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Développement de méthodes d'extraction et d'analyse multi-résidus pour le suivi de contaminants organiques polyaromatiques et de métabolites oxygénés dans les sédiments

Ingrid Brito-Berger

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Normandie Université

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Pour obtenir le diplôme de doctorat

Spécialité Chimie

Préparée au sein de l'Université de Rouen Normandie

Développement de méthodes d'extraction et d'analyse multi-résidus pour le suivi de contaminants organiques et de métabolites oxygénés dans les sédiments.

Présentée et soutenue par

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Normandie Université

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Développement de méthodes d'extraction et d'analyse multi-résidus pour le suivi de contaminants organiques et de métabolites oxygénés dans les sédiments.

Résumé

Dans ce travail, deux méthodes d'extraction multi-résidus de contaminants présents dans des sédiments ont été développées. Dans la première partie de cette étude, une méthode a été développée pour l'extraction simultanée de deux familles de métabolites oxygénés d'hydrocarbures aromatiques polycycliques (HAP), les quinones et les HAP hydroxylés (OH-HAP). Une approche chimiométrique a permis de déterminer les paramètres influant sur l'extraction assistée par micro-ondes (MAE) et une zone de compromis a été trouvée pour extraire de manière optimale les deux familles de composés. Deux méthodologies d'analyses chromatographiques ont été développées et validées pour analyser les extraits, puis comparées, à savoir la chromatographie liquide haute performance couplée aux détections UV et fluorimétrique (HPLC-UV-Fluo) et la chromatographie en phase gazeuse couplée à un spectromètre de masse par impact électronique (CPG-SM). En CPG-SM, des réactions de silylation des OH-HAP et d'acétylation des quinones ont dû être mises au point, afin d'abaisser les limites de détection (LD), en particulier pour les ortho-quinones. En HPLC-UV-Fluo, les LD étaient plus faibles qu'en CPG-SM, surtout pour les OH-HAP détectés en Fluo et l'analyse était plus rapide, sans processus de dérivation; mais la détection n'étant pas sélective, l'identification des analytes s'est avérée hasardeuse. Le choix s'est donc porté sur la CPG-SM pour une analyse plus fiable des deux familles de composés de matrices sédimentaires naturellement contaminées. Dans la deuxième partie de ce travail de thèse, une nouvelle méthodologie d'extraction a été développée et validée, basée sur la dispersion en phase solide de la matrice solide (MSPD), capable d'extraire mais aussi de purifier l'échantillon, méthodologie par ailleurs simple et rapide. Deux familles de composés ont été extraits simultanément à partir de sédiments, les HAP et les polychlorobiphényles (PCB). Un certain nombre de paramètres ont été optimisés, tels la nature des agents dispersants, le temps de broyage, le volume et la nature du mélange de solvants d'élution. Dans un deuxième temps, l'introduction des OH-HAP dans le processus analytique a amené à coupler à la MSPD une autre méthode d'extraction/purification beaucoup plus sélective, basée sur les polymères à empreintes moléculaires (MIP). En effet, les interférents polaires, restés piégés par l'agent dispersant polaire dans la première cartouche contenant le sédiment broyé, devaient être élués afin de libérer les OH-HAP, qui à leur tour devaient être retenus sélectivement dans un MIP empreint pour les phénols, pour fournir une élution finale exempte d'autres composés. Il a été montré que ces MIPs pouvaient extraire sélectivement les OH-HAP de faible et de haut poids moléculaire, mais il fallait choisir soigneusement le solvant de percolation pour ne pas endommager le polymère. Cependant, la difficulté principale a été de désorber les OH-HAP fortement retenus par le sédiment par liaison hydrogène. Cela a pu être réalisé pour les OH-HAP légers, en utilisant un mélange de solvants avec un effet de relargage par un sel, mais pas pour les OH-HAP lourds, trop fortement adsorbés sur la matrice sédimentaire. Par ailleurs, il a fallu utiliser une grande quantité de polymère à empreinte moléculaire à cause de la compétition pour les sites de reconnaissance entre les OH-HAP et des composés phénoliques.

Mots clés

Sédiment; HAP; PCB; quinones; HAP hydroxylés; Chromatographies en phase liquide et gazeuse ; Dérivation ; Extraction assistée par micro-ondes; Dispersion de la matrice en phase solide; Polymères à empreintes moléculaires.

Development of multiresidual extractions and analytical methodologies for polyaromatic organic contaminants and oxygenated metabolites in sediments.

Summary

In this work two multiresidual methods for extracting contaminants from sediments were developed. In the first part of this study, a method was developed for extracting simultaneously two groups of oxygenated metabolites of polycyclic aromatic hydrocarbons (PAHs), quinones and hydroxylated PAHs (hydroxy-PAHs). A chemometric approach allowed us to determine the influential parameters on microwave assisted extraction (MAE), and a compromise could be found for extracting quantitatively both families of compounds. Two chromatographic analytical methodologies were developed and validated for analysing the extracts: high performance liquid chromatography coupled with fluorimetric and ultraviolet detection (HPLC-UV-FLD) and gas chromatography coupled with an electronic impact mass spectrometer (GC-MS). Using GC-MS, reactions of silylation of hydroxy-PAHs and of acetylation of quinones had to be developed, to decrease detection limits (LOD), particularly for ortho-quinones. Using HPLC-UV-FLD, LODs were lower than using GC-MS, particularly for hydroxy-PAHs detected by FLD, and the analysis was faster, without derivatization; but the detectors were not selective, and identification of analytes was doubtful. Choice was done to favour GC-MS for a more reliable analysis of the two families of compounds extracted from naturally contaminated sediments. In the second part of this thesis work, a new fast and simple extraction methodology was developed and validated, based on matrix solid phase dispersion (MSPD), capable of extracting and purifying simultaneously sediment samples. Two families of compounds were simultaneously extracted from sediments, PAHs and polychlorobiphenyls (PCBs). Many parameters were optimized, as the nature of dispersing agents, the time of grinding, the volume and nature of elution solvent mixtures. In a second step, hydroxy-PAHs were introduced in the analytical process, which led us to add another more selective extraction/purification method to MSPD, based on molecularly imprinted polymers (MIPs). Indeed polar interfering compounds, trapped by the polar dispersant in the first cartridge containing the blended sediment, had to be eluted to release hydroxy-PAHs, which in turn had to be selectively retained by the polymer, imprinted for phenols, to provide a final eluate free from other polar compounds. It was demonstrated that those MIPs could selectively extract low and high molecular weight hydroxy-PAHs, but appropriate percolating solvents had to be chosen to avoid polymer damages. However, the main difficulty was to desorb hydroxy-PAHs strongly retained by the sediment matrix through hydrogen bonds. It could be achieved for light hydroxy-PAHs, using a mixture of eluting solvents with salting-out effect, but not for heavy hydroxy-PAHs which stayed strongly sorbed on the sediment matrix. Furthermore we needed to use high amounts of imprinted polymer because of the competition for recognition sites between hydroxy-PAHs and phenolic compounds.

Key words

Sediment; PAHs; PCBs; quinones; hydroxylated PAHs; high performance liquid chromatography and gas chromatography; Derivatization; Microwave assisted extraction; Matrix solid phase dispersive extraction; Molecularly imprinted polymers.

Abbreviations/ Nomenclature

9,10-AQ	9,10-Anthracenequinone
ASE	Assisted Solvent Extraction
ATSDR	Agency for Toxic Substances and Disease Registry
BaA	Benz[<i>a</i>]anthracene
BaP	Benzo[<i>a</i>]pyrene
BbF	Benzo[<i>b</i>]fluoranthene
BCR	European Union Community Bureau of Reference
BkF	Benzo[<i>k</i>]fluoranthene
1,4-BQ	1,4-Benzoquinone
BSTFA	N,O-bis(trimethylsilyl)trifluoroacetamide
BSA	N,O-bis(trimethylsilyl)acetamide
C18	Octadecylsilyl silica
CCD	Central Composite Design
CMC	Critical Micelle Concentration
CYP-450	Cytochrome P-450
d_p	diameter
DAD	Diode Array Detector
DBOFB	4,4'-dibromooctafluorobiphenyl
DDT	Dichlorodiphenyltrichloroethane
DNA	Deoxyribonucleic acid
ECD	Electron Capture Detector
EI	Electron Impact
EPA	Environmental Protection Agency
EU	European Union
FDA	Food and Drug Administration
FLD	Fluorescence
FID	Flame Ionization
FMASE	Focused Microwave-Assisted Soxhlet Extraction
GC	Gas Chromatography
GPC	Gel Permeation Chromatography

HMW	High molecular weight
HO•	Hydroxyl Radicals
HPLC	High Performance Liquid Chromatography
HPSE	High-Pressure Soxhlet Extraction
HRMS	High Resolution Mass Spectrometer
K_{ow}	Octanol-water partition coefficient
L	Liter
LC-APPI-MS	Liquid Chromatography-Atmospheric Pressure Photoionization-Mass Spectrometry
LLE	Liquid-Liquid Extraction
LOD	Limits of detection
LOQ	Limits of quantification
MAE	Microwave Assisted Extracton
MA-MSPD	Magnetically Assisted Matrix Solid Phase Dispersion
mg	Miligram
MI-MSPD	Molecular Imprinted Matrix Solid-Phase Dispersion
MIPs	Molecularly Imprinted Polymers
MI-SBSE	Molecular Imprinted Stir-Bar Sorptive Extraction
MI-SPE	Molecularly Imprinted SPE
MI-SPME	Molecular Imprinted Solid-Phase Microextraction
MS	Mass Spectrometry
MSPD	Matrix Solid Phase Dispersion
MSTFA	N-methyl-N-(trimethylsilyl)trifluoroacetamide
MTBSTFA	N-tertbutyldimethylsilyl-N-methyltrifluoroacetamide
MW	Molecular Weight
NIOSH	National Institute for Occupational Safety and Health
nMIP	Nano-Sized Molecularly Imprinted Polymers
NIST	National Institute of Standards and Technology
1,2-NQ	1,2-Naphthoquinone
1,4-NQ	1,4-Naphthoquinone
$O_2^{\bullet-}$	Superoxide Anion Radicals
2-OHFluo	2-Hydroxyfluorene
2-OHNaph	2-hydroxynaphthalene or 2-naphtol
OH-PAHs	Hydroxy-PAHs or hydroxylated PAHs

9-OHPhen	9-hydroxyphenanthrene or 9-Phenanthrol
1-OHpyr	1-Hydroxy pyrene
OM	Organic Matter
Oxy-PAHs	Oxygenated PAHs
<i>P.</i>	<i>Pseudomonas</i>
PAHs	Polycyclic Aromatic Hydrocarbons
PBDEs	Polybrominated Diphenyl Ethers
PCBs	Polychlorinated Biphenyls
PCDDs	Dibenzo- <i>p</i> -dioxins
PCDFs	Dibenzo-furans
PerD12	Perylene D12
PFE	Pressurized Fluid Extraction
PhenD10	Phenanthrene D10
PHSE	Pressurized Hot-solvent Extraction
PHWE	Pressurized Hot-water Extraction
PLE	Pressurized Liquid Extraction
PM	Particulate material
POP	Persistent organic pollutants
9,10-PQ	9,10-Phenanthrenequinone
QuEChERS	Quick, Easy, Cheap, Effective, Rugged, and Safe
<i>R.</i>	<i>Rhodococcus</i>
RESS	Rapid Expansion of Supercritical Solvent
RNA	Ribonucleic acid
ROS	Reactive oxygen species
RSD	Relative Standard Deviations
RSM	Response Surface Methodology
SCF	Scientific Committee for Food
SDS	Sodium Dodecyl Sulfate
SFE	Supercritical Fluid Extraction
SFEE	Supercritical Fluid Extraction of Emulsions
SFG	Supercritical Fluid Chromatography
SIM	Selected Ion Monitoring
SPLE	Selective Pressurized Liquid Extraction
SHWE	Superheated Water Extraction

S/N	Signal-to-Noise
SPE	Solid Phase Extraction
SWE	Subcritical Water Extraction
TBSCl	Tert-butyldimethylchlorosilane
TEF	Toxic Equivalent Factor
TFA	Trifluoroacetic Acid
TMAB	Tetramethyl Ammonium Bromide
TMCS	Trimethylchlorosilane
TOF	Time-of-Flight
UASE	Ultrasound-Assisted Soxhlet Extraction
UPLC	Ultra-Performance Liquid Chromatography

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Introduction générale - General introduction

La notion de pollution de l'environnement correspond à l'introduction dans différents compartiments environnementaux (air, eau, sols...) de substances ou de nuisances ayant un impact négatif sur les organismes vivants et d'une manière générale sur les écosystèmes. Les contaminations peuvent être d'origine naturelle (feux, volcans...) mais elles sont, de nos jours, principalement liées aux activités humaines (origine anthropique). Parmi les substances induisant des effets négatifs, certaines sont particulièrement préoccupantes, de par leur toxicité à faible dose, leur persistance et leur dissémination dans plusieurs compartiments de l'environnement. Parmi les contaminants organiques, les polychlorobiphényles (PCB) font par exemple partie d'une liste de polluants organiques persistants (POP), établie suite à la convention de Stockholm de mai 2001, polluants devant être très restreints voire éliminés de par leur dangerosité. Les hydrocarbures aromatiques polycycliques (HAP) ont également été répertoriés en tant que POP par le protocole d'Aarhus de 1998. Malheureusement, si un certain nombre de polluants a été identifié depuis quelques décennies et a un statut réglementaire bien défini (comme par exemple le règlement européen 850/2004), un nombre croissant de contaminants peut être qualifié de « polluants émergents » : ces polluants émergents ont été plus récemment identifiés et les données sur leur présence et leurs effets sur les écosystèmes sont parcellaires. Parmi les polluants émergents apparaissent de plus en plus les produits de dégradation des POP, obtenus suite à des voies de dégradation naturelles (photodégradation, biodégradation...) ou suite à des traitements de remédiation de la pollution (bioremédiation augmentée, électroremédiation...). Les comportements environnementaux des produits de transformation des POP et leur impact écotoxicologique sont peu connus et parfois très différents de ceux des composés parents, car ils ont subi d'importantes modifications structurales. Il s'avère donc nécessaire de détecter, d'identifier et de doser ces polluants issus de processus de dégradation, dans des environnements variés, mais aussi dans des mélanges variables.

Si les polluants émergents sont actuellement plus recherchés dans les milieux aquatiques, car ils sont susceptibles d'avoir un impact direct sur la faune aquatique de par leur bioaccessibilité, il s'avère que d'autres compartiments environnementaux sont tout aussi préoccupants. Les sédiments constituent en particulier un milieu solide particulière complexe susceptible de stocker à plus ou moins long terme un certain nombre de POP, mais également de les transformer par des processus biogéochimiques (diagénèse). Mais ce piégeage n'est pas définitif car en cas de perturbation des systèmes (crues, dragages, arasement de barrages...), les

POP accumulés dans les sédiments, ou leurs produits de dégradation, peuvent à nouveau être remis en suspension en milieu aqueux et ainsi constituer des sources continues de pollution aquatique, contrariant ainsi les efforts réalisés pour atteindre le bon état des milieux aquatiques. Par ailleurs, il ne faut pas négliger le développement de nouvelles méthodes de remédiation de la pollution, visant par exemple à décontaminer les sédiments de dragage afin de pouvoir les valoriser ultérieurement. Si ces méthodes ne sont pas encore utilisées à l'échelle industrielle, il faudra pourtant s'assurer à l'avenir que les processus de décontamination mettant en œuvre des réactions d'oxydoréduction (procédé Fenton, électroremédiation...) ne conduiront pas à la formation de substances dans les sédiments encore plus toxiques que les composés parents.

Dans ce contexte, les études menées dans ces travaux de thèse ont visé à mettre au point de nouvelles méthodes d'extraction et d'analyse pour le suivi de contaminants organiques polyaromatiques et de certains de leurs métabolites oxygénés dans des sédiments. Il s'agissait tout particulièrement de développer des méthodes d'analyses multi-résidus, c'est-à-dire permettant une analyse simultanée de plusieurs familles de contaminants, si possible faciles et rapides à mettre en œuvre.

Une étude bibliographique, développée dans le chapitre I, permet dans un premier temps de comprendre les propriétés physico-chimiques, les sources et la dissémination dans l'environnement, ainsi que la toxicité des contaminants ciblés, à savoir :

- les PCB et les HAP en tant que POP ;
- et certains produits de transformation des HAP, en tant que polluants émergents, à savoir les HAP hydroxylés (OH-HAP) et des HAP carbonylés (de type quinones).

Le chapitre I permet également de compiler les différentes techniques d'extraction et d'analyse existantes visant à séparer, identifier et doser ces contaminants dans des matrices solides particulaires.

Dans le chapitre II est exposé le développement d'une méthode permettant d'extraire simultanément deux familles de HAP oxygénés (oxy-HAP), à savoir les OH-HAP et des HAP carbonylés (plus particulièrement les quinones), puis de les analyser par deux voies possibles : la chromatographie en phase gazeuse couplée à la spectrométrie de masse (CPG-SM) ou la chromatographie liquide haute performance couplée aux détecteurs UV et fluorimétrique (HPLC-UV-Fluo). La première partie du chapitre expose la mise au point et la validation des méthodes d'analyses chromatographiques, avec un focus particulier sur l'amélioration des méthodes de dérivation des oxy-HAP pour leur analyse en CPG-SM, à savoir : la silylation des OH-HAP et l'acétylation des quinones. La deuxième partie du chapitre II traite de la mise au point de la technique d'extraction multi-résidus, par extraction assistée par micro-ondes ou

MAE (microwave-assisted extraction), à partir d'une matrice sédimentaire dopée. L'optimisation est réalisée par chimiométrie : un premier plan d'expérience doit permettre de déterminer les facteurs influant sur l'extraction MAE, un deuxième plan d'expérience doit permettre de trouver le meilleur compromis pour une extraction optimale des deux familles d'oxy-HAP. Enfin, la méthode analytique complète est appliquée à des sédiments naturellement contaminés en HAP, donc susceptibles de contenir des oxy-HAP, et une discussion suit sur l'emploi de la meilleure méthode analytique, en fonction de sa rapidité et de sa fiabilité.

Le chapitre III décrit quant à lui la mise au point en deux temps d'une méthode d'extraction multi-résidus, rapide et très facile à mettre en œuvre, à savoir l'extraction par dispersion de la matrice solide ou MSPD (matrix solid phase dispersive extraction). Dans une première partie du chapitre III, est décrite l'optimisation de la technique d'extraction et de purification par MSPD pour deux familles de contaminants, à savoir les HAP et les PCB. La MSPD est ensuite validée et appliquée à un sédiment certifié et comparée à la MAE, permettant ainsi de dégager les avantages de la MSPD en termes de taux de recouvrement, de rapidité de mise en œuvre et d'économies. Dans un deuxième temps, cette technique d'extraction est étendue aux OH-HAP. Mais il est nécessaire d'employer pour cela une méthodologie couplée à la MSPD afin d'extraire sélectivement les OH-HAP parmi d'autres composés oxygénés non désirés, à savoir l'extraction sur phase solide employant des polymères à empreintes moléculaires ou MIP (molecularly imprinted polymers).

Une conclusion générale est enfin exposée, résumant les principales avancées de ces travaux, et les perspectives envisagées.

Chapter I

**Analytical tools for analyzing oxygenated PAHs (hydroxy-,
carbonyl-) from environmental particulate matter**

This first chapter brings general information about the environmental contaminants studied in this thesis, including polycyclic aromatic hydrocarbons (PAHs), polychlorobiphenyls (PCBs), but more particularly oxygenated PAHs (oxy-PAHs), among which quinones (carbonyl-PAHs) and hydroxylated PAHs (hydroxy-PAHs). Their sources and toxicity, their structure and chemical properties are presented, showing the importance of studying them in the environment, particularly in sediments. The extraction processes from solid matrices and the chromatographic techniques that are used for identifying and quantifying them are presented, emphasizing the new ones that will be developed in this study.

I.1 Polycyclic aromatic compounds and oxygenated derivatives

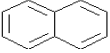
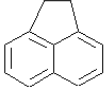
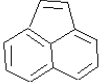
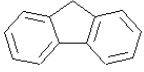
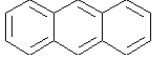
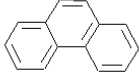
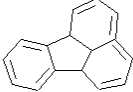
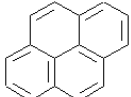
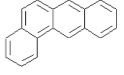
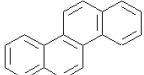
I.1.1 Polycyclic aromatic compounds

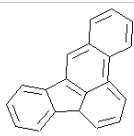
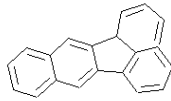
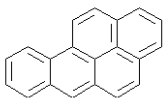
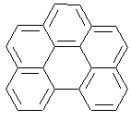
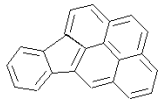
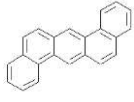
I.1.1.1 Polycyclic aromatic hydrocarbons (PAHs)

a. Structure and chemical properties

Polycyclic aromatic hydrocarbons (PAHs) are toxic ubiquitous environmental contaminants, composed of two or more fused aromatic rings in linear, angular or cluster arrangements^{1, 2}. These polyaromatic compounds are chemically very stable and are difficult to degrade or biodegrade. There are thousands of PAHs in the environment, although only some of them are monitored by agencies as US Environmental Protection Agency (US-EPA), Agency for Toxic Substances and Disease Registry (ATSDR), European Union (EU), EU Scientific Committee for Food (SCF) (Table I.1)³⁻⁵, Agency for Toxic Substances and Disease Registry⁶. Among the chemical-physical parameters concerning PAHs, the octanol-water partition coefficient (K_{ow}) is very important to predict the sorbed amount of these nonpolar organic compounds in sediments, soils or dissolved organic matter, but also their bioaccumulation in organisms⁷. Indeed substances with high $\log K_{ow}$ values, that are also stable and persistent such as PAHs, tend to sorb and accumulate in the organic matter (OM) of sediments and soils, because of their low affinity with water⁸. Heavier molecular weight (HMW) PAHs have higher $\log K_{ow}$ values (Table I.1), showing their tendency to adsorb more in sediment matrices, making them more difficult to extract. PAHs have very low water solubility and with the increase of the number of aromatic rings, this factor decreases (Table I.1). All PAHs have also high boiling points and very low vapor pressures (Table I.1), which can partially explain their persistence in soils and sediments⁹.

Table I.1: General information about the 16 priority PAHs, monitored by the US Environmental Protection Agency (US EPA), Agency for Toxic Substances and Disease Registry (ATSDR), European Union (EU) and EU Scientific Committee for Food (SCF) ¹⁰⁻¹²

Monitoring Institutes	Molecular structure	Formula	Mole Weight (g mol ⁻¹)	Boiling Point (°C)	Log K _{ow} ¹³	Aqueous Solubility (g·L ⁻¹) at 25°C	Vapor Pressure (atm) at 25°C
EPA	 Naphthalene	C ₁₀ H ₈	128	218	3.3	3.2 x 10 ⁻²	1.1 x 10 ⁻⁴
EPA ATSDR	 Acenaphthene	C ₁₂ H ₁₀	154	279	3.9	3.9 x 10 ⁻³	3.3 x 10 ⁻⁵
EPA ATSDR	 Acenaphthylene	C ₁₂ H ₈	152	280	4.1	1.6 x 10 ⁻²	1.2 x 10 ⁻⁶
EPA ATSDR	 Fluorene	C ₁₃ H ₁₀	166	295	4.2	1.9 x 10 ⁻³	8.3 x 10 ⁻⁶ to 1.1 x 10 ⁻⁵
EPA ATSDR	 Anthracene	C ₁₄ H ₁₀	178	342	4.5	4.3 x 10 ⁻⁵ to 7.5 x 10 ⁻⁵	3.5 x 10 ⁻⁹
EPA ATSDR	 Phenanthrene	C ₁₄ H ₁₀	178	340	4.6	1.2 x 10 ⁻³	1.5 x 10 ⁻⁷
EPA ATSDR	 Fluoranthene	C ₁₆ H ₁₀	202	384	5.2	2.6 x 10 ⁻⁴	1.6 x 10 ⁻¹¹
EPA ATSDR	 Pyrene	C ₁₆ H ₁₀	202	404	5.2	1.4 x 10 ⁻³	3.2 x 10 ⁻⁹ to 6.0 x 10 ⁻⁹
EPA ATSDR EU SCF	 Benz(a)anthracene	C ₁₈ H ₁₂	228	438	5.7	9.4 x 10 ⁻⁶	1.4 x 10 ⁻¹⁰ to 3.9 x 10 ⁻¹¹
EPA ATSDR EU SCF	 Chrysene	C ₁₈ H ₁₂	228	448	5.8	2.0 x 10 ⁻⁶ to 6.3 x 10 ⁻⁶	8.2 x 10 ⁻¹²

EPA ATSDR EU SCF	 Benzo(b)fluoranthene	C ₂₄ H ₁₄	252	481	6.4	1.5 x 10 ⁻⁶	6.6 x 10 ⁻¹⁰
EPA ATSDR EU SCF	 Benzo(k)fluoranthene	C ₂₄ H ₁₄	252	480	6.4	8.0 x 10 ⁻⁷	2.6 x 10 ⁻¹²
EPA ATSDR EU SCF	 Benzo(a)pyrene	C ₂₀ H ₁₂	252	496	6.2	1.6 x 10 ⁻⁶	7.2 x 10 ⁻¹²
EPA ATSDR EU SCF	 Benzo(g,h,i)perylene	C ₂₂ H ₁₂	276	550	6.6 ¹⁴	2.6 x 10 ⁻⁸	1.3 x 10 ⁻¹³
EPA ATSDR EU SCF	 Indeno(1,2,3 - c,d)pyrene	C ₂₂ H ₁₁	276	536	6.7	2.2 x 10 ⁻⁸	1.3 x 10 ⁻¹³
EPA ATSDR EU SCF	 Dibenzo(a,h)anthracene	C ₂₂ H ₁₄	278	524	6.5	2.5 x 10 ⁻⁶	1.3 x 10 ⁻¹¹

b. Sources and occurrence in the Environment

PAHs are produced by all the processes of incomplete combustion of organic substances, like incomplete combustion of fossil fuels, industrial processes (coke and carbon production, petroleum processing, aluminum sintering, among others), heating of residences (furnaces, fireplaces and stoves, gas and oil burners), cooking of meat (grill, barbecue), power and heat generation (coal, oil, wood and peat power plants), modes of transport (gasoline and Diesel engines), incineration of wastes. Natural sources as forest fires and volcano eruptions are also responsible of PAH emissions, but the emissions by these sources are not so significant than anthropogenic sources^{2, 15, 16}. PAHs produced by all the sources mentioned previously are pyrogenic PAHs, produced at high temperatures and are generally emitted in the atmosphere, before their deposition onto solid surfaces such as soils or sediments. But some PAHs found in the environment are from petrogenic or diagenic sources (natural processes of diagenesis or biosynthesis): they have been produced at low temperatures and are generally directly found in soils or sediments. Some PAHs are emitted by petroleum leaks from industrial accidents, or accidents in the transport of petroleum¹⁷. Some PAHs can be found in high concentrations in

sites contaminated by coal tar and creosote¹⁸. Some PAHs are also produced commercially for specific uses, as acenaphthene, acenaphthylene, anthracene, phenanthrene, naphthalene, fluoranthene and pyrene. In the case of naphthalene, it is the only one used directly, as moth repellent¹⁹.

After being emitted in the atmosphere, PAHs can travel long distances before being deposited to soils or sediments, water or vegetation². Most of the PAHs are present in the air in the particulate phase, particularly the heavier ones having molecular weights (MW) ≥ 202 g mol⁻¹ and very low vapor pressures (Table I.1). They are attached with breathing particles with an aerodynamic diameter smaller than 2.5 μ m. But some PAHs can be found also in the gaseous phase, particularly the lighter ones having MW ≤ 202 g mol⁻¹ and having the higher vapor pressures (Table I.1)^{1,20}. The deposition of atmospheric PAHs onto soils or sediments can be made by dry (fall of agglomerated dust) or wet (rain, snow) mechanisms²¹.

PAHs are persistent because of their polyaromatic stable structures and they can be strongly adsorbed in the organic matter (humus) layer of sediments and soils²². With the increase of the number of aromatic rings (about 4 or 5), lipophilicity and environmental persistence increase, so they can accumulate¹⁷. Thus they can be found at very high concentrations, of hundreds mg kg⁻¹, in soils of industrialized countries²¹. PAHs are adsorbed to the particular matter more easily than in aqueous media, thanks to their low solubility, that can lead to a concentration 1000 times higher in sediments than in the water column²³. Hydrophobic compounds of low volatility such as PAHs make aqueous leaching and volatilization not effective for their dissipation in sediments and soils^{22,23}. They continue to be buried in the sediments until some process can transfer or transform them: hence they can be transferred to living organisms (microbes, fungi, animals or plants) where they can more or less bio-accumulate^{24,25}; they can also be re-suspended in the water column after dredging operations or erosion processes; ultimately they can be degraded through photo-oxidation, chemical oxidation or microbial degradation^{26,27}.

c. Toxicity of PAHs

PAHs are regarded as priority pollutants by agencies such as the US-EPA and the European Environmental Agency, because of their toxic properties associated to their persistence in the environment^{17,28}. Benzo[a]pyrene (BaP) is classified by the International Agency for Research on Cancer (IARC) in Group 1 (carcinogenic for humans) and benz[a]anthracene (BaA)²⁹, benzo[b]fluoranthene (BbF) and benzo[k]fluoranthene (BkF) in Group 2B (possible carcinogens)³⁰. Among all the PAHs, BaP is one of the most measured and studied due to its carcinogenic properties and its high toxic equivalent factor (TEF)¹. In the case of PAHs, TEFs

measure toxicity relative to that of BaP and may vary from 0.001 to 1. BaP have the highest value among them, of 1. It is also the case for dibenz[a, h]anthracene.

In the case of human beings, they can be exposed to PAHs through respiratory tract, dermal contact and food ingestion. The ingestion of contaminated food (as mollusks, crustaceans, oil, margarine, grilled, smoked or barbecued meat) is the major source of contamination for humans that lead to a regulation by the European Commission of the level of PAHs that are permitted in certain foods, in areas with a high level of contamination^{15, 31}. After they are inside the human body, they are metabolized by the family of cytochrome P-450 (CYP-450) enzymes, transformed in more polar compounds such as hydroxylated PAHs, which can be bound to DNA (as adducts) in the body^{16, 32}. This process can be the beginning of carcinogenesis. In human body, PAHs are metabolized faster than some other polyaromatic contaminants, as polychlorinated biphenyls (PCBs)³³. Some of the metabolized and un-metabolized PAHs could be detected in human urine, and also in feces¹⁶.

I.1.1.2 Polychlorinated Biphenyls (PCBs)

a. Structure and chemical properties

Persistent organic pollutants (POP) are considered as a major problem, due to their toxicity and persistence in the environment. After their emission, these compounds can be found far from their emission source, thanks to their resistance to breakdown in the air³⁴. Dioxins, dichlorodiphenyltrichloroethane (DDT), polychlorinated dibenzo-p-dioxins and -furans (PCDD/Fs), polybrominated diphenyl ethers (PBDEs) and polychlorinated biphenyls (PCBs) belong to the list of POPs³⁵. In the case of PCBs, they are 209 different congeners, with different levels of chlorination (1 to 10) and substitution positions, but about 130 different individual PCBs are found in commercial PCB products (Figure I.1)³⁴.

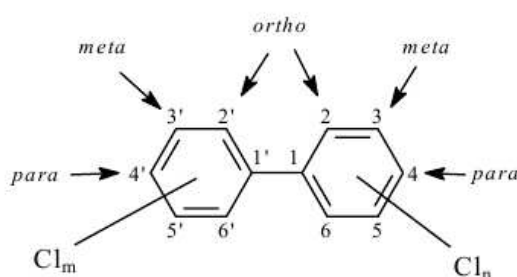


Figure I.1 Molecular structure of PCBs and position by IUPAC¹⁰

PCBs are composed by a biphenyl with chlorine atoms substituting some hydrogens, having the general formula of $C_{12}H_{(10-m-n)}Cl_{(m+n)}$, in which $m+n$ represents the number of chlorine atoms in the two rings (Figure I.2) ³⁶.

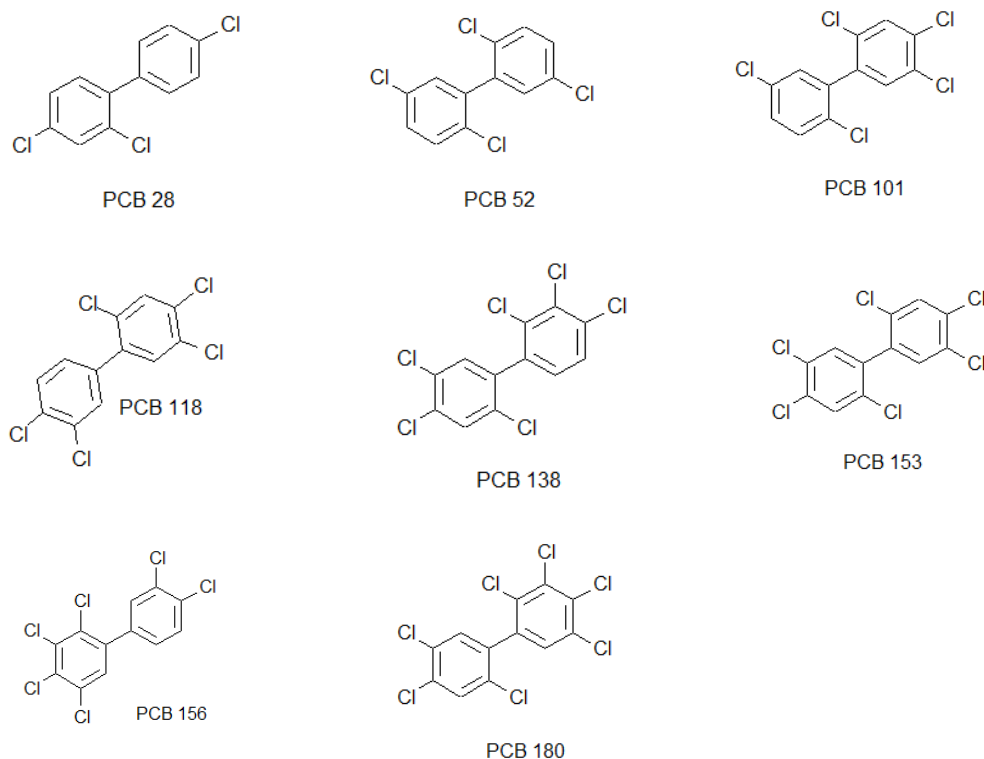


Figure I.2 Structural formula of the 7 PCB indicators (recommended by the European Union Community Bureau of Reference (BCR)) and PCB156.

The more chlorinated PCBs have the lower vapor pressures, and the higher melting and boiling points (Table I.2). The solubility of these compounds in water may vary from 0.0012 to 4830 $\mu\text{g L}^{-1}$, that is very low, so these compounds are lipophilic ¹⁰.

Table I.2: Chemical properties of different groups of PCBs ³⁷

Homologue group	Formula	Number of isomers	Mole weight (g mol ⁻¹)	Vapor pressure (Pa) (25°C)	Melting Point (°C)	Boiling Point (°C)
Monochlorobiphenyl	C ₁₂ H ₉ Cl	3	188.7	1.1	25.0-77.9	285
Dichlorobiphenyl	C ₁₂ H ₈ Cl ₂	12	223.1	2.4 x10 ⁻¹	24.4-149.0	312
Trichlorobiphenyl	C ₁₂ H ₇ Cl ₃	24	257.6	5.4x10 ⁻²	28.0-87.0	337
Tetrachlorobiphenyl	C ₁₂ H ₆ Cl ₄	42	292.0	1.2x10 ⁻²	47.0-180.0	360
Pentachlorobiphenyl	C ₁₂ H ₅ Cl ₅	46	326.4	2.6x10 ⁻³	76.5-124.0	381
Hexachlorobiphenyl	C ₁₂ H ₄ Cl ₆	42	360.9	5.8x10 ⁻⁴	77.0-200.0	400
Heptachlorobiphenyl	C ₁₂ H ₃ Cl ₇	24	395.3	1.3x10 ⁻⁴	83.0-149.0	417
Octachlorobiphenyl	C ₁₂ H ₂ Cl ₈	12	429.8	2.8x10 ⁻⁵	159.0-162.0	432
Nonachlorobiphenyl	C ₁₂ HCl ₉	3	464.2	6.3x10 ⁻⁶	182.8-206.0	445
Decachlorobiphenyl	C ₁₂ Cl ₁₀	1	498.7	1.4x10 ⁻⁶	305.9	456

b. Sources and occurrence in the Environment

PCBs are xenobiotic mixtures that have been exclusively synthesized by humans, and have been used in agriculture and industrial activities since the 1930's. Due to their dielectric properties and their chemical and physical stability (they are largely resistant to breakdown by acids, bases and heat), they were used for capacitors and transformers, but also in coatings, sealants, flame-retardants, paints, cutting oils and inks. First restrictions in the use of these compounds started in the 1970's for closed systems (transformers and capacitors) and the forbiddance in production and use became effective in the 1980's in many European countries. However they can still be found in elevated concentrations (soil, milk, animal tissues, etc.) in some places because of the persistence of their congeners ³⁷⁻⁴¹. Another contamination source is in the inappropriate handling of waste material, leaking from materials as transformers, hydraulic systems and condensers, vaporization or open burnt products that contain these contaminants ⁴². Sediments become the major reservoir of PCBs which continuously release these compounds into the water; they can bio-accumulate in the aquatic organisms and enter in the food chain. Soils are also an important reservoir of PCBs ⁴³.

PCBs have been added as priority contaminants in lists from U.S EPA and OSPAR since 2002 ⁴². The Stockholm Convention for POPs particularly chose six PCBs (PCB-28, PCB-52, PCB-101, PCB-138, PCB-153 and PCB-180) (Figure I.2) for their characterization, because of their high concentrations found in food, environment and human fluids or tissues. However the list of priority congeners analyzed may vary according to the situation or country, the European

Union Community Bureau of Reference (BCR) recommending 7 PCB indicators, for example (Figure I.2).

c. Toxicity of PCBs

As PCBs are highly soluble in lipids, it increases the bioaccumulation in human bodies, especially because the metabolization and elimination of these compounds is slower than their absorption^{37, 42}. One of the main contacts of the humans with PCBs is through food ingestion (especially from animal origin as meat, eggs and dairy products), but there are other exposure sources as the air and soil. Some PCBs (coplanar PCBs) have been identified as “dioxin-like” with relative toxicities 100–1000 times higher than those associated with non-planar PCB congeners (non dioxin-like). These PCBs, like dioxins/furans, have toxic equivalency factors ranging from 0.00003 to 0.1 (PCB-126 being the most toxic).

PCBs may cause neurological problems, reproductive, sexual dimorphism, immunological, liver effects, cardiovascular, skin effects, diabetes, carcinogenic, thyroid effects, etc.^{38, 40, 44, 45}. PCBs can be transferred to the adipose tissue of an infant, through breastfeeding from their mothers or from the placenta, and in this case the effects can be both permanent and transient.

I.1.2 Oxygenated derivatives of PAHs

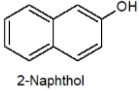
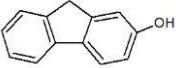
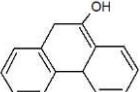
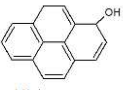
After processes as chemical redox reactions, photochemical reactions or aerobic and anaerobic biodegradation, PAHs can be transformed in various forms of metabolites as, nitrated-PAHs, alkyl-PAHs, azaarenes and oxygenated compounds (oxy-PAHs) such as epoxides, monohydroxy- or dihydroxy-PAHs, dihydrodiols, ketones, quinones, aldehydes, phenols or carboxylic acids⁴⁶. Once in the human body, the PAHs can be also metabolized into a mixture of quinones, phenols, dihydrodiols, triols and tetrols⁴⁷. Among all these various derivatives of PAHs, hydroxylated and carbonylated PAHs are also ubiquitous contaminants which are suspected to be more toxic than their parent PAHs. So they need to be studied in various environmental compartments as emerging contaminants.

I.1.2.1 Hydroxylated PAHs

a. Structure and chemical properties

Hydroxylated PAHs (hydroxy-PAHs or OH-PAHs) are organic compounds composed by at least two fused aromatic rings with at least one hydroxyl group attached, so being more polar than their parent PAHs⁴⁸. Indeed their log K_{ow} values are lower than those of their respective parent PAHs (Tables I.1 and I.3). Likewise the PAHs, the water solubility generally decreases with the increase of aromatic rings and the boiling point increases^{49, 50}.

Table I.3 Structural formula and chemical properties of the studied hydroxy-PAHs ^{47, 49}

Molecular structure	Mole weight (g mol ⁻¹)	Parent PAH	Boiling Point (°C)	Melting Point (°C)	Solubility in water (g L ⁻¹) at 20°C	Log Kow ⁴⁸
 2-Naphthol	144.2	Naphthalene	285	122-123	7.6 x 10 ⁻¹	2.7-2.9
 2-Hydroxy fluorene	182.2	Fluorene	332	168	5.0 x 10 ⁻³	3.2-3.5
 9-Phenanthrol	194.2	Phenanthrene	363	143-146	8.7 x 10 ⁻³	3.7-4.1
 1-Hydroxy pyrene	218.3	Pyrene	407	176-180	1.7 x 10 ⁻²	3.1-3.4

b. Sources and toxicity

Hydroxy-PAHs are produced from the oxidation of the PAHs. They are intermediate products from the metabolization of the PAHs (phase I) which are thereafter transformed in sulfates or glucuronide conjugates (phase II), as shown in Figure I.3 ⁵². So the metabolization process produces more polar compounds, that can be next conjugated with glutathione, glucuronic acid or sulfuric acid, by uridine 5'-diphosphate(UDP)-glucuronyl transferases or sulfotransferases enzymes, which should facilitate the excretion (by urine, feces and bile) of these contaminants ^{51, 53}.

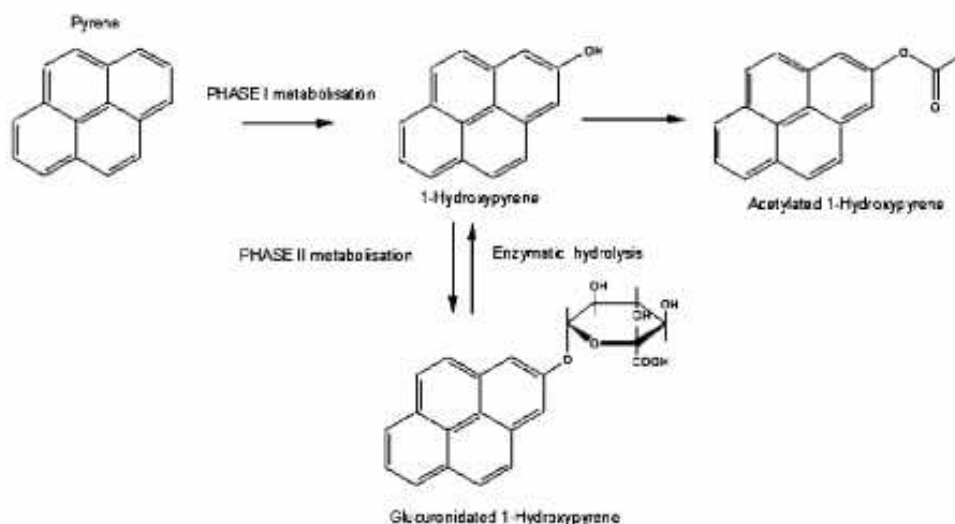


Figure I.3 Example of the metabolization of pyrene in its hydroxylated metabolite ⁵²

One of the enzymes responsible for the primary oxidation of PAHs during the phase I is the cytochrome CYP-450 monooxygenase that will form first epoxides with the addition of an oxygen on a double bond. Other oxygenated reactive metabolites of PAHs are next produced (hydroxy, diols, quinones). Some of them are even more cancerogenous and toxic than their parent PAH, in that they can directly react with the proteins, RNA or even the DNA (forming adducts) and cause cell mutations ⁵⁰. Hydroxy-PAHs with less than 4 aromatic rings, such as hydroxylated pyrene, naphthalene, phenanthrene or chrysene, are used as biomarkers for measuring parent PAHs exposition in human and animal bodies. The most measured one is 1-hydroxypyrene (1-OHPyr): it is the main pyrene metabolite formed in mammals ⁵⁴⁻⁵⁶. However, others have also been used for a larger representation of the different PAHs, for measuring the total PAH exposure, as: 2-hydroxynaphthalene (or 2-naphtol) (2-OHNaph) or various hydroxyfluorenes (used as biomarkers for tobacco smoke exposure), 4-hydroxyphenanthrene and 9-hydroxyphenanthrene (9-OHPhe) ^{15, 32, 57, 58}. The interest in measuring phenanthrene metabolites has grown also for the biological monitoring of PAHs, for example in the diet of smokers and non-smokers; smoking causes the apparition of a large variety of metabolites, that can be found in concentrations $> 1-10 \text{ ng L}^{-1}$, most being excreted in urine preferably than feces ⁵⁸. Most of the excretion of the high boiling point oxygenated PAHs are made from the feces, less than 1% are from urine; metabolites of PAHs with two or three rings are excreted preferentially with urine ⁵⁹.

As hydroxy-PAHs are oxidation products of PAHs, it is expected that these compounds can be found in the same contaminated matrices, like in sediments and soils. They were actually found in the range of $36.0-400.7 \text{ ng g}^{-1}$ (sum of the studied hydroxylated PAHs) in different types of sediments, e.g. mangrove systems, which are sites with abundant organic matter and

anoxic/reducing conditions, favorable for maintaining organic contaminants as PAHs and PCBs^{57,60,61}. Hydroxy-PAHs were also found in tropical soils (known to be less polluted with PAHs), in a mineral topsoil and in a certified soil for PAHs (European Reference Material ERM-CC013a). It appeared that in this ERM soil, the hydroxy-PAHs were detected in the range 0.5 – 130 ng g⁻¹, but they were particularly difficult to extract, with low recoveries, and it was reported that one of the reasons was a great sorption to soil particles (greater than for PAHs)⁶². 9-OHPhe was also detected in a Luvisol soil, at a concentration of 63 ng g⁻¹ in the first 7 days after being spiked with PAHs, but at 21 days, it was 140 ng g⁻¹. So the level of hydroxy-PAHs can evolve with time, depending on the biodegradation activity of soil microorganisms. They are generally intermediate products in degradation pathways, hydroxy-PAHs are not so persistent than their parent PAHs. Such a production of these contaminants was not observed in a sediment with a higher quantity of PAHs⁶³. Indeed, according to Verrhiest et al.⁶⁴, a high concentration of PAHs in the sediment could eliminate the microorganisms responsible for their degradation or inhibit the bacterial division, which could interfere in the production of the hydroxy-PAHs at the end. At the level of 30 mg kg⁻¹ of PAHs in soils, it was not noticed any effect over the bacterial density, but a negative effect was noticed at 300 mg kg⁻¹ PAHs^{63,64}. PAHs can be degraded by numerous ways, one of them is from the metabolization by bacterial consortia⁶⁵. Low molecular weight PAHs such as fluorene, phenanthrene and acenaphthene could be degraded by microorganisms through their metabolism, leading to hydroxy-PAHS. In the case of fluorene, the biodegradation was made by bacteria of the genus *Rhodococcus* (*R. rhodochrous* 172, *R. opacus* 4a and 557, and *R. rhodnii* 135), the genus *Arthrobacter* sp. strain F101 and two strains of the genus *Pseudomonas* (*P. fluorescens* 26k and 17k)^{66,62}. Degradation from the bacteria *Rhodococcus* can lead to three different paths from the metabolites 9-hydroxyfluorene (9-OHFluo) and 2-hydroxyfluorene (2-OHFluo). The proposed dead-end product was coumarin (3,4-dihydrocoumarin) (Figure I.4).

