Universitat de Girona

DEVELOPMENT OF ANALYTICAL METHODOLOGIES FOR THE DETERMINATION OF EMERGING CONTAMINANTS AND PHOSPHORUS COMPOUNDS

Carme Valls Cantenys

Dipòsit legal: Gi. 2038-2014
http://hdl.handle.net/10803/284742

Universitat de Girona

DOCTORAL THESIS

DEVELOPMENT OF ANALYTICAL METHODOLOGIES FOR THE DETERMINATION OF EMERGING CONTAMINANTS AND PHOSPHORUS COMPOUNDS

Carme Valls i Cantenys

2014

Doctoral program in Experimental Sciences and Sustainability

Supervised by Dr. Victòria Salvadó Martín and Dr. Mònica Iglesias Juncà

This manuscript has been presented to opt for the doctoral degree from the University of Girona

Universitat de Girona

Dr. Victòria Salvadó Martín and Dr. Mònica Iglesias Juncà, of University of Girona,

WE DECLARE:

That the thesis titles *Development of analytical methodologies for the determination of emerging contaminants and phosphorus compounds***,** presented by Carme Valls Cantenys to obtain a doctoral degree, has been completed under our supervision and meets the requirements to opt for an International Doctorate.

For all intents and purposes, we hereby sign this document.

Dr. Victòria Salvadó Martín

Dr. Mònica Iglesias Juncà

Girona, 23rd of July 2014

The development of this thesis has been funded by two research projects from the Spanish Government:

- "Evaluación de la eficacia de las plantas de tratamiento de aguas residuales en la eliminación de compuestos orgánicos emergentes de las aguas para su reutilización" (CTM2008-06847-C02-02).

- "Comportamiento y eliminación de contaminantes orgánicos emergentes en sistemas de tratamientos de aguas. Evaluación de sistemas terciarios" (CTM2011-28765-C02-02).

Carme Valls i Cantenys gratefully acknowledge a PhD research grant from the Spanish Ministry of Science and Innovation (Ref. BES2009-024892) and two research mobility grants from the Spanish Ministry of Science and Innovation. Part of the experimental work of this thesis was performed in:

- Instituto de Investigación y Análisis Alimentario, Universidad de Santiago de Compostela, Santiago de Compostela, Spain.

- Technologiezentrum Wasser (TZW-DVGW), Karlsruhe, Germany.

The research described in this thesis has result in the publication of two scientific papers and three more that are in preparation:

- Valls-Cantenys, C., Iglesias, M., Todolí, J. L., Salvadó, V. *Speciation of phosphorus oxoacids in natural and waste water samples*. Journal of Chromatography A 1231 (2012) 16-21

- Valls-Cantenys, C., Villaverde-de-Sáa, E., Rodil, R., Quintana, J. B., Iglesias, M., Salvadó, V., Cela, R. *Application of polydimethylsiloxane rod extraction to the determination of sixteen halogenated flame retardants in water samples.* Analytica Chimica Acta 770 (2013) 85-93

- Valls-Cantenys, C., Iglesias, M., J. L., Salvadó, V. *Two polydimethylsiloxane rod extraction-based methods for the sensitive determination of phenolic compounds in water samples*. Sent to Journal of Separation Science (July 2014)

- Valls-Cantenys, C., Iglesias, M., J. L., Salvadó, V. *The Influence of the operating conditions of inductively coupled plasma-mass spectrometry on the sensitivity of phosphorus oxoanion speciation analysis.* In preparation

- Valls-Cantenys, C., Scheurer, M., Iglesias, M., Salvadó, V., Brauch, H. J., Sacher, F. *Development of a multiresidue methodology using liquid chromatography-tandem mass spectrometry for the analysis of water samples*. In preparation

A la meva família,

AGRAÏMENTS

Aquest és un moment molt esperat ja que si estic escrivint això vol dir que la redacció de la tesi ja està bastant enllestida i per tant que el final d'aquesta aventura és més pròxima que mai..... En molts aspectes ha sigut un camí llarg i no sempre fàcil, no ens enganyarem, en d'altres molt constructiu tant a nivell professional com personal. Existeix un abans i un després en tot això; són moltes les persones que al llarg d'aquests anys n'han format part i a les quals els hi vull dedicar aquests agraïments.

En primer lloc, voldria agrair a les meves directores de tesi el fet d'haver-me permès viure aquesta experiència. Mònica, tu vas saber veure les meves aptituds i vas confiar en elles. Sempre m'has sabut guiar per tal que aprengués al màxim i poder-ne treure el major profit de tot plegat. Has estat disponible i disposada a ajudar-me en tots els problemes que han anat sorgint, que no han estat pocs, fins i tot en els personals. Ets una bona persona amb un gran instint, segueix-lo sempre!! I gràcies per tot! Victòria, a tu t'haig d'agrair la oportunitat que em vas donar, gràcies a tu he pogut seguir i aconseguir aquest somni, que molta gent desitja i que no a tothom se li permet. Sense el teu suport i els teus múltiples consells no hagués pogut arribar fins aquí. Moltes gràcies!!

També voldria agrair el suport rebut dels companys de l'Àrea de Química Analítica: Nela, Clàudia, Eva, Enriqueta, Juanma, Víctor, Anna, Dolors, Ester, Aida, Mònica A., Subha, Santanu, Geerke, Raquel, Marta i Rubén. A vosaltres Nela i Enriqueta voldria agrair-vos tots els consells i ajuda "instrumental" que m'heu proporcionat. Anna, Dolors i Aida, aquest camí el vam començar juntes amb el màster, i encara que a vegades no semblava possible, ara totes ja estem arribant al final. Anna i Dolors vosaltres ja heu fet un pas més enllà, i heu format unes famílies molt maques (Jan tenim ganes de veure't!!). Ester, saps que tinc un especial record per a tu, m'has ajudat moltíssim i encara que ja hagis acabat ho continues fent, sempre disposada a donar un cop de mà!! Hem compartit molts bons moments i molts riures; i també llàgrimes i ganes de tirar la tovallola, però per sort hem resistit i seguit endavant!! Sempre m'has dit

que existeix llum al final del túnel, i aviat et donaré la raó (encara no, que les coses es poden torcí fàcilment!! ;P). I a tu Aida, també et tinc moltes coses a agrair, el teu positivisme i bon humor omplien el despatx del Parc i ajudaven a passar fins i tot els dies més durs. Treballar al teu costat ha sigut molt fàcil, sempre buscant el consens i evitant disputes, sort n'hem tingut de tu!! Hehe. Un dels records més macus que m'emporto d'aquests anys és el de les Nenes del Parc: el cafè amb llet de les 11h, les decoracions de Nadal, els sopars analítics en petit comitè, dormir al pis de l'Aida, les manualitats múltiples que sempre acabàvem fent,... Vam formar una petita família, que espero que sapiguem mantenir per molts anys!!! As a part of the "Nenes del Parc" I would like to mention Subha. At the end of your research you came to the Parc and together with Aida we spent lot of time having breakfast, reading the horoscope, smoking, listening your advice (lots of them!!),... You are a great friend and I wish you a happy life with Priyanka! Santanu, with you I didn't spent so much time as with Subha, but enough to know that you are a kind person and I wish good luck in the future. I hope in few years I can come to India to visit you!! Marta i Rubén vosaltres sou el futur dels becaris d'analítica, hehe, només dir-vos que endavant, que amb esforç tot s'aconsegueix i que la recompensa val la pena!!

Una parte de esta Tesis se realizó en el grupo de Química Analítica, nutrición y bromatología de la Universidad de Santiago de Compostela. Tengo que agradecer a Rafael, Tito y Charo todo lo que me enseñasteis en técnicas de microextracción y la buena acogida que tuve en el grupo y en la ciudad. Y a Eugenia y Inés, toda la ayuda recibida en el laboratorio. Al principio de mi estancia en Santiago de Compostela hubo una persona que con su temprana visita me ayudo muchísimo, y esa eres tu Geerke. No sé si lo recuerdas, pero el hecho de que antes de que me fuera de Girona ya tuvieras el billete para visitarme me ayudo a coger las fuerzas que en ese momento me faltaban para irme. Muchas gracias!!

Another part of this Thesis was developed in the TZW in Karlsruhe. I would like to thank you to Dr. Brauch, Dr. Sacher and Dr. Scheurer for all the

help received, especially with the apartment, and all the knowledge you provide me.

Durant aquests anys, altres persones han format part de la meva vida i per tant en certa forma d'aquesta tesis. A vosaltres Bet i Fedra, gràcies pels cafès a mitja tarda, que es van convertir en quedades de cap de setmana ara que sou mames d'aquestes nenes tant maques. Cristina i Patt, gràcies per les birretes de tarda, pels sopars i per les sortides nocturnes que tant bé m'han anat per desconnectar de tot, i per ser tant úniques com sou. Marta, gràcies pels teus immensos consells i per confiar sempre en mi. Des de sempre m'has dit que apuntés molt alt, que hi podia arribar. Gràcies!

I ara arriba el moment de donar les gràcies a les persones que amb el seu suport incondicional han permès que arribés on sóc. Gràcies Mama, gràcies Papa, gràcies Josep i gràcies Txus. Sempre meu fet costat en totes les decisions que he pres, per a bé o per a malament, sempre sou allà per ajudar-me a aixecar quan caic, o per celebrar-ne els èxits. Papa i Mama, sé que encara ara no enteneu ben bé què hi he anat a fer tants anys a Girona però no per això meu deixat d'ajudar. Papa, han sigut molts els matins que t'aixecaves molt aviat per portar-me a l'estació a buscar el tren, per tal que pogués anar a estudiar, sempre amb les preses de que el tren marxaria sense mi!! Gràcies. Mama, moltes gràcies per als milers de dinars que m'has preparat i em prepares, per aixecar-te aviat per venir-me a despertar per tal que estudies i per les milers de vegades que m'has escoltat i aconsellat quan ho volia engegar tot a pastar fang. Josep i Txus, gràcies pel vostre suport, per ajudar-me a buscar solucions quan jo no en trobava, i per animar-me a seguir els meus somnis sabent que sempre us tindria al meu costat. A tots vosaltres, moltes gràcies per haver confiat sempre en mi, molt més del que jo he fet en mi mateixa. I falteu els petits de la casa, Isaac i Tomàs, que amb la vostra innocència i enginy m'aconseguiu treure un somriure fins i tot en el dies més amargs.

I a tu, Jordi, què dir-te que no sàpigues... La nostra història va començar al mateix moment que aquesta tesi; tu la vas veure néixer, l'has vist créixer i ara en veus el resultat final. Tu també ets un dels pilars essencials

en aquesta història, i estic segura que sense el teu suport res de tot això hagués estat tant "fàcil". Des del principi vas apostar per mi, i en les meves capacitats. Amb el teu bon humor has aconseguit animar-me fins i tot en els moments més durs. Has estat al meu costat sempre, m'has encoratjat a seguir endavant i no defallir, i m'has ajudat per sobre de les teves possibilitats. Les estades fora han estat una realitat gràcies a tu, al teu suport, a les innumerables hores al telèfon i a les teves múltiples visites, fins i tot a terres veïnes!! Ets una de les millors persones que he conegut a la vida, sempre disposat a ajudar a tothom i amb un cor que no et cap al pit. Ets únic, increïble i màgic, i desitjo que aquest camí que vam començar fa cinc anys ens porti lluny, molt lluny però sempre un al costat de l'altre. Moltes gràcies per tot, pel que has fet i pel que fas cada dia per mi. No canviïs mai. T'estimo!

Moltes gràcies a tots els que heu format part de la història de la meva vida fins al dia d'avui.

Carme

LIST OF ABBREVIATIONS

10,11-DHC	10,11-dihydro-10,11-dihydroxy-carbamazepine
4-AA	4-aminoantipyrine
4-AAA	4-acetylaminoantipyrine
4-FAA	4-formylaminoantipyrine
4-MAA	4-methylaminoantipyrine
4MBT+5MBT	4-Methylbenzotriazole + 5-Methylbenzotriazole
AA	Acetic anhydride
ACN	Acetonitrile
APCI	Atmospheric pressure chemical ionisation
APE	Alkylphenol ethoxylates
BDE-100	2,2’,4,4’,6-pentabromodiphenyl ether
BDE-153	2,2’,4,4’,5,5’-hexabromodiphenyl ether
BDE-154	2,2’,4,4’,5,6’-hexabromodiphenyl ether
BDE-183	2,2’,3,4,4’,5’,6-heptabromodiphenyl ether
BDE-197	2,2’,3,3’,4,4’,6,6’-octabromodiphenyl ether
BDE-209	Decabromodiphenyl ether
BDE-47	2,2’,4,4’-tetrabromodiphenyl ether
BDE-99	2,2’,4,4’,5-pentabromodiphenyl ether
BFR	Brominated flame retardant
BPA	Bisphenol A
BSTFA	Bis(trimethylsilyl)trifluoroacetamide
BTBPE	1,2-bis(2,4,6-tribromophenoxy)ethane
CAR	Carboxen
CE	Capillary electrophoresis
CEn	Collision energy

CI	Chemical ionisation
CW	Carbowax
CxP	Collision cell exit potential
DAD	Diode array detector
DBDPE	Decabromodiphenyl ethane
DEHTBP	Bis (2-ethylhexyl)-3,4,5,6-tetrabromo-phthalate
DLLME	Dispersive liquid-liquid microextraction
DP	Dechlorane plus
DPo	Declustering potencial
DRP	Dissolved reactive phosphorus
DVB	Divinylbenzene
ECD	Electron capture detector
ED	Electrochemical detection
EDC	Endocrine disrupting compounds
Eh	Redox potencial
EHTBB	2-ethylhexyl-2,3,4,5-tetrabromobenzoate
EI	Electron impact ionisation
ESI	Electrospray ionisation
FR	Flame retardants
GC	Gas chromatography
HBCD	Hexabromocyclododecanes
HLB	Hydrophilic-lipophilic balanced
HPLC	High performance liquid chromatography
HS	Headspace
IC	Ion chromatography
ICM	Iodinated X-ray contrast media
ICP-AES	Inductively coupled plasma- atomic emission spectrometer / spectrometry

ICP-MS	Inductively coupled plasma-mass spectrometer / spectrometry
ILC	Isotope-labelled compounds
IS	Internal standard
LC	Liquid chromatography
LD	Liquid desorption
LLE	Liquid-liquid extraction
LOD	Limit of detection
LOQ	Limit of quantification
MDL	Method detection limits
MeOH	Methanol
MilliQ	Doubly deionised water
MRM	Multiple reaction monitoring
MS	Mass spectrometer / Mass spectrometry
MSD	Mass selective detector
NCI	Negative chemical ionisation
NP	4-*n*-Nonylphenol
NSAID	Non-steroidal anti-inflammatory drugs
Octbias	Octopole bias voltage
OP	4-*n*-Octylphenol
ORC	Octopole collision-reaction cell
PA	Polyacrylate
PBDE	Polybrominated diphenyl ether
PCI	Positive chemical ionisation
PCP	Pentachlorophenol
PDMS	Polydimethylsiloxane
PES	Polyethersulfone
PP	Polypropylene

QPbias	Quadrupole bias voltage
RF	Radio frequency
R_S	Resolution
RSD	Relative standard deviation
R_T	Retention time
SBR	Signal/ background ratio
SBSE	Stir bar sorptive extraction
SDVB	Styrene-divinylbenzene
SICA	Single-injection calibration approach
SIM	Selective ion monitoring
SPE	Solid phase extraction
SPME	Solid phase microextraction
SS	Surrogate standard
STP	Sewage treatment plant
TBBPA-A	Tetrabromobisphenol A
TCP	2,4,6-Trichlorophenol
TD	Thermal desorption
TP	Transformation products
TTBPP	Tris(tribomoneopenthyl)phosphate
WFD	Water Framework directive
WHO	World health organization
WW	Wastewater
WWTP	Wastewater Treatment Plant

INDEX OF FIGURES

INDEX OF TABLES

CONTENTS

RESUM

La contaminació de l'aigua per compostos d'origen antropogènic és un problema crucial ja que pot afectar l'equilibri ecològic del medi aquàtic, així com a la salut humana. El nombre d'aquests compostos que es detecten al medi ambient creix contínuament i està relacionat amb l'ús intensiu, principalment a la agricultura i a la indústria, de productes sintètics. A demés, els efluents de les plantes de tractament d'aigües residuals, que estan dissenyades per eliminar només matèria orgànica, són una altra font d'entrada d'aquests contaminants al medi aquàtic. Per això, és necessari controlar la presència dels contaminants, ja siguin regulats o emergents, a les aigües que puguin posar en risc la salut humana i el medi ambient. La determinació dels contaminants orgànics és complicada degut a la gran varietat de compostos a considerar i a la falta de mètodes apropiats per a determinar-ne la seva concentració a nivells traça. Per altra banda, la presència d'alguns nutrients, com les espècies de fòsfor, en medis aquàtics també s'ha de considerar i monitoritzar atès que una quantitat excessiva pot afectar als ecosistemes (per exemple, l'abundància de compostos de fòsfor està relacionada amb l'eutrofització). A més a més, per tal d'aconseguir comprendre la dinàmica del fòsfor en el medi natural és necessari un coneixement detallat del seu cicle i dels processos d'oxidació i reducció (redox) que experimenta, així com disposar de mètodes per a la determinació d'espècies inorgàniques de fòsfor, oxidades i reduïdes, en aigües.

L'objectiu general d'aquesta tesi és el desenvolupament de metodologies analítiques senzilles, eficients i sensibles per a determinació de espècies de fòsfor i microcontaminants orgànics en mostres d'aigua. En el cas dels microcontaminants, atès que es troben a concentracions molt baixes (μg L^{-1} o ng L^{-1}) en les aigües, serà necessari, aplicar un mètode de preconcentració abans de la seva determinació instrumental.

En primer lloc, s'han desenvolupat dos mètodes d'especiació per a la determinació de oxoanions de fòsfor simples (ortofosfat, fosfit i hipofosfit) i condensats (pirofosfat i tripolifosfat). En ambdós mètodes la separació de les espècies s'ha dut a terme per cromatografia de líquids d'alta resolució (HPLC) utilitzant una columna

d'intercanvi iònic. En el primer mètode desenvolupat es va utilitzar com a detector un plasma induït per alta freqüència acoblat a un espectròmetre d'emissió atòmica (ICP-AES); no obstant, per tal de millorar la sensibilitat, en el segon mètode aquest detector es va substituir per un de plasma induït per alta freqüència acoblat a un espectròmetre de masses (ICP-MS).

El pH i la força iònica de la fase mòbil han sigut les variables més crítiques a l'hora d'aconseguir la resolució adient per la separació de les cinc espècies de fòsfor. Aquesta es va aconseguir amb una fase mòbil de nitrat amònic i aplicant un gradient on s'incrementava la força iònica mentre es disminuïa el pH. També es va observar que per afavorir l'elució de tripolifosfat, que era l'espècie més retinguda, calia addicionar metanol.

En el mètode on s'emprava ICP-AES per a la detecció, es va estudiar l'efecte del sistema de nebulització i l'addició de metanol en el senyal dels analits, observant un increment significatiu en la sensibilitat amb l'addició d'un 2% (v/v) de metanol a la mostra, mentre que els millors resultats en sensibilitat es van aconseguir amb el nebulitzador babington a temperatura ambient. En aquestes condicions, els límits de detecció es trobaven en el rang 1 - 5 mg P L^{-1}, i els límits de quantificació en el rang 3 - 15 mg P L^{-1}. La exactitud i la precisió del mètode de HPLC-ICP-AES es varen avaluar analitzant mostres d'aigua fortificades (aixeta i efluent de depuradora) degut a la falta de materials de referència que continguin totes aquestes espècies de fòsfor. Les recuperacions obtingudes indiquen que el mètode desenvolupat es pot aplicar a la determinació d'espècies de fòsfor en matrius complexes tals com efluents de depuradora.

Per tal de millorar els límits de detecció del mètode de especiació es va decidir acoblar HPLC a un ICP-MS. En general, l'ICP-MS és més sensible que ICP-AES; no obstant, la detecció de ^{31}P es troba limitada per l'elevat potencial de ionització d'aquest element i la presència d'interferències espectrals. Per tal de solventar aquesta limitació es poden portar a terme diferents estratègies, una d'elles és l'ús de cel·les de col·lisió/reacció (ORC). En el nostre estudi, es van avaluar diversos paràmetres instrumentals, incloent els relacionats amb l'ús de ORC. L'addició d'un

2% de metanol a la fase mòbil permet incrementar fins a cinc vegades la sensibilitat, independentment de si s'usa o no la ORC. Altres paràmetres com la potència (RF), la distància de la torxa, el cabal de gas portador, el cabal d'He o el voltatge de les lents també van ser sistemàticament estudiats. Les millors condicions per a la detecció de fòsfor, que permeten reduir-ne les limitacions, són les següents: utilitzant la ORC pressuritzada amb He a un cabal de 1.5 mL min^{-1} i aplicant una potencia de 1300 W a una distància de torxa de 6 mm. En aquestes condicions es van obtenir límits de detecció de entre 7 a 30 μg L^{-1} per a les cinc espècies. Aquests valors són comparables als obtinguts per altres autors. Les recuperacions obtingudes en l'anàlisi de mostres d'aigua fortificades (80% a 103%) indiquen la viabilitat del mètode desenvolupat per a l'anàlisi d'aigües tractades de depuradora. L'aplicabilitat de la calibració mitjançant una única injecció (SICA) per a l'especiació de oxoanions de fòsfor també es va estudiar comparant els resultats obtinguts en l'anàlisi d'aigües tractades de depuradora mitjançant les dues metodologies de calibració, obtenint resultats molt similars.

Recentment, la comunitat científica està fent grans esforços en la miniaturització de les tècniques analítiques per tal de reduir el consum de dissolvents, els costos energètics i els residus. Les tècniques de microextracció, com per exemple la microextracció en fase sòlida (SPME), la microextracció en una única gota (SDME) i la extracció per sorció en barra (SBSE) han guanyat importància degut a que compleixen amb aquests requeriments. Una altra tècnica de microextracció que està esdevenint popular en els últims anys es basa en l'ús, com a sorbent, de materials purs en forma de tubs o cordons, el que permet reduir el cost del dispositiu i evitar problemes d'efecte memòria.

En aquesta tesi, s'han desenvolupat dues metodologies analítiques, utilitzant cordó de polidimetilsiloxà (PDMS) com a material sorbent, per a la determinació de compostos que actuen com a disruptors endocrins. Concretament, s'han seleccionat dues famílies de compostos, els retardants de flama halogenats i els compostos fenòlics. Per als retardants de flama halogenats, degut a que són compostos volàtils, la determinació es va portar a terme mitjançant cromatografia de gasos acoblada a espectrometria de masses amb ionització química negativa (GC-NCI-MS). Es van

estudiar les condicions que afectaven a l'extracció (modificador orgànic, força iònica, volum de mostra i cinètica) i a la desorció (dissolvent d'elució i volum) dels compostos del cordó de PDMS. Es va observar que calia addicionar un 40% de MeOH al vial d'extracció per evitar la adsorció dels compostos a les parets de vidre del vial, i també que l'addició d'un 4% de NaCl a la solució millorava l'extracció. Els factors d'enriquiment obtinguts, en un temps d'extracció de 15 hores, variaven entre 108 i 840 quan el volum de mostra era de 100 mL. La desorció dels analits es va dur a terme en 300 μL d'acetat d'etil durant quinze minuts amb sonicació. En aquestes condicions, l'eficiència d'extracció va ser de entre el 9 i el 70%, i els límits de detecció es trobaven entre els 0.4 i els 10 ng L^{-1}. Els possibles efectes matriu es van corregir amb l'addició de patrons interns a la mostra (surrogates), que també es va utilitzar com a patrons interns en la detecció instrumental.

Després de validar el mètode, es va aplicar a l'anàlisi de diferents mostres d'aigua, incloent mostres d'aigua de riu, ria i mar, lixiviats d'abocador, influent i efluents de depuradora urbana i efluents de depuradora d'una indústria tèxtil. A les mostres de depuradora i als lixiviats es van detectar el BDE-47, el BDE-99, el BDE-100 i el BDE-197 a concentracions per sobre dels 2887 ng L^{-1}. De entre els nous retardants de flama desenvolupats per a substituir els polibromats de difenil èter, el bis(2-etilhexil)-3,4,5,6-tetrabromo-ftalat (DEHTBP) va ser detectat en aigües superficials (mar, riu i ria) i el 1,2-bis(2,4,6-tribromofenoxi)eta (BTBPE) va ser detectat en la mostra de lixiviat.

El segon mètode desenvolupat on s'emprava un cordó de PDMS per extreure els compostos d'interès es va aplicar a determinació de cinc compostos fenòlics (bisfenol A (BPA), 2,4,6-triclorofenol (TCP), pentaclorofenol (PCP), 4-*n*-nonilfenol (NP) i 4-*n*-octilfenol (OP)) en aigües. En aquest cas, degut a la polaritat i volatilitat dels compostos seleccionats, es va utilitzar com a tècnica de separació la cromatografia de líquids. La detecció es va portar a terme amb un detector de diodes en fila. Per a aquests compostos, es van desenvolupar dues metodologies: una aplicada als compostos fenòlics (mètode directe) i l'altre aplicada als seus derivats acetilats. La derivatització per acetilació es va portar a terme principalment per millorar l'extracció del BPA mitjançant el cordó de PDMS. Per a tots aquest

compostos, també es van estudiar els paràmetres que afecten a l'extracció i a la desorció. Es va observar que el pH era important per a l'extracció dels compostos fenòlics, mentre que no tenia un paper rellevant en l'extracció dels derivats fenòlics acetilats, tot i que sí que era un factor a controlar en la reacció de derivatització. L'addició d'un modificador orgànic a la mostra no va representar cap millora, mentre que la força iònica de la mostra afectava de manera important l'extracció dels compostos fenòlics, i quasi no tenia efecte sobre l'extracció dels seus derivats acetilats. L'estudi de les condicions de derivatització es va centrar en l'efecte de la quantitat d'agent derivatitzant i del pH.

En el cas del mètode directe, es van obtenir límits de detecció en l' interval de 0.6 - 2 µg L^{-1} per a tots els compostos, excepte per al BPA (9.5 µg L^{-1}). Amb el mètode basat en la derivatització del compostos fenòlics, es van aconseguir límits de detecció més baixos (0.3 - 0.9 µg L^{-1}) per a tots els compostos. La precisió es va avaluar a dos nivells de concentració, 5 µg L^{-1} i 15 µg L^{-1}, i es van obtenir RSD en el rang 4 - 17% per al mètode directe i de entre el 2% i el 9% per al mètode amb derivatització. En ambdós casos són precisions dins el mateix dia i entre dies. Finalment, el mètode desenvolupat es va aplicar a l'anàlisi de mostres d'aigua de riu, fortificades a tres nivells de concentració, obtenint recuperacions entre el 60.2% i el 131.7% per al mètode directe i entre el 76.6% i el 108.2% per al mètode amb derivatització.

Malgrat que l'ús de tècniques de microextracció s'ha incrementat en els darrers anys, l'extracció en fase sòlida (SPE) continua essent la tècnica més usada en metodologies multiresidu, tal i com és el cas de la metodologia desenvolupada a la última part de la tesi. El principal desafiament que representa l'ús de SPE en el nostre mètode multiresidu és l'amplia varietat de propietats físico-químiques i l'elevada polaritat d'alguns dels compostos d'interès, especialment dels productes de transformació. És per això, que l'elecció del sorbent més apropiat per l'extracció de tots els compostos a analitzar és un punt essencial en el desenvolupament de metodologies multiresidu.

S'ha desenvolupat un mètode multiresidu mitjançant extracció per SPE seguit de cromatografia líquida acoblada a espectrometria de masses en tàndem (LC-MS/MS) per a la determinació d'un grup representatiu de 35 analits, incloent compostos farmacèutics de diferents famílies (p.ex. analgèsics i antiinflamatoris, contrastadors de raig X iodats, ß-bloquejadors, ...), inhibidors de la corrosió i plaguicides, així com dels seus metabòlits i productes de transformació. És important remarcar que existeixen poques metodologies multiresidu que incloguin plaguicides, productes farmacèutics i diferents contrastadors de raig X iodats, ja que en moltes de les metodologies existents, només es determina la iopromida en representació del grup de compostos utilitzats com a contrastadors de raig X.

En aquest cas es varen estudiar diversos paràmetres relacionats tan amb la separació cromatogràfica dels compostos com amb la seva extracció amb el sorbent més apropiat. Es van provar quatre cartutxos de SPE amb diferents mecanismes de retenció, obtenint-se els millors resultats amb el cartutx Oasis HLB, que conté un sorbent de fase inversa hidrofílica-lipofílica equilibrat, que és el que millor s'ajusta a les necessitats requerides donada la diferent naturalesa dels nostres compostos. Per als compostos més polars, es va comprovar que el pH de la mostra tenia un paper important en l'eficiència d'extracció i per tant, es van estudiar diferents valors de pH. Finalment, es va seleccionar un pH de 7 com a compromís entre les recuperacions obtingudes per a les diferents espècies. El volum d'elució també es va investigar en el rang de 4 a 10 mL, però no es observar cap efecte per a la majoria dels compostos, excepte per al diclofenac, per al qual la recuperació augmentava al augmentar el volum d'elució, seleccionant-se finalment un volum de 8 mL.

Per tal de corregir els efectes matriu, es van utilitzar un total de 20 patrons interns (surrogates) que s'afegien a la mostra al començament del procés. Les recuperacions obtingudes es trobaven entre el 79% i el 134% per a aigua d'aixeta i entre el 66.5% i el 144 % per a aigua superficial. Els límits de quantificació del mètode es trobaven entre 1.7 i 11 ng L^{-1}, per a la majoria dels compostos, excepte per al iomeprol, l'àcid amidotrizoic i el iohexol que van ser de 22, 25.5 i 17.9 ng L^{-1} respectivament. Finalment, el mètode es va aplicar a l'anàlisi de 56 mostres reals, incloent aigua de riu i aigua d'influent i efluent de depuradora. Tots els compostos farmacèutics

estudiats, incloent els metabòlits i els productes de transformació, van ser detectats, en concentracions variables, en les mostres d'aigua analitzades, obtenint les concentracions més altes en les mostres d'influent i efluent de depuradora. El 1H-benzotriazol, que és un inhibidor de la corrosió, va ser detectat en totes les mostres analitzades, amb una concentració mediana més gran (1715 ng L^{-1}, 16800 ng L^{-1} i 8700 ng L^{-1} en mostres d'aigua de riu, influent i efluent de depuradora, respectivament) respecte a la resta de compostos analitzats. En el cas dels plaguicides, almenys sis dels compostos estudiats van ser detectats en totes les 56 mostres d'aigües analitzades, i en tots els casos, dels plaguicides que es troben inclosos a la directiva, les concentracions trobades eren inferiors a la concentració màxima permesa que estableix l'estàndard de qualitat mediambiental.

RESUMEN

La presencia de compuestos de origen antropogénico en las aguas es un problema crucial ya que puede afectar el equilibrio ecológico del medio acuático, así como a la salud humana. El número de estos compuestos detectados en el medio ambiente aumenta continuamente y está relacionado con el creciente uso, principalmente en la agricultura y en la industria, de productos sintéticos. A demás, los efluentes de las estaciones depuradoras de aguas residuales, diseñadas para eliminar materia orgánica, son otra fuente de entrada de estos contaminantes al medio acuático. Por todo ello, es necesario controlar la presencia de contaminantes estén o no regulados, en las aguas que puedan poner en riesgo la salud humana y el medio ambiente. La determinación de contaminantes orgánicos en las aguas es complicada debido a la gran variedad de compuestos a considerar ya la falta de métodos apropiados para su determinación a niveles traza. Por otra parte, hay que tener en cuenta y controlar la presencia de nutrientes, como las especies de fósforo, en medios acuáticos ya que su excesiva concentración puede afectar a los ecosistemas (por ejemplo, la abundancia de compuestos de fósforo está relacionada con la eutrofización). Además, para comprender la dinámica del fósforo en el medio natural es necesario conocer en detalle su ciclo, incluyendo los procesos de oxidación y reducción (redox), y disponer de métodos apropiados para la determinación de las especies inorgánicas de fósforo en aguas.

El objetivo general de esta tesis es el desarrollo de metodologías analíticas sencillas, eficientes y sensibles para la determinación de especies de fósforo y microcontaminantes orgánicos en muestras de agua. En el caso de los microcontaminantes, debido a las muy bajas concentraciones (μg L^{-1} o ng L^{-1}) en que se encuentran en medio acuático, será necesario aplicar un método de preconcentración, antes de la determinación instrumental.

En esta tesis, se han desarrollado, en primer lugar, dos métodos de especiación para la determinación de oxoaniones de fósforo simples (ortofosfato, fosfito y hipofosfito) y condensados (pirofosfato y tripolifosfato). En ambos métodos, la separación de las especies de fósforo se realizo por cromatografía de líquidos de alta

resolución (HPLC) utilizando una columna de intercambio iónico. En el primer método desarrollado se utilizo un espectrómetro de emisión atómica con plasma inducido por alta frecuencia (ICP-AES); no obstante, para mejorar la sensibilidad, en el segundo método, este detector se sustituyó por un espectrómetro de masas con plasma de acoplamiento inducido (ICP-MS).

En la separación de las cinco especies de fósforo, el pH y la fuerza iónica de la fase móvil fueron las variables más críticas para la obtención de la resolución apropiada. Esta se consiguió con una fase móvil de nitrato amónico y con un gradiente donde se incrementaba la fuerza iónica mientras se disminuía el pH. Además se observo que la adición de metanol favorecía la elución del tripolifosfato, que era la especie más retenida.

En la detección con ICP-AES, se estudió el efecto de parámetros instrumentales, como el sistema de nebulización, y el de la adición de metanol en la señal de los analitos. Con la adición de un 2% (v/v) de metanol en la muestra, se observó un incremento significativo en la sensibilidad, mientras que con el nebulizador Babington a temperatura ambiente también mejoro la sensibilidad. En estas condiciones, se obtuvieron límites de detección en el intervalo de 1 a 5 mg P L^{-1}, y límites de cuantificación en el intervalo de 3 a 15 mg P L^{-1}. La exactitud y precisión del método de HPLC-ICP-AES se evaluó analizando muestras de agua fortificadas (grifo y efluente de depuradora) debido a la falta de materiales de referencia que contengan todas estas especies de fósforo. Las recuperaciones obtenidas indican que el método desarrollado se puede aplicar para la especiación de fósforo en matrices complejas tales como efluentes de depuradora.

