre en place un protocole d'extraction et de quantification robuste du Cr(III) et Cr(VI) dans des cuirs, tout en évitant les interconversions.