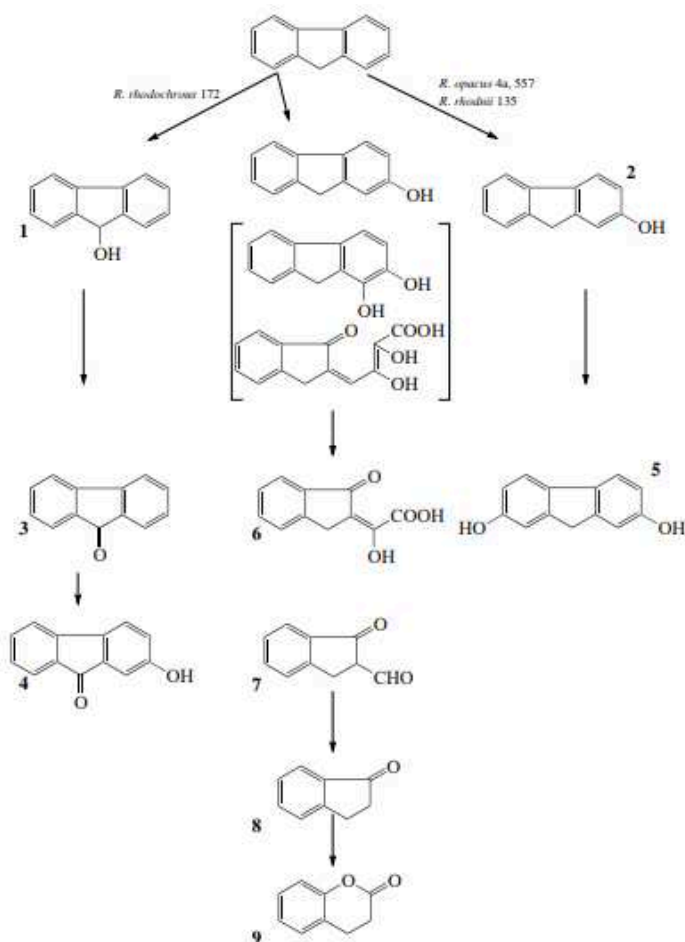


Figure I.4 Example of pathways in the transformation of fluorene by *Rhodococcus* ⁶⁶

In the case of acenaphthene, it was oxidized by *Beijerinckia* sp. forming 1-hydroxy-acenaphthene that will lead to two paths: one ending with acenaphthenediol and the other with acenaphthenequinone ⁶⁷. For phenanthrene the genus *Pseudomonas* had success degrading it into metabolites as 9-OHPhe. This metabolite and a trihydroxyphenanthrene were the only ones detected ⁶⁵.

I.1.2.2 Carbonyl PAHs

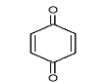

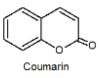
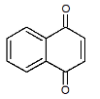
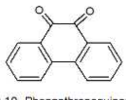
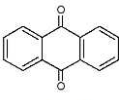
a. Structures and chemical properties

Among the oxygenated metabolites of PAHs, carbonyl PAHs contain one or more carbonyl groups and can be ketones, aldehydes but also diones, which are called in this case quinones ⁶⁸. According to IUPAC, compounds containing the carbonyl group are commonly used in the restricted sense of aldehydes and ketones, although it actually includes carboxylic acids and derivatives such as esters. So coumarin (in fact 3,4-dihydrocoumarin) can be considered as a carbonyl-PAH, as well as quinones (Table I.4). Carbonyl-PAHs are known for their lower vapor

pressure than their parent PAHs ⁴⁶. They are also more polar than their parent PAHs, their log K_{ow} values being lower (Tables I.1 and I.4).

Coumarins are composed by a benzene ring and a pyrone ring. Inside this class, there are four classifications: simple (hydroxylated, alkoxyated and alkylated derivatives of their parent compounds), furanocoumarins (5-membered furan ring attached to benzene ring), pyranocoumarins (6-membered furan ring attached to benzene ring) and the pyrone-substituted coumarins (substitution on pyrone ring, especially in the 3-C or 4-C positions) ^{69, 70}. 3,4-dihydrocoumarin (called coumarin in this study) has a very low water solubility compared to two-rings quinones (Table I.4) ⁷¹.

Table I.4 Structural formula and chemical properties of the studied carbonyl-PAHs ⁷²

Molecular structure	Parent PAHs	Boiling Point (°C)	Melting Point (°C)	Water solubility (g L ⁻¹)	Vapor pressure (atm) at 25 °C	Log K_{ow}
 1,4- Benzoquinone	Benzene	180	116	11.1	1.3×10^{-4}	0.2
 1,2- Naphthoquinone	Naphthalene	-	145	1.8×10^{-1}	1.3×10^{-7}	2.1
 Coumarin	Low molecular weight PAHs	297-299	71	1.9×10^{-3}	1.3×10^{-6}	1.4
 1,4- Naphthoquinone	Naphthalene	100	126-128	3.5	2.4×10^{-6}	1.7
 9,10- Phenanthrenequinone	Phenanthrene	360	206-207	0.4×10^{-3}	1.1×10^{-9}	2.5
 9,10- Anthraquinone	Anthracene	380	286	1.3×10^{-3}	1.5×10^{-10}	3.4

b. Sources and toxicity

Quinones are products of oxidation of the PAHs through photochemistry, biological oxidation (during biodegradation) or chemical oxidation with O_3 , OH^\bullet or NO_3^- . Carbonyl-PAHs can be also produced during the incomplete combustion of fossil fuels (diesel, gasoline, charcoal, coal, etc.), waste or biomass (crop residue, wood burnt, wheat, rice, oak, etc.). In this case, they are emitted in the atmosphere ^{46, 68, 73, 74}. Carbonyl-PAHs are considered as dead-end products in many biological and chemical degradation paths (Figure I.4). Likewise their parent PAHs, some

quinones are persistent in the atmosphere, but also in soils or sediments, being one of the most persistent group amongst PAH metabolites ^{18, 57}.

Quinones can be more toxic and carcinogenic than PAHs, because they do not need enzymatic activation, they act as direct mutagenic and cancerogenous compounds ^{57, 68}. They are capable of generating reactive oxygen species (ROS) (superoxide anion radicals ($O_2^{\bullet-}$), hydroxyl radicals (HO^{\bullet}) and their derivatives like hydrogen peroxide) responsible for oxidative stress and causing cell injurie ^{46, 75, 76}. Others effects of quinones are depletion of glutathione and generation of protein and DNA adducts ⁷³.

Quinones can be found in the atmosphere (particularly urban aerosols) but also in soils, sediments, sewage sludge, diesel particulate matter, fly ash, animal tissues, infant foods ^{57, 75, 77}. As they have a lower vapor pressure than their parent PAHs (below 10 Pa or 10^{-4} atm, Table I.4), they may be more sorbed to the particular matter than be present in the gaseous phase ⁴⁶. One of the most abundant quinone found in combustion emissions, anthracenedione (or anthraquinone, 9,10AQ), has been classified in the 2B group by IARC, which means that it can be carcinogen for humans; 1,2-naphthoquinone (1,2NQ) can induce the mitochondria to produce H_2O_2 that lead to an oxidative stress ^{68, 73}. Because of their constant presence in environment and their toxicity that can be higher than their parent PAHs, quinones need to be investigated.

Coumarin (3,4-dihydrocoumarin, also called 2H-1-benzopyran-2-one) is the second most abundant natural metabolite ⁷⁸. It is metabolized through the cytochrome P-450 system ⁷⁹. Coumarin can be found in diverse locations as plants, essential oils (cinnamon bark oil, cassia leaf oil, lavender oil etc.), foods (green tea, fruits and chicory), in microorganisms and some animal species ^{71, 78}. Coumarin (and its derivatives) has been used in clinical medicine because of its properties for preventing diseases, so it is generally not considered toxic at low concentrations. Coumarin and derivatives have antioxidant properties ⁸⁰, and also anti-coagulant, anti-tumor, anti-inflammatory ⁷¹, antibacterial ⁸¹, antifungal, antidepressant and anti-HIV properties ⁸², and can be used for the inhibition of tumors induced by benzo[a]pyrene and 7,12-dimethylbenz[a]anthracene ⁸³. Coumarin can be used also in the industry, as fragrant fixers, food additive, flavor/odor stabilizer in tobaccos, enhancer of natural oils, odor masker of paints and rubbers and cosmetics products ^{84, 85}.

Spite all the benefic effects cited previously, coumarin has been classified by the US Food and Drug Administration (FDA) in 1954 in the category 1, as carcinogenic and hepatotoxic ^{79, 86}. Coumarin may be a toxic substance that may provoke tumors in the rat's and dog's liver according to the US National Institute for Occupational Safety and Health (NIOSH) ⁶⁹. Because of this, it was banned in 1950's by the US FDA ⁷⁹ and recommended to be taken out in the UK

in 1965⁸⁴. However it has been also reported that coumarin is not a genotoxic agent, non-mutagenic which represents no risk for human through food and cosmetics⁸⁵. Because of this controversy, the presence of coumarin has been still allowed in Europe in food by the Council of Europe since 1974⁷⁹.

I.2 Sample preparation for extraction of target contaminants from particulate matter

I.2.1 Environmental particulate matter

Particulate matter is generally defined as the sum of all the microscopic solid and liquid particles, of human or natural origin, that remains suspended in a medium such as air or water. These particles vary greatly in size, composition, and origin. Atmospheric particulate matter may be in the form of fly ash, soot, dust, fog, fumes, pollen etc. Suspended particulate matter can also be found in rivers or seawater and is composed of mineral and organic fractions. Sedimentation requires that the water flow is slow enough to permit the particulate matter to settle to the bottom.

Particulate material (PM) in sediments is composed by important components as silts, clays and sand, classified as a function of their diameter (d_p). Clays are the finest particles ($d_p \leq 2 \mu\text{m}$), silts are also fine particles ($2 \mu\text{m} < d_p \leq 63 \mu\text{m}$) and sands are the coarser ($d_p > 63 \mu\text{m}$). Sediments are also composed of iron and manganese oxides and carbonates as inorganic components, but also of organic matter (OM). Sediments are products of mechanical or chemical breakdown of rocks that have been transported by the wind, water or ice and accumulate in rivers, lake, dunes or in the sea. OM comes from the decomposition of plants, living organisms and microbial residues that will constitute humic substances (fulvic acids, humic acids, humin)^{87,88}. Diagenesis of the sedimentary OM lead to the transformation of the biopolymers in geopolymers and then in kerogens: this maturation change the composition of OM, leading to more glassy and carbonaceous matter, with a higher aromatic character. Other external carbonaceous material can enter in the composition of OM: it can be unburned coal, black carbon or soot that come from deposits due to anthropogenic combustion activities.

Organic and inorganic contaminants have the tendency to accumulate in the sedimentary matrix. The finest particles (clays, silts) and the organic matter are considered as the most important adsorption agents for metallic or organic contaminants that can confer to the sediment a hazardous character^{89,90}. Clays are composed of a mixture of silicates, allophane, iron and aluminum oxides (aluminum silicates), with inter-layer cations^{91,92}. They have the highest specific surface compared to the other sediments components, and a negative net surface charge

⁹³. Electrostatic force attracts a diversity of cations (cation exchange capacity (CEC) measuring the ability of clays to attract cations such as K^+ or Ca^{2+} ...) but also polarized organic molecules. The siloxane surface of clays contains also reactive sites with hydroxyl groups that can attract organic compounds via strong hydrogen bonds. Silts are also a mixture of diverse components as clay minerals, quartz, micas and feldspar, and have a high specific surface (even if lower than clays), negatively charged ⁹³⁻⁹⁵. For sand, the most common constituent is silica (silicon dioxide). It has a hydrophilic character and the contaminants are bound to the surface by hydrogen bonds. Generally, specific surface of silica in sediments is low and its capacity to sorb polarized organic compounds is quite low compared to clay particles. At last, organic matter is composed in majority of humic substances (as previously mentioned) which can sorb high amounts of hydrophobic organic compounds; for example, 100 g of humus acid can retain 2 g of hydrophobic compounds by adsorption ⁹⁶. OM has a high specific area and interact with the contaminants through Van der Waals forces, such as London dispersion forces with amorphous OM or π - π forces with more glassy forms of OM (such as soot).

In the case of lipophilic organic contaminants such as PAHs, PCBs, organochlorine pesticides or polychlorinated dibenzo-p-dioxins and furans (PCDDs and PCDFs), they tend to strongly sorb to OM, particularly to mature OM such as kerogens or soot, that make difficult to extract them ⁹⁷. In the case of metals, they are particularly sorbed onto the negatively charged clayey fraction. But the fine clayey fraction has also a non-negligible contribution to organic contaminants sorption through the clayey-humic complex ⁹⁸. The contamination of the sediments is problematic because re-suspended PM can contaminate the living organisms in water and the habitat around it ⁹⁷. So it is important to develop and monitor techniques able to extract and quantify the contaminants in order to characterize the degree of contamination and to apply remediation strategies.

I.2.2 Extraction methods from particulate matter

I.2.2.1 Classical extraction methods

a. Soxhlet extraction

Created in 1879 by Franz Ritter von Soxhlet, the Soxhlet extraction is a reflux method that has been one of the most used techniques for years, first used for the determination of the fat in milk ⁹⁹. The important factors to consider for extraction are mass transfer, solubility and matrix effects ¹⁰⁰. The disadvantages of using Soxhlet extraction are that: (i) it requires a high amount of organic solvent, which is not good for the environment and may demand time for a further sample concentration by evaporation; (ii) it takes too much time: several tens of cycles are

necessary to obtain good extraction recoveries that can take 8 to 72 hours, depending on the solid matrix. Nevertheless Soxhlet has been widely used for extraction of compounds as pesticides, PCBs, PAHs and their metabolites, from biological or various environmental matrices (sediment, soils, sewage sludge, soot, plant materials) ^{89, 99-101}. Recent adjustments were made to automatize and improve the Soxhlet extraction process: Focused Microwave-Assisted Soxhlet Extraction (FMASE), High-Pressure Soxhlet Extraction (HPSE), hot Soxhlet, Ultrasound-Assisted Soxhlet Extraction (UASE) ^{100, 102}.

b. Ultrasonic extraction

Ultrasonic extraction is not a reflux method but may be also efficient for the extraction of trace organics from sediments, soils and plants. It has been used for the extraction of contaminants as pesticides and PAHs ^{103, 104}. The extraction by ultrasound is made by a high frequency sound energy applied from a dispositive of ultrasound probe, cup horn systems or by an ultrasonic bath ^{105, 106}. Ultrasounds can be strong enough to disrupt the matrix and facilitate the extraction ¹⁰⁴⁻¹⁰⁷. Ultrasound-assisted extraction has advantages as relatively high extraction efficiency (even if generally lower than Soxhlet extraction), it does not require an expensive equipment, is easy to operate, with little sample preparation ¹⁰³. Searching to improve the capacity of extraction and reducing the extraction time and solvent consumption, other techniques involving the ultrasound were created as: ultrasound-assisted Clevenger distillation, continuous ultrasound-assisted extraction, and combination of ultrasound with microwave and supercritical fluid extraction ¹⁰⁷.

I.2.2.2 Enhanced extraction methods

a. Pressurized Liquid Extraction (PLE)

Pressurized liquid extraction (PLE) is also known as assisted solvent extraction (ASE), pressurized hot-solvent extraction (PHSE) or pressurized fluid extraction (PFE). It is an automated process that is faster than conventional extractions and uses less solvent, so it is considered as a greener technology when compared to conventional techniques ^{108, 109}. Another advantage is the capacity of automation, that allows a higher reproducibility ¹⁰⁹. But it can be also considered as a disadvantage, because the necessity to buy such an equipment make the process more expensive. When used just with water as solvent, it is called subcritical water extraction (SWE), superheated water extraction (SHWE) or pressurized hot-water extraction (PHWE) ¹⁰⁹. This process consists in submitting the solvent to a high temperature and a high pressure but always lower than the critical point to keep it in the liquid state. The high pressure

will keep the solvent lower than the boiling point even at high temperatures which helps the solvent to penetrate into the matrix and to solubilize the analytes, improving the extraction ¹¹⁰. There are two types of extraction processes, the static and the dynamic, which can occur successively in several cycles¹¹⁰. For the extraction the important parameters to consider are the temperature, time of extraction, type of solvent, flushing volume and number of extraction cycles ^{102, 110}. PLE is used for analyses of bioactive compounds from plants ¹⁰⁹, persistent organic pollutants in the environment ¹¹⁰, phenolic compounds ¹¹¹, etc. More recently, another step as the clean-up was introduced to the PLE process, with the use of adsorbents as Florisil, acidic/neutral/basic alumina or silica, which is called selective pressurized liquid extraction (SPLE) ¹¹².

b. Supercritical fluid extraction (SFE)

Supercritical fluid extraction (SFE) started to be used in the 1970's for extraction of compounds as caffeine from tea and coffee ¹¹³ and today is very used in the industry ¹¹⁴. It is considered as a green extraction process that uses very few amounts of organic solvents. The extraction fluid is used above its critical temperature and pressure ¹⁰⁹. The supercritical fluid has low viscosity and high diffusivity, as gaseous phases, and has a high solubility power as liquid phases, that enhances the extraction ¹¹⁵. One of the most used fluids is carbon dioxide for being harmless for human and the environment, and for having a moderate critical temperature (31.2°C) and pressure (72.9 atm) ¹¹⁵. The advantages of this process is the low extraction time, the utilization of very low contents of co-solvents (modifiers) generally recognized as safe, the high efficiency in recovery, the possibility to couple directly with a method of analyze as gas chromatography (GC) or supercritical fluid chromatography (SFC) ¹¹⁵, leading to a good reproducibility and higher selectivity than the other extraction methods ¹¹⁶. But, likewise PLE, the equipment is expensive that increases the cost of extractions. Some of the parameters that have to be considered are the temperature and pressure of the fluid (which determine its solubility capacity), the nature and percentage of co-solvent added to CO₂, the extraction time (static and dynamic) and the flow rate ^{109, 117}. SFE can be also used with rapid expansion of supercritical solvent (RESS) in a method which is called supercritical fluid extraction of emulsions (SFEE) ^{118, 119}.

c. Microwave Assisted Extraction (MAE)

As shown previously, the automation of the extraction processes was increasing with the past decades due to the necessity to decrease the time of extraction ¹²⁰. Among the automated processes, the technique using microwaves in a laboratory was first used by Abu-Samra et al.

in 1975 for analyses of trace metals from biological samples ¹²¹. Eleven years later, microwaves were used for organic extraction by Ganzler et al. ¹²² and Lane and Jekins ^{123, 124}. This process was used for various matrices as biological or environmental, for diverse compounds as PAHs, phenols, PCBs, metals and pesticides ¹²⁰. The advantages of MAE are: (i) a considerable decrease of the extraction time because of the in-situ sample heating by the microwaves instead of the conventional convection heating, allowing a temperature increase in less time ¹²⁵; (ii) the significant decrease of the solvent consumption and loss of volatile compounds; (iii) the capacity for multiple simultaneous extractions through automation and (iv) decrease of the cost of the equipment compared to SFE and PLE. For this process, parameters as time, temperature, nature of solvent, volume and matrix characteristic are important ^{120, 126}. There is two types of commercial microwaves, the open vessel and the closed one ¹²⁰.

An important factor to consider when using MAE is the dielectric loss coefficient that is the ability of the solvent to convert microwave energy into thermal energy ¹⁰⁰. The microwaves can affect the solvent by dipole rotation or ionic conduction, or by a combination of the two, called interfacial polarization which will depend on the solvent used ^{100, 120, 125}. In the ionic conduction, the ions transit between the vacancies (point defects) in the crystal lattice of the solid sample when excited by an electromagnetic field ^{120, 127}. With the resistance of the sample to the transfer, the solvent is heated because of the friction between them ¹²⁷. For the dipole rotation effect, the dipoles line up by rotation with an electric field and waves, causing molecularly friction and collisions, and consequently heat. Normally these phenomena happen at 2450 MHz in commercial systems ^{120, 125}. The higher is the polarization of the solvent dipole, the higher is the capacity to absorb microwave energy and heating is faster. For example, solvents as water, methanol and acetonitrile are highly polar and are heated very quickly. On the contrary, apolar solvents (hexane, toluene...) have no dipole that makes more difficult or impossible to heat them ¹⁰⁰. The high temperatures used in MAE will increase the solubility of the analytes in the solvent, brake the analyte-matrix bonds, increase the mass transfer and diffusion, reduce the viscosity and interfacial tension of the solvent and even improve selectivity because temperature will change the dielectric constant of the solvent ¹¹¹.

I.2.2.3 Standard extraction methods for PAHs/PCBs

For regulated contaminants such as PAHs or PCBs, there is already various standard methodologies developed for their extraction from various environmental matrices. One of them (EPA-3540C method) is the extraction of PAHs/PCBs from soils, sediments, sludges, clays and waste solids using a Soxhlet apparatus, using 300 mL of an acetone:hexane mixture (1:1 (v:v)) during 16-24 h, with 4-6 cycles per hour. An alternative has been proposed, with an

automated Soxhlet extraction system (EPA-3541 method): 50 mL of an hexane:acetone (1:1 (v:v)) is used to extract the semi-volatile compounds, with a temperature of 140°C for 60 minutes (when in “boiling” position), and another 60 minutes in “rising” position. After the solute can be concentrated, cleaned (Method EPA-3600) and analyzed (Method EPA-8000 and EPA-8270) ¹²⁸.

Another methodology is the extraction of semivolatile organic compounds such as organophosphorus pesticides, organochlorine pesticides, chlorinated herbicides, phenoxyacid herbicides, substituted phenols, PAHs, PCBs, and PCDDs/PCDFs, from soils, clays, sediments, sludges, and solid wastes using microwave assisted extraction. The method EPA-3546 uses elevated temperature and pressure conditions (i.e., 100-115°C and 50-175 psi) in a closed vessel containing the sample and organic solvent(s), and microwave irradiation for 10-20 min. For PAHs/PCBs extracted from soils, the solvent used is 25 mL of a mixture of hexane:acetone 1:1 (v/v) ¹²⁹. No purification methodology is introduced after the extraction process that can lead to further problems in the quantification analyses using chromatography, with distorted peaks. It can be noted here that there is no standardized methodologies to extract and quantify emerging contaminants such as oxy-PAHs, from soils or sediments.

I.2.2.4 Matrix solid phase dispersive extraction (MSPD)

a. General principles of MSPD

Extraction through matrix solid phase dispersion (MSPD) was used first in 1989 by Barker et al. for isolating drugs from tissues ¹³⁰. It is a quite recent and simple technique that uses a dispersant for assisting the disruption of the solid matrix particles, for improving the extraction ¹³¹. It has been used for solid, semi-solid and viscous samples ¹³². The disruption is improved by grinding the solid material with a mechanical force like a pestle and mortar ¹³³. The most used dispersants are particles of octadecylsilyl silica (C₁₈) ¹³⁴, Florisil ¹³⁵, alumina ¹³⁶, silica gel ¹³⁷ or diatomaceous earth ¹³⁸. For improving the extraction of non-polar, non- or semi-volatile organic compounds, in matrices as sediments, soils, sewage sludge etc., a drying agent can be added, e.g. Na₂SO₄. Indeed the presence of water can interfere in the extraction of the non-polar compounds and make the co-elution of the polar ones ^{100, 135}. The choice of the solid dispersant will depend on the nature of the analytes to be extracted and on the competitive adsorption between the impurities of the matrix and the target compounds ¹³⁹. The solid sample is first mixed through blending with dispersants; after the crushed mixture is packed into an empty column between two polypropylene frits in which the target compounds will be eluted by a proper solvent (Figure I.5) ¹³⁴.

It is a fast method that does not require high quantities of solvent and sample and any expensive equipment ¹³¹. Another advantage of the method is that it makes the clean-up of the sample at the same time as the extraction, with the addition of a sorbent layer which has to adsorb interfering compounds ¹³⁶. One of the disadvantages is the quite time-consuming stage of disruption of the sample and possible inhomogeneity in column packing ¹³¹.

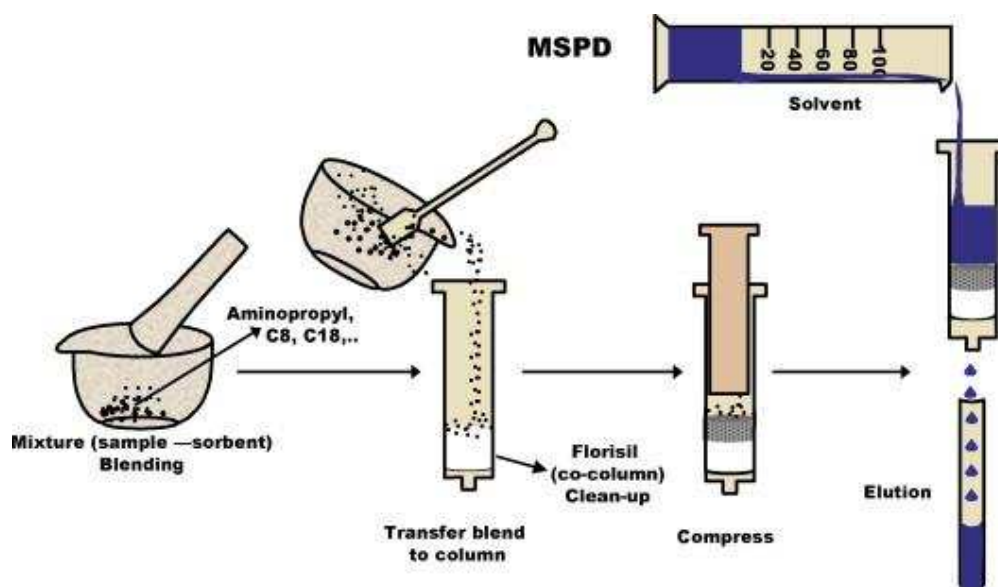


Figure I.5 Schema of the MSPD process ¹⁴⁰

MSPD has been used for extraction of diverse contaminants as pesticides ¹⁴¹, polycyclic aromatic hydrocarbons ²², polychlorinated biphenyls ¹⁴², drugs ¹³⁹, active pharmaceutical substances ¹³⁸, etc. It has been used for matrices as plants, moss ¹³¹, food ^{137,141}, soils, sediments, animal tissues, cosmetics and human biological samples ¹⁴³. An adaption of the method is the magnetically assisted matrix solid phase dispersion (MA-MSPD), that uses a magnetic sorbent for homogenizing the mixture and does not need the step of packing in a column ¹⁴⁴.

b. MSPD for PAHs, PCBs and oxy-PAHs

PAHs and PCBs have to be monitored frequently and with relatively high flow, due to their ubiquity in the environment, which demands cheap and fast methods ^{145, 146}. As reported previously, MSPD has been used for the extraction and clean-up of a variety of compounds in a variety of matrices, including PCBs and PAHs. Extractions with this technique are cheaper, faster, less laborious and consume less quantity of solvent than traditional techniques as Soxhlet or Ultrasound-assisted extraction, and do not need a supplementary step for clean-up after extraction, as pressurized liquid extraction ¹⁴⁷. MSPD has been used for extraction of PAHs from soils ¹⁴⁷, moss ¹³¹, bivalves ³¹, sewage sludge ¹⁴⁸, honey ¹⁴⁹, asphalt binder ¹⁵⁰, dust ¹⁵¹, etc.

According to Concha-Grana et al.¹³¹ the dispersant that is the most used for PAHs is composed of C₁₈ particles, but other ones as silica, Florisil, alumina and diatomaceous earth have been also reported¹³¹.

For PCBs, MSPD showed satisfactory recoveries for samples as fish muscle tissue^{152, 153}, mussel¹⁵⁴, edible vegetables oils¹⁵⁵, peanuts and soybeans¹⁴². Likewise PAHs, a drying agent (e.g. anhydrous sodium sulphate) and a dispersive agent (similar as used for PAHs, as Florisil¹⁵⁴ and C₁₈^{152, 153}; or not-conventional as sulfuric acid-impregnated silica^{142, 155}) are added to the sample to help extraction; afterwards the mixture is crushed in a mortar for a few minutes and introduced in a cartridge that could have or not a co-column with agents as Florisil¹⁵³, silica¹⁵⁵ or a mixture of Florisil and C₁₈¹⁴², to better sorb the interfering compounds. The n-hexane is generally used to elute PAHs or PCBs because of their low polarity. Extraction solvents can be used pure¹⁵² or in mixtures with acetone or dichloromethane^{153, 142, 155}.

Spite of the growth of interest in the analysis of oxygenated metabolites of PAHs, there is not a lot of studies about their extraction using MSPD. Olmos-Espejel et al. used MSPD for extraction of some PAHs and their dihydrodiol and hydroxy metabolites from algae²⁸. Usually the metabolites of PAHs are sensitive to light, pH, temperature and oxidants in air, and are found only at trace levels in the environment that make analyses of these compounds not so easy²⁸. So, new fast and sensitive analytical methods, including the extraction step, have to be developed for oxy-PAHs²⁸.

I.2.3 Selective extractions using molecular imprinted polymers

I.2.3.1 Molecularly Imprinted Polymers (MIPs) for PAHs

After the solvent extraction step, extracts are generally purified and fractionated using column fractionation or solid phase extraction (SPE) to remove impurities¹⁵⁶. The clean-up step allows eliminating a majority of the interfering compounds that can mask or disturb the detection of the analytes of interest during the analytical step. So clean-up processes improve accuracy, eliminating matrix effects, but this step is quite long and it can lead to subsequent losses of the more volatile compounds, so that this time-consuming step is sometimes bypassed in some analytical methods¹⁵⁷. A lot of cleanup methods have been already used after the extraction step of the PAHs as solid phase extraction (SPE) or gel permeation chromatography (GPC) with adsorbents as C₁₈ or molecularly imprinted polymers (MIPs)¹⁵⁸.

MIPs can be used as a clean-up method that uses molecularly recognition for extracting selectively the target compounds, with the possibility to concentrate the analytes in the eluate at the same time^{159, 160}. MIPs are produced by a polymerization of the templates and monomers

in a solution, in presence of an initiator and a cross-linker (Figure I.6). After, the templates are removed and cavities with the similar shape, size and spatial distribution than the target analytes are left in place ^{159, 161}.

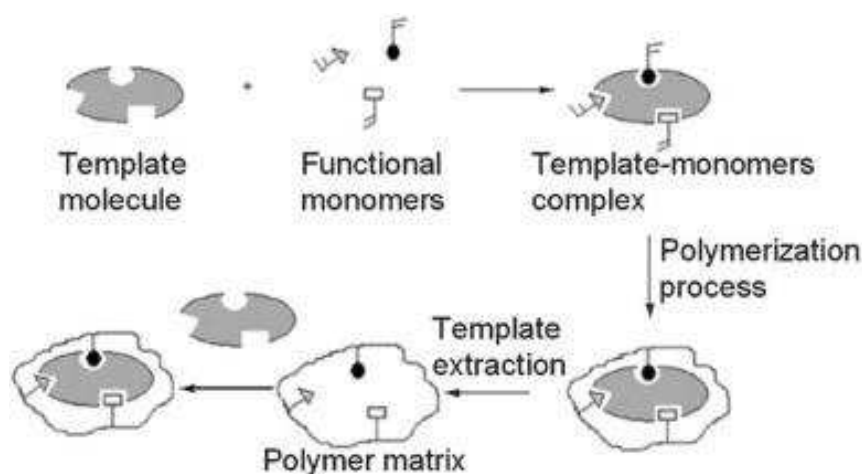


Figure I.6 polymerization of a MIP ¹⁶²

MIPs have the advantages of high selectivity recognition, low cost, stability for stocking, easy manipulation, resistance to high temperatures and pressure and they can be appropriate for a wide variety of compounds ^{161, 163}. Among the different uses of the MIPs, is molecularly imprinted SPE (MIP-SPE) ¹⁶⁴, molecular imprinted solid-phase microextraction (MIP-SPME), molecular imprinted stir-bar sorptive extraction (MIP-SBSE) and molecular imprinted matrix solid-phase dispersion (MIP-MSPD) ¹⁴³. MIP-MSPD has already been used for analyses of steroids ¹⁶⁵, β -estradiol ¹⁵⁹, quinolones ¹⁶⁶, fluoroquinolones ¹⁴³, tetracyclines ¹³⁹, enrofloxacin ¹⁶⁷, etc. Applications can be found in various matrices as milk ¹⁵⁹, serum ¹⁶⁶, fish sample ¹⁴³, etc.

Because PAHs are lipophilic and have no pronounced functional groups, it is difficult to imprint MIPs, in which the bonds between the functional monomers and templates are made with hydrophobic and π - π interactions, weaker and less selective than non-covalent interactions as hydrogen bonds ^{158, 163}. Between all the PAHs, selective MIPs for benzo[a]pyrene or pyrene were the most used. For synthesis of the MIPs it was reported the use of templates made from only one PAH, but used to extract simultaneously the two to six ringed PAHs ^{168, 169}. Unfortunately, an imprint made for a low molecular weight PAH is not necessarily suitable for high molecular weight PAHs ¹⁵⁸. A multi-molecular technique proposed to include more PAHs (six to sixteen PAHs) as templates for imprinting a selective MIP ¹⁷⁰⁻¹⁷².

I.2.3.2 Molecularly Imprinted Polymers (MIPs) for oxy-PAHs

In the same way that MIPs were used for PAHs, they were produced also for the selective extraction and clean-up of their metabolites. A MIP was made using 1-hydroxypyrene (1-OHPyr) as template for its detection from human urine, the analyte being connected to the polymer cavities by hydrogen bonds and ionic interactions. When testing it with 1-hydroxyphenanthrene (1-OHPhe), a competition was demonstrated, due to the 1-hydroxy position which diminishes a little the final recovery, but not significantly for invalidating the method ¹⁷³. It was reported the production of a MIP using 1-OHPyr as template in which 90% removal of the template was achieved ¹⁷⁴. Molecules as 1-naphthol (1-OHNaph) and phenol were used for testing the selectivity of this MIP towards 1-OHPyr, which was successful, having a retention for 1-OHPyr in the MIP higher than the others ¹⁷⁴. Nano-sized molecularly imprinted polymers (nMIP), using multi-template imprinting based on precipitation polymerization, were synthesized for the analysis of 1-OHNaph, 2-OHFluo, 9-OHFluo, 9-OHPhe and 1-OHPyr in human urine ¹⁷⁵. In another case, a MIP imprinted for phenols was used for the clean-up of 2-OHFluo, 9-OHFluo, 9-OHPhe and 1-OHPyr after MAE extraction from soils ⁶³.

I.3 Chromatographic tools for separation and quantification of PAHs, PCBs and oxy-PAHs

I.3.1 Gas chromatography (GC)

I.3.1.1 Analyses of PAHs and PCBs

Gas chromatography is an analytical method that uses a column to separate vaporized mixtures of volatile or semi-volatile compounds. The mixture is transported with a mobile gas phase in which the separation is made thanks to interactions with the column stationary phase. The most used carrier gas is helium, because it is an inert gas. Various detection systems can be used to detect the eluted analytes. The most used detectors in the analysis of PAHs are mass spectrometry (MS) and flame ionization (FID) ¹⁷⁶; although being more expensive, MS is more and more used because it allows identification through mass spectra ¹⁷⁷. Tandem MS/MS and high resolution mass spectrometer (HRMS) are more efficient in the selective analysis of very low concentrations of PAHs ¹⁷⁸. The mass spectrometer generally uses an electron impact (EI) interface for ionizing PAHs that is used in positive ion mode and 70 eV energy. When tandem MS/MS is used, argon or helium are applied as collision gases. Among the columns used for PAH analysis is the typical DB-5MS of 30 m long and 0.25 mm diameter, with 5% phenyl-95% methyl-polysiloxane as stationary phase (0.25 μ m film thickness) ¹⁷⁹; other stationary

phases, more specialized for PAHs, can be used (DB-EUPAH column¹⁸⁰), with longer (40, 60 m) or more or less thick stationary phases^{177, 181, 182}. Among the surrogate or internal standards used for quantifying PAHs in GC-MS are reported the deuterated PAHs (naphthalene-D8, acenaphthylene-D10, phenanthrene-D10, chrysene-D12, perylene-D12)³³.

Gas chromatography was used also for the analysis of PCBs using mass spectrometer detectors (MS, HRMS, TOF-MS)^{183, 184} or an electron capture detector (ECD), or a micro-electron capture detector (μ ECD)¹⁸⁵. ECD detector is selective for halogenated compounds and is more sensitive than MS detectors for PCBs, but it does not allow identification. The same columns as those used for PAHs can be used for PCBs¹⁸³, but other columns (Rtx-PCB) are more adapted for a better selectivity and separation of dioxin-like PCBs. For PCBs analyzed in GC-MS, 4,4'-dibromooctafluorobiphenyl (DBOBF), PCB-24, PCB-27, PCB-67, PCB-77, PCB-89 and PCB-169 were used as surrogate standards^{33 186} and PCB-209 as internal standard for quantification¹⁸⁵, because they are not found in environmental matrices or commercial solutions of PCBs.

I.3.1.2 Analyses of oxy-PAHs

Analysis of oxygenated metabolites of PAHs can be difficult due to their instability to light, temperature, pH and air oxidants, but also due to their very low concentration in the environment, which demands a fast separation method and a sensitive detection method²⁸. The oxygenated PAHs are generally analyzed in GC-MS using the same types of columns than those used for PAHs^{187, 188}. However, for analysis of oxy-PAHs in gas chromatography, derivatization processes need generally to be applied because of their thermal instability. For the hydroxylated PAHs, it was used various derivatization agents to convert the active hydrogen of the -OH functional group, to prevent thermal decomposition and to increase volatility. Silylation reagents were: N-tertbutyldimethylsilyl-N-methyltrifluoroacetamide (MTBSTFA) containing 1% of tert-butylchlorosilane¹⁸⁷, N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA)^{63,188}, N-methyl-N-(trimethylsilyl)trifluoroacetamide (MSTFA)¹⁸⁹, N,O-bis(trimethylsilyl)acetamide (BSA) with 5% trimethylchlorosilane (TMCS)¹⁹⁰. The silylation derivatization improves the sensitivity but also the selectivity of the analysis. Acetylation was also used for hydroxy-PAHs with acetic acid anhydride and potassium hydrogen carbonate as reagents⁵², but this derivatization reaction is used principally for quinones.

Indeed direct determination of some quinones by GC-MS has been reported as unsuitable because of their low volatility and possible thermal degradation, particularly if carbonyl groups are in ortho position¹⁹¹. Quinones are converted to their diacetyl derivatives generally using acetic anhydride as derivatization agent and zinc particles^{68, 76, 192}. With the exception of

anthraquinone, acenaphthenequinone, methyl-anthraquinone, dimethyl-anthraquinone, 5,12-naphthacenequinone, benz[a]anthracene-7,12-dione, the other quinones showed a significant improvement in the sensitivity of their GC analysis after acetylation, probably because they were almost fully converted^{76, 192}.

I.3.2 Liquid chromatography

I.3.2.1 Analyses of PAHs and PCBs

In high performance liquid chromatography (HPLC), a mixture of compounds solubilized in a liquid is eluted by a liquid mobile phase through pumping, and according to the interactions between the liquid and the stationary phase (filling the chromatographic column), the separation can be made¹⁹³. HPLC systems are often coupled to UV detectors, which are suitable for PAH analysis with a quite good sensitivity at 254 nm. HPLC coupled with a tunable absorbance UV detector can be used to separate and detect the 16 priority PAHs, generally using C₁₈ silica grafted stationary phases (with classical columns of 4.6 mm diameter, 250 mm long and particles of 5 µm diameter), more or less designed for PAHs¹⁹⁴. Generally, the mobile phase used for separating PAHs is a mixture of acetonitrile and water. Used with ultra-performance liquid chromatography (UPLC), systems can be fitted with a diode array detector (DAD) but shorter columns (5-10 mm long) with smaller particles (2 µm) or core-shell particles must be used^{195, 196}. Fluorescence detection can also be used for PAHs and can be 10-1000 times more sensitive than UV detection. This detection is relatively selective as only fluorescent compounds, with the appropriate couple of excitation and emission wavelengths can be detected¹⁹⁷⁻²⁰⁰. But the drawback is that in the case of the 16 priority PAHs analysis, acenaphthylene is not fluorescent and cannot be detected. Spite having the advantage over fluorescence detection to allow identification of the analytes with a quite certainty, the MS coupled to HPLC is still less sensitive than the fluorescence detector²⁰¹⁻²⁰³.

Concerning PCBs, they are not often analyzed using liquid chromatography. HPLC coupled to UV detection can be used, but also UPLC²⁰⁴. Liquid chromatography atmospheric pressure photoionization-mass spectrometry (LC-APPI-MS/MS) system, with a trifunctional alkyl C₁₈ bonded phase column, allows the simultaneous determination of diverse compounds including PCBs²⁰⁵.

I.3.2.2 Analyses of oxy-PAHs

HPLC has the advantage over GC, for the analysis of oxygenated PAHs, of not requiring derivatization since oxy-PAHs are not heated. For hydroxy-PAHs, liquid chromatography was more often used coupled to a fluorescence detector⁶³, although UV-DAD can also be used²⁰⁶

or tandem MS/MS ²⁰⁷. Nevertheless, fluorescence detector is the most sensitive, although it does not allow identification. Columns used are generally C₁₈ bonded silica phases ⁶³, with acetonitrile or methanol as co-solvents in water for the mobile phase ²⁰⁸. For quinones, HPLC can be used with reversed-phase C₁₈ columns and coupled to an UV detector ^{209, 210}. Unlike hydroxy-PAHs, the quinones are not fluorescent, that make not possible to detect them with a fluorimeter. Chemiluminescence detection could also be used for a more sensitive detection of quinones ²¹¹.

I.4 Conclusion

Among all the environmental matrices, it appears very important to be able to analyze ubiquitous and carcinogenic pollutants as PAHs and their oxygenated derivatives, and PCBs, from soils and sediments, which act as sinks for these contaminants. With notorious damaging for human health, these persistent compounds can enter in contact with humans through the sediments, by their transferring to plants and animals. In the case of the polar derivatives of PAHs, they can be carried more easily into the aquatic system because of their higher solubility. Diverse extraction techniques have been used for years to extract the organic contaminants from solid particles, as the traditional Soxhlet or the ultrasonic extractions, until more faster and “green” methods tended to replace them, as MAE, SFE, or ASE. Now there is a growing interest for developing new sample preparation methods, fast, easy to handle, and able to facilitate multi-residual analysis, so simultaneous analysis of different families of compounds. Another interest in developing analytical methods is to achieve good chromatographic separations of mixed families of analytes, but also to reach low detection limits. It is why two strategies were developed in this study, to extract and analyze simultaneously different families of contaminants from sediment matrices:

- A first sample preparation methodology was developed to extract simultaneously hydroxy-PAHs and carbonyl-PAHs from sediments using MAE, which has never been done in the past. A chemometric approach was performed to optimize the extraction step and different chromatographic methodologies (GC-MS, HPLC coupled to UV and fluorescence detectors) were tested and compared to quantify them at trace levels;
- A second sample preparation methodology was elaborated, not only to extract PAHs and PCBs, but also hydroxy-PAHs. MSPD was optimized in order to extract for the first time PAHs and PCBs simultaneously, but also to allow sample purification at the same time. As hydroxy-PAHs and PAHs/PCBs were not of the same polarity, we attempted to extract the lipophilic compounds together in a first step, using MSPD, and hydroxy-

PAHs in a second step. Thus MIPs were coupled to MSPD to obtain a selective extraction of hydroxy-PAHs alone, without the other interfering polar compounds eluting also from sediments.

Résumé du chapitre I

Outils pour analyser les PCB, HAP et HAP oxygénés (hydroxylés ou carbonylés) dans des matrices environnementales particulières

Ce chapitre donne des informations générales sur les propriétés et les techniques d'analyse des contaminants environnementaux étudiés dans ces travaux de thèse, incluant les polychlorobiphényles (PCB), les hydrocarbures aromatiques polycycliques (HAP) et certains HAP oxygénés (oxy-HAP), tels les HAP carbonylés (parmi lesquels les quinones) et les HAP hydroxylés (OH-HAP).

I.1 Composés aromatiques polycycliques et dérivés oxygénés

I.1.1 Composés aromatiques polycycliques

Les HAP sont des contaminants ubiquitaires composés d'au moins deux cycles aromatiques juxtaposés. Parmi les centaines de HAP que l'on peut retrouver dans l'environnement, seulement certains d'entre eux sont suivis prioritairement, dont les 16 HAP « prioritaires » définis par l'US-EPA. Les HAP sont des composés lipophiles, possédant une faible solubilité dans l'eau et un coefficient de partage octanol/eau élevé (qui augmente avec le nombre de cycles aromatiques), qui permet de prédire une adsorption importante dans les milieux comportant de la matière organique (tels les sédiments) mais aussi leur bioaccumulation dans les organismes vivants. En effet, les HAP sont également des hydrocarbures aromatiques très stables chimiquement et donc persistants. Les HAP ont également de hauts points d'ébullition, ainsi que de très faibles pressions de vapeur qui diminuent avec le nombre de cycles aromatiques : les HAP de faible poids moléculaire sont semi-volatils, et sont partagés entre la phase gazeuse et particulaire de l'atmosphère ; ceux de haut poids moléculaire sont non volatils et sont essentiellement retrouvés dans la phase particulaire. Les HAP sont produits par des procédés de combustion incomplets, essentiellement d'origine anthropique, mais peuvent avoir aussi des sources naturelles (feux de forêt, volcans...). Après avoir été émis dans l'atmosphère, ils sont déposés par voie sèche ou humide sur les surfaces liquides ou solides. Dans les milieux aquatiques, ils sont majoritairement liés aux particules en suspension et ont donc tendance à s'accumuler dans les sédiments. Les HAP sont des substances toxiques et certains sont classés comme substances probablement cancérigènes. Le benzo[a]pyrene (BaP) a le facteur de toxicité le plus élevé et est le plus souvent mesuré en tant qu'indicateur de la présence des HAP. Les êtres humains sont essentiellement exposés par voie respiratoire ou par ingestion d'aliments.

Une fois ingérés, les HAP sont métabolisés par une famille d'enzymes de type cytochrome P-450 et transformés en composés plus polaires, parmi lesquels les HAP hydroxylés, qui se fixent alors à l'ADN, amorçant ainsi des processus de cancérogénèse.

Les polychlorobiphényles (PCB) sont des polluants organiques persistants composés d'un biphényle substitué par 1 à 10 atomes de chlore, cette famille comportant ainsi 209 congénères. Ils sont lipophiles, de très faible solubilité dans l'eau, et possèdent de hauts points d'ébullition et de très faibles pressions de vapeur. Les PCB sont des xénobiotiques uniquement synthétisés par l'Homme depuis le début du 20^e siècle. De par leurs propriétés diélectriques et leur grande stabilité chimique et thermique, ils ont été utilisés en grande quantité dans les transformateurs électriques, en tant que retardateurs de flamme... Ils sont interdits dans les pays européens depuis les années 80, mais suite à des fuites ou des déversements sauvages, on les retrouve toujours dans l'environnement, particulièrement accumulés dans les sédiments ou bioaccumulés dans des organismes vivants. Les PCB parviennent dans les organismes vivants essentiellement par ingestion d'aliments et s'accumulent d'autant plus que les organismes sont en haut de la chaîne alimentaire (bioamplification). Les PCB coplanaires (ressemblant à la structure des dioxines) sont 100 à 1000 fois plus toxiques que les non coplanaires.

I.1.2 Dérivés oxygénés des HAP

Parmi les dérivés oxygénés des HAP, les HAP hydroxylés contiennent au moins une fonction hydroxyle et sont donc plus polaires et un peu plus solubles dans l'eau que leurs composés parents. Les OH-HAP sont produits au cours de processus d'oxydation des HAP : photo-oxydation, oxydation chimique ou biochimique. Les HAP sont naturellement métabolisés dans les organismes vivants en différents HAP oxygénés, parmi lesquels les OH-HAP, qui sont parmi les premiers intermédiaires de métabolisation (phase I) avant d'être conjugués (phase II) afin d'être plus facilement excrétés. Les OH-HAP sont plus toxiques et cancérigènes que les HAP car ils sont des agents cancérigènes et mutagènes directs, formant des adduits avec l'ADN, sans activation enzymatique préalable. Ils sont mesurés dans les organismes en tant que biomarqueurs de l'exposition aux HAP. Cependant, ils sont potentiellement présents dans d'autres matrices environnementales contenant des HAP, telles les sols ou sédiments, ou leur taux évolue selon l'activité des microorganismes. En effet, un certain nombre de microorganismes des sols ont pu être identifiés comme capables de dégrader les HAP (essentiellement ceux de faible poids moléculaire), conduisant à la formation d'OH-HAP, certains d'entre eux étant plus ou moins persistants.