Para mejorar los límites de detección del método de especiación se decidió acoplar HPLC a un ICP-MS. En general, el ICP-MS es más sensible que ICP-AES; no obstante, la detección de ^{31}P se ve limitada por el elevado potencial de ionización que presenta y la presencia de interferencias espectrales. Para solventar esta segunda limitación, se pueden utilizar diferentes estrategias, una de ellas es el uso de celdas de colisión/reacción (ORC). En nuestro estudio, se evaluaron diversos parámetros instrumentales, incluyendo los relacionados con el uso de ORC. La adición de un

2% de metanol a la fase móvil permite incrementar hasta cinco veces la sensibilidad, independientemente de si se usa o no la ORC. Otros parámetros como la potencia (RF), la distancia de la antorcha, el caudal de gas portador, el caudal de He o el voltaje de las lentes también fueron estudiados sistemáticamente. Las limitaciones relacionadas con la detección de fósforo se minimizaron operando en las siguientes condiciones: utilizando la ORC presurizada con He a un caudal de 1.5 mL min^{-1} y aplicando una potencia de 1300 W a una distancia de antorcha de 6 mm. En estas condiciones se obtuvieron límites de detección en el rango de 7 a 30 μg L^{-1} para las cinco especies. Estos valores son comparables a los obtenidos por otros autores. Las recuperaciones obtenidas en el análisis de muestras de agua fortificadas (80% a 103%) indican la viabilidad del método desarrollado para el análisis de aguas tratadas de depuradora. La aplicabilidad de la calibración mediante una única inyección (SICA) para la especiación de oxoaniones de fósforo también se estudió, mediante la comparación de los resultados obtenidos en el análisis de aguas tratadas de depuradora aplicando las dos metodologías de calibración, obteniéndose resultados muy similares.

En los últimos años, la comunidad científica está haciendo grandes esfuerzos para miniaturizar las técnicas analíticas con el objetivo de reducir el consumo de disolventes, los costes energéticos y la generación de residuos. Como resultado de esta estrategia, se han incrementado las aplicaciones de las técnicas de microextracción, como por ejemplo la microextracción en fase sólida (SPME), la microextracción en una única gota (SDME) y la extracción por sorción en barra (SBSE). Otra técnica de microextracción que está siendo cada vez más utilizada es la que se basa en el uso de materiales puros, en forma de tubos o cordones, como material de adsorción, permitiendo reducir los costes del dispositivo y evitando problemas de efecto memoria.

En esta tesis, se han desarrollado dos metodologías analíticas utilizando cordón de polidimetilsiloxano (PDMS) como material de sorción para la determinación de compuestos que actúan como disruptores endocrinos. Concretamente, se han seleccionado dos familias de compuestos, los retardantes de llama halogenados y los compuestos fenólicos. Para los retardantes de llama halogenados, debido a que son

compuestos volátiles, la determinación se llevó a cabo mediante cromatografía de gases acoplada a espectrometría de masas con ionización química negativa (GC-NCI-MS). Se estudiaron las condiciones que afectaban a la extracción (modificador orgánico, fuerza iónica, volumen de muestra y cinética) y la desorción (disolvente de desorción y volumen) de los compuestos del cordón de PDMS. Se observó que era necesaria la adición de un 40% de MeOH al vial de extracción para evitar la adsorción de los compuestos en las paredes de vidrio del vial, así como que la adición de un 4% de NaCl mejoraba la extracción. Se consiguieron factores de enriquecimiento de entre 108 y 840 en un tiempo de extracción de 15 horas con un volumen de muestra de 100 mL. La desorción de los analitos se realizo con 300 μL de acetato de etilo durante quince minutos con sonicación. En estas condiciones, la eficiencia de extracción fue de entre el 9 y el 70%, y los límites de detección se encontraban en el intervalo entre los 0.4 y los 10 ng L^{-1}. Los posibles efectos de la matriz se corrigieron con el uso de patrones internos que se añadieron al inicio del proceso analítico (surrogate), y que también se utilizaron como patrones internos en la determinación instrumental.

Una vez validado el método, se aplico al análisis de diferentes muestras de agua, incluyendo muestras de agua de río, ría y mar, lixiviados de vertedero, influyente y efluentes de depuradora urbana y efluentes de depuradora de una industria textil. En las muestras de depuradora y de los lixiviados se detectaron concentraciones de BDE-47, BDE-99, BDE-100 y BDE-197 superiores a 2887 ng L^{-1}. De entre los nuevos retardantes de llama desarrollados para substituir a los polibromados de difenil éter, el bis(2-etilhexil)-3,4,5,6-tetrabromo-ftalato (DEHTBP) fue detectado en aguas superficiales (mar, río y ría) y el 1,2-bis(2,4,6-tribromofenoxi)eta (BTBPE) fue detectado en la muestra de lixiviado.

El segundo método desarrollado basado en extracción en cordón de PDMS se aplicó a la determinación de cinco compuestos fenólicos (bisfenol A (BPA), 2,4,6-triclorofenol (TCP), pentaclorofenol (PCP), 4-*n*-nonilfenol (NP) y 4-*n*-octilfenol (OP)). En este caso, debido a la polaridad y volatilidad de los compuestos seleccionados, se utilizó la cromatografía de líquidos como técnica de separación con un detector de diodos en fila (DAD). Para estos compuestos, se desarrollaron

dos metodologías: una aplicada a los compuestos fenólicos (método directo) y el otro aplicado a sus derivados acetilados. La derivatización por acetilación se llevó a cabo principalmente para mejorar la extracción del BPA mediante el cordón de PDMS. Para todos estos compuestos, también se estudiaron los parámetros que afectan a la extracción ya la desorción. Se observó que el pH era importante para la extracción de los compuestos fenólicos, mientras que no tenía un papel relevante para la extracción de los derivados fenólicos acetilados, aunque sí que era esencial para la reacción de derivatización. La adición de un modificador orgánico en la muestra no representó ninguna mejora, mientras que la fuerza iónica de la muestra afectaba de manera importante la extracción de los compuestos fenólicos, y casi no tenía efecto sobre la extracción de sus derivados acetilados. También se estudiaron las condiciones de derivatización como la cantidad de agente derivatizante o el pH.

En el caso del método directo, se obtuvieron límites de detección en el rango de 0.6 - 2 µg L^{-1} para todos los compuestos, excepto para el BPA (9.5 µg L^{-1}). Con el método basado en la derivatización de los compuestos fenólicos, se obtuvieron límites de detección más bajos (0.3 - 0.9 µg L^{-1}) para todos los compuestos. La precisión se evaluó a dos niveles de concentración, 5 µg L^{-1} y 15 µg L^{-1}, y se obtuvieron RSD en el rango 4 -17% para el método directo y de entre el 2% y el 9% para el método con derivatización. En ambos casos, las precisiones se evaluaron dentro del mismo día y entre días. Finalmente, el método desarrollado se aplicó al análisis de muestras de agua de río, fortificadas a tres niveles de concentración, y se obtuvieron recuperaciones entre el 60.2% y el 131.7% para el método directo y entre el 76.6% y el 108.2% para el método con derivatización.

A pesar del incremento en el uso de técnicas de microextracción, la extracción en fase sólida (SPE) sigue siendo la técnica más usada en el desarrollo de metodologías multiresiduo, como es el caso de la metodología desarrollada en la última parte de la tesis. La variedad de propiedades físico-químicas y la elevada polaridad que pueden presentar los compuestos de interés y, especialmente sus productos de transformación, es el principal inconveniente que hay que resolver en el uso de SPE en métodos multiresiduo. Es por ello, que la selección del absorbente de SPE más apropiado es un punto crítico en el desarrollo de las metodologías multiresiduo.

Se ha desarrollado un método multiresiduo mediante extracción por SPE seguido de cromatografía líquida acoplada a espectrometría de masas en tándem (LC-MS/MS) para la determinación de un grupo representativo de 35 analitos, incluyendo compuestos farmacéuticos de diferentes familias (p.ej. analgésicos y antiinflamatorios, contrastadores de rayos X yodados y ß-bloqueadores), inhibidores de la corrosión y plaguicidas, así como sus metabolitos y productos de transformación. Es importante remarcar que existen pocas metodologías multiresiduo que incluyan plaguicidas, productos farmacéuticos y diferentes contrastadores de rayos X yodados, ya que en la mayoría de las metodologías desarrolladas sólo se determina la iopromida.

En este caso se estudiaron diversos parámetros relacionados con la separación cromatográfica de los compuestos y su extracción con el absorbente más apropiado. Se probaron cuatro cartuchos de SPE con diferentes mecanismos de retención, obteniendo los mejores resultados utilizando el cartucho Oasis HLB, que contiene un absorbente de fase inversa equilibrado hidrofílico-lipofílico, que es el que mejor se ajusta a la naturaleza diversa de los compuestos seleccionados. Se comprobó que el pH de la muestra tenía un papel importante en la eficiencia de extracción para los compuestos más polares, y por tanto, se estudiaron diferentes valores de pH. Finalmente, se seleccionó un pH de 7 como compromiso entre las recuperaciones obtenidas para los diferentes compuestos. El volumen de elución también se varió en el rango de 4 a 10 mL, pero no se observó ningún efecto para la mayoría de los compuestos, excepto para el diclofenaco, cuya recuperación aumentaba al aumentar el volumen de elución, y seleccionándose finalmente un volumen de 8 mL.

Para corregir los efectos matriz, se utilizaron un total de 20 patrones internos que se añadieron al inicio del proceso de análisis (surrogate). Las recuperaciones obtenidas se encontraban entre el 79% y el 134% para agua de grifo y entre el 66 5% y el 144% para agua superficial, obteniéndose límites de cuantificación del método entre 1.7 y 11 ng L^{-1}, para la mayoría de los compuestos, excepto para el iomeprol, el ácido amidotrizoico y el iohexol que fueron de 22, 25.5 y 17.9 ng L^{-1} respectivamente. Finalmente, el método se aplicó al análisis de 56 muestras, incluyendo agua de río y agua de influyente y efluente de depuradora. Todos los

compuestos farmacéuticos estudiados, incluyendo sus metabolitos y productos de transformación, fueron detectados, con un amplio rango de concentraciones, en las muestras de agua analizadas, obteniendo las concentraciones más altas en las muestras de influyente y efluente de depuradora. El 1H-benzotriazol, que es un inhibidor de la corrosión, fue detectado en todas las muestras analizadas, presentando a demás la concentración mediana más grande (1715 ng L^{-1}, 16800 ng L^{-1} y 8700 ng L^{-1}en agua de río, influyente y efluente de depuradora, respectivamente) respecto al resto de compuestos analizados. Con respecto a los plaguicidas, al menos seis de los compuestos estudiados fueron detectados en todas las 56 muestras de aguas analizadas. Para los plaguicidas incluidos en la directiva las concentraciones encontradas fueron inferiores a la concentración máxima permitida que establece el estándar de calidad medioambiental.

SUMMARY

The presence of anthropogenic chemical substances in water resources is of major concern since they can affect the ecological balance of aquatic systems, as well as human health. The number of these pollutants entering into the environment is steadily increasing due to the wide use of many synthetic products mainly in agricultural and industrial practices. Moreover, wastewater treatment plants (WWTPs) are only designed for the elimination of organic matter and their effluents are another source of entry of contaminants into aquatic environment. Hence, it is necessary to control the presence in water bodies of compounds such as regulated and emerging contaminants that can pose a health and environmental risk. The determination of organic contaminants is complicated by the great variety of compounds which need to be considered and the lack of suitable methods to reveal their presence at trace levels. The presence of nutrients like phosphorus species in aquatic systems have to be also considered since their excessive presence can affect ecosystems (e.g. the overabundance of phosphorus compounds causes eutrophication). Moreover, a full understanding of the dynamics of phosphorus in the natural environment requires expanded knowledge of its oxidation-reduction (redox) cycle including the determination of inorganic phosphorus species (speciation analysis) in waters.

The general objective of this thesis is the development of simple, efficient and sensitive analytical methodologies for the determination of phosphorus species and organic micropollutants in water samples. In the second case, as the compounds are found at low concentrations (μg L^{-1} or ng L^{-1} level), a preconcentration method is also required.

Firstly, two speciation methods for the determination of simple (orthophosphate, phosphite and hypophosphite) and condensate (pyrophosphate and tripolyphosphate) phosphorus oxoanions have been developed. In the two methods, the separation of species was carried out by liquid chromatography (HPLC) using an anion exchange column. An inductively coupled plasma-atomic emission spectrometer (ICP-AES) was the detector employed in the first methodology developed. In order to improve

the sensitivity, this detector was substituted by an inductively coupled plasma mass spectrometer (ICP-MS) in the second method.

The pH and the ionic strength of the mobile phase were the most critical variables in order to obtain an appropriate resolution in the separation of all inorganic phosphorus species. This was achieved using ammonium nitrate aqueous solutions with gradient elution and increasing ionic strength while decreasing the pH. Moreover, methanol was added to promote the elution of tripolyphosphate which was the most retained species.

When the detection was carried out by ICP-AES, the instrumental parameters such as the nebuliser system and the effect of the methanol addition on the analyte signal were studied. A significant increase on the sensitivity is obtained by the addition of 2% (v/v) of MeOH to the sample solution while the use of a babington nebulizer at room temperature gave also the best sensitivity. In these conditions, limits of detection (LOD) in the range 1 - 5 mg P L^{-1} and limits of quantification (LOQ) in the range 3 - 15 mg P L^{-1} were obtained. The accuracy of the HPLC-ICP-AES developed method was evaluated by analysing spiked water samples (tap and effluent wastewater), since no reference materials containing all the phosphorus species are available. The recoveries obtained indicate that the method developed can be applied to the speciation analysis of phosphorus in complex matrices such as effluent wastewaters. In order to improve the detection limits of the speciation method, we decided to couple the HPLC with an ICP-MS as, in general, ICP-MS is more sensitive than ICP-AES. However, the detection of ^{31}P is limited by its high ionisation potential and the presence of spectroscopic interferences. Different strategies can be performed to overcome this last limitation; one of them is the use of a collision/reaction cell (ORC). In our study, we evaluated a wide range of instrumental parameters, including those related with the use of an ORC. The presence of a 2% (v/v) of methanol in the mobile phase represented a five-fold increase in the sensitivity independently of using or not the ORC. Other parameters such as RF power, sampling depth, carrier gas flow rate, He flow rate and lens voltages have been also systematically studied. The limitations related with phosphorus detection were minimised under the following conditions: an ORC

pressurised with He at a flow rate of 1.5 mL min^{-1} and applying an RF of 1300 W at a sampling depth of 6 mm. In these conditions, LODs in the range of 7 - 30 μg P L^{-1} for the five species were obtained. These values were comparable to those calculated by other authors. The recoveries obtained in the analysis of spiked wastewater samples (80% to 103%) indicate the feasibility of using the developed method in the analysis of treated wastewater. The applicability of the single-injection calibration approach (SICA) in the speciation analysis of phosphorus oxoanions was also studied by comparing the results obtained in analysing wastewater samples using both calibration methods, which resulted in good agreement.

In recent years, scientific community is making a great effort in the development of analytical techniques using miniaturised equipments and reducing amounts of solvents, energy costs and wastes. As a result, microextraction techniques, such as solid phase microextraction (SPME), single drop microextraction (SDME) and stir bar sorptive extraction (SBSE), are gaining importance. Another microextraction technique that has become popular over the last few years is based on the use of bulk material, such as rods or tubes, as the sorbent, reducing the cost of the device and avoiding carryover problems.

Thus, we have developed two analytical methodologies for the determination of endocrine disrupting compounds using polydimethylsiloxane (PDMS) rod as sorptive extraction material. Two families of compounds, halogenated flame retardants and phenolic compounds have been selected. In the case of volatile halogenated flame retardants (FRs), gas chromatography coupled to negative chemical ionisation-mass spectrometry (GC-NCI-MS) was used for their determination. The conditions affecting the extraction (organic modifier, ionic strength, kinetics and sample volume) and desorption (solvent and volume) of these compounds from the PDMS rod were studied. The addition of 40% of MeOH to the extraction vial was necessary to avoid adsorption of the compounds into the glass walls, as well as the addition of 4% of NaCl. The selected extraction volume was 100 mL achieving enrichment factors ranged from 108 to 840, with an extraction time of 15 h. Analyte desorption was performed in 300 μL of ethyl acetate during fifteen-minutes with sonication. Under these conditions, extraction efficiencies in

the 9 to 70% range and detection limits ranged from 0.4 to 10 ng L^{-1} were obtained. Possible matrix effects were corrected by using surrogate internal standards, which were also used as instrumental internal standards.

After the method validation, different real samples, including river, ria and sea waters, landfill leachate, influent and effluent wastewater from an urban WWTP, and effluent wastewater from a textile industry, were analysed. BDE-47, BDE-99, BDE-100 and BDE-197 were detected in wastewater and landfill leachate samples at concentration levels up to 2887 ng L^{-1}. Among the FRs designed to replace polybrominated diphenyl ethers, bis(2-ethylhexyl)-3,4,5,6-tetrabromo-phthalate (DEHTBP) was detected in surface water samples (sea, river and ria) and 1,2-bis(2,4,6-tribromophenoxy)ethane (BTBPE) in the landfill leachate.

The second PDMS rod extraction method was applied to the determination of five phenolic compounds (bisphenol A (BPA), 2,4,6-trichlorophenol (TCP), pentachlorophenol (PCP), 4-*n*-nonylphenol (NP) and 4-*n*-octylphenol (OP)). In this case, due to the polarity and lower volatility of the selected compounds, liquid chromatography separation was used. The detection was carried out using a diode array detector. For these compounds, two extraction methodologies were developed: one was applied to the determination of phenolic compounds (direct method) and the second was applied to the determination of their acetylated-derivatives. Acetylation derivatisation was carried out mainly because BPA, which is the most polar of the five compounds included in the study, was not successfully extracted by the PDMS rod. Parameters affecting the extraction and desorption of the compounds have been studied. We observed that, for the extraction of phenolic compounds, the pH was an important parameter, whereas it was not for the extraction of their acetylated-derivatives. However, it was an essential parameter in performing the derivatisation reaction. The addition of an organic modifier to the sample did not show any effect, while the ionic strength had an important effect in the extraction of phenolic compounds but only slightly affected the extraction of the acetylated ones. The derivatisation conditions, such as the amount of derivative reagent and pH, were also adjusted to the best values. In the case of the direct method, limits of detection in the 0.6 - 2 μg L^{-1} range were obtained for all compounds except BPA (9.5 μg L^{-1}). With

the derivatisation-based method, lower limits of detection (0.3 - 0.9 μg L^{-1}) were obtained for all the compounds. The precision was evaluated at two concentration levels, 5 μg L^{-1} and 15 μg L^{-1}, and RSD (%) values in the range of 4 - 17% were achieved for both intra and inter-day for the direct method and in the 2 - 9% range for the derivatisation-based method. Finally, the developed methods were applied to the analysis of river water samples spiked at three concentration levels obtaining recoveries of between 60.2% and 131.7% for the direct method, and of between 76.6% and 108.2% for the derivatisation-based method.

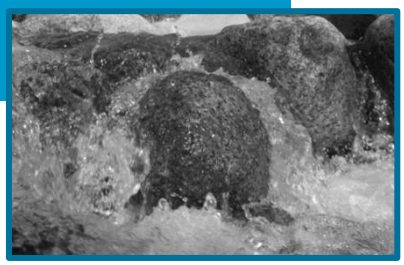
Despite the increasing use of the microextraction techniques, solid phase extraction (SPE) is the most used sample treatment technique in multiresidue methods. This is the case of the developed method described in the last part of this thesis**.** The main challenge of using SPE enrichment in our multiresidue method is the wide range of physicochemical properties and the high polarity of many of the target compounds and especially of their transformation products. Therefore the selection of the most appropriate sorbent is a crucial point in the development of the multiresidue method. A multiresidue method for the determination of a representative group of 35 analytes, including pharmaceuticals of different families (i.e. analgesic/anti-inflammatory drugs, iodinated contrast media, ß-blockers...), corrosion inhibitors, and several pesticides, as well as metabolites and transformation products, has been developed using SPE followed by liquid chromatography-tandem mass spectrometry (LC-MS/MS). Few multiresidue methods include pesticides, pharmaceuticals and diverse iodinated contrast media as, in most of them, iopromide is the only iodinated compound selected.

Several parameters related to the chromatographic separation of these compounds and their extraction by the most appropriate sorbent were studied. Four cartridges with different retention mechanism were tested. The Oasis HLB cartridge, which has a hydrophilic-lipophilic balanced reversed-phase sorbent, was the one that gave the best results for the range of selected compounds. The pH of the sample has an important effect on the extraction efficiency of the more polar compounds, and, hence, different pHs were assayed. Finally, a pH of 7 was selected as a compromise between the recoveries obtained for the different compounds. The elution volume

was also investigated in the range 4 to 10 mL but it was found to only affect the recovery of diclofenac, which increased as elution volume increased. An elution volume of 8 mL was finally selected.

In order to correct matrix effects a total of 20 surrogate/internal standards were used. Recoveries in the range of 79 and 134% and of 66 and 144% were obtained for tap and surface water, respectively. Method quantification limits (MQL) were in the 1.7 - 11 ng L^{-1} range for most compounds, but higher for iomeprol, amidotrizoic acid and iohexol (22, 25.5 and 17.9 ng L^{-1}, respectively). Finally, the method was applied to the analysis of 56 real water samples including river water and influent and effluent wastewaters. All the studied pharmaceuticals, including metabolites and transformation products, have been detected in a wide range of concentrations in the water samples analysed. The highest concentrations were obtained in influent and effluents of WW samples. 1H-benzotriazole, a corrosion inhibitor, was found in all the samples analysed and present the highest median concentration (1715 ng L^{-1}, 16800 ng L^{-1} and 8700 ng L^{-1} for river, influent WW and effluent WW, respectively) with respect the rest of the compounds analysed. With regard to pesticides, at least six of the studied compounds were detected in all the 56 samples. In all the cases, the pesticides included in the directive were found below the maximum allowable concentration established in the environmental quality standard.

CHAPTER 1. INTRODUCTION



The presence of both inorganic and organic anthropogenic chemical substances in water resources is of major concern since they can affect the ecological balance of aquatic systems as well as human health. Since World War II, more than 100,000 synthetic chemicals have been introduced for domestic, industrial and agricultural use [1]. Despite many of these substances having extremely beneficial functions, it is essential that they do not contaminate the water supplies as this is an important human exposure pathway to infectious pathogens, toxins and carcinogenic organic and inorganic contaminants.

Phosphorus compounds and other nutrients are examples of inorganic contaminants whose speciation must be determined in order to fully understand the pathways by which they spread, provoking eutrophication, and to design wastewater treatment systems.

On the other hand, the capacity of certain persistent and emerging organic pollutants to be bioaccumulated and to induce lethal toxicity in living organisms even at low concentrations results in loss of biodiversity and presents a threat to human health.

In an attempt to protect the quality of water bodies against the risk of non-controlled disposal of organic pollutants, specific legislation such as the European Water Framework Directive (WFD, 2000/60/EC) [2] are currently in force in the European Union. Ecotoxicity studies have resulted in an increasing number of chemicals being banned but new chemicals are also constantly being synthesized and introduced to the market. The so-called emerging contaminants are a large, relatively new group of as yet unregulated compounds constituted by among others, pharmaceuticals, personal care products, plasticizers, surfactants and herbicides about which there is relatively little information in relation to their ecotoxicological effects [3].

Agriculture and industrial activities are the main contributors to the contamination of surface and ground waters, by direct discharge, runoff and lixiviation of the compounds. As conventional wastewater treatment plants (WWTPs) are only designed for the elimination of organic matter, their effluents are another source of entry of contaminants into aquatic environments. Reclamation and reuse of water are an increasing priority in water resource management due to the limitation of water

supplies [4, 5]. However, the presence of contaminants in these waters is potentially a major problem. According to Spanish legislation [6], the reuse of treated waste water is allowed for agricultural and municipal irrigation, industrial purposes and environmental aims, such as forest irrigation or aquifer recharge. Provided that this water meets the minimum quality criteria determined for each particularly use. Nevertheless, this legislation fails to take into account contamination by organic micropollutants. The determination of organic contaminants, both regulated and emerging contaminants, is complicated by the great variety of compounds which need to be considered and the trace level at which they are present. However, it is necessary to control compounds that are known to pose a health risk and those called emerging contaminants, whose potential for harm still remain to be determined.

Nutrients and organic compounds in waters are normally determined by chromatographic techniques and usually a preconcentration step is required when the compounds are present at trace and ultra-trace concentration levels.

This chapter is divided into two different parts: in the first part the physicochemical properties and characteristics of phosphorus compounds and organic pollutants are described as well as their distribution in different environmental compartments, especially water. In the second part, the most frequently used analytical methodologies in determining the compounds of the interest in this thesis are discussed.

1.1 NUTRIENTS

The term nutrient refers to chemical species that are actively taken up by organisms and used to maintain their functions (life, growth and reproduction). They can be divided into two groups: macro-nutrients (e.g. nitrogen, phosphorus and oxygen) and micro-nutrients (e.g. cobalt, fluorine and iodine). This thesis focuses on the development of analytical methodologies for the speciation of inorganic phosphorus compounds. **Table 1.1** shows the list of studied compounds.

1.1.1 Inorganic phosphorus compounds

Phosphorus (P) is a highly reactive, non-metallic element that is an essential nutrient for many living organisms and plays a vital role in cell physiology and biochemistry. It is frequently the growth limiting element in freshwater, coastal, and estuarine systems [7]. Phosphorus can be found in mineral, organic (such as phospholipids, nucleic acids and proteins), gaseous, particulate and colloidal forms as well as in the form of dissolved species (DRP, dissolved reactive phosphorus) [8-12]. Inorganic phosphorus can exist in five different oxidation states (+V, +III, +I, 0, −III) and DRP is widely believed to be fully oxidised phosphorus (orthophosphate) although reduced forms of phosphorus, including trivalent (III) phosphite and univalent (I) hypophosphite are both potential contributors to measured DRP concentrations. Recent biochemical evidence suggests that reduced forms of phosphorus (phosphite and hypophosphite) may, like orthophosphate, provide living organisms with nutrients. A full understanding of the dynamics of phosphorus in the natural environment requires expanded knowledge of its oxidation-reduction (redox) cycle. Phosphorus oxoanions are present in the environment as both simple and condensate compounds.

1.1.1.1 Simple phosphorus oxoanions

In this group, we include orthophosphate, phosphite and hypophosphite, which are the specific compounds that are studied in this thesis.

Orthophosphate [P(V)], or orthophosphoric acid when it is in a protonated form, is the most stable phosphorus compound and is assumed to be the predominant form in the environment. Although orthophosphate is considered to be the only form that can be uptaken by plants, reduced phosphorus species, trivalent phosphite and univalent hypophosphite have also been detected in sewage treatment plants and anaerobic sediments [8].

Phosphite [P(III)], or phosphorous acid when it is protonated, is widely used as a fungicide and fertilizer as it is an indirect source of phosphorus for plants. It is more efficient than phosphate due to its greater mobility and solubility (1000 times higher

at similar pH and temperature) in the soil, reducing the energetic and economic costs [7, 13]. Other researchers have demonstrated that phosphite can be transformed to biologically available phosphorus through biological or abiotic pathways [14-16]. Phosphite is also used as a fumigant because it is toxic to insects at relatively low concentrations.

Hypophosphite [P(I)], or hypophosphorous acid when protonated, is used as a flame retardant [17]. Like phosphite, it is thought that it can be transformed into orthophosphate. **Figure 1.1** shows where the different phosphorus species are found in the environment.

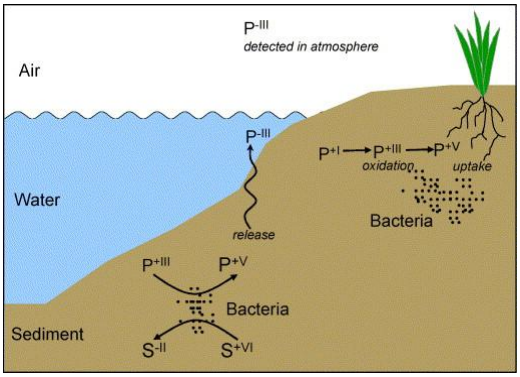


Figure 1.1 The different environmental compartments where phosphorus species are found. (From reference [8])

Figure 1.2 is a graphical representation of the equilibrium between chemical species both as a function of pH and redox potential (Eh or pE). The pH of natural waters can range from 4 to10 and can have Eh values of between +1000 and −600mV. In these conditions, the thermodynamic stable species are $H_2PO_4^-$ and HPO_4^{2-}, and therefore based on equilibrium considerations reduced phosphorus species are unlikely to be found in natural waters. However, they have been detected in sewage plants and sediments. Furthermore, standard solutions of phosphorus in the +III (phosphite) and +I (hypophosphite) oxidation states are stable for at least 15 days in aerobic laboratory conditions and with high Eh [9]. In some acidic aquatic environments such as those associated with acid mine drainage, the fully protonated form of phosphite, H_3PO_3, is thermodynamically stable. Therefore, reduced

phosphorus species could be found in certain environmental conditions and should be considered in speciation analysis.

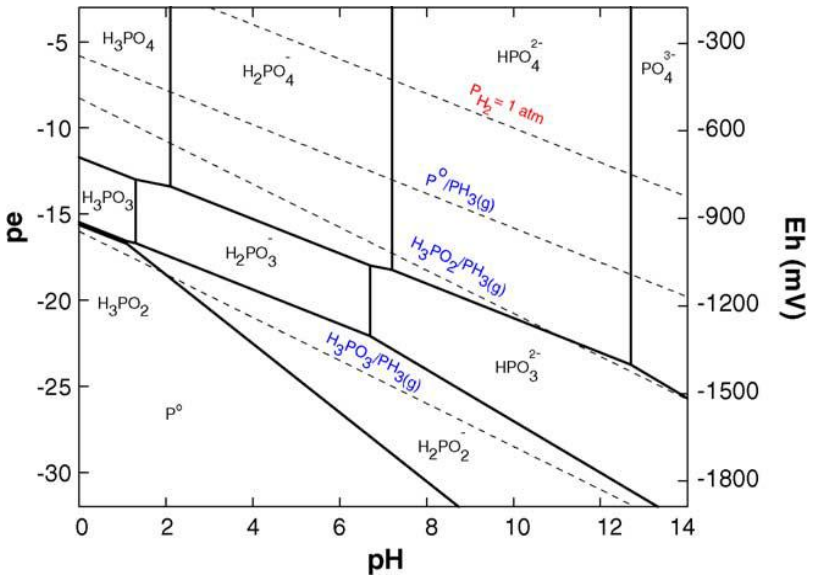


Figure 1.2 Eh/pE-pH diagram for phosphorus species in water. For calculations involving equilibrium between reduced P species and phosphine (i.e., dashed lines including PH_3), an equilibrium concentration of 10^{-6} M for the reduced compound was assumed. (From reference [8]).

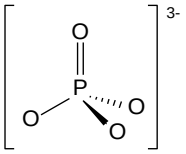
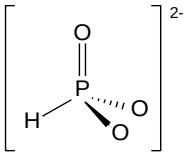
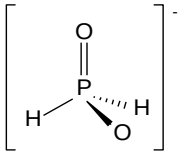
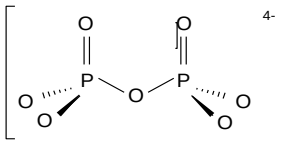
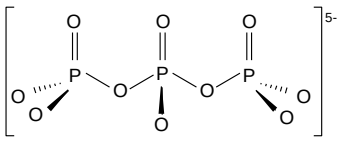
1.1.1.2 Condensed phosphorus oxoanions

A class of phosphate oligomers, generally referred to as condensed phosphate, are important compounds that are commercially produced for a variety of uses (e.g., detergent builders, preservatives, sequestering agents, acidulants) [18]. These include, among others, pyrophosphate and tripolyphosphate, both with a phosphorus oxidation state of +5. Although all these species can produce orthophosphate by hydrolysis, the kinetics of the hydrolysis is compound dependent.

Pyrophosphate has also a biological origin as it is generated in mammalian cells and regulates intracellular functions and extracellular crystal deposition inhibiting the formation of calcium oxalate and hydroxyapatite [19]. Pyrophosphate and phytic acid (myoinositol hexaphosphate) are considered to be natural inhibitors of urolithiasis [11].

Hence, there is a growing interest in the development of speciation methodologies of simple and condensed phosphorus oxoanions in environmental, biological and food samples to establish the distribution and bioavailability of the different species [10-12] as well as to establish the phosphorus cycle.

Table 1.1 Structures of the selected phosphorus oxoanions.

COMPOUND NAME	OXIDATION STATE	FORMULA	STRUCTURE	PKA	REFERENCE
Orthophosphate (phosphoric acid)	+5	PO_4^{3-}		2.2 7.2 12.2	[20]
Phosphite (phosphorous acid)	+3	HPO_3^{2-}		1.5 6.8	[20]
Hypophosphite (hypophosphorous acid)	+1	$H_2PO_2^-$		1.3	[20]
Pyrophosphate	+5	$P_2O_7^{4-}$		0.9 2.3 6.7 9.4	[20]
Tripolyphosphate	+5	$P_3O_{10}^{5-}$		0.5 1.0 2.4 6.6 9.4	[20]

1.2 ORGANIC MICROPOLLUTANTS

Micropollutants are toxic, persistent and bioaccumulative substances that can be both of synthetic or natural origin. Despite their ubiquity in the environment, current WWTPs are not specifically designed to eliminate them and many of these compounds are returned to aquatic environments affecting both ecosystems and human health [21].

Micropollutants are commonly present in waters at trace concentrations ranging from a few ng L^{-1} to several µg L^{-1}. Therefore, there is a need to develop sensitive analytical methods to monitor all these compounds, which is one of the aims of this thesis. To this end, we have developed different analytical methodologies to determine endocrine disrupting compounds including brominated flame retardants, pharmaceuticals and pesticides.