Parmi les produits de transformation des HAP, les HAP carbonylés contiennent un ou plusieurs groupes fonctionnels aldéhydes ou cétones. Les diones sont appelées des quinones. Les

coumarines (fonction pyran-2-one) sont toutefois incluses dans les HAP carbonylés. Les quinones sont plus polaires et un peu plus solubles dans l'eau que leurs composés parents, mais ont des pressions de vapeur plus faibles. Les HAP carbonylés sont non seulement produits au cours de processus d'oxydation (photo-oxydation, oxydation chimique par l'ozone ou oxydation biochimique par les organismes), mais également au cours de processus de combustion incomplète. On les retrouve donc potentiellement non seulement dans les organismes vivants, les sols ou sédiments, mais également sur les particules atmosphériques, ou certains peuvent être particulièrement persistants. Les quinones sont généralement plus toxiques et cancérigènes que leurs composés parents, car elles ne nécessitent pas d'activation enzymatique pour former des adduits avec l'ADN et qu'elles peuvent générer des composés oxygénés hyper-réactifs causant des dommages cellulaires. En revanche, la coumarine (3,4-dihydrocoumarin), second métabolite le plus abondant retrouvé dans les organismes vivants, n'est pas toxique à faible dose et peut même avoir des propriétés anti-oxydantes. Cependant, à plus forte teneur, elle peut avoir des effets cancérigènes.

I.2 Méthodes de préparation de l'échantillon pour extraire les contaminants cibles de matrices particulières

I.2.1 Matériaux environnementaux particuliers

La matière particulaire est un ensemble de particules solides et liquides microscopiques, variant en taille, composition et origine. Il existe des particules solides en suspension dans l'air, mais également dans l'eau. Ces dernières peuvent s'agréger dans l'eau et former des sédiments. La composition des sédiments est complexe, contenant des argiles, limons, sables (classés selon leur granulométrie) mais également des phases minérales telles des oxydes de fer ou de manganèse, des carbonates, ou bien encore de la matière organique. Les composés lipophiles persistants (HAP, PCB...) tendent à s'adsorber fortement et à s'accumuler dans les sédiments, particulièrement dans la phase organique et sur les particules fines (argiles).

I.2.2 Méthodes d'extraction des matrices particulières

Afin de caractériser une contamination d'une matrice particulaire solide, il est nécessaire de mettre en œuvre une première étape dans le processus analytique, à savoir l'extraction. Une des méthodes d'extraction classique est l'extraction par Soxhlet. Mais même si cette technique d'extraction par reflux de solvants est efficace, c'est une technique très longue qui nécessite une quantité importante de solvants organiques. Une autre méthode facile d'emploi est l'extraction par solvants assistée par ultra-sons. Elle est cependant considérée comme moins efficace que l'extraction Soxhlet.

Afin de diminuer la durée d'extraction, la consommation de solvants organiques et d'augmenter l'efficacité d'extraction, d'autres méthodes ont été développées ces dernières décennies. Parmi elles, l'extraction assistée par solvants sous pression (ASE ou PLE) est très employée pour extraire les HAP, PCB et certains oxy-HAP de matrices solides. L'extraction par fluides supercritiques (SFE) est également très efficace et plus sélective, mais elle est moins employée car l'investissement est plus coûteux. L'extraction assistée par micro-ondes (MAE), bien que moins employée que la PLE, a l'avantage d'être aussi très rapide, peu consommatrice en solvants. Elle consiste à chauffer quasi instantanément la matrice à extraire en convertissant l'énergie micro-ondes en énergie thermique, grâce à l'emploi d'un solvant ou d'un mélange de solvants possédant un moment dipolaire. La MAE n'a jamais été employée pour extraire simultanément différentes familles de contaminants tels les OH-HAP et les HAP carbonylés. Enfin, une technique d'extraction a été développée encore plus récemment, dont l'objectif était principalement le faible coût d'investissement et de mise en œuvre, ainsi que la rapidité. Il s'agit de l'extraction par dispersion de la matrice solide (MSPD). Elle consiste à introduire des agents solides dispersants au sein de la matrice solide séchée à extraire, et de les broyer ensemble dans un double but : certains agents particuliers permettent le transfert des contaminants ciblés de la matrice déstructurée vers des sites de sorption moins énergétiques ou plus accessibles ; d'autres agents adsorbent des composés de polarité inverse de celle des composés cibles, ces interférents étant ainsi immobilisés. Dans une deuxième étape, les composés cibles sont extraits grâce à un mélange de solvants éluants de polarité proche de la leur, tandis que les interférents restent dans la matrice solide, l'éluat étant ainsi purifié avant l'analyse. La MSPD n'a jamais été développée pour extraire et analyser simultanément différentes familles de POP, tels les HAP et PCB.

I.2.3 Extractions sélectives utilisant des polymères à empreintes moléculaires

La majorité des techniques d'extraction ne sont pas ou très peu sélectives. Cependant, une technique d'extraction sur phase solide (SPE) permet d'extraire de manière très sélective les composés ciblés, éliminant non seulement les composés interférents de polarité inverse (purification) mais également les composés de même polarité mais de structure chimique différente. Les extractions SPE utilisant des matériaux de type polymères à empreintes moléculaires (MIP) reposent sur le principe d'une reconnaissance de taille, de forme et de structure chimique du ou des composés cibles. Les MIPs sont peu employés pour les HAP car les interactions avec les sites de reconnaissance moléculaire sont peu sélectives. En revanche, les MIPs sont employés avec succès pour certains OH-HAP, car les interactions par liaison hydrogène sont plus sélectives. Les MIPs sont employés essentiellement pour l'analyse de OH-

HAP dans des fluides biologiques, quasiment pas pour l'analyse de matrices environnementales.

I.3 Outils chromatographiques pour la séparation et le dosage des HAP, PCB et oxy-HAP

I.3.1 La chromatographie en phase gazeuse

Les mélanges de HAP, même si certains d'entre eux sont peu volatils, peuvent être séparés et analysés en chromatographie en phase gazeuse (CPG) à l'aide de colonnes apolaires classiques de type polyméthylphénylsiloxanes de 30 m de long. Pour améliorer la résolution de certaines paires critiques de HAP, des colonnes plus spécialisées pour les HAP ont été conçues, ou des colonnes de longueur plus longue sont employées. Le détecteur le plus employé est le spectromètre de masse (SM) (désormais devant le détecteur à ionisation de flamme (FID)), généralement avec une source à impact électronique, employant un simple quadripôle ou deux quadripôles en tandem. Les étalons de suivi ou de dosage employés sont généralement des HAP perdeutérés.

Les PCB peuvent eux aussi être séparés et analysés en CPG couplée à un spectromètre de masse, bien que le détecteur ECD à capture d'électrons soit bien plus sensible. Les mêmes colonnes que celles employées pour les HAP peuvent être utilisées pour les PCB, mais certaines colonnes sont plus sélectives pour séparer les PCB de type dioxines. Certains PCB de type dioxine peuvent être employés comme étalons de suivi ou de dosage, car on ne les retrouve pas dans les formulations commerciales et donc dans l'environnement.

Les analyses de HAP hydroxylés ou de quinones sont quant à elles plus délicates en CPG, car ces composés sont plutôt thermolabiles et trop peu volatils. Ils nécessitent donc une étape préliminaire de dérivation avant analyse en CPG, les colonnes employées étant les mêmes que pour les HAP. Concernant les OH-HAP, la fonction alcool est généralement silylée à l'aide de différents types d'agents de silylation commercialisés. Bien connue, cette étape de silylation dépend toutefois des composés hydroxylés et de la matrice à analyser et peut être améliorée en jouant sur le temps de réaction et sur l'ajout de catalyseurs. Concernant les quinones, surtout celles dont les groupes cétones sont en position ortho, c'est plutôt une réaction d'acétylation qui permet de les rendre plus stables et plus volatiles. L'acétylation se fait généralement à l'aide d'anhydride acétique en présence de zinc. La réaction de dérivation se fait en plusieurs étapes et nécessite une étape finale de purification. Bien moins utilisée que la silylation des OH-HAP, la dérivation des quinones nécessite encore une optimisation des différentes étapes.

I.3.2 La chromatographie en phase liquide

Les HAP peuvent être séparés et analysés par chromatographie en phase liquide haute performance (HPLC) ou ultra-performance (UPLC) à l'aide de colonnes dont la phase stationnaire est apolaire (phase greffée en C₁₈) et dont le diamètre de particules est plus généralement de 5 µm ou moins. La phase mobile employée est généralement constituée d'eau et d'acétonitrile. Le détecteur UV est encore largement employé pour détecter les HAP, le SM beaucoup moins, mais c'est le détecteur fluorimétrique qui est de loin le plus sensible.

Concernant les PCB, ils sont peu analysés en HPLC car les phases stationnaires en C₁₈ ne sont en général pas assez efficaces et résolutive pour certaines paires critiques de PCB. Le détecteur le plus employé est l'UV, devant le SM. Les PCB ne sont pas fluorescents.

Enfin, l'analyse des oxy-HAP en HPLC ne nécessite pas de dérivation. Ils peuvent être séparés sur les mêmes types de colonnes que celles employées pour les HAP (C₁₈), avec des phases mobiles constituées d'eau et d'acétonitrile ou de méthanol. Les quinones peuvent être détectées à l'aide d'un détecteur UV ou par chimioluminescence. Les OH-HAP peuvent être quant à eux détectés en UV et par fluorimétrie, mais tous n'ont pas de bons rendements quantiques de fluorescence.

I.4 Conclusion

Les sédiments accumulent certains polluants organiques toxiques et persistants, tels les HAP et PCB, pouvant conduire à la formation de composés oxygénés potentiellement plus toxiques, obtenus par transformation biologique ou à l'issue de procédés de traitements. Il s'agit donc de mettre au point de nouvelles techniques d'extraction et d'analyse multi-résidus, permettant de les analyser et les doser simultanément de manière plus rapide, fiable et sensible.

Chapter II

Optimization of the simultaneous extraction of hydroxy- and carbonyl-PAHs using microwave assisted extraction and choice of chromatographic analytical tools

Publications and communications in relation to this chapter:

- Experimental designs for optimizing multi-residual microwave-assisted extraction and chromatographic analysis of oxygenated (hydroxylated, quinones) metabolites of PAHs in sediments, I. Berger-Brito, N. Machour, C. Morin, F. Portet-Kotalo, *Chromatographia* 81 (2018) 1401-1412. **PUBLICATION.**
- I. Berger, N. Machour, F. Portet-Kotalo, Analytical Tools for Multi-residue Analysis of some Oxygenated Metabolites of PAHs (Hydroxylated, Quinones) in Sediments. ICEMA 2016: 18th International Conference on Environmental Monitoring and Analysis (Germany, Berlin). 19-20 may 2016. **FLASH ORAL COMMUNICATION.**
- I. Berger-Brito, N. Machour, C. Morin, F. Portet-Kotalo, Experimental design for the optimisation of multi-residual analysis of oxygenated metabolites of PAHs (hydroxylated, quinones) in sediments. ICCE 2017 : 16th International Conference on Chemistry and the Environment (Oslo, Norway). 18-22 June 2017. **ORAL PRESENTATION**
- I. Berger, N. Machour, F. Portet-Kotalo, Analytical developments for the analysis of oxygenated metabolites of PAHs (Hydroxylated, Quinones) in sediments. Journée de l'Ecole Doctorale Normande de Chimie (Caen, France). 13 June 2016. **POSTER.**
- I. Berger-Brito, N. Machour, C. Morin, D. Martin, F. Portet-Kotalo, Experimental design for the optimization of multi-residual analysis of oxygenated metabolites of PAHs (hydroxyl, quinones) in sediments. SEP 2017: 12ème Congrès de l'Association Francophone des Sciences Séparatives (Paris, France). 28-30 Marsh 2017. **POSTER**

In this chapter, a methodology for the simultaneous extraction of two groups of oxygenated metabolites of PAHs (hydroxy-PAHs and carbonyl-PAHs, having different chemical properties) was developed using for the first time the microwave assisted extraction (MAE). This extraction method was optimized using a chemometric approach in order to find the factors influencing the extraction and to find a compromise to extract quantitatively the two families of compounds. Two analytical methodologies for their separation, quantification and detection (HPLC coupled to UV and FLD detectors or GC coupled to MS detector) were developed and compared. With HPLC-UV-FLD, the detection of the two families of compounds can be made directly and simultaneously and fluorimetry can achieve very low limits of detection. Concerning the use of GC-MS, MS is a more selective detector than UV or FLD, which allows identification, but it needs to optimize derivatization methods to detect hydroxy-PAHs and carbonyl-PAHs at trace levels.

II.1 Optimization of chromatographic tools for the analysis of oxy-PAHs

Before extracting contaminants from a solid matrix, it is important to validate optimal analytical tools for their quantification. The contaminants were analyzed with two chromatographic instruments, namely gas and liquid chromatographers, taking into consideration the advantages and disadvantages of both methods and of their detection equipment: gas chromatography coupled with a mass spectrometer (GC-MS) and liquid chromatography coupled with a fluorimeter (FLD) and an UV detector (HPLC-UV-FLD).

II.1.1 Liquid chromatography

II.1.1.1 Chromatographic conditions

Initially, the optimal wavelengths for the detection of quinones and hydroxy-PAHs standards were determined using a Varian Cary Eclipse fluorescence spectrophotometer from Agilent Technologies and an ultraviolet/visible UV-1650PC spectrophotometer from Shimadzu, for finding the optimal fluorescence emission/excitation wavelengths and the optimal UV absorbance wavelength of each compound. Quinones were not fluorescent enough and could only be detected by UV absorbance. The optimal wavelength was 254 nm for all the target quinones (Table II.1), but it was 271 nm for the coumarin. The target hydroxy-PAHs (Table II.1) had high fluorescence quantum efficiencies, except 9-hydroxyfluorene, which was removed from the study. The determined optimal wavelengths were chosen for the wavelengths programming using the HPLC apparatus Ultimate 3000 from Thermofisher (Villebon-sur-Yvette,

France), coupled to a fluorescence detector (FLD) and an UV/visible detector. The HPLC system was equipped with a Nucleodur column C₁₈ PAH (100 × 4 mm, d_p = 3 μm) from Macherey-Nagel (Hoerdt, France), for the separation of the compound mixtures. Carbonyl-PAHs were detected thanks to the UV detector and hydroxy-PAHs thanks to the fluorescence detector. The wavelengths programming is described in Table II.1. The optimum separation was performed at a constant flow of 1 mL min⁻¹, at 30°C and with a mobile phase gradient set as follows: 0 - 0.5 min 40% of acetonitrile in water, 0.5 - 6.5 min linear increase until 100% of acetonitrile, keeping it 0.5 min constant and turning to 40% acetonitrile during 15 min.

Table II.1 Retention times and optimal wavelengths of the target carbonyl- and hydroxy-PAHs by HPLC-UV-FLD

Retention time (min)	Compounds	Abbreviation	Wavelengths for detection (nm)
0.66	1,4-Benzoquinone	1,4-BQ	254 ^a
0.94	1,2-Naphthoquinone	1,2-NQ	254 ^a
1.11	Coumarin	-	271 ^a
1.51	1,4-Naphthoquinone	1,4-NQ	254 ^a
2.21	9,10-Phenanthrenequinone	9,10-PQ	254 ^a
3.54	9,10-Anthracenequinone	9,10-AQ	254 ^a
1.85	2-Naphthol	2-OHNaph	220/350 ^b
2.71	2-Hydroxyfluorene	2-OHFluo	270/320 ^b
3.13	9-Phenanthrol	9-OHPhen	245/390 ^b
3.91	1-Hydroxypyrene	1-OHPyr	240/400 ^b

a UV detection

b FLD detection

The lighter hydroxy-PAHs (of lower mole weight) were the first to elute due to their lower affinity for the apolar stationary phase (Figure II.1). The same was observed for the carbonyl-PAHs, in which 1,4-BQ (see Table II.1 for the abbreviations used for oxy-PAHs) was the first to elute and 9,10-AQ was the last one (Figure II.2).

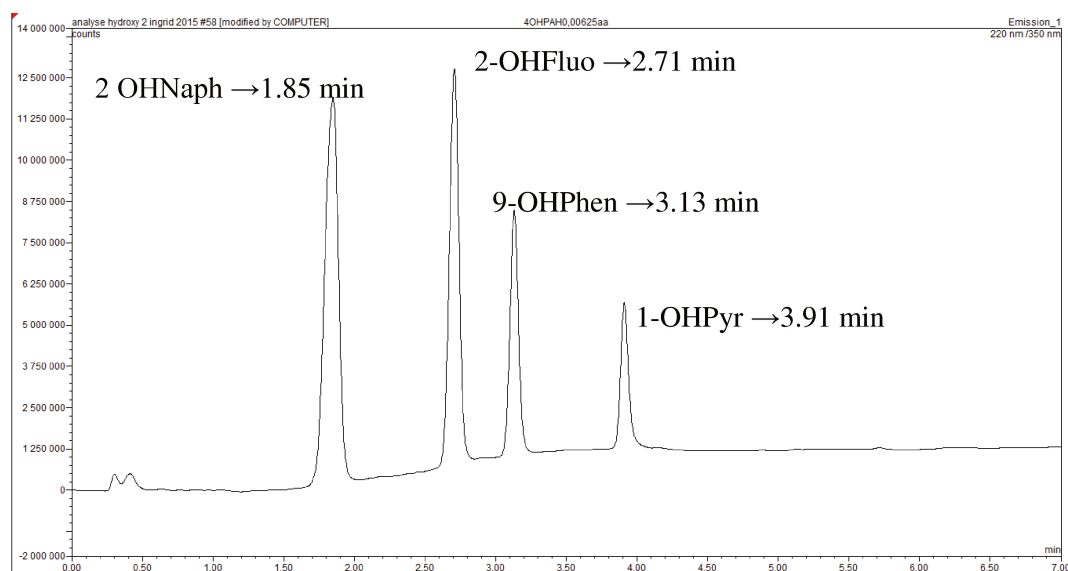


Figure II.1 Separation of the four hydroxy-PAHs, detected with FLD detector ($0.00625 \text{ mg L}^{-1}$)

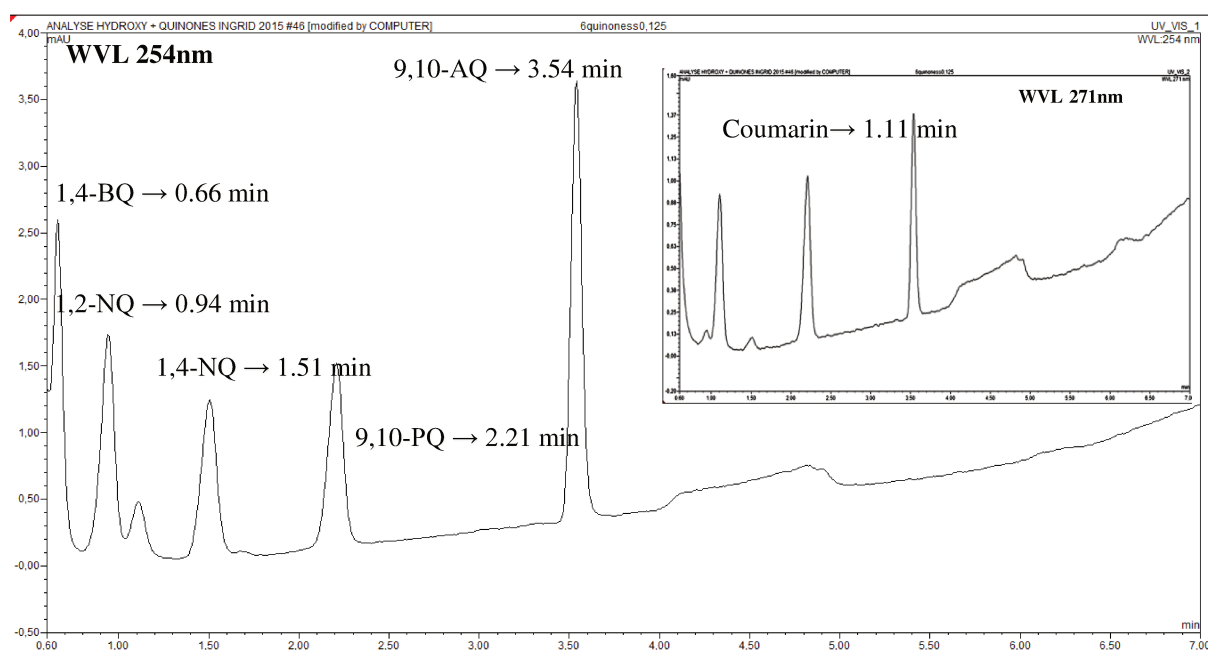


Figure II.2 Separation of the five quinones and coumarin, detected with UV detector (0.125 mg L^{-1})

II.1.1.2 Calibration curves, limits of detection and quantification

a. Mathematical methodology for determining LOD/LOQ

Calibration curves were established using 5-6 levels of concentrations, using an external calibration methodology for the 5 quinones, the coumarin and the 4 hydroxy-PAHs. The tests were made in triplicate for the following concentrations: 0.0125 mg L^{-1} was the standard stock solution for hydroxy-PAHs, then dilutions by factors 2, 5, 10, 16 and 20 were done. For carbonyl-PAHs the standard stock solution was prepared at 0.125 mg L^{-1} and then dilutions by factors 2, 5, 10, 16 and 20 were done. Calibration curves were obtained through mathematical

linear regressions, with equations linking the area of the chromatographic peaks and their concentration, in the form: $\text{Area} = a_1 \times [C] + a_0$. Aberrant points were eliminated using regression residuals, and the results can be seen in the Table II.2. All the correlation coefficients were $R^2 > 0.990$. Limits of detection (LOD) and quantification (LOQ) were calculated from the linear regression of the calibration curves using the equations $\text{LOD} = 3.3 \times S_y / a_1$ and $\text{LOQ} = 10 \times S_y / a_1$ where a_1 is the slope of the linear regression of the calibration curve and S_y is the standard error at the y intercept of the regression line.

Table II.2 Method performance characteristics: calibration curves, linearity range, correlation coefficient R^2 , limit of detection (LOD) and limit of quantification (LOQ) obtained by the mathematical methodology.

Compounds	Calibration curve	Linearity range ($\mu\text{g L}^{-1}$)	R^2	LOD ($\mu\text{g L}^{-1}$)	LOQ ($\mu\text{g L}^{-1}$)
2-OHNaph	$y = 273374357 x + 7785$	[1.25-12.5]	0.996	0.3	0.9
2-OHFluo	$y = 162914598 x - 5899$	[1.25-12.5]	0.995	0.3	1.0
9-OHPhen	$y = 106654058 x - 8634$	[1.25-12.5]	0.996	0.3	1.0
1-OHPyr	$y = 73004648 x - 18612$	[0.625-12.5]	0.999	0.2	0.6
1,4- BQ	$y = 1.0847 x + 0.0011$	[7.81-12.5]	0.997	2.4	8.0
1,2-NQ	$y = 1.0709 x + 0.0006$	[12.5-125.0]	0.996	2.9	9.6
Coumarin	$y = 0.6583 x + 0.0012$	[12.5-125.0]	0.996	2.8	9.2
1,4-NQ	$y = 1.3445 x + 0.0038$	[25.0-125.0]	0.991	4.8	16.2
9,10-PQ	$y = 1.0169 x - 0.0001$	[12.5-125.0]	0.997	2.6	8.4
9,10-AQ	$y = 2.3656 x - 0.0035$	[12.5-125.0]	0.998	3.1	10.2

It appears from the results shown in Table II.2 that the limits of detection of hydroxy-PAHs obtained through FLD detection are ten times lower than those of carbonyl-PAHs obtained through UV detection.

b. Signal-to-noise methodology for determining LOD/LOQ

The LOD and LOQ were also calculated using a more conventional methodology that estimates the limit of detection as three times the signal-to-noise (S/N) ratio (Equation 1) of a blank sample (which gives the height of the noise), and the limit of quantification as ten times the S/N ratio (Equation 2), according to the IUPAC criterion:

Equation 1 Calculation of the limit of detection (LOD) by S/N methodology

$$LOD = \frac{\text{Height of noise} \times 3 \times \text{lower concentration}}{\text{Height of peak of lower concentration}}$$

where the height of the signal of the analyte obtained at its lower concentration is compared to the height of the noise of a blank sample (in the same area of the chromatogram).

Equation 2 Calculation of the limit of quantification (LOQ) by S/N methodology

$$LOQ = \frac{\text{Height of noise} \times 10 \times \text{lower concentration}}{\text{Height of peak of lower concentration}}$$

This methodology gave significantly lower LODs and LOQs (about 4 times lower for quinones and 20 times lower for OH-PAHs) than the mathematical one (Table II.3). It is possible that working with only three replicates at each concentration level is not enough for the mathematical modeling and that the standard error S_y at the y intercept was overestimated, leading to high values of LOD and LOQ. In any case, LODs and LOQs are generally given using the S/N methodology in the literature and only LODs and LOQs obtained from the S/N methodology will be compared in the following studies.

Table II.3 Limit of detection (LOD) and limit of quantification (LOQ) of quinones and hydroxy-PAHs obtained by the S/N methodology

Compounds	LOD ($\mu\text{g L}^{-1}$)	LOQ ($\mu\text{g L}^{-1}$)
2-OHNaph	0.0091	0.0305
2-OHFluo	0.0130	0.0433
9-OHPhen	0.0191	0.0637
1-OHPyr	0.0094	0.0315
1,4- BQ	0.7631	2.5436
1,2-NQ	0.8203	2.7344
Coumarin	1.6164	5.3879
1,4-NQ	1.0758	3.5861
9,10-PQ	1.0915	3.6384
9,10-AQ	0.4292	1.4308

LODs obtained by the S/N methodology for carbonyl-PAHs analyzed in HPLC-UV are ranging from 0.4 to 1.6 $\mu\text{g L}^{-1}$ and contrast with LODs obtained for hydroxy-PAHs analyzed in HPLC-FLD, which are markedly lower, ranging from 0.009 to 0.019 $\mu\text{g L}^{-1}$. This is due to the fluorescence detection, which is known to be particularly more sensitive than UV, or MS detections.

II.1.2 Gas chromatography

II.1.2.1 Gas chromatography-mass spectrometry without derivatization

a. Chromatographic conditions

For the analyses of oxy-PAHs performed using a gas chromatograph (GC), the tests were made with a GC (model 6850) coupled to a mass spectrometer (GC-MS) (model 5975C) from Agilent Technologies. At first, the ramp of temperature was optimized, and the mass spectra were found in full SCAN mode to identify the compounds with the NIST mass spectra library and determine the main ions. Thereafter the detection was conducted in selected ion monitoring (SIM) for obtaining a better sensitivity for quantification (Table II.4). 1 μ L of a mixture containing the 5 non-derivatized quinones, the coumarin, the 4 non-derivatized hydroxy-PAHs and 2 internal standards (perdeuterated Phenanthrene-D10 (PhenD10) and Perylene-D12 (PerD12)), were injected in the splitless mode at 285°C and separated with the column Zebron ZB – SemiVolatiles (60 m length x 0.25 mm i.d. x 0.25 μ m film thickness) from Phenomenex (Le Pecq, France). The oven program started at 50°C (1.1 min) to 190°C (at 40°C min⁻¹) followed by an increase to 300°C (at 6°C min⁻¹), staying at 300°C during 4 min, under a constant carrier gas (He) flow of 1.4 mL min⁻¹ and temperature of the transfer line was set at 300°C.

Table II.4 Information on the detected ions in GC-MS (SIM mode) for the non derivatized oxy-PAHs

Groups (min)	m/z (Dalton)	Compounds	Retention time (min)
6→8	81;108;110	1,4-BQ	6.77
	158;130;102	1,4-NQ	7.90
8→11	146;118	Coumarin	8.07
	144;115	2-OHNaph	8.59
	158;102	1,2-NQ	9.65
11→15	188	PhenD10	11.31
	152;181;182	2-OHFluo	12.85
	208;180;152	9,10-AQ	13.58
15→19	194;165	9-OHPhen	15.47
	208;180;152	9,10-PQ	16.64
19→27	218;189	1-OHPyr	20.30
	264	PerD12	25.29

In Figure II.3 is reported the typical chromatogram of the mixture containing the 10 oxy-PAHs. It can be noted that two quinones (9,10-PQ and 1,2-NQ) are missing, that couldn't be detected

in the concentration ranges that were prepared, which were: 0.5 mg L⁻¹, 1 mg L⁻¹, 2 mg L⁻¹, 5 mg L⁻¹, 8 mg L⁻¹ and 10 mg L⁻¹. The two missing quinones are diketones in ortho position, which are known to be more affected by thermal degradation ¹⁹¹. We can also note on the chromatogram (Figure II.3) pronounced peak tailing, particularly for hydroxy-PAHs.

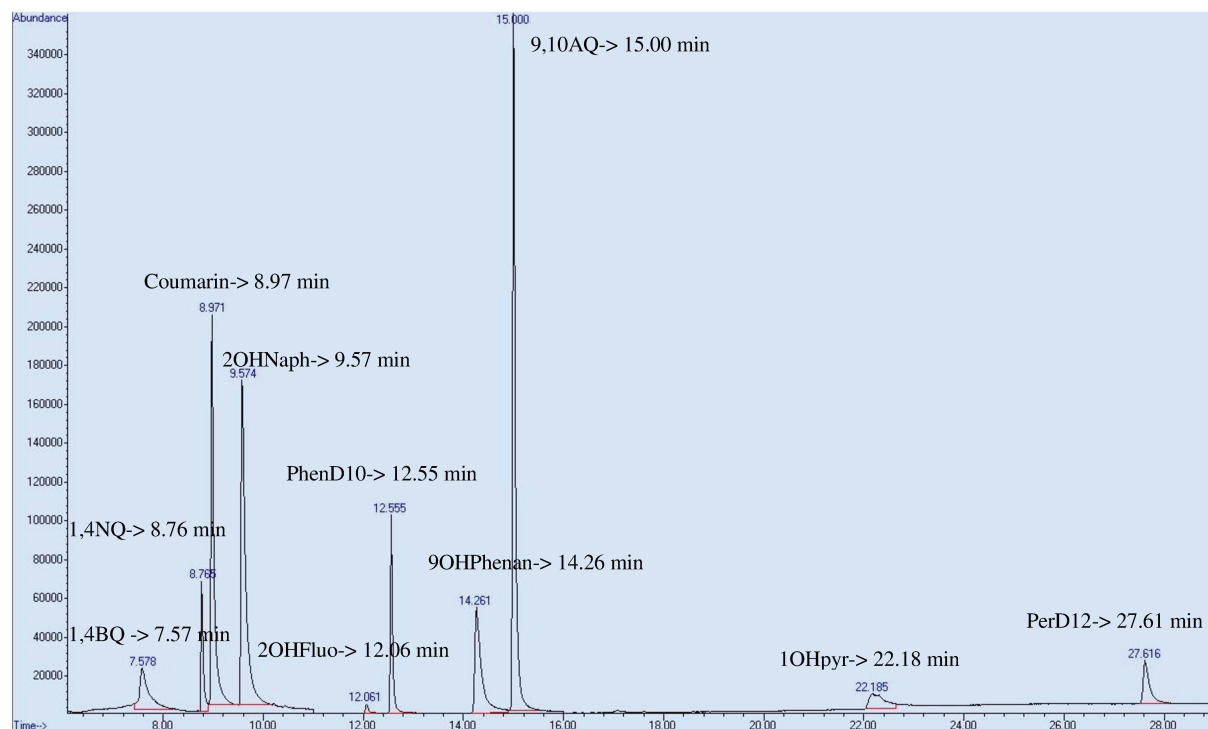


Figure II.3 GC-MS analysis of the 10 oxy-PAHs (5 mg L⁻¹) and two internal standards (1 mg L⁻¹) without any derivatization step.

b. Calibration curves, limits of detection and limits of quantification

Calibration curves were established using 6-7 levels of concentrations (0.5 mg L⁻¹, 1 mg L⁻¹, 2 mg L⁻¹, 5 mg L⁻¹, 8 mg L⁻¹ and 10 mg L⁻¹), using the internal calibration methodology, to avoid the problems due to the low reproducibility of the liquid injection. The internal standard PhenD10 (1 mg L⁻¹) was used for all the oxy-PAHs, except for 1-OHpyr which was quantified thanks to the higher molecular weight internal standard PerD12 (1 mg L⁻¹).

The calibration curves of the oxy-PAHs were obtained using the same mathematical methodology than for liquid chromatography, using triplicates at each concentration levels. Equations of the calibration curves were in the form: Area (oxy-PAH)/Area (Internal standard) = $a_1 \times [C_{oxyPAH}]$, with the concentration of the internal standards being 1 mg L⁻¹ and the intercept at the origin a_0 being neglected (and not forced to zero) (Table II.5). Aberrant points were eliminated using regression residuals, but linear correlation coefficients were not all satisfactory, some of them being $R^2 < 0.990$ (Table II.5). The LOD and LOQ were calculated with the two methodologies mentioned previously that is using S_y , the standard error at the y intercept of the regression line, or the signal-to-noise methodology. The limits were 30-620

lower calculating LODs/LOQs with the signal-to-noise methodology (excepting for 1,4-NQ), but in general, LODs and LOQs were high: GC-MS direct analysis of oxy-PAHs was not sensitive enough (Table II.5). For 1,2-NQ and 9,10-PQ, just a small peak was detected at the highest concentration of 10 mg L⁻¹, this is why a linearity curve was not made for these compounds.

Table II.5 Method performance characteristics: Calibration curves, linearity range, correlation coefficients R², limit of detection (LOD) and limit of quantification (LOQ) obtained with the two methodologies

Compounds	Calibration curve	Linearity Range (mg L ⁻¹)	R ²	LOD (mg L ⁻¹) ^a	LOQ (mg L ⁻¹) ^a	LOD (mg L ⁻¹) ^b	LOQ (mg L ⁻¹) ^b
1,4- BQ	y = 0.0739 x	[5.0-10.0]	0.9236	1.2274	4.0915	0.0151	0.0504
1,2-NQ	-	> 10.0	-	> 10.0	> 33.33	-	-
1,4-NQ	y = 2.6204 x	[2.0-10.0]	0.9834	0.558	1.859	0.1276	0.4252
9,10-PQ	-	> 10.0	-	> 10.0	> 33.33	-	-
9,10-AQ	y = 5.8784 x	[1.0-10.0]	0.9952	0.2543	0.8477	0.0082	0.0275
Coumarin	y = 0.4971 x	[1.0-10.0]	0.9953	0.2929	0.9762	0.0022	0.0073
2-OHNaph	y = 0.8167 x	[1.0-10.0]	0.9978	0.1822	0.6075	0.0014	0.0047
2-OHFluo	y = 0.6311 x	[1.0-10.0]	0.9943	0.3118	1.0394	0.0005	0.0015
9-OHPhen	y = 0.2000 x	[2.0-10.0]	0.9821	0.5986	1.9953	0.0166	0.0555
1-OHPyr	y = 0.6777 x	[2.0-10.0]	0.9908	0.3776	1.2586	0.0046	0.0153

a LOD and LOQ calculated with the standard deviation at the y-intercept

b LOD and LOQ calculated with the signal-to-noise methodology

II.1.2.2 Gas chromatography- mass spectrometry with derivatization

a. Silylation of hydroxy-PAHs

For the analysis of hydroxy-PAHs by GC-MS, a methodology of derivatization was employed to improve the detection sensitivity. It is well known that a silylation reaction on the OH group favors the formation of a silylated derivative that is less sensitive to thermal degradation and is more volatile, which is favorable for GC analysis²¹² (Figure II.4). For the silylation of hydroxy-PAHs, the best solvent and the best reaction time were tested, adapting the process of silylation from the article of Baltrons et al.⁶³. Acetonitrile was chosen as the dilution solvent according to the possibility to use it in MAE a posteriori. The silylation reagent was BSTFA (N,O-Bis(trimethylsilyl)trifluoroacetamide), an usual reagent for silylation of hydroxyl groups that promotes the derivatization of sterically hindered hydroxyl groups; silylation can be improved by adding TMCS (trimethylchlorosilane), a silylation catalyst that increases the reactivity of

BSTFA. One silyl group of the BSTFA molecule replaces the active hydrogen of the hydroxyl group of the hydroxy-PAHs (Figure II.4).

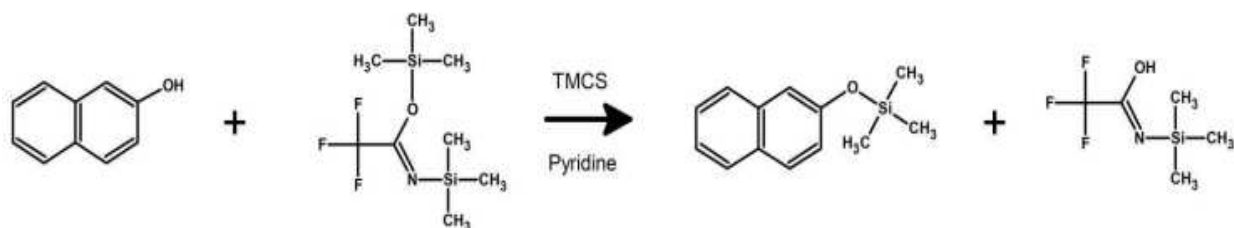


Figure II.4: Reaction of silylation of an hydroxy-PAHs using BSTFA and TMCS as catalyser ²¹³

The derivatization reagents (BSTFA/TMCS 99/1%) were used for silylating the four hydroxy-PAHs at 60°C, varying the reaction time in the range 5-60 min. The duration of silylation did not significantly influence the peak areas of the silylated 2-OHNaph, 2-OHFluo or 9-OHPhen ($p > 0.05$, Fisher test, $n = 3$ replicates). But the silylation of 1-OHPyr was significantly improved at $t = 5$ min compared to 30, 45 and 60 min ($p < 0.05$, Fisher test, $n = 3$) (Figure II.5).

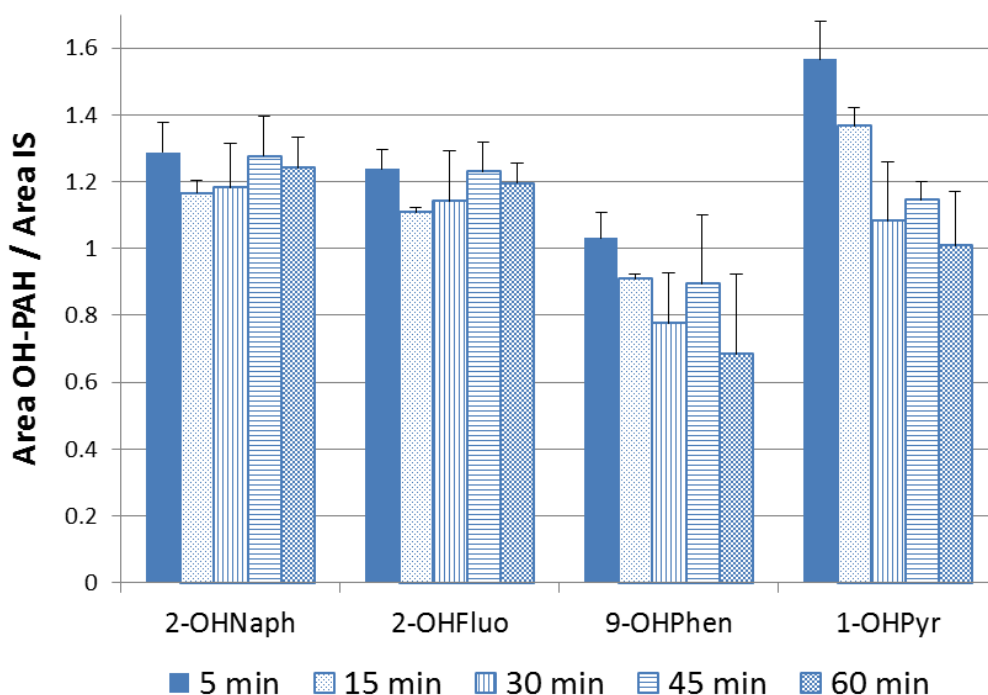


Figure II.5 Means of the areas of hydroxy-PAHs on Areas of Internal standard ($n = 3$) in the optimization of the silylation time (5-60 min)

Moreover, increasing the silylation time tended to decrease the repeatability calculated from the peak areas, which was not favourable. So 5 min of silylation was chosen for the following experiments. Furthermore, Kumirska et al. ²¹⁴ demonstrated that adding a mixture of ethyl acetate and pyridine to their derivatization reagents could improve the derivatization of some hydroxylated drugs. Ethyl acetate, as a neutral organic solvent, improves the dissolution of the analytes and pyridine as a Lewis base activates the acid compounds and drives the reaction in

the product direction ²¹⁵. Therefore, a volume of pyridine and a volume of ethyl acetate were added in the silylation process of hydroxy-PAHs, with (BSTFA+TMCS)/ethyl acetate/pyridine in the proportions 2/1/1. The areas of the hydroxy-PAHs were not modified significantly in the range 0.2-1 mg L⁻¹ (p > 0.05, Fisher test, n = 3 replicates) but the linearity of the calibration curves was better (considering the linear correlation coefficients for each hydroxy-PAH), and also the LODs and LOQs were decreased by factors 2.4-4.9. Consequently, the addition of the ethyl acetate-pyridine mixture was more favourable for the silylation of hydroxy-PAHs at the lowest concentrations. Kumirska et al. showed that prolonging the reaction time in the presence of an ethyl acetate-pyridine mixture could reduce the efficiency of silylation and increasing the reaction temperature over 60°C did not improve its efficiency ²¹⁵. So we did not change the temperature and the silylation time. In our best conditions, 1000 µL of sample (diluted in acetonitrile) were silylated adding 50 µL of BSTFA/TMCS 99/1, 25 µL pyridine and 25 µL ethyl acetate at 60°C during 5 min with a constant stirring. After, a volume of 10 µL of the internal standards (PhenD10 and PerD12 at 100 mg L⁻¹) was added to 990 µL of the silylated sample and injected in GC-MS.

To be sure of the efficiency of the silylation process, tests for determining the derivatization yields (%) were made in quintuplicate (with a concentration of 5 mg L⁻¹); the selected m/z ions of the silylated and non-silylated compounds were searched on the same chromatogram (Figure II.6). Silylation of the OH-PAHs in our optimized conditions could be considered as quantitative and reproducible (Table II.6), the residuals of the non-derivatized compounds being negligible.

Table II.6 Tests in quintuplicate at 5 mg L⁻¹ for the determination of the silylation yields (%)

Compounds	Silylated			Non-silylated		
	Mean (%)	Standard Deviation (%)	Relative standard deviation (RSD) %	Mean (%)	Standard Deviation (%)	Relative standard deviation (RSD) %
2-OHNaph	152.1	11.3	7.4	5.1	6.6	129.6
2-OHFluo	127.5	5.9	4.6	4.0	3.3	83.0
9-OHPhen	106.1	5.0	4.7	4.5	3.8	84.2
1-OHPyr	143.0	11.4	8.0	8.3	7.1	85.6

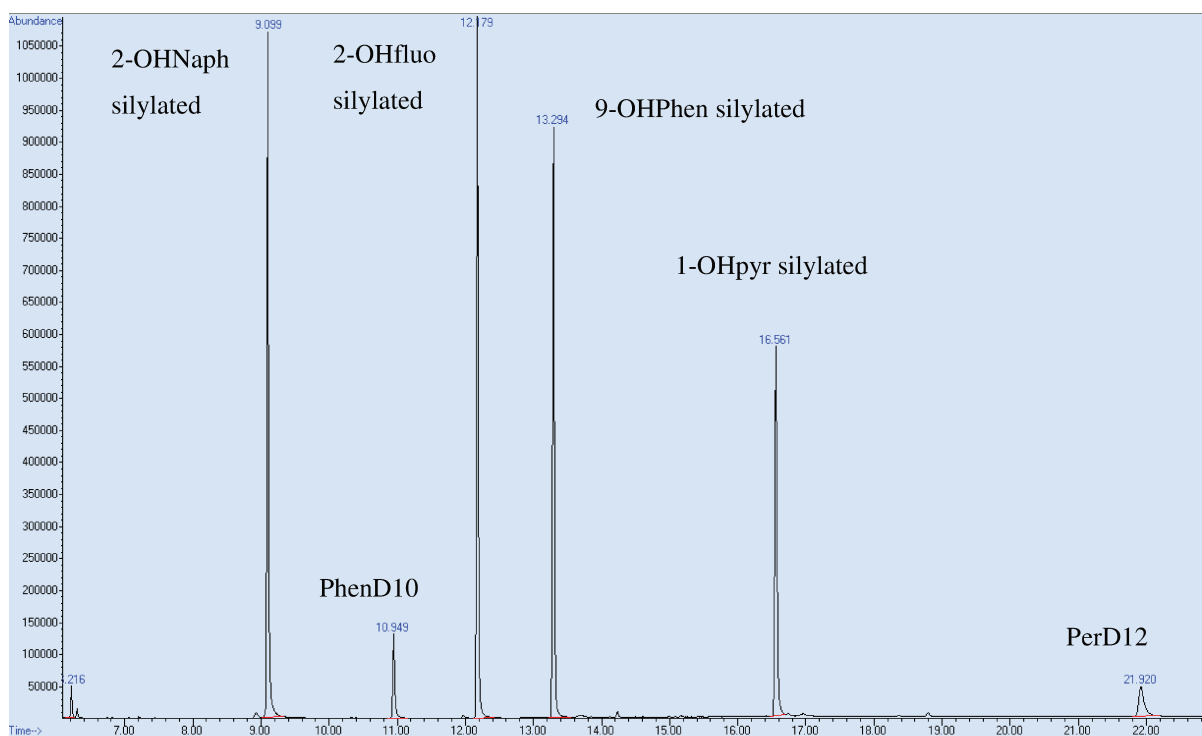


Figure II.6: Chromatogram of the 4 silylated OH-PAHs (5 mg L⁻¹) and 2 internal standards (1 mg L⁻¹). Not silylated OH-PAHs are not visible (at trace levels).

b. Acetylation of quinones

Among the carbonyl-PAHs studied, it must be recalled that coumarin is not a quinone (that is a dione structure) but is a benzopyrone which is thermally stable and does not need derivatization to be analysed by GC-MS.

Concerning the derivatization of quinones, the methodology of Cho et al.¹⁹² and Sousa et al.¹⁹¹ was first applied and then modified. It consisted in converting the carbonyl moieties to their diacetylated derivatives, which are known to be more resistant to high temperatures and confer higher volatility to the derivatives, by a reaction with acetic anhydride in the presence of zinc powder, in two steps (Figure II.7). In this reaction there is the introduction of two acetyl groups consecutive to the rupture of the two ketone double bonds. The reduction of the ketone groups is accompanied by an oxidation of the Zn.

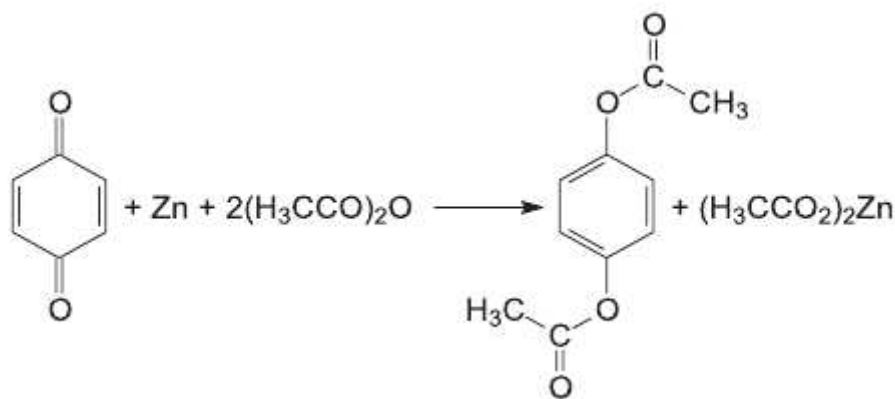


Figure II.7: Reaction of acetylation of quinones using acetic anhydride and Zn powder ¹⁹¹

At the beginning, the derivatization reaction was not necessary optimized. It was performed in these conditions: 400 μL of acetic anhydride (reagent) were added to 200 μL of the solution containing the quinones at 1 mg L^{-1} , and 0.15 g of zinc was added; the reaction was done at 80°C for 15 minutes with a constant stirring. The solution was cooled for about 5 minutes at ambient temperature and 0.15g of Zn was added again, for a new step of 15 min reaction at 80°C; the reactor was cooled again for 5 minutes. Thereafter a liquid-liquid extraction (LLE) was performed to eliminate the polar residues of the reaction. For the LLE, 1 mL of water and 3 mL of pentane were mixed with the products of the reaction, and centrifuged for 10 minutes at 2000 rpm. The aqueous phase (bottom phase) was discarded and the organic phase (upper phase) was recovered, evaporated under a nitrogen flow (after adding 40 μL of octan-1-ol as solvent keeper), re-dissolved in 158 μL of acetonitrile; at the end, 2 μL of the two internal standards (PhenD10 and PerD12 at 100 mg L^{-1}) were added to the 198 μL of the final solution, which was analyzed in GC-MS.

To optimize the derivatization of quinones, different amounts of Zn were tested, in the range 0.1-0.25 g. Adding higher quantities of Zn presented better results on the areas of acetylated derivatives, but all the results had large relative standard deviations (RSD), that might be due to the heterogeneity of the Zn powder (Table II.7).

Table II.7 Influence of different amounts of Zn added to the derivatization process (0.1-0.25 g) on the mean areas of acetylated quinones (divided by the area of the internal standards) (C=1 mg L⁻¹) (n = 3)

Quinones	0.1 g Zn		0.15 g Zn		0.2 g Zn		0.25 g Zn	
	Mean areas	RSD %	Mean areas	RSD %	Mean areas	RSD %	Mean areas	RSD %
1,4-BQ acetylated	0.09	35.9	0.16	75.5	0.17	38.2	0.16	7.2
1,2-NQ acetylated	0.05	118.5	0.16	111.4	0.16	16.2	0.18	19.6
9,10-PQ acetylated	0.05	116.5	0.24	115.8	0.16	4.2	0.50	54.9

Therefore, tests with sifted Zn were made, and the improvement of the acetylation efficiency was particularly notable for 0.1 g and for 0.25 g of Zn added during the derivatization process. The two best results, using 0.1 g or 0.25 g of Zn, were compared with or without Zn sifting: the addition of 0.1 g sifted Zn showed higher results compared to the addition of 0.25 g not sifted Zn (Table II.8). So, the lower quantity of Zn (0.1 g) was chosen for the other optimization steps of the process. It must be underlined that it was particularly crucial to use the finest and the most homogeneous particles of Zn to obtain suitable reproducibility on quantification.

Table II.8 Influence of different amounts of Zn, sifted or not, added to the derivatization process (0.1-0.25 g) on the mean areas of acetylated quinones (divided by the area of the internal standard) (C=1 mg L⁻¹) (n=3)

Quinones	0.1 g sifted Zn		0.25 g sifted Zn		0.1 g not-sifted Zn		0.25 g not-sifted Zn	
	Mean area	RSD %	Mean area	RSD %	Mean area	RSD %	Mean area	RSD %
1,4-BQ acetylated	0.23	50.1	0.16	22.3	0.09	35.9	0.16	7.21
1,2- NQ acetylated	0.20	20.5	0.12	1.1	0.05	118.5	0.18	19.6
9,10-PQ acetylated	0.34	30.6	0.14	6.1	0.05	116.5	0.49	54.9

Different volumes of the acetic anhydride reagent were also tested, in the range 400-800 μ L. Adding more reagent was only favorable for the acetylation of 1,4-BQ, but not for the other quinones. So 400 μ L was kept for the other tests. The duration of the two steps of reaction was also tested, in the range 5-30 min. Decreasing the reaction time from 2 \times 30 min to 2 \times 5 min increased slightly the acetylation of all the quinones, but the reproducibility on quantification (n=3) was worse with a reaction time of 5 min for the two steps. Therefore, an intermediate reaction time of 2 \times 15 min was kept for the other tests.

At last, the liquid-liquid extraction step used to remove the polar impurities and the excess of anhydride acetic was modified, increasing the volume of water (1000-1500 μ L) or replacing pentane (3 mL) by dichloromethane (3 mL) (Figure II.8).

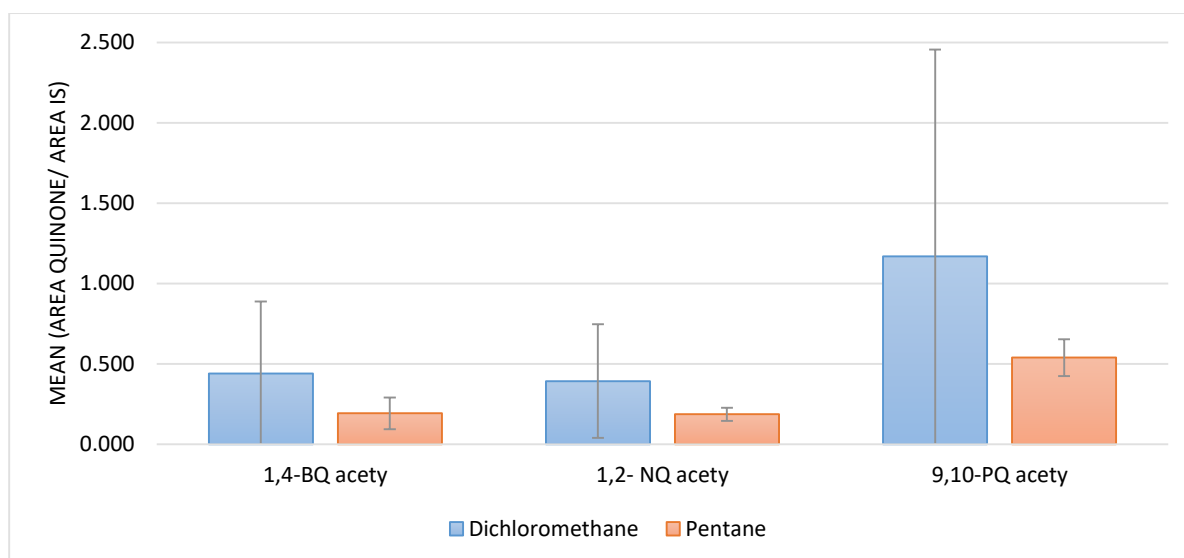


Figure II.8: Comparison of the mean areas of quinones (divided by the areas of internal standards) after centrifugation and recovery in 3mL of pentane or 3 mL methylene chloride (n=3).

Varying the volume of water did not change the recoveries of quinones, but replacing the pentane by dichloromethane significantly increased their recoveries. Probably the upper layer of the too volatile pentane was lost during the centrifugation step (10 min at 2000 rpm) whereas methylene chloride, found in the bottom part, could be quantitatively recovered.

The mean areas of the three quinones increased considerably after changing pentane by dichloromethane in the LLE process, but the standard deviation also (Figure II.8). Further tests were made using dichloromethane as the organic solvent for LLE, but paying very close attention to the recovery of the whole bottom phase; thus the huge standard deviation was posteriorly corrected.

So, in our best conditions, 0.1 g of Zn fine powder and 400 μL of acetic anhydride were added to 1000 μL of sample (containing the quinones diluted in acetonitrile), reacting during 15 min at 80°C. After 5 min cooling, 0.1 g of Zn was added again and the reaction went on 15 min at 80°C. After 5 min cooling again, 1000 μL of water and 3000 μL of dichloromethane were added, vortexed and centrifuged for 10 min at 2000 rpm. The lower organic phase fraction was carefully recovered whereas the upper aqueous phase was discarded. The organic phase was evaporated under a gentle nitrogen flow after addition of 60 μL of octan-1-ol (solvent keeper). At last, 10 μL of the two internal perdeuterated standards (100 mg L^{-1}) were added and the volume was completed with 930 μL acetonitrile that is the same volume than initially sampled, before injection in the GC-MS system.

The derivatization reaction yield (%) of the quinones was established on five experiments (at 5 mg L^{-1}), following the target m/z ions of the derivatized and not derivatized quinones on the same chromatogram (Figure II.9). Table II.9 shows that quinones were quantitatively

acetylated, excepting 9,10-AQ which result is significantly smaller as compared with the other ones. Similar results were obtained by Sousa et al ¹⁹¹, even for 9,10-AQ.

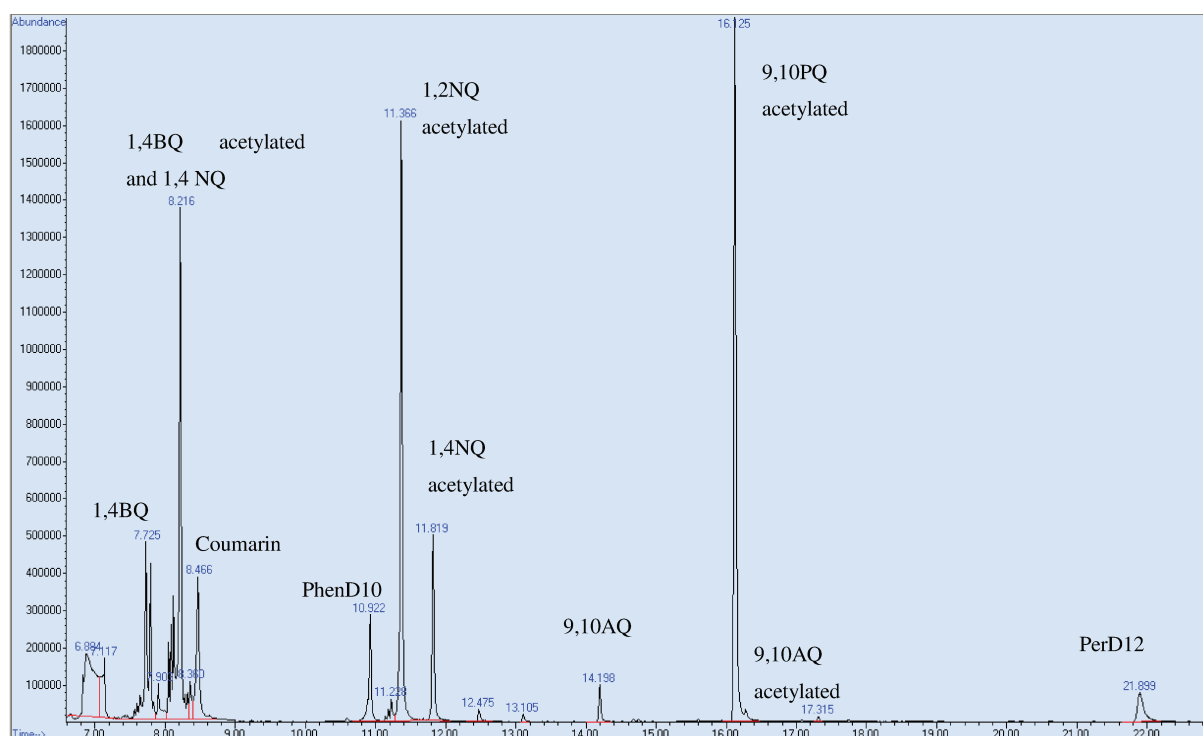


Figure II.9: Chromatogram of 5 acetylated quinones and coumarin (5 mg L^{-1}), 3 non-acetylated quinones (the two others are not visible, at trace levels) and 2 internal standards (1 mg L^{-1})

Table II.9 Acetylation yields (%) of the quinones

Quinones	This work (%)	Sousa et al. (%) ¹⁹¹
1,4-BQ acetylated	99.3	100.0
1,2-NQ acetylated	97.8	99.7
1,4-NQ acetylated	96.4	95.8
9,10-PQ acetylated	102.3	97.8
9,10-AQ acetylated	37.1	23.9

c. Chromatographic conditions, calibration curves and limits of detection and of quantification

Derivatized quinones and hydroxy-PAHs were analyzed in GC-MS with the same oven programming. The oven program started at 50°C (1.1 min) to 200°C (at $40^\circ\text{C min}^{-1}$) followed by an increase to 300°C (at $10^\circ\text{C min}^{-1}$) during 6 min, under a constant He carrier gas flow of 1.4 mL min^{-1} . The temperature of the transfer line was set at 300°C and the detection of the analytes was first conducted in full SCAN mode to obtain the complete mass spectra of the derivatives and confirmation of their retention times; thereafter they were analyzed in selected ion

monitoring (SIM) for better sensitivity, with the selected masses that are shown in Table II.10. It must be mentioned that PhenD10 was used as an internal standard for all the derivatized compounds, excepting the silylated 1-OHPyr and the acetylated 9,10-PQ and 9,10-AQ which were quantified using PerD12 as an internal standard.

Table II.10 Information on the detected ions in GC-MS in SIM mode for the derivatized oxy-PAHs

Groups (min)	m/z (Dalton)	Compounds	Retention times (min)
6→9	110; 194	1,4-BQ acetylated	8.22
	118; 146	Coumarin	8.47
	201, 216	2-OHNaph silylated	8.06
	188	PhenD10	10.93
9→12	160; 244	1,2-NQ acetylated	11.37
	160; 244	1,4-NQ acetylated	11.82
	239; 254	2-OHFluo silylated	10.89
	251; 266	9-OHPhen silylated	11.95
12→21	290	1-OHPyr silylated	15.01
	152; 210; 294	9,10-PQ acetylated	16.13
	152; 210; 294	9,10AQ acetylated	16.23
	264	PerD12	21.89

Internal calibration curves were done using triplicates at each concentration levels, to determine LODs and LOQs with the mathematical and the S/N procedures. First, for hydroxy-PAHs, standard solutions were derivatized in the range 0.18 mg L⁻¹, 0.45 mg L⁻¹, 0.91 mg L⁻¹, 1.82 mg L⁻¹, 2.73 mg L⁻¹ and 3.64 mg L⁻¹. In the optimized conditions of silylation, LODs were improved for all the hydroxy-PAHs by factors from 1.4 to 3.6 comparing the results obtained by the mathematical method, and from 2.3 to 7.3 using the signal-to-noise methodology (Tables II.5 and II.11).

Table II.11 Method performance characteristics for silylated hydroxy-PAHs: Calibration curves, linearity range, correlation coefficients R^2 , limits of detection (LOD) and limits of quantification (LOQ) obtained with the two methodologies.

Hydroxy-PAH	Calibration curves	Linearity Range (mg L ⁻¹)	R^2	LOD (mg L ⁻¹) ^a	LOQ (mg L ⁻¹) ^a	LOD (mg L ⁻¹) ^b	LOQ (mg L ⁻¹) ^b
2-OHNaph silylated	$y = 1.164x$	[0.5-5.0]	0.992	0.135	0.451	0.0003	0.0009
2-OHFluo silylated	$y = 1.131x$	[0.5-5.0]	0.997	0.090	0.300	0.0018	0.0059
9-OHPhen silylated	$y = 0.869x$	[1.0-5.0]	0.989	0.167	0.557	0.0023	0.0075
1-OHPyr silylated	$y = 4.645x$	[1.0-5.0]	0.981	0.215	0.716	0.0009	0.0030

a LOD and LOQ calculated with the standard deviation at the y-intercept

b LOD and LOQ calculated with the signal-to-noise methodology

The chromatogram of the four silylated hydroxy-PAHs can be seen in Figure II.10. It can be noted that peak tailing was considerably reduced after the derivatization (compared to Figure II.3).

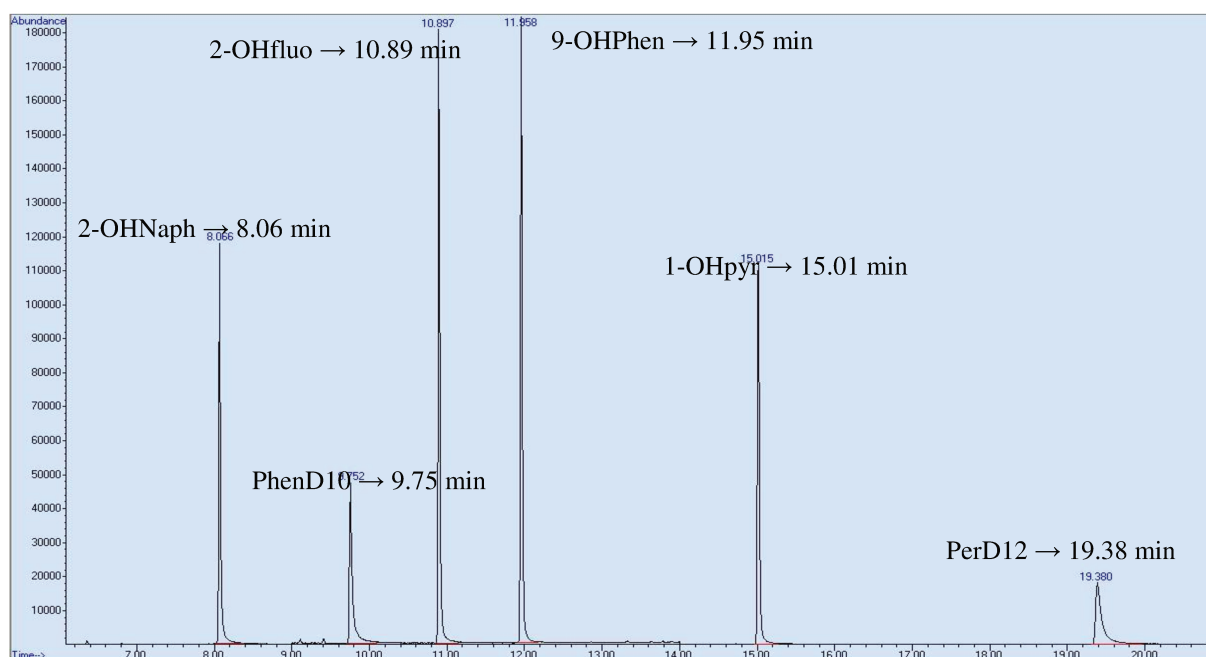


Figure II.10 Chromatogram of the four silylated hydroxy-PAHs ($C = 4 \text{ mg L}^{-1}$) and the two internal standards ($C = 1 \text{ mg L}^{-1}$)

Second, for the acetylated quinones and the coumarin, the concentrations used to establish the calibration curves (always in triplicates at each concentration level) were: 0.2 mg L^{-1} , 0.5 mg L^{-1} , 1.0 mg L^{-1} , 2.0 mg L^{-1} , 3.0 mg L^{-1} , 4.0 mg L^{-1} and 5.0 mg L^{-1} . Likewise the hydroxy-PAHs, LODs and LOQs were calculated using the two methodologies (mathematical and S/N procedures), with lower values obtained for the methodology using the signal-to-noise ratio (lowered by factors of 10 – 320) (Table II.12). The optimal acetylation process improved considerably the LODs and LOQs for the quinones, by factors of 3 – 52 (Tables II.5 and II.12). The higher beneficiary contribution of acetylation to the decrease of LODs and LOQs was for

the two ortho-quinones 1,2-NQ and 9,10-PQ, which could not be analyzed in GC-MS without derivatization. 9,10-AQ was an exception, that had its best result without acetylation. Indeed, as demonstrated in Table II.9, acetylation of 9,10-AQ is not complete and there was no advantage to analyze it in GC-MS after derivatization. Coumarin presented any change on its LOD and LOQ values, an expected result due to the fact that it is not a quinone.

Table II.12 Method performance characteristics for carbonyl-PAHs: Calibration curves, linearity range, correlation coefficients R², limits of detection (LOD) and limits of quantification (LOQ) obtained with the two methodologies.

Carbonyl-PAHs	Calibration curves	Linearity Range (mg L ⁻¹)	R ²	LOD (mg L ⁻¹) ^a	LOQ (mg L ⁻¹) ^a	LOD (mg L ⁻¹) ^b	LOQ (mg L ⁻¹) ^b
1,4-BQ acetylated	y = 1.210 x	[1.0-5.0]	0.972	0.288	0.962	0.0009	0.0030
Coumarin	y = 0.279 x	[1.0-5.0]	0.972	0.287	0.956	0.0075	0.0251
1,2-NQ acetylated	y = 1.314 x	[1.0-5.0]	0.986	0.192	0.639	0.0013	0.0045
1,4-NQ acetylated	y = 0.719 x	[1.0-5.0]	0.992	0.208	0.692	0.0033	0.0109
9,10-PQ acetylated	y = 2.464 x	[1.0-5.0]	0.983	0.229	0.763	0.0038	0.0128
9,10-AQ acetylated	y = 0.058 x	[2.0 – 5.0]	0.960	0.435	1.448	0.0444	0.1479

a LOD and LOQ calculated with the standard deviation at the y-intercept

b LOD and LOQ calculated with the signal-to-noise methodology

The chromatogram of the five acetylated quinones and the coumarin can be seen in Figure II.11. As mentioned before, all the peak areas were improved, with the exception of 9,10-AQ that had a smaller signal.

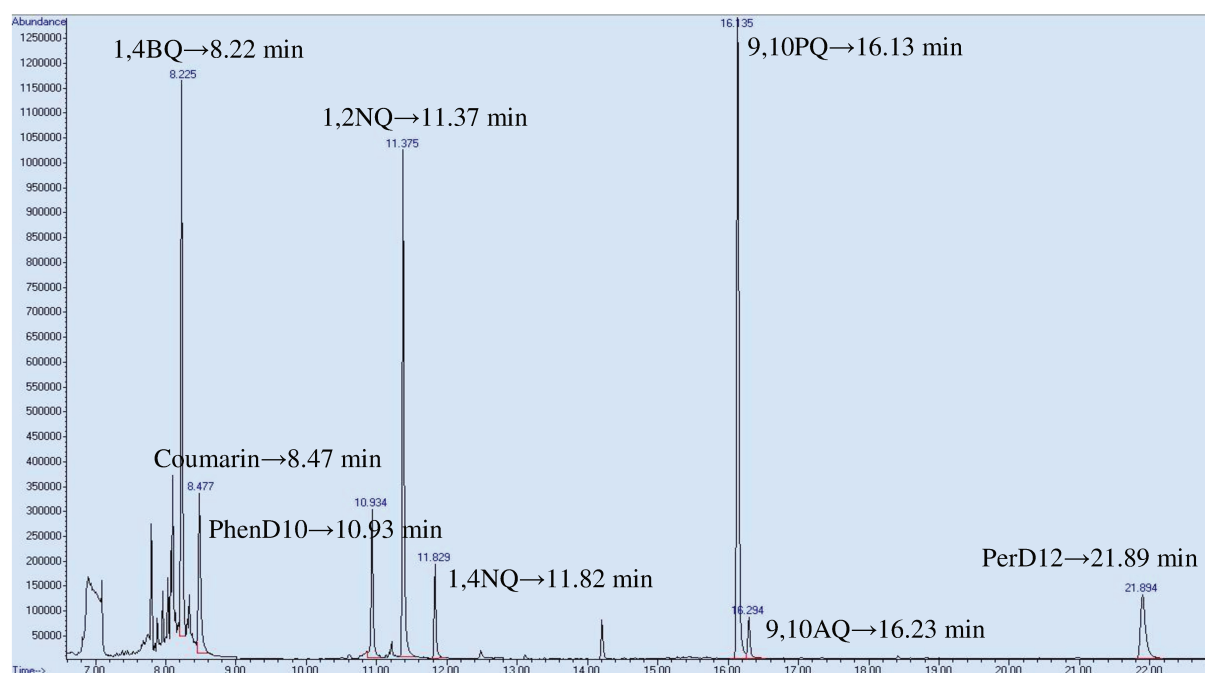


Figure II.11 Chromatogram of the five acetylated quinones and coumarin (C = 4 mg L⁻¹) and the two internal standards (C = 1 mg L⁻¹)

II.2 Optimization of microwave assisted extraction

Once the separation and detection methods were optimized, the main aim of this chapter could be developed: it consisted in optimizing the simultaneous MAE extraction of the two families of oxy-PAHs, having different chemical properties and probably different extraction behavior. In this part, diverse parameters as the nature and volume of extraction solvent, temperature and time of the extraction were tested, first one factor at a time. But after realizing that optimization was difficult considering the two families of compounds together, with too much possibly influencing factors, a chemometric approach was planned, to decrease the quantity of tests and be able to find the optimal parameters by mathematically modeling the responses (recovery yields).

II.2.1 Preliminary experiments to define the experimental domain

Microwave-assisted extractions were performed using a MAE MARS X equipment (CEM Corporation, Matthews, USA), with only two fixed parameters: the weight of the sediment sample was 1.0 g, and the amount of oxy-PAHs to extract was constant. A model sediment was prepared, mixing 19.5% of kaolinite (IMERYYS, Poigny, France), 73% of silt (collected from surface formations covering the chalk plateau in Normandy, France), 5% of sand (SIKA, Hostun, France) and 2.5% of organic matter (VEOLIA, France). The granulometric composition of the model sediment was representative of a natural sediment dredged from a harbor of Normandy (France). The model sediment was preliminary spiked with a volume of 100 μL of the oxy-PAHs (all prepared at 100 mg L^{-1} in acetone) and was evaporated for 2 hours under a fume-hood (with agitation) to eliminate the solvent. Thereafter 1 g of this spiked sediment was introduced into a PTFE flask for MAE, with 10-30 mL of organic solvent, and heated using a power of 300 W for one flask (600 W for 3 simultaneous flasks, 1200 W for 6 simultaneous flasks) at 80-120°C for 10-30 min. Once cooled, the extracted solutions were filtered with Teflon PTFE filters (0.2 μm) obtained from Fisher Scientific (Illkirch, France). Then, 1000 μL were taken to perform silylation, and 1000 μL for acetylation derivatization, before subsequent analysis by GC-MS.

Many preliminary tests were made changing only one parameter at a time, to better define the experimental domain. The study of Baltrons et al.⁶³, realized previously in the laboratory, was a starting point to test different parameters that could influence the MAE extraction of oxy-PAHs, knowing that this study dealt only with the extraction of hydroxy-PAHs. So the first tests were made changing just the temperature of extraction, from 80°C-120°C, using 90%

CH₃CN/10% toluene as an extraction solvent, as recommended in the article of Baltrons et al.⁶³ (Figure II.12).

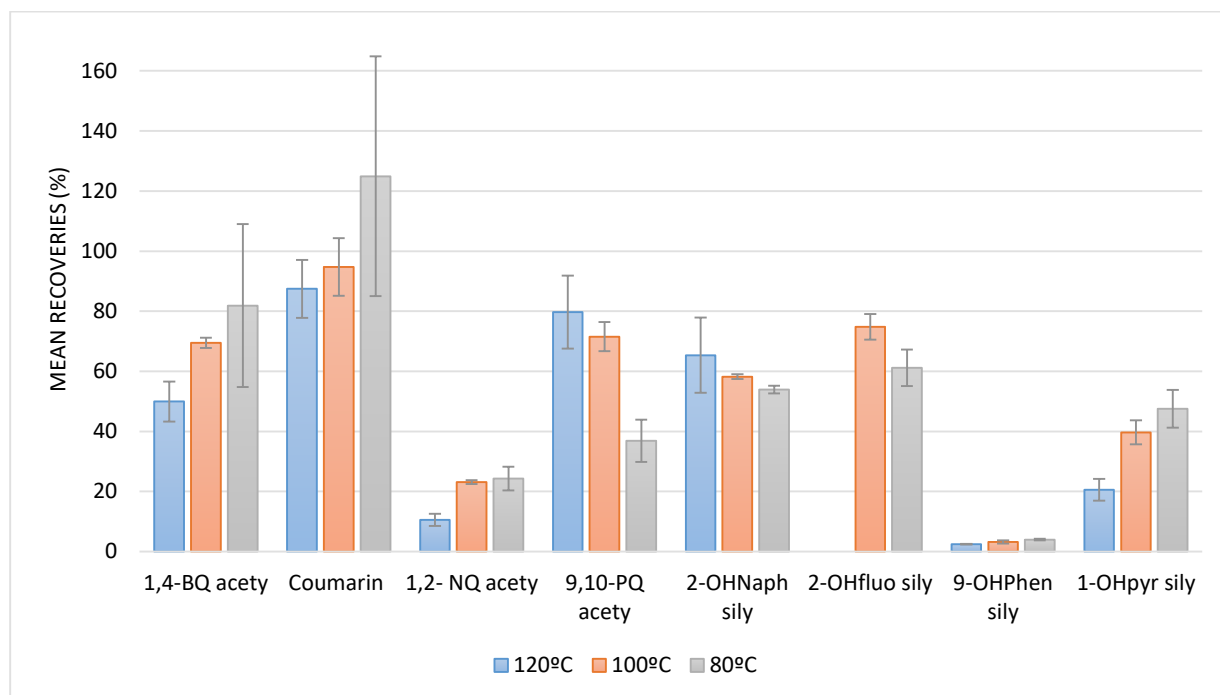


Figure II.12 Recoveries (%) of derivatized oxy-PAHs after MAE (n=3) using 10 mL of a 90% acetonitrile/10% toluene mixture and 30 minutes extraction, with different temperatures. GC-MS analysis.

What appears first in Figure II.12 is that the recoveries were not in the same order of magnitude as a function of the compounds: some of them could be quantitatively extracted, others were more difficult to extract. The best recoveries were generally found for the lower extraction temperature of 80°C, except for the heavier quinone (9,10-PQ) and the 2-OHNaph. However, the standard deviations were important at 80°C as compared to 100°C that had also fairly good recoveries for all the compounds.

So other tests varying the nature of the extraction solvent were made at 100°C (Figure II.13). The tested solvents were acetonitrile/toluene, pure acetonitrile and acetonitrile/dichloromethane, because they appeared as the best solvents for extracting hydroxy-PAHs in the study of Baltrons et al.⁶³. Figure II.13 shows that there is not a particular solvent, among the three tested, that is the best for extracting all the oxy-PAHs. For quinones, the mixture 90% acetonitrile/10% dichloromethane allowed obtaining better recoveries for 1,4-BQ and coumarin, but 90% acetonitrile/10% toluene allowed obtaining a greater recovery for 9,10-PQ. Moreover, for the hydroxy-PAHs, 90% acetonitrile/10% dichloromethane was the best extraction solvent for all of them but RSDs were the highest.

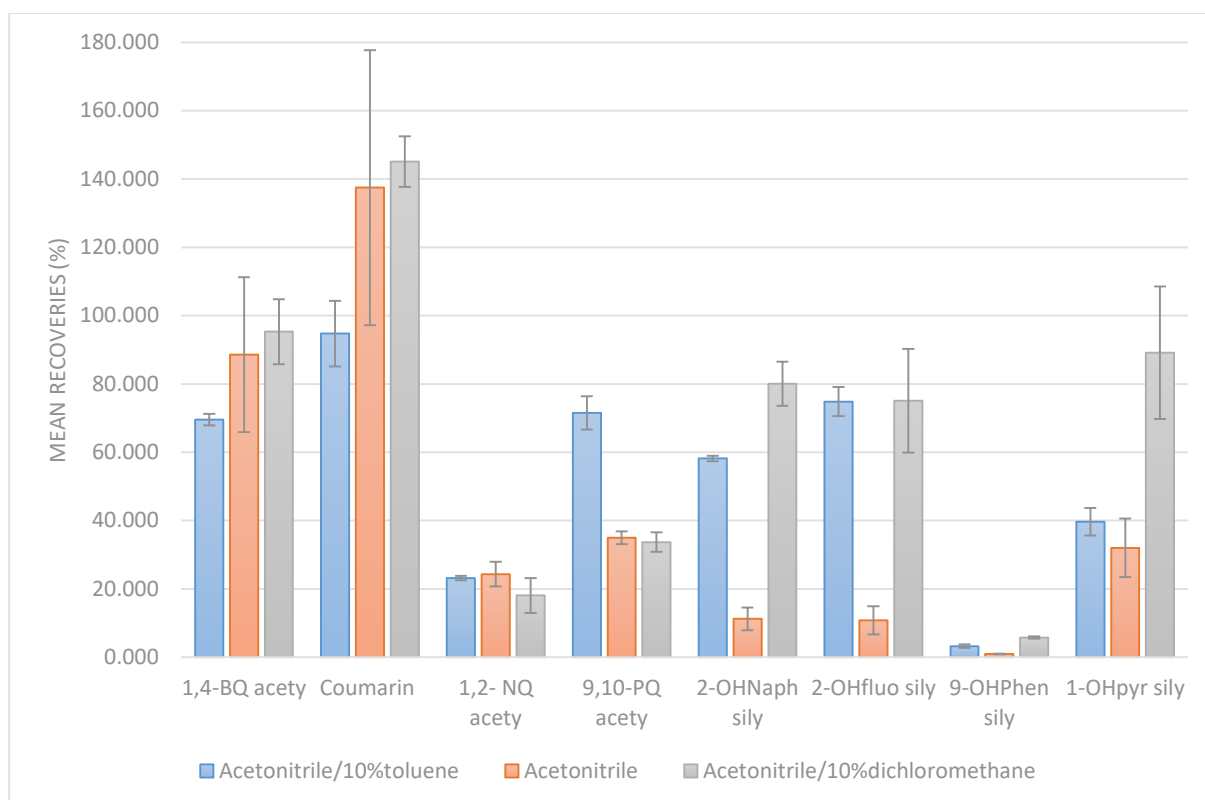


Figure II.13 Recoveries (%) of derivatized oxy-PAHs after MAE (n=3) using 10 mL of different solvent mixtures and 30 minutes extraction, at 100°C. GC-MS analysis.

Tests were also made using 10 mL of a 50% acetone/50% toluene mixture at 120°C, which is the best solvent mixture for extracting native PAHs (chapter III.2.2.2), but recoveries were significantly poorer than using the acetonitrile/toluene mixture. In general, 90% acetonitrile/10% toluene at 100°C had fairly good results, so a volume of 20 mL and 10 min extraction were tried and showed better recoveries than using 10 mL of solvent. Therefore, it could be also a benefit to varying the volume of the extracting solvent. Tests with varying the extraction duration from 10 to 30 min were not conclusive.

What appears from these first sets of experiments is that it will be difficult to find the best compromise for extracting simultaneously the two families of oxy-PAHs with the same solvent mixture. It appears also that other factors could more or less influence the extraction recovery yields, and that it is difficult to predict in which sense and with which magnitude. So two experimental designs were built to diminish the number of experiments necessary to find the optimal MAE extraction conditions for the two families of oxy-PAHs. A first experimental design was built to screen the influential factors on MAE extraction and verify if there were interactions between the parameters; the second design was built to model the responses and to find the optimal extraction conditions for the two families of oxy-PAHs.

II.2.2 Principle of factorial designs

We saw previously that many factors such as temperature, extraction duration, volume and nature of extraction solvents can more or less modify the MAE extraction yields¹²⁶. So when a new methodology must be optimized, it is important to understand what are the factors, among all, which significantly influence the results, how the factors interfere each other and where is the optimal area for obtaining the best results. Univariate studies do not allow estimating the interactions between the studied parameters and do not allow modelling the responses considering all the influencing factors simultaneously.

Factorial designs or statistical modelling explores all the possible combinations of variables with a minimum of analyses. The advantages compared to manipulations changing one variable by one, is to observe the interaction between different factors without increasing the number of experiments²¹⁶. Usually, a first-degree polynomial model is made when many factors have to be studied and screened, called a fractional factorial design. In this modelling, when many factors have to be screened, some of them can be aliased with the intention to decrease the quantity of experiments. In general, a 2^{n-p} modelling is made, considering that “2” is the number of studied levels (called (-1) and (+1) levels), “n” is the number of factors to be studied and “p” is the number of aliased factors, to reduce the test matrix. As an example, when a 2^{n-1} experimental design is made, it means that only the 1/2 fraction of the 2^n test matrix will be made²¹⁷. An example of a mathematical model of the first degree is represented in Equation 3 for a model 2^3 :

Equation 3 Mathematical equation of a model 2^3 (polynomial of the first degree)

$$Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{23}X_2X_3 + b_{123}X_1X_2X_3$$

where Y is the experimental response, X_i are the factors studied, b_0 is the average response, b_i are the main effects for each factor (or estimates), b_{ij} and b_{ijk} are the second and third order interactions between the different factors. Analysis of variance (ANOVA) allows testing for the statistical significance of the estimates. If a p-value < 0.05 (95% confidence level) is found, it demonstrates that the variance of the estimate is significantly different from the variance of the residuals, and so the factor is really influencing the Y response.

Once the influent factors have been found, a higher order experimental design can be performed, capable of modelling the response surface. The most popular experimental designs for Response Surface Methodology (RSM) are the Box-Wilson and Box-Benhken²¹⁸. Quadratic terms are introduced in RSM designs, allowing identifying if the behavior of the parameter is linear or not (Equation 4)²¹⁹. The Box-Wilson is more known as the Central

Composite Design (CCD) ²²⁰, that is composed by a factorial design at two levels (± 1), a star design (at levels $\pm\alpha$) and a centered design (0 level) ²¹⁹:

Equation 4 Mathematical formula of a quadratic model for a model 2² (polynomial of the second degree)

$$Y = b_0 + b_1X_1 + b_2X_2 + b_{12}X_1X_2 + b_{11}X_1^2 + b_{22}X_2^2$$

In a 2ⁿ experimental design for screening influent factors, only two levels -1 and +1 are used and the variables can be quantitative or qualitative. In the RSM modeling, -1 and +1 levels are chosen symmetrically to the original value of the center point ²¹⁹. The center points are added to be able to do an estimation of the variance (an uniform variance on the overall experimental domain is hypothesized) and check in the linearity of the model ²²¹. Higher will be the number of center points (more than three), lower will be the influence of errors and better will be the mathematical prediction (Table II.13) ²¹⁹. For the estimation of the quadratic terms, “star” points are necessary and the levels are calculated by 2^{n/4} (Table II.13). With results obtained at 5 levels (- α , -1, 0, +1, + α), polynomial equations of the second degree (and even of the third degree) can be obtained. To know which mathematical model is the best (linear, quadratic?), the correlation coefficient R² of the model and the adjusted correlation coefficient R²_{adj} must show a good fit between the model and the experimental values, so they must tend towards 1.

Table II.13 Number of points used for RSM, depending on the number of studied factors ²¹⁸

Factors (n)	Number of edge points (-1/+1)	Number of star points (-α/+α)	Number of center points (0)	α value
2	4	4	5	1.41
3	8	6	6	1.68
4	16	8	7	2

II.2.3 Results of the screening design

A fractional factorial design 2⁴⁻¹ was built in order to screen the influence of four factors on the extraction recoveries: temperature T (F1), volume of solvent V (F2), nature of the co-solvent added to acetonitrile co-Solv (F3) and extraction time t (F4). These 4 factors appeared as possible important factors influencing the MAE extraction in the preliminary experiments. F4 was aliased with the interaction F1 \times F2 \times F3 in order to decrease the total number of experiments, assuming that interactions between F1, F2 and F3 would not be significant and that F4 might be the less influential factor. The screening design involved 8 runs at the two coded levels -1 and +1 and seven central points to estimate the variance (Table II.14), which represented a total of 15 experiments performed randomly. The experimental levels were

chosen thanks to the preliminary experiments. Unlike the other factors, the nature of extraction solvent was a nominal factor (Table II.14).

Table II.14 Experimental matrix for the two-level fractional factorial experimental design, with the 4 factors studied, the coded levels and the experimental levels into brackets

Two-level fractional factorial design 2^{4-1}			
F ₁ (Temperature) (°C)	F ₂ (Volume) (mL)	F ₃ (co-solvent nature) *	F ₄ = F ₁ × F ₂ × F ₃ (duration) (min)
-1 (80)	-1 (10)	-1 (10% CH ₂ Cl ₂)	-1 (10)
-1 (80)	-1 (10)	+1 (10% toluene)	+1 (30)
-1 (80)	+1 (30)	-1 (10% CH ₂ Cl ₂)	+1 (30)
-1 (80)	+1 (30)	+1 (10% toluene)	-1 (10)
+1 (120)	-1 (10)	-1 (10% CH ₂ Cl ₂)	+1 (30)
+1 (120)	-1 (10)	+1 (10% toluene)	-1 (10)
+1 (120)	+1 (30)	-1 (10% CH ₂ Cl ₂)	-1 (10)
+1 (120)	+1 (30)	+1 (10% toluene)	+1 (30)
0 (100)	0 (20)	0 (0% co-solvent)	0 (20)
0 (100)	0 (20)	0 (0% co-solvent)	0 (20)
0 (100)	0 (20)	0 (0% co-solvent)	0 (20)
0 (100)	0 (20)	0 (0% co-solvent)	0 (20)
0 (100)	0 (20)	0 (0% co-solvent)	0 (20)
0 (100)	0 (20)	0 (0% co-solvent)	0 (20)
0 (100)	0 (20)	0 (0% co-solvent)	0 (20)

* Co-solvent added to acetonitrile

Responses (recovery yields, calculated in %) were obtained from GC-MS but also from HPLC-UV-FLD quantifications: 1 g of spiked model sediment was extracted through MAE using a power of 300 W (one flask at a time randomly). Once cooled and filtered, 1000 µL were taken to perform silylation, and 1000 µL for acetylation, before subsequent analyses by GC-MS. The residual volume was used for HPLC-UV-FLD analyses. MAE extracted solutions were evaporated to dryness (after addition of 50 µL DMSO as a solvent keeper) with a MiVac duo concentrator (Genevac, Ipswich, UK). The temperature was set at 42°C, and the pressure ramp consisted in decreasing the pressure to 200-30 mBar in 1 h, thereafter staying 1h30 at this pressure. The residual volume was then completed with 950 µL of acetonitrile and injected in the HPLC system. Results from the quantification in GC-MS and HPLC-UV-FLD (recoveries

(%) for each oxy-PAH) were handled by using JMP 13 software (SAS institute, Cary, USA) for statistical calculations and modelling.

In a first time, the individual recoveries of each carbonyl-PAH and hydroxy-PAH, and the overall recoveries of each family were examined by GC-MS after their derivatization. Concerning the overall recovery of the carbonyl-PAHs, the correlation coefficient of the model and the adjusted correlation coefficient were respectively $R^2 = 0.91$ and $R^2_{adj} = 0.77$ which showed a good fitting between the model and the experimental values. The analysis of variance (ANOVA) showed a p-value = 0.028 < 0.05 (95% confidence level): it demonstrated that the variance corresponding to the linear regression obtained from the measured responses (recoveries) was significantly different from the variance of the residuals. The most influential factors and interactions (p-value < 0.05) were in the order (from the lower p-value to the higher): $V > T \times V > V \times \text{co-Solv} > \text{co-Solv}$. The volume was the most influential factor on the overall extraction yields, the temperature appeared as an influential factor but only through its interaction with the volume. Cosolvent appeared to be an active factor as regards recoveries but its influence was weaker than the other influential factors (higher p-value, but < 0.05). It should be noted that extraction time did not modify significantly the recoveries in the experimental space studied (p-value > 0.05).

Individually, the extraction behavior of coumarin was particular because ANOVA did not show any influential factor (p-value = 0.61) probably because the variance corresponding to the error was too high. The peak area corresponding to 9,10-AQ was generally too low (near the limit of quantification) and the high variance prevented us to find influential factors as regards its extraction. The cube plot representation obtained from the modeling software (Figure II.14) showed two optimal conditions as regards the overall extraction of quinones: the first one was reached for $V = -1$, $T = -1$ and $\text{co-Solv} = +1$; the second optimum was reached at $V = +1$, $T = +1$ and $\text{co-Solv} = -1$. It must be underlined that at any level of extraction time, recovery results were practically the same. So Figure II.14 shows that if the lowest volume is selected, the temperature need to be set at the lowest level and the extraction solvent must be the mixture acetonitrile/toluene 90/10 (v/v). On the contrary, if the highest volume is selected, the temperature must be set at the highest level and the mixture acetonitrile/dichloromethane 90/10 (v/v) should be used as extraction solvent.

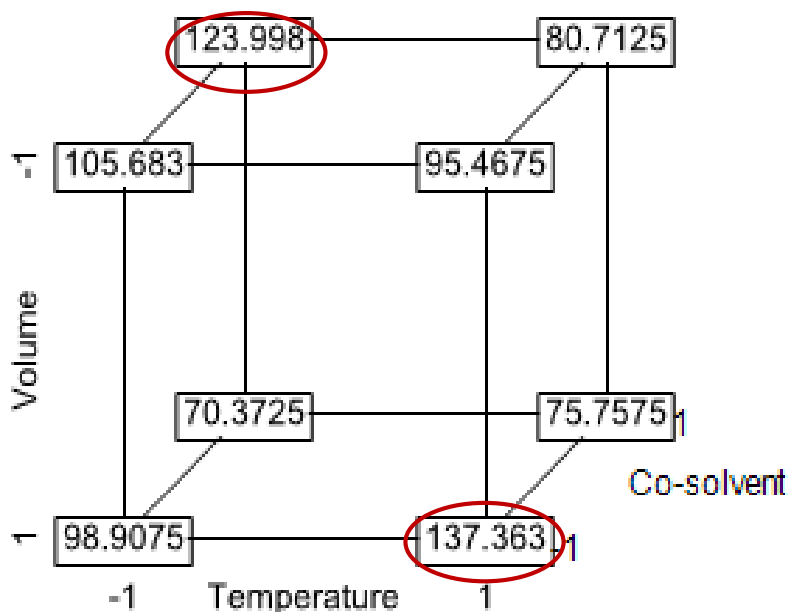


Figure II.14 Representation by a cube plot of the calculations of the overall recoveries of carbonyl-PAHs as a function of the levels of co-solvent, temperature and volume (duration being fixed at -1 level). Surrounded values correspond to optimal areas.

Concerning the overall extraction recoveries of the hydroxy-PAHs, the correlation coefficient and the adjusted correlation coefficient were $R^2 = 0.90$ and $R^2_{adj} = 0.78$, respectively, and the analysis of variance showed a p -value = $0.014 < 0.05$, which demonstrated that the influential factors found in this study were responsible of the variation of the extraction yields. The most influential factors and interactions (p -value < 0.05) were in the order (from the lower p -value to the higher): $V > T > T \times \text{co-Solv}$. The extraction time was not an influential factor (p -value > 0.05). Individually, the extraction behavior of 9-OHPhen was particular and no influential factor was found (p -value > 0.05), probably because of the high experimental variance. The cube plot representation (Figure II.15) showed that the overall extraction of hydroxy-PAHs could be better at the levels $V = 0$, $T = -1$ and $\text{co-Solv} = -1$. So Figure II.15 shows that if a mid-volume is selected, the temperature need to be set at the lowest level and the extraction solvent must be the mixture acetonitrile/methylene chloride 90/10 (v/v).

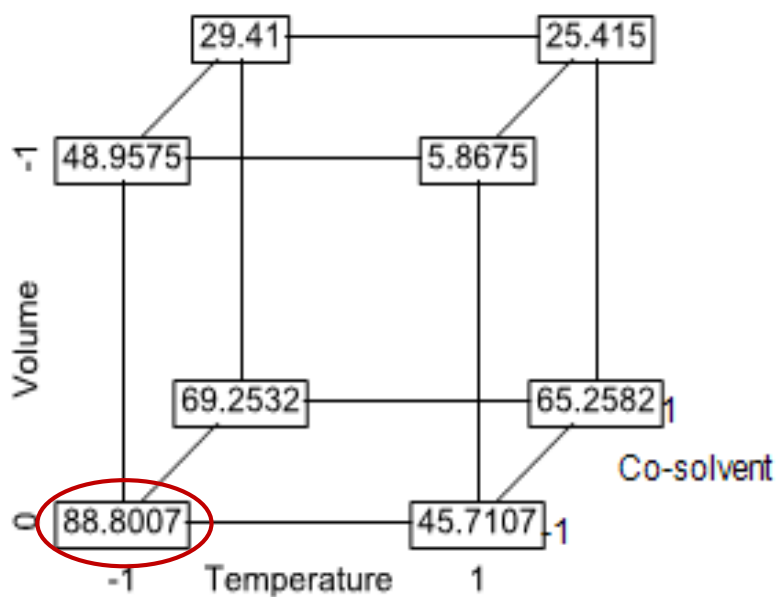


Figure II.15 Representation by a cube plot of the calculations of the overall recoveries of hydroxy-PAHs as a function of the levels of co-solvent, temperature and volume (duration being fixed at -1 level). Surrounded value corresponds to an optimal area.

Concerning the results obtained from the MAE extraction of the two families of oxy-PAHs, followed by an evaporation and an analysis by HPLC-UV-FLD, the modelling was unsatisfactory. The overall recoveries of the two families of compounds and the individual recoveries could not be satisfactorily explained, the adjusted correlation coefficient being generally low and the analysis of variance unsatisfactory (p -value > 0.05). Even if the data were not correctly explained, major trends could be revealed: the volume was the most influential factor for extracting carbonyl- and hydroxy-PAHs, and the experimental domains where the extraction recoveries could be the best were the same than those found with GC-MS as an analytical tool.

To conclude with these sets of experiments, the screening design showed us that the most influential factors for extracting simultaneously quinones and hydroxy-PAHs were, first, the volume of solvent, second the temperature and third, the nature of extraction solvent but only in interaction with the two other main factors. The duration was never an influential factor. So our new objective was to find the best extraction area, now considering only the volume and temperature as varying factors, fixing the extraction time at the lowest level to save time. The chosen extraction solvent was the mixture acetonitrile/dichloromethane 90/10 (v/v) (-1 level) because it was the less influential factor and it appeared in the best extraction domains for the two families of oxy-PAHs.