1.2.1 Endocrine disrupting compounds

Endocrine disrupting compounds (EDCs) are natural or synthetic compounds that alter the functioning of the endocrine system in humans or animals, often through mimicking or blocking endogenous hormones. Specifically, they interfere with the ability of the body to regulate growth, development and metabolism, among other functions. EDCs can contribute to a wide range of diseases, including obesity, diabetes, cancer, heart disease and reproductive health problems [22]. Various types of natural and synthetic chemical compounds with different functionalities have been identified as EDCs and can be classified into several categories [23, 24]:

- Hormones (natural and synthetic estrogens or steroids)
- Pharmaceuticals and personal care products
- Industrial chemicals
- Pesticides
- Combustion by-products
- Surfactants

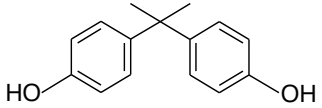
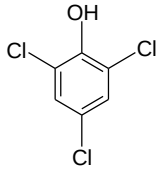
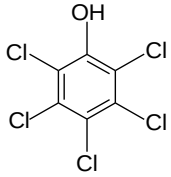
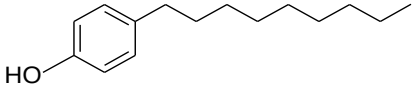
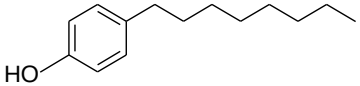
The group of the EDCs that have industrial applications includes phenols, alkylphenolic compounds, polybrominated diphenyl ethers and phthalates among others. In this section we will first focus on phenols and alkylphenolic compounds before discussing polybrominated diphenyl ethers (PBDEs).

Phenols and alkylphenols are compounds that contain a hydroxyl group bonded to a benzene ring, which differ in their substituents (e.g. halogens, alkyl groups, etc.). Phenolic compounds occur as natural and synthetic compounds and are involved in many industrial processes, such as the manufacture of plastics, resins, surfactants, drugs and antioxidants, and used as preservatives and disinfectants. Phenols are also breakdown products from natural organic compounds such as humic substances, lignins and tannins. Phenol and substituted phenols are considered to be priority pollutants even when they are present at low concentrations [25, 26]. In this thesis, five phenolic compounds have been studied: bisphenol A, 2,4,6-trichlorophenol, pentachlorophenol, 4-*n*-nonylphenol and 4-*n*-octylphenol. Their structures are summarised in **Table 1.2**.

1.2.1.1 Bisphenol A

Bisphenol A (BPA), 2,2-bis(4-hydroxyphenyl)propane, is one of the most studied EDCs. It is a plasticizer used in the manufacturing of polycarbonate plastics as well as epoxy resins from which a wide variety of products are generated such as the packaging of food and drink, dental sealants, baby-milk bottles, powder paints and optical lenses [27]. Its world-wide production is estimated to be at 2 x 10^6 metric tons [28]. The main way of exposure to BPA comes from drinking water in industrialised regions but it can also leach out of containers into the food or beverage which is then a source for human exposure. Consequently, it is present in a great variety of matrices and is under review as a candidate to be included in the priority substances list of the European Union [26].

Table 1.2 Structures of the selected phenolic compounds.

COMPOUND NAME	FORMULA	STRUCTURE	MOLECULAR WEIGHT	pK_A	LOG K_{OW}	REFERENCE
Bisphenol A	$C_{15}H_{16}O_2$		228.3	9.8 10.4	4.04	[29, 30]
2,4,6-Trichlorophenol	$C_6H_3Cl_3O$		197.4	6.23	3.48	[30, 31]
Pentachlorophenol	C_6HCl_5O		266.3	4.9	4.69	[29, 30]
4-*n*-nonylphenol	$C_{15}H_{24}O$		220.4	7.2	5.74	[29, 30]
4-*n*-octylphenol	$C_{14}H_{22}O$		206.3	10.31	5.30	[30]

1.2.1.2 Trichlorophenol and pentachlorophenol

The main pathways for the introduction of chlorophenols into the environment are through agricultural and industrial activities. Although some chlorophenols are now banned, others are still widely used as fungicides, biocides, pesticides, preservatives, and disinfectants. They can also be formed during the degradation of some pesticides and triclosan (a bactericide product), and during the chlorination of drinking water. Moreover, the use of chlorine in pulp and paper mills leads to the formation of chlorophenols, which can be found in the effluents of these factories [25, 32, 33]. Chlorophenols, which accumulate in the environment , have a toxicity that depends on the pH and the number of chlorine atoms in the molecule, with pentachlorophenol being the most toxic [34]. 2,4,5-trichlorophenol, 2,4,6-trichlorophenol and pentachlorophenol have been classified by the World Health Organization (WHO) as compounds suspected of having carcinogenic properties [32].

For these reasons, we have selected two of them (2,4,6-trichlorophenol (TCP) and pentachlorophenol (PCP)) as target compounds to be determined in water. Moreover, pentachlorophenol is included as a priority pollutant in the WFD 2008/105/EC [26].

1.2.1.3 Nonylphenol and octylphenol

Alkylphenol ethoxylates (APEs) are non-ionic surfactants that have been widely used for over 40 years in domestic and industrial applications, primarily in the manufacture of plastics, elastomers, agrochemicals, paper and pulp. These compounds enter into the environment mainly through the effluents from urban and industrial wastewater treatment plants [35]. Their structure consists of a non-polar hydrocarbonate chain and a polar ethoxy unit attached to an aromatic ring located in the *para* position. Among APEs, nonylphenol ethoxylates represent approximately 80% of the total worldwide production and octylphenol ethoxylates most of the remaining 20% [36]. APEs are biodegraded by removal of ethoxy groups to alkylphenols (AP), which are more lipophilic and toxic than their parent compounds [27, 37]. Nonylphenols (NP) and octylphenols (OP) are APs that have been reported

to bioaccumulate in the lipids of aquatic organisms and so have been included in the European Union list of priority substances to be monitored in surface waters [26].

1.2.2 Brominated flame retardants

Flame retardants (FRs) are a diverse group of chemicals, mainly based on bromine, chlorine or phosphorus compounds, which are found in a broad range of commercial products including children's sleepwear, televisions, computers, plastics, electronics, building insulation, and furniture. These compounds can be added to polymers without being chemically bound to them or bonded through a chemical reaction. Therefore, those FRs that are simply additives can migrate out of the product over time reaching the environment. In the case that FRs are chemically bound to the polymers, their non-reacted residues can also be released into the environment.

Polybrominated organic compounds represent about 25% of the chemicals used as FRs in plastic materials. Most of them have shown to be persistent, lipophilic, and bioaccumulative in animals and humans and have been found in locations far from where they were originally produced or used [38].

Brominated flame retardants (BFRs) can be either aromatic, aliphatic or cycloaliphatic compounds and can contain between 50% and 85% of bromine by weight. Among the 75 different commercially produced classes of BFRs [39], polybrominated diphenylethers, tetrabromobisphenol A (TBBPA-A) and hexabromocyclododecanes (HBCDs) stereoisomers are the most frequently used and those which have been most studied. PBDEs exist in a theoretical variety of 209 congeners, containing between 1 and 10 bromine atoms. They are the most commonly detected BFR group in food and environmental samples due to their widespread use as additives [40].

The commercial technical mixtures of PBDEs (penta-BDE and octa-BDE mix), used in plastics, polyurethane foam and adhesives, have been banned for European markets since 2004 and deca-BDE mix have been banned since 2008 [41-44].

Moreover, PBDEs are listed as priority substances within the European Union WFD [26]. In spite of the hazard that the use of these compounds represents for human

health and the environment, the global consumption of BFRs increased to 410,000 tonnes in 2008 [39]. Due to the necessity to meet fire safety standards on commercial products, new FRs have recently been introduced in the market. These novel FRs, also called non-PBDE FRs, include 2-ethylhexyl-2,3,4,5-tetrabromobenzoate (EHTBB) and bis(2-ethylhexyl)-3,4,5,6-tetrabromo-phthalate (DEHTBP) [45, 46]. Dechlorane plus (DP) can also be considered a non-PBDE FR because, despite being a chlorinated FR that has been used for over 40 years, it has only recently been identified as a relevant pollutant of the environment and biota [47]. In this thesis, an analytical methodology for the determination of nine PBDEs (BDE-47, BDE-85, BDE-99, BDE-100, BDE-153, BDE-154, BDE-183, BDE-197 and BDE-209) and seven non-PBDEs FRs (EHTBB, DBDPE, DEHTBP, BTBPE, TTBPP and the two isomers of DP) in water samples is presented. **Table 1.3** shows the structure of the selected FRs.

Table 1.3 Structures of the selected flame retardants.

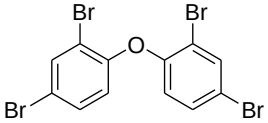
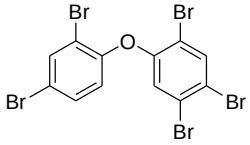
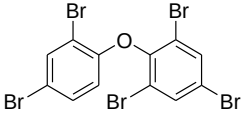
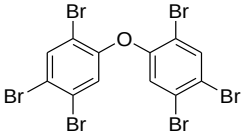
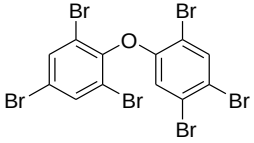
COMPOUND NAME	FORMULA	STRUCTURE	MOLECULAR WEIGHT	LOG K_{OW}	SOLUBILITY (g L^{-1})	REFERENCE
2,2',4,4'-tetrabromodiphenyl ether (BDE-47)	$C_{12}H_6Br_4O$		485.8	6.7	2.5×10^{-4}	[46]
2,2',4,4',5-pentabromodiphenyl ether (BDE-99)	$C_{12}H_5Br_5O$		564.7	7.3	6.2×10^{-5}	[46]
2,2',4,4',6-pentabromodiphenyl ether (BDE-100)	$C_{12}H_5Br_5O$		564.7	7.1	7.3×10^{-5}	[46]
2,2',4,4',5,5'-hexabromodiphenyl ether (BDE-153)	$C_{12}H_4Br_6O$		643.6	7.9	1.6×10^{-5}	[46]
2,2',4,4',5,6'-hexabromodiphenyl ether (BDE-154)	$C_{12}H_4Br_6O$		643.6	7.7	1.9×10^{-5}	[46]

Table 1.3 Structures of the selected flame retardants. (continued)

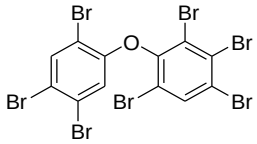
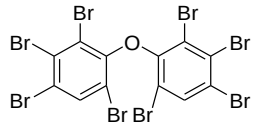
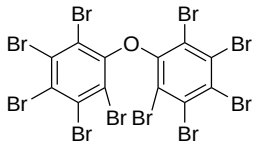
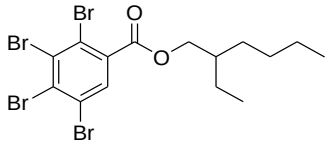
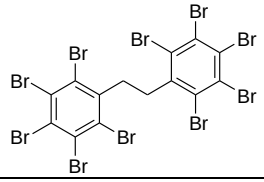
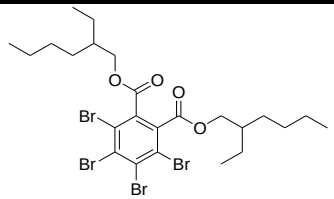
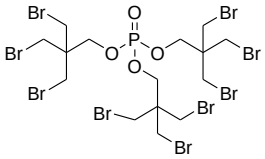
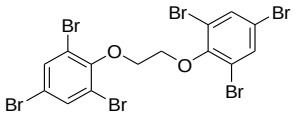
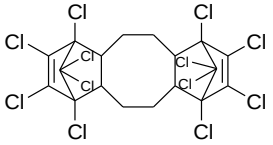
COMPOUND NAME	FORMULA	STRUCTURE	MOLECULAR WEIGHT	LOG K_{OW}	SOLUBILITY ($g\ L^{-1}$)	REFERENCE
2,2',3,4,4',5',6-heptabromodiphenyl ether (BDE-183)	$C_{12}H_3Br_7O$		722.5	8.2	5.6×10^{-6}	[46]
2,2',3,3',4,4',6,6'-octabromodiphenyl ether (BDE-197)	$C_{12}H_2Br_8O$		801.4	8.4	1.9×10^{-6}	[30, 48]
Decabromodiphenyl ether (BDE-209)	$C_{12}Br_{10}O$		959.2	9.5	1.4×10^{-7}	[46]
2-ethylhexyl-2,3,4,5-tetrabromobenzoate (EHTBB)	$C_{15}H_{18}Br_4O_2$		549.9	7.28	1.6×10^{-4}	[46]
Decabromodiphenyl ethane (DBDPE)	$C_{14}H_4Br_{10}$		971.2	11.7	9.5×10^{-8}	[46]

Table 1.3 Structures of the selected flame retardants. (continued)

COMPOUND NAME	FORMULA	STRUCTURE	MOLECULAR WEIGHT	LOG K_{OW}	SOLUBILITY ($g\ L^{-1}$)	REFERENCE
Bis (2-ethylhexyl)-3,4,5,6-tetrabromo-phthalate (DEHTBP)	$C_{24}H_{34}Br_4O_4$		706.1	9.3	3.3×10^{-6}	[46]
Tris(tribomoneopenthyl)phosphate (TTBPP)	$C_{15}H_{24}Br_9O_2$		1018.5	7.31	8.0×10^{-6}	[30, 48]
1,2-bis(2,4,6-tribromophenoxy)ethane (BTBPE)	$C_{14}H_8Br_6O_2$		687.6	8.3	1.6×10^{-5}	[46]
Bis(hexachlorocyclopentadien) cyclooctane (DP)	$C_{18}H_{12}Cl_{12}$		653.7	9.1	4.4×10^{-8}	[30, 48]

1.2.3 Pharmaceuticals

Pharmaceuticals are chemicals used for the diagnosis, prevention and treatment of diseases in humans and animals. The production of pharmaceutical substances for human and veterinary use is of several thousand tonnes per year worldwide. In the EU, about 4000 pharmaceutical active compounds are currently in use. These compounds have a wide variety of structures, functional groups, and physicochemical properties [49, 50].

They are commonly excreted, via urine and faeces, as parent compounds and metabolites and are continuously discharged into domestic wastewaters. For example, a diuretic such as hydrochlorothiazide is mainly excreted unchanged (90-95%) but only about 50% of the lipid regulator bezafibrate is excreted as the parent compound [51]. Given that conventional WWTP are not designed to completely remove these pollutants, they have become crucial emerging contaminants and their presence in environmental waters involves possible oestrogenic and ecotoxicological effects [52-54].

Pharmaceuticals can be classified into different groups depending on their use including analgesics and anti-inflammatories, anti-epileptics, lipid regulators, X-ray contrast agents, β-blockers, antibiotics and hypertension regulators.

1.2.3.1 Analgesics and anti-inflammatories

Analgesics are used to alleviate pain by acting on the peripheral and central nervous systems. Analgesics are divided into narcotics, non-narcotics and non-steroidal anti-inflammatory drugs (NSAIDs) [55]. Diclofenac and ibuprofen, the second anti-inflammatory drug most consumed in Spain [56], are NSAIDs. They are both anionic compounds and are commonly detected in waters.

Metamizole or dipyrone is a potent analgesic and antipyretic, included in the non-narcotic group. During its metabolisation, dipyrone is rapidly hydrolyzed to 4-methylaminoantipyrine (4-MAA), which is further transformed to 4-formylaminoantipyrine (4-FAA), 4-aminoantipyrine (4-AA) and 4-acetylaminoantipyrine (4-AAA). These four main metabolites represent ~60% of the

administered dose excreted in urine [57]. All of them are neutral compounds. Dipyrone is banned in many countries of the European Union; however it is still commercialized in Spain [56].

1.2.3.2 Anti-epileptics

Anti-epileptic drugs are a diverse group of pharmaceuticals used in the treatment of epilepsy, neuropathic pain and bipolar disorder as they can also act as mood stabilizers. Carbamazepine and lamotrigine, neutral compounds, and gabapentin, an anionic compound, are some of the anti-epileptic drugs most commonly detected in waters [58, 59].

1.2.3.3 Lipid regulators

Lipid regulators are drugs used to prevent high blood cholesterol and other cardiovascular problems. Bezafibrate, an acidic pharmaceutical drug, is extensively used as a lipid regulator with an estimated annual consumption in developed countries of several hundred of tonnes [60]. This compound has been detected in different kind of waters as it is persistent [51, 61, 62].

1.2.3.4 X-ray contrast agents

X-ray contrast agents are chemical compounds used in X-ray based imaging techniques such as computed tomography and radiography. Nowadays, the most commonly used are iodinated X-ray contrast media (ICM) which are derivatives of 2,4,6-triiodobenzoic acid. ICM can be divided in ionic and non-ionic, depending on their side chain functional moieties. For example, amidotrizoic acid is negatively charged at pH 7, while iohexol, iomeprol or iopamidol are uncharged at the same pH [63]. The worldwide consumption of ICM is around 3000 tonnes/year [64]. Normal doses are up to 200 g per application, and the unmetabolised compound is excreted within 24 hours. ICM compounds are not eliminated in wastewater treatment plants due to their stability and hydrophilic character and, hence, they are continuously discharged into the aquatic environment [63-65].

1.2.3.5 β-blockers

β-blockers are a class of drugs that block the action of stress hormones such as epinephrine (adrenaline) and norepinephine (noradrenaline) by interfering with the binding to their receptors and weakening their effects.

Metoprolol and Sotalol are examples of β-blockers that are prescribed to treat hypertension and cardiac arrhythmias, respectively. Sotalol is mainly excreted as parent compound via urine (75 - 80%) but in faeces, the percentage of unmetabolised compound is reduced to a 12%. In the case of metoprolol, only a low percentage (3 - 10%) of this drug is excreted unchanged [66]. As these compounds are cationic and soluble in water, they have been detected in environmental waters [3, 61, 67].

1.2.3.6 Antibiotics

An antibiotic is an agent that inhibits or abolishes the growth of microorganisms, such as bacteria, fungi, or protozoa. Antibiotics are one of the most consumed pharmaceuticals worldwide and are classified according to their chemical structure, as β-lactams, macrolides, fluoroquinolones, aminoglycosides, sulfonamides and tetracyclines [55]. The degree of antibiotic metabolisation ranges from 10% to 90% depending on the compound. As the metabolised compounds are often more water soluble than the parent compounds [68], and they are continuously introduced into the environment, antibiotics and their metabolites are frequently detected in water samples. Sulfamethoxazole, a sulphonamide bacteriostatic antibiotic, have been found in different water matrices [21, 69, 70]. Acetyl-sulfamethoxazole, its major human metabolite, represents more than 50% of an administered sulfamethoxazole dose in human excretion and is more toxic to humans than the parent compound [68]. The concentration of this metabolite in WWTP influents is 2.5 - 3.5 times higher than the parent compound [71].

1.2.3.7 Hypertension regulator

Anti-hypertensive are a class of drugs used to treat the high blood pressure. Several classes of antihypertensive drugs: diuretics, anti-adrenergics, direct-acting

vasodilators, calcium-channel blockers, angiotensin-converting–enzyme inhibitors, and angiotensin-receptor blockers can be used. Diuretics that act by inhibiting the ability of kidney to retain unnecessary water and salts are the oldest and least expensive class of drugs used to treat hypertension. Hydrochlorothiazide, a diuretic drug, is a neutral compound, frequently found in water samples at high ng L^{-1} concentrations [51, 61, 72].

1.2.4 Pesticides

Pesticides are chemical substances or biological agents used in agriculture to control pests. All chemical pesticides share the common property of blocking a vital metabolic process of the organisms to which they are toxic. The different classes of pesticides, based on target organisms, are [73]:

- Acaricide
- Algicide
- Avicide
- Bactericide
- Disinfectant
- Fungicide
- Herbicide
- Insecticide
- Larvicide
- Molluscicide
- Nematicide
- Piscicide
- Rodenticide

Among them, herbicides, insecticides, and fungicides represent the most used categories. Insecticides could be subdivided according chemical families in: organochlorides (e.g. DDT), organophosphates and carbamates. In the case of

herbicides, the subgroups are: phenoxy and benzoic acid herbicides (e.g. pentachlorophenol), triazines (e.g. Atrazine), ureas (e.g. Diuron) and chloroacetanilides (e.g. metolachlor). However, some extensively used herbicides as Glyphosate are not included in this classification [73].

Intensive agricultural practices have highly increased the consumption of pesticides resulting in their dispersion in all environmental compartments. The medium and high polarity and thermal stability of modern pesticides facilitated their distribution in the aquatic compartment [74, 75]. Consequently, several pesticides have been include in the list of the European WFD of priority substances to be monitored [26]. Nine herbicides and five of their metabolites have been studied in this thesis: isoproturon, chloridazon, atrazine, desethylatrazine, simazine, terbuthylazine, metazachlor, metazachlor-C-metabolite, metazachlor-S-metabolite, metolachlor, metolachlor-C-metabolite, metolachlor-S-metabolite, chlortoluron and diuron.

1.2.5 Corrosion inhibitors

Corrosion inhibitors refers to a group of chemical compounds that are used to prevent corrosion of metallic surfaces through the formation of a coating, often a passivation layer, which prevents the access of the corrosive substance to the metal. Benzotriazoles are one of the classes of corrosion inhibitors more highly produced, 9000 tonnes/year, as they have broad applications in various industrial processes [76]. The main representative compounds of this group are 1H-benzotriazole and tolytriazole, a mixture of the isomers 4- and 5-methyl-1H-benzotriazole. They are widely used as anticorrosives (e.g. in aircraft deicing fluids, automotive antifreeze formulations, brake fluids, metal-cutting fluids, dishwashing agents), printing inks, and as UV-light stabilizer in plastics [77]. 1H-benzotriazole and tolytriazole are soluble in water and present resistant to biodegradation. They are partially removed in wastewater treatment plant, allowing them to be found in aquatic environments [78, 79].

Table 1.4, Table 1.5 and Table 1.6 summarise the main physicochemical properties of the selected pharmaceutical, pesticides and corrosion inhibitors compounds.

Table 1.4 Structures of the selected pharmaceutical compounds.

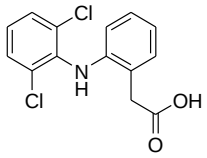
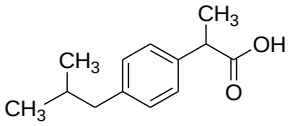
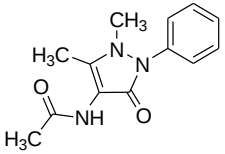
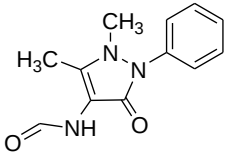
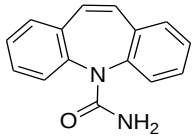
COMPOUND NAME	FORMULA	STRUCTURE	MOLECULAR WEIGHT	pK_A	LOG K_{OW}	REFERENCE
Diclofenac	$C_{14}H_{11}C_{12}NO_2$		296.2	4.2	4.3	[80, 81]
Ibuprofen	$C_{13}H_{18}O_2$		206.3	4.9	3.8	[30, 80]
N-acetyl-4-aminoantipyrine (4-AAA)	$C_{13}H_{15}N_3O_2$		245.3	12.5	0.2	[30, 81]
N-formyl-4-aminoantipyrine (4-FAA)	$C_{12}H_{13}N_3O_2$		231.3	12.6	0.1	[30, 81]
Carbamazepine	$C_{15}H_{12}N_2O$		236.3	13.9	2.5	[80, 82]

Table 1.4 Structures of the selected pharmaceutical compounds. (continued)

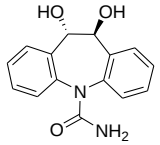
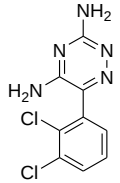
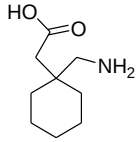
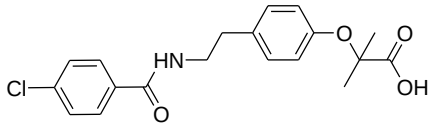
COMPOUND NAME	FORMULA	STRUCTURE	MOLECULAR WEIGHT	pK_A	LOG K_{OW}	REFERENCE
10,11-dihydro-10,11-dihydroxy-carbamazepine (10,11-DHC)	$C_{15}H_{14}N_2O_3$		270.3	12.8	0.8	[30, 81]
Lamotrigine	$C_9H_7Cl_2N_5$		256.1	5.3	2.6	[83]
Gabapentin	$C_9H_{17}NO_2$		171.2	4.6 9.9	-1.3	[30]
Bezafibrate	$C_{19}H_{20}ClNO_4$		361.8	3.6	4.3	[80, 83]

Table 1.4 Structures of the selected pharmaceutical compounds. (continued)

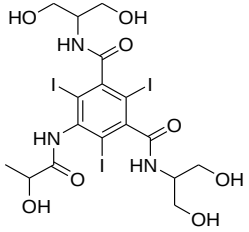
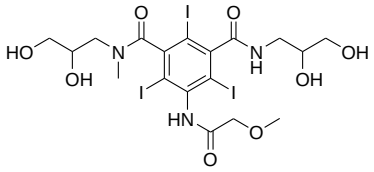
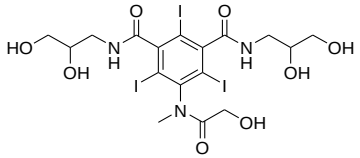
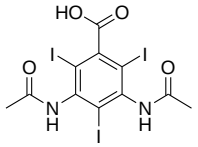
COMPOUND NAME	FORMULA	STRUCTURE	MOLECULAR WEIGHT	pK_A	LOG K_{OW}	REFERENCE
Iopamidol	$C_{17}H_{22}I_3N_3O_8$		777.1	11 12 12.9	-2.1	[30, 69]
Iopromide	$C_{18}H_{24}I_3N_3O_8$		791.1	11 12.2	-0.4	[30]
Iomeprol	$C_{17}H_{22}I_3N_3O_8$		777.1	11.7 12.6 13.3	-3.1	[69]
Amidotrizoic acid	$C_{11}H_9I_3N_2O_4$		613.9	2.1 11.8 12.5	2.9	[30]

Table 1.4 Structures of the selected pharmaceutical compounds. (continued)

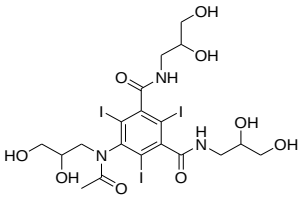
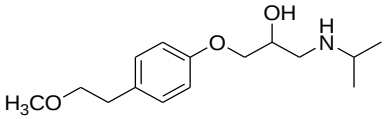
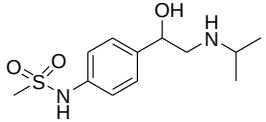
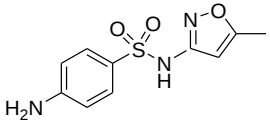
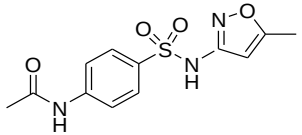
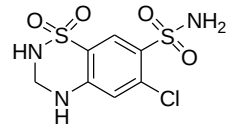
COMPOUND NAME	FORMULA	STRUCTURE	MOLECULAR WEIGHT	pK_A	LOG K_{OW}	REFERENCE
Iohexol	$C_{19}H_{26}I_3N_3O_9$		821.1	11.7 12.6	-4.2	[69]
Metoprolol	$C_{15}H_{25}NO_3$		267.4	9.6	1.9	[83]
Sotalol	$C_{12}H_{20}N_2O_3S$		272.4	9.4 10.1	-0.4	[30]
Sulfamethoxazole	$C_{10}H_{11}N_3O_3S$		253.3	1.8 5.6	0.9	[80, 83]
Acetyl-sulfamethoxazole	$C_{12}H_{13}N_3O_4S$		295.3	0.4 5.9 13.9	0.9	[30]
Hydrochlorothiazide	$C_7H_8ClN_3O_4S_2$		297.7	9.1 9.8 11.3	-0.6	[30]

Table 1.5 Structures of the selected corrosion inhibitors compounds.

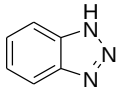
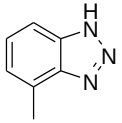
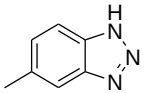
COMPOUND NAME	FORMULA	STRUCTURE	MOLECULAR WEIGHT	pK_A	LOG K_{OW}	REFERENCE
1H-benzotriazole	$C_6H_5N_3$		119.1	8.6	1.2	[78]
4-methylbenzotriazole	$C_7H_7N_3$		133.1	8.9	1.9	[78]
5-methylbenzotriazole	$C_7H_7N_3$		133.1	8.9	1.9	[78]

Table 1.6 Structures of the selected pesticides compounds.

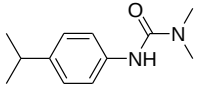
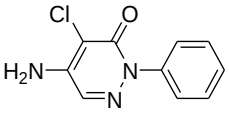
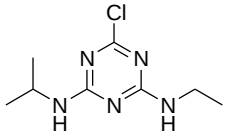
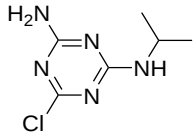
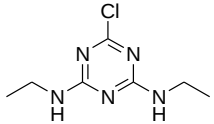
COMPOUND NAME	FORMULA	STRUCTURE	MOLECULAR WEIGHT	pK_A	LOG K_{OW}	REFERENCE
Isoproturon	$C_{12}H_{18}N_2O$		206.3	13.8	2.6	[30]
Chloridazon	$C_{10}H_8ClN_3O$		221.6	-	1.1	[30]
Atrazine	$C_8H_{14}ClN_5$		215.7	1.7	2.6	[75]
Desethylatrazine	$C_6H_{10}ClN_5$		187.6	2.1	1.5	[69]
Simazine	$C_7H_{12}ClN_5$		201.7	1.6	2.2	[75]

Table 1.6 Structures of the selected pesticides compounds. (continued)

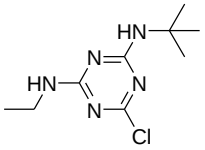
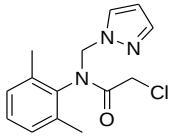
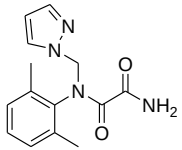
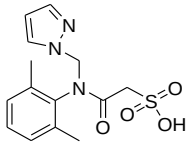
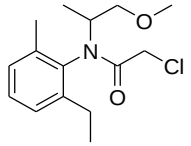
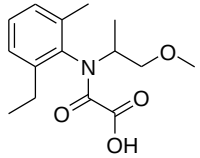
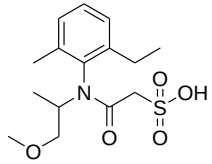
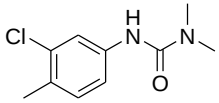
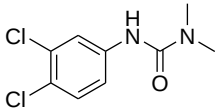
COMPOUND NAME	FORMULA	STRUCTURE	MOLECULAR WEIGHT	pK_A	LOG K_{OW}	REFERENCE
Terbuthylazine	$C_9H_{16}ClN_5$		229.7	3.2	2.5	[30]
Metazachlor	$C_{14}H_{16}ClN_3O$		277.7	1.8	2.9	[30]
Metazachlor-C-metabolite (N-(2,6-dimethylphenyl)-N-(1H-pyrazol-1-ylmethyl)oxalamide)	$C_{14}H_{16}N_4O$		272.3	1.8 12.9	1.6	[30]
Metazachlor-S-metabolite (N-(2,6-dimethylphenyl)-N-(1H-pyrazol-1-ylmethyl)aminocarbonylmethyl-sulfonic acid)	$C_{14}H_{17}N_3O_4S$		323.3	1.8 13.6	0.4	[30]

Table 1.6 Structures of the selected pesticides compounds. (continued)

COMPOUND NAME	FORMULA	STRUCTURE	MOLECULAR WEIGHT	pK_A	LOG K_{OW}	REFERENCE
Metolachlor	$C_{15}H_{22}ClNO_2$		283.5	-	3.5	[30]
Metolachlor-C-metabolite (N-(2-Ethyl-6-methylphenyl)-N-(2-methoxy-1-methylethyl)oxalamic acid)	$C_{15}H_{21}NO_4$		279.3	3.3	2.9	[30]
Metolachlor-S-metabolite (N-(2-Ethyl-6-methylphenyl)-N-(2-methoxy-1-methylethyl)aminocarbonylmethylsulfonic acid)	$C_{15}H_{23}NO_5S$		329.4	13.7	2.1	[30]
Chlortoluron	$C_{10}H_{13}ClN_2O$		212.7	13.5	2.4	[30]
Diuron	$C_9H_{10}C_{l2}N_2O$		233.1	13.2	2.5	[30, 69]

1.3 ANALYTICAL METHODOLOGIES

As explained above, human and economic developments cause the introduction of tones of biologically active chemicals into the environment due to the extensive use of them in agricultural and industrial practices. On one hand, phosphorous compounds regulate trophic status of natural environments and thus, their monitoring in natural waters is essential. On the other hand, most of these chemicals and specially those listed as priority pollutants are hydrophobic and persistent in the environment and it has been demonstrated to be toxic for humans. Moreover, there are new compounds such as pharmaceuticals, personal care products, plasticizers, surfactants and herbicides that are dispersed in large amounts to the environment from domestic, commercial and industrial uses. Their characteristic is that they do not need to persist in the environment to cause negative effects because they are continually being released into the environment.

These emerging contaminants are not commonly monitored in the environment even though many of them have been identified as endocrine disrupting chemicals which can interfere with the normal functioning of the endocrine systems of many aquatic and terrestrial organisms. The presence of such anthropogenic chemicals in water resources can affect ecosystems as well as human health and, hence, there has been an increasing interest in the development of fast, accurate and sensitive analytical methodologies to determine regulated and emerging contaminants in water samples. In the case of regulated compounds, it is necessary to develop sensitive methods in order to meet the low detection limits required by legislations.

The complexity of matrices and the huge amount of different organic and inorganic pollutants, present at very low levels, are some of the main analytical challenges to detect and quantify these pollutants in environmental samples.

A complete analytical process, involving many steps, is normally carried out for the determination of pollutants. In general, the steps of an analytical process are show in **Figure 1.3**.

Figure 1.3 Diagram of an analytical process.

In this section, we will focus on two important steps of the analytical process: the sample treatment and the instrumental measurement. Moreover, the sample treatment will be exclusively focused on organic micropollutants.

1.3.1 Sample preparation techniques

Sample preparation is one of the most critical steps in the determination of trace pollutants in environmental matrices. The basic concept of sample-preparation methods is to convert a real matrix into a sample suitable for analysis and it represents two-thirds of the total analysis time [84]. Moreover, the main sources of error of the full analytical process are associated to this step.