II.2.4 Full factorial design for modeling response surfaces (RSM)

II.2.4.1 Results of RSM obtained after gas chromatography-mass spectrometry analyses

In the second stage of the study, temperature T (F1 factor) and volume V (F2 factor) were chosen to be optimized, according to the previous results, in a larger experimental space, considering the instrumental constraints (Table II.15). Extraction time was set at 10 min and extraction solvent was the mixture acetonitrile/dichloromethane 90/10%.

Table II.15 Experimental matrix for the central composite design (with star points at +/- α levels), with the factors studied, the coded levels and the experimental levels into brackets

Central composite design 2^2

F ₁ (Temperature) (°C)	F ₂ (Volume) (mL)
-1 (80)	-1 (15)
-1 (80)	+1 (35)
+1 (120)	-1 (15)
+1 (120)	+1 (35)
- α (72)	0 (25)
+ α (128)	0 (25)
0 (100)	- α (11)
0 (100)	+ α (39)
0 (100)	0 (25)
0 (100)	0 (25)
0 (100)	0 (25)
0 (100)	0 (25)
0 (100)	0 (25)
0 (100)	0 (25)

The second order central composite design 2^2 involved 4 runs at two coded levels -1 and +1, one 0 central point with 5 repetitions to estimate the variance and required 4 experiments at - α and + α (with $|\alpha|=1.41$) levels to estimate the effects of the squared terms if the response was not linear on the defined experimental space. Different mathematical models were tested which allowed for the adjustment of the experimental response (recovery yields) obtained from the 13 experiments. Parametric second and/or third order equations could be obtained in order to model the variation of the measured response as a function of influential factors.

Concerning the overall recovery of the carbonyl-PAHs obtained after their GC-MS analysis, the correlation coefficient and the adjusted correlation coefficient were respectively $R^2 = 0.83$

and $R^2_{adj} = 0.78$ for the best fitted model, with a good p-value = 0.0007 (< 0.05) for the analysis of variance. The most influential factors and interactions were in the order: T^3 (p-value: 0.0003) > V^2 (p-value: 0.0216) > $T \times V$ (p-value: 0.0469), with an optimum as regards extraction obtained for $T = 1.41$ (128°C) and $V = 0.78$ (33 mL) levels. All the single recoveries of the carbonyl-PAHs were well fitted with the overall recovery ($R^2 = 0.75-0.95$) except 9,10-AQ which behaviour was different from the others, with erratic values, probably because the derivatization step was not favorable for this quinone. So 9,10-AQ was removed from the modelling.

For the overall recovery of the hydroxy-PAHs achieved by using GC-MS analysis, we obtained $R^2 = 0.82$ and $R^2_{adj} = 0.72$ for the best model, with a p-value = 0.0093 for the analysis of variance. The most influential factors and interactions were in the order: T (p-value: 0.0097) > V^2 (p-value: 0.0117) > $T \times V$ (p-value: 0.0401) > T^2 (p-value: 0.0753), with an optimum for the extraction with $T = 0.46$ (110°C) and $V = 0.38$ (29 mL) levels. We can note that the optima were different for the two families of oxygenated compounds, with a high temperature less favorable for the MAE extraction of hydroxy-PAHs. The single recoveries of all the hydroxy-PAHs were well fitted with the overall recovery ($R^2 = 0.84 - 0.97$), except for 9-OHPhen which behavior was different, with low recovery yields, and difficult to model because of its high variance.

Prediction profiler with desirability function was performed to find the optimal extraction conditions for the two families of oxygenated compounds together. Obviously, the optimal area was a compromise, particularly for hydroxy-PAHs for which the model fitting appeared less satisfactory ($R^2 = 0.70$ and $R^2_{adj} = 0.58$, p-value < 0.05). The equations 5 and 6 obtained for modelling the response surface of the overall extraction yields were:

Equation 5 Mathematical equation modeling the extraction recovery of carbonyl-PAHs

$$\text{Recovery (\%)} = 57.06 + 6.11 \times T^3 - 5.02 \times V^2 + 5.52 \times T \times V$$

Equation 6 Mathematical equation modeling the extraction recovery of hydroxy-PAHs

$$\text{Recovery (\%)} = 77.10 + 5.34 \times T^3 - 8.16 \times V^2 - 8.75 \times T \times V$$

Figure II.16 gives the prediction profiles. It allowed predicting the best values for the MAE overall extraction recoveries that were $75.2 \pm 8.7 \%$ for carbonyl-PAHs and $90.7 \pm 14.6 \%$ for hydroxy-PAHs. These calculated good values could be obtained with an extraction temperature at the +1.41 level (so 128°C) and a volume at +0.12 level, meaning 26 mL.

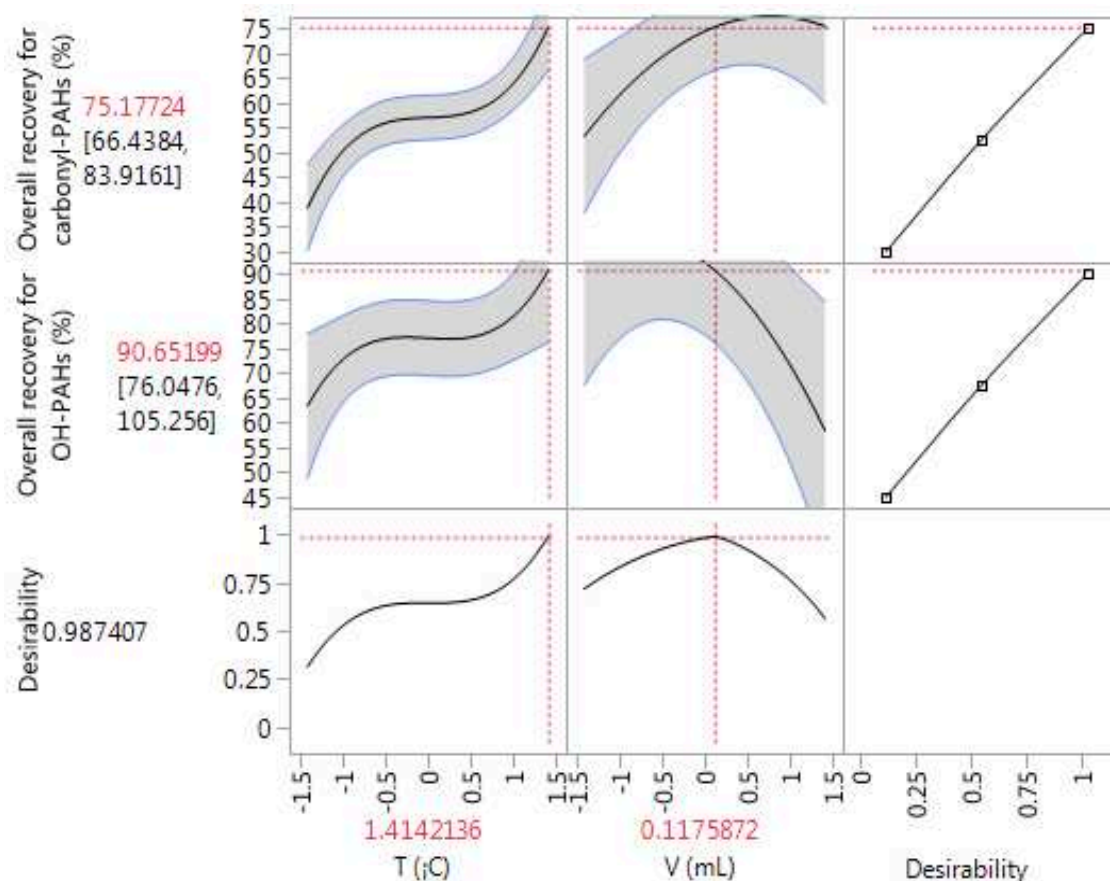


Figure II.16 Optimum prediction profiles for quinones and hydroxy-PAHs

II.2.4.2 Results of RSM obtained after liquid chromatography-fluorescence/ultraviolet analyses

Modelling was also attempted for the results obtained from HPLC-UV-FLD analyses consecutive to MAE extractions, so without the derivatization steps. For the overall recovery of the carbonyl-PAHs, we obtained a good modeling with $R^2 = 0.96$ and $R^2_{adj} = 0.90$, with a p-value = 0.0039 for the analysis of variance. The most influential factors and interactions were in the order (from the lower p-value to the higher): $V^3 > T \times V > V^2 > T$, with an optimum of extraction for $T = 1.41$ (128°C) and $V = 1.41$ (39 mL) levels. Surprisingly, modeling was different to that obtained with GC-MS analyses, mainly because the extraction volume became the most influential factor (V^3) instead of the temperature (T^3) in the previous modeling. So we supposed that the evaporation step between MAE extraction and HPLC analysis could introduce a bias on the results, because the only factor that differed between the 13 different samples was the volume to evaporate (the temperature and the pressure ramp were the same for all).

Furthermore, concerning the hydroxy-PAHs, it was not possible to obtain a good modeling of the results, the best one giving $R^2 = 0.57$, $R^2_{adj} = 0.27$ and p-value = 0.214 > 0.05. No influential

factor could be highlighted because the variance was so large that the observations were linked to the measurement errors. Again, we suspected that the evaporation step was responsible for this important bias on measurements.

So eight analyses of solutions containing carbonyl- and hydroxy-PAHs were performed in HPLC-UV-FLD, with or without the evaporation step. First, for the carbonyl-PAHs, the mean areas were significantly different ($p < 0.05$, $n=8$) between the two sets of experiments and the relative standard deviations ($n=8$) were slightly higher after the evaporation step (RSD = 0.6-5.5% without evaporation, 4.4-6.6% with evaporation). We concluded that the evaporation step actually introduced a significant bias on the results for carbonyl-PAHs, which could modify the mathematical model, increasing the effect of the volume on results obtained from HPLC-UV analyses. Second, for the hydroxy-PAHs, the mean concentrations were not significantly different ($p = 0.155$) between the two sets of experiments, but the RSD ($n=8$) were drastically increased after the evaporation step (RSD = 12.5-13.7% without evaporation, 20.9-23.9% with evaporation). Therefore, the evaporation step was actually liable for the significant errors introduced in the quantification of hydroxy-PAHs in HPLC-FLD and the impossibility to model the responses using the results of the experimental design.

II.2.5 Method validation

II.2.5.1 Optimal conditions of extraction and validation

Although HPLC-FLD allowed us obtaining lower LODs and LOQs for hydroxy-PAHs and allowed direct analysis without derivatization, it was not possible to obtain good modelling from MAE-HPLC results, as discussed previously, so the best conditions of extraction were chosen from the results obtained after MAE-GC-MS experiments. So the temperature of MAE extraction was set to 128°C, the volume of solvent was 26 mL (for 1 g sediment), the solvent was acetonitrile/dichloromethane 90/10 (v/v) and the extraction time was 10 min.

Five experiments were done the same day for obtaining the intra-day repeatability (or intra-day variation) of the two methods: MAE-GC-MS and MAE-HPLC-UV-FLD. Five other experiments were done on different days for obtaining the reproducibility (intermediate precision or inter-day variation) of the two methods. Detailed results for repeatability and reproducibility can be found in Table II.16 and Table II.17, respectively.

Table II.16 Mean extraction recoveries (%) and their repeatability after MAE-GC-MS or MAE-HPLC-UV-FLD experiments, using the optimal MAE extraction conditions.

	Repeatability for MAE-GC-MS experiments							Repeatability for MAE-HPLC-UV-FLD experiments						
	Extraction recoveries (%) (n=5)					Mean ± SD (%)	RSD (%)	Extraction recoveries (%) (n=5)					Mean ± SD (%)	RSD (%)
1,4-BQ	89.2	93.0	79.4	86.0	86.6	86.8±5.0	5.8	29.7	40.0	34.4	40.8	40.7	37.1±4.9	13.2
1,2-NQ	19.5	20.2	20.7	13.2	13.0	17.3±3.9	22.4	5.8	9.1	12.4	6.1	6.8	8.0±2.7	34.2
1,4-NQ	114.8	139.3	73.4	148.8	138.0	122.9±30.4	24.7	99.5	113.1	102.5	115.7	113.8	108.9±7.4	6.8
9,10-PQ	113.8	141.1	98.1	124.2	106.3	116.7±16.7	14.3	86.8	102.0	93.1	97.5	97.5	95.4±5.7	6.0
9,10-AQ	129.1	155.5	88.2	111.8	*	121.1±28.4	23.4	106.4	119.0	108.2	120.6	120.6	115.0±7.1	6.1
Coumarin	100.4	101.2	71.4	87.9	80.5	88.3±12.8	14.5	114.9	130.4	120.2	132.3	133.6	126.3±8.3	6.5
2-OHNaph	84.5	85.2	65.1	84.5	84.9	80.8±8.8	10.9	112.8	129.5	111.9	118.4	96.8	113.9±11.9	10.4
2-OHFluo	86.9	120.0	95.6	121.4	118.0	108.4±16.0	14.8	129.4	152.2	139.0	135.1	114.0	133.9±14.0	10.4
9-OHPhen	9.9	8.1	8.5	14.3	15.4	11.3±3.4	30.1	0.5	0.3	0.3	*	0.81	0.48±0.24	50
1-OHPyr	83.0	71.4	51.4	75.9	73.0	70.9±11.8	16.6	85.0	84.5	89.7	87.2	78.9	85.1±4.0	4.7

* Aberrant value

Table II.17 Mean extraction recoveries (%) and their reproducibility after MAE-GC-MS or MAE-HPLC-UV-FLD experiments, using the optimal MAE extraction conditions.

	Reproducibility for MAE-GC-MS experiments							Reproducibility for MAE-HPLC-UV-FLD experiments						
	Extraction recoveries (%) (n=5)					Mean ± SD (%)	RSD (%)	Extraction recoveries (%) (n=5)					Mean ± SD (%)	RSD (%)
1,4-BQ	97.7	105.3	88.6	68.5	96.1	91.2±14.0	15.4	42.8	44.5	26.6	39.8	37.6	38.3±7.1	18.4
1,2-NQ	17.8	15.5	13.7	13.2	20.3	16.1±3.0	18.6	16.1	13.5	11.4	19.0	20.0	16.0±3.6	22.7
1,4-NQ	101.7	123.6	103.7	145.8	115.1	118.0±17.9	15.2	96.4	110.8	103.4	96.7	101.1	101.7±5.9	5.8
9,10-PQ	75.4	125.0	100.5	83.8	103.4	97.6±19.2	19.7	92.3	97.3	86.9	86.6	87.5	90.1±4.7	5.2
9,10-AQ	121.8	*	*	207.1	177.4	168.8±43.3	25.7	103.9	114.4	108.0	103.8	106.2	107.3±4.4	4.1
Coumarin	94.2	82.7	86.5	64.0	79.5	81.4±11.2	13.8	109.1	122.2	118.7	110.7	113.7	114.9±5.5	4.8
2-OHNaph	99.7	91.0	104.1	88.5	96.5	96.0±6.3	6.6	116.9	97.9	101.4	93.5	117.6	105.5±11.1	10.6
2-OHFluo	105.7	113.9	124.3	109.0	108.0	112.2±7.2	6.4	119.1	109.6	105.8	104.9	131.2	114.1±11.1	9.7
9-OHPhen	23.7	4.9	23.4	10.5	14.6	24.2±15.0	62.0	27.9	12.7	19.9	11.2	20.1	18.4±6.7	36.5
1-OHPyr	55.3	59.7	109.0	60.2	85.4	73.9±22.9	31.0	116.3	84.6	91.4	65.7	96.5	90.9±18.4	20.2

* Aberrant values

The mean values of the overall extraction of the carbonyl-PAHs after GC-MS analyses were 92.2% (mean intra-day variation = 18.5%) and 95.5% (mean inter-day variation = 18.1%). Without considering 9,10-AQ, the mean extraction values were 86.4% (mean intra-day variation = 17.5%) and 80.9% (mean inter-day variation = 16.5%). These experimental mean overall extraction values are in the prediction interval (in the higher limit: $66.4 \leq 75.2 \leq 83.9$) obtained from the modelling related to the overall recovery of carbonyl-PAHs, 9,10-AQ being not included in the modelling. Table II.17 shows actually that some values from 9,10-AQ could be aberrant.

In the case of hydroxy-PAHs, the mean values of their overall extraction after GC-MS analyses were 67.9% (mean intra-day variation = 18.1%) and 76.6% (mean inter-day variation = 26.5%). These experimental values are in the prediction interval obtained from the modelling, even if

they were in the lower limit ($76.0 \leq 90.7 \leq 105.3$), but it is recalled that the joint modelling was less robust for hydroxy-PAHs.

Concerning the mean values of the overall extractions obtained after HPLC-UV-FLD analyses, they were 81.8% (mean intra-day variation = 12.2%) and 78.2% (mean inter-day variation = 10.2%) for the carbonyl-PAHs, and 83.3% (mean intra-day variation = 18.9%) and 82.2% (mean inter-day variation = 19.3%) for the hydroxy-PAHs.

Good results were obtained for the single MAE extraction and analysis of the oxygenated compounds, with 81.4-118% extraction recoveries for carbonyl-PAHs and 73.9-112.2% extraction recoveries for hydroxy-PAHs after GC-MS analyses, and 90.1-114.9% recoveries for carbonyl-PAHs and 90.9-114.1% recoveries for hydroxy-PAHs after HPLC-UV-FLD analyses. These results are as good as - or even better than - those obtained with a PLE extraction procedure for quinones from atmospheric particulate matter (9,10-PQ 64-84%) or soils (40% 1,4-NQ, 80% 9,10-AQ)^{222, 223} and even better than those obtained with a PLE extraction procedure for hydroxy-PAHs from sediments (2-OHNaph 91-97%, 2-OHFluo 42-66%, 1-OHPyr 32-68%) or soils (40% 2-OHNaph)^{222, 224}.

The good results obtained for the overall recoveries masked the fact that 1,2-NQ and 9-OHPhen could not be quantitatively extracted, with recoveries in the range 4.3-24.2% and low reproducibility (Table II.17). This is not due to the derivatization steps, nor to the high injection temperature and oven temperature in GC, since results were also not satisfactory using the HPLC method for which no heating was applied. 1,2-NQ is rarely measured in solid environmental matrices in literature and when it is the case, extraction recoveries are not mentioned^{191, 225}. Also, 9-OHPhen is more rarely measured than 2- or 3-OHPhen in solid matrices, but these phenanthrene metabolites can actually show lower recoveries than the other hydroxy-PAHs²²⁴. Musa Bandowe and Wilcke²²² also noted that some quinones (notably 1,2-NQ, that was lost during the analytical procedure) or some hydroxy-PAHs (notably 9-OHPhen) could have very low extraction recoveries from soils (using PLE), due to their instability.

II.2.5.2 Comparison of the two analytical methods

The two optimized methods of extraction and analysis (MAE-GC-MS vs MAE-HPLC-UV-FLD) were compared in terms of various criteria of performance. First, the mean overall recoveries were compared (Table II.18). The overall recoveries of carbonyl-PAHs and hydroxy-PAHs were statistically the same (p -value > 0.05) whatever the way of their chromatographic analysis. However individually, some differences could be noted: the analysis by MAE-GC-MS of 9,10-AQ was less reliable than by MAE-HPLC-UV and 1,4-BQ gave significantly lower recoveries by MAE-HPLC-UV than by MAE GC-MS (Table II.17).

Second, the RSDs (reproducibility) were relatively high for both processes (Table II.18), but not unusual: values found for RSDs in validation processes, where the overall analytical process is considered (spiking, sample preparation, extraction, chromatographic analysis) are usually close to 15-20%. For HPLC-FLD/UV, the evaporation step can also increase the error and for GC-MS, it is the derivatization step.

Table II.18 Comparison of various criteria of performance for the extraction and analysis of carbonyl-PAHs and hydroxy-PAHs using the two optimized methods

	MAE-derivatization-GC-MS		MAE-evaporation-HPLC-UV-FLD	
	Carbonyl-PAHs	Hydroxy-PAHs	Carbonyl-PAHs	Hydroxy-PAHs
Mean overall recovery (%) (n=5)	95.5	76.6	78.2	82.2
Mean reproducibility (%) (n=5)	18.1	26.5	10.2	19.3
Detection limits (ng g ⁻¹)	0.9-44.4	0.3-2.3	0.4-1.6	0.009-0.019
Total duration (min)	595 min for 6 samples ~ 155 / 75 min per sample (carbonyl-PAHs / hydroxy-PAHs respectively)		258 min for 6 samples ~ 45 min per sample	
Solvent consumption (mL)	30 mL per sample		49 mL per sample	

Third, we noted previously that GC-MS was slightly less sensitive than HPLC-UV for carbonyl-PAHs, particularly for 9,10-AQ, but above all, GC-MS was 30-140 times less sensitive than HPLC-FLD for hydroxy-PAHs (Table II.18). However, MS allowed for structural identification unlike UV or FLD detection. Moreover, the presence of the target compounds in extracts could be carried out taking into account both retention time and m/z ratio selected in SIM mode using GC-MS. This confirmation was not possible in the case of HPLC-UV-FLD, UV and FLD detectors being not enough selective for identification confirmation.

Fourth, we compared the overall duration of the two analytical processes. For six samples extracted at the same time using MAE (10 min sample preparation + 10 min extraction + 15 min cooling and filtering), 3 samples could be acetylated or silylated at the same time (this represents 90 min × 2 acetylations - including 10 min of centrifugation and 5 min for evaporation of each sample - and 10 × 2 min silylation) and the twelve samples could be finally

analysed with GC-MS (12 × 30 min). It represents a total of 595 min for 6 samples, approximately 155 min and 75 min per sample acetylated and silylated, respectively (Table II.18). Now, for 6 samples extracted using MAE, they could be evaporated at the same time using the MiVac concentrator (120 min) and then analysed simultaneously for hydroxy- and carbonyl-PAHs with HPLC-UV-FLD (8 min analysis + 15 min equilibrating between each sample). It represents a total of 258 min, so approximately 45 min per sample (Table II.18). Consequently, MAE-HPLC-UV-FLD analyses take half as long as GC-MS analyses.

At last, organic solvent consumption was evaluated. The method using HPLC was more solvent-consuming due to the use of acetonitrile in the mobile phase (Table II.18).

II.2.6 Analysis of naturally contaminated sediments

II.2.6.1 Extraction of the non-spiked model sediment (blank)

A procedural blank was analysed in triplicate for being sure of the values found during the analyses performed on the spiked model sediment. It involved extracting the non-spiked model sediment with the optimized MAE process and to analyse the extracts by GC-MS (after acetylation and silylation steps) and by HPLC-UV-FLD (after evaporation). According to Gratz et al. ¹⁸, method blanks can frequently contain the presence of naphthalene (ubiquitous contaminant) and phenanthrene, which can justify the presence of their metabolites in the analyses. In a method blank, it is tolerated that just two of the target compounds can be found with a concentration three times greater than the limit of detection. A target compound in a blank sample can be also tolerated if the concentration of the analyte in analyzed samples is ten times greater than the concentration of the analyte in the blank ²²⁶.

The results of the MAE extraction and analysis of the model sediment are presented in the Table II.19. As can be seen, target oxy-PAHs could be found in this non-spiked sample, particularly hydroxy-PAHs. Fortunately, the concentrations spiked in the model sediment for the experiments (experimental designs) were of 10 000 ng g⁻¹, which made the values found in the “blanks” acceptable (Table II.19). Solvent blanks (from acetonitrile) were also analyzed by GC-MS and HPLC-UV-FLD, and no presence of the target compounds was found. Another possibility is that the inorganic materials used to create the model sediment (clay, silt, sand) were not real blanks and could be slightly contaminated. It could be also the case of the added organic matter, which was the result of the degradation of plant material. The furnisher (VEOLIA) told us that plants were not contaminated but it is not so sure. So we can suppose that the model sediment was not a real blank sample but it can be considered as a “pseudo”

blank sample. So, this slight contamination was taken into account in our further experiments, removing it from the quantification results.

Table II.19 Mean amounts of carbonyl- and hydroxy-PAHs in the non-spiked model sediment (procedural blank).

Compounds	MAE-HPLC- UV/FLD		MAE-GC-MS	
	Mean amount (n=3) (ng g ⁻¹)	SD (ng g ⁻¹)	Mean amount (n=3) (ng g ⁻¹)	SD (ng g ⁻¹)
2-OHNaph	26.1	19.0	73.9	27.0
2-OHFluo	8.7	6.0	43.2	16.8
9-OHPhen	1.7	0.3	6.2	10.7
1-OHPyr	0.8 *	-	-	-
1,4-BQ	-	-	-	-
Coumarin	-	-	-	-
1,2-NQ	-	-	437.2	135.1
1,4-NQ	-	-	-	-
9,10-PQ	-	-	1142.6	371.5
9,10-PQ	-	-	-	-

* Appeared in just one analysis

II.2.6.2 Extraction of naturally contaminated sediments

A naturally contaminated sediment, called Tancarville sediment, was a dredged material and was collected from a disposal site of a harbour from Normandy (France). It contained PAHs at a level approximately 10 times the geochemical background (the sum of the sixteen priority PAHs being $2144 \pm 63 \text{ ng g}^{-1}$ ²²⁷), which is not a high contamination. This sediment was analysed for the oxy-PAHs with the two procedures. Table II.20 gives the results from three experiments, each made from 1 g of dried Tancarville sediment. Using the methodology MAE-GC-MS, just 1,4-BQ could be quantified with values higher than those found in the “pseudo-blank” (Table II.19). In the case of 9,10-PQ, its concentration was lower than that found in the procedural blank. Strangely, the values were not confirmed in HPLC-UV although LOD and LOQ were of the same order of magnitude than in GC-MS for quinones (Table II.18). Concerning the hydroxy-PAHs, the four target compounds could be quantified with HPLC-FLD, but not with GC-MS, which seems normal, as FLD is much more sensitive than MS (Table II.18). The values found were all lower than the “pseudo” analytical blank. They were in the 0.8-2.6 ng g⁻¹ range, so at levels 20 to 80 times lower than their parent PAHs in the Tancarville sediment. It seems normal to find these oxy-PAHs at concentrations well under those of their parent PAHs, because they are only some oxygenated metabolites among others and some of them transform with time and are less persistent than their parent PAHs.

Table II.20 Mean amounts (Q) (ng g⁻¹) of carbonyl-PAHs and hydroxy-PAHs measured in the Tancarville contaminated sediment, using MAE-GC-MS and MAE-HPLC-UV-FLD. Comparison with mean amounts of native PAHs *

Oxygenated compounds	Mean Q ± SD (ng g ⁻¹) (n=3) MAE-GC-MS	Mean Q ± SD (ng g ⁻¹) (n=3) MAE-HPLC-UV-FLD	Native PAHs	Mean Q ± SD (ng g ⁻¹) (n=5) MAE-GC-MS ²²⁸
1,4-BQ	149 ± 79	< LOD	Naph	53 ± 4
1,2-NQ	< LOD	< LOD	Acy	19 ± 7
1,4-NQ	< LOD	< LOD	Ace	37 ± 1
9,10-PQ	620 ± 271	< LOD	Fluo	38 ± 1
9,10-AQ	< LOD	< LOD	Phen	125 ± 2
Coumarin	< LOD	< LOD	Ant	101 ± 5
2-OHNaph	< LOD	2.4 ± 1.4	Flt	227 ± 6
2-OHFluo	< LOD	1.4 ± 0.2	Pyr	202 ± 6
9-OHPhen	< LOD	0.8 ± 0.4	B[a]ant	156 ± 3
1-OHPyr	< LOD	2.6 ± 0.7	Chry	152 ± 3
			B[b]flt	290 ± 5
			B[k]flt	103 ± 7
			B[a]pyr	169 ± 6
			I(123cd)pyr	240 ± 3
			Db[ah]ant	54 ± 1
			B[ghi]per	178 ± 3

* Integral values, not corrected with the concentrations of the method blank

Another contaminated sediment was analyzed, which is the reference material BCR 535 (European Commission) with 7 certified values of PAHs at high concentrations, the sum of the seven PAHs exceeding 12 000 ng g⁻¹ (Table II.21). We recall here that no reference material was certified for oxy-PAHs. Among all the compounds analyzed in the reference material, just the parent PAH (pyrene) of 1-OHPyr was certified and could be compared with the value of its metabolite.

The measured concentrations of the target oxy-PAHs are shown in the Table II.21. Concerning 1-OHPyr, it could be quantified by GC-MS and its value was significantly higher than in the “pseudo” analytical blank (Table II.18) and 23 times lower than the concentration of its parent PAH in the sediment. The three other target hydroxy-PAHs could be also quantified by GC-MS, at levels much higher (65-454 ng g⁻¹) than those found in the slightly contaminated sediment of Tancarville. We can note that 3 of these hydroxy-PAHs could be also quantified

by HPLC-FLD, but the concentrations were significantly lower than those found in GC-MS, excepting 9-OHPhen. It does not appear logical as FLD is much more sensitive than MS. In the same way, 1,2-NQ, 1,4-NQ, 9,10-PQ and coumarin could be quantified by GC-MS but not by HPLC-UV while they were well above their UV detection LOQ.

Table II.21 Mean amounts (Q) (ng g⁻¹) of carbonyl-PAHs and hydroxy-PAHs measured in the certified sediment BCR 535 using MAE-GC-MS and MAE-HPLC-UV-FLD. Comparison with mean amounts of certified native PAHs *

Oxygenated compounds	Mean Q ± SD (ng g ⁻¹) (n=3) MAE-GC-MS	Mean Q ± SD (ng g ⁻¹) (n=3) MAE-HPLC-UV-FLD	Native PAHs	Mean Q ± SD (ng g ⁻¹) ²²⁹
1,4-BQ	363 ± 69	462 ± 75	Pyrene	2520 ± 180
1,2-NQ	94 ± 45	< LOD	Benz[a]anthracene	1540 ± 100
1,4-NQ	115 ± 3	< LOD	Benzo[a]pyrene	1160 ± 100
9,10-PQ	229 ± 84	< LOD	Benzo[e]pyrene	1860 ± 130
9,10-AQ	585 ± 405	696 ± 49	Benzo[b]fluoranthene	2290 ± 150
Coumarin	161 ± 50	< LOD	Benzo[k]fluoranthene	1090 ± 150
2-OHNaph	49 ± 3	12 ± 10	Indeno[1,2,3-cd]pyrene	1560 ± 140
2-OHFluo	64 ± 2	8 ± 3		
9-OHPhen	65 ± 27	62 ± 20		
1-OHPyr	108 ± 14	0		

* Integral values, not corrected with the concentrations of the method blank

The disparity between the results from GC-MS and HPLC-UV/FLD made us doubt on the reliability of the HPLC-UV-FLD. Indeed, as discussed previously, the confirmation of the compounds obtained from GC-MS analysis was made not only according to their retention times, but also with their selected m/z ions, obtained after their derivatization; it was not the case for HPLC-UV-FLD where the target compounds could be determined only with their retention times, which is not enough to confirm them.

A second certified reference sediment CRM 104 (Sigma-Aldrich) was also tested for the detection of the oxygenated derivatives of PAHs (Table II.22). This sediment was certified for the sixteen priority PAHs (but not for the oxy-PAHs), which represented a sum of 4140 ng g⁻¹ that is twice the contamination of the sediment of Tancarville.

Table II.22 Mean amounts (Q) (ng g⁻¹) of carbonyl-PAHs and hydroxy-PAHs measured in the certified sediment CRM104 using MAE-GC-MS and MAE-HPLC-UV-FLD. Comparison with mean amounts of native PAHs *

Oxygenated compounds	Mean Q ± SD (ng g ⁻¹) (n=3) MAE-GC-MS	Mean Q ± SD (ng g ⁻¹) (n=3) MAE-HPLC-UV-FLD	Native PAHs	Mean Q ± SD (ng g ⁻¹)
1,4BQ	557 ± 105	1168 ± 119	Acenaphthene	522 ± 26
1,2NQ	85 ± 12	200 ± 26	Acenaphthylene	355 ± 18
1,4NQ	218 ± 3	< LOD	Anthracene	480 ± 24
9,10PQ	217 ± 23	< LOD	Benz[a]anthracene	103 ± 5
9,10AQ	144 ± 20	98 ± 65	Benzo[a]pyrene	240 ± 12
Coumarin	45 ± 6	< LOD	Benzo[b]fluoranthene	305 ± 15
2-OHNaph	59 ± 33	16 ± 23	Benzo[g,h,i]perylene	133 ± 7
2-OHFluo	65 ± 23	9 ± 8	Benzo[k]fluoranthene	189 ± 10
9-OHPhen	38 ± 12	6 ± 2	Chrysene	138 ± 7
1-OHPyr	72 ± 30	< LOD	Dibenz[a,h]anthracene	76.2 ± 4
			Fluoranthene	325 ± 16
			Fluorene	226 ± 11
			Indeno[1,2,3-cd]pyrene	228 ± 11
			Naphthalene	282 ± 14
			Phenanthrene	348 ± 17
			Pyrene	191 ± 10

* Integral values, not corrected with the concentrations of the method blank

All the target oxygenated derivatives could be detected and quantified by GC-MS and they were found well below the concentration of their parent PAHs. Again the concentrations found in HPLC-UV-FLD did not correspond to those found with GC-MS. 1,4-BQ and 1,2-NQ had greater values at the wavelength 271 nm what is abnormal for them and could signify that maybe another compound eluted at the same retention times, which gives the high concentrations found. Again, the concentrations of hydroxy-PAHs found in HPLC-FLD were significantly lower than those found in GC-MS. HPLC-UV-FLD seems not a so reliable analytical method than GC-MS.

II.3 Conclusion

The objective of this chapter II was to propose a new complete methodology for extracting simultaneously and analyzing two families of oxygenated metabolites of PAHs (hydroxy-PAHs and carbonyl-PAHs) from a sediment matrix. Microwave assisted extraction has never been used in the past to extract simultaneously hydroxy-PAHs and carbonyl-PAHs (among which quinones) from this kind of environmental matrix. It is why a rigorous methodology was employed to optimize the extraction step, using a chemometric approach. A fractional factorial design, carried out for screening influential factors on MAE extraction, showed that Volume and Temperature were the most influential factors, acetonitrile/dichloromethane 90/10 (v/v) was the best solvent for extracting the two families of compounds simultaneously and the extraction time did not modify significantly the extraction yields. So the minimal extraction time (10 min) was chosen to save time. A central composite design allowed finding the optimal values for temperature (128°C) and volume (26 mL), values that were a good compromise to obtain high extraction recoveries for the two families of oxygenated compounds. Excepting 1,2-NQ and 9-OHPhen, individual recoveries were in the range 73.9-118%.

Two chromatographic analytical tools (GC-MS vs HPLC-UV-FLD) were used to analyse the extracts obtained from MAE. Derivatization of hydroxy-PAHs and quinones were necessary to analyse them by GC-MS, due to the possible thermal instability of certain oxy-PAHs. Silylation of hydroxy-PAHs could be improved, particularly for the low concentrations, improving the derivatization duration and adding catalysts. Acetylation of quinones was optimized in order to analyse them by GC-MS, particularly for ortho-quinones which are thermolabile. LODs were decreased 2.7 to 52 fold after their acetylation, with the highest benefit for ortho-quinones. There was no real advantage to use HPLC-UV instead of GC-MS in terms of detection sensitivity for carbonyl-PAHs, excepting 9,10-AQ which had no advantage to be derivatized for GC-MS analysis. In contrast there was an advantage in using HPLC-FLD instead of GC-MS in terms of hydroxy-PAH detection sensitivity because LODs were reduced 30- to 140-fold. Another benefit of the HPLC-UV-FLD analytical tool was a 50% reduction of the total analysis time because derivatizations were not necessary.

Despite these advantages, analyses in HPLC-UV-FLD appeared less reliable to analyse oxy-PAHs in naturally contaminated sediment matrices, due to the low selectivity of the detection, and due the apparition of interferences that may adversely disturb the results from quantification. So, spite of the improved sensitivity, less laborious and reduced analytical time of HPLC-UV-FLD, GC-MS after derivatization can be considered a best analytical method for its reliability, its capacity of identification of the compounds (and the second advantage that it

consumes less solvent than the first method) to analyze simultaneously carbonyl-PAHs and hydroxy-PAHs at trace levels from naturally contaminated sediment matrices.

Although this new analytical methodology has been demonstrated reliable, some laboratories cannot invest in MAE extraction apparatus for extracting contaminants from solid matrices such as soils or sediments. So the further chapter presents a low-cost and quick methodology for extracting a mixture of different families of contaminants from a sediment matrix.

Résumé du chapitre II

Optimisation de l'extraction simultanée des HAP hydroxylés et carbonylés par extraction assistée par micro-ondes et choix des outils d'analyse chromatographique.

Ce chapitre décrit tout le processus expérimental ayant mené à l'optimisation par plans d'expérience d'une méthode d'extraction assistée par micro-ondes (MAE), méthode multi-résidus permettant d'extraire simultanément de matrices sédimentaires deux familles de HAP oxygénés (hydroxylés et carbonylés). Deux voies d'analyses chromatographiques ont été expérimentées pour leur séparation et leur quantification, à savoir la CPG couplée à la spectrométrie de masse et l'HPLC couplée aux détecteurs UV et fluorimétriques. La méthode d'analyse globale a été validée et appliquée sur des sédiments naturellement contaminés et une discussion a permis de faire un choix parmi les deux modes d'analyses chromatographiques.

II.1 Optimisation des outils chromatographiques pour l'analyse des HAP oxygénés

II.1.1 HPLC couplée aux détecteurs UV et fluorimétriques

Les longueurs d'onde optimales de détection en UV de 5 quinones et de la coumarine, ainsi que de 4 OH-HAP en fluorimétrie, ont tout d'abord été déterminées par spectroscopie. Par la suite, la séparation du mélange des deux familles d'oxy-HAP a été optimisée sur colonne C₁₈ (100 × 4 mm, d_p = 3 μm), avec un gradient d'acétonitrile et d'eau et une programmation des longueurs d'onde en UV et en fluorimétrie (Fluo). La séparation des 10 composés est réalisée en moins de 4 min.

Des droites d'étalonnage ont été établies pour une quantification de chaque composé par étalonnage externe, chaque point de la gamme étant réalisé en triplicat. Cela a permis de calculer les limites de détection (LD) et de quantification (LQ) à l'aide d'une méthodologie mathématique. Ces limites ont également été calculées par la méthode plus conventionnelle du rapport signal sur bruit (S/B). Les deux méthodologies ont donné des résultats significativement différents, les LD/LQ les plus faibles étant obtenues par la méthode S/B. Par cette dernière méthode, les LD des HAP carbonylés obtenues en HPLC-UV vont de 0,4 à 1,6 μg L⁻¹, tandis que celles des HAP hydroxylés, obtenues en HPLC-Fluo, sont comprises entre 0,009 et 0,019 μg L⁻¹ et sont sensiblement plus basses.

II.1.2 CPG couplée à la spectrométrie de masse

L'analyse simultanée des deux familles d'oxy-HAP a été réalisée en premier lieu sans dérivation préalable, sur une colonne de polyméthylphénylsiloxane (60 m × 0,25 mm diamètre × 0,25 µm d'épaisseur de film), avec une phase mobile composée d'hélium. Une rampe de température a permis une analyse en moins de 28 min des 10 composés. Le spectromètre de masse (SM) d'ionisation par impact électronique et de type simple quadripôle, a permis une identification des composés en mode de balayage d'une large gamme de masses (SCAN). La mise au point d'une programmation des masses sélectionnées en fonction du temps d'élution des composés a permis une détection plus sensible en mode sélection d'ions (SIM). Les pics chromatographiques des composés hydroxylés se sont avérés trainants. Des droites d'étalonnage ont été établies pour une quantification de chaque composé par étalonnage interne (les étalons internes étant des HAP perdeutérés), chaque point de la gamme étant réalisé en triplicat afin de déterminer les LD/LQ par la méthode mathématique. Celles-ci se sont avérées significativement plus élevées que les LD/LQ déterminées par la méthode S/B. Les deux ortho-quinones se sont avérées impossibles à quantifier dans la gamme de concentrations des autres composés, leurs LQ/LD étant trop élevées.

La dérivation des OH-HAP par silylation avec un réactif de type BSTFA/TMCS a été mise en œuvre pour obtenir une meilleure symétrie de pics et une meilleure sensibilité de l'analyse. Les meilleures conditions de durée de dérivation ont été recherchées et l'ajout de catalyseurs a permis de diminuer les LD/LQ de facteurs compris entre 2,3 et 7,3. Les rendements de dérivation se sont avérés de l'ordre de 100%.

La dérivation des quinones a été réalisée par acétylation à l'aide d'anhydride acétique et de particules de zinc. Toutes les étapes de la dérivation ont été améliorées (qualité du Zn, durée des étapes, volumes de réactifs), ainsi que l'étape de purification des dérivés par extraction liquide/liquide. Les LD/LQ ont été diminuées de facteurs compris entre 3 et 52, les meilleurs résultats étant obtenus pour les deux ortho-quinones. En revanche, la 9,10-antraquinone s'est avérée défavorisée par l'étape de dérivation, son rendement de dérivation étant de l'ordre de 37%, contre 96-102% pour les autres quinones.

Par la méthode S/B, les LD des OH-HAP sont comprises entre 0,3 et 2,3 µg L⁻¹, tandis que celles des quinones sont comprises entre 0,9 et 3,8 µg L⁻¹, à l'exception de la 9,10-antraquinone dont la LD est plus élevée (44 µg L⁻¹).

II.2 Optimisation par plans d'expériences de l'extraction assistée par micro-ondes

II.2.1 Expériences préliminaires

Des expériences préliminaires ont été menées pour tenter d'extraire simultanément et quantitativement les HAP hydroxylés et carbonylés de sédiments dopés par extraction assistée par micro-ondes. Ces expériences, menées de manière univariée, ont permis de définir les limites du domaine expérimental mais n'ont pas permis de dégager un compromis permettant de réaliser une extraction optimale des deux familles d'oxy-HAP.

II.2.2 Principe des plans d'expérience

Quand une nouvelle méthodologie doit être optimisée, il peut s'avérer important de comprendre quels facteurs influent sur les résultats et comment les facteurs interfèrent entre eux. C'est donc par une approche chimiométrique que l'extraction simultanée par MAE des deux familles d'oxy-HAP a été ensuite envisagée, pour minimiser le nombre d'expériences et comprendre au mieux les phénomènes. Un premier plan d'expériences, plan factoriel fractionnaire (ou plan de screening) a été envisagé pour rechercher les facteurs influents en un minimum d'expériences. Un second plan factoriel complet en étoile a ensuite été envisagé pour modéliser mathématiquement les surfaces de réponse et obtenir les zones optimales.

II.2.3 Plan de screening pour trouver les facteurs influents

Parmi les facteurs à considérer, 4 d'entre eux ce sont dégagés, à savoir : (i) la nature du solvant d'extraction, ou plus exactement la nature du co-solvant à mélanger avec le solvant de base acétonitrile (noté co-Solv) ; (ii) le volume du solvant d'extraction (V) ; (iii) la température d'extraction (T) et (iv) la durée de l'extraction (t). Un plan factoriel fractionnaire 2^{4-1} a été construit, avec un facteur aliasé pour réduire le nombre d'expériences, 2 niveaux d'étude (-1 et +1) et des points centraux (0) pour évaluer la variance du système. Les réponses étudiées étaient les rendements d'extraction globaux des deux familles d'oxy-HAP, ainsi que les rendements d'extraction individuels de chacun des 10 composés. Ces réponses étaient obtenues d'une part suite aux analyses en CPG-SM après dérivation (silylation ou acétylation), d'autre part suite aux analyses en HPLC-UV-Fluo après reconcentration par évaporation. Suite aux analyses en CPG-SM, il s'est avéré que les facteurs les plus influents pour les deux familles de composés étaient V et T, et enfin co-Solv, mais en interaction avec T ou V. La durée de l'extraction n'apparaissait pas influente. Après une modélisation linéaire des réponses, il s'est avéré que le mélange acétonitrile/dichlorométhane 90/10 était le mieux adapté pour extraire les deux familles

d'oxy-HAP, mais qu'il fallait refaire un plan d'expérience plus adapté pour trouver la zone d'extraction optimale en fonction de T et V.

II.2.4 Plan factoriel complet pour modéliser les surfaces de réponse

Le second plan d'expérience, un plan factoriel complet en étoiles, a été conçu pour examiner l'influence des deux facteurs les plus influents, T et V, sur les rendements d'extraction, mais en intégrant 5 niveaux d'étude ($-\alpha$, -1 , 0 , $+1$, $+\alpha$) pour pouvoir réaliser des modélisations quadratiques. Le mélange acétonitrile/dichlorométhane 90/10 a été fixé, ainsi que la durée d'extraction la plus courte (10 min). Suite aux analyses en CPG-SM, il s'est avéré que des modélisations quadratiques étaient nécessaires pour une meilleure correspondance entre le modèle mathématique et les réponses, et que les deux zones optimales d'extraction pour les deux familles d'oxy-HAP ne correspondaient pas. Une zone de compromis a été dégagée, correspondant à une température d'extraction de 128°C et un volume de solvant de 26 mL, avec une prédiction d'extraction de $75,2 \pm 8,7$ % pour les HAP carbonylés et $90,7 \pm 14,6$ % pour les HAP hydroxylés. Les analyses effectuées en HPLC-UV-Fluo ont quant à elles donné des résultats de modélisation différents, avec un impact plus important du volume sur les rendements d'extraction des HAP carbonylés et une impossibilité de modéliser les réponses pour les HAP hydroxylés, du fait d'une variance trop importante. Ces biais ont pu être expliqués par l'impact de l'étape d'évaporation des échantillons avant leur analyse chromatographique. Par conséquent, ce sont les conditions optimales d'extraction MAE modélisées après analyse CPG-SM qui ont été considérées pour la suite des études.

II.2.5 Validation de la méthode

La méthode d'extraction MAE a été validée, en déterminant sa répétabilité, sa reproductibilité (ou fidélité intermédiaire, en considérant différents jours de manipulation) et en comparant les rendements d'extraction réels avec ceux obtenus par la modélisation. Les rendements globaux d'extraction se sont bien retrouvés dans les intervalles de prédiction, et les rendements individuels dans une fourchette de 81,4-118,0% pour les HAP carbonylés et 73,9-112,2% pour les OH-HAP analysés en CPG-SM, contre 90,1-114,9% et 90,9-114,1% pour les HAP carbonylés et les OH-HAP, respectivement, mais analysés en HPLC-UV-Fluo. Seuls la 1,2-naphtoquinone et le 9-hydroxyphenanthrène n'ont pu être extraits quantitativement (4,3-24,2%), mais le même type de résultats a pu être observé dans la littérature avec d'autres procédés d'extraction.

Bien que l'extraction MAE associée à l'analyse en CPG-SM ou en HPLC-UV-Fluo donne des rendements globaux d'extraction non significativement différents statistiquement, les deux

méthodologies ont été comparées sur d'autres critères. La MAE-CPG-SM s'est avérée moins répétable et reproductible que la MAE-HPLC-UV-Fluo ; les LDs/LQs étaient équivalentes pour les HAP carbonylés détectés en SM ou en UV, mais très nettement plus basses pour les OH-HAP détectés en Fluo plutôt qu'en SM. La durée totale des analyses était par ailleurs deux fois plus courte en utilisant l'HPLC plutôt que la CPG. Cependant, l'utilisation de la SM permettait d'être plus sélectif dans la détection des analytes et d'être plus fiable dans la confirmation de la présence d'un analyte, par croisement des temps de rétention et des masses sélectionnées.

II.2.6 Analyse de sédiments naturellement contaminés

Les deux méthodologies complètes d'analyse ont été appliquées sur des sédiments naturellement contaminés. Auparavant, un « échantillon blanc » a été extrait, à partir du sédiment modèle reconstitué non dopé. Ce « blanc analytique » s'est avéré être un pseudo-blanc, car certains oxy-HAP ont été détectés ou quantifiés à l'état de traces. Par la suite, un sédiment non certifié (Tancarville), collecté dans la chambre de dépôt d'un port Normand, ainsi que deux sédiments certifiés (quant à la présence de HAP), les sédiments BCR535 et CRM104, ont été analysés. On rappelle qu'aucun matériau particulaire n'est certifié quant à la présence des quinones ou d'OH-HAP. Dans tous les cas, l'analyse en HPLC-UV-Fluo a montré des résultats différents et globalement plus faibles que ceux obtenus en CPG-SM, résultats remis en cause du fait de l'impossibilité de confirmer l'identité des analytes en UV ou en Fluo. Le sédiment de Tancarville, peu contaminé en HAP, s'est montré très peu contaminé en oxy-HAP (essentiellement des quinones) ; tous les composés oxygénés cibles, carbonylés ou hydroxylés, ont été quantifiés dans les deux autres sédiments, celui contenant le plus de HAP natifs contenant globalement plus de métabolites oxygénés.

II.3 Conclusion

Deux méthodes d'analyse chromatographique ont été validées pour détecter et quantifier 6 HAP carbonylés et 4 HAP hydroxylés à l'état de traces, à savoir en HPLC-UV-Fluo et en CPG-SM ; la méthode d'analyse en CPG-SM a nécessité l'optimisation de procédures de dérivation, à savoir la silylation des OH-HAP et l'acétylation des quinones. Deux plans d'expériences ont permis de déterminer une zone de compromis pour extraire simultanément et de manière optimale par MAE les deux familles de HAP oxygénés. Par la suite, des sédiments naturellement contaminés ont été analysés, mais la procédure MAE-HPLC-UV-Fluo s'est avérée moins fiable pour l'identification et la quantification des composés que la procédure MAE-CPG-SM.

Chapter III

Optimization of the simultaneous extraction of PAHs, PCBs and hydroxy-PAHs from sediments using MSPD and MIPs

Publications and communications in relation to this chapter:

- Determination of multi-class polycyclic aromatic compounds in sediments by modified matrix solid phase dispersive extraction, I. Berger-Brito, Y. Tian, N. Machour, C. Boulangé-Lecomte, A. Benamar, F. Portet-Koltalo, submitted to Journal of Chromatography A. **PUBLICATION.**
- F. Portet-Koltalo, Y. Tian, I. Berger, C. Boulanger-Lecomte, A. Benamar, N. Machour, Advantages of Matrix Solid Phase Dispersive (MSPD) Extraction Associated to MIPs versus MAE Liquid Extraction for the Simultaneous Analysis of PAHs, PCBs and some Hydroxylated PAHs in Sediments. ICEMA 2016: 18th International Conference on Environmental Monitoring and Analysis (Germany, Berlin). 19-20 May 2016. **ORAL PRESENTATION.**

In this chapter III, a new extraction methodology is presented, the matrix solid phase dispersive (MSPD) extraction, that will have to extract simultaneously different families of lipophilic polyaromatic contaminants (PAHs and PCBs), but that will have also to perform the clean-up of the sample at the same time. An attempt will be made to add to this process the extraction and analysis of a third family of contaminants that are the more polar hydroxy-PAHs. In this case, a method of selective extraction will be added after the MSPD first extraction step, using molecularly imprinted polymers (MIPs). The challenge will be to find optimal conditions that allow extracting the first group of compounds (PAHs/PCBs) without the polar impurities, and then extracting selectively the hydroxy-PAHs without damaging the MIPs. For their separation and detection, PAHs and PCBs will be analyzed by GC-MS and hydroxy-PAHs by HPLC-FLD.

III.1 Simultaneous analysis of PAHs and PCBs with GC-MS

III.1.1 Chromatographic conditions

To save time, the choice was made to analyze simultaneously the 16 priority PAHs and a mixture of 7 PCBs (PCB-28, PCB-52, PCB-101, PCB-138, PCB-153, PCB-180 and PCB-209) using GC-MS (Table III.1). Six of these PCBs are the PCB indicators recommended by the BCR, the PCB-209 being not in this list (see chapter I.1.1.2). For the separation, a Zebron ZB – Semi-Volatiles column (from Phenomenex) of 60 m long, longer than those generally used for PAH separation, was chosen to obtain a better efficiency and enough resolution ($d_c = 0.25 \text{ mm} \times 0.25 \text{ }\mu\text{m}$ film thickness). The stationary phase (95% methyl - 5% phenylarylene) has been designed by the furnisher to improve the critical pair resolution of benzo[b]- and benzo[k]-fluoranthene. 1 μL of mixtures containing the 16 PAHs and 7 PCBs at different concentrations in toluene, 3 surrogate standards at 1 mg L^{-1} (Fluoranthene D10, PCB 156 and 7-methylbenzo[a]pyrene) and 2 internal standards at 1 mg L^{-1} (Phenanthrene D10 and Perylene D12), was injected (in the splitless mode at 285°C) into a gas chromatograph (model 6850) coupled to a mass spectrometer (model 5975C) from Agilent Technologies. Fluoranthene D10 was chosen as a surrogate standard to follow the extraction efficiency and correct the quantification of low molecular weight PAHs, from naphthalene to chrysene; 7-methylbenzo[a]pyrene was chosen for high molecular weight PAHs, from Benzo[b]fluoranthene to Benzo[g,h,i]perylene, and PCB-156 for the 7 PCBs (knowing that this PCB is a dioxin-like PCB that is generally not found in the environment). Phenanthrene D10 was chosen as an internal standard for the quantification of low molecular weight PAHs, from

Naphthalene to Chrysene, and for PCBs whereas Perylene D12 was chosen for high molecular weight PAHs, from Benzo[b]fluoranthene to Benzo[g,h,i]perylene.

Table III.1 Mass program in the SIM mode for analyzing 16 PAHs and 7 PCBs (with surrogate and internal standards) in GC-MS

Groups (min)	m/z (Dalton)	Compounds	Retention time (min)
6.1→7.8	128	Naphthalene	6.48
7.8→11.8	153; 154	Acenaphthene	8.31
	152	Acenaphthylene	8.58
	166	Fluorene	9.55
11.8→15.8	188	Phenanthrene D10	12.00
	178	Phenanthrene	12.08
	178	Anthracene	12.24
	256; 258	PCB28	13.18
	290; 292	PCB52	14.22
15.8→21.8	212	Fluoranthene D10	16.46
	202	Fluoranthene	16.63
	324; 326; 328	PCB101	17.25
	202	Pyrene	17.64
	360; 362	PCB153	20.31
	360; 362	PCB138	21.27
21.8→26.8	360; 362	PCB156	22.76
	228	Benz[a]anthracene	23.03
	228	Chrysene	23.18
	394; 396; 398	PCB180	23.56
26.8→33.4	252	Benzo[b]fluoranthene	27.51
	252	Benzo[k]fluoranthene	27.61
	496; 498; 500	PCB209	28.55
	252	Benzo[a]pyrene	28.72
	264	Perylene D12	28.94
	266	7-methylbenzo[a]pyrene	30.73
33.4→39	276	Indeno[1,2,3cd]pyrene	33.65
	278	Dibenz[a,h]anthracene	33.81
	278	Benzo[g,h,i]perylene	35.01

The oven program started at 60°C (1.2 min) to 190°C (at 40°C min⁻¹) followed by an increase to 240°C (at 4°C min⁻¹) and finally to 305°C (at 6°C min⁻¹) during 12 min, under a constant carrier gas flow of 1.4 mL min⁻¹. The temperature of the transfer line was set at 300°C and the detection of the analytes was conducted in selected ion monitoring (SIM), with the selected masses shown in Table III.1. The chromatogram obtained from the optimal program used for

the 16 PAHs and 7 PCBs with their surrogate and internal standards in the SIM mode can be seen in Figure III.1.

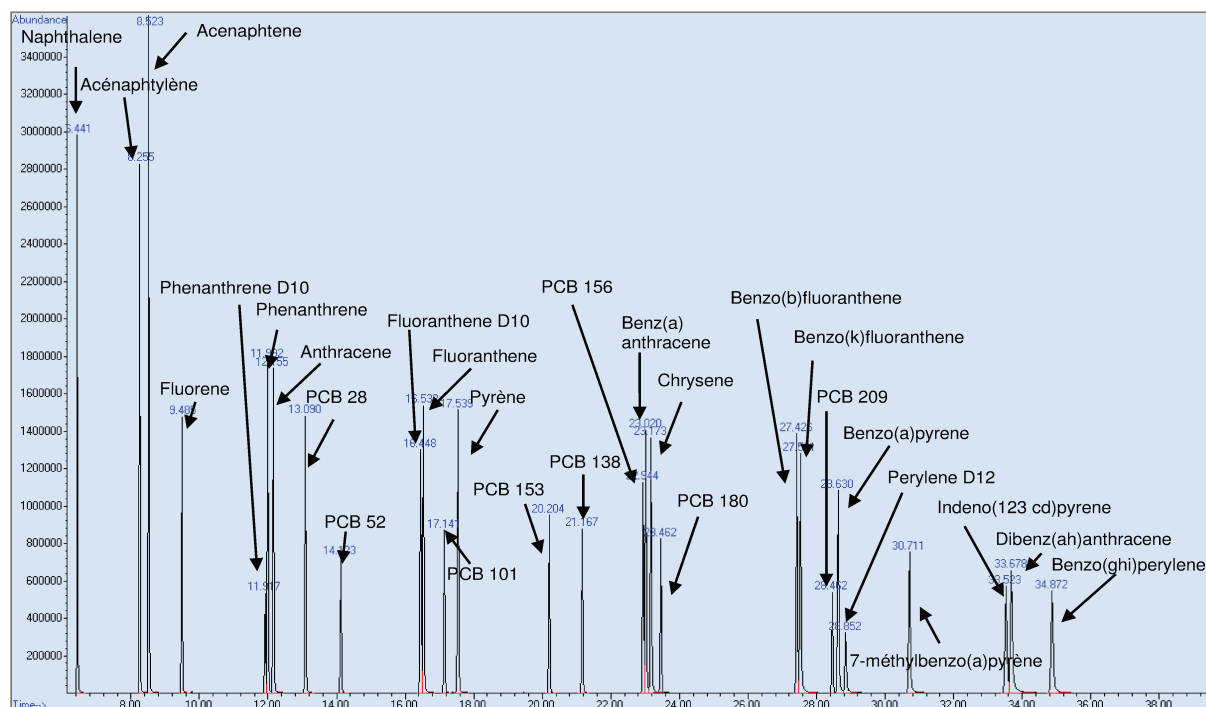


Figure III.1 Chromatogram in SIM mode of the 16 PAHs, 7 PCBs, 2 internal standards and 3 surrogate standards in GC-MS

III.1.2 Linearity, limits of detection (LOD) and limits of quantification (LOQ)

Calibration curves were established using 7 levels of concentrations, using the internal calibration methodology for the 16 PAHs and 7 PCBs. Equations of calibration curves were in the form: $\text{Area (compound)} / \text{Area (Internal standard)} = a_1 \times [C_{\text{compound}}]$, with the concentration of the internal standards being fixed at 1 mg L^{-1} and the intercept at the origin a_0 being neglected. The analyses were made in triplicate for each level of concentration, the range being: $0.1\text{-}3.0 \text{ mg L}^{-1}$ for PAHs and PCBs. Aberrant points were eliminated using regression residuals. The results can be seen in the Table III.2. All the correlation coefficients R^2 were > 0.990 excepting for two PCBs. Limits of detection (LOD) and quantification (LOQ) were calculated from the linear regression of the calibration curves using the equations $\text{LOD} = 3.3 \times S_y / a_1$ and $\text{LOQ} = 10 \times S_y / a_1$ where a_1 is the slope of the linear regression of the calibration curve and S_y is the standard error at the y intercept of the regression line. LOD and LOQ were also calculated using the S/N methodology (Table III.2): the limits were 60-459 times lower for PAHs, calculating them with the signal-to-noise methodology rather than the mathematical method, and 76-208 times lower for PCBs.

Table III.2 Method performance characteristics: Calibration curves, linearity range, correlation coefficients R², limit of detection (LOD) and limit of quantification (LOQ)

Compounds	Calibration curve	Linearity Range (mg L ⁻¹)	R ²	LOD (mg L ⁻¹) ^a	LOQ (mg L ⁻¹) ^a	LOD (mg L ⁻¹) ^b	LOQ (mg L ⁻¹) ^b
Naphthalene	y = 1.027 x	[0.2-3.0]	0.997	0.052	0.172	0.00011	0.00037
Acenaphthene	y = 1.236 x	[0.1-3.0]	0.999	0.028	0.095	0.00023	0.00076
Acenaphthylene	y = 1.621 x	[0.2-3.0]	0.999	0.039	0.129	0.00016	0.00053
Fluorene	y = 0.746 x	[0.2-3.0]	0.999	0.037	0.123	0.00061	0.00202
Phenanthrene	y = 1.145 x	[0.2-3.0]	0.998	0.043	0.144	0.00032	0.00106
Anthracene	y = 1.120 x	[0.1-3.0]	0.999	0.026	0.088	0.00044	0.00147
Fluoranthene	y = 1.232 x	[0.2-3.0]	0.999	0.040	0.133	0.00035	0.00117
Pyrene	y = 1.233 x	[0.1-3.0]	0.999	0.031	0.105	0.00036	0.00120
Benz[a]anthracene	y = 1.125 x	[0.2-3.0]	0.998	0.044	0.146	0.00029	0.00097
Chrysene	y = 1.145 x	[0.2-3.0]	0.998	0.046	0.152	0.00026	0.00086
Benzo[b]fluoranthene	y = 1.102 x	[0.2-3.0]	0.998	0.043	0.142	0.00022	0.00073
Benzo[k]fluoranthene	y = 1.174 x	[0.2-3.0]	0.999	0.038	0.126	0.00024	0.00081
Benzo[a]pyrene	y = 1.025 x	[0.1-3.0]	0.999	0.032	0.105	0.00029	0.00095
Indeno[1,2,3,cd]pyrene	y = 0.825 x	[0.5-3.0]	0.995	0.065	0.217	0.00055	0.00183
Dibenz[a,h]anthracene	y = 1.129 x	[0.5-3.0]	0.993	0.080	0.265	0.00046	0.00155
Benzo[g,h,i]perylene	y = 0.902 x	[0.5-3.0]	0.995	0.073	0.243	0.00060	0.00201
PCB28	y = 1.010 x	[0.2-3.0]	0.997	0.058	0.194	0.00039	0.00130
PCB52	y = 1.010 x	[0.2-3.0]	0.997	0.058	0.194	0.00077	0.00255
PCB101	y = 0.666 x	[0.2-3.0]	0.998	0.048	0.159	0.00060	0.00201
PCB153	y = 0.728 x	[0.2-3.0]	0.997	0.053	0.177	0.00044	0.00146
PCB138	y = 0.670 x	[0.2-3.0]	0.997	0.053	0.177	0.00046	0.00154
PCB180	y = 0.682 x	[0.5-3.0]	0.989	0.104	0.348	0.00050	0.00167
PCB209	y = 0.454 x	[0.5-3.0]	0.987	0.115	0.383	0.00078	0.00259

a LOD and LOQ calculated with the standard deviation at the y-intercept

b LOD and LOQ calculated with the signal-to-noise methodology

III.2 Extraction of PAHs and PCBs using MSPD

III.2.1 Optimization steps

III.2.1.1 Influence of elution solvent

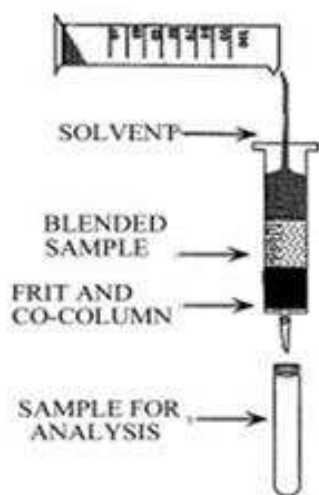
The matrix solid phase dispersive extraction methodology was tested for 5 representative PAHs (as a function to their number of condensed aromatic rings, so the 3 to 5 ring-PAHs phenanthrene, fluoranthene, pyrene, benzo[k]fluoranthene and benzo[a]pyrene) and 5 target PCBs (varying as a function of their chlorine atom number, so the 3 to 7 chlorinated PCB 28, PCB 52, PCB 101, PCB 138 and PCB 180), and the optimization of extraction was made as a function of different parameters, as the nature of elution solvent, the volume of elution solvent, the time of grinding and the nature of different types of dispersants and adsorbents. To avoid the granulometric and geometric variability of naturally contaminated sediments, the extraction tests were made with the model sediment already described in chapter II.2.2, containing 73% silt, 19.5% clay, 5% sand, and 2.5% of organic matter, with a pH of 8.4. Indeed the granulometry of a sediment can change according to the seasons and the fluvial flow, and the finest particles (clays) make it difficult to homogenize. So the optimization step must be done from a relatively homogeneous material. A quantity of 100 g of sediment was spiked with 2.5 mg kg⁻¹ of each of the 5 PAHs (Σ_5 PAHs= 12.5 mg kg⁻¹) and 0.5 mg kg⁻¹ of each of the 7 PCBs (Σ_7 PCBs= 3.5 mg kg⁻¹). The sediment was spiked with solutions made in acetone (10 mL of 0.025 g of each PAH) or hexane (10 mL of 0.005 g of each PCB), staying one day under agitation under a fume-hood for solvent evaporation and at least staying for one week in the dark for aging (with regular agitation). Spiked sediments were left in the fridge, no more than one month, because some PAHs can be degraded even if sediments are dry.

The MSPD extraction optimization started studying the nature of elution solvent necessary for an optimal and a simultaneous elution of PAHs and PCBs from the sediment. Solvents with a high polarity were avoided, because it could cause problems in the dissolution of the PAHs (apolar) and PCBs (not very polar), and volatile solvents were preferably chosen due to the steps of evaporation. The tests were made in triplicate with the following initial process: 0.5 g of the model sediment (spiked with 15 μ L of the surrogate standards fluoranthene D10 and 7-methylbenzo[a]pyrene at 100 mg L⁻¹ and 15 μ L of PCB 156 at 100 mg L⁻¹) was mixed with 1.0 g of Florisil (magnesium silicate particles) which is used as a dispersing agent but also as a sorbent for polar interfering compounds; 1 g of sodium sulfate (desiccant) was added and the mixture was grinded for 5 minutes.

The objective for using a desiccant was to diminish the water interferences that could be prejudicial to the analyses^{148, 230}. Indeed sodium sulfate has a high capacity of water adsorption and Florisil is also good for the adsorption of polar compounds²³¹. Florisil was already used in other studies for MSPD extraction of PAHs and associated with sodium sulfate, it diminished the interfering polar residues^{148, 149}. Alumina, diatomaceous earth, silica can also be used for MSPD. Florisil gave better recoveries and lower interferences than silica according to Pena et al.¹⁴⁸. Moreover, as mentioned before, Florisil retains strongly the polar compounds, especially when using less polar organic solvents, as hexane, used in our study¹⁵⁴. It is why Florisil associated with sodium sulfate were used as dispersants in this first step of our studies.

A co-column containing a sorbent for polar interfering compounds can be added at the bottom of the MSPD cartridge, to ensure a higher matrix clean-up (Figure III.2). Silica, aluminum and Florisil have been already used as co-columns in other MSPD studies, presenting a decrease in the matrix interfering compounds¹⁴⁸. So, in our case, a co-column of 1 g Florisil was added at the bottom of the MSPD cartridge, the solid mixture blended previously being put above it, and the elution by 10 mL of solvent was performed using a SPE extraction system (Phenomenex) (Figure III.2).

a



b

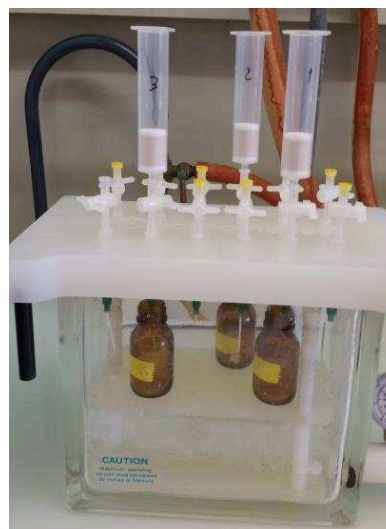


Figure III.2: a/ Constitution of the MSPD cartridge, with the co-column of Florisil at the bottom followed by the layer of blended sediment. b/ SPE elution system.

The final solutions were evaporated using the multi-evaporator MiVac, adding 60 μL of octanol as solvent-keeper and rediluting the residual volume with 1440 μL of toluene. From this solution, 990 μL were taken, 10 μL of the internal standards (Phenanthrene D10 and Perylene D12 at 100 mg L^{-1}) were added and after analyzed in GC-MS.

Hexane, dichloromethane and mixtures of solvents with hexane/dichloromethane 50/50 (v/v) or hexane/acetone 50/50 (v/v) or 25/75 (v/v) were tested for eluting PAHs and PCBs ¹⁴⁸. It can be observed in the Figure III.3 that the solvents with a medium polarity (containing dichloromethane or acetone) improved the extraction of PAHs and PCBs, compared to pure hexane. The best choice was hexane/acetone 50/50 or hexane/acetone 25/75 (v/v), which gave similar results and which allowed for the extraction of 60% of the 5 PAHs (Σ_5 PAHs) and more than 90% of the 7 PCBs (Σ_7 PCBs). The mixture hexane/acetone 25/75 (v/v) was not selected for the further studies because it was too polar and it may desorb more polar interfering compounds. It can be noted that even if less efficient, methylene chloride (pure or mixed with hexane) can also be interesting to elute PAHs and PCBs from the sediment matrix (Figure III.3).

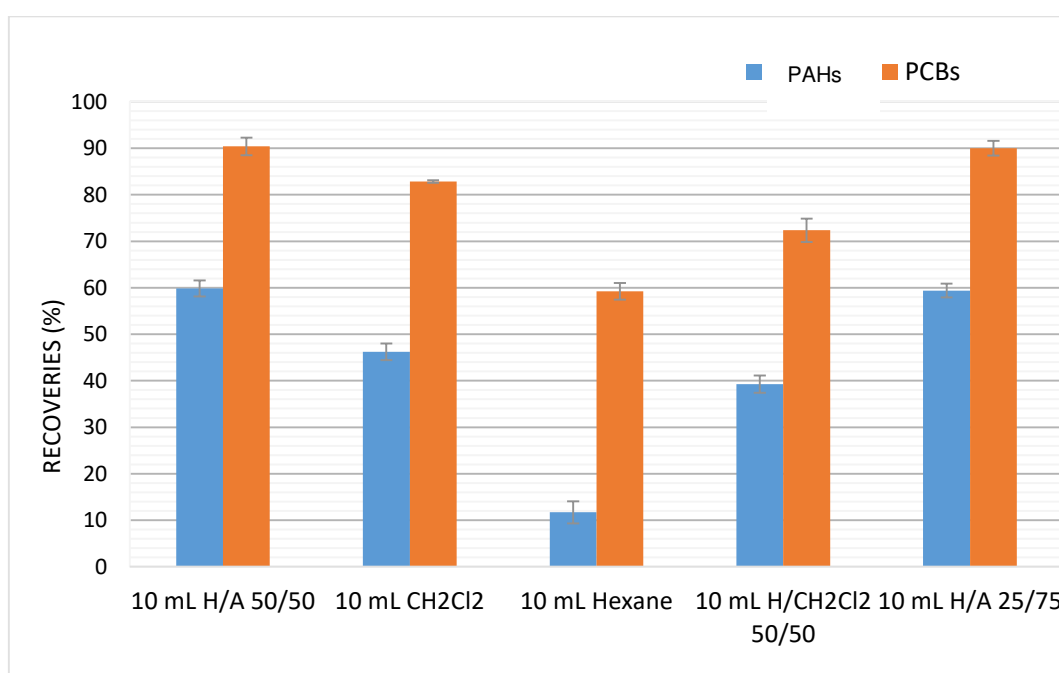


Figure III.3 Extraction recoveries for the Σ_5 PAHs and Σ_7 PCBs using different elution solvents (V= 10 mL). H: hexane; A: acetone; CH₂Cl₂: dichloromethane (Adsorbent: 1g Florisil, time of grinding: 5 min)

For the optimization of the elution volume, tests were performed in the same previous conditions, with the solvent hexane/acetone 50/50, using: 5 mL, 8 mL, 10 mL and 15 mL. In this range of volumes, the elution volume did not seem to have any influence on the recovery of PAHs and PCBs (Figure III.4). An elution volume of 5 mL was slightly better, but with a slightly higher standard deviation for PCBs.

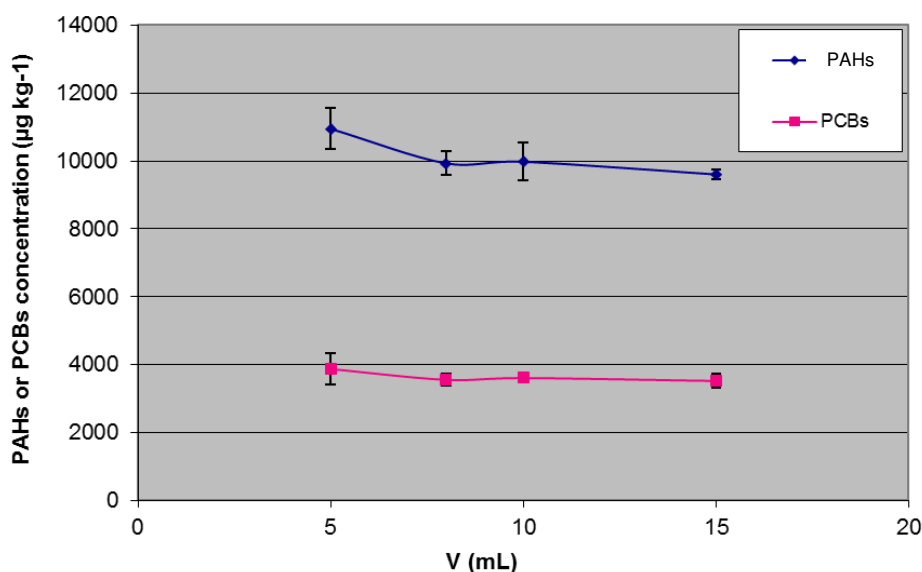


Figure III.4 Evolution of the concentration of the Σ_5 PAHs and Σ_7 PCBs extracted from the sediment as a function of the volume of the elution mixture acetone/hexane 50/50 (v/v)

III.2.1.2 Influence of the nature of the dispersing agent

Using the hexane/acetone 50/50 (v/v) solvent mixture for PAH/PCB elution, MSPD tests were made now with adding another dispersing agent to Florisil: 0.5 g of an apolar dispersant was added in addition to the 1 g of the polar Florisil. The following combinations of dispersant/adsorbent particles were chosen: C₁₈/Florisil, 3-chloropropyl/Florisil and 4-benzylchloride/Florisil. Florisil particles, C₁₈ bonded silica particles, 3-chloropropyl or 4-benzylchloride bonded silica particles act as dispersing agents because they favor the disruption of the sediment particles when grinding the solid mixture. After disruption of the sediment matrix, some apolar contaminants sequestered inside the matrix (particularly inside the glassy fraction of organic matter) can be released, and C₁₈, 3-chloropropyl, 4-benzylchloride bonded particles favor their sorption onto the non-polar or slightly-polar particle surface through Van der Waals interactions. Thereafter de-sequestered PAHs and PCBs may be better eluted by a solvent from the surface of the dispersing agents. C₁₈ bonded particles have been already used for improving MSPD extraction of PAHs from fish samples²³², moss¹³¹, sewage sludge¹⁴⁸, etc., but not for PCBs. In contrast, 3-chloropropyl and 4-benzylchloride bonded particles have never been tested before for MSPD extractions and we considered that the possible Van der Waals interactions with these particles might favor the transfer of the target sequestered non polar analytes from the sediment matrix to the sorbent particles. Florisil has obviously another objective, as discussed previously: it favors the adsorption of the polar interfering compounds present in the sediment, allowing their elimination from the extracts after the elution step. It helps sample purification.

The Figure III.5 shows that the combination of the two dispersants did not present a significant improvement in the extraction recovery of PAHs in relation to the utilization of Florisil alone. 3-chloropropyl bonded particles gave only slightly better results. Based on the extraction recovery mean, $59.2 \pm 7.2\%$ of the Σ_5 PAHs were extracted with the combination of 3-chloropropyl and Florisil particles. In the case of the PCBs, two mixtures, 3-chloropropyl/Florisil and C₁₈/Florisil particles, gave slightly better results. When using the 3-chloropropyl/Florisil particles mixture, the mean recovery for the Σ_7 PCBs was $86.5 \pm 3.9\%$.

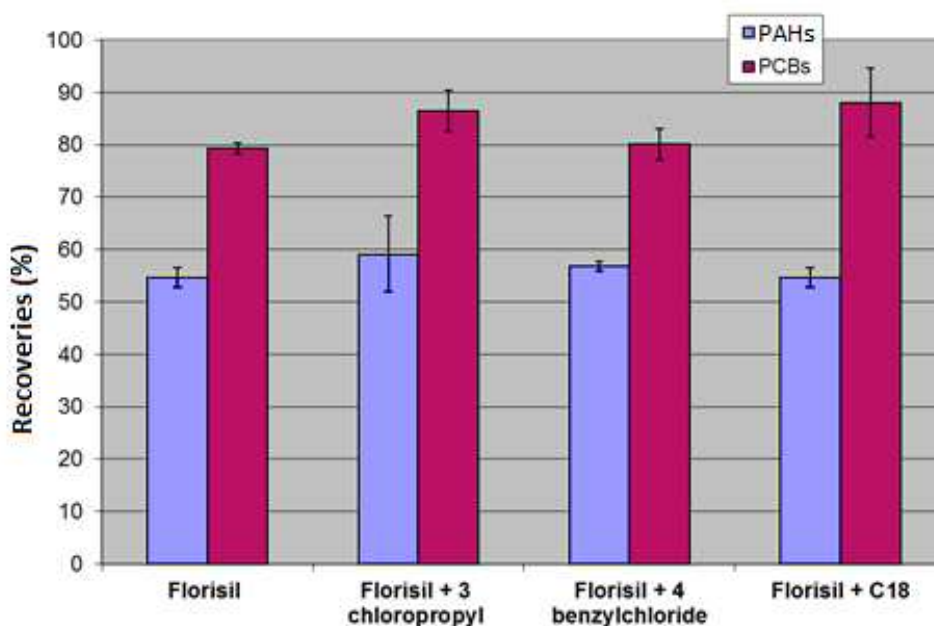


Figure III.5 Extraction recoveries for the Σ_5 PAHs and Σ_7 PCBs using different combination of dispersants (1 g Florisil for 0.5 g of the other dispersing agents). $V_{\text{elution}} = 10$ mL acetone/hexane 50/50 (v/v), time of grinding: 5 min

III.2.1.3 Influence of the crushing time

The time of grinding was also taken into consideration to optimize the process, in the range 2-10 minutes. The 3-chloropropyl/Florisil particles mixture was kept for the MSPD tests and the best elution volume was chosen, that is 5 mL of hexane/acetone 50/50 (v/v). The Figures III.6 a and b demonstrate that with the increase of the grinding time, an increase in the extracted amounts of PAHs and PCBs could be observed.

Ten minutes grinding allowed obtaining the best extraction values. A longer grinding time might probably still improve the results, but a too long grinding time is time- and operator energy-consuming. So, the time of 10 minutes was chosen, with mean extraction values of $66.4 \pm 1.4\%$ for the Σ_5 PAHs and $110.5 \pm 6.7\%$ for the Σ_7 PCBs.

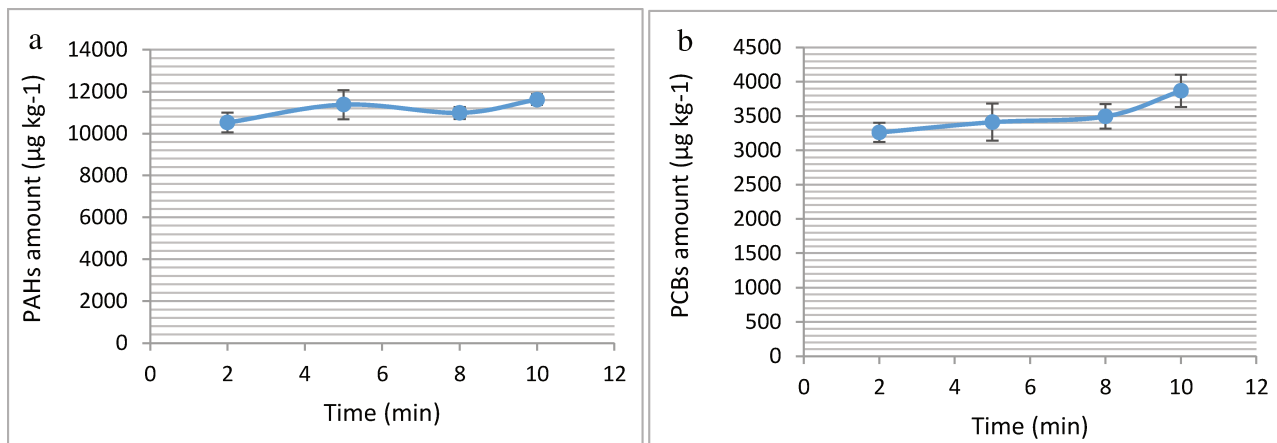


Figure III.6 Concentration of extracted analytes as a function of time of grinding (a) Σ_5 PAHs and (b) Σ_7 PCBs. 1 g Florisil for 0.5 g of 3-chloropropyl particles. $V_{\text{elution}} = 5 \text{ mL}$ acetone/hexane 50/50 (v/v)

III.2.2 Validation of MSPD on a standard sediment

The MSPD extraction process was optimal in these conditions: 0.5 g of pre-contaminated (PAHs/PCBs) dry sediment was spiked with the three surrogate standards and was mixed with 1 g of Florisil, 1 g of Na_2SO_4 and 0.5 g of 3-chloropropyl particles and grinded for 10 minutes. The solid mixture was introduced in a SPE cartridge with 1 g of Florisil at the bottom, and pressed. PAHs/PCBs were eluted with 5 mL of hexane/acetone 50/50 (v/v); the volume was evaporated, completed, spiked with the two internal standards and analyzed by GC-MS. Tests to evaluate the repeatability and reproducibility of the MSPD method were made with 5 repetitions, carried out the same day for intra-day repeatability and at different days for the reproducibility (intermediate precision or inter-days variation). A certified reference sediment was used to compare the results from the MSPD extraction method with the certified data, but also with another extraction method (MAE extraction method).

III.2.2.1 MSPD repeatability, reproducibility and detection/quantification limits

a. Repeatability and reproducibility

The results of the 5 intra-day repetitions showed that the MSPD method could extract the Σ_5 PAHs and Σ_5 PCBs with good efficiencies and good repeatability (2.4-2.8% relative standard deviations), with mean extraction amounts of $10832.4 \pm 258.5 \mu\text{g kg}^{-1}$ ($61.9 \pm 1.5\%$ recovery) and $3316.1 \pm 93.6 \mu\text{g kg}^{-1}$ respectively ($94.8 \pm 2.7\%$ recovery) from the model sediment. The results of the 5 inter-days tests gave an evaluation of the reproducibility (intermediate precision) of the extractions using MSPD. The Σ_5 PAHs and Σ_5 PCBs were extracted from the model sediment with a mean amount of $11055.3 \pm 616.0 \mu\text{g kg}^{-1}$ ($63.2 \pm 3.5\%$ recovery) and $3518.8 \pm 221.7 \mu\text{g kg}^{-1}$, respectively ($100.5 \pm 6.3\%$ recovery).

b. Detection and quantification limits

The limits of detection and quantification of the whole extraction method were evaluated from the analysis of a blank sample that was the non-spiked model sediment. The model sediment was extracted with the same overall MSPD process, to determine the blank sample noise. LODs and LOQs were calculated with the S/N methodology.

Table III.3 shows the values of LOD/LOQ, calculated by the S/N methodology, obtained for the target analytes found in the solvent extracts. It appears that the values are higher than the values of LOD and LOQ calculated by the S/N methodology presented in the Table III.2, which were calculated from standard solutions. LODs are 6-16 times higher for PAHs after the MSPD process and 70-340 times higher for PCBs. It is probably due to matrix effects that increase the noise signal of the blank sample and so increase significantly the LOD, particularly for PCBs. Table III.3 shows also the LODs/LOQs of the analytes calculated after their extraction from the sediment matrix. LODs ranged from 0.026 to 0.109 ng for 1 g sediment for PCBs and from 0.006 to 0.022 ng for 1 g sediment for PCBs. Table III.3 also shows the values achieved by Pena et al. for PAHs in soils using also MSPD. The LOD/LOQ obtained in our study were better for the three first PAHs ²².

As mentioned before, the blank was made with the model sediment and the m/z of each compound was called in MS for being sure of their presence or not. Some PAHs were detected (Table III.4) at low concentrations: as supposed in the chapter II, the reconstituted model sediment contained actually some PAHs. It may explain the presence of some oxygenated metabolites of PAHs in the model sediment, which can be considered only as a pseudo-blank sample (Table II.19).

Table III.3: Limits of detection and quantification of the 5 PAHs and the 7 PCBs after MSPD extraction
($Q_{\text{sediment}} = 0.5 \text{ g}$, $n=3$ replicates)

Compounds	LOD (mg L^{-1}) ^a	LOQ (mg L^{-1}) ^a	LOD (ng g^{-1}) ^b	LOQ (ng g^{-1}) ^b	LOD (ng g^{-1}) ^c	LOQ (ng g^{-1}) ^c
Phenanthrene	0.003 ± 0.001	0.009 ± 0.003	0.008 ± 0.003	0.026 ± 0.008	0.05	0.2
PCB 28	0.009 ± 0.002	0.029 ± 0.006	0.026 ± 0.006	0.087 ± 0.018	-	-
PCB 52	0.015 ± 0.004	0.050 ± 0.013	0.045 ± 0.011	0.150 ± 0.038	-	-
Fluoranthene	0.002 ± 0.001	0.006 ± 0.002	0.006 ± 0.002	0.019 ± 0.006	0.1	0.5
PCB 101	0.010 ± 0.002	0.033 ± 0.008	0.030 ± 0.007	0.099 ± 0.024	-	-
Pyrene	0.002 ± 0.001	0.006 ± 0.002	0.006 ± 0.002	0.018 ± 0.006	0.04	0.1
PCB 153	0.013 ± 0.003	0.042 ± 0.011	0.038 ± 0.010	0.126 ± 0.033	-	-
PCB138	0.014 ± 0.003	0.045 ± 0.011	0.041 ± 0.010	0.136 ± 0.034	-	-
PCB 180	0.016 ± 0.005	0.053 ± 0.018	0.048 ± 0.016	0.158 ± 0.054	-	-
Benzo[k]fluoranthene	0.006 ± 0.002	0.020 ± 0.007	0.018 ± 0.006	0.061 ± 0.020	0.004	0.01
PCB 209	0.036 ± 0.012	0.121 ± 0.039	0.109 ± 0.035	0.364 ± 0.116	-	-
Benzo[a]pyrene	0.007 ± 0.003	0.024 ± 0.008	0.022 ± 0.008	0.073 ± 0.024	0.004	0.02

a LOD/LOQ in solution

b LOD/LOQ from the solid matrix

c LOD/LOQ from Pena et al.²²

Table III.4: Means values (n=3) of the PAHs found in the matrix blank (non spiked model sediment)

Compounds	Peak height	Area	Concentration (mg L^{-1})	Concentration (ng g^{-1})
Naphthalene	26675 ± 788	54403 ± 5127	0.307 ± 0.022	0.920 ± 0.065
Acenaphthene	1611 ± 145	2424 ± 176	0.011 ± 0.001	0.032 ± 0.003
Fluorene	1042 ± 146	1923 ± 604	0.008 ± 0.003	0.023 ± 0.008
Phenanthrene	7965 ± 1321	19263 ± 3621	0.093 ± 0.016	0.278 ± 0.048
Anthracene	456 ± 113	1012 ± 319	0.005 ± 0.002	0.014 ± 0.006
Fluoranthene	988 ± 644	2578 ± 1143	0.011 ± 0.005	0.034 ± 0.015
Benzo[a]anthracene	497 ± 366	1470 ± 1094	0.007 ± 0.005	0.021 ± 0.016
Chrysene	466 ± 302	1140 ± 784	0.005 ± 0.004	0.016 ± 0.011
Benzo[b]fluoranthene	295 ± 199	1060 ± 755	0.014 ± 0.010	0.041 ± 0.030
Benzo[k]fluoranthene	115 ± 103	394 ± 281	0.005 ± 0.003	0.014 ± 0.010

III.2.2.2 MSPD validation using a naturally contaminated standard sediment and comparison with MAE extraction

a. MAE extraction of PAHs and PCBs

The optimization of the MAE extraction of PAHs and PCBs has been already done for previous studies. The optimal conditions are reported here: 5 g sediment samples are dried through freeze-drying and thereafter spiked with the three surrogate standards (15 μL at 100 mg L^{-1}). They are introduced inside the PTFE MAE vessels and mixed with 40 mL acetone/toluene 50/50 (v/v) solvent. They are extracted through MAE at 130°C during 35 min with a 300 W power for one extraction (600 W for 3 or 4 simultaneous extractions, or 1200 W for 5 or 6 simultaneous extractions). After cooling, extracts are filtered through a 0.2 μm PTFE filter and evaporated to dryness, after addition of 60 μL octanol as solvent keeper. The volume is completed with toluene to 1.5 mL and 990 μL are taken, mixed with 10 μL of the two internal standards and 1 μL is finally injected in the GC-MS system.

These extraction conditions were applied to a standard sediment CRM104, furnished by Sigma-Aldrich and certified (values revised in 2017) for the 16 priority PAHs (Table III.5).

Table III.5 Means of certified and extracted amounts of the 16 priority PAHs from the reference sediment CRM104 through MAE extraction

PAHs	Certified values (2017) ($\mu\text{g kg}^{-1}$)	Mean extracted amounts (n=4) ($\mu\text{g kg}^{-1}$)	Recovery yields (%)
Naphthalene	282±14	167±31	59.2
Acenaphthene	522±26	405±45	77.6
Acenaphthylene	355±18	469±48	132.0
Fluorene	226±11	230±25	101.8
Phenanthrene	348±17	340±40	97.6
Anthracene	480±24	466±54	97.0
Fluoranthene	325±16	301±34	92.7
Pyrene	191±9	196±22	102.5
Benz[a]anthracene	103±5	127±16	123.2
Chrysene	138±7	150±18	108.7
Benzo[b]fluoranthene	305±15	344±37	112.8
Benzo[k]fluoranthene	189±9	212±23	111.9
Benzo[a]pyrene	240±12	258±29	107.5
Indeno[1,2,3,cd]pyrene	228±11	275±33	120.5
Dibenzo[a,h]anthracene	76±4	92±10	121.0
Benzo[g,h,i]perylene	133±7	150±18	112.7

Table III.5 shows that except for naphthalene, which is the most volatile PAH and is easily lost during the evaporation step, all the PAHs were quantitatively extracted (77.6-132%) using this MAE extraction process.

b. Validation of the MSPD extraction and comparison with MAE extraction

MSPD extraction method was validated using a certified reference sediment (CNS391) provided by Merck-Fluka. It is a sediment collected from a fresh water river that contains certified amounts of PAHs, PCBs and pesticides. The values from the certificate of analysis, established in 2013, are listed in the Table III.6.

We must underline here that only fifteen of the 16 priority PAHs were considered in this part of the study: naphthalene was removed from the discussion because its mean values, obtained from 3 different extraction tools, were considerably lower than those mentioned by the certificate of analysis of CNS391. The 3 different extraction tools were: our optimized MSPD extraction methodology, our MAE extraction methodology, but the conventional Soxhlet extraction was also tested (8 h extraction with 100 mL dichloromethane). In the three cases, naphthalene recoveries were lower than 10%. We saw previously that for the certified reference material CRM104, MAE gave correct results for naphthalene, even if not quantitative (59.2% extraction yield). The problem with the CNS391 certified sediment could be explained observing the date of the certificate of analysis: the certified data were listed in 2013 and the certificate has not been reconsidered for many years, while it is well known that the lower molecular weight PAHs can be volatilized or degraded, even in cold and dry storage conditions. It was probably the case for naphthalene in CNS391. Considering now the CRM104 standard sediment, certified values are regularly reconsidered (it was done only few months before our analyses, Table III.5) and we can consider that the certified amounts for naphthalene were more reliable.

When comparing the mean amounts obtained for the MSPD extraction of PAHs with the mean values obtained from the certified sediment, it can be observed that the mean extracted amount of the Σ_{15} PAHs ($3241.6 \pm 210.1 \mu\text{g kg}^{-1}$) was lower than the mean certified value ($3985 \mu\text{g kg}^{-1}$) (Table III.6). In the Table III.6 it is possible to observe that with the MSPD extraction method, the extraction of the lighter PAHs (acenaphthene, acenaphthylene, phenanthrene and anthracene) and benzo[a]pyrene gave better extraction values than those listed in the certificate. Unfortunately, all the values of PAHs were not in the intervals of prediction of the certificate and had mean extracted amounts lower than the values of the certificate: it was particularly the case for high molecular weight PAHs. However, the global extraction recovery of the 15 PAHs using MSPD was satisfactory, being 81.4%.

MSPD extraction was compared to the already validated MAE extraction. Table III.6 gives the results obtained from the MAE extraction of the reference material CNS391. It appears that, as MSPD extraction, lots of results were not in the intervals of prediction of the certificate. Unlike MSPD, MAE extraction was slightly less efficient for extracting low molecular weight PAHs but slightly better for high molecular weight PAHs. However, the global extraction recovery of the 15 PAHs using MAE was less satisfactory than MSPD, being 73.6%.

Table III.6 Certified amounts of PAHs from the standard sediment CNS391 and means of extracted amounts (and standard deviation) for 15 of the priority PAHs extracted by MSPD or MAE from CNS391 reference material

PAHs	Certified values (CNS391) ($\mu\text{g kg}^{-1}$)	Mean amounts from MSPD (n=5) ($\mu\text{g kg}^{-1}$)	MSPD recovery yields * (n=5) (%)	Mean amounts from MAE (n=5) ($\mu\text{g kg}^{-1}$)	MAE recovery yields * (n=5) (%)
Acenaphthene	29.9±6.4	57.6±5.5	192.7	56.4±4.6	188.7
Acenaphthylene	53.4±10.8	73.7±14.5	138.0	27.2±4.4	51.0
Fluorene	409.0±42.3	229.7±20.3	56.2	116.4±6.8	28.5
Phenanthrene	660.0±34.5	842.5±85.1	127.7	633.4±17.6	96.0
Anthracene	15.0±3.4	33.3±2.4	222.1	31.9±2.2	212.4
Fluoranthene	557.0±29.5	546.2±11.9	98.1	526.7±15.8	94.6
Pyrene	331.0±31.6	117.0±3.2	35.3	96.7±7.3	29.2
Benz[a]anthracene	338.0±26.6	169.0±5.0	50.0	167.4±7.5	49.5
Chrysene	376.0±13.1	355.1±11.2	94.4	358.8±14.7	95.4
Benzo[b]fluoranthene	210.0±8.1	182.7±7.9	87.0	192.2±8.2	91.5
Benzo[k]fluoranthene	300.0±11.6	213.0±9.8	71.0	238.8±10.0	79.6
Benzo[a]pyrene	38.2±4.8	22.7±3.1	59.7	8.7±0.5	23.0
Indeno[1,2,3,cd]pyrene	235.0±12.0	154.6±9.6	65.8	179.9±5.6	76.6
Dibenzo[a,h]anthracene	294.0±11.8	207.5±12.9	70.6	256.9±12.4	87.4
Benzo[g,h,i]perylene	139.0±10.1	40.9±3.2	29.4	42.3±3.3	30.4
Σ 15 PAHs	3985.3	3245.4	81.4	2933.8	73.6

* Recoveries obtained in relation to the means given by the certificate

The Table III.7 shows that the mean amounts of PCBs extracted by MSPD were significantly higher than the mean amounts listed in the certificate. The extraction of the PCBs were higher using the MSPD method whatever the PCB, from the less chlorinated (3 Cl) to the more chlorinated (7 Cl). Likewise PAHs, the values of PCBs did not enter in the interval of prediction of the certified sediment, but this time the values were higher than the prediction, which shows a strong capacity for MSPD extraction, with a mean extraction recovery of 165.3%.