The main goals of sample preparation method are:

- to remove potential interferences;
- to increase the concentration of an analyte;
- if necessary, to convert an analyte into a more suitable form;
- to provide a robust and reproducible method that is independent of variations in the sample matrix.

Another goal of the sample treatment is to have the sample and the analytes in the chemical and physical conditions required for instrumental analysis using the selected technique, e.g. sample solvent miscible in the mobile phase in liquid chromatography or derivatisation of polar or thermally unstable compounds for gas chromatography, etc. [85].

It is important to mention that a sample treatment step is not always necessary, especially with aqueous samples.

Current sample treatment developments in environmental analysis are focussed towards the automation of the analysis, the development of more selective or

specific methodologies and the use of lower amounts of solvents or non-solvents. The first two objectives are related to the improvement of the robustness of the entire analytical procedure.

Extraction and preconcentration of analytes from water samples was traditionally, performed by liquid-liquid extraction (LLE). Other techniques that have been extensively used are solid phase extraction (SPE) and solid phase microextraction (SPME). Recently, new alternative techniques such as stir bar sorptive extraction (SBSE) and rod extraction have been developed taking advantage of the SPME principles. In the following sections these extraction and preconcentration techniques will be discussed in detail.

1.3.1.1 Liquid-liquid extraction

LLE is based on the partition of the dissolved analytes between the aqueous sample and an immiscible organic solvent according to their partition coefficients. Despite its simplicity, some disadvantages such as the need for large volumes of organic solvents, most of them toxic; the formation of emulsions which favours the loss of analytes; the fact that it cannot be applied to highly polar compounds without a derivatisation step and finally it is difficult to automate.

LLE has been applied for the preconcentration of brominated flame retardants in water samples [86] or NP, OP and BPA in river water samples [87], using in both cases dichloromethane as extraction solvent. Nowadays, it is not commonly used.

In the last few decades it has become increasingly important to develop environmentally friendly techniques which minimise the use of organic solvents, as is the case of liquid phase microextraction [88] and dispersive or hollow fibre liquid phase microextraction [35, 89].

1.3.1.2 Solid phase extraction

SPE or liquid-solid extraction is based on the retention of the analytes on a sorbent (solid phase) which is filling a cartridge or deposited on a disk. The advantage of this technique is that it allows the extraction and preconcentration of the analytes to

be performed in one step. In addition, SPE is an easily automatable method. Other advantages of SPE over LLE are the high concentration factors achieved and a shorter sample preparation time.

The method of operation can be divided in five steps (**Figure 1.4**). Firstly, the sorbent is conditioned by applying a conditioning solution to the cartridge and the aqueous sample is then passed through the sorbent allowing the analytes to be trapped. A washing step is performed in order to eliminate matrix components. The loaded cartridge is sometimes dried with N_2 gas before performing analyte elution with small volumes of a solvent or a solvent mixture.

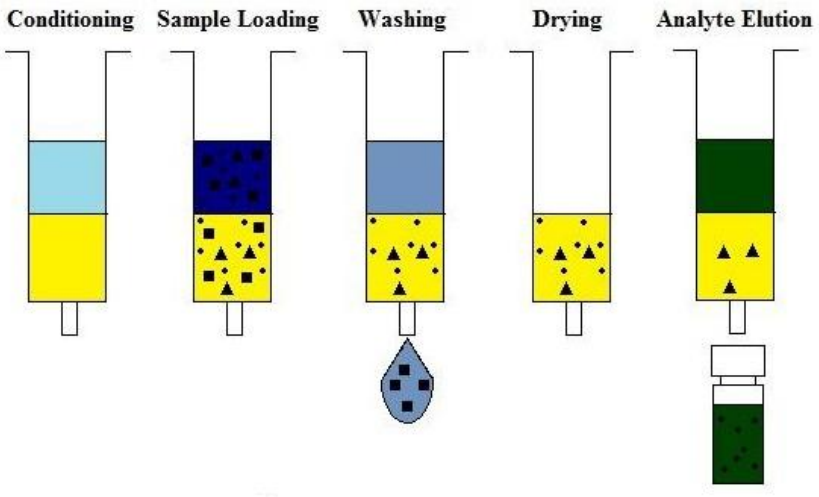


Figure 1.4 Steps in solid phase extraction.

In the development of a SPE method different parameters must be studied in order to obtain high extraction efficiencies. The choice of the sorbent is critical since it controls selectivity, affinity and capacity. Other parameters that can affect extraction are the chemical composition of the sample (pH, salts, etc), the washing solution and its volume and the solvent and elution volume.

SPE sorbents, mainly used for organic compounds, are silica based particles and can be divided into three classes [90, 91]:

- Normal phase sorbents: they have polar functional groups, e.g. cyano, amino and diol or unmodified silica.

- Reversed phase sorbents: they have non-polar functional groups, e.g. octadecyl, octyl and methyl.
- Ion exchange sorbents: having either cationic or anionic functional groups, which interact with opposite charged compounds.

Currently, there are different commercial sorbents that combine some of the classes mentioned above, as well as sorbents based on polymeric materials bearing different functional groups, immunosorbents and molecularly-imprinted polymers, among other new developments [92].

The limitations of SPE are that this technique requires a labour intensive method development and is time-consuming due to their multiple steps, which also difficult sample traceability and increase the probability of sample contamination [84]. SPE has extensively been used for the extraction and preconcentration of the organic micropollutants studied in this thesis from different environmental matrices. For example, SPE was applied in the extraction of phenolic compounds such as 2,4,6-TCP [93], TCP and PCP [94, 95], and TCP, PCP, NP and OP [96] from water samples. SPE was also applied to the extraction of BFR from water samples [97-99]. Moreover, SPE is generally the method of choice to extract different classes of analytes in multiresidue methodologies. Bono-Blay et al. used an Oasis HLB cartridge for the extraction of pesticides, BPA, NP and OP, together with other compounds [100] and López-Serna et al. determined 95 pharmaceuticals and transformation products by using SPE as the sample treatment method [72].

1.3.1.3 Solid phase microextraction

SPME is an extraction technique which, similar to SPE, is based on the equilibrium partitioning of the analytes between an aqueous or gaseous sample and a stationary phase coated on a fused silica fibre (**Figure 1.5**). The fibre is exposed to the sample either by direct immersion or headspace extraction. Liquid and thermal desorption can then be applied to desorb the analytes from the fibre. Therefore, SPME is a suitable technique for the treatment of liquid and gaseous samples, which requires the use of small amounts of organic solvents or none at all for elution. Depending on the nature of the target compound, several commercial coatings are available (**Table**

1.5), but polydimethylsiloxane (PDMS) which is a non-polar polymeric phase, is the most extensively used.

The limitations of this technique are short fibre lifetime, high cost, fragility, and carry-over effects [90, 101, 102]. SPME was applied to the extraction of PBDE from water samples [103, 104] and a polydimethylsiloxane/divinylbenzene (PDMS/DVB) fibre was used in the extraction of chlorophenols (TCP and PCP, among others) [33]. Hernandez et al. applied SPME to extract herbicides [105] and Antoniou et al. determined pharmaceutical and endocrine disrupting compounds with this technique [106].

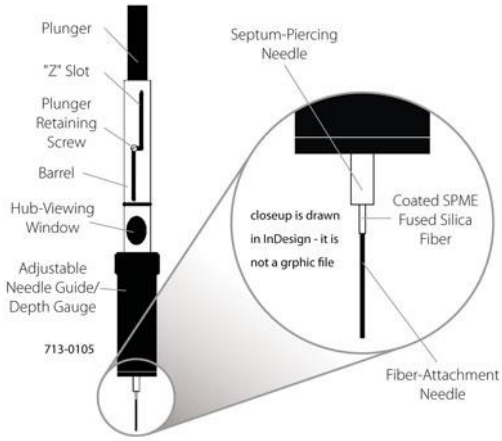


Figure 1.5 Schematic representation of a SPME fibre. (From reference [107])

Table 1.7 Commercially available SPME fibre coatings and its applications. (From reference [108])

ANALYTE TYPE	FIBRE COATING
Volatiles Non-polar high molecular weight compounds Non-polar semi-volatiles compounds	PDMS
Polar semi-volatiles compounds	PA
Volatiles, amines and nitro-aromatic compounds Amines and polar compounds	PDMS/DVB
Alcohols and polar compounds	CW
Gases and low molecular weight compounds	CAR/PDMS
Flavour compounds Trace compounds	DVB/CAR/PDMS

PDMS: polydimethylsiloxane, PA: polyacrylate, DVB: divinylbenzene, CW: Carbowax, CAR: Carboxen

1.3.1.4 Stir bar sorptive extraction

SBSE devices are magnetic stir bars incorporated into a glass jacket coated with an absorbent material, sharing the same sorptive principle of SPME. Typically, the sorptive phase is PDMS, although new commercial and in-house coatings have been developed in recent years [109, 110]. The advantage of SBSE over SPME is that the former contains a higher amount of PDMS, which results in greater recoveries and increased sample capacities given that the efficiency of the extraction process is highly dependent on both the surface area and the amount of sorbent [111, 112]. Furthermore, SBSE devices are less fragile than SPME fibres. However, carryover problems are frequent as the expensive coated magnetic bars are typically reused.

As in SPME, SBSE consists of two principals steps: extraction and desorption (**Figure 1.6)**.

1.3.1.4.1 Extraction step

During extraction, the polymer-coated stir bar is immersed in the liquid sample and stirred over time under controlled conditions until equilibrium is achieved.

Afterwards, the stir bar is removed, rinsed with distilled water and dried with a paper tissue. The extraction step is affected by several variables: stirring time and speed affect kinetics while the pH, the presence of organic modifiers and ionic strength of the sample affect the equilibrium with the analytes. Volatile and semi-volatile compounds can also be extracted by exposing the coated stir bar to the vapour phase of a liquid matrix in a headspace vial, while the liquid is shaken to promote the partition of the compounds between the liquid and vapour phases.

1.3.1.4.2 Desorption step

Desorption of the analytes can be performed by elution with a solvent or by thermal desorption. In thermal desorption (TD), the stir bar is placed into a glass tube heated at temperatures ranging between 150 and 300 ºC. TD can only be applied in the desorption of thermally stable compounds prior to gas chromatography (GC) analysis, which requires the addition of an expensive module to the GC setup. In the case of liquid desorption (LD), the stir bar is placed inside a vial and covered with the selected desorption solvent until equilibrium between the sorbent and the liquid phases is achieved. The solvent containing the analytes is then ready for analysis by liquid chromatography (LC), capillary electrophoresis (CE) or GC. LD is suitable for semi-volatile, non-volatile and thermo-labile compounds.

There are several applications of SBSE reported in the literature. Margoum et al. used SBSE with direct immersion for the extraction of pesticides followed by LD [111]. The same methodology was applied in the determination of TCP, PCP, NP, BPA and ibuprofen, among other compounds [113] and for the determination of PBDE [114]. The determination of BPA, NP and OP by GC was carried out by Tan et al. through direct immersion SBSE and TD [115].

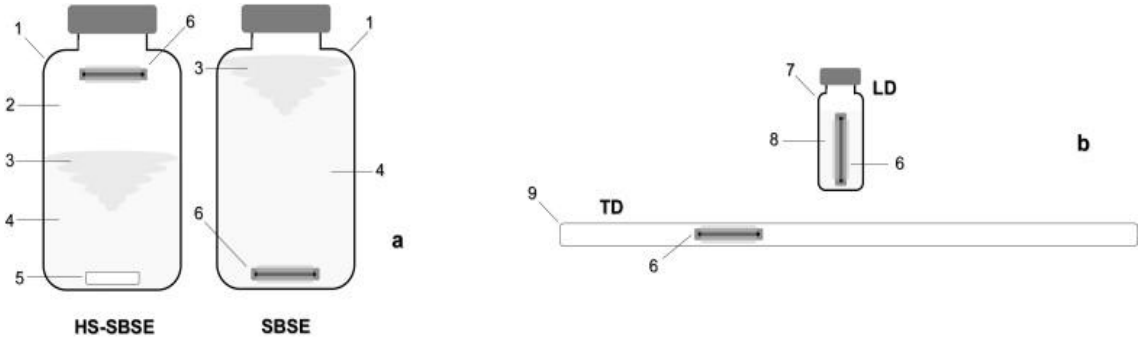


Figure 1.6 Schematic representation of the SBSE extraction (a) and desorption (b). Legend: 1. sampling flask; 2. headspace; 3. vortex; 4. sample; 5.Teflon magnetic stir bar; 6. stir bar; 7. vial; 8. desorption solvent; 9. thermal desorption glass tube. (From reference [116])

1.3.1.5 Silicone rod extraction

Silicone rod extraction and silicone tube extraction are methodologies that have been developed as low-cost alternatives to SBSE devices. In addition, considering that the rods and tubes are of single use, they avoid the carryover problems of SBSE. The sorption mechanism of silicone rods and silicone tubes are similar to SPME and SBSE but with the advantage of being inexpensive, flexible and robust. As we can see in **Figure 1.7,** different thickness and types of fibre materials are commercially available. Despite being a relative new technique, different bulk materials, such as PDMS, polyethersulfone (PES), polypropylene (PP) and polyparaphenylene terephthalamide, have been used [117-119]. PDMS was the first material employed [120] and remains the most widely used fibre [121]. The operational conditions are exactly the same as those described for SBSE (see **section 1.3.1.4**). In this thesis we focus our attention on the applications of rod materials.

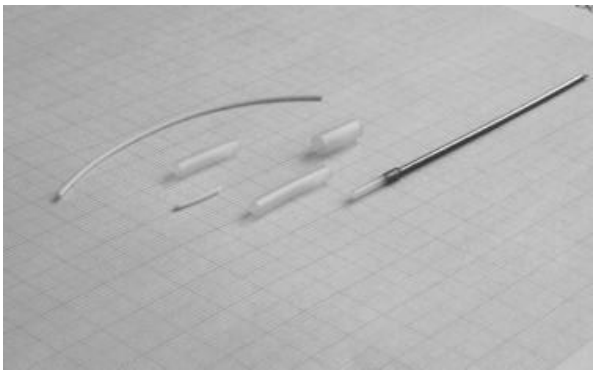


Figure 1.7 Different kinds of silicone rods and silicon tubes. (From reference [121])

Silicone rods have been applied in the extraction of PBDEs [122], sunscreen compounds [123], pesticides [124], NP and other organic pollutants [118] in water samples.

1.3.2 Instrumental analytical techniques

After sample treatment, the following step in the analytical process is the measurement using the most appropriate instrumental technique provided with a detection system that meets the requirements of uncertainty and sensitivity of the analytical problem. When the objective is to determine a group of analytes or when the detection system lacks selectivity, chromatographic techniques, such as gas chromatography and liquid chromatography, could be used to separate out the components of a sample so as to determine them properly.

As will be shown, several chromatographic methods have been developed for the determination of the compounds included in this thesis. The selection of the most appropriate chromatographic technique is clear for some specific compounds, but uncertain for others. In these latter cases, different methods can be developed taking into account the matrix of the sample and the availability of specialised instrumentation.

1.3.2.1 Gas chromatography

Gas chromatography (GC) is a widely used technique for the analysis of thermo-stable and volatile or semi-volatile compounds. When using this analytical technique and for the analysis of water samples, a sample preparation step is compulsory. These preparation techniques have been explained in detail in **section 1.3.1**.

Different detectors can be used in gas chromatography, thermal conductivity, electron capture or nitrogen phosphorus detector, among others. Mass spectrometer detectors (MS) coupled to a gas chromatograph instrument have become widely used for the analysis of environmental samples although in some cases MS detectors are less sensitive than some conventional ones. In single MS detectors, parent ions, formed in the ion source, are monitored for each compound, meanwhile in MS/MS detectors mass transitions (after fragmentation of the parent ion) can be monitored, improving the selectivity and sensitivity of the method. Two ionisation sources could be used when coupling with GC: electron impact ionisation (EI) and chemical ionisation (CI). In the first case, an electron impact with the molecule generating an ion. The second case is a weak ionisation source, in which the mass charge is transferred from a reactive gas (e.g. CH_4). The formed ions can be positive (positive chemical ionisation (PCI)) or negative (negative chemical ionisation (NCI)) [125].

GC-MS [126, 127] and GC-MS/MS [46, 128] are used for the determination of PBDEs and new brominated flame retardants as they are non-polar compounds. Electron capture detector (ECD) can also be used as these compounds are halogenated [104, 122].

For phenolic endocrine disrupting compounds, the vast majority of published methods use gas chromatography separation. However, since phenolic compounds are polar, a derivatisation step is required to convert the compounds into non-polar derivates. Different derivatisation strategies can be considered depending on the nature of the target compound and the extraction and determination technique employed. Derivatisation can be performed *in-situ* or *in-tube*. In the first case derivatisation occurs in the aqueous sample before, or simultaneously with, the extraction step. The most used is acetylation, which involves the addition of a

reagent, such as acetic anhydride, into the aqueous sample. In the second case the derivatisation takes places after the extraction in the GC injection port. The more frequently used are sylilation with e.g. bis(trimethylsilyl)trifluoroacetamide (BSTFA) and esterification with diazomethane or pentafluorobenzylbromide. Another option is the addition of tetraalkylammonium salts, which improve the extraction efficiency by ion-pair formation, followed by a derivatisation step in the injector port of the GC [113, 129].

Gatidou et al. [27] determined NP, OP and BPA by GC-MS analysis after derivatisation with BSTFA. A GC-MS method was also developed by Kawaguchi et al. [130] for the determination of BPA, PCP, NP and OP with acetylation derivatisation; De Morais et al. [33] used the same derivatisation reaction for the determination of chlorophenols by GC- ECD; and Quintana et al.[113] selected a silylation derivatisation for the determination of endocrine disrupting compounds, pharmaceuticals and pesticides.

Pharmaceutical and pesticide compounds can also be determined by GC-MS after derivatisation of the acidic compounds [75, 131-133]. Robles-Molina et al. analysed several pharmaceuticals and pesticides compounds but only the non-polar ones were determined by GC-MS/MS [134]. Benzotriazoles can be also determined by GC-MS [135].

The coupling of an inductively coupled plasma-mass spectrometry detector (ICP-MS) to a gas chromatograph allowed the sensitive determination of organophosphorus pesticides [136, 137].

1.3.2.2 Liquid chromatography

Liquid chromatography (LC) is the most appropriate technique for the determination of thermo-labile, semi-volatile and non-volatile compounds, and macromolecules. Although the column efficiency in LC is less than in gas chromatography, the resolution can be increased by modifying the composition of the mobile phase. LC techniques can be divided according to the retention mechanism.

Reverse phase partition and anionic exchange are the separation mechanisms used in the LC methods developed in this thesis. As well in the case of GC, different detection systems can be applied, such as spectrophotometry and conductivity.MS detectors can also be coupled to LC through an interface, being this combination one of the most widely used today due to its universality and sensibility. Electrospray ionisation (ESI) is the interface commonly used for polar compounds due to its high sensitivity for this type of compounds while atmospheric pressure chemical ionisation (APCI) is used for medium-polarity and low-polarity compounds. In addition, when these interfaces are used, matrix effects can produce ion suppression or enhancement of the ion signal. To overcome these problems standard addition or internal standard calibration can be used [138].

Moreover, with soft ionisation techniques such as ESI the peaks corresponding to isobaric compounds can be overlapped in the mass spectra and the use of a tandem mass spectrometry is highly recommended in order to improve the specificity in quantitative analysis. A tandem mass spectrometer detector consist of two quadrupole mass analysers in series, with a (non-mass resolving) radio frequency (RF) quadrupole between them to act as a cell for collision-induced dissociation. The first (Q_1) and third (Q_3) quadrupoles serve as mass filters. Precursor ions selected in Q1 are dissociated in the collision cell in the presence of an inert gas such as Ar, He, or N_2. Resulting fragments are passed through to Q_3 where they may be filtered or scanned. As has been explained for GC, the detection can be carried out by monitoring one or a small group of ions (MS) or a transition (MS/MS).

Apart from the detection systems described above, alternative coupling, such as with ICP based instruments, can also be performed.

For the determination of inorganic ionic species, ion chromatography (IC) with suppressed conductivity detectors is the chromatographic technique traditionally employed. Nowadays, IC can also be carried out using different kinds of detection systems.

Liquid chromatography using an ionic exchange column is one of the most selected separation methods in speciation studies including the determination of inorganic

phosphorus species, among others. Phosphite and orthophosphate were determined in geothermal waters by IC with conductivity detection and LC-MS [139], the determination of hypophosphite, phosphite and orthophosphate in water samples can be carried out by LC methods with either conductivity [9] or MS detection [140]. The coupling of an ICP-MS detector to a LC system allowed the determination of glyphosate, phosphate and aminomethylphosphonic acid [141, 142].

Phenolic compounds are generally determined by LC using diode array (DAD) and MS detection. LC-DAD methods were developed for the determination of chlorophenols, BPA, NP and OP [25, 143, 144], whereas LC-MS was used in determining BPA, NP, OP, TCP and PCP in water samples [29]. LC-MS/MS has been also used for the determination of NP, OP and BPA [145].

Pharmaceuticals are also widely determined by LC, due to the acidic character of some of these compounds. LC-MS and LC-MS/MS methodologies have been developed for the determination of pharmaceuticals in environmental waters [59, 146]. Iodinated X-ray contrast media are generally determined by LC-MS/MS [63, 147, 148], but can be also determined by LC-ICP-MS [65]. Corrosion inhibitors (benzotriazoles) are also generally determined by LC-MS [77] or LC-MS/MS [78, 79] as well as pesticides [149, 150].

1.3.2.3 Inductively coupled plasma based methods

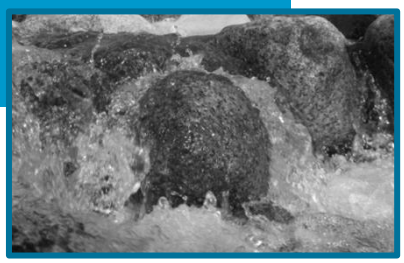
Inductively coupled plasma (ICP) is an energy source used to atomise and/or ionise an analyte as the plasma torch can reach temperatures of up to 8000 K, which allows the atomisation and ionisation of most of the existing elements [85]. Different detection systems such as atomic emission spectrometers (AES) o mass spectrometers (MS) can be coupled to the ICP source to perform analytical measurements.

In ICP-AES, the function of the plasma is to produce excited atoms and ions that emit electromagnetic radiation at characteristic wavelengths. Whereas in ICP-MS the function of the plasma is to produce ions that then will be separated and quantified using a MS.

The advantages of ICP-AES and ICP-MS detectors are the low limits of detection, especially in ICP-MS, the wide linear dynamic range that can be obtained, the multielement capability, and the high selectivity. However, they suffer from spectral and non-spectral interferences. The different approaches that can be applied to overcome this point in the case of phosphorus determination will be explained in more detail in **Chapter 4**. An important advantage of ICP based techniques coupled to a chromatographic technique in speciation analysis, is that allows the determination and quantification of an element independently of the molecular environment of the compound being analysed.

Phosphorus speciation analysis in water was already performed by coupling an ICP-AES to a LC instrument [151, 152]. Moreover, analytical methodologies based on ICP-MS coupled to LC were developed for the determination of glyphosate and phosphate [153] and for the speciation of four phosphorus pesticides in water [154]. ICP-MS has also been used as an element-specific detector for IC in the determination of different anions including orthophosphate [155]. The determination of DNA nucleotids [156] by LC-ICP-MS and the determination of pyrophosphate and phytic acid in biological fluids by SPE-ICP-MS [11] are some of the more recently methodologies reported for the determination of phosphorus compounds. The methodologies for phosphorus determination based on GC coupled to ICP are scarce [136].

CHAPTER 2. OBJECTIVES



Given the harmful effects that endocrine disruptors, flame retardants, pesticides and pharmaceutical residues have on human health and the environment, it is essential to control their presence and concentration in water. Moreover, to understand phosphorus cycle and the role of the different phosphorus species on eutrophication it is necessary to establish sensitive methods for the speciation of reduced phosphorus species together with orthophosphate and condensate phosphorus oxoanions species in water. As a contribution to these aims, the objective of this thesis is to develop simple and efficient analytical methodologies for the determination of organic micropollutants and phosphorous species in water samples.

The specific objectives are:

1. To develop an HPLC-ICP-based methodology for the speciation analysis of phosphorus oxoanions (hypophosphite, phosphite, orthophosphate, pyrophosphate and tripolyphosphate) in water samples.

2. To develop different methodologies using PDMS rod extraction followed by liquid desorption and chromatographic separation for the determination of endocrine disrupting compounds in water samples:

    a. Simultaneous determination of flame retardants in water samples using GC-NCI-MS detection. Target compounds were selected in order to cover the most representative polybrominated diphenylethers and their recent replacement chemicals.

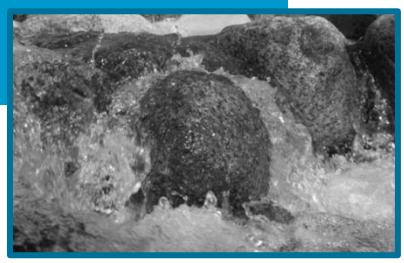
    b. Determination of bisphenol A, 2,4,6-trichlorophenol, pentachlorophenol, 4-*n*-nonylphenol and 4-*n*-octylphenol in water samples using HPLC-DAD detection.

    c. Determination of bisphenol A, 2,4,6-trichlorophenol, pentachlorophenol, 4-*n*-nonylphenol and 4-*n*-octylphenol with *in-situ* acetylation prior to PDMS rod extraction and HPLC-DAD detection.

3. To develop a sensitive SPE-LC-MS/MS multiresidue method for the determination of 35 compounds (pharmaceutical compounds, including five

ICM, pesticides and corrosion inhibitors) and some of their transformation products in different water matrices.

CHAPTER 3. METHODS



In this thesis different analytical methodologies based on chromatographic separation followed by the determination of the analytes with different detection systems have been developed. The materials, instrumentation and reagents employed in studying each analytical system were different although the chemical and operational conditions involved in the different steps of each analytical procedure were systematically studied as follows:

- Study of the separation and detection conditions.
- Study of the preconcentration and clean-up conditions (when required).
- Validation of the method (analytical figure of merit, matrix effect).
- Application of the developed method to the analysis of water samples.

This chapter will be divided in three sections, according to the specific objectives of this thesis: speciation analysis of phosphorus oxoanions, PDMS rod extraction of endocrine disrupting compounds and multiresidue SPE extraction.

3.1 SPECIATION ANALYSIS OF PHOSPHORUS OXOANIONS

For the speciation analysis of phosphorus oxoanions, two HPLC-ICP based methods have been developed: one coupled to ICP-AES and the other coupled to ICP-MS.

3.1.1 Standards and reagents

Standard stock solutions of phosphorus oxoanions were prepared by dissolving the appropriate amounts of anhydrous sodium dihydrogen phosphate (Panreac Química, Barcelona, Spain), sodium hypophosphite monohydrate and sodium pyrophosphate tetrabasic decahydrate (Fluka Chemie, Buchs, Switzerland), sodium phosphite dibasic pentahydrate (Sigma-Aldrich, Steinheim, Germany) and pentasodium tripolyphosphate hexahydrate (Sigma-Aldrich, Steinheim, Germany), in doubly deionised water (MilliQ) obtained from a Millipore water purification system (18.2 $M\Omega\ cm^{-1}$, Millipore, Bedford, MA, USA).

Standard stock solutions of 1000 mg P L^{-1} were stored in amber glass bottles at 4 ºC [9]. The stability of stock standard solutions based on storage time was studied for periods of up to 15 days. Diode array spectrophotometer was used to register the absorption spectra of phosphite, pyrophosphate and tripolyphosphate; phosphate and hypophosphite do not have a maximum absorbance above 190 nm. Results showed that the stock standard solutions were stable for periods up to 15 days. Based on these results and previous studies [9] stock solutions were prepared every 7-15 days.

Intermediate solutions were prepared every two days by dilution of the stock solutions with MilliQ water acidified with nitric acid at pH 2, whereas working solutions at μg P L^{-1} level were prepared daily.

Ammonium nitrate was prepared with the appropriate amount of Suprapure® nitric acid (Merck, Darmstadt, Germany) and ammonia (Panreac Química, Barcelona, Spain). The pH was adjusted with nitric acid and ammonia. HPLC-grade methanol (Carlo Erba, Rodano, Italy) was used. Acetic - acetate, formic - formiate and citric - citrate buffers were prepared dissolving appropriate amounts of acetic acid (Fluka Chemie, Buchs, Switzerland), formic acid (Sigma-Aldrich, Steinheim, Germany) and citric acid (Panreac Química, Barcelona, Spain), respectively, and with the addition of ammonia to obtain the desired buffer solution. Mobile phases were prepared in MilliQ water and filtered through a disposable 0.20 μm cellulose nitrate membrane filter (Whatman, Dassel, Germany). The solutions were degassed in an ultrasonic bath prior to use.

3.1.2 Instrumentation

3.1.2.1 High-performance liquid chromatography

The high performance liquid chromatography (HPLC) system (P2000 Thermo Separation products) consisted of an online degasser, an HPLC binary pump and a Rheodyne Series 7725 manual injector valve with a 20 μL (ICP-AES) and a 100 μL (ICP-MS) injection loop. The chromatographic column was a PRP-X100 anion exchange column (150 mm x 4.1 mm, 5 μm, Hamilton), preceded by a PRP-X100 guard column (Hamilton) to retain the impurities.

3.1.2.2 Inductively coupled plasma-atomic emission spectrometry

The chromatographic system has been hyphenated to a Liberty series II ICP-AES (Varian) (**Figure 3.1**). The end of the chromatographic column was directly connected to the ICP-AES nebulizer using a simple Teflon tube. The ICP-AES was equipped with a photomultiplier tube detector and either a Babington nebulizer coupled to a double-pass spray chamber or a concentric nebulizer together with a thermostated double-pass spray chamber. **Table 3.1** shows the experimental conditions employed in the detection step. Some other parameters, such as adjustment of the phosphorus emission wavelength, were optimised daily for highest sensitivity. Peaks integration has been carried out with WinFaas 1.0 [157].

Figure 3.1 HPLC-ICP-AES system.

Table 3.1 ICP-AES operating parameters.

ICP-AES OPERATING PARAMETERS	
Integration time (s)	0.50
Power (kW)	1.20
Plasma flow (L min^{-1})	13.5
Auxiliary flow (L min^{-1})	1.50
Wavelength (nm)	213.618

3.1.2.3 Inductively coupled plasma-mass spectrometry

The chromatographic system was also coupled to an Agilent 7500c ICP-MS (Agilent Technologies, Palo Alto, CA) (**Figure 3.2**) using a Teflon tube. The ICP-MS which uses a quadrupole as a mass analyser was equipped with a Babington nebulizer, a double-pass spray chamber, an octopole collision-reaction cell (ORC) and an electron multiplier detector. **Table 3.2** shows the experimental conditions employed in the detection step. Sensitivity and oxide level (*m/z* 156/140) were checked daily using a calibration solution containing lithium (*m/z* 7), cobalt (m/z 59), yttrium (*m/z* 89), thallium (*m/z* 205) and cerium (*m/z* 140). Data analysis Masshunter software (Agilent Technologies, Palo Alto, CA) and WinFAAS 1.0 were used for peak integration.

Table 3.2 ICP-MS operating parameters.

ICP-MS OPERATING PARAMETERS	
RF forward power (W)	1300
Plasma gas flow rate (L min^{-1})	15
Auxiliary gas flow rate (L min^{-1})	0.9
Carrier gas flow rate (L min^{-1})	1.1
Makeup gas flow rate (L min^{-1})	0
Sampling depth (mm)	6
Torch	Standard quartz
Nebulizer	Babington
Spray chamber	Double-pass quartz, 2 ºC
Sampling and skimmer cones	Nickel
Isotope monitored	*m/z* 31
Detector mode	Pulse
Dwell time (s per isotope)	0.3
ORC gas	He 1.5 mL min^{-1}

Figure 3.2 HPLC-ICP-MS system.

3.1.3 Chromatographic separation

Four different mobile phases (50 mM acetic-acetate, 100 mM formic-formiate, 15 mM citric-citrate, 10 mM ammonium nitrate) at different pHs were tested in order to achieve the chromatographic separation of the five phosphorus oxoanions. These experiments were performed in isocratic mode. As tripolyphosphate was not eluted in these conditions, a gradient elution and the addition of methanol as organic modifier were then tested.

3.1.4 Samples

The developed speciation methods were applied to the analysis of tap water and effluent wastewater samples. All samples were collected in 1L amber glass bottles which had been thoroughly rinsed with the sample before collection. Samples were transported to the laboratory under refrigeration and then stored at 4 ºC. Before analysis, the samples were filtered through 0.45 μm cellulose nitrate membrane filters. The two effluent waters were taken from two WWTP, Castell-Platja d'Aro and Blanes, which have population equivalents of 175,000 and 109,000, respectively. Castell-Platja d'Aro WWTP exclusively treats domestic residues whereas Blanes WWTP has some industrial input. In both cases, they are equipped with conventional secondary biological units, although the two plants differ in that the secondary treatment in Blanes also includes a nitrification-denitrification unit.

3.2 PDMS ROD EXTRACTION OF ENDOCRINE DISRUPTING COMPOUNDS

The polydimethylsiloxane (PDMS) rod preconcentration technique has been applied to two groups of compounds: flame retardants and phenolic compounds. In the first case, the determination was carried out by gas chromatography-mass spectrometry, and in the second case the determination was performed by liquid-chromatography-diode array detection. Moreover, for the second group of compounds, two methodologies have been developed, one for the phenolic compounds and another for their acetylated derivatives.

3.2.1 Standards and reagents

3.2.1.1 Flame retardants compounds

Standard solutions of 2,2´,4,4´-tetrabromodiphenyl ether (BDE-47) 100%, 2,2´,3,4,4´-pentabromodiphenyl ether (BDE-85) 98.5%, 2,2´,4,4´,5-pentabromodiphenyl ether (BDE-99) 99.2%, 2,2´,4,4´,6-pentabromodiphenyl ether (BDE-100) 100%, 2,2´,4,4´,5,5´-hexabromodiphenyl ether (BDE-153) 99.3%, 2,2´,4,4´,5,6´-hexabromodiphenyl ether (BDE-154) 100%, 2,2´,3,4,4´,5´,6-heptabromodiphenyl ether (BDE-183) 100%, 2,2´,3,3´,4,4´,6,6´-octabromodiphenyl ether (BDE-197) 98.1%, decabromodiphenyl ether (BDE-209) in isooctane (AccuStandard, New Haven, CT, USA), 50 μg mL^{-1}, were used. Solid standards of 2-ethylhexyl-2,3,4,5-tetrabromobenzoate (EHTBB) 97.3%, decabromodiphenyl ethane (DBDPE) technical grade, bis(2-ethylhexyl)tetrabromophtalate (DEHTBP) 99.5%, 1,2-bis(2,4,6-tribromophenoxy)ethane (BTBPE) 100% and tris(tribromoneopenthyl)phosphate (TTBPP) 97.3% (AccuStandard) were also employed and the two isomers of bis(hexachlorocyclopentandien)cyclooctane (DP) (*syn* and *anti*), 100 μg mL^{-1} each in isooctane, were acquired from Cambridge Isotope Laboratories (Andover, MA, USA). As internal standards (IS), 3,3´,4,4´-tetrabromodiphenyl ether (BDE-77) 100% and 2,2´,3,4,4´,5,6-heptabromodiphenyl ether (BDE-181) 98%, 50 μg mL^{-1} in isooctane (AccuStandard) and [$^{13}C_{12}$]decabromodiphenyl ether (^{13}C-BDE-209)> 98%, 25 μg mL^{-1} in toluene, (Wellington Laboratories, Guelph, Canada) were purchased. Diluted solutions of

analytes and IS were made in acetone when used to prepare spiked water samples and in isooctane when injected directly in the GC-MS. Isooctane, hexane, cyclohexane, toluene and methanol, chromatographic analysis grade, were purchased from Merck (Darmstadt, Germany). Acetone pestinorm, ethyl acetate and sodium chloride were acquired from VWR Prolabo (Mollet de Vallès, Spain). Deionised water was obtained with a MilliQ Gradient A-10 system (Millipore, Bedford, MA, USA).

3.2.1.2 Phenolic compounds

Standard solutions of 2,4,6-trichlorophenol and 4-*n*-nonylphenol (Dr. Ehrenstorfer GmbH, Augsburg, Germany), pentachlorophenol, bisphenol A and 4-*n*-octylphenol (Sigma-Aldrich, Steinheim, Germany) were diluted with methanol (MeOH, HPLC grade, Carlo Erba, Rodano, Italy) in order to prepare working solutions of the analytes. Other reagents used are acetonitrile (ACN), sodium chloride (Carlo Erba, Rodano, Italy), acetic acid (Sigma-Aldrich, Steinheim, Germany), ethanol absolute, ammonia (Panreac Química, Castellar del Vallès, Spain), hydrochloric acid (37%) (Merck, Darmstadt, Germany), acetic anhydride (AA) and potassium carbonate anhydrous (Fisher Chemical, Loughborough, United Kingdom). Ultra-pure water (MilliQ), which has a conductivity of 18.2 MΩ cm^{-1} was obtained from a Millipore water purification system (Millipore, Bedford, MA, USA).

For both methodologies PDMS cord with a diameter of 2 mm (Goodfellow Cambridge Ltd., Huntingdon, England) was used.

3.2.2 Instrumentation

3.2.2.1 Gas chromatography

Chromatographic analysis of flame retardants was performed on an Agilent 7890A gas chromatograph equipped with a 7683B automatic sampler and a split/splitless injection port coupled to a 5975C mass selective detector (MSD) (Agilent Technologies, Palo Alto, CA, USA) in selective ion monitoring (SIM) mode using negative chemical ionisation with CH_4 as reagent gas.

GC separation was performed on a DB-5HT capillary column of 15 m (length) x 0.250 mm (I.D.) x 0.1 μm (film thickness) (J & W Scientific, Folsom, CA, USA) using helium as carrier gas at a constant flow rate of 1 mL min^{-1}. The oven temperature program was as follows: the initial temperature of 80 ºC was held for 1.5 min, first ramp at 60 ºC/min until 220 ºC, second ramp at 30 ºC/min to 250 ºC, third ramp at 5 ºC/min to 270 ºC and fourth ramp at 10 ºC/min to 325 ºC (held for 5 min). The total runtime was 19.33 min and the solvent delay was 3.9 min. Standard solutions and sample extracts were injected in the splitless mode (splitless time: 1.5 min) with an injection volume of 1 μL. Injector, transfer line, MS source and MS quadrupole temperatures were set at 300 ºC, 280 ºC, 150 ºC and 150 ºC, respectively. Several analytes and internal standard (IS) having particular target ions were grouped in time windows, defined by the corresponding retention times, with dwell times from 30 to 50 ms. For each compound one quantifier and two qualifier ions were recorded (see **Table 3.3**).

3.2.2.2 Liquid Chromatography

Chromatographic analysis of phenolic compounds was performed using an Agilent 1200 Series LC system (Agilent Technologies, CA, USA) with a diode array detector (Agilent 1290 Infinity) and LC3D ChemStation software (Agilent Technologies) for data acquisition and control of the instrument. The analytes were separated in a C18 Zorbax Eclipse Plus column (50 mm x 2.1 mm, 1.8 μm particle size) (Agilent Technologies) using a mobile phase in gradient mode consisting of acidified acetonitrile and water. Different gradients and mobile phase compositions have been tested before obtaining the final conditions. The chromatographic separation and detection conditions for the two developed methods, both the direct and derivatisation-based, are set out in **Table 3.4**. The flow rate of the mobile phase was set at 300 μL min^{-1}.

Table 3.3 GC-NCI-MS retention times and *m/z* values. Underlined *m/z* values were used for quantification and the others as qualifiers.

TIME SEGMENT	COMPOUND	RETENTION TIME (MIN)	*m/z*
1	BDE-47	5.04	79.0, 81.0, 160.7
1	BDE-77*	5.27	79.0, 81.0, 325.7
1	BDE-100	5.56	79.0, 81.0, 160.7
1	BDE-99	5.74	79.0, 81.0, 160.7
1	EHTBB	5.78	79.0, 81.0, 356.6
1	BDE-85	6.11	79.0, 81.0, 160.7
1	BDE-154	6.35	79.0, 81.0, 160.7
1	BDE-153	6.72	79.0, 81.0, 160.7
2	BDE-183	8.08	79.0, 81.0, 561.4
2	BTBPE	8.60	79.0, 81.0, 251.7
2	BDE-181*	8.97	79.0, 81.0, 561.4
3	DEHTBP	9.55	383.6, 79.0, 81.0
3	*syn*-DP	9.62	653.6, 651.6, 655.6
3	*anti*-DP	10.04	653.6, 651.6, 655.6
3	BDE-197	10.09	408.5, 79.0, 81.0
4	BDE-209	14.26	486.5, 484.5, 482.5
4	^{13}C-BDE-209*	14.26	494.5, 496.5, 492.5
5	TTBPP	14.50	938.4, 936.4, 940.4
5	DBDPE	15.32	79.0, 81.0, 493

*Internal standards

Table 3.4 HPLC-DAD separation and detection conditions.

		DIRECT METHOD	DERIVATISATION-BASED METHOD
Gradient		0 – 2 min: 40% A; 3 – 9 min: 50% A; 12 – 14 min: 60% A; 16 min: 40% A	0 min: 40% A; 1.5 min: 45% A; 6 min: 50% A; 8 – 9 min: 55% A; 11 – 19 min: 80% A; 22 min: 40% A
Wavelength (nm) / Retention Time (min)	BPA	280 / 1.6	265 / 9.7
	TCP	293 / 4.3	278 / 9.1
	PCP	302 / 9.1	250 / 14.0
	NP	280 / 16.1	260 / 16.1
	OP	280 / 16.8	260 / 16.5

A (acetonitrile) and B (water), both containing 0.5% v/v acetic acid for the direct method, and 0.3% v/v acetic acid in mobile phase B for the derivatisation-based method.

3.2.3 PDMS rod extraction

10 mm PDMS rods were cut from the PDMS cord with a scalpel. These were then cleaned and stored in ethanol and, immediately prior to use, dried with a lint-free tissue.

In order to find the best extraction/desorption conditions for the preconcentration of the target compounds several parameters affecting both steps were systematically studied. The main parameters to be studied in the extraction process are sample volume, ionic strength (salting out effect), pH of the sample, addition of an organic modifier and extraction time and, in the case of desorption, the desorption solvent and its volume, desorption time and sonication. The selected conditions for the extraction/desorption are described in **Chapter 4**.

3.2.3.1 Flame retardants

To prepare samples for analysis applying the best conditions, aliquots of 100 mL of water (56 mL of sample) containing 40% (volume/volume (v/v)) of methanol and 4% of sodium chloride (except in sea and ria water samples),were placed inside 120 mL glass crimp-cap vials containing a magnetic stirrer. The PDMS rod (10 mm) was exposed directly to the water sample under magnetic stirring at room temperature

overnight, for a 15 h period. After the extraction step, the rod was removed from the vial using tweezers, washed with MilliQ water, dried with a lint-free tissue and placed inside a 400 μL insert situated inside a 2 mL clear screw-cap vial. For solvent desorption 300 μL of ethyl acetate was added to the insert, the vial was closed and sonicated for 15 min. After the desorption period the vial was opened, the rod removed and the organic solvent containing the analytes evaporated to dryness with a gentle stream of nitrogen and reconstituted with 50 μL of isooctane. This isooctane extract was stored in the freezer until injection into the GC-NCI-MS system for analysis.

3.2.3.2 Phenolic compounds

In a typical assay, 10 mm of PDMS rod was immersed in a 125 mL amber vial containing 50 mL of water and 15% w/v of NaCl. The vial was then closed and the extraction was performed for 16 h. The experiments were performed three times using a five-point orbital shaker (MultiMix D, Ovan, Badalona, Spain) at room temperature. After extraction, the PDMS rod was removed with clean tweezers, washed with MilliQ water, and then dried with a lint-free tissue. The rod was then placed into a "tapered" glass insert containing 100 μL of MeOH. The vial was closed allowing the desorption process to take place for 45 min at room temperature. The PDMS rod was removed and 5 μL of the extract was then injected in the liquid chromatograph.

3.2.3.3 Derivatisation procedure and extraction of acetylated phenolic compounds

Acetylation in aqueous media was the derivatisation method selected to reduce the polarity of the phenolic compounds by replacing the hydroxyl group with an acetate group. The derivatisation reagent (AA) was added to the aqueous sample after basification of the media with K_2CO_3. The amounts of AA and K_2CO_3 required for the derivatisation reaction had been studied.

In a typical assay, 50 mL of water was placed in a 125 mL amber vial containing a magnetic stirrer. 100 mg of K_2CO_3 and 400 μL of AA were added to the water

sample and the resulting solution was then stirred until the bubbles were eliminated. The extraction and desorption procedures are the same as those explained for phenolic compounds (**section 3.2.3.2**), but in this case desorption step was performed during 30 minutes at room temperature. All the experiments were performed in triplicate.

3.2.4 Samples

3.2.4.1 Flame retardants

Several types of water samples have been considered in this study: river, ria, sea, landfill leachate and wastewater samples. Ria and sea water were taken from a coastal area on the north-western coast of Spain. Urban influent and effluent wastewater samples were taken from an urban sewage treatment plant (STP) located in the northwest of Spain and receiving the discharges from a ca.100,000 inhabitants city. Industrial wastewater samples were obtained from a textile industry located in Catalonia (Spain) and landfill leachate water samples were taken in the Valencian Community (Spain).

All samples were collected in amber glass bottles, previously rinsed with methanol and ultrapure water, and stored in the dark under refrigerated conditions (4 ºC) for a maximum of 48 h. Filtration or centrifugation of samples was not performed to avoid losses of analytes (see section 4.2.2.4.1).

3.2.4.2 Phenolic compounds

Water samples from the Ter River, located in the northeast of Spain, were collected in 1 L amber glass bottles that had previously been rinsed in the river water. Samples were transported to the laboratory under refrigeration and then stored at 4 ºC.

The water samples were spiked with different concentration levels of the phenolic compounds. Recovery experiments were performed by analysing these spiked water samples. All the experiments were carried out in duplicate.

3.3 MULTIRESIDUE SPE EXTRACTION

In order to monitor the presence of several organic micropollutants in aquatic environments, a multiresidue SPE extraction method followed by LC-MS/MS determination has been developed.

3.3.1 Standards and reagents

Diclofenac, ibuprofen, N-acetyl-4-aminoantipyrine, carbamazepine, lamotrigine, gabapentin, bezafibrate, iohexol, amidotrizoic acid, metoprolol, sulfamethoxazole, hydrochlorothiazide, 1H-benzotriazole, and 5-methylbenzotriazole were obtained from Sigma-Aldrich (Steinheim, Germany). Iomeprol, sotalol, acetyl-sulfamethoxazole, isoproturon, chloridazon, atrazine, simazine, terbuthylazine, metazachlor, metolachlor, chlortoluron, and diuron were purchased from Dr. Ehrenstorfer (Augsburg, Germany). Iopamidol, iopromide (USP, Basel, Switzerland), N-formyl-4-aminoantipyrine (LGC GmbH, Wesel, Germany), 10,11-dihydro-10,11-dihydroxy-carbamazepine (Campro Scientific GmbH, Berlin, Germany), and 4-methylbenzotriazole (Chemos GmbH, Regenstauf, Germany), metazachlor-C-metabolite (BH479-4) (BASF, Ludwigshafen, Germany), metazachlor-S-metabolite (BH479-8), metolachlor-C-metabolite (CGA51202/351916), and metolachlor-S-metabolite (CGA380168/354743) (all from Syngenta, Basel, Switzerland) were also used. Isotopically labelled compounds, used as surrogate/internal standards, were ibuprofen-d_3, carbamazepine-d_{10}, diclofenac-d_4, gabapentin-d_4, clofibric acid-d_4, phenazon-d_3, pentoxifylline-d_5 (all from CDN Isotopes Inc., Pointe-Claire, Quebec, Canada); sulfamethoxazole-^{13}C-d_6 (Cambridge Isotope laboratories Inc., Tewksbury, MA, USA); sotalol-d_6, isoproturon-d_6, diuron-d_6 (all from Dr. Ehrenstorfer, Augsburg, Germany); clenbuterol-d_9 (Cerilliant, Round Rock, Texas, USA); amidotrizoic acid-d_6, benzotriazole-d_4, hydrochlorothiazide-^{13}C-d_2 (all from Campro Scientific GmbH, Berlin, Germany); iomeprol-d_3 (Chiron, Trondheim, Norway); iopamidol-d_8, iohexol-d_5 (all from Toronto Research Chemicals, Toronto, Ontario, Canada); and metolachlor-d_6 (Syngenta, Basel, Switzerland). Desmethoxyiopromide (MSP, Shoreview, Minnesota, USA) was also used as surrogate/internal standard. All

compounds used as surrogate/internal standards were added to the samples before extraction and were used for the quantification of the analytes.

Solvents and additives used for sample preparation and for mobile phase preparation were of HPLC grade. Methanol was provided by LGC (Wesel, Germany); acetonitrile and acetic acid were purchased from VWR Chemicals (Darmstadt, Germany); formic acid and ammonium acetate were obtained from Merck (Darmstadt, Germany); ammonium formate was supplied by Sigma-Aldrich (Steinheim, Germany); and hydrochloric acid was obtained from Carl Roth (Karlsruhe, Germany). MilliQ water was provided by an arium® 611 UV system (Sartorius AG, Göttingen, Germany).

Stock solutions of the individual standards were prepared by dissolving each compound in methanol at concentrations between 100 and 1000 mg L^{-1}. Standard mixtures, at different concentrations, were prepared by appropriate dilution of the stock solutions in water/methanol (80:20).

3.3.2 Instrumentation

Separation of the analytes was performed using an Agilent 1290 Infinity Series LC system (Agilent Technologies, Santa Clara, CA, USA). Different reversed phase HPLC columns were tested: XBridge BEH C18 (150 mm x 2.1 mm, particle size 3.5 µm, Waters, Milford, MA, USA), Gemini C18 (250 mm x 2.0 mm, particle size 5 µm) and Luna C18 (250 mm x 2.0 mm, particle size 5 µm) (both from Phenomenex, Aschaffenburg, Germany), and Eclipse Plus C18 (50 mm x 2.1 mm, particle size 1.8 µm, Agilent Technologies, Santa Clara, CA, USA). The separation was done in gradient mode at a flow rate of 200 µL min^{-1}. Eluent A was a 2 mM ammonium formate solution in MilliQ water acidified at pH 5 with formic acid and eluent B was a 2 mM ammonium formate solution in methanol. Depending on the detection sensitivity to the compounds, different injection volumes were used. An injection volume of 40 µL was used for iopromide, iomeprol, iohexol, iopamidol, amidotrizoic acid, hydrochlorothiazide and ibuprofen, and 3 µL for the rest of the compounds. The liquid chromatography instrument was coupled to a 5500 AB Sciex triple quadrupole MS (Framingham, MA, USA) with an electrospray source. MS

parameters such as declustering potential (DPo), collision energy (CEn) and collision cell exit potential (CXP) were adjusted by direct injection of the individual standard solutions of each compound. All these conditions were studied in detail in **Chapter 4**. **Table 3.5** shows a summary of the selected conditions. All transitions were recorded using the Scheduled MRM^{TM} algorithm. The analyses were done in the negative ionisation mode for ibuprofen, 4-AAA, hydrochlorothiazide, metazachlor-S-metabolite, metolachlor-C-metabolite and metolachlor-S-metabolite and in the positive ionisation mode for the other compounds. All data were acquired and processed using Analyst software. For quantitative purposes, multiple reaction monitoring (MRM) was used and two characteristic transitions of $[M+H]^+$ or $[M-H]^-$ were monitored for each compound, except for iopromide and iohexol which one of the transitions was $[M+H]^+$ and the other one was $[M+NH_4]^+$.

In order to monitor the entire analytical process from the sample preparation to the instrument performance, 20 compounds were used as surrogate/internal standards (SS/IS). The addition of SS/IS allows the correction of analyte losses during SPE performance and matrix effects during ESI ionisation. In this last case, matrix effect can cause signal suppression or, less frequently an enhancement of the analyte signal. When available, the deuterated compound was selected as SS/IS. When no isotope-labelled reference compound was available, the SS/IS was selected based on similar chemical structure, retention time and SPE and MS response. The SS/IS used for each compound is shown in **Table 3.5**.

3.3.3 Sample extraction

The SPE cartridges tested for the sample extraction were Oasis HLB (Waters, Milford, MA, USA) a hydrophilic-lipophilic balanced (HLB) reversed-phase sorbent; Bakerbond SDB (J.T. Baker, Center Valley, PA, USA) a non-functionalized styrene-divinylbenzene (SDVB) polymer; Bond Elut PPL (Agilent Technologies, Santa Clara, CA, USA) a non-polar functionalized SDVB material; and Isolute ENV+ (Biotage, Uppsala, Sweden), a hydroxylated SDVB material. The SPE cartridges were connected to a SPE manifold (J.T. Baker, Center Valley, PA, USA), conditioned with 7.5 mL of MeOH and equilibrated with 7.5 mL of MilliQ water.

Sample volumes of 50 mL were passed through the cartridge and afterwards the cartridge was dried using a stream of N_2 gas for 45 min. The analytes were eluted and extracts were reduced to dryness under a gentle flow of N_2 gas and reconstituted to 200 µL with water/MeOH (80/20). 3 µL or 40 µL of this solution was injected in the chromatographic system.

3.3.4 Samples

River water samples were collected from the German rivers Echaz, Rems, Schussen, Stockacher Aach, Danube, Wutach, Kinzig, Enz, Körsch, Glems, Neckar, Jagst, Kocher, Würm, Rhine, Leimbach, Kraichbach and Wutach. Influent and effluent waste water samples were collected in several small and medium size WWTPs in the Southwest of Germany and in Girona, Spain. Influent wastewater (WW) samples were diluted 10 times and effluent WW samples were diluted 5 times with MilliQ water. Surface water samples were taken as grab samples. Wastewater samples in Germany were collected with automated sampling devices as twenty-four hours composite samples. On the contrary, wastewater samples from Spain were taken as grab samples.

Table 3.5 Compounds analysed, classified by their chemical group, and their retention time, HPLC-ESI-MS/MS parameters and surrogate/internal standard used. **In bold** transitions used for quantification.

THERAPEUTIC GROUP OR CLASS	COMPOUND	RETENTION TIME (MIN)	ESI MODE	PRECURSOR ION	PRODUCT ION	DPO/CEN/CXP (V)	SURROGATE/INTERNAL STANDARD	PRECURSOR ION / PRODUCT ION
Analgesic/anti-inflammatory and TP	Diclofenac	19.1	+	**296**	**213.8**	26/37/5	Diclofenac-d_4	300/217
				296	249.8	26/19/6		
	Ibuprofen	21.5	-	**204.9**	**160.9**	-41/-10/-6	Ibuprofen-d_3	208/164
				204.9	159.4	-41/-10/-5		
	N-acetyl-4-aminoantipyrine (4-AAA)	8.9	+	**246**	**83.1**	41/37/5	Phenazon-d_3	192/59
				246	103.9	41/29/5		
	N-formyl-4-aminoantipyrine (4-FAA)	8.8	+	**231.9**	**82.9**	41/29/5	Phenazon-d_3	192/59
				231.9	103.9	41/29/5		
Anti-epileptic and TP	Carbamazepine	15	+	**237**	**193.9**	31/25/5	Carbamazepine-d_{10}	247/204
				237	164.9	31/55/5		
	10,11-dihydro-10,11-dihydroxy-carbamazepine (10,11-DHC)	11.5	+	**270.9**	**180.2**	26/39/5	Carbamazepine-d_{10}	247/204
				270.9	236.1	26/17/5		
	Lamotrigine	10.7	+	**256**	**211**	161/37/14	Pentoxifylline-d_5	284/138
				256	73.8	161/107/12		
	Gabapentin	7.9	+	**172.1**	**154.1**	51/19/10	Gabapentin-d_4	176/158
				172.1	137.2	51/23/8		
Lipid regulator	Bezafibrate	15.6	+	**362**	**138.8**	31/35/5	Clofibric acid-d_4	217/131
				362	121.1	31/41/5		
X-ray contrast agents	Iopamidol	5.7	+	**777.5**	**558.8**	231/31/16	Iopamidol-d_8	781/562
				777.5	386.9	231/51/28		
	Iopromide	7.3	+	**791.6**	**572.8**	216/33/16	Desmethoxyiopromide (DMI)	762/543
				808.6	791.8	36/17/20		
	Iomeprol	6.3	+	**777.6**	**404.9**	221/55/32	Iomeprol-d_3	781/535
				777.6	531.8	221/41/14		
	Amidotrizoic acid	6.5	+	**614.6**	**233**	156/59/26	Amidotrizoic acid-d_6	639/368
				614.6	360.9	120/46/16		
	Iohexol	6.2	+	**821.6**	**803.7**	221/29/22	Iohexol-d_5	827/809
				838.6	821.8	71/15/8		

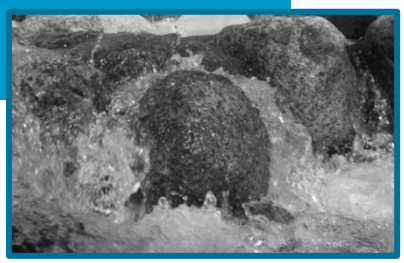
Table 3.5 Compounds analysed, classified by their chemical group, and their retention time, HPLC-ESI-MS/MS parameters and surrogate/internal standard used. **In bold** transitions used for quantification. (continued)

THERAPEUTIC GROUP OR CLASS	COMPOUND	RETENTION TIME (MIN)	ESI MODE	PRECURSOR ION	PRODUCT ION	DPO/CEN/CXP	SURROGATE/INTERNAL STANDARD	PRECURSOR ION / PRODUCT ION
ß-blocker	Metoprolol	10.2	+	**268.1**	**116.2**	46/27/5	Clenbuterol-d_9	288/206
				268.1	56.2	46/43/5		
	Sotalol	6.8	+	**273**	**133.1**	51/35/5	Sotalol-d_6	279/261
				273	213	51/39/5		
Antibiotic	Sulfamethoxazole	8.8	+	**254.1**	**156.1**	51/23/12	Sulfamethoxazole-d_4	260/162
				254.1	65.1	51/55/5		
	Acetyl-sulfamethoxazole	9.3	+	**296**	**65**	36/55/5	Sulfamethoxazole-d_4	260/162
				296	134.1	36/35/10		
Hypertension regulator	Hydrochlorothiazide	6.9	-	**295.7**	**268.7**	-75/-26/-15	Hydrochlorothiazide-^{13}C-d_2	299/270
				295.7	204.8	-75/-32/-9		
Corrosion inhibitor	1H-benzotriazole	9.8	+	**120**	**64.9**	66/33/12	Benzotriazole-d_4	124/69
				120	92.1	66/27/16		
	4-Methylbenzotriazole + 5-Methylbenzotriazole (4MBT+5MBT)	11.6	+	**134**	**77**	71/39/14	Benzotriazole-d_4	124/69
				134	79	71/29/14		
Pesticides and metabolites	Isoproturon	16.8	+	**207.2**	**72.1**	101/29/12	Isoproturon-d_6	213/78
				207.2	46.1	101/31/20		
	Chloridazon	10.8	+	**222**	**77**	136/53/12	Pentoxifylline-d_5	284/138
				222	65	136/55/12		
	Atrazine	16.8	+	**216.1**	**174.1**	101/27/12	Carbamazepine-d_{10}	247/204
				216.1	68	101/51/10		
	Desethylatrazine	11.8	+	**188.1**	**146**	71/25/22	Phenazon-d_3	192/59
				188.1	79.1	71/37/14		
	Simazine	14.5	+	**202.1**	**104**	86/37/18	Phenazon-d_3	192/59
				202.1	68.1	86/47/10		
	Terbuthylazine	19.4	+	**230.1**	**174**	66/25/12	Isoproturon-d_6	213/78
				230.1	68	66/53/10		
	Metazachlor	16.7	+	**278**	**210**	51/17/12	Phenazon-d_3	192/59
				278	134	51/33/12		

Table 3.5 Compounds analysed, classified by their chemical group, and their retention time, HPLC-ESI-MS/MS parameters and surrogate/internal standard used. **In bold** transitions used for quantification. (continued)

THERAPEUTIC GROUP OR CLASS	COMPOUND	RETENTION TIME (MIN)	ESI MODE	PRECURSOR ION	PRODUCT ION	DPO/CEN/CXP	SURROGATE/INTERNAL STANDARD	PRECURSOR ION / PRODUCT ION
Pesticides and metabolites	Metazachlor-C-metabolite (BH479-4)	10.4	+	**274**	**134**	61/31/10	Clofibric acid-d_4	217/131
				274	161.9	61/13/12		
	Metazachlor-S-metabolite (BH479-8)	10.5	-	**322.1**	**120.7**	-130/-30/-7	Clofibric acid-d_4	217/131
				322.1	147.8	-130/-32/-15		
	Metolachlor	21.6	+	**284.1**	**252**	76/23/6	Metolachlor-d_6	290/258
				284.1	176	76/37/12		
	Metolachlor-C-metabolite (CGA51202/351916)	15.1	-	**278.1**	**206**	-70/-16/-15	Clofibric acid-d_4	217/131
				278.1	173.7	-70/-24/-13		
	Metolachlor-S-metabolite (CGA380168/354743)	14.8	-	**328.1**	**79.9**	-120/-55/-5	Clofibric acid-d_4	217/131
				328.1	120.8	-120/-32/-19		
	Chlortoluron	15.9	+	**213.1**	**72**	81/31/12	Isoproturon-d_6	213/78
				213.1	46	81/29/20		
	Diuron	16.8	+	**233**	**71.9**	101/33/12	Diuron-d_6	239/78
				233	46.2	101/29/6		

CHAPTER 4. RESULTS AND DISCUSSION



The results obtained from the different developed methodologies are presented in this chapter. It is divided in three sections, corresponding to the different families of the target compounds and the methodologies used.

4.1 SPECIATION ANALYSIS OF PHOSPHORUS OXOANIONS

4.1.1 Precedents

Although there are different phosphorus species, with different oxidation states, common environmental analysis are performed as orthophosphate or total phosphorus, through acid hydrolysis and digestion transforming condensed and organic phosphorus species to orthophosphate. In these cases, the determination is carried out by molecular absorption spectrometry (UV) or by ion chromatography with conductivity detection [12, 158-160].

Phosphorus speciation analysis is generally performed by IC [18, 140] with different detectors: suppressed conductivity detector, indirect UV absorption or Fluorescence [12], requiring in some cases a pre or post column derivatisation to increase the detection selectivity. Separation of phosphorus species can also be done by CE using conductivity, UV and MS detectors [161].

ICP-AES or ICP-MS detection provides excellent analytical characteristics in elemental speciation analysis, including high sensitivity (specially ICP-MS) and selectivity, a wide linear range, and high compatibility with the most used mobile phases in HPLC [120, 162]. ICP detectors can be coupled to classical IC [141, 154, 163], HPLC [152, 164-167] and, in the case of organophosphorus pesticides and phosphonate flame retardants, to GC [137, 168].

However, the determination of phosphorus by ICP-MS is limited by its high first ionisation potential (10.5 eV), resulting in low phosphorus ionisation degree taking place in the plasma, as well as the presence of polyatomic interferences. The use of high resolution instruments and the introduction of collision/reaction cell devices can help to overcome spectroscopic interferences [169]. When using pressurised reaction cells, two possible approaches can be considered: either to remove the interfering ions from the *m/z* of interest or to remove the analyte ion to a non-

interfered *m/z*. In this latter case, the processes which are most generally used are hydrogen atom-transfer and oxidation reactions. Phosphorus oxidation to $^{31}P^{16}O^{+}$ has been used in different studies [170, 171]. However, many ICP-MS instruments are unable to be set up for the performance of oxidation reactions. Recent studies explore the option of measuring phosphorus at *m/z* 47 without the addition of oxidation gases [16]. In order to increase the degree of ionisation in the plasma for elements that have a low level of ionisation, the addition of carbon-containing compounds has been proposed [172, 173]. However, in the case of phosphorus determination by ICP-MS, according to published data, it may not be possible to improve some of the analytical parameters such as SBR [174-176].

Another advantage of ICP-based detection systems is that they are elemental techniques whose responses should be independent of the molecular species, making it possible to obtain a complete calibration curve from a single injection. In the single-injection calibration approach (SICA) a solution containing different concentrations of the compounds is injected into the system and the peak areas obtained for each compound are plotted against the mass content of the element measured [177, 178].

Until now, phosphorus speciation analysis has been focused in different types of phosphorus species; speciation of simple oxoanions (orthophosphate, phosphite and hypophosphite) in water samples [9, 140, 163], or speciation of condensate phosphates [18, 179]. However, there is a lack of speciation studies which include the determination of simple oxoanions and condensed oxoanions. Therefore in this thesis a speciation methodology, including simple and condensate oxoanions has been developed.

The results obtained in the development of the speciation analysis methods are divided according to the detection system used. Moreover, the study of the chromatographic separation conditions is described in the section dedicated to the development of the LC-ICP-AES method, meanwhile the same chromatographic separation are used with ICP-MS detection

4.1.2 Phosphorus speciation using HPLC-ICP-AES

For the development of the speciation method we started with the study of the detection conditions, followed by the study of the chromatographic separation.

4.1.2.1 ICP-AES detection

The first step in the method development is to study and adjust the conditions affecting P detection by ICP-AES such as the addition of methanol to the solution and the type of nebulizer.

4.1.2.1.1 Addition of methanol

Different studies concluded that the addition of small amounts of methanol in the samples improved their nebulisation and, moreover, other studies reported an increase in sensitivity of some atomic emission lines in ICP-AES due to the presence of carbon containing compounds [180, 181]. The energy transfer from metastable carbon atoms is considered as a possible pathway of enhancement emission intensity. However, it is also reported that the addition of large quantities of organic solvent can cool the plasma and therefore reduce analyte sensitivity [176]. For this reason, a study of the effect of methanol addition on the ICP-AES response to the phosphorus detection was done. To perform this study, different calibration curves with different amounts of methanol were prepared. The results are shown in **Figure 4.1**. With the addition of methanol to a P standard solution (10 mg P L^{-1}) an increase in the sensitivity of phosphorus detection was observed, as well as an increase in the signal/ background ratio (SBR) (12, 16 and 16 for 0, 2 and 4% (v/v) of methanol, respectively). From these results, the addition of 2% (v/v) of MeOH to the sample solution is the option selected because a significant increase on the sensitivity is obtained without observing neither degradation of the plasma nor reduction of the signal.

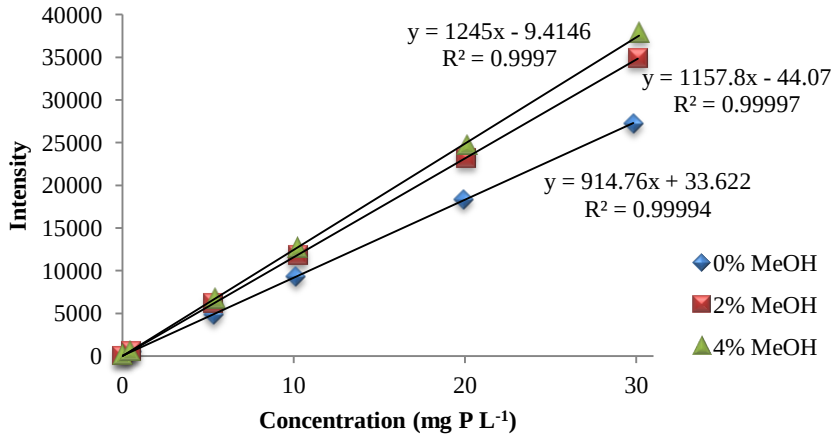


Figure 4.1 Effect of the addition of methanol in phosphorus ICP-AES response.

4.1.2.1.2 Sensitivity for the different phosphorus species

Previous studies have proved that different species with the same content of an element can contribute differently to the ICP-AES response [182]. To investigate this, five calibration curves, corresponding to each phosphorus species, were obtained. The obtained slopes (mean ($\pm tS_b$)) were 820 (±9), 837 (±14), 849 (±8), 825 (±10) and 812 (±17) corresponding to orthophosphate, phosphite, hypophosphite, pyrophosphate and tripolyphosphate, respectively. The calculated slopes are very similar with confidence intervals overlapped in the majority of cases. This result can be explained by the fact that any of the studied species is volatile or semi-volatile and their behaviour is very similar during nebulisation and vaporisation on the ICP-AES detector.