Table III.7 Certified amounts of PCBs from the standard sediment CNS391 and means of extracted amounts and standard deviation for 6 of the PCB indicators extracted by MSPD or MAE from CNS391 reference material

PCBs	Certified values (CNS391) ($\mu\text{g kg}^{-1}$)	Mean amounts from MSPD (n=5) ($\mu\text{g kg}^{-1}$)	MSPD recovery yields * (n=5) (%)	Mean amounts from MAE (n=5) ($\mu\text{g kg}^{-1}$)	MAE recovery yields * (n=5) (%)
PCB 28	44.9±3.3	52.1±1.1	116.0	47.8±1.9	106.4
PCB 52	64.6±4.2	98.4±7.8	152.4	89.8±4.1	139.1
PCB 101	45.7±3.1	69.8±4.1	152.7	54.0±4.0	118.1
PCB 153	50.1±2.6	101.7±13.1	203.1	54.6±2.3	109.0
PCB 138	34.6±2.7	86.2±4.5	249.2	52.0±3.3	150.2
PCB 180	54.7±3.0	78.8±8.5	144.0	48.2±1.6	88.1
Σ 6 PCBs	294.6	487.0	165.3	346.3	117.6

* Recoveries obtained in relation to the means given by the certificate

The results achieved from the extraction of PCBs by MSPD can be surprising, so they were compared with MAE extraction of the reference material, performed in the same conditions than those applied for PAHs. Table III.7 shows that results obtained using MAE were not far from those listed in the certificate, but with a better global recovery (117.6%). It must be mentioned that MAE or MSPD extractions are compared with conventional extraction methods for PCBs, developed in the beginning of the 90': ultrasound-assisted extraction (method 3550A of US-EPA) or Soxhlet extraction (method 3541 of US-EPA) (the results listed in the certificate are a combination of these methods, as mentioned in the certificate). However, when examining these methods, it is mentioned that the method 3550A extracts less than 50% of the organochlorine compounds and that the method 3541 is not appropriate when concentrations of PCBs are too low. So, it is not so surprising that the MSPD extraction method allows achieving concentration values two times higher than the values given by the certificate of analysis.

c. MSPD extraction of a naturally contaminated sediment (comparison with MAE)

Using the optimal extraction conditions of MSPD and the conditions validated for PAHs extraction from MAE, tests were made from a naturally contaminated sediment from Tancarville (France) (Table III.8). Comparing the two methodologies, it is visible that MSPD had higher extraction recoveries for the majority of the contaminants, both PCBs and PAHs (except for anthracene and PCB101), which confirm the previous results.

Table III.8 Mean amounts of 16 PAHs and 6 PCBs in Tancarville sediment, obtained from MSPD and MAE extraction

Compounds	Mean amounts from MSPD (ng g⁻¹) (n=3)	Mean amounts from MAE (ng g⁻¹) (n=3)
Naphthalene	175.2±11.6	23.4±0.9
Acenaphthene	34.5±2.4	14.6±3.0
Acenaphthylene	35.6±1.5	5.7±0.8
Fluorene	27.8±1.1	13.7±1.1
Phenanthrene	191.3±5.2	63.2±1.6
Anthracene	38.2±1.4	49.5±0.5
Fluoranthene	150.0±8.7	109.3±5.4
Pyrene	146.9±9.9	102.7±5.3
Benz[a]anthracene	91.5±2.0	71.8±2.7
Chrysene	74.0±0.6	56.4±2.6
Benzo[b]fluoranthene	160.1±3.7	106.0±5.3
Benzo[k]fluoranthene	48.6±2.4	32.2±0.8
Benzo[a]pyrene	81.0±7.7	59.1±6.3
Indeno[1,2,3,cd]pyrene	5.6±0.4	4.2±0.9
Dibenzo[a,h]anthracene	94.6±1.5	65.9±5.3
Benzo[g,h,i]perylene	91.0±4.9	63.6±6.0
Σ 16 PAHs	1445.8±55.8	841.3±40.4
PCB28	7.4 ± 0.7	4.4 ± 0.1
PCB 52	16.1 ± 1.4	5.2 ± 0.5
PCB 101	15.5 ± 1.2	22.9 ± 3.6
PCB 153	36.3 ± 2.4	15.8 ± 0.9
PCB 138	36.5 ± 1.6	18.2 ± 0.5
PCB 180	63.2 ± 2.1	45.4 ± 2.3
Σ 6 PCBs	174.9 ± 6.3	112.1 ± 4.3

III.2.3 Advantages of MSPD in terms of purification

The extractions performed in MSPD presented significant improvement in the extraction of the lighter PAHs in comparison with MAE extraction (although it was also the case for the global results). When comparing the analyses made after MSPD or MAE extractions (Figure III.7), it can be noted that there were much more interfering peaks in chromatograms obtained from MAE (Figure III.7 a) than in the chromatograms obtained from MSPD extractions (Figure III.7

b), especially in the beginning of the chromatograms. This can be explained by the capacity of MSPD to purify the sample at the same time than the extraction and obviously by the lack of any purification step in the analytical process using MAE.

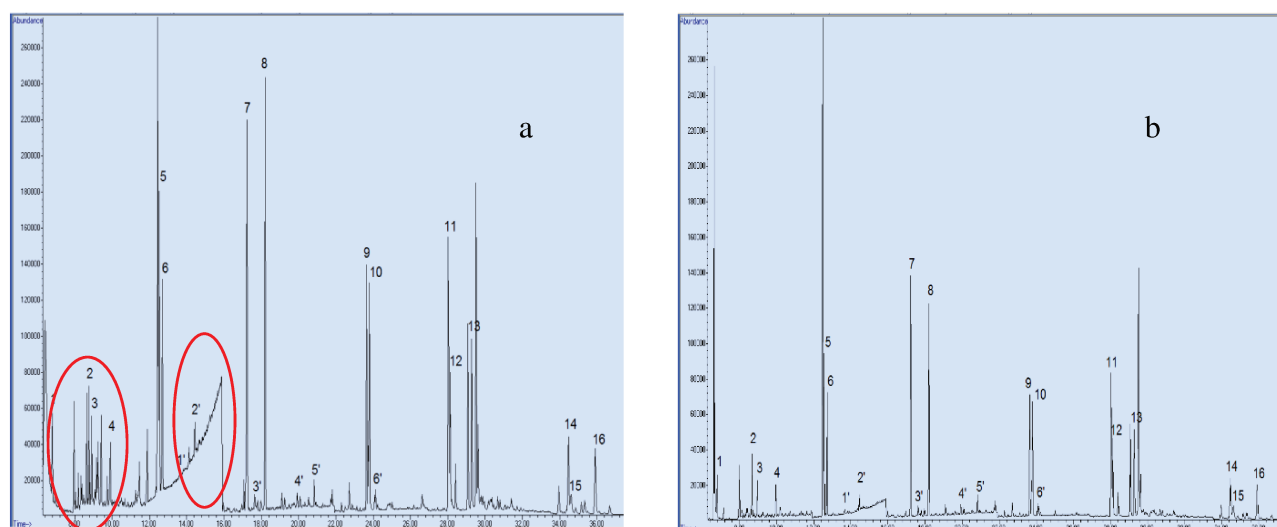


Figure III.7 Chromatogram of PAHs/PCBs after a MAE (a) extraction and MSPD (b)

III.3 Selective extraction of hydroxy-PAHs using MSPD-MIPs

III.3.1 Description of the different steps of the process

The MSPD methodology was optimized for PCBs/PAHs, but another group of contaminants potentially present into the sediment was added to the study, that is the more polar group of hydroxy-PAHs. The tests began with the same optimal conditions achieved for PAHs/PCBs cited previously, using the same model sediment as a matrix to be extracted (73% silt, 19.5% clay, 5% sand and 2.5% of organic matter). Hydroxy-PAHs were not added immediately (when the sediment is prepared) with PAHs and PCBs, because there was a possibility that these compounds would degrade quickly in the sediment, which would alter the results of the extraction. In fact, the hydroxy-PAHs spiking was made one day before the experiments, with a solution of 4 hydroxy-PAHs in acetone at 100 mg L^{-1} let for 2 hours with constant stirring under a fume-hood (for evaporating the solvent) and kept after in the fridge.

The first step of the process consisted in the extraction of PAHs/PCBs alone, in the optimal conditions already found: 0.5 g of sediment (with $15 \mu\text{L}$ of the surrogate standards: fluoranthene D10 and 7-methylbenzo[a]pyrene at 100 mg L^{-1} + $15 \mu\text{L}$ of a PCB 156 solution at 100 mg L^{-1}), 1 g of Florisil, 1 g of sodium sulphate and 0.5 g of 3-chloropropyl bonded particles were crushed together for 10 minutes. At the bottom of the MSPD cartridge 1 g of Florisil was added followed by the sediment mixture and the elution was performed with 5 mL of a solvent (called solvent mixture n°1) composed of 50% hexane/50% acetone (v/v) with the SPE extraction system

(Phenomenex). The final solutions were evaporated to dryness with the MiVac system after the addition of 60 μL of octanol, and after re-diluted in 1440 μL of toluene. From this solution 990 μL were taken, 10 μL of the internal standards (Phenanthrene D10 and Perylene D12 at 100 mg L^{-1}) were added and 1 μL was injected in GC-MS for the analysis of PAHs/PCBs.

The second step consisted in the elution of the hydroxy-PAHs from the sediment cartridge, which was made with different volumes and mixtures of more polar solvents (called solvent mixture n°2). For this, the MSPD cartridge was coupled with a pre-conditioned MIP cartridge (Figure III.8).



Figure III.8: Coupling between the MSPD cartridge containing the sediment and the MIP-phenolic, to selectively sorb OH-PAHs.

The MIP cartridge (3 mL cartridge, 100 mg sorbent) is a commercial one, designed for phenolic compounds (AffiniMIP-SPE-Phenolics from AffiniSEP, France), which are structurally close to hydroxy-PAHs. We can note here that these MIPs has been already successfully used to extract hydroxy-PAHs from soils ⁶³. MIPs were used at this step of the process because a lot of more polar (than PAHs/PCBs) compounds, previously considered as interfering compounds for the analysis of PAHs/PCBs, could be potentially eluted as the same time as hydroxy-PAHs and could interfere on the chromatogram. So the target hydroxy-PAHs had to sorb preferentially inside the selective MIP-Phenolics, which is used to purify the eluate (Figure III.9).

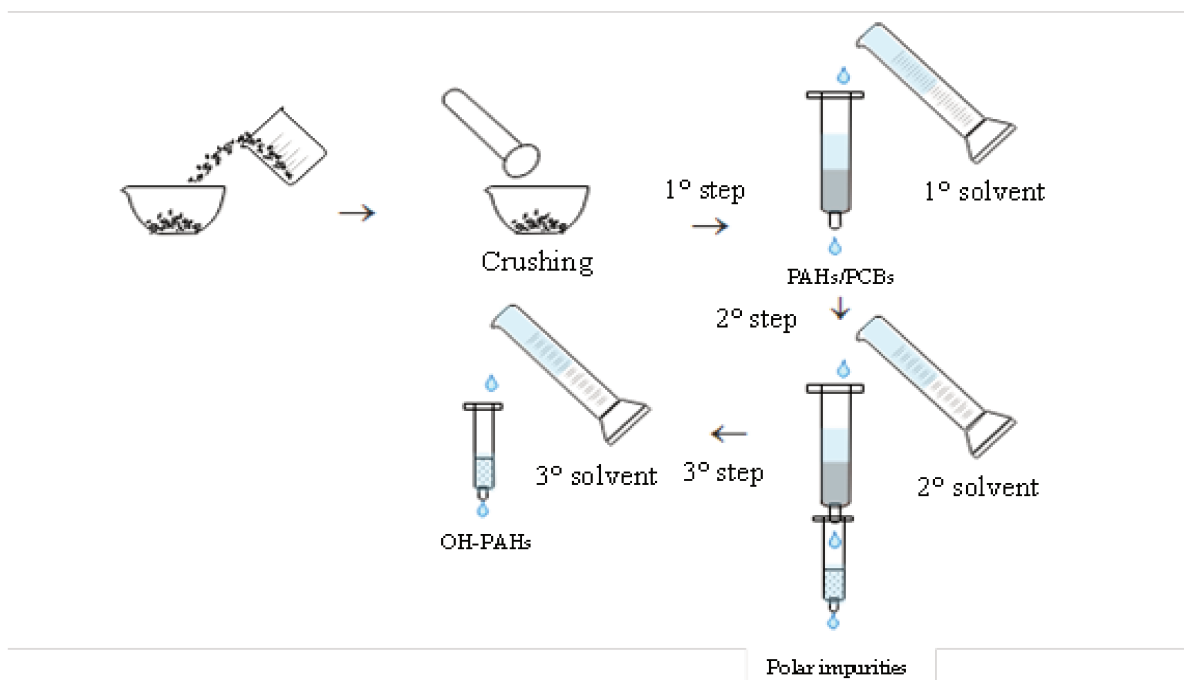


Figure III.9 Main steps of the matrix solid phase dispersion extraction for PAHs/PCBs and coupling with molecularly imprinted polymer extraction for hydroxy-PAHs

In the final third step, the MIP cartridge was dried under a nitrogen flow and the elution of the target hydroxy-PAHs was made with a volume of a 98% methanol/2% acetic acid (v/v) mixture (called solvent mixture n°3) and the hydroxy-PAHs were analyzed in HPLC-FLD (see chapter II.1.1.1). The addition of the MIP actually allowed purifying the eluate analyzed in HPLC-FLD, as observed when comparing the chromatograms in Figures III.10 and III.11, in which there was much more interfering peaks in the absence of the MIP.

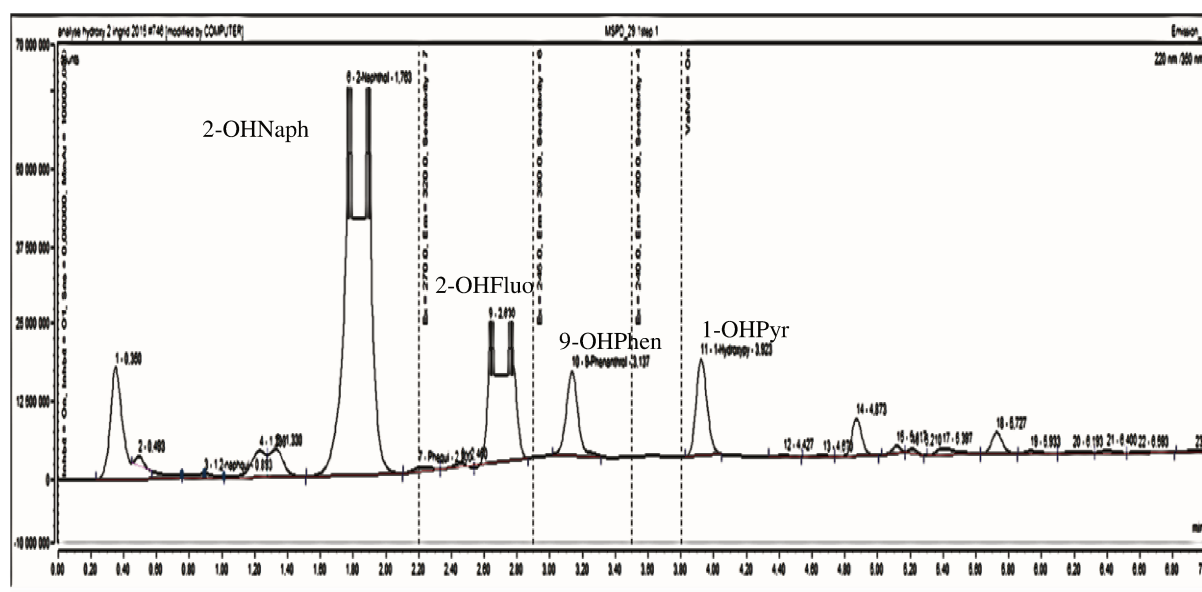


Figure III.10 Chromatogram of 4 OH-PAHs after an extraction from the model sediment using the MSPD process and without the use of a MIP (note: peaks of 2-OHNaph and 2-OHFuo were saturated).

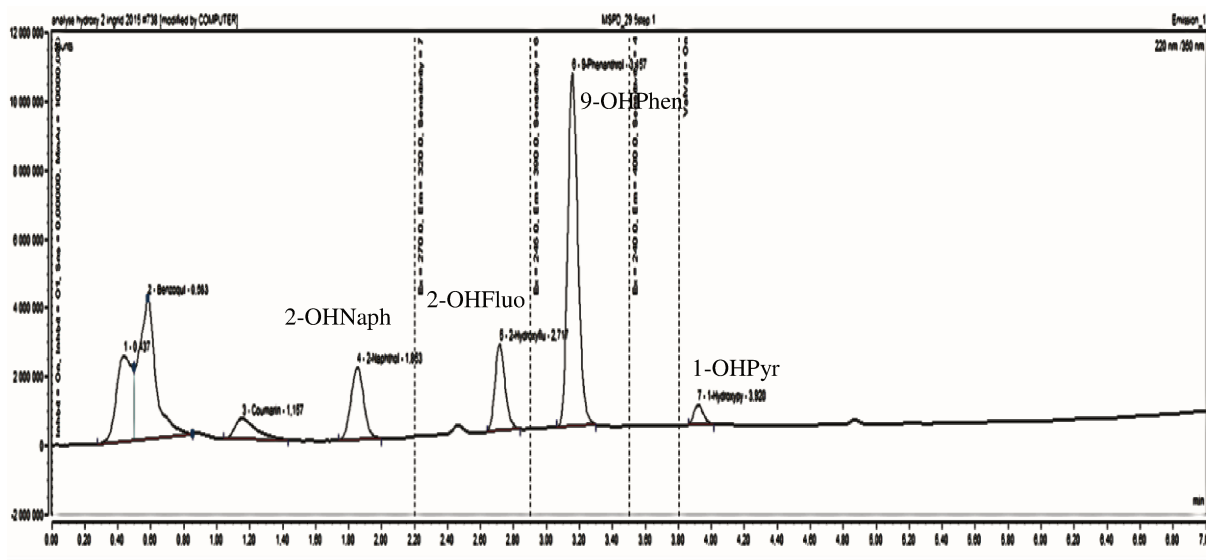


Figure III.11 Chromatogram of 4 OH-PAHs after an extraction from the model sediment using the MSPD process and with the use of a MIP

III.3.2 Choice of percolation and elution solvents compatible with MIPs

III.3.2.1 Tests on various percolation and elution solvents

After the elution of the PAHs/PCBs, the MSPD cartridge was dried under a nitrogen flow to avoid interference of the solvents used in the first step for the elution of PAHs/PCBs (mixture n°1) and solvents used for the second step of elution of the more polar compounds (mixture n°2). During this time, the MIP cartridge was conditioned. Conditioning with the proper solvent activates the imprinted cavities, swelling it properly, to make sure that the size and the chemical groups in the cavities are in the best conditions for receiving the target molecules, which improves the capacity of recognition of the sites for the target molecules^{233, 234}. The MIP-Phenolic was conditioned according to the instructions of the manufacturer: if the solvent mixture n°2 was composed of a less polar solvent and a more polar solvent, the MIP was first conditioned percolating 3 mL of the less polar solvent and then 3 mL (x 2) of the more polar solvent, never leaving the cartridge dry. In the second step, the MSPD cartridge is then connected with the MIP cartridge and the elution of the polar compounds from the MSPD cartridge (and so the simultaneous percolation through the MIP cartridge) is performed with a given volume of the solvent mixture n°2. In the initial tests, the solvent mixture n°2 was composed of 4 mL of 90% CH₃CN/10% toluene (v/v), a mixture already used in the study of Oriol et al.⁶³ but for MAE extraction of hydroxy-PAHs from soils.

The third and last step consisted in the elution of the hydroxy-PAHs from the MIP cartridge. The MIP-Phenolics cartridge was previously dried under a nitrogen flow for the same reason than the MSPD cartridge, and the elution was made with a given volume of a solvent mixture

n°3. At the beginning we used the same solvent mixture than used in the study of Oriol et al. ⁶³, that was 6 mL of 98% methanol/2% acetic acid (v/v). The complete scheme, with the steps mentioned previously, is presented in the Figure III.12.

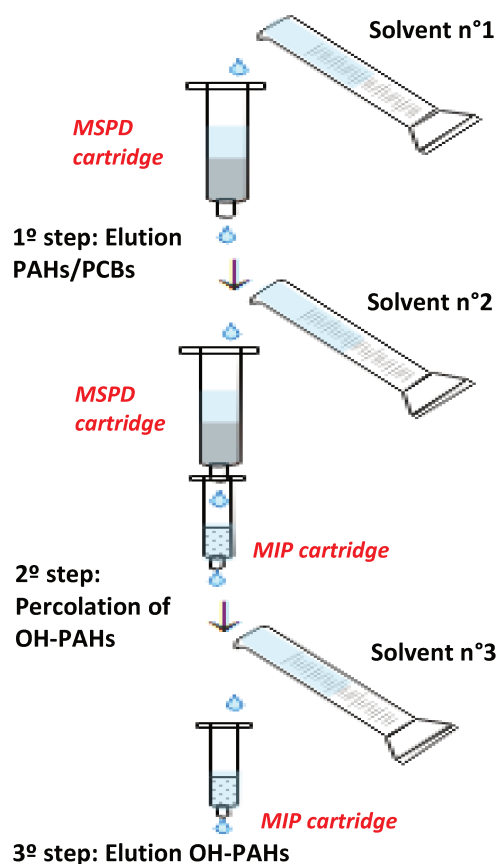


Figure III.12 Scheme of the three steps of the elution of OH-PAHs: the first one is the elution of PAHs/PCBs without OH-PAHs, the second one is the transfer of OH-PAHs from the MSPD cartridge and simultaneous percolation through the MIP and the third is the elution of the OH-PAHs from the MIP.

Next, hydroxy-PAHs were analyzed in HPLC-FLD and some results, obtained varying the volume or the solvent mixture n°2, are presented in the Table III.9.

Varying the volume of the percolation solvent (mixture n°2) from 4 to 6 mL allowed improving the recovery of 9-OHPhen, but not those of the three other OH-PAHs. Nevertheless a volume of 6 mL was chosen for the other tests. The addition of 1 mL supplementary in the final elution solvent (mixture n°3) presented an improvement for three of the hydroxy-PAHs, so a volume of 7 mL for the final elution solvent was chosen for the other tests.

Table III.9 Recoveries of the 4 hydroxy-PAHs after the extraction from the MSPD cartridge (solvent mixture n°1: 6 mL 50% hexane/50% acetone) and the elution from the MIP-Phenolic (n=3)

Percolation solvent (mixture n°2)	Volume (mL)	Final elution solvent (mixture n°3)	2-OHNaph (%)	2-OHFluo (%)	9-OHPhen (%)	1-OHPyr (%)
90% acetonitrile/10% toluene	4	6 mL 98% methanol/2% acetic acid	12.5 ± 0.2	15.2 ± 0.5	26.4 * ± 0.9	2.5 ± 0.2
	5		11.0 ± 1.7	12.7 ± 1.4	20.9 * ± 1.7	4.9 ± 2.5
	6		9.2 ± 0.1	12.7 ± 0.1	44.7 * ± 4.2	2.8 ± 0.2
	6	7 mL methanol/2% acetic acid	11.7 ± 0.2	16.1 ± 0.2	35.4 * ± 7.5	4.7 ± 0.3
	6 **		2.7 ± 0.1	4.1 ± 2.0	22.6* ± 0.4	9.4 ± 2.0
Acetonitrile	6	7 mL methanol/2% acetic acid	11.9 ± 0.2	14.4 ± 0.3	10.4 ± 1.2	4.8 ± 0.3
90% acetonitrile/10% methanol	6	7 mL methanol/2% acetic acid	0.4 ± 0.1	0.3 ± 0.1	0.9 ± 0.1	0.3 ± 0.1
80% acetonitrile/20% toluene	6	7 mL methanol/2% acetic acid	0.7 ± 0.1	0.4 ± 0.1	35.6 * ± 11.6	0.1 ± 0.1

* There is a doubt on these values that seem too high: when toluene is used in the percolating solvent n°2, a more or less intense interfering compound appears at the same retention time than 9-OHPhen in HPLC-FLD analysis.

** MSPD and MIP cartridges were directly coupled in the first step of the process

Moreover, changing the nature of the percolation solvent (without toluene in acetonitrile, with 20% toluene instead of 10%, or changing toluene to methanol) did not present any improvement compared to the mixture n°2 90% acetonitrile/10% toluene (Table III.9). As we thought that a part of hydroxy-PAHs could be lost at the first step of the process, at the same time as the elution of PAHs/PCBs (although Florisil was added to prevent it), we coupled the two cartridges at the beginning of the process. But Table III.9 shows that the results were better only for 1-OHPyr but not for the three other hydroxy-PAHs. Coupling the MIP cartridge at the first step was not favorable probably because the MIP was not conditioned properly.

Also, in some tests, the introduction of the polymer constituting the MIP cartridge (in the form of powder) was tested in the step of grinding instead of using the cartridge, directly at the first step: 0.5 g of the imprinted polymer was added to the sediment, at the same time than the other dispersing agents, and the mixture was grinded. But the results obtained at the end of the process (all other things being equal) were lower than using the MIP cartridge, with recoveries ranging between 0.72% and 6.13%. The step of grinding could have decrease the capacity of recognition of the imprinted polymer. Vasapollo et al. actually said that when grinding, the loading capacity

of the polymer could decrease due the fact that some interaction sites are destroyed during this process ²³⁵.

III.3.2.2 Influence of the first solvent mixture on PAHs/PCBs/hydroxy-PAHs results

As the final results were not satisfactory, tests were made to see if hydroxy-PAHs could be lost at the beginning of the process, with the elution of PAHs/PCBs from the MSPD cartridge. Tests were made with the same MSPD initial process, using Florisil and the same dispersing agents, and the co-column of sorbent at the bottom of the cartridge, but without the MIP cartridge.

Table III.10 Recoveries (%) of hydroxy-PAHs in the eluting solvent (mixture n°1) from the MSPD cartridge (n=2)

Elution solvent (mixture n°1)	Volume (mL)	Difference	2-OHNaph (%)	2-OHFluo (%)	9-OHPhen (%)	1-OHPyr (%)
50% hexane/ 50% acetone	5*		51.0 ± 9.1	58.8 ± 6.0	1.8 ± 0.1	1.7 ± 1.1
	6		62.6 ± 9.1	70.2 ± 9.8	2.4 ± 0.4	8.0 ± 0.7
CH ₂ Cl ₂	6		0.9 ± 0.1	2.5 ± 0.1	1.1 ± 1.0	0.0
50% hexane/50% CH ₂ Cl ₂	6		6.6 ± 0.7	5.7 ± 0.9	0.1 ± 0.1	0.2 ± 0.2
		Sediment without any additives **	19.6	23.6	0.5	0.0

* Test done in triplicate

** Just one extraction performed

Table III.10 shows that we could observe a huge loss of the two lighter hydroxy-PAHs (2-OHNaph and 2-OHFluo) from the MSPD sediment cartridge, at the beginning of the process, that could be responsible of the low results at the end of the process, when the mixture n°1 composed of 50% hexane/50% acetone was used for the elution of PAHs/PCBs. Losses were in the range 51-59% and were even higher when the volume of the eluting solvent was increased (63-70% losses). To avoid the loss of the lighter and more polar hydroxy-PAHs at the beginning, a less polar solvent was tested as solvent mixture n°1. Acetone (Polarity index P = 5.1) was replaced by dichloromethane (P = 3.1). We must remind that dichloromethane was actually a good solvent mixture to elute PAHs and PCBs from the MSPD cartridge (Figure III.3). Using pure dichloromethane as an eluting solvent from the MSPD cartridge decreased drastically the loss of the two lighter hydroxy-PAHs (less than 2.5%), but it was not good for PAH recoveries (Table III.11). Using the mixture composed of 50% hexane/50% dichloromethane was a good solution to avoid the loss of the two lighter hydroxy-PAHs (less than 6.6%) at the first step of the process, keeping good recoveries for PAHs (Table III.11), even if a little lesser than using hexane/acetone (Table III.11 and Figure III.3). Recoveries of PCBs were always satisfactory (Table III.11).

Table III.11 Recoveries (%) of PAHs/PCBs at the first step with diverse eluting solvent mixtures (n°1)

PAHs/ PCBs	6 mL 50% hexane/50% acetone (separated cartridges) (%) (n=2)	6 mL 50% hexane/50% acetone (cartridges together) (%) (n=2)	6 mL 100% CH ₂ Cl ₂ (%) (n=2)	6 mL 50% hexane/50% CH ₂ Cl ₂ (%) (n=2)
Phenanthrene	76.1 ± 0.3	84.4 ± 0.7	52.8 ± 25.5	76.0 ± 1.5
Fluoranthene	78.3 ± 0.8	80.5 ± 0.8	56.1 ± 28.2	74.1 ± 1.2
Pyrene	75.9 ± 1.2	78.2 ± 0.4	55.1 ± 27.3	71.4 ± 0.9
Benzo[k]fluoranthene	76.0 ± 1.5	78.0 ± 2.7	56.6 ± 25.1	73.7 ± 4.0
Benzo[a] pyrene	63.5 ± 0.9	66.9 ± 0.1	44.2 ± 18.6	58.8 ± 3.9
PCB28	82.2 ± 1.0	82.1 ± 9.4	115.5 ± 65.4	89.9 ± 2.8
PCB52	81.2 ± 0.1	80.8 ± 7.7	119.1 ± 64.8	72.3 ± 2.1
PCB101	84.6 ± 1.9	83.9 ± 8.2	126.0 ± 74.0	73.7 ± 8.1
PCB153	91.4 ± 6.5	87.1 ± 5.8	138.0 ± 71.9	77.3 ± 5.8
PCB138	91.2 ± 2.4	88.6 ± 5.4	139.7 ± 79.9	79.7 ± 6.6
PCB180	79.1 ± 6.3	82.3 ± 1.0	123.4 ± 71.4	75.7 ± 1.5
PCB209	86.1 ± 0.8	96.4 ± 19.5	133.0 ± 77.8	100.0 ± 7.3

At last, losses of hydroxy-PAHs were measured (using the hexane/dichloromethane solvent mixture n°1) when all the additives used in the MSPD process were removed (Table III.10). We can see that a more important part of the lighter hydroxy-PAHs was lost, not retained by the Florisil and the other additives.

Table III.12 shows the results of the overall process when 6 mL 50% hexane/ 50% CH₂Cl₂ was chosen now as the solvent mixture n°1. Unfortunately, there was not a big change in the hydroxy-PAH recoveries at the end of the process, with the substitution of acetone by dichloromethane.

Table III.12 shows that replacing toluene by acetone, methanol or water in the solvent mixture n°2, to allow a better desorption of polar compounds from the Florisil sorbent, decreased the recoveries. In the other hand, increasing the percolation volume (from 6 mL to 20 mL) of the best solvent mixture n°2 (found for the moment) did not allow a significant increase of the recoveries (Table III.12).

Table III.12 Recoveries of the 4 hydroxy-PAHs after the extraction from the MSPD cartridge and the elution through the MIP-Phenolic (n=2)

Elution solvent for PAHs/PCBs (mixture n°1)	Percolation solvent (mixture n°2)	2-OHNaph (%)	2-OHFluo (%)	9-OHPhen (%)	1-OHPyr (%)
6 mL 50% hexane/ 50% CH ₂ Cl ₂	6 mL 90% acetonitrile/ 10% toluene	1.3 ± 0.2	1.2 ± 0.3	23.7 *± 2.2	0.8 ± 0.7
	20 mL 90% acetonitrile/ 10% toluene	1.7 ± 0.1	1.2 ± 0.2	27.8 *± 2.0	1.9 ± 0.1
	6 mL 90% acetonitrile/ 10% acetone	1.4 ± 0.1	2.4 ± 0.2	0.5 ± 0.1	1.2 ± 0.1
	6 mL 99% acetonitrile/1% methanol **	1.5	2.0	1.0	2.3
	6 mL 40% acetonitrile/ 60%water **	3.3	2.1	0.4	0.9

* There is a doubt on these values that are too high: when toluene was used in the percolating solvent n°2, a more or less intense interfering compound appears at the same retention time than 9-OHPhen in HPLC-FLD analysis.

** Tests not done in duplicate (n= 1)

At this step of the study, we could find the solvent mixture n°1 to quantitatively desorb PAHs and PCBs from the MSPD cartridge without a substantial loss of the more polar hydroxy-PAHs. We found a solvent mixture n°3 to quantitatively desorb hydroxy-PAHs when they are sorbed in the MIP-Phenolics cartridge⁶³. The main difficulty remains in desorbing hydroxy-PAHs from the sediment cartridge and to transfer them quantitatively to the MIP cartridge, with the appropriate solvent mixture n°2.

III.3.2.3 Compatibility of solvents with the MIP cartridge

Tests were made just with the MIP cartridge to verify the compatibility of various percolation solvents (mixtures n°2) with the imprinted polymer, incompatibility being a possible explanation for the low final results (Table III.13). Co-solvents added to acetonitrile were chosen to develop different selectivity, such as proton acceptors, proton donors or developing dipole interactions.

Tests were made with conditioned MIPs, with conditions previously described and depending on the tested solvent mixture. The four hydroxy-PAHs were directly spiked in the solvent mixture and percolated through the MIP cartridge. Then the MIP was dried and eluted with 7 mL 98% methanol/2% acetic acid (solvent mixture n°3).

Table III.13 Recoveries (%) of the 4 hydroxy-PAHs percolated with the solvent mixture n°2 through the MIP-phenolic and eluted with 7 mL 98% methanol/2% acetic acid (n=1)

Percolation solvent (mixture n°2)	Volume (mL)	2-OHNaph (%)	2-OHFluo (%)	9-OHPhen (%)	1-OHPyr (%)
90% acetonitrile/ 10% toluene	6*	101.5	98.6	119.7	126.9
90% acetonitrile/ 10% acetone	6*	98.9	83.70	89.6	117.0
90% acetonitrile/10% dichloromethane	6*	76.5	78.3	72.1	91.3
70% acetonitrile/30% ethyl acetate	6	78.7	78.1	76.4	61.8
50% acetonitrile/50% ethyl acetate	6	67.1	64.2	35.4	6.4
50% hexane/50% acetone	20	52.6	77.8	112.4	115.4
50% acetonitrile/50% toluene	20	20.5	18.6	261.0 **	50.0
50% toluene/50% acetone	20	17.0	19.1	183.2 **	52.6
99% acetonitrile/1% methanol	20	35.5	97.9	122.6	101.7
	6	111.0	132.0	114.8	95.1
98% acetonitrile/2% methanol	20	24.3	41.8	100.0	102.9
	6	97.9	120.4	98.3	83.4
95% acetonitrile/5% methanol	20	29.2	26.4	57.3	105.1
	6	19.3	26.2	53.9	96.5
99.5% acetonitrile/0.5% butanol	6	28.0	38.4	76.5	115.7
	20	29.3	43.0	68.4	144.7
99% acetonitrile/1% butanol	6	36.8	45.8	77.2	97.8
	20	31.6	41.2	70.9	88.3
98% acetonitrile/2% butanol	6	47.4	57.3	81.3	97.0
	20	31.5	43.3	60.8	90.5
95% acetonitrile/5% butanol	6	42.5	52.7	77.9	94.8
	20	37.5	51.5	83.6	96.5

* Tests made in duplicate

** These values are too high: when toluene is used in the percolating solvent n°2, a more or less intense interfering compound appears at the same retention time than 9-OHPhen in HPLC-FLD analysis.

Table III.13 shows that some mixtures of solvents were particularly compatible with the MIP material and gave quantitative results for the four hydroxy-PAHs. The mixtures with acetonitrile and 10% toluene or 10% acetone gave excellent results; adding 10% dichloromethane or 30% ethyl acetate to acetonitrile gave little less quantitative results, but always satisfactory. As expected, the solvents that use a high quantity of alcohol (methanol or butanol) were not good for the MIP, due to the fact that MIP contains imprinted cavities for phenolic compounds, so alcohols could enter in competition with the target compounds for the recognition sites of the polymer which provokes a huge loss of the target compounds in the

percolation step. Adding 1% or 2% of methanol to acetonitrile was an exception: there was not enough alcohol solvent for competition with the MIP recognition sites, so the target compounds could interact with them and results were quantitative. Table III.13 also shows that in a general manner, using a too large percolation volume (20 mL instead of 6 mL) was not favorable for the recovery of the two lighter hydroxy-PAHs. MIP breakthrough volume was probably exceeded for these two hydroxy-PAHs which are less intensively retained into the polymeric phase than the two other heavier hydroxy-PAHs.

III.3.3 Use of different sorbents or new additives for improving hydroxy-PAHs recoveries

Although some interesting solvent mixtures (n°2) could be found to percolate quantitatively hydroxy-PAHs through the MIP cartridge without damaging the MIP material, they did not gave good results at the end of the whole process (Table III.12) if we tried to use them to transfer the hydroxy-PAHs from the MSPD cartridge to the MIP cartridge. So we supposed that Florisil could be a too strong sorbent for hydroxy-PAHs. So deactivated silica was also tested to replace Florisil. For the deactivation of the silica, the material was put in the oven at 180°C for a period of about 20 hours. Next, it was weighted and a quantity of 10% of water was added to the silica, which was put in the desiccator ²³². The deactivation molecules occupy the higher energetic sites of silica, reducing its adsorption capacity and increasing the reproducibility ¹³¹, which could be observed by a slightly higher loss of the hydroxy-PAHs with comparison to the use of Florisil.

Table III.14 shows that replacing Florisil by the deactivated silica led to better results for the recoveries of PAHs and PCBs at the first step of the process. Silica not deactivated gave too high values for some PAHs and PCBs. Indeed Pena et al. reported that the use of silica made the co-elution of apolar impurities higher ⁶³. This problem was corrected with the deactivation of the silica with 10% of water ²³³.

Table III.14 Recoveries (%) of PAHs/PCBs at the first step of the process with various dispersants/sorbents for polar compounds

PAHs/ PCBs	Florisil (%) (n=2)	Not deactivated Silica (%) (n=1)	Deactivated Silica (%) (n=2)
Phenanthrene	76.0 ± 1.5	156.3	117.5 ± 8.4
Fluoranthene	74.1 ± 1.2	129.6	108.8 ± 1.6
Pyrene	71.4 ± 0.9	116.3	92.2 ± 1.5
Benzo[k]fluoranthene	73.7 ± 4.0	82.8	96.2 ± 6.6
Benzo[a] pyrene	58.8 ± 3.9	66.0	65.7 ± 5.3
PCB28	89.9 ± 2.8	116.7	76.3 ± 2.5
PCB52	72.3 ± 2.1	136.6	80.5 ± 2.0
PCB101	73.7 ± 8.1	144.7	81.7 ± 3.0
PCB153	77.3 ± 5.8	100.0	80.9 ± 4.7
PCB138	79.7 ± 6.6	97.9	81.9 ± 3.3
PCB180	75.7 ± 1.5	87.0	128.5 ± 1.9
PCB209	100.0 ± 47.3	71.9	112.1 ± 7.2

Moreover, an addition of 1 mL KOH saturated in methanol ($C = 50 \text{ g L}^{-1}$) was tested at the beginning of the process, when the sediment mixture is crushed with the dispersing agents, to favor the breaking of hydrogen bonds between the sediment surface and the hydroxylated compounds. We thought that KOH might liberate hydroxy-PAHs by two ways: first, by the hydrolysis of the sediment organic matter labile ester bonds, that will break-down some bonds of the macromolecular humic network. It could not only facilitate the accessibility of the solvent but also the liberation of the organic compounds that were bound by hydrolysable bonds to the soil organic matter. The other possibility might be that the introduction of alkaline conditions to the sediment could enhance the negative charged carboxyl, phenolic and hydroxyl functional groups of the humic macromolecules, causing repulsion between them, what extends the macromolecules and provides an easier access to the molecules, promoting the release of the contaminants and reducing hydrogen bond ability²³⁶.

At last, an addition of salt into the percolation solvent was also tested to promote a salting-out effect.

Table III.15 shows some results obtained from various tests using deactivated silica instead of Florisil. It shows that even when using percolation solvents (mixtures n°2) compatible with MIPs (6 mL 90% acetonitrile/10% toluene, or 99% acetonitrile/1% methanol or 70% acetonitrile/30% ethyl acetate), final results staid very low. Unfortunately, adding KOH in the initial step of crushing did not show any improvement. More hydroxy-PAHs were found in the percolated solvent through the MIP cartridge, showing that KOH was not favorable to the selective sorption of hydroxy-PAHs into the imprinted polymer.

Table III.15 Recoveries of the 4 hydroxy-PAHs after the extraction from the MSPD cartridge with 6 mL 50% CH₂Cl₂/50% hexane and the elution through the MIP-Phenolic (n=2)

Percolation solvent (mixture n°2)	Differences	2-OHNaph (%)	2-OHFluo (%)	9-OHPhen (%)	1-OHPyr (%)
6 mL 90% acetonitrile/10% toluene	1 g deactivated silica	2.2 ± 2.0	3.1 ± 3.2	10.0 ± 0.2	1.1 ± 0.3
	1 g deactivated silica + 1 mL KOH	0.3 ± 0.2	0.5 ± 0.1	8.4 ± 1.8	0.7 ± 0.2
	1 g deactivated silica * + MSPD/MIP not coupled **	9.0	4.2	8.6	0.4
6 mL 99% acetonitrile/1% methanol	1 g not deactivated silica one **	5.4	10.9	1.1	4.5
	1 g deactivated silica	6.5 ± 0.1	14.3 ± 1.4	1.5 ± 0.1	3.0 ± 0.2
6 mL 70% acetonitrile/30% ethyl acetate	1 g deactivated silica	0.5 ± 0.2	1.4 ± 0.2	0.5 ± 0.1	1.3 ± 0.1
	1 g deactivated silica + 1 mL KOH	0.3 ± 0.3	0.1 ± 0.1	-	0.6 ± 0.1
10 mL 99% acetonitrile/1% methanol + 1g NaCl	1 g deactivated silica	1.1 ± 0.2	2.7 ± 0.4	2.1 ± 0.3	2.0 ± 0.2
	1 g deactivated silica + MSPD/MIP not coupled *	20.0 ± 2.1	25.9 ± 3.6	5.2 ± 0.4	2.3 ± 1.7

* Elution made without coupling the MSPD cartridge and the MIP cartridge in the second step

** Tests not made in duplicate (n= 1)

Using 99% acetonitrile/1% methanol as a percolation mixture n°2 gave better results for the two lighter hydroxy-PAHs (Table III.15). Also, when the two cartridges were not coupled at the second step of percolation, the results were higher: in this case, after the elution of PAHs and PCBs, the percolation solvent (mixture n°2) passing through the MSPD cartridge was recovered in a flask and thereafter passed through the MIP cartridge. It is possible that in this case the flow was more constant in the MIP cartridge and favored the sorption of the target compounds to the imprinted polymer.

The results started to be improved for the lighter hydroxy-PAHs with the addition of a lot of NaCl in the percolation solvent n°2 (Table III.15), as in the method Quick, Easy, Cheap, Effective, Rugged, and Safe (QuEChERS), where NaCl is used to promote the effect of salting-out, when used in an aqueous medium ²³⁶. Acetonitrile is one of the most used solvents in QuEChERS due to the facility, when using the correct salt as sulfate of magnesium (MgSO₄) or sodium chloride (NaCl), to be separated from water, but other solvents can be used as ethyl acetate, methanol or acetone ²³⁷. So, the solvent mixture n°2 composed of 99% acetonitrile/1% methanol was tested with a large addition of NaCl (10 g for 10 mL solvent). Without water, the dissolved Na⁺ and Cl⁻ ions in the organic solvent could interact with the sediment organic matter and be responsible for the improvement of the results for lighter hydroxy-PAHs.

Another improvement in the results was achieved when separating the MSPD cartridge from the MIP cartridge (Table III.15). As mentioned previously, the solvent mixture n°2 was first percolated through the MSPD cartridge and recovered into a flask; thereafter this mixture was percolated through the MIP cartridge (after the MIP was conditioned). As discussed previously, the flow was probably better controlled when the two cartridges were not coupled, but also, the time taken by the solvent passing through the MSPD cartridge was too long and may cause a MIP drying, which may cause the retraction of the pores, consequently diminishing the selectivity of the MIP material and letting the hydroxy-PAHs pass through.

III.3.4 Percolation solvents for improving hydroxy-PAHs transfer from MSPD to MIP cartridge

III.3.4.1 Desorption from the MSPD cartridge

As discussed previously, the main challenge was to use a solvent mixture that was strong enough to desorb the hydroxy-PAHs from the sediment cartridge (after a first step of elution of PAHs and PCBs) and at the same time should be compatible with the MIP-Phenolics material, for a subsequent selective analysis of hydroxy-PAHs. Indeed depending on the percolating solvent used, it could swell or shrink the polymeric imprinted pores, and the compounds would pass direct through it, without any selective retention.

We considered the solvent mixture (n°2) composed of 99% acetonitrile/1% methanol to study its capacity to desorb the hydroxy-PAHs from the MSPD cartridge, knowing that this solvent was compatible with the MIP-Phenolic. Several volumes of this solvent mixture were tested to see if there was any influence of the volume on the desorption of the contaminants from the sediment (Figure III.13). In these tests, sediment was mixed and crushed in the same conditions than previously described, but using deactivated silica instead of Florisil (we recall that the use of deactivated silica instead of Florisil gave better results for the recoveries of PAHs and PCBs).

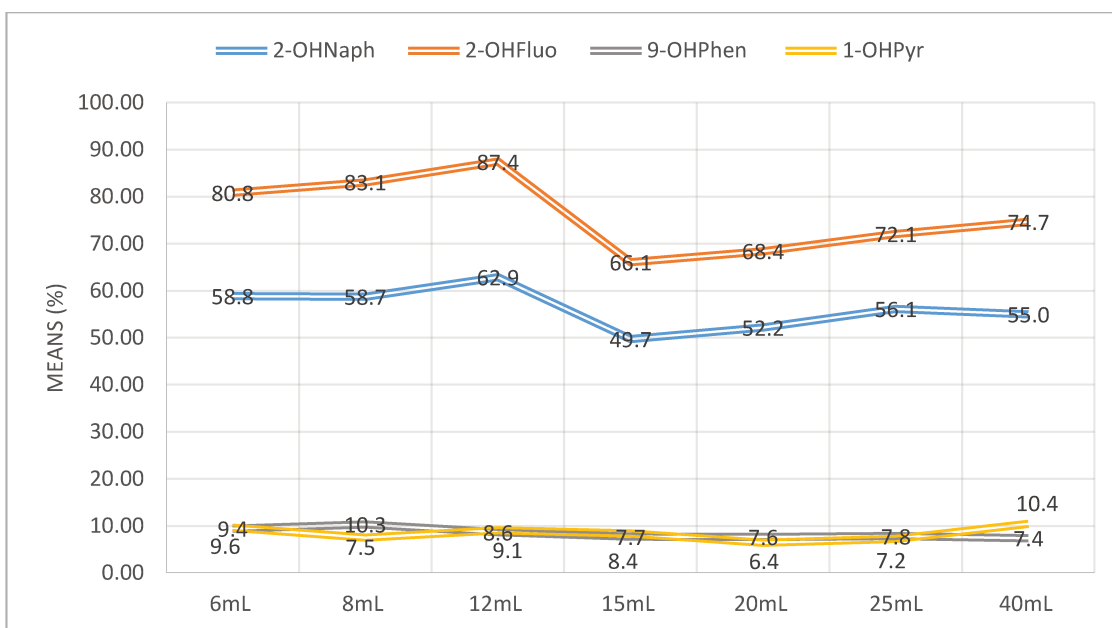


Figure III.13 Extraction recoveries of the 4 hydroxy-PAHs from the MSPD cartridge using different volumes of 99% acetonitrile/1% methanol (A co-column of deactivated silica was not added in these series of tests).

As shown in Figure III.13, we could not see any significant influence of the solvent volume on desorption of hydroxy-PAHs from the sediment mixture. So low volumes (6-12 mL) of a 99% acetonitrile/1% methanol solvent mixture were enough to desorb more than 80% of 2-OHNaph, 60% of 2-OHFluo, but less than 10% of 9-OHPhen or 1-OHPyr. Thus the low results found for the two lighter hydroxy-PAHs when the MIP cartridge was coupled to the MSPD cartridge (6.5-14.3% recoveries, see Table III.15) were not really a problem of desorption from the sediment cartridge, as good desorption recoveries could be achieved: 60-80% when no deactivated silica co-column was added to the sediment mixture and 30-45% when the silica co-column was added. In the case of the heavier hydroxy-PAHs, it is obvious that the main problem was to desorb them from the sediment before transferring them to the MIP cartridge, as less than 10% could be desorbed from the sediment cartridge, even in the absence of the deactivated silica co-column.