4.1.2.1.3 Nebuliser system

The behaviour of babington and concentric pneumatic nebulisers was also compared. The basic function of a nebuliser is to convert a sample solution to an aerosol. It is important for a nebuliser to generate an extremely fine mist because only the smallest droplets are efficiently transported into the plasma where they undergo desolvation, vaporisation, and atomisation and/or ionisation.

The babington nebulizer was evaluated at room temperature (25 ºC) and the concentric nebulizer, which has the option to be used with a thermostatic chamber, was evaluated at four temperatures (25 ºC, 30 ºC, 40 ºC and 50 ºC). In **Figure 4.2** it can be observed that an increase in temperature caused an increase in intensity. This can be explained in terms of an increase in the aerosol transport efficiency caused by an enhancement in the solvent evaporation. However, background signal also increased. At room temperatures (25 - 30 ºC) the sensitivity improvement was not important whereas above 40 ºC the plasma became highly inestable. Under these conditions (room temperatures), the obtained sensitivities were slightly lower than those furnished by the babington nebulizer adapted to the double pass spray chamber. For these reasons and for simplicity, the use of a babington nebulizer at room temperature was selected.

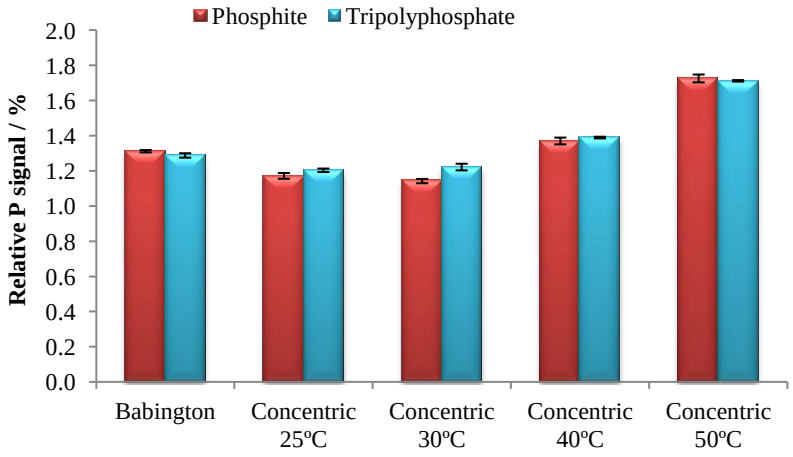


Figure 4.2 Relative phosphorus signal with two different nebulizers at different temperatures, for selected compounds (For the rest of the compounds the behaviour was the same). Bars correspond to the emission signal divided by the phosphorus concentration of each species.

4.1.2.2 Chromatographic separation

The first point to achieve the chromatographic separation was the selection of the column. Checking previous published studies, we found that PRP-X100 was an anion exchange column commonly used in speciation analyses of phosphorus and other anions [12, 183-185]. In addition, its polymeric support allows working at a

wide pH range, from 1 to 13. For these reasons we decided to use this column in our method.

4.1.2.2.1 Choice of the mobile phase

The retention of anion species on HPLC anion exchange column is dependent on the pK_a of the species and mobile phase variables (pH, ion strength, flow rate and temperature). We tested four different mobile phases with different pHs. As shown in **Table 4.1**, with acetic-acetate buffer (pK_a 4.75) and by injecting a sample with the five species we only obtained one peak. Then we tested formic-formate buffer (pK_a 3.75), a chromatogram with 3 peaks corresponding to orthophosphate, phosphite and hypophosphite was obtained. However, orthophosphate and phosphite appeared nearly overlapped. In previous works [153, 154], the use of citric-citrate buffer (pK_a 2.94) was described for phosphorus speciation. With this buffer three separated peaks were obtained (orthophosphate, phosphite and hypophosphite) but it was not possible to elute the other two species (pyrophosphate and tripolyphosphate). Pyrophosphate and tripolyphosphate are the most acidic species (for pK_a values see **Table 1.1**) and at the working pH they are only partially protonated, so they are strongly retained in the column. Therefore, we tested ammonium nitrate [141] as a mobile phase. The main advantage of this mobile phase was that retention times were lower than those obtained using other mobile phases. Furthermore, it is easy and faster to prepare, there are not dissolved solids and a lower pH can be obtained. With an ammonium nitrate mobile phase, pyrophosphate eluted efficiently however tripolyphosphate was still retained. For this reason, we decided to study additional variables affecting the chromatographic separation.

Table 4.1. Comparison of eluents for the separation of orthophosphate, phosphite, hypophosphite, pyrophosphate and tripolyphosphate.

ELUENT		ORTHOPHOSPHATE		PHOSPHITE		HYPOPHOSPHITE		PYROPHOSPHATE		TRIPOLYPHOSPHATE
	pH	R_T (min)	R_s Orthophosphate/ Phosphite	R_T (min)	R_s Phosphite/ Hypophosphite	R_T (min)	R_s Hypophosphite/ Pyrophosphate	R_T (min)	R_s Pyrophosphate/ Tripolyphosphate	R_T (min)
50 mM acetic-acetate	4.75	4.9	-	4.9	-	4.9	-	-	-	-
100 mM formic-formate	3.30	4.8	0.43	5.0	1.05	5.7	-	-	-	-
15 mM citric-citrate	2.30	5.4	1.79	7.3	1.44	9.3	-	-	-	-
10 mM Ammonium nitrate	2.00	2.5	1.30	3.6	0.85	4.4	9.67	16.2	-	-

R_T: retention time; $R_s = \frac{(R_{Ta} - R_{Tb})}{\frac{1}{2}(W_a + W_b)}$ (resolution); W: peak width

4.1.2.2.2 Effect of the pH of the mobile phase

During the method development we observed that pH had an important role in the chromatographic separation. As previously indicated, the studied species had a strong acidic character, and a low pH of the mobile phase is needed to elute efficiently all the compounds from the column. A study of the effect of the pH was done. The study was performed with a pH from 1.8 to 2.3 with MilliQ water and nitric acid as a pH modifier. At pH lower than 1.8 the polymeric support can suffer structural changes whereas at pH higher than 2.3 the chromatographic separation was degraded. The results shown in **Table 4.2** demonstrated the importance of the pH in the chromatographic separation and that low pHs allow the elution of pyrophosphate. However, the elution of tripolyphosphate was still not achieved at this pH range and orthophosphate, phosphite and hypophosphite resolution was degraded at low pH. These results suggested that a gradient elution was necessary.

Table 4.2 Effect of the pH on the chromatographic separation with MilliQ water and nitric acid as a pH modifier.

pH	R_S Orthophosphate/ Phosphite	R_S Phosphite/ Hypophosphite	R_T (min) Hypophosphite	R_T (min) Pyrophosphate
2.3	4.25	3.30	19.6	-
2.2	3.86	2.47	15.4	-
2.1	3.10	1.74	11.9	-
2.0	2.46	1.66	7.7	35.0
1.9	2.00	1.29	6.0	27.9
1.8	1.70	1.00	5.0	25.4

R_T: retention time; $R_s = \frac{(R_{Ta} - R_{Tb})}{\frac{1}{2}(W_a + W_b)}$ (resolution)

4.1.2.2.3 Gradient design

The main challenges of the chromatographic separation were the elution of tripolyphosphate and the reduction of pyrophosphate retention time. Previous studies revealed that when the mobile phase consisted of monovalent anions, high concentrations of them are required to elute highly retained anions [12]. In our case,

NO_3^- was the exchanger anion. Based on this premise, a pH and an ionic strength gradient were investigated. Moreover, as the addition of small amounts of organic modifiers to the mobile phase allows the elution of highly retained anions on anionic exchange chromatographic systems [18, 169] and the addition of methanol increases the sensitivity of phosphorus detection by ICP-AES, methanol was incorporated to the mobile phase composition.

The first mobile phase tested consisted of 4 mM ammonium nitrate at pH 2.2 with 2% (v/v) MeOH (A), lower pH and higher ionic strength was not desirable because it would have negative consequences for the orthophosphate, phosphite and hypophosphite resolution. Mobile phase (B) (with 2% (v/v) MeOH) had a pH value of 1.8 based on the results present on **Table 4.2**. The results obtained with several gradient times and varying the ammonium nitrate concentration in mobile phase B are shown in **Table 4.3**. It can be observed in this table that tripolyphosphate is finally eluted by increasing the ionic strength. The selected ammonium nitrate concentration was 200 mM and the selected gradient was: 100% A from 0 to 6 min; 100% A to 100% B from 6 to 9 min; 100% B from 9 to 13 min; and 100% B to 100% A from 13 to 15 min.

Table 4.3 Effect of ammonium nitrate concentration in mobile phase using a gradient elution with an initial mobile phase of 4 mM ammonium nitrate at pH 2.2. Orthophosphate and phosphite retention times are not shown because the objective of this study was the elution of pyrophosphate and tripolyphosphate.

NH_4NO_3 (mM) CONCENTRATION	R_T (MIN) HYPOPHOSPHITE	R_T (MIN) PYROPHOSPHATE	R_T (MIN) TRIPOLYPHOSPHATE
15	8.4	17.8	-
30	9.6	14.0	-
80	7.5	13.9	20.3
100	5.9	9.7	12.3
200	5.2	9.1	9.9

R_T: retention time

Flow rate is another important variable for chromatographic separations. Therefore, two flow rates were tested: 1 mL min^{-1} and 1.5 mL min^{-1}. Higher flow rate were not

considered because were not compatible with the ICP-AES. At 1.5 mL min^{-1} peak shape was better, total chromatogram time was smaller and resolution was not worse than at 1 mL min^{-1}, so we finally chose this higher flow rate.

4.1.2.3 Calibration

The linearity of the standard curves was investigated in the range from limit of quantification (LOQ) to 40 mg P L^{-1} with the selected conditions. The peak area of the analyte was used for quantification. Linear regression curves (n=5) were obtained with determination coefficients higher than 0.99 for all the analytes (**Table 4.4**).

Table 4.4 Linearity, LODs and LOQs (n=5).

ANALYTE	SLOPE±tS_B	INTERCEPT±tS_A	r^2	LOD (mg P L^{-1})	LOQ (mg P L^{-1})
Orthophosphate	1164±55	-600±1500	0.9993	1	5
Phosphite	1140±120	-800±2900	0.997	3	9
Hypophosphite	1320±240	-500±5500	0.990	5	15
Pyrophosphate	1384±66	-1200±1600	0.9993	1	4
Tripolyphosphate	1383±81	-5000±2000	0.9996	1	3

Limits of detection (LOD) in the range 1 - 5 mg P L^{-1} and LOQ in the range 3 - 15 mg P L^{-1} were obtained. These values were calculated as three and ten times the regression residual standard deviation ($\sigma_{y/x}$), respectively [186]. The intra-day and inter-day precision were evaluated by analysing a 20 mg P L^{-1} solution of each analyte. RSD values between 1 - 6% and 2 - 7%, respectively, were achieved. A chromatogram at this concentration level is displayed in **Figure 4.3**.

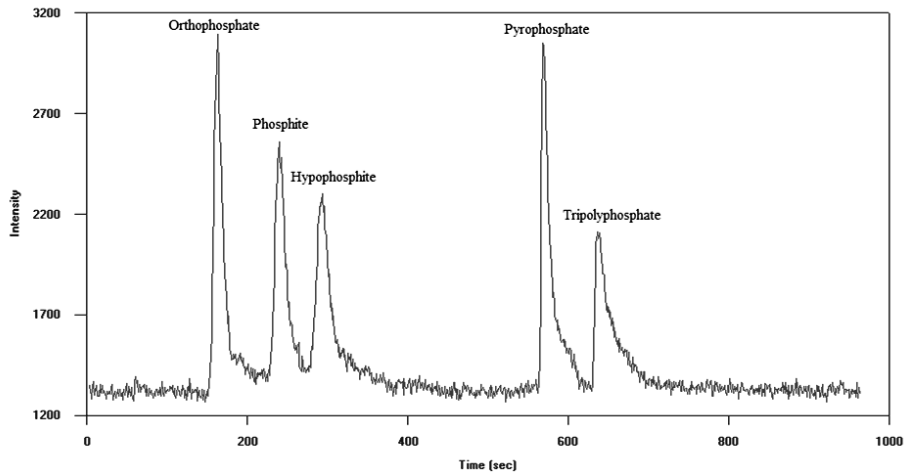


Figure 4.3 Chromatogram of MilliQ water spiked at 20 mg P L^{-1} of each species.

4.1.2.4 Efficiency of the method

Finally, the accuracy of the HPLC-ICP-AES developed method was evaluated by analysing spiked water samples due to the lack of reference materials containing all the phosphorus species. Tap water and effluent wastewater have been spiked at two concentration levels (5 mg P L^{-1} at the low level and 20 mg P L^{-1} at the high level) (n=3). Previously, both types of samples were analysed in order to ensure that any of the phosphorus species were already present resulting in the detection of orthophosphate in effluent wastewater. However, its quantification was not possible. The recoveries obtained are shown in **Table 4.5**. Therefore, intensity values from spiked effluent wastewater have been corrected with the intensity obtained from non-spiked effluent wastewater before calculation of the recoveries. For tap water recoveries ranged from 90.8% to 126.8% and from 91.5% to 142.1% for effluent wastewater. The recoveries obtained indicate that the method developed can be applied to the speciation analysis of phosphorus oxoanions in complex matrices such as effluent wastewaters.

Table 4.5. Recoveries from spiked water at 5 and 20 mg P L^{-1} by HPLC-ICP-AES (n=3).

		RECOVERY % (RSD, %)				
Water type	Spiked level (mg L^{-1})	Orthophosphate	Phosphite	Hypophosphite	Pyrophosphate	Tripolyphosphate
Tap water	5	110 (10)	120 (5)	100 (8)	90 (10)	126 (7)
	20	106 (5)	105 (6)	103 (1)	106 (4)	103 (3)
Effluent wastewater	0	< LOQ	n.d.	n.d.	n.d.	n.d.
	5	119 (7)	125 (7)	142 (5)	113 (3)	142 (4)
	20	110 (13)	111 (5)	91 (8)	112 (5)	103 (1)

4.1.3 Phosphorus speciation using HPLC-ICP-MS

Although the separation of the five phosphorus species was achieved, the sensitivity and detection limits were not good enough to determine real concentrations of some of the phosphorus species in water samples.

The use of an ICP-MS as a detector is considered a technique of choice in trace element speciation. However, its application to the determination of phosphorus is compromised by its high first ionisation potential (10.5 eV) and the presence of polyatomic interferences.

4.1.3.1 Study of the ICP-MS operating conditions

Different parameters affecting the determination of ^{31}P by ICP-MS were carefully studied in order to improve analytical sensitivity. The low degree of ionisation in the plasma source, reported at around 33% [187] and the presence of spectroscopic interferences ($^{14}N^{16}O^{1}H^{+}$ and $^{15}N^{16}O^{+}$) were two issues that needed addressing. The use of an ICP-MS equipped with an ORC can reduce spectroscopic interferences and maximise the signal-background ratio. The operating conditions to be controlled were sample uptake rate, nebulizer gas flow rate, sampling depth, and applied RF power [188]. In our case, the sample uptake rate could not be adjusted since it was set by the HPLC mobile phase flow rate.

In order to remove interfering ions from the *m/z* of interest, collision gases are added to the ORC. Helium and hydrogen, which are the most commonly used collision gasses, were investigated in the pressurised cell using a 100 μg L^{-1} phosphorus solution containing 2% (v/v) of MeOH, as this was the amount added to the mobile phase. As is reported in the literature [141, 142], the use of collision gasses reduces both the background and the signal and so it is necessary to find a balance between the two. Ramp flows of He and H_2 were tested, and He at a flow rate of 2.5 mL min^{-1} was found to give the best SBR as the minimum background equivalent concentration (BEC, μg L^{-1}) was obtained. We then studied parameters affecting the determination of *m/z* 31 by comparing vented and pressurised cell conditions.

4.1.3.1.1 Addition of methanol

The addition of small amounts of carbon-containing compounds, such as methanol, has been reported to enhance the ionisation degree of analytes with high ionisation potentials in ICP-MS. The mechanisms proposed as explaining this effect are (a) improvement in ionisation due to the charge transfer reaction from the positive carbon species to analyte atoms, (b) the improvement in nebulisation processes as a result of methanol being more volatile than water and (c) the shift of the zone of maximum ion density in the plasma [188, 189]. Calibration curves with 2% (v/v) of methanol and without methanol were calculated using vented and pressurised cell conditions. The results showed a five-fold increase in the sensitivity independently of the cell conditions used (**Figure 4.4**). The SBR obtained analysing an 8 μg P L^{-1} solution was twice as large when using methanol (1.6 and 1.9 without methanol, and 3.4 and 3.9 with 2% of methanol when vented and pressurised cell conditions were used, respectively). Our results differ from those obtained by Kovacevic et al. [174] and by Carr et al. [176]. In the first case, the authors found that SBR decreases with the addition of large amounts of methanol. In the second case, the authors concluded that the sensitivity of phosphorus detection by ICP-MS decreased significantly when methanol was used together with certain buffer solutions. However, in both cases, the amounts of methanol studied were higher than those included in the present study.

Therefore, from our results the presence of 2% of methanol in the mobile phase has a positive effect on the determination of ^{31}P by ICP-MS, enhancing the sensitivity and the SBR of the detection.

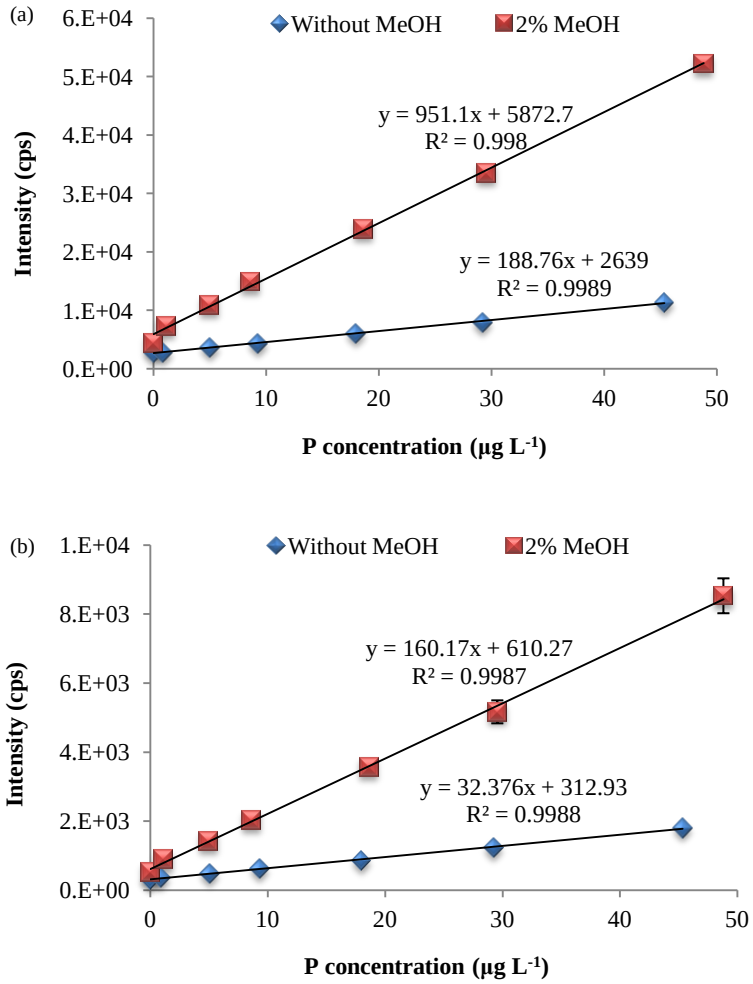


Figure 4.4 Effect of the addition of methanol in ^{31}P ICP-MS response with a vented cell (a) and a pressurised cell (He: 2.5 mL min^{-1}) (b).

4.1.3.1.2 Radio frequency power (RF) and sampling depth

Before study the best operational conditions for the phosphorus determination ion lens parameters were adjusted in order to increase the SBR. The signal of 100 µg L^{-1} phosphorus solution containing 2% of methanol was measured using the mobile phase B (200 mM of NH_4NO_3 and 2% of MeOH) as the background.

RF powers of 1300, 1400 and 1500 W were tested with sampling depth distances ranging from 4.5 to 7 mm and with a 1.1 L min^{-1} flow rate of carrier gas. **Figure 4.5a** shows the experimental SBR values obtained using vented cell conditions. The maximum SBR (9.6) was obtained at 1400 W and 6 mm. Using pressurised cell conditions (**Figure 4.5b**), a higher SBR (24.1) was obtained at 1300 W and 6 mm. These results would indicate that elements with high ionisation energies, such as phosphorus, require longer residence times inside the plasma. This can be done by increasing the sampling depth.

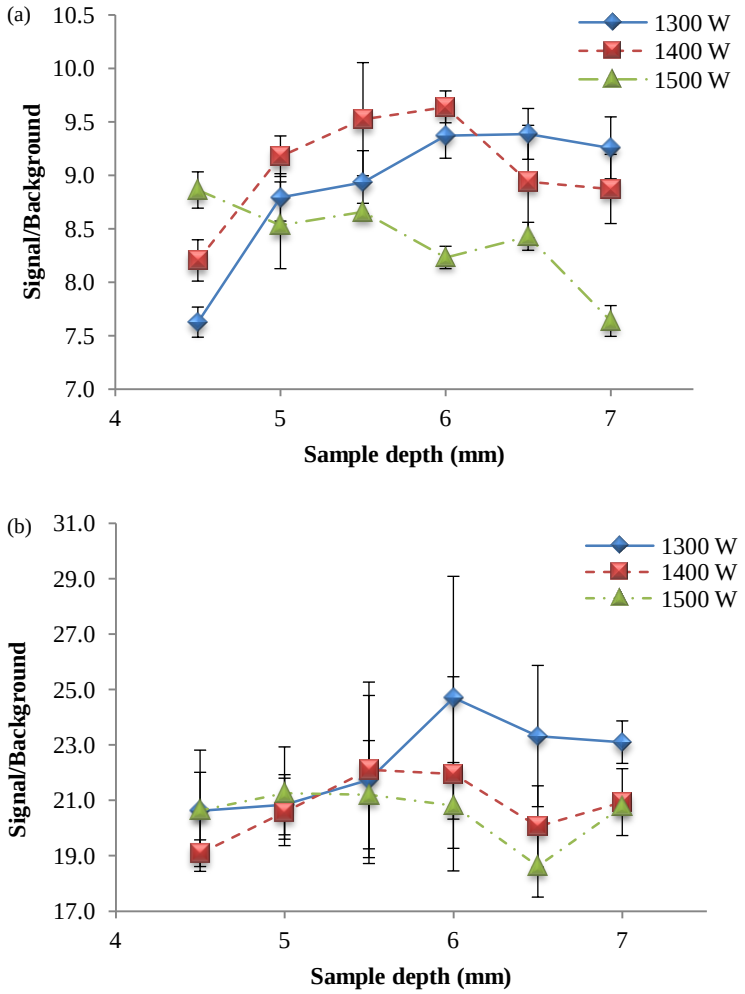


Figure 4.5 Effect of the sample depth at different RF powers (n=3) using a vented cell (a) and a pressurised cell (He: 2.5 mL min^{-1}) (b).

4.1.3.1.3 Carrier gas

The effect of the carrier gas flow rate was also investigated by varying it from 0.9 to 1.25 L min^{-1}. The highest SBR value using vented cell conditions was obtained between 1.1 and 1.15 L min^{-1} (**Figure 4.6**). In order to select the maximum SBR value, the flow was then varied from 1.05 to 1.15 L min^{-1}. No significant SBR differences were obtained in this range and a carrier gas flow rate of 1.12 L min^{-1} was finally selected. A maximum SBR value of 28.1 was found with pressurised cell conditions and a carrier gas flow rate of 1.1 L min^{-1} (**Figure 4.6**). The use of lower carrier gas flow rates improved the energy transfer into the plasma but at the same time can reduce the nebulisation efficiency decreasing the sensitivity.

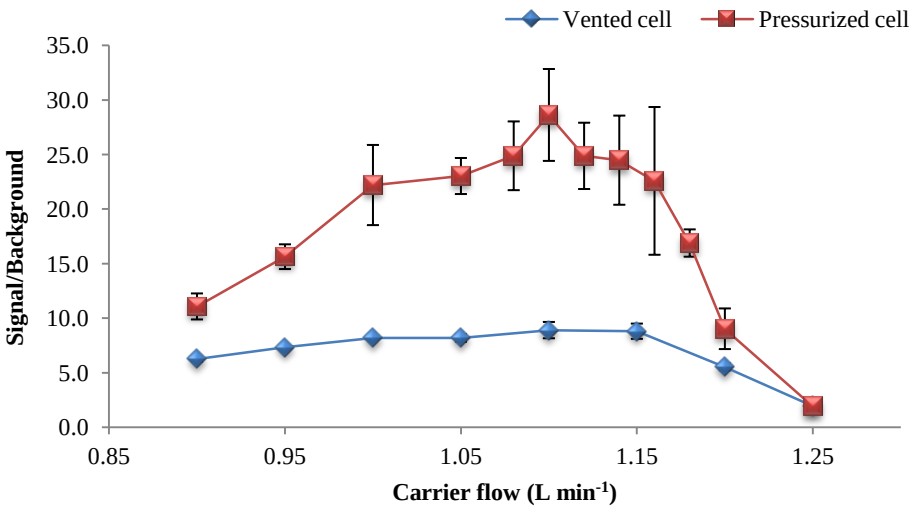


Figure 4.6 Effect of the carrier gas flow rate on SBR using a vented cell and a pressurised cell (He: 2.5 mL min^{-1}).

Comparing all the results obtained with vented and pressurised cell conditions, it can be seen that the highest SBR values were found under pressurised cell conditions. We, therefore, decided to conduct a more careful study of the operating conditions with the pressurised cell.

4.1.3.1.4 He flow rate

The effect of He flow rate on the sensitivity of phosphorus determination was studied in the conditions of the chromatographic separation and using the

instrumental conditions which gave the highest SBR. A solution containing 100 µg L^{-1} of each phosphorus species (phosphite, hypophosphite, pyrophosphate and orthophosphate) and 500 µg P L^{-1} of tripolyphosphate was injected into the HPLC-ICP-MS system using different He flow rates in the ORC. The results showed that the maximum SBR for all the species was achieved with a He flow rate of 1.5 mL min^{-1} (**Figure 4.7**). Given this result, 1.5 mL min^{-1} of He was selected for further experiments.

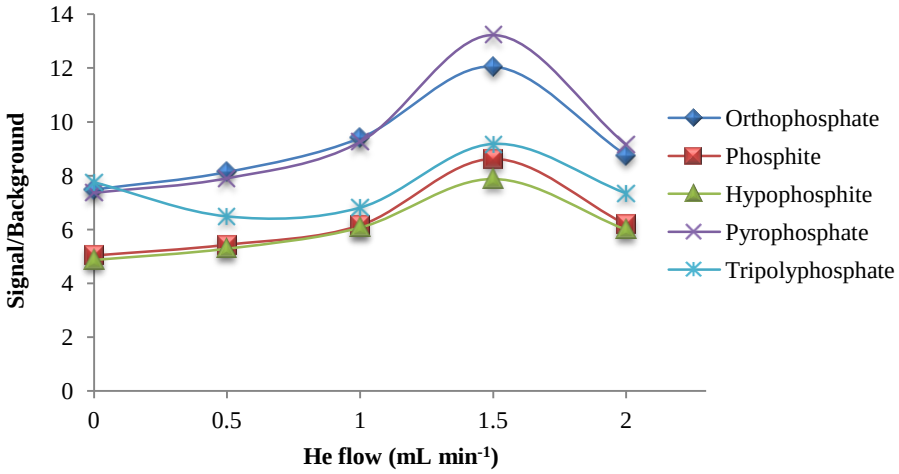


Figure 4.7 Effect of the He flow rate on SBR under pressurised cell conditions with HPLC-ICP-MS.

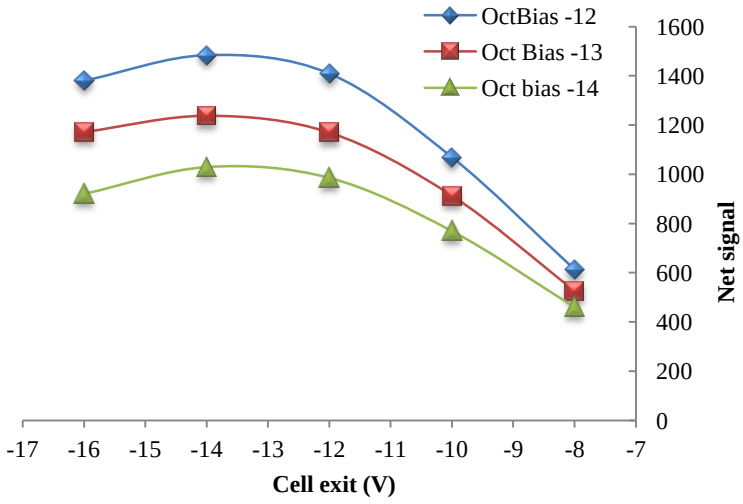


Figure 4.8 Cell exit and Octopole bias voltage effect on net signal at helium 1.5 mL min^{-1}.

4.1.3.1.5 Lens voltage

Quadrupole bias voltage (QPbias), octopole bias voltage (Octbias) and cell exit voltage values are important parameters to be studied when ORC is used in ICP-MS. In our case, and taking into account previous experiments, QPbias voltage was fixed at -10 V whereas Octbias voltage was tested at -14, -13 and -12 V and cell exit voltage was varied from -17 to -5 V. All voltages were chosen following manufacturer's recommendations. The maximum value of the net signal (the difference between phosphorus and background signals) was achieved operating at -12 V for Octbias and at -14 V for the cell exit (**Figure 4.8**). In these voltage conditions, an increase in the phosphorus signal was obtained whereas the background signal decreased or remained the same.

The effect of RF power and sample depth on SBR values was again studied under the selected He flow rate and with the mentioned OctBias and cell exit voltages. The same results were obtained as before.

A chromatogram obtained under the selected conditions is shown in **Figure 4.9**.

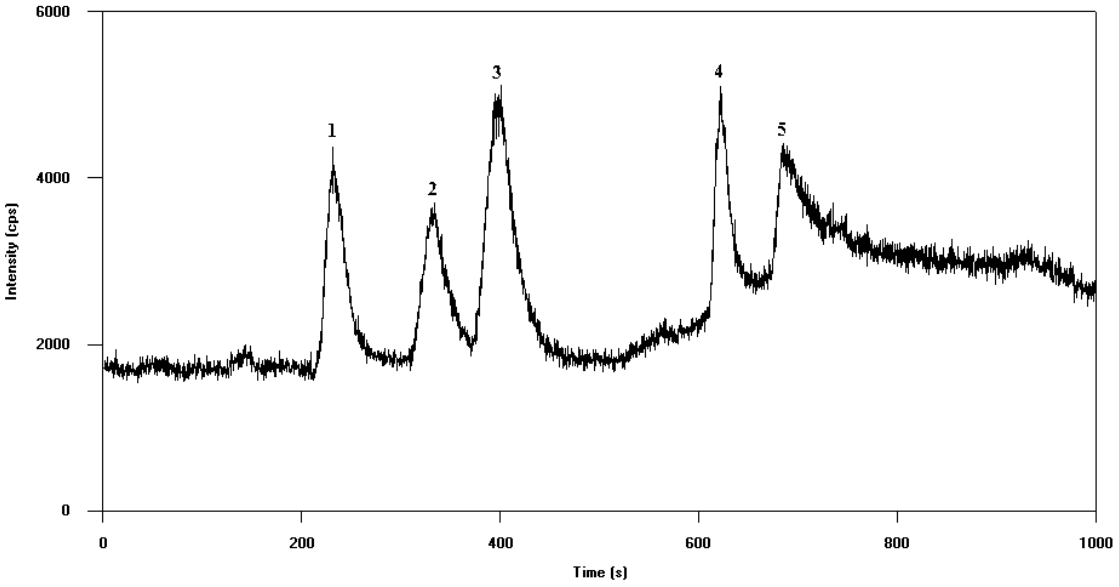


Figure 4.9 Chromatogram of MilliQ water spiked at 100 µg P L^{-1} of orthophosphate, phosphite, hypophosphite and pyrophosphate, and at 500 µg P L^{-1} of tripolyphosphate. 1. Orthophosphate, 2. Phosphite, 3.Hypophosphite, 4.Pyrophosphate, 5.Tripolyphosphate.

4.1.3.2 Calibration

Operating the pressurised cell and the ICP-MS instrument under the best conditions in order to achieve the maximum SBR, the linearity of the standard curves, plotting peak areas against concentrations, was investigated in the LOQ - 600 μg L^{-1} range, although the linear range could be larger. The determination coefficients (r^2), slopes and intercepts are given in **Table 4.6**. The method was linear for all compounds, with r^2 greater than 0.997.

Table 4.6 Linearity, LODs and LOQs for conventional calibration method (n=7).

ANALYTE	RT (MIN)	SLOPE ± tS_B	INTERCEPT ± tS_A	R^2	LOD (μg P L^{-1})	LOQ (μg P L^{-1})
Orthophosphate	3.8	1400 ± 40	8000 ± 12000	0.9994	14	46
Phosphite	5.5	1200 ± 20	7000 ± 5000	0.9998	7	23
Hypophosphite	6.7	1400 ± 50	13000 ± 21000	0.9990	24	81
Pyrophosphate	10.3	1200 ± 40	22000 ± 14000	0.9991	18	60
Tripolyphosphate	11.4	1200 ± 90	86000 ± 30000	0.997	30	101

RT: Retention time; S_b: Standard deviation of the slope; S_a: Standard deviation of the intercept

Precision was evaluated by determining the relative standard deviation (RSD) at two concentration levels: 100 μg L^{-1} and 400 μg L^{-1} (n=3). Intra-day and inter-day precision ranged from 1% to 5% and from 0.2% to 4% at both levels, respectively. The LOD and LOQ were calculated as three and ten times the regression residual standard deviation ($\sigma_{y/x}$), respectively [186]. LODs in the 7 - 30 μg P L^{-1} range and LOQs in the 23 - 101 μg P L^{-1} range were obtained. A comparison of the LODs of the developed method with those reported in the literature is summarised in **Table 4.7**. As can be seen, the values obtained are comparable to those calculated using different methodologies. The use of a SICA [177] could drastically reduce the analysis time and cost. Unlike ICP based detectors, other detectors such as the conductivity one provide different sensitivity as a function of the phosphorous compound, and thus SICA cannot be applied. For the application of this calibration approach it is necessary to have statistically similar sensitivities. This similarity was demonstrated in the case of the different phosphorus oxoanions by applying an ANOVA test to the slopes (**Table 4.6**) obtained with the conventional calibration

method (F_{Value}=3.06 < F_{Tab}=3.48). In applying this method, a standard solution containing 93 μg P L^{-1} of orthophosphate, 200 μg P L^{-1} of phosphite, 350 μg P L^{-1} of hypophosphite, 400 μg P L^{-1} of pyrophosphate and 700 μg P L^{-1} of tripolyphosphate was analysed and the peak areas obtained for each compound were plotted against the phosphorus content, obtaining a full calibration. The calibration curve showed a good determination coefficient (r^2=0.998), and a LOD of 32 μg P L^{-1} and a LOQ of 106 μg P L^{-1} were calculated. The standard solution was injected five times and the precision for each compound was calculated obtaining RSD values ranging from 1.4 to 5.0%.

Table 4.7 Comparison of calculated LOD (µg P L^{-1}) of different phosphorous species.

ORTHOPHOSPHATE	PHOSPHITE	HYPOPHOSPHITE	PYROPHOSPHATE	TRIPOLYPHOSPHATE	OTHER SPECIES	CALCULATION METHOD	REFERENCE
11	12	26				a	[9]
20			30	50		b	[179]
	4.5					c	[190]
1			2.3	39		Not mentioned	[191]
					1-6.5	Not mentioned	[192]
					8-10	c	[166]
10	7	20	20	30		d	This study

a. three times the SD of the noise of ultrapure water injection. b. Lowest concentration of the analyte that could be distinguished from a blank. c. Peak height response which is three times the average background signals from blank samples. d. three times the regression residual standard deviation ($\sigma_{y/x}$).

4.1.3.3 Efficiency of the method

Due to the lack of water reference material containing the five phosphorus oxoanions included in the present study, the accuracy of the method was determined by analysing spiked wastewater samples. Two samples were collected at the outlet of the secondary units of two WWTPs and were analysed prior to fortification. As orthophosphate was detected in both samples, this species was not included in the quality control procedure. The two effluent wastewater samples, from Castell-Platja d'Aro and Blanes plants, were spiked at two concentration levels for each species (200 μg P L^{-1} at the low level and 500 μg P L^{-1} at the high level). The recoveries obtained (**Table 4.8**) ranged from 82% to 101% at the low level and from 80% to 103% at the high level, which indicates the feasibility of using the developed method in the analysis of treated wastewater samples.

Table 4.8 Recoveries from spiked effluent wastewater samples at 200 and 500 μg P L^{-1} of each species.

		RECOVERY (RSD, %) N=3			
Water type	Spiked level (μg L^{-1})	Phosphite	Hypophosphite	Pyrophosphate	Tripolyphosphate
Effluent WW Blanes	200	95 (2)	101 (2)	82 (2)	92 (4)
	500	98 (2)	97 (4)	87 (1)	87 (3)
Effluent WW Castell	200	99 (4)	100 (2)	95 (8)	85 (8)
	500	100 (0.3)	103 (1)	94 (2)	80 (5)

Blanes: Conductivity 1433 μS cm^{-1}, pH 7.2, COD 38 mg L^{-1}; Castell: Conductivity 1144 μS cm^{-1}, pH 7.6, COD 31 mg L^{-1}.

4.1.3.4 Analysis of wastewater samples

Two effluent wastewater samples were analysed using conventional calibration. The two samples were filtered and injected, and a single peak corresponding to orthophosphate was obtained in both cases. Due to the high concentration of this species, water samples were diluted four times and analysed again resulting in concentrations of 1969 μg P L^{-1} in Blanes wastewater and 997 μg P L^{-1} in Castell-

Platja d'Aro wastewater (**Table 4.9**). These results are in agreement with those obtained by the standard official method [193, 194].

The same wastewater samples were then analysed applying the single-injection calibration approach in order to evaluate this method's feasibility. The samples were diluted and analysed three times. The orthophosphate concentrations determined using both calibration systems were statically compared by applying the F- and t-tests. No significant differences were found between the results obtained with the conventional calibration and the SICA showing this calibration approach to be useful for phosphorus oxoanions speciation analysis (**Table 4.9**).

Table 4.9 Analysis of wastewater samples using conventional calibration and single-injection calibration approach (SICA).

		ORTHOPHOSPHATE			
Water type	Calibration method	Concentration ($\mu g\ P\ L^{-1}$)	RSD (%) (n=3)	F-value[a]	t-value[a]
Effluent WW Blanes	Conventional	1969	1	12.6	1.39
	SICA	1913	4		
Effluent WW Castell	Conventional	997	2	29.8	1.8
	SICA	1093	8		

[a] Tabulated F-value for two degrees of freedom at P (0.95) is 39 and tabulated t-value for four degrees of freedom at P (0.95) is 2.78.

4.2 PDMS ROD EXTRACTION OF ENDOCRINE DISRUPTING COMPOUNDS

4.2.1 Precedents

In recent years, a large number of studies have investigated the development of analytical methods and occurrence of endocrine disrupting compounds (EDCs) (flame retardants and phenolic compounds) in several matrices such as biological samples [195-199], air and dust [200-202], sediments, soils and sewage sludge [203-207], food [36, 208], and water [145, 202, 208, 209].

Polluted water is one of the main exposure pathways both for humans and wildlife. However, direct determination of EDCs in aqueous matrices is not possible due to their trace level and thus, a sample extraction/preconcentration step is required.

Stir bar sorptive extraction (SBSE) has been extensively used for the extraction of phenolic compounds from water samples [113, 210-213], and some applications have been developed for FRs [114, 214]. As has been explained in the introduction, polydimethylsiloxane (PDMS) rods have been proposed as an alternative to the use of SBSE.

The most applied instrumental technique for flame retardants is GC, due to in general they are volatile compounds. The detection can be done either by electron capture detector or mass spectrometry detector [46, 99, 215]. However, seeing the degradation problems that have sometimes experienced certain congeners, i.e. BDE-209, and the different nature of brominated flame retardants different than those included in this thesis, LC based methods can be also used [97, 216, 217].

For phenolic compounds separation can be carried out by LC or GC using different detection systems such as MS [145, 218], DAD [31], ECD [33], and electrochemical detection (ED) [219]. In the case of LC-MS analysis, low detection limits can be achieved but the presence of matrix components in the samples may produce signal suppression or enhancement when ESI is used. Furthermore, this technique needs to

be performed by highly skilled technicians and requires the use of isotope-labelled compounds (ILC) as internal standards [220].

In this thesis, the use of PDMS rod has been applied to the extraction and preconcentration of brominated flame retardants, including as a novelty PBDE and non-PBDE, prior their analysis by GC/MS. PDMS rod has also been applied for the first time to extract and preconcentrate bisphenol A, nonylphenol, octylphenol, trichlorophenol and pentachlorophenol, before performing HPLC-DAD analysis. In the following sections the results obtained in the development of the analytical methodologies for the determination of both groups of endocrine disruptor compounds are discussed. For each group, the results are divided in those obtained in the study of the sample treatment conditions and in those related to the chromatographic analysis.

4.2.2 Flame retardants

4.2.2.1 Study of PDMS rod desorption conditions

For the study of the PDMS rod desorption conditions, several parameters were adjusted, such as desorption solvent and time, and the necessity to improve the desorption by applying sonication.

4.2.2.1.1 Desorption solvent

Different desorption solvents were tested: hexane, cyclohexane, isooctane, toluene and ethyl acetate. Duplicate extractions were performed with MilliQ water samples spiked at 0.5 μg L^{-1}. A 10 mm rod was placed in a glass vial containing MilliQ water spiked with the compounds, and 10% of MeOH achieving a final volume of 20 mL. The vial was closed and the extraction period was set at 5 h. After the extraction period, three consecutive desorptions of 10 minutes each were done with 100 μL of solvent, which is the minimum volume that covers the sorbent material completely in a 200 μL insert. Nevertheless, according to the following order: hexane > cyclohexane > toluene > isooctane > ethyl acetate, an increase in the size of the PDMS rod inside the insert due to swelling was observed, causing a decrease in the volume of desorption solvent that could be recovered, which could; in turn, affect

the precision of the method. This effect was more important when hexane, cyclohexane and toluene were used. On the other hand, for isooctane and ethyl acetate similar analyte recoveries were obtained. Ethyl acetate is more volatile than isooctane and therefore could be further concentrated. For this reason it was the selected desorption solvent.

4.2.2.1.2 Desorption time and sonication

To study the effect of desorption time and sonication, desorption times of 5, 10 and 15 min were tested with or without sonication. As shown in **Figure 4.10**, the best recoveries were obtained after 15 minutes of desorption in an ultrasonic bath for the most hydrophobic FRs, whereas desorption of the lightest compounds was not affected by sonication. Moreover, three consecutive desorptions with 100 µL were necessary for a complete elution of the analytes. Hence, in order to simplify the method, a comparison was carried out between three consecutive desorptions with 100 µL and one single desorption with 300 µL. The obtained results showed no significant difference, so, for practical reasons, one desorption with 300 µL of ethyl acetate was selected. Finally, the solvent was evaporated to dryness and the sample was reconstituted in 50 µL of isooctane for injection in the GC-NCI-MS system.

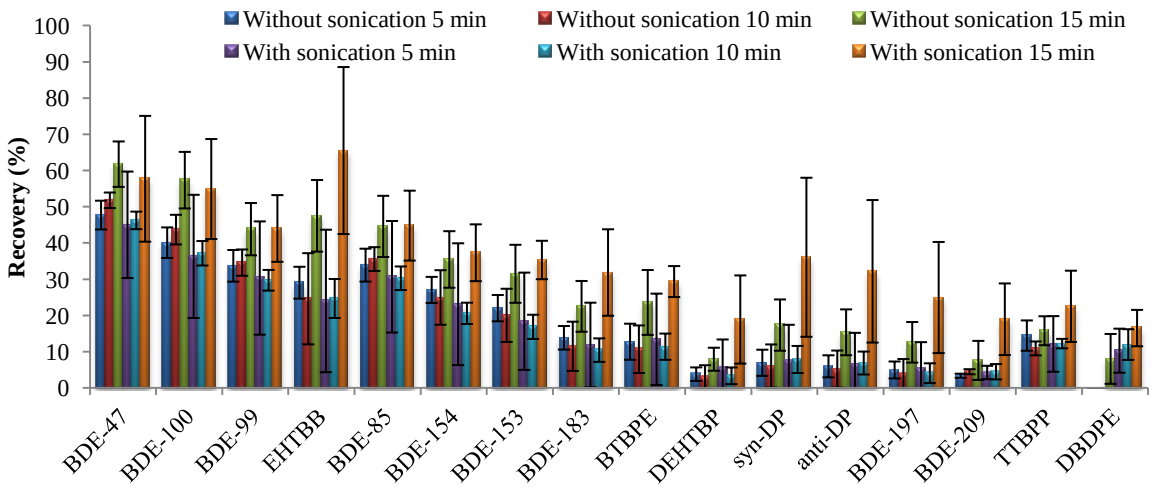


Figure 4.10 Influence of time and sonication in PDMS desorption. Bars correspond to recoveries obtained with three consecutive desorptions using 100 µL ethyl acetate each (n=2).

4.2.2.2 Study of PDMS rod extraction conditions

4.2.2.2.1 Organic modifier

The target FRs are very lipophilic and, therefore, prone to be adsorbed onto the walls of the extraction vial [221]. However, the addition of methanol may increase their solubility in water and avoid losses. To this end, different percentages of methanol (from 0 to 40%) were added to the samples. Methanol values higher than 40% were not considered since they could lead to salt precipitation in sea water samples. These experiments were performed in triplicate with an extraction time of 5 h and a total volume of 20 mL and spiking the samples at 0.5 μg L^{-1} level. Desorption was carried out in the previously adjusted conditions. As depicted in **Figure 4.11**, no significant differences on the recoveries were observed with the addition of methanol between 0 and 10%, except for DBDPE. However, a further increase, 20 - 40% methanol, represented a significant increase in the recoveries for most of the compounds with the exception of BDE-47, DEHTBP, and DBDPE. Thus, 40% was selected as the best methanol content.

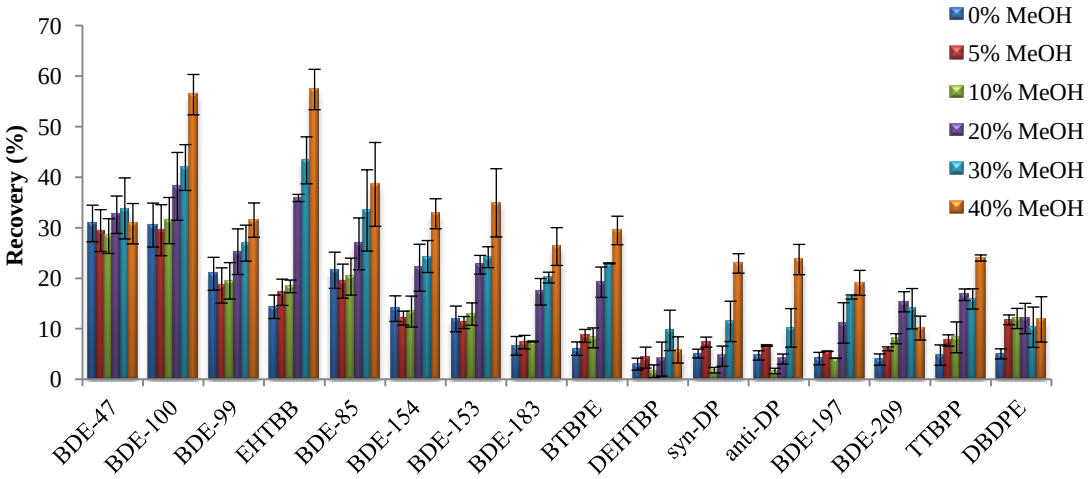


Figure 4.11 Influence of the addition of methanol in the extraction yield (n=3).

4.2.2.2.2 Ionic strength

The effect of the ionic strength on the extraction efficiency was studied at three levels of NaCl: 0, 5 and 10%. Higher NaCl amounts were not considered due to

saturation of the methanolic solution. These experiments were performed using MilliQ water, spiked at 0.5 μg L^{-1}, with 40% of MeOH at a final volume of 20 mL, for 5h. The extraction efficiency was slightly increased by the addition of 5% NaCl and then slightly decreased or were not affected at 10% NaCl (**Figure 4.12**). Thus, 4% of NaCl (6% referred to the sample, similar to sea water) in combination with a 40% of methanol were adopted as best extraction conditions, so that addition of salt needed not to be performed with marine samples.

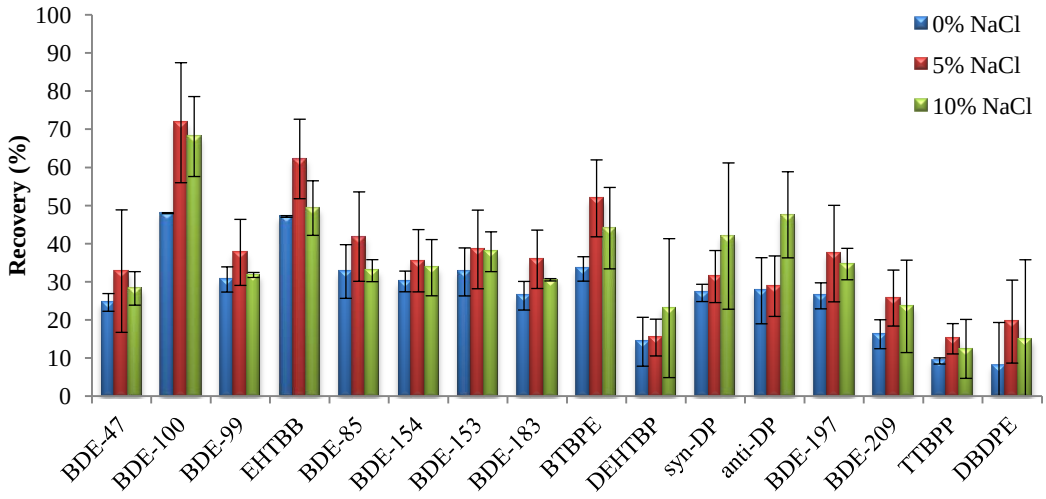


Figure 4.12 Influence of the addition of NaCl in the extraction yield (n=2).

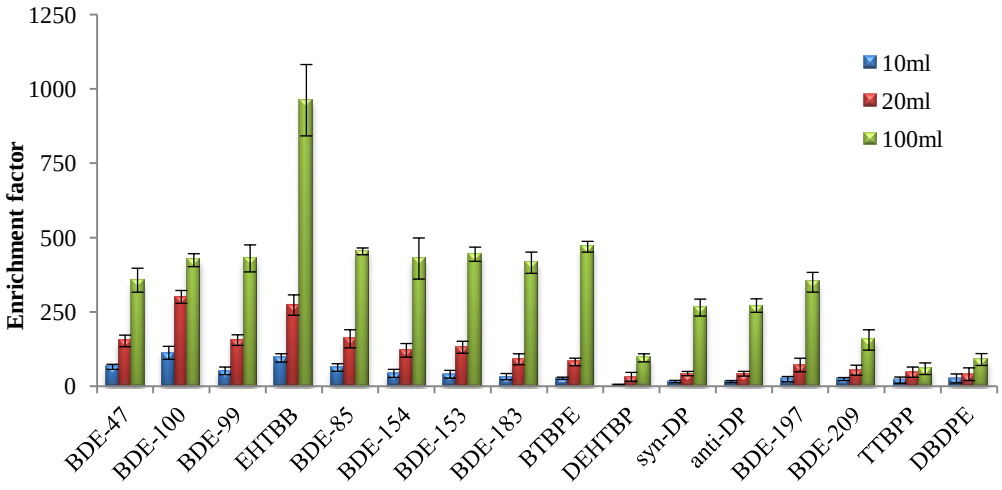


Figure 4.13 Enrichment factors obtained for different sample volumes (n=4).

4.2.2.2.3 Sample volume

The sample volume is another important parameter in sorptive extraction. Thus, three different volumes were evaluated, in accordance with typical head space crimp-cap vials: 10, 20 and 100 mL. These experiments were performed with fortified MilliQ water samples (10 ng of each analyte) with the addition of 40% MeOH and 4% NaCl, and stirring the solution at 500 rpm for 22 hours, in order to get close to equilibrium conditions for the different volumes. Analytes desorption was again performed using 300 μL of ethyl acetate in an ultrasonic bath for 15 min. The results of these experiments showed that concentration factors increased as the sample volume is raised from 10 mL to 100 mL (**Figure 4.13**). Thus, the sample volume was set at 100 mL in order to maximise method sensitivity.

4.2.2.2.4 Extraction time

As a final step, extraction time profiles were evaluated by triplicate experiments with 100 mL of spiked MilliQ water (0.1 μg L^{-1}) containing 40% MeOH and 4% NaCl, with a stirring speed of 500 rpm for a period of time between 30 min and 1440 min (24 h). Desorption was performed with 300μL of ethyl acetate in an ultrasonic bath for 15 min. The extraction profiles obtained were very similar for all compounds, with equilibrium not being achieved even after 1440 min, as exemplarily shown in **Figure 4.14** for some FRs. Therefore, a sampling time of 900 min (15 h, overnight extraction) was selected as a compromise between method sensitivity and sample treatment time. Although, this extraction time is relatively long, many samples can be extracted simultaneously in an unattended way, using a multiposition magnetic stirrer, and therefore this extraction step can be performed in a very simple and convenient manner.

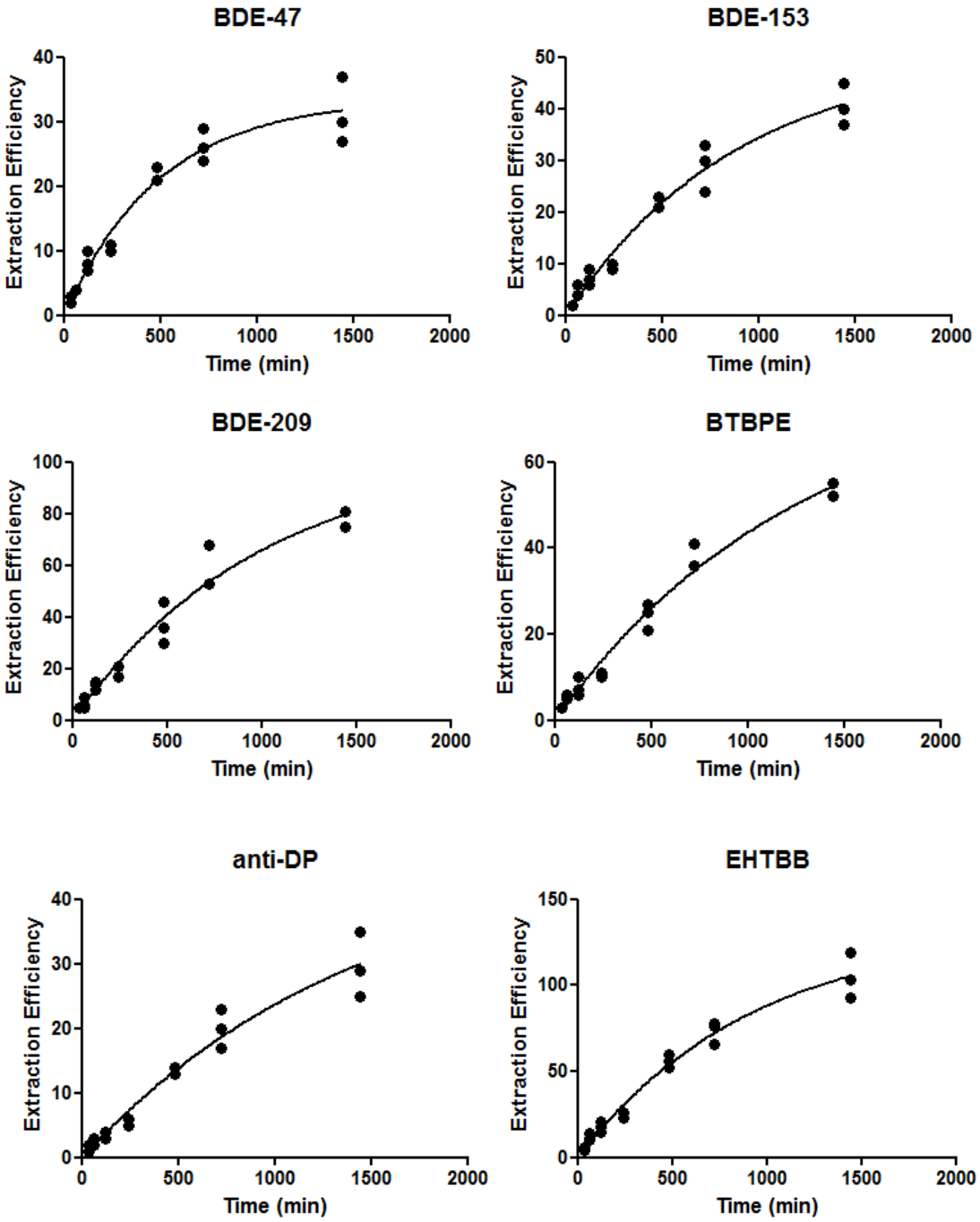


Figure 4.14 Extraction time profiles (n=4) for BDE-47, BDE-153, BDE-209, EHTBB, BTBPE and *anti*-DP.

4.2.2.3 Method performance

4.2.2.3.1 Linearity and precision

Linearity was evaluated by analysing duplicate MilliQ water samples spiked at six concentration levels in the range from the LOQ to 500 ng L^{-1}. BDE-77, BDE-181 and ^{13}C-BDE-209 were used as internal standards. For PBDEs, the internal standard for each congener was selected based on the ISO22032:2006 [222]. In the remaining cases, BDE-77 was selected as surrogate standard for TTBPP whereas BDE-181 was used for BTBPE, EHTBB, DEHTBP and DBDPE and ^{13}C-BDE-209 was employed for *syn* and *anti* DP. Each surrogate standard was selected according to best correction of matrix effects and added to the calibration solution at 100 ng L^{-1}.

As shown in **Table 4.10**, the method was linear for all compounds, with determination coefficients (r^2) higher than 0.997. The precision of the experimental procedure was evaluated by calculating the relative standard deviation (RSD, %) at two concentration levels: 50 ng L^{-1} and 500 ng L^{-1} (n=6). Results (**Table 4.10**) showed RSD values between 8% and 22% at the low concentration level and between 2% and 16% at the highest level, except for DBDPE with a RSD value of 23%. The obtained values are in the same range compared to those reported in the literature (most of them only considering PBDEs) by other preconcentration techniques, such as SPE, SPME or SBSE (**Table 4.11**). So, the precision of the method was deemed acceptable keeping in mind that different portions of manually cut sorptive materials were used.

Table 4.10 Method performance figures for flame retardants.

COMPOUND	LINEARITY (R^2)	LOD (ng L^{-1})	EXTRACTION EFFICIENCY (%) (N=6)	ENRICHMENT FACTOR	REPEATABILITY (RSD %) (N=6)	
					50 ng L^{-1}	500 ng L^{-1}
BDE-47	0.9998	0.4	27 ± 2	324	13	3
BDE-100	0.9998	0.6	39 ± 1	468	12	3
BDE-99	0.9999	0.6	32 ± 3	384	15	2
EHTBB	0.9999	1.3	70 ± 6	840	18	15
BDE-85	0.9998	0.9	27 ± 3	324	14	2
BDE-154	0.9999	0.7	32 ± 3	384	16	5
BDE-153	0.9999	0.7	31 ± 3	372	11	6
BDE-183	0.9999	2.2	31 ± 4	372	14	4
BTBPE	0.9998	4.9	38 ± 6	456	8	9
DEHTBP	0.9997	10.0	15 ± 3	180	13	16
syn-DP	0.9995	0.4	24 ± 3	288	18	15
anti-DP	0.9997	0.9	25 ± 3	300	18	15
BDE-197	0.9997	0.6	22 ± 2	264	15	9
BDE-209	0.9999	5.0	14 ± 2	168	15	7
TTBPP	0.9970	7.0	9 ± 1	108	22	13
DBDPE	0.9981	5.0	9 ± 1	108	22	23

4.2.2.3.2 Limits of detection and extraction efficiency

The method limit of detection was defined as the amount of the analyte for which the signal-to-noise ratio is equal to 3 and was estimated by extrapolating the results obtained analysing the MilliQ sample spiked at the lowest concentration. As shown in **Table 4.10**, LODs were all at the low nanogram per litre, ranging from 0.4 to 10 ng L^{-1}.Thus, the proposed PDMS rod extraction-GC-NCI-MS method provides good LODs when compared to those of the published methods (**Table 4.11**). Particularly, when novel FRs are considered as the only developed method, based on SPE, reports LODs as high as 270 ng L^{-1}.

The extraction efficiency of the preconcentration method was also calculated by comparing the GC-MS peak areas of an extract of a MilliQ water sample spiked at 50 ng L^{-1} with those of a standard solution prepared in isooctane. Calculated extraction efficiencies ranged from 9 to 70%, leading to enrichment factors between 108 and 840 (**Table 4.10**).

Table 4.11 Comparison of different methods for the determination of brominated flame retardants in water samples.

COMPOUNDS [A]	METHODS[B]	RELATIVE RECOVERIES (%)	PRECISION (RSD, %)	LOD (ng L^{-1})	REFERENCES
PBDEs(3 to 154)	HS-SPME-GC-EI-MS/MS	74-117	1-26	0.02-0.19	[103]
PBDEs (47 to 206)	SBSE-LD-GC-EI-MS	65-117	not reported	0.3-203	[114]
PBDEs (28 to 154)	SBSE-TD-GC-EI-MS	85-106	6-11	0.6-1.9	[214]
PBDEs (47 to 154)	PDMS rod-GC-ECD	71-107	3-12	0.09-1.5	[122]
PBDEs (28 to 154)	SPE-DLLME-GC-ECD	66-94	4-8	0.03-0.15	[223]
PBDEs (28 to 209)	SPE-GC-EI-MS/MS	87-124	2-17	0.5-130	[46]
PBDEs (47 to 209)	PDMS rod-GC-NCI-MS	76-114	2-16	0.4-5	This work
EHTBB, BTBPE, DBDPE	SPE-GC-EI-MS/MS	61-119	2-27	0.5-270	[46]
EHTBB, BTBPE, DEHTBP, DP, TTBPP, DBDPE	PDMS rod-GC-NCI-MS	53-130	13-23	0.4-10	This work

[a] Parentheses values correspond to the range of PBDE congeners considered.

[b] HS: headspace; EI: electron ionisation; LD: liquid desorption; TD: thermal desorption; ECD: electron capture detection; DLLME: dispersive liquid-liquid microextraction.

4.2.2.4 Application to the analysis of real samples

4.2.2.4.1 Effect of suspended particulate matter

Usually, sample filtration is performed in order to remove particles and colloidal organic matter that can interfere with the extraction procedure. However, such step can lead to serious biases when analysing hydrophobic analytes due to a possible adsorption on filters and glassware resulting in analyte loses during filtration. For this reason, the effect of two different procedures, namely filtration and centrifugation, were evaluated by comparing the responses obtained for raw wastewater samples which were spiked with 250 ng L^{-1} of all the compounds before or after the elimination of the suspended particulate matter. The results of these tests showed that both filtration and centrifugation led to a significant underestimation of the concentrations of FRs: between 10% and 83%. BDE-209, TTBPP and DBDPE peaks completely disappear of the chromatogram after filtration or centrifugation of the water sample. These results can be explained by the fact that the analytes, which present a low solubility in water, remain completely or partially adsorbed on the glass walls, on the filter or on the particles itself. Therefore, samples were analysed directly without any pre-treatment, which represents an advantage in terms of time consumption and sample manipulation effort compared to other preconcentration techniques, such as SPE. However, it contributes to a higher matrix effects during extraction as is explained below.

4.2.2.4.2 Matrix effects and trueness evaluation

The extraction efficiency of sorptive extraction techniques can be affected by the composition of the sample matrix. High levels of dissolved or suspended organic matter contained in water samples may compete with the sorptive material for the analytes and the extraction efficiency could change from sample to sample. Therefore, matrix effects were estimated by comparing the responses obtained analysing MilliQ water with those of other water samples (sea, river, effluent and influent wastewaters). All samples were spiked with the compounds at 100 ng L^{-1} prior to performing the sorptive process. Non-spiked samples of all these types of waters were also analysed and considered for calculations. The highest matrix

effects were obtained for influent wastewater, since this is the sample with the highest organic content. It is necessary to remember the impossibility of eliminate suspended solids without dramatic analyte losses. Nevertheless, the relative recovery (compared to MilliQ), was scarcely affected by the composition of the sample when surrogate internal standards were used (**Figure 4.15**). Thus, relative recoveries between 70 and 130% except for DBDPE (50 - 70%) were obtained with all the sample matrices. A chromatogram obtained from a sea water sample spiked at 50 ng L^{-1} is presented in **Figure 4.16**.

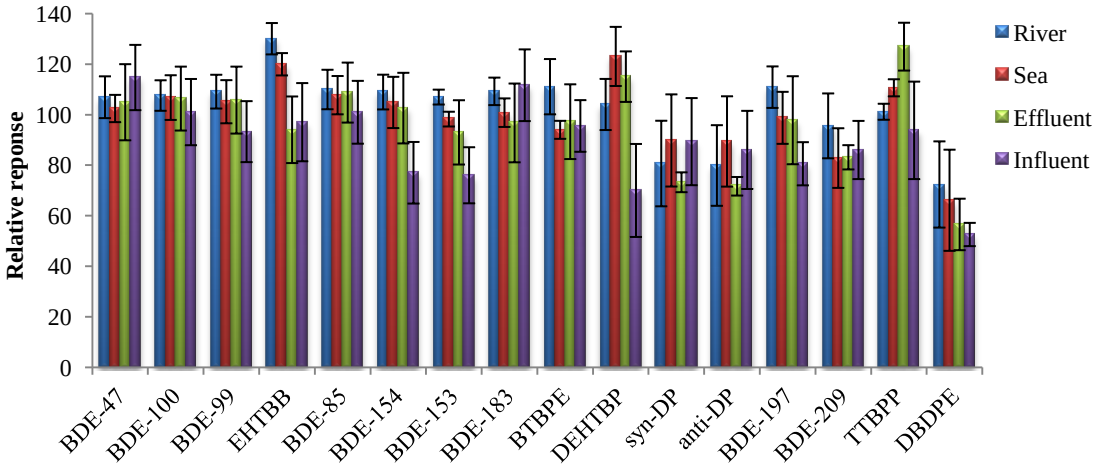


Figure 4.15 Surrogate internal standard corrected recoveries (n=3), relative to MilliQ.

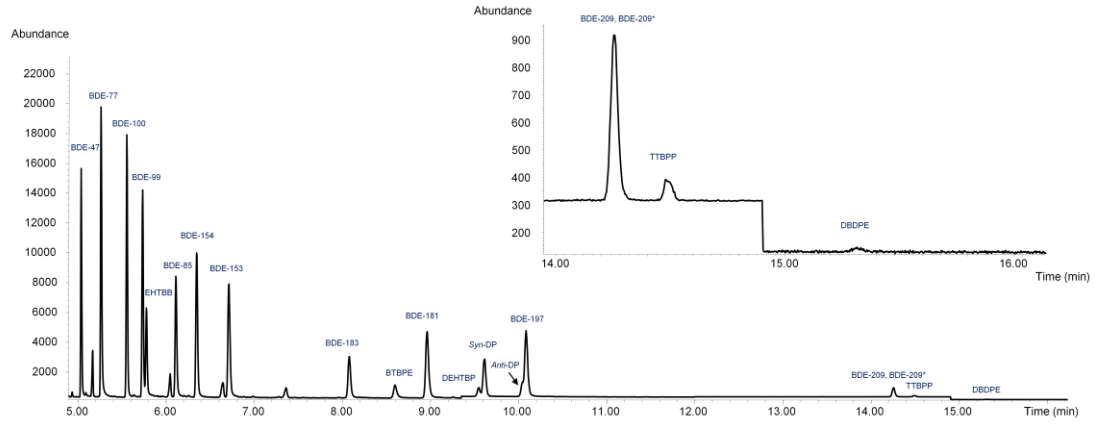


Figure 4.16 Total ion chromatogram of a sea water sample spiked with 50 ng L^{-1} of target flame retardants and internal standards.