Other solvents were used to desorb hydroxy-PAHs from the MSPD cartridge that were also compatible with the MIPs. Using 6 mL of 90% acetonitrile/10% acetone or just 6 mL acetonitrile showed lower results for the desorption from the MSPD cartridge, even for the two lightest hydroxy-PAHs, with only 8.5-15.5% desorption for the 90% acetonitrile/10% acetone mixture and 21.5-29.5% desorption for the acetonitrile alone (Figures III.14 a and b) (here the silica co-column was added to the MSPD cartridge). As one of the main problem was to be capable to break the interactions between the contaminants and the sediment, it seems clear that the best solvent mixture to do this was the mixture containing acetonitrile and 1% methanol. Methanol could be more able to break hydrogen bonds that were responsible of the strong

sorption of the hydroxy-PAHs. But as discussed previously, mixtures containing more than 1-2% methanol in acetonitrile could not be used without damages on the MIP cartridge (Table III.13).

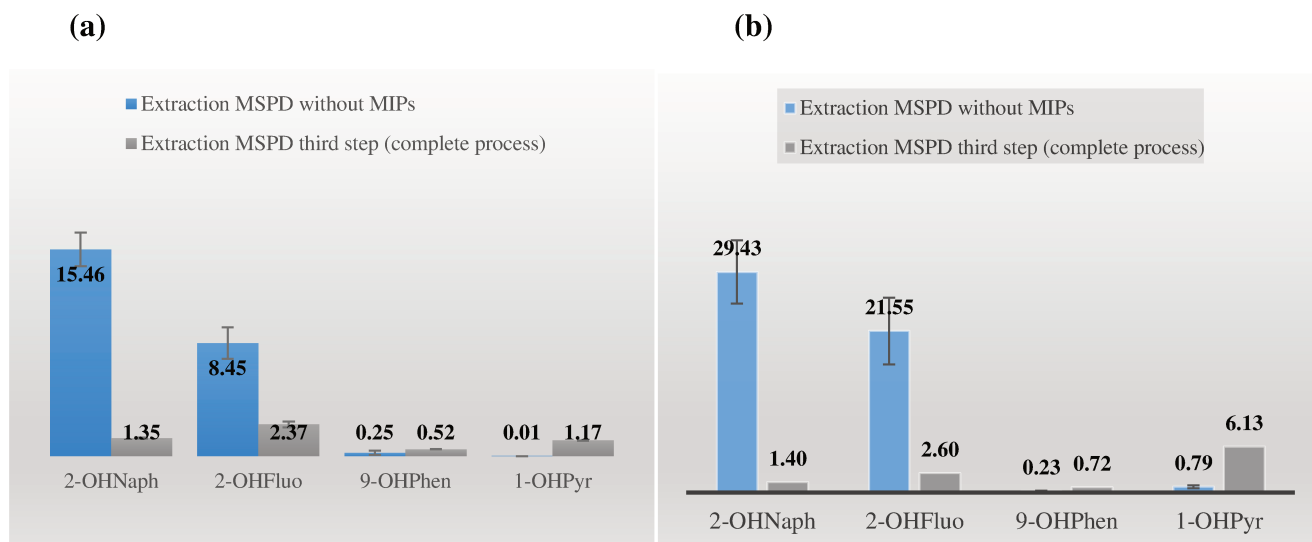


Figure III.14 Comparison between the recoveries obtained after the complete process (MSPD+MIPs) and just the desorption from the MSPD cartridge (containing the deactivated silica co-column) (a) 6 mL of 90% acetonitrile/10% acetone (b) 6 mL pure acetonitrile, as solvent mixtures n°2

Unfortunately, even quite good recoveries obtained after the desorption from the MSPD cartridge were lost in the second phase when the MIP cartridge was coupled to the sediment cartridge (Figure III.14), that could be a sign that the imprinted polymer could be damaged and was not capable of retaining these compounds during the percolation step. As discussed previously, one possibility was that the MIP cartridge was drying, even for a few seconds, during the moment that the percolation solvent was passing from the MSPD cartridge to the pre-conditioned MIP cartridge; another phenomena could be that the eluted volume from the MSPD cartridge contains high amounts of hydroxylated aromatic interfering compounds (polyphenols) that might compete with the target hydroxy-PAHs for the imprinted sites.

Other combinations of percolation solvents (acetonitrile with 2% methanol, or 30% ethyl acetate, or 0.5% butanol...) were tested to desorb hydroxy-PAHs from the MSPD cartridge, without any improvement. The addition of KOH in the sediment before crushing was tested again to see if it could break the hydrogen bonds. Compared to the results shown in the Figure III.14 b, Table III.16 shows that the two lighter hydroxy-PAHs were actually better desorbed from the MSPD cartridge when 500 μ L or 1 mL of methanolic KOH was spiked to the sediment and crushed with the other additives. Mechanically crushing with KOH actually promoted desorption of hydroxy-PAHs from the sediment cartridge, by breaking some strong bonds. In contrast, adding 2% of methanolic KOH to acetonitrile and percolating the mixture through the

sediment cartridge gave low results (Table III.16). Alkaline percolation conditions, when adding 1% of the strong base n-butylamine (pKa = 10.8) to acetonitrile, did not help in better desorbing hydroxy-PAHs. At last, Table III.16 shows that percolating with pure methanol improved the desorption of the two lighter hydroxy-PAHs from the MSPD cartridge (containing the silica co-column) compared to the mixture 99% acetonitrile/1% methanol, but the desorption was not complete and the results for the two heavier hydroxy-PAHs stayed very poor.

Table III.16 Recoveries (%) of the 4 hydroxy-PAHs from the MSPD cartridge using various elution conditions (n=2)

	Volume (mL)	Differences	2-OHNaph (%)	2-OHFluo (%)	9-OHPhen (%)	1-OHPyr (%)
Acetonitrile	6	500 μ L KOH spiked	37.3 \pm 4.5	34.2 \pm 6.8	-	0.2 \pm 0.2
		1 mL KOH spiked	36.7 \pm 5.0	50.5 \pm 5.8	0.1 \pm 0.1	1.3 \pm 1.2
98% acetonitrile/2% KOH in methanol	6		26.1 \pm 5.0	20.8 \pm 5.5	0.1 \pm 0.1	0.7 \pm 0.2
99% acetonitrile/1% n-butylamine	6		20.5 \pm 0.1	15.4 \pm 0.2	0.1 \pm 0.1	0.2 \pm 0.1
Methanol	6		55.8 \pm 0.1	55.6 \pm 1.4	4.3 \pm 0.5	2.0 \pm 0.1
99.5% CH ₃ OH/0.5% TFA	6		54.8 \pm 1.0	61.0 \pm 1.2	3.3 \pm 0.2	0.2 \pm 0.1

Acidifying the percolation solvent with TFA was neither the solution (Table III.16). TFA, as a strong acid (pKa = 0.23), could not be tested in higher amounts to avoid damages on the HPLC column. Elution with methanol or acidified methanol showed a strong turbid color in the eluate²²². Indeed Pena et al. had the same problem with residuals, using this solvent with silica as dispersant²². It is a fact that methanol or acidified methanol could desorb higher amounts of polar interfering compounds from the sediment matrix than the other less polar solvent mixtures tested, but it was anyway not quantitative for our target hydroxy-PAHs. Moreover, pure methanol is not compatible with the retention of hydroxy-PAHs on the MIP-Phenolic.

Other additives such as salts were tested, added to the percolation solvent, to help desorption of hydroxy-PAHs from the sediment matrix by a salting-out effect, without using high amounts of methanol. Compared to the results of the Figure III.14 b, Table III.17 shows that adding 1 g NaCl and 1.5 g sodium citrate into 10 mL acetonitrile considerably favored the desorption of the two lighter hydroxy-PAHs, but the results stayed very low for the two other hydroxy-PAHs. In spite of the quite good recoveries obtained with sodium citrate, it was not very soluble in organic solvents. So it was replaced by a cationic compound, the tetramethyl ammonium bromide (TMAB), which was tested as an additive into acetonitrile and gave quite similar

results without the problems of pressure (Table III.17). TMAB probably sorbed on the negatively charged surface of the sediment, helping in displacing the less strongly sorbed hydroxy-PAHs. Unfortunately the strongly sorbed heavier hydroxy-PAHs were not desorbed.

Table III.17 Recoveries (%) of the 4 OH-PAHs from the MSPD cartridge using elution solvents with additives such as surfactants and/or salts (n=2)

Percolation solvent (mixture n°2)	Volume (mL)	2-OHNaph (%)	2-OHFluo (%)	9-OHPhen (%)	1-OHPyr (%)
CH ₃ CN + NaCl + sodium citrate	10	52.1 ± 5.3	60.8 ± 7.6	4.0 ± 0.8	1.9 ± 0.4
CH ₃ CN + 100 mM tetramethyl ammonium bromide	6	51.2 ± 6.4	52.5 ± 18.4	3.6 ± 0.4	2.3 ± 0.5
99% CH ₃ CN/1% CH ₃ OH + NaCl	10	67.0 ± 3.5	60.9 ± 9.4	4.3 ± 0.1	1.7 ± 0.3
90% CH ₃ CN/10% ethyl acetate + NaCl	10	51.6 ± 0.3	44.7 ± 2.0	3.7 ± 0.3	1.0 ± 0.1
90% CH ₃ CN/10% acetone + NaCl	10	51.5 ± 4.0	44.8 ± 5.1	4.3 ± 0.2	1.9 ± 0.3
90% CH ₃ CN/10% toluene + NaCl	10	39.7 ± 0.4	40.5 ± 0.7	*	2.1 ± 0.1
99% CH ₃ CN/ 1% CH ₃ OH + NaCl + SDS	6	50.7 ± 3.9	61.7 ± 0.4	3.8 ± 1.0	1.6 ± 0.4
89%CH ₃ CN/ 10%water/1%CH ₃ OH + SDS + NaCl	10	62.5 ± 0.1	72.1 ± 4.8	6.0± 0.5	3.5 ± 0.9

* Aberrant value > 100%: the use of toluene contributes to the apparition of an interfering compound that elutes at the same retention time than 9-OHPhen in HPLC-FLD.

Table III.17 shows that adding 10 g NaCl to 10 mL of the 99% acetonitrile/1% methanol solvent mixture also favored the desorption of the two lighter hydroxy-PAHs, reaching 60.9-67.0% desorption levels even when the deactivated silica co-column was added to the MSPD cartridge.

Table III.17 also shows that adding NaCl to other solvent mixtures containing ethyl acetate, acetone or toluene instead of methanol gave actually interesting results for the desorption of the lighter hydroxy-PAHs, but always less interesting than using methanol into acetonitrile.

Table III.17 shows that adding a surfactant, the sodium dodecyl sulfate (SDS) at 81.84 mM (10xCMC) to the eluting mixture composed of 99% acetonitrile/1% methanol and NaCl did not improve the results compared with the same elution solvent without SDS. SDS is an anionic surfactant that showed good results in the extraction of PAHs from sediments, so it was tested for the hydroxy-PAHs. Anionic surfactants are far less sorbed on the anionic surface of sediment particles than cationic and nonionic ones^{90, 238}, forming more micelles in solution at only ten times their critical micellar concentration (CMC)²³⁹. Surfactants have a high capacity of extraction of the fulvic and humic acids and it is supposed that the hydroxy-PAHs are highly sorbed in these fractions of the organic matter. The lack of improvement of the extraction of

hydroxy-PAHs when adding SDS to the solvent mixture n°2 could be due to the fact that SDS micelles could not form in mixtures of organic solvents. It might be necessary to add water to see an effect of SDS micelles on hydroxy-PAHs desorption. Table III.17 shows actually that adding 10% water to the solvent mixture composed of acetonitrile, methanol, NaCl and SDS gave not only better desorption results for the two lighter hydroxy-PAHs, but also, for the first time, for the heavier hydroxy-PAHs. However, due to the difficulty to pass the water through the MSPD cartridge (which is impregnated of the elution mixture n°1 composed of hexane and dichloromethane), only a low quantity of water could be used, just sufficient to produce the aqueous micelles of SDS. Moreover, in the aqueous medium, there was also the salting-out effect due to NaCl ^{240, 241}.

At last, another solution for desorbing the two heavier hydroxy-PAHs was tried, which consisted in making a gradient of different solvent mixtures and mixing them at the end of the percolating step, before their simultaneous introduction into the MIP cartridge. Table III.18 shows some tests performed in two or three steps, only for desorption of the hydroxy-PAHs from the MSPD cartridge.

The first set of experiments consisted in percolating through the MSPD cartridge a very polar solvent mixture composed of methanol and TFA (5 mL) to desorb the two lighter hydroxy-PAHs and second, a less polar solvent mixture (5 mL, composed of acetonitrile and dichloromethane) to favor the desorption of the less polar hydroxy-PAHs. The results were satisfactory for the lightest hydroxy-PAHs but not for the two others (Table III.18). So a first step was added to these elution conditions: 5 mL of a methanolic solution saturated with KOH was percolated first and the two other steps were the same. Here, for the first time, the recovery of 1-OHPyr was significantly improved, thanks to the addition of KOH. Unfortunately, these elution conditions were not compatible with a selective retention of hydroxy-PAHs on the MIP cartridge. We could not eliminate KOH by a preliminary evaporation of this complex solvent mixture, before solvent change and percolation through the MIP.

Table III.18 Recoveries (%) of the 4 hydroxy-PAHs from the MSPD cartridge using a gradient of solvents for the percolation mixture (n=2)

Percolation solvent (mixtures n°2) by steps	Volume (mL)	2-OHNaph (%)	2-OHFluo (%)	9-OHPhen (%)	1-OHPyr (%)
1°: 99.5% CH ₃ OH/0.5% TFA 2°: 90% CH ₃ CN/10% CH ₂ Cl ₂	5 + 5	61.4 ± 1.2	77.0 ± 24.0	6.6 ± 0.1	2.4 ± 0.2
1°: KOH in methanol 2°: 99.5% CH ₃ OH/0.5% TFA 3°: 90% CH ₃ CN/10% CH ₂ Cl ₂	5 + 5 + 5	65.4 ± 1.7	71.5 ± 5.5	7.1 ± 1.9	9.4 ± 3.3
1°: 89% CH ₃ CN/10% water/1%CH ₃ OH + NaCl + SDS 2°: 99% CH ₃ CN/1% CH ₃ OH + NaCl 3°: 90% CH ₃ CN/10% acetone	5 + 5 + 5	60.3 ± 1.8	61.0 ± 3.6	6.0 ± 0.4	2.1 ± 0.1
1°: CH ₃ CN 2°: 89% CH ₃ CN/10% water/1%CH ₃ OH + NaCl + SDS	5+5	79.3 ± 4.1	80.0 ± 2.4	8.1 ± 1.6	5.6 ± 1.1
1° : KOH in methanol * 2°: CH ₃ CN 3°: 89% CH ₃ CN/10% water/1%CH ₃ OH + NaCl + SDS	5+5	45.5 ± 1.4	55.4 ± 4.0	0.1 ± 0.0	3.1 ± 0.1

* 1 mL of the KOH in methanol spiked at the step of crushing

In a second set of experiments, we percolated the solvent mixture that gave good results for the lighter hydroxy-PAHs (composed of acetonitrile, 1% methanol, 10% water, NaCl and SDS), followed by a less polar solvent mixture (without water and SDS), followed again by a less polar solvent (composed of acetonitrile and acetone) (Table III.18). This solvent gradient did not improve the hydroxy-PAHs desorption compared to the single elution with the first elution solvent (Table III.17).

At last, we reversed the gradient polarity of the percolation solvents: we started with an elution with pure acetonitrile to better eliminate the residues of hexane and dichloromethane still present in the MSPD cartridge even after the drying step. Thereafter we percolated the solvent mixture composed of acetonitrile, 1% methanol, 10% water, NaCl and SDS. Table III.18 shows that the desorption of the two lighter hydroxy-PAHs was for the first time quantitative, reaching nearly 80%, but the two other hydroxy-PAHs could not be quantitatively desorbed from the MSPD cartridge (5.6-8.1%). Instead of what was expected, adding KOH in the crushing step to this sequence of elution solvents did not showed the best results (Table III.18). Finally, the use of this solvent gradient, constituting the solvent mixture n°2, presented the best results until now, even if it was not satisfactory for the heaviest hydroxy-PAHs.

III.3.4.2 Final results of the whole process in the best conditions

Even if the step of hydroxy-PAHs desorption from the MSPD cartridge was not optimized for the two heavier hydroxy-PAHs, we used the best conditions of elution already found on the whole process, with adding the step of the selective sorption of hydroxy-PAHs on the MIP cartridge.

In each further experiment, 0.5 g sediment was mixed with 1 g deactivated silica, 1 g sodium sulfate and 0.5 g 3-chloropropyl bonded particles; surrogate standards were added and the mixture was crushed during 10 min, then introduced above a layer of 1 g deactivated silica in a SPE cartridge and compacted. PAHs and PCBs were eluted by 6 mL of the solvent mixture n°1, composed of 50% hexane/50% dichloromethane, and analyzed in GC-MS. The MSPD cartridge was dried under a nitrogen flow and then different percolating solvents or gradients of solvents (mixtures n°2) were tested, with directly coupling or not the MIP-Phenolics cartridge (containing 100 mg of polymeric phase). After drying the MIP cartridge, hydroxy-PAHs were eluted with 7 mL of a 98% methanol/2% acetic acid mixture (n°3), and analyzed in HPLC-FLD.

The first percolating solvent mixture n°2 to be tested was composed of acetonitrile, 1% methanol, 10% water, NaCl and SDS, as Table III.16 showed that it was one of the best solvent mixtures to desorb the two lighter hydroxy-PAHs from the sediment cartridge. It was tested in two situations: first, the MSPD and MIP cartridges were directly coupled during the percolation of the solvent mixture n°2, second they were not connected. Table III.19 shows that the final results were bad, particularly when the two cartridges were connected together. The disconnection of the MIP from the MSPD cartridge during the elution of the second step improved the results of the last step, which showed that the cartridge could have been damaged when coupling them, what affected the selectivity of the MIP. However, even when the two cartridges were disconnected, the hydroxy-PAHs were far from reaching the values obtained after their elution from the first cartridge, that were 62-72% (Table III.17): they continued to leave during the percolation step through the MIP cartridge in a huge quantity.

Table III.19 Recoveries (%) of the 4 hydroxy-PAHs from the MIP-Phenolics cartridge using different percolation solvent mixtures n°2 (n=2)

Percolation solvent (mixture n°2)	Volume (mL)	Differences	2-OHNaph (%)	2-OHFluo (%)	9-OHPhen (%)	1-OHPyr (%)
89% CH ₃ CN/10% water/1% CH ₃ OH + SDS + NaCl	10	MSPD/MIP coupled	0.9 ± 0.6	0.1 ± 0.1	0.0 ± 0.0	0.0 ± 0.0
		MSPD/MIP not coupled	5.2 ± 0.5	7.9 ± 1.1	0.1 ± 0.2	0.0 ± 0.0
1°: CH ₃ CN 2°: 89% CH ₃ CN/10% water/1%CH ₃ OH + NaCl + SDS	5+5	MSPD/MIP not coupled	7.8 ± 0.6	11.8 ± 0.3	0.8 ± 0.2	1.1 ± 0.3

Unfortunately, using the best gradient of solvents (as mixture n°2), which allowed eluting the lightest hydroxy-PAHs at 79.3-80.0% levels from the MSPD cartridge (Table III.18), and disconnecting the two cartridges, gave also final results very lower than expected. Table III.19 shows that they reached only 7.8-11.8% recovery, which was not so better than the first results obtained without any optimization of each step (Table III.9), and that is not better than the results obtained with the percolating mixture n°2 composed of 10 mL acetonitrile/methanol 99/1% with 1 g NaCl (Table III.15).

We supposed previously that one of the problems encountered with the MIPs could be that eluting polar interfering compounds, with chemical structures such as phenols, polyphenols and perhaps catechols, could compete and saturate the retention sites and prevent from the quantitative retention of hydroxy-PAHs. Indeed, the template used to build the commercial MIP-Phenolics was phenol and not specifically a hydroxylated polyaromatic compound. Consequently the recognition for the sites was preferentially in favor of phenols or polyphenols. So a commercial MIP-Phenolics cartridge containing 500 mg polymeric phase instead of 100 mg (so containing more retention sites) was used to verify this hypothesis²⁴². The best solvents or solvent gradients (mixtures n°2) capable of desorbing quantitatively the lighter hydroxy-PAHs from the sediment cartridge were tested for the percolation through the bigger MIP. But in this case, the MSPD cartridge and the MIP cartridge were not coupled after the elution of PAHs/PCBs, because it was a more favorable configuration, as discussed previously.

Table III.20 shows that changing for the bigger MIP improved considerably the final elution of the two lightest hydroxy-PAHs, the loss in the percolation step through the MIP actually decreasing drastically. Recoveries were increased 4 to 9 times, dropping from 5-11% to 39-59%. Unfortunately, recoveries were not improved for the two heavier hydroxy-PAHs, as the

problem was not the competition for the imprinted sites but their desorption from the sediment matrix.

Table III.20 Recoveries (%) of the 4 hydroxy-PAHs from the bigger MIP-Phenolics cartridge (500 mg polymeric phase) using different percolation solvent mixtures n°2 (MSPD and MIP cartridges not coupled) (n=2)

Percolation solvent (mixture n°2)	Volume (mL)	Differences	2-OHNaph (%)	2-OHFluo (%)	9-OHPhen (%)	1-OHPyr (%)
1°: CH ₃ CN 2°: 89% CH ₃ CN/10% water/1% CH ₃ OH + NaCl + SDS	5+5		38.6 ± 0.8	48.8 ± 0.7	3.5 ± 0.2	0.5 ± 0.0
99% CH ₃ CN/1% CH ₃ OH + NaCl	10	No co-column	47.3 ± 9.1	59.4 ± 17.5	1.1 ± 0.4	0.5 ± 0.1
90% CH ₃ CN/10% toluene	10		32.9 ± 0.1	36.7 ± 0.1	32.4 * ± 2.2	0.0 ± 0.0
99%CH ₃ CN/1% CH ₃ OH + NaCl **	10	- Bigger MIP (2x) - Heat 30 min at 35°C	76.6 ± 6.0	64.9 ± 3.4	0.0	0.0
1°: CH ₃ CN 2°: 89% CH ₃ CN/10% water/1% CH ₃ OH + NaCl + SDS ^b	5+5	- Bigger MIP (2x) - Heat 30 min at 35°C	67.3 ± 1.9	47.6 ± 6.7	0.0	0.0

* There is a doubt on this value that seems too high: when toluene was used in the percolating solvent n°2, a more or less intense interfering compound appeared at the same retention time than 9-OHPhen in HPLC-FLD analysis.

** 10 mL of 98% CH₃OH/2% acetic acid used for the final elution

In a last attempt to better desorb the two heavier hydroxy-PAHs, we tried to heat the sediment cartridge after the first step of PAHs/PCBs elution, in order to break hydrogen bonds. The MSPD cartridge was put 30 min in an oven at 35°C. Moreover, to improve the recoveries of the two lighter hydroxy-PAHs, the amount of imprinted polymeric material was increased again, being 2 × 500 mg now. Table III.20 shows that in these conditions, 10 mL of the mixture n°2 composed of 99% CH₃CN/1% CH₃OH + 1 g NaCl gave the best results. The two lighter compounds that were successfully desorbed from the sediment were retained almost entirely into the MIP cartridge and successfully eluted with 10 mL of 98% CH₃OH/2% acetic acid mixture n°3, giving 64.9-76.6% final recoveries. In the case of the two heavier hydroxy-PAHs, it was still not possible to desorb them from the sediment, despite the heating step, what made impossible to have good results in the final elution from the MIP.

III.4 Conclusion

Following our results, matrix solid phase dispersive extraction (MSPD) appears as a simple, low-cost and efficient extraction method that showed satisfactory results for extracting simultaneously, for the first time, PAHs and PCBs from sediments. It consisted in adding dispersing agents (Florisil, and an original dispersant composed of 3-chloropropyl bonded particles) and a desiccant (sodium sulfate) to the dried sediment and to crush the mixture 10 min. After the addition of a Florisil layer under the crushed sediment mixture layer, put into a SPE cartridge, the target analytes were successfully eluted with a solvent mixture composed of hexane and acetone 50%/50%. Moreover, MSPD was capable of performing the clean-up step at the same time, something put in evidence when comparing to the analyses after MAE. MSPD saves time and labor, thus eliminating a great part of polar interfering compounds possibly responsible of incorrect results at the chromatographic analytical step. The optimized MSPD extraction method was validated using a certified standard reference sediment and showed a mean extraction yield of 81.4% for the sum of 15 priority PAHs, which was better than the optimized MAE extraction for PAHs (73.6%). Concerning the sum of 6 PCB indicators, the mean extraction yield reached 165.3%, which was better than the results obtained by MAE (117.6%) and was significantly higher than expected. But values obtained from our optimized MSPD extraction method were compared to certified values obtained with old conventional extraction methods using Soxhlet or ultrasound-assisted extraction which could extract less than 50% of the organochlorine compounds or were not appropriate for analyzing low concentrations of PCBs.

Another challenge of this study was to add to the developed MSPD methodology another step, which could allow analyzing another family of contaminants that was a mixture of hydroxylated metabolites of PAHs. The idea was to elute in a second step some of the sorbed polar interfering compounds from the MSPD cartridge and to percolate them through a coupled MIP cartridge containing imprinted polymers selectively retaining phenolic compounds. This MIP-Phenolics material proved to be efficient to retain hydroxy-PAHs, from naphthol to pyrenol, but only some combinations of solvents could be used to avoid damages and keep the selectivity of the imprinted material.

A new solvent mixture could be found to quantitatively elute PAHs/PCBs from the MSPD cartridge without great losses of hydroxy-PAHs at the first step: hexane/dichloromethane 50%/50%. A new dispersing and sorbing agent (deactivated silica), replacing Florisil, could be found to improve PAHs/PCBs recoveries, being less strong for hydroxy-PAHs retention. For the third and final step, an optimal solvent could be found for the elution of the 4 hydroxy-

PAHs from the MIP cartridge (98% methanol/2% acetic acid). But the main difficulty was to find an appropriate solvent mixture to transfer all the strongly sorbed hydroxy-PAHs retained on the MSPD cartridge to the MIP cartridge at the second step of the process. Different mixtures of solvents with diverse polarities were tested, adding surfactants, salts, acid, bases, but with not a lot of success, particularly for the two heavier hydroxy-PAHs.

At the end, the best results were found using 1 g of deactivated silica for the co-column, 6 mL of 50% hexane/50% CH₂Cl₂ for the solvent mixture n°1, for desorbing quantitatively PAHs and PCBs from the sediment cartridge; 10 mL of 99% acetonitrile/1% methanol + NaCl 100 g L⁻¹ (solvent mixture n°2) was used to elute the hydroxy-PAHs from the MSPD cartridge, which was finally not connected to the MIP cartridge. This eluate was then percolated through the pre-conditioned MIP cartridge (containing 1 g of imprinted polymer, instead of 0.1 g) and 10 mL of 98% methanol/2% acetic acid (solvent mixture n°3) was used at the final elution step. In these conditions, 76.6% 2-OHNaph and 64.9% 2-OHFluo could be selectively recovered. But recoveries for the two heavier hydroxy-PAHs were really unsatisfactory. Further tests have to be done for the optimization of the complete MSPD+MIP process, with the bigger MIP, trying to find a solution to break the strong hydrogen bonds between the sediment and the heavy hydroxy-PAHs. Thereafter the steps of optimization, the method would have to be validated.

Résumé du chapitre III

Optimisation de l'extraction simultanée des HAP, PCB et HAP hydroxylés des sédiments en utilisant la MSPD et les MIPs.

Ce chapitre présente l'optimisation d'une nouvelle méthode d'extraction multi-résidus, l'extraction par dispersion de la matrice solide (nommée MSPD), permettant d'extraire et de purifier simultanément deux familles de contaminants, les HAP et les PCB, d'une matrice sédimentaire. Cette méthode, de faible coût, facile et rapide d'emploi, a été validée sur un sédiment certifié et comparée à la MAE. Cependant, une étape a été ajoutée, dans le but d'extraire et analyser sélectivement une autre famille de contaminants, les HAP hydroxylés. Elle consiste à coupler à la MSPD l'emploi de polymères à empreintes moléculaires (MIP) permettant une extraction sélective des OH-HAP par reconnaissance structurale.

III.1 Analyse simultanée de HAP et PCB par CPG-SM

III.1.1 Conditions chromatographiques

Les 16 HAP prioritaires et 7 PCB indicateurs ont été analysés simultanément en CPG, sur une longue colonne (60 m) apolaire, afin d'avoir une bonne résolution de certaines paires critiques. Les 28 composés (comportant les étalons de suivi et les étalons internes) ont été analysés en moins de 35 minutes, et ont été identifiés en SM en mode full SCAN. Une programmation en mode sélection d'ions SIM a permis de les détecter en optimisant le rapport signal/bruit.

III.1.2 Linéarité, limites de détection et de quantification

Les droites d'étalonnage interne ont été réalisées en triplicats et ont permis de déterminer la linéarité et de calculer les LDs/LQs par une méthode mathématique, qui s'est avérée moins favorable que le calcul réalisé par la méthode S/B.

III.2 Extraction des HAP et PCB en utilisant la MSPD

III.2.1 Etapes d'optimisation

L'extraction par dispersion de la matrice solide (MSPD) a été optimisée à l'aide d'un sédiment modèle sec reconstitué, dopé par une quantité connue de HAP et PCB, laissé au minimum 1 semaine pour favoriser les interactions analytes/sédiment. Pour commencer le processus d'optimisation de la MSPD, 1 g de particules solides de Florisil ont été rajoutées à 0,5 g de sédiment, pour favoriser le piégeage des interférents polaires, ainsi que 1 g de sulfate de sodium

comme desséchant, et le tout a été broyé. Le broyat a été introduit dans une cartouche SPE, en ayant pris soin de mettre en dessous une couche de 1 g de Florisil (co-colonne) pour mieux piéger les interférents polaires. Plusieurs mélanges de solvants ont été testés pour éluer les HAP et PCB. Le meilleur s'est avéré être un mélange d'hexane/acétone 50/50. Le volume d'élution s'est avéré un facteur peu influent, un volume de 5 mL étant suffisant pour atteindre de bons rendements d'extraction. Plusieurs agents dispersants particuliers ont été testés pour favoriser la rupture des interactions analytes apolaires/sédiment, à savoir : des particules greffées par des greffons C₁₈, 3-chloropropyles ou 4-chlorophényles. L'ajout de 0,5 g d'agent dispersant contenant des greffons 3-chloropropyles s'est avéré légèrement meilleur pour l'extraction des HAP et PCB. Enfin, un temps de broyage allongé à 10 min a favorisé l'extraction des composés d'intérêt.

III.2.2 Validation de la MSPD sur un sédiment certifié

La répétabilité et la reproductibilité (précision intermédiaire sur différents jours) de l'extraction MSPD ont été évaluées sur le sédiment reconstitué et se sont avérées très satisfaisantes (< 6%). Les LDs/LQs ont été calculées par la méthode S/B, en considérant comme « échantillon blanc » le sédiment reconstitué non dopé. Il s'est avéré que ce dernier n'était pas totalement vierge de HAP. Les LDs étaient comprises entre 0,006-0,022 ng g⁻¹ pour les HAP et 0,026-0,109 ng g⁻¹ pour les PCB, ce qui était mieux, concernant quelques HAP, que les résultats trouvés dans la littérature.

La méthode d'extraction MSPD a été validée sur un sédiment CNS391, certifié pour les HAP et les PCB, et a été comparée à l'extraction MAE. L'extraction des HAP par MAE avait été au préalable validée sur un sédiment certifié pour les HAP, CRM104, donnant une moyenne de 105±18% d'extraction des HAP. La méthode MSPD appliquée au sédiment CNS391 a donné un rendement d'extraction moyen des HAP de 81,4 par rapport aux valeurs du certificat (bien que certaines valeurs puissent être remises en cause, de par leur ancienneté d'analyse), tandis que la MAE donnait un rendement d'extraction moyen de 73,6. Concernant les PCB, le rendement d'extraction moyen par MSPD était de 165,3%, contre 117,6% par MAE. La valeur obtenue pour les PCB est surprenante, mais elle est à comparer avec des méthodes d'extraction des PCB anciennes et non optimales (par Soxhlet et ultra-sons). De fait, l'extraction par MSPD apparaît plus efficace que l'extraction par MAE, tant pour les HAP que pour les PCB.

III.3 Extraction sélective des HAP hydroxylés en utilisant le couplage MSPD-MIPs

III.3.1 Description des différentes étapes du processus

Dans une première étape, les HAP et PCB sont extraits de la cartouche contenant le sédiment préparé pour la MSPD dans les conditions optimales décrites dans le chapitre précédent. Mais une troisième famille de contaminants, les OH-HAP, sont susceptibles d'être extraits ultérieurement en associant à la première cartouche « MSPD » une seconde cartouche SPE contenant un polymère à empreintes moléculaires (MIP). Ce MIP commercial (MIP-Phenolics) est empreint pour retenir sélectivement des composés de type phénols, de structure très proche des OH-HAP. La première étape du processus consiste donc à éluer les HAP et PCB de la première cartouche MSPD, à l'aide d'un mélange de solvants n°1, sans éluer les interférents polaires comprenant les OH-HAP. La seconde étape consiste à transférer certains interférents polaires, comprenant les OH-HAP, de la première cartouche MSPD vers la seconde cartouche MIP, à l'aide d'un mélange de solvants n°2. Au cours de cette étape, le MIP retient sélectivement les OH-HAP. La troisième étape consiste à éluer quantitativement les OH-HAP retenus sur le MIP, à l'aide d'un mélange de solvants n°3. Les OH-HAP sont ensuite analysés en HPLC-FLD (voir partie II.1.1).

III.3.2 Choix des solvants de percolation et d'élution compatibles avec les MIPs

Plusieurs mélanges de solvants n°2 ont été testés (acétonitrile pur, acétonitrile/toluène 90/10, acétonitrile/méthanol 90/10...), avec des résultats de récupération très faibles pour les 4 OH-HAP à l'issue du processus complet. On pouvait penser que les OH-HAP étaient en partie perdus lors de la première étape d'élution des HAP/PCB. L'utilisation du solvant d'élution n°1 acétone/hexane 50/50 provoquait de fait la perte de plus de 50% des 2 OH-HAP les plus légers. Ce mélange n°1 a été remplacé par le mélange dichlorométhane/hexane 50/50, avec une perte des OH-HAP inférieure à 7% à la première étape, tout en conservant des rendements d'extraction des HAP/PCB excellents.

Plusieurs mélanges de solvants ont été testés pour voir si le problème ne venait pas d'une incompatibilité du mélange de solvants n°2 avec le matériau du MIP. Effectivement, certains mélanges de solvants ne convenaient pas au matériau polymérique, qui perdait ses capacités de rétention sélective, en particulier les mélanges comportant une forte proportion d'alcools ; en revanche, les mélanges acétonitrile/méthanol, s'ils n'excédaient pas 2% de méthanol, pouvaient convenir. Il a été montré que d'autres mélanges de solvants permettaient une rétention à plus de 72% des 4 OH-HAP, en utilisant 7 mL du mélange n°3 composé de méthanol et d'acide

acétique 98/2 pour les éluer. Toutefois, même en utilisant de tels mélanges compatibles avec le MIP, les résultats finaux d'extraction des 4 OH-HAP n'étaient toujours pas satisfaisants.

III.3.3 Utilisation de différents adsorbants ou de nouveaux additifs pour améliorer le recouvrement des OH-HAP

Le Florisil, en tant qu'agent adsorbant les interférents polaires, a été remplacé par de la silice désactivée un peu moins rétentive, pour permettre une meilleure désorption des OH-HAP. La silice désactivée a été introduite comme agent dispersant dans le processus MSPD, mais également comme co-colonne. Il s'est avéré que la récupération des HAP/PCB à la première étape était meilleure qu'en utilisant le Florisil ; la silice désactivée a été conservée pour la suite des travaux.

Le KOH a été ajouté à l'étape de broyage du sédiment pour favoriser la rupture des liaisons hydrogène entre les OH-HAP et le sédiment, mais sans succès, le KOH altérant la capacité de rétention du MIP. Une grande quantité de NaCl (100 g L⁻¹) a été rajoutée dans le solvant n°2 composé d'acétonitrile/méthanol 99/1, pour provoquer un effet de relargage. Cela a conduit à une augmentation significative de l'extraction des deux OH-HAP légers, en particulier lorsque les deux cartouches MSPD/MIP n'étaient pas directement couplées. Mais les résultats restaient peu satisfaisants.

III.3.4 Solvants de percolation pour améliorer le transfert des OH-HAP de la cartouche MSPD vers la cartouche MIP

L'étape de désorption des OH-HAP de la cartouche MSPD contenant le sédiment s'est donc avérée cruciale, avant leur percolation sur la cartouche MIP. On a pu montrer un comportement différent entre les 2 OH-HAP légers (2-naphtol et 2-fluorenonol) et les 2 OH-HAP plus lourds (9-phenanthrol et 1-hydroxypyrene). Les OH-HAP légers pouvaient être relativement facilement désorbés de la cartouche MSPD à l'aide d'un mélange acétonitrile/méthanol 99/1, tandis que les 2 OH-HAP lourds restaient piégés à plus de 90%. L'ajout de co-solvants basiques (butylamine) ou d'additifs acides (TFA) n'ont pas permis d'améliorer la désorption des OH-HAP de la matrice sédimentaire. D'autres additifs au mélange de solvant n°2 ont été testés, avec une amélioration de la désorption des 2 OH-HAP légers, à savoir le citrate de sodium, le bromure de tétraméthyl ammonium, l'agent tensioactif SDS (dodécyl sulfate de sodium). Le mélange composé d'acétonitrile, de méthanol, d'eau (89/1/10) et de SDS (à 10 fois sa concentration micellaire critique) a permis de désorber 62 à 72% des OH-HAP légers de la cartouche MSPD, mais moins de 6% des OH-HAP lourds.

Des gradients d'élution ont été tentés, pour permettre une élution des OH-HAP légers avec des mélanges plus polaires, et une élution des OH-HAP lourds avec des solvants moins polaires. Dans le meilleur des cas, pratiquement 80% des OH-HAP légers ont pu être relargués de la cartouche MSPD, contre moins de 8,1% des OH-HAP lourds.

Cependant, même en utilisant les meilleurs mélanges ou gradients de solvants n°2 permettant un relargage quasi-quantitatif des OH-HAP légers de la cartouche MSPD, les rendements d'extraction de la cartouche MIP restaient inférieurs à 12%. On a supposé que les interférents polaires relargués, ayant des structures proches des OH-HAP (phénols, polyphénols, catéchols...) saturaient les cavités du polymère, empêchant leur sorption et diminuant considérablement leur extraction. Par conséquent, des cartouches MIPs contenant une plus grande quantité de polymère (500 mg et 2x500 mg, contre 100 mg) ont été testées. Les meilleurs résultats obtenus avec le procédé complet (MSPD et MIP associés mais non directement couplés) l'ont été avec le mélange n°2 constitué de 99% CH₃CN/1% CH₃OH + NaCl, en utilisant le MIP contenant 2x500 mg de polymère : les résultats ont permis une extraction sélective de 59% et 77% des deux OH-HAP légers, mais l'extraction des deux OH-HAP lourds s'est avérée quasi-nulle.

III.4 Conclusion

Une nouvelle méthode d'extraction MSPD a été optimisée pour extraire simultanément des HAP et des PCB d'une matrice sédimentaire, en broyant le sédiment en présence d'agents particuliers dispersants, à savoir (i) le Florisil pour retenir les interférents polaires et (ii) des particules greffées de 3-chloropropyles pour mieux désorber les analytes apolaires ; le mélange est introduit dans une cartouche et élué à l'aide d'acétone/hexane 50/50. De fait, l'extraction par MSPD est apparue plus efficace que l'extraction par MAE, pour les HAP et surtout les PCB, tout en étant moins chère, plus rapide et facile d'emploi, et permettant également une purification de l'échantillon.

En second lieu, une étape a été ajoutée dans le but d'extraire une troisième famille de composés, les OH-HAP, à l'aide d'une seconde cartouche MIP. Le mélange n°1 de solvants hexane/dichlorométhane 50/50 s'est avéré meilleur pour éluer les HAP et PCB de la première cartouche MSPD, en évitant la perte des OH-HAP. Le Florisil a été remplacé par de la silice désactivée pour permettre un meilleur transfert des OH-HAP vers la cartouche MIP. Différents mélanges de solvants de percolation (n°2) ont été testés pour vérifier leur compatibilité avec le MIP et ensuite trouver le solvant d'élution (n°3) des OH-HAP du MIP. L'obtention d'un mélange de solvants n°2 permettant une bonne désorption des OH-HAP légers de la cartouche MSPD, et l'emploi d'une plus grande quantité de polymère empreint, ont permis d'atteindre des rendements d'extraction convenables pour les OH-HAP légers, mais pas pour les OH-HAP

lourds, extrêmement difficiles à désorber du sédiment. La technique couplée MSPD-MIP pour une analyse multi-résiduelle de trois familles de contaminants doit encore être optimisée et validée.

General conclusions and perspectives

In this study, it was pointed out that some groups of molecules, such as PCBs, PAHs and some of their oxygenated metabolites, can affect the ecosystems health and the human beings in general. Because of their persistence and toxicity, numerous analytical procedures have been developed for many years, with the objectives to be able to monitor them and establish risk assessment in different environmental compartments, to assess the effectiveness of environmental policy and to make appropriate decisions regarding site clean-up and remediation. As number of pollutants have to be monitored, new multi-residual analytical methodologies become excellent strategy to get this goal. One of the difficult tasks in multi-residual analyses of solid environmental matrices is to find a fast and not complicated extraction tool, efficient for all the families of target contaminants although they have different physical-chemical properties.

In this study two new multi-residual extraction methods were developed to quantify different families of contaminants at trace levels in sediment matrices. The first one was optimized for the simultaneous extraction of two groups of oxygenated metabolites of PAHs: quinones and hydroxy-PAHs. Microwave assisted extraction was used for the first time for the fast and simultaneous extraction of the two groups of oxy-PAHs. The separation and the detection of these compounds could be made with two optimized methods: GC-MS and HPLC-FLD/UV. For GC-MS, its advantage was to be selective in terms of detection, but derivatization steps had to be optimized and used for both groups (silylation for hydroxy-PAHs and acetylation for quinones) to increase their detectability; in the case of ortho-quinones, it was even impossible to quantify them at trace levels without derivatization. For the HPLC-FLD/UV method, it was possible to separate and detect both groups at the same time, using UV detection for quinones and FLD detection for hydroxy-PAHs. HPLC-UV-FLD was not only faster than GC-MS, because derivatizations were not necessary, but also markedly lower LODs could be achieved using FLD detection compared to MS. However the disadvantage of HPLC-UV-FLD was that analyzing after extraction from natural complex matrices, it was uncertain that it was really the compound and not an interfering compound that could co-elute, because of the reduced selectivity of the UV and FLD detectors.

For the optimization of the MAE extraction methodology, a chemometric approach was chosen because a “conventional” optimization one factor by one appeared long and complex, the two groups of compounds having different extraction behaviors. A screening design was performed to find the parameters (and their interactions) which could significantly influence the extraction recoveries of the two groups of compounds. Thereafter a full star factorial design was performed to model and find the optimal extraction values. A compromise was found for the two families, which allowed obtaining quantitative results. The MAE optimized method was tested for a natural sediment and a certified one (certified for parent PAHs, not for oxy-PAHs because certified materials do not exist for them). We were able to quantify even in very small quantities compounds of both groups and it appeared that GC-MS was more reliable as HPLC-UV-FLD as a chromatographic tool.

In the future, this method (MAE-GC-MS) could be used for the extraction, separation and detection of various quinones and hydroxy-PAHs from sediments treated by an electrokinetic remediation process. Indeed electrokinetic remediation is a recent efficient method to eliminate PAHs from contaminated sediments through their electroosmotic migration via their incorporation into micelles of neutral surfactants. But being an electrochemical process, oxidation phenomenon also occurs at the anode side that can lead to the degradation of PAHs and to the formation of oxygenated metabolites. It appears important to follow their apparition and to correlate them not only to PAH decontamination but also to the evolution of the toxicity of the treated material. It is also true for other remediation processes, such as enhanced bioremediation, which consist in using bacteria or fungi consortia that biodegrade PAHs but lead to more or less toxic oxidized metabolites.

It can be added here that the whole analytical methodology could be improved by using GC-MS/MS, as we noted the difficulty to certify the identity of the oxidized metabolites extracted from complex environmental matrices. Using a tandem MS, with the development of a MRM method, could allow a most reliable identification of the target compounds not only based on retention times but also on their MRM transitions, which are more specific.

In the second part of this study, a fast, cheap and less laborious method was developed to extract and purify simultaneously PAHs and PCBs in a first step, and hydroxy-PAHs in a second step, using matrix solid phase dispersive extraction. In the first step, the volume and nature of elution solvent, the nature of dispersive agents and the time of grinding were considered as influent factors which were optimized for obtaining a successful extraction of PAHs and PCBs, eliminating the most part of polar interfering compounds. A validation of the MSPD method was performed with a certified standard sediment, and extraction results were 81.4% for the sum of the 15 priority PAHs and 165.3% for the sum of 6 PCBs indicators. The high values of

the PCBs extraction were explained by the fact that the values mentioned on the sediment certificate were obtained using old extraction methods as Soxhlet and ultrasound-assisted extraction, and that they were not capable to extract the totality of these chlorinated contaminants. MSPD appeared more powerful than MAE extraction for PAHs, but more particularly for PCBs with significantly higher extraction values, and also had the advantage of the purification during the extraction. It seems now important to test the optimized MSPD process for sediments with very different compositions than our studied sediments, particularly marine sediment which contain salts. In the future, it could be also interesting to test this MSPD methodology, adding the family of organochlorine pesticides, which are also potential persistent and toxic pollutants found in sediments.

Introducing the extraction of hydroxy-PAHs in a second step led us to modify the initial MSPD methodology: more particularly, the dispersing agent sorbing the polar interfering compounds was changed, because it had now to develop less strong hydrogen bonds with hydroxylated compounds; the nature of PAHs/PCBs eluting solvent was also changed to avoid significant losses of hydroxy-PAHs at the first step of the process. With these changes, PAHs and PCBs were always quantitatively extracted from the MSPD cartridge. But the main challenge was to find a solvent mixture for desorbing hydroxy-PAHs from the sediment in the second step, and analyzing them in HPLC-FLD without all the polar interfering compounds. So a commercial MIP-Phenolics, whose polymer was imprinted for phenols, was added to the process, to selectively sorb and analyze hydroxylated polyaromatics. The solvents used for desorbing the hydroxy-PAHs from the sediment should be compatible with the MIP polymer to avoid recognition sites damages. Hydroxy-PAHs were considerably retained in sediment fractions such as organic matter (containing fulvic and humic acids) and the fine clay particles (composed of phyllosilicates and so hydroxyl groups), and even the combination of acids, bases, surfactants and salts with mixtures of more or less polar solvents was not able to desorb the heavier hydroxy-PAHs from the grinded sediment. The best results were only achieved for the lighter hydroxy-PAHs, using a mixture of solvent containing low amounts of methanol (not to compromise recognition) and using salting-out effect, associated with a higher amount of imprinted polymeric material.

In a direct continuation of this study, the optimization of the best conditions found for extracting and analyzing low molecular weight hydroxy-PAHs still have to be made (best volume of percolation solvent and volume of elution solvent from the bigger MIP have to be found...); but more important, a solvent mixture that could be capable of desorbing the heavier hydroxy-PAHs from the sediment still have to be found. Inserting a heating resistive wire around the

MSPD cartridge during the second step of hydroxy-PAHs elution might be an economic solution to heat the cartridge, favoring hydrogen bonds breaking.

At last, to avoid the problem of competition for the recognition sites between the hydroxy-PAHs and the phenols or polyphenols also present in the sediment matrix that occurred with the commercial MIP-Phenolics a MIP made specifically for the hydroxy-PAHs could be produced. Also, the production of MIPs for other families of PAH oxygenated metabolites (among which quinones) may permit successive analyses, recuperating the eluate from a first selective MIP retaining one family and percolating it through a second MIP cartridge, selective for another family. At last, it is obvious that analyzing all these oxygenated metabolites using HPLC-FLD and/or HPLC-UV is not the best analytical tool: LC coupled to tandem MS/MS would not only allow quantification at trace levels but also a more reliable identification.

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