4.2.2.4.3 Analysis of water samples

The developed methodology was applied to the analysis of different water samples, including sea, river and ria, a landfill leachate, influent and effluent wastewater from an urban wastewater treatment plants and effluent wastewater from a textile industry. The obtained concentrations with the corresponding standard deviations are compiled in **Table 4.12**.

Among the novel FRs, similar concentrations of DEHTBP were obtained in sea, river and ria water (2.1, 2.2 and 1.3 ng L^{-1} respectively), but this FR was not found in any other sample. BTBPE was detected only in the landfill leachate sample at 64 ng L^{-1}. PBDEs were not detected in surface water. However, BDE-197 was found at 8 and 0.5 ng L^{-1} in the influent and effluent sewage, respectively, and BDE-47 was also found in the influent urban WW sample (1.8 ng L^{-1}). The most contaminated samples were the landfill leachate and the textile wastewater. In the first sample, besides BTBPE, BDE-47, BDE-99, BDE-100 were found at concentrations between 8 and 33 ng L^{-1}. In the case of the sample taken from the textile industry, BDE-47 and BDE-100 were observed at 68 and 44 ng L^{-1}, respectively, whereas BDE-99 occurred at a much higher concentration of 2887 ng L^{-1}. These observed concentrations agree with those previously observed in water samples for PBDEs (except BDE-99) [224]. DEHTBP has been previously detected in wastewater samples at the same level found in this work [225]. Although BTBPE was recently found in precipitation samples at very low ng L^{-1} concentrations [215], this was the first time it is reported in landfill leachate.

Table 4.12 Average concentration (ng L^{-1}, n=3) and standard deviations obtained in the analysis of real water samples. EHTBB, BDE-85, BDE-154, BDE-153, BDE-183, *syn*-DP, *anti*-DP, BDE-209, TTBPP and DBDPE were not detected in any of the analysed samples.

	SEA	RIVER	RIA	EFFLUENT WW	INFLUENT WW	LANDFILL LEACHATE	TEXTILE INDUSTRY
BDE-47	< LOD	< LOD	< LOD	< LOD	1.8 (0.6)	33 (9)	68 (4)
BDE-100	< LOD	< LOD	< LOD	< LOD	< LOD	8 (6)	44 (5)
BDE-99	< LOD	< LOD	< LOD	< LOD	< LOD	25 (6)	2900 (500)
BTBPE	< LOD	< LOD	< LOD	< LOD	< LOD	64 (20)	< LOD
DEHTBP	2.1 (0.5)	2.2 (0.9)	1.3 (0.3)	< LOD	< LOD	< LOD	< LOD
BDE-197	< LOD	< LOD	< LOD	0.5 (0.2)	8 (3)	< LOD	< LOD

4.2.3 Phenolic compounds

The PDMS rod preconcentration technique was also applied to phenolic compounds, another group of endocrine disrupting compounds. In this case, extraction and desorption was performed as before, but the determination was performed by LC-DAD. Moreover, an *in-situ* derivatisation reaction was investigated to favour the extraction of the most polar compounds.

4.2.3.1 Study of PDMS rod extraction and desorption conditions for phenolic compounds

In order to find the best conditions of extraction and desorption for the preconcentration of the phenolic compounds, a systematic study of several parameters affecting the two steps was undertaken. In preliminary experiments, methanol and ethyl acetate proved to be highly efficient desorption solvents. As there were no significant differences in the results obtained between the two solvents, methanol was selected as this is the most suitable solvent for liquid chromatographic analysis.

Parameters of the aqueous solutions such as pH and ionic strength were studied. To perform these experiments, a 10 mm-PDMS rod was immersed in 20 mL of aqueous

solution containing 300 μg L^{-1} of all the phenolic compounds for 5h. After this period, the rod was exposed to 300 μL of MeOH for 15 min.

The sorption of the phenolic compounds by the PDMS rod strongly depends on the pH of the aqueous solution as to favour their extraction the compounds must be in neutral form. The pH of the aqueous solution was adjusted to three different values (3.0, 5.6 and 9.0) with HCl or ammonia solutions. The best results in terms of peak area for BPA, TCP and PCP were obtained at pH 3, especially in the case of PCP, whose peak area increased 115 times with respect to the values obtained at pH 9. However, a slight reduction of peak areas was obtained for NP and OP at this pH. Thus, considering that at pH 3 an increase of the peak area was observed for BPA, which is the compound that present the lowest peak area, this pH was finally selected as a compromise.

Another factor that can favour the sorption of the phenolic compounds by the PDMS rod is the addition of salt (salting out effect). This effect was studied by adding sodium chloride to the aqueous solution until the final salt concentration ranged between 0% and 25% (w/v). As can be seen in **Figure 4.17**, the peak areas of BPA and TCP increased when the percentage of NaCl was increased whereas in the cases of NP and OP, a decrease in the peak areas was observed at salt concentrations higher than a 15% of NaCl. The peak area of PCP decreased at 25% NaCl while no significant differences were observed in the 10 - 20% NaCl range. The salting out effect initially seemed to favour the extraction of all the compounds but the increase in the viscosity of the solution reduced the diffusion of NP, OP and PCP to the PDMS rod [226]. Thus, the addition of 15% NaCl to the aqueous solution was selected as the best condition.

In the case of the desorption process, the volume of solvent and the desorption time were studied. On testing three different volumes (100, 200 and 300 μL) of MeOH it was found that the enrichment factor increased as the desorption volume decreased. Therefore, the desorption volume was set at 100 μL, which is the minimum volume necessary to cover 10 mm of PDMS rod. Desorption time was then modified in the

range from 15 min to 60 min. As is shown in **Figure 4.18**, the peak areas of the analytes increased until 45 min, so this time was selected as the desorption time.

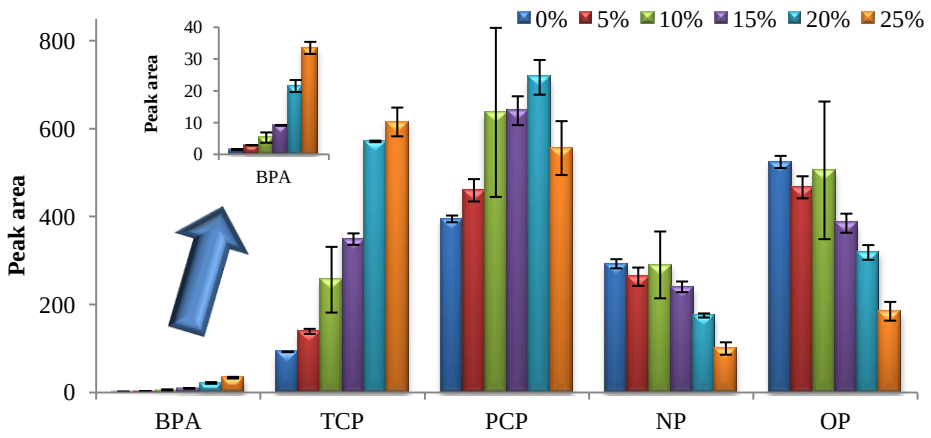


Figure 4.17 Effect of the addition of NaCl on the peak areas of phenolic compounds (n=3).

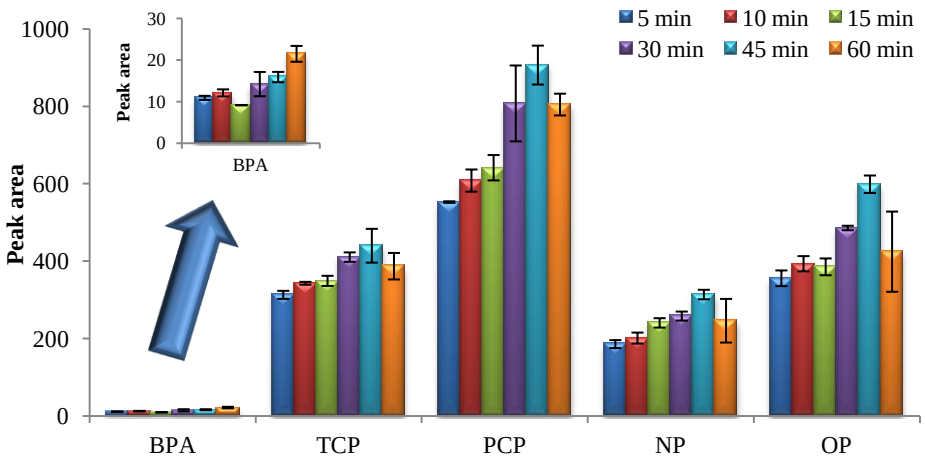


Figure 4.18 Effect of the desorption time on the peak areas of phenolic compounds (n=3).

After establishing the best chemical conditions of the aqueous solutions, the volume of desorption solvent and desorption time, the extraction process was improved by studying the effect of the sample volume, the extraction time and the amount of sorbent. To this end, different sample volumes (20, 50 and 100 mL) were tested. The experiments were conducted for a 24 h period in order to reach equilibrium conditions for each of the different volumes. As can be seen in **Figure 4.19**, the

concentration factors for PCP, NP and OP increase when the sample volume rises and, in the case of BPA and TCP, concentration factors increase until 50 mL. For practical reasons 50 mL was selected as the sample volume for the following experiments. Five different extraction periods (1, 4, 8, 16, and 24 h) were studied in the conditions described above. Equilibrium was reached at an extraction time of 16 h for BPA, TCP and PCP whereas it was nearly achieved for NP and OP. We chose to use an extraction time of 16 h, as this corresponds to an overnight period. Different PDMS rod sizes (5, 10 and 15 mm) were also tested using 200 µL as desorption volume, in order to fully immerse the rod. Peak areas generally increased as PDMS size increased although no significant differences were observed between 10 and 15 mm. Consequently, 10 mm of PDMS rod was selected allowing a desorption volume of 100 µL to be used and for higher enrichment factors to be achieved.

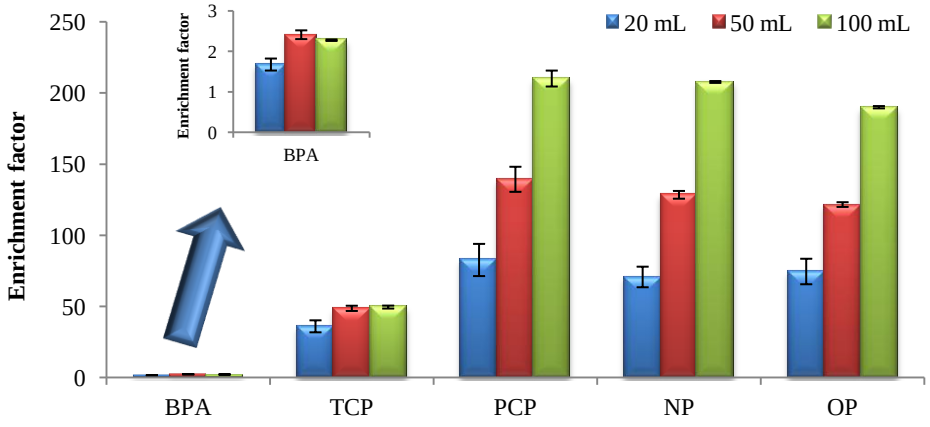


Figure 4.19 Enrichment factors obtained with different sample volumes (n=3).

4.2.3.2 Study of PDMS rod extraction for acetylated phenolic compound

Although the extraction and preconcentration of the phenolic compounds by PDMS rods were performed in the most favourable conditions, the LOD obtained for BPA was high. This high LOD can be explained by the low extraction efficiency of the PDMS rod for this compound (0.9 %), a finding that Kim et al. [31] also made in reaching the conclusion that PDMS polymer was not suitable for BPA extraction.

Therefore, we decided to introduce a derivatisation step in order to decrease the polarity of the compounds and so increase their affinity towards the sorbent. To this end, different derivatisation reactions were considered [211, 212]. Acetylation in aqueous media was selected as the best derivatisation method due to its efficiency, simplicity and speed and the fact that it can be performed directly in the water sample.

In situ acetylation involves the addition of a reagent, such as acetic anhydride (AA), into the aqueous sample in an alkaline medium to ensure the deprotonation of the compounds. Therefore, the amounts of AA and K_2CO_3 need to be adjusted in order to obtain the highest peak areas in the chromatogram. Different amounts of K_2CO_3 (100, 300, 500, 700, and 1000 mg) and AA (100, 200, 300, 400, and 500 μL) were added into a 50 mL MilliQ sample containing 100 μg L^{-1} of the analytes. Then, the extraction was carried out using the same conditions as in the case of non-derivatised compounds. As can be seen in **Figure 4.20**, the volume of the derivatisation agent and the amount of K_2CO_3 were interrelated parameters. With large amounts of K_2CO_3 and low volumes of AA, the peak areas of the compounds were low indicating that derivatisation was not complete. In general, the best results were obtained when combinations of 100 mg or 300 mg of K2CO3 and 300 μL or 400 μL of AA were used. It should be noted that BPA derivatisation increases the peak area of this compound to the same order of magnitude as the other compounds studied here. TCP and NP were the compounds with the smallest peak areas and so to maximise these areas we decided to perform the derivatisation reaction with 100 mg of K_2CO_3 and 400 μL of AA.

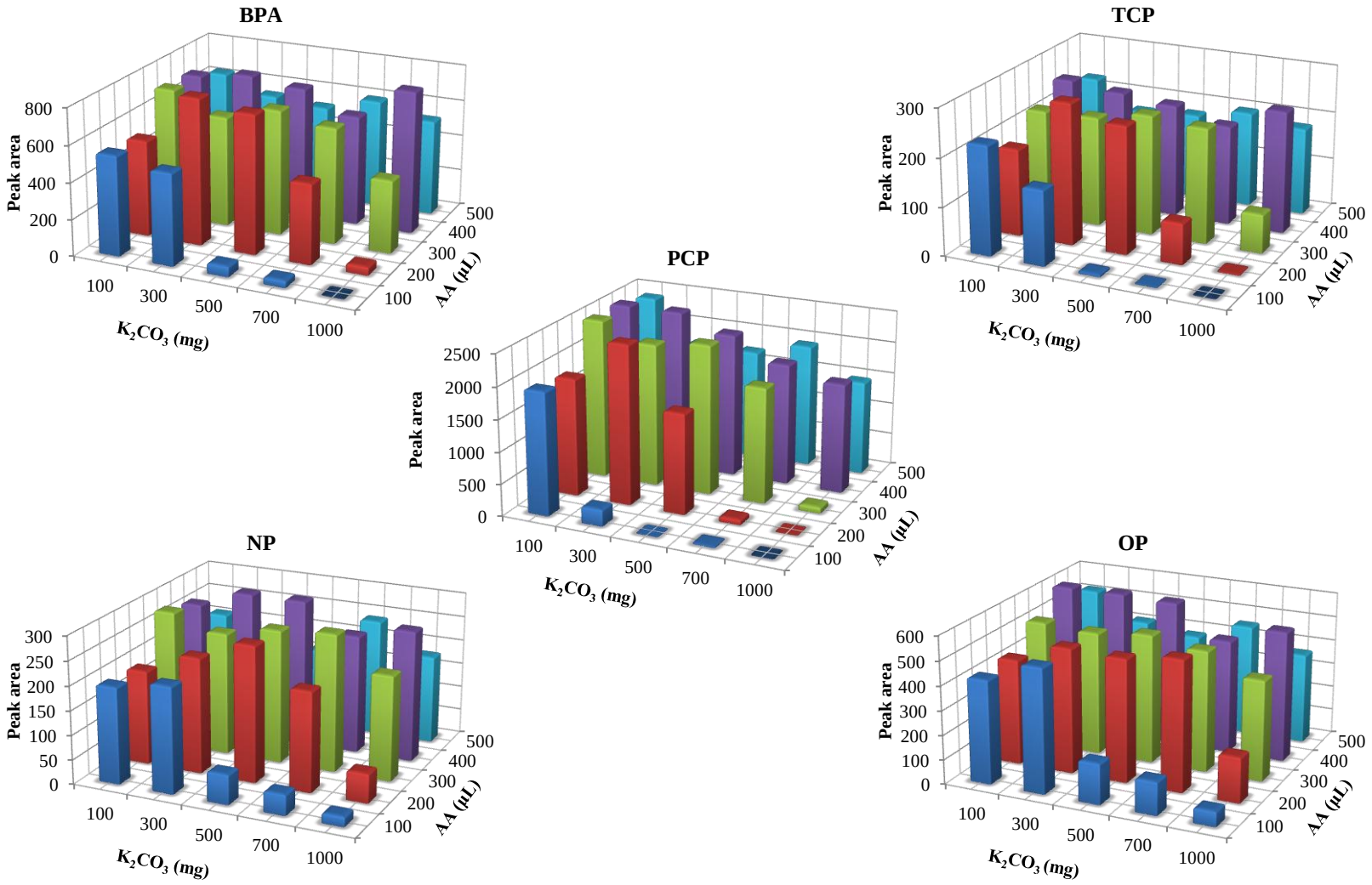


Figure 4.20 Effect of the amount of K_2CO_3 and acetic anhydride (AA) on peak areas (n=3).

After derivatisation, the polarity of the compounds changed and it was necessary to study the most important parameters affecting the extraction and desorption of the analytes (the chemical conditions of the aqueous solution and the extraction and desorption times).

Low pH was the most suitable condition for the extraction of phenolic compounds without derivatisation. However, a basic medium of the aqueous solution was required to ensure that the acetylation reaction would take place. To this end, the pH was adjusted by adding K_2CO_3 as described previously (pH before derivatisation was around 12). The effect of ionic strength was studied again by the addition of NaCl (5%, 10%, 15% and 20% (w/v)) to 50 mL of a water sample spiked at 100 μg L^{-1}. A reduction of PCP, NP and OP peak areas were observed when the percentage of NaCl was increased. For the rest of the compounds, no significant differences were observed and the absence of salt was selected for further experiments. These results are in agreement with the previously described positive and negative salting out effect on phenol acetate peak areas [213].

The kinetics of extraction and desorption processes for the acetylated compounds were then studied. Six desorption times (5, 10, 15, 30, 45, and 60 min) and five extraction times (1, 4, 8, 16, and 24 h) were tested. Extraction time profiles were very similar to those obtained with non-derivatised analytes and equilibrium was reached after 16 h for all the analytes whereas in the desorption process, the maximum peak areas were attained at 30 min (**Figure 4.21**).

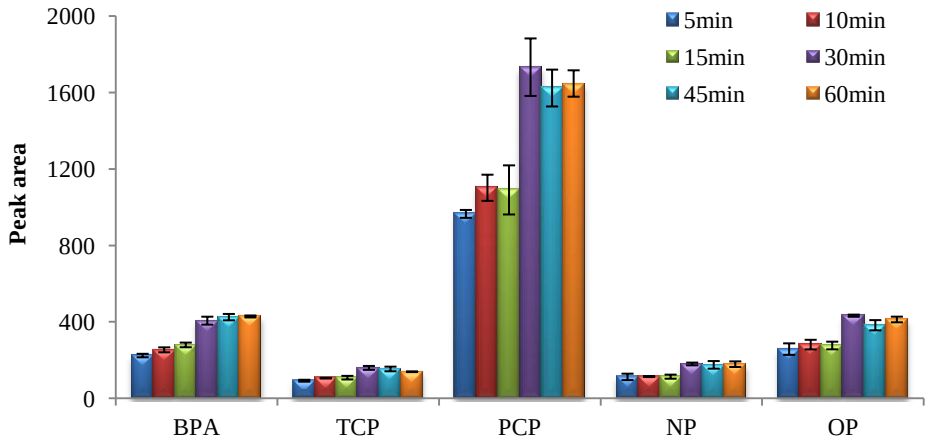


Figure 4.21 Effect of the desorption time on the peak areas of derivatised compounds (n=3).

4.2.3.3 Method performance

4.2.3.3.1 Phenolic compounds

The linearity of the direct method was evaluated by analysing MilliQ water samples spiked at six concentration levels ranging from 2 to 74.7 μg L^{-1} of OP, NP, TCP, and PCP and from 31.7 to 662.7 μg L^{-1} of BPA (**Table 4.13**). The method was linear for all compounds and determination coefficients (r^2) were higher than 0.990. The LODs, calculated using the Hubaux and Vos method [227], ranged from 0.6 μg L^{-1} to 2 μg L^{-1} except for BPA, which was 9.5 μg L^{-1}. The LODs for PCP, NP and OP can be improved by increasing the sample volume to 100 mL (**Figure 4.19**). The precision of the method, expressed as RSD, was evaluated by replicate analysis (n=6) of MilliQ samples spiked at two concentration levels (5.0 and 15.0 μg L^{-1} except for BPA, which was 50 and 150 μg L^{-1}). Intra-day precision was in the range of 4 - 17% at both levels and inter-day precision was between 5% and 10% at both levels. A chromatogram corresponding to the phenolic compounds is presented in **Figure 4.22**.

Table 4.13 Linear range, LODs and precision obtained for the five phenolic compounds by PDMS rod extraction with the direct and derivatisation-based methods.

COMPOUND	LINEAR RANGE (MG L^{-1})		LOD (μg L^{-1})		INTRA-DAY PRECISION (RSD, %) (N=6)				INTER-DAY PRECISION (RSD, %) (N=6)			
	Direct method	Derivatisation-based method	Direct method	Derivatisation-based method	Direct method		Derivatisation-based method		Direct method		Derivatisation-based method	
					5 μg L^{-1}	*15 μg L^{-1}*	*5 μg L^{-1}*	*15 μg L^{-1}*	*5 μg L^{-1}*	*15 μg L^{-1}*	*5 μg L^{-1}*	*15 μg L^{-1}*
BPA	31.7 - 662.7	2 - 20.3	9.5	0.6	8[a]	4[b]	4	4	9[a]	10[b]	2	5
TCP	4.0 - 61.7	1 - 20.1	1.2	0.3	6	16	4	8	5	9	5	7
PCP	2.0 - 57.9	3 - 20.2	0.6	0.9	13	5	2	8	10	8	6	8
NP	6.7 - 62.7	1.7 - 20.2	2	0.5	10	7	6	6	8	10	9	8
OP	4.7 - 74.7	2.7 - 20.5	1.4	0.8	17	7	6	9	10	10	5	8

[a] for BPA concentration was 50 μg L^{-1}, [b] for BPA concentration was 150 μg L^{-1}

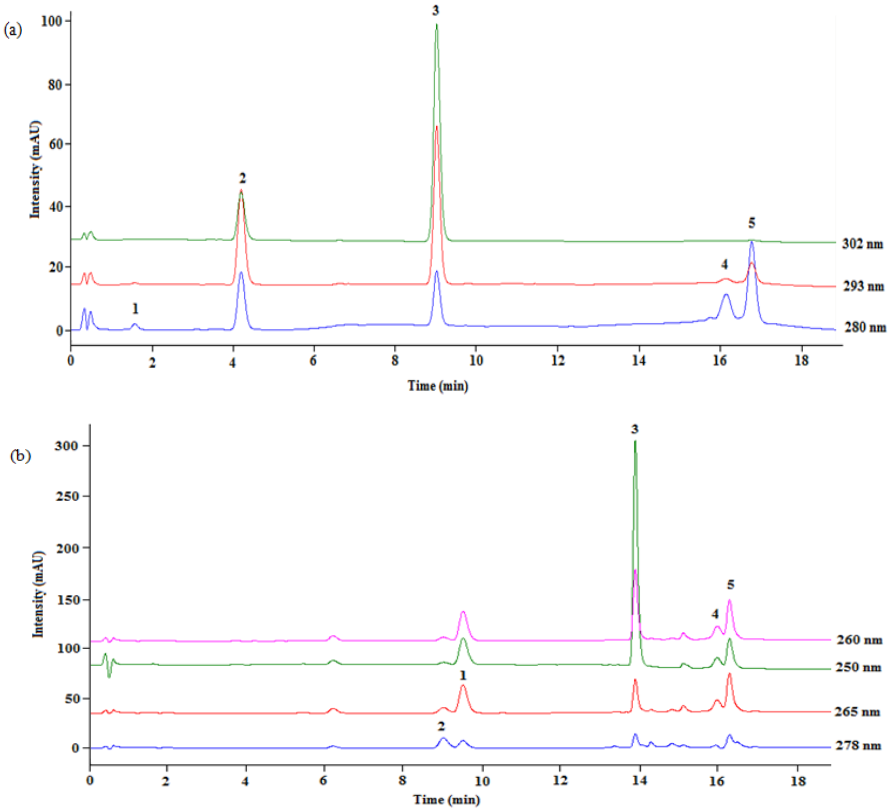


Figure 4.22 Chromatograms of MilliQ water spiked at 100 µg L^{-1} for non-derivatised (a) and derivatised (b) compounds. *1* BPA, *2* TCP, *3* PCP, *4* NP, *5* OP.

4.2.3.3.2 Acetylated compounds

The linearity of the derivatisation-based method was evaluated by analysing water samples containing all the compounds in the 1 - 20.5 µg L^{-1} concentration range. The method was linear for all of the compounds and determination coefficients (r^2) were higher than 0.988. LODs were calculated as described for phenolic compounds, and the values obtained were between 0.3 µg L^{-1} and 0.9 µg L^{-1}, which represent an enhancement of the sensitivity over the direct method (**Table 4.13**). These values can be improved by concentrating higher volumes of the samples (the performance of the method was studied using 50 mL of water) or by reducing the volume of desorption solvent. The comparison of our results with those of other methodologies using the same detection technique (DAD) has the difficulty that the group of compounds determined are different. However, when we have been able to compare the same compounds, we have found that our LODs were lower than those obtained

by Gadzała-Kopciuch et al. [37], who obtained LODs of 60 μg L^{-1} and 40 μg L^{-1} for NP and OP, respectively, using SPE extraction, Campíns-Falcó et al. [228] who obtained a LOD of 1.5 μg L^{-1} for BPA by coupling in-tube solid-phase microextraction to a capillary LC-DAD, Sharma et al. [25] who obtained a LOD of 3.9 μg L^{-1} for PCP, using HS-SDME and Hu et al. [229] who found a LOD of 0.45 μg L^{-1} for TCP using an amino modified multi-walled carbon nanotubes/PDMS coated stir bar to extract phenols from water samples. Using a new monolithic SCSE, Huang et al. [210] obtained a LOD of 0.69 μg L^{-1} for BPA which is the same achieved in our method. Although Sheng et al. [230] have achieved a lower LOD for BPA, they did not use commercially available rods and their study was limited to just one compound. The precision of the method, expressed as RSD, was evaluated by replicate analysis (n=6) of MilliQ samples spiked at two concentration levels (5.0 and 15.0 μg L^{-1}). Intra-day and inter-day precision were in the range of 2 - 9% at both levels in both cases. In **Figure 4.22** a chromatogram corresponding to the acetylated compounds is presented.

4.2.3.4 Efficiency of the method

In order to study possible matrix effects, the developed methods were applied to the analysis of river water samples. First, a blank of the river water was run to confirm that none of these compounds were present. When the direct method was applied, the river water sample was spiked at three concentration levels (5, 20 and 60 μg L^{-1}) of TCP, PCP, NP, and OP, and 50, 200 and 600 μg $L^{-1}$of BPA, obtaining recoveries in the 60.2% - 95.7% range at the lowest concentration level, 69.0% - 131.7% for the medium concentration level, and 64.3% - 96.8% for the highest concentration level. In order to test the derivatisation-based method, river water samples were spiked with all the compounds at three concentration levels (5, 10 and 15 μg L^{-1}). The recoveries obtained were in the 76.7% - 108.2% range at the lowest level, 82.6% - 94.5% at the medium level and 77.9% - 88.6% at the highest level. The derivatisation-based method presented better results in terms of a reduction in the matrix effects and increased sensitivity and precision.

4.3 MULTIRESIDUE SPE EXTRACTION

4.3.1 Precedents

In order to improve our control over the water quality, it is necessary to develop single-run, fast, sensitive and reliable analytical methods for the determination of untargeted organic contaminants, metabolites and transformation products (TPs). One of the difficulties in achieving this goal is that the physicochemical properties of these contaminants are very different from one to another and so the developed multiresidue methods have to be of broad application. The main advantages of multiresidue methods are the reduction of analysis time and solvent consumption, and as a result the cost of the analysis is also reduced. Nowadays, several multiresidue methodologies have been developed for the determination of pharmaceuticals from different therapeutic classes, including metabolites and transformation products [50, 72, 231, 232]. For the determination of iodinated X-ray contrast media (ICM) compounds several methodologies have also been reported [233-235]. Ens et al. have recently proposed a method to determine seven X-ray contrast media and three artificial sweeteners [236]. However, there are few methods allowing the determination of pharmaceuticals compounds which included ICM and, in most of them, iopromide is the only compound of this group selected [59, 61, 67]. Multiresidue methods for the determination of pesticides are well established [74, 149, 237], as well as combination of pharmaceuticals and metabolites [81, 238], but methodologies with the incorporation of pesticides and pharmaceuticals including ICM are really scarce [69].

Solid phase extraction (SPE) is the most used sample treatment technique in multiresidue methods. The low environmental concentrations of most analytes require an enrichment step, although the sensitivity of instrumental techniques such as LC-MS/MS is high. The main challenge for the enrichment step in multiresidue methods is the wide range of physicochemical properties and the high polarity of many of the target compounds especially the TPs. Therefore, the selection of the most appropriate sorbent is a crucial point in the development of this type of methods.

Some authors have suggested the use of different cartridges, with different polarity or structural properties, in a unique multiresidue method [51, 239, 240], but this represents an increase in the analysis time and costs.

Many analytical techniques have been developed for the determination of pharmaceuticals and/or pesticides in water samples. Gas chromatography-mass spectrometry (GC-MS) has been more extensively used for the determination of pesticides and other non-polar compounds [75, 150, 237, 241], whereas liquid chromatography-tandem mass spectrometry (LC-MS/MS) has been extensively used for the determination of pharmaceuticals [59, 138, 242] although, depending on the nature of the pharmaceutical compound, they can also be determined by GC-MS [106]. In general, LC-MS/MS is the technique of choice for multiresidue determination comprising pharmaceuticals and pesticides [81, 243], because it allows the determination of compounds having diverse polarity, including non-volatile and/or thermolabile compounds.

First at all, the chromatographic conditions to separate and determine all the compounds by LC-MS/MS were studied.

4.3.2 LC-MS/MS analysis

The parameters (intensity, peak area, peak shape and retention time) affecting both chromatographic analysis and MS/MS detection were studied to improve analyte separation and sensitivity. Water and methanol were chosen as the basic components of the mobile phases due to its suitability to MS detection, although acidic compounds were weakly retained on reversed phase columns. To overcome this problem, the effect of the addition of ammonium acetate or ammonium formate, at concentrations ranging from 2 to 20 mM, and of the addition of acetic or formic acid to the mobile phase were investigated by analysing a 80:20 (Water/MeOH) standard solution containing 25 μg L^{-1} of each compound. We had to consider that the use of additives can cause the reduction of the signal intensities in the MS. A gradient from 5% to 95% of MeOH in 43 min and a flow rate of 0.2 mL min^{-1} were used. When the concentration of ammonium formate was increased from 2 mM to 20 mM the intensity and retention time of the analytes decreased, especially those

corresponding to ICM compounds. The background signal also increased hampering the detection of ibuprofen. The retention times obtained by adding 20 mM of ammonium acetate to the mobile phases were similar to those obtained by adding 2 mM ammonium formate. In general, the intensities were lower with ammonium formate, but its addition provided better peak shape and lower background signal compared to ammonium acetate. Therefore 2 mM ammonium formate was selected as the additive composition.

The addition of 0.1% of acetic acid to both mobile phases resulted in a worst separation of the ICM peaks and higher background hampering the detection of ibuprofen. In contrast, the addition of 0.1% of formic acid (pH = 3) to the mobile phases caused an increase on the pesticides peak areas whereas, in the case of negative ionised compounds, a decrease on their peak areas was observed. The detection of ibuprofen was again not possible in these conditions (0.1% of formic acid at pH 3). Finally, it was detected when formic acid was added to the aqueous mobile phase until setting the pH at 5. No differences in the peak areas were found for the rest of compounds in these conditions, except for the negative ionised ones which peak areas slightly decreased. Moreover, for ICM compounds, the formation of narrower peaks led to an increase in sensitivity with respect to that obtained at pH=3. As this result represent and important improvement in the sensitivity of the detection, especially for ICM compounds, this mobile phase composition was selected for further experiments.

Once the mobile phase composition was established, several reverse phase columns with different sizes and particle diameter were tested (see **Chapter 3**). The best peak shapes were obtained using a Luna C18 column, and therefore this one was the selected column to perform chromatographic analysis. Different gradients and column temperatures (40 ºC, 50 ºC and 60 ºC) were also tested. Although a complete separation is not necessary when MS/MS detection is used due to its high selectivity, it is preferable to increase the sensitivity and to reduce the total analysis time. Finally, the gradient chosen was 95% 2 mM ammonium formate in MilliQ water acidified at pH 5 (A) at the beginning; 50 % A at minute 6; 5 % A from minute 25 to minute 31; and returning to the initial conditions at minute 32. The best resolution

was obtained by keeping the column temperature at 60 ºC. Moreover, an improvement on ICM and ibuprofen peak shapes was also observed at this temperature.

Ion source conditions were also studied with the objective of increasing the sensitivity of detection. Both ESI and APCI ion sources were checked and peak areas greater than one order of magnitude were obtained with ESI over APCI. Ion source temperature (300 ºC, 400 ºC and 550 ºC), ion spray voltage (3500 V, 4500 V and 5500 V) and different pressures of curtain gas and ion source gas were investigated, getting no important differences with respect to the default values (Ion source temperature was 550 ºC, ion spray voltage was 4500 V, curtain gas was 40 psi and ion source gases GS1 and GS2 were 60 and 75 psi, respectively)

4.3.3 Solid phase extraction

Four cartridges with different sorbents were tested to perform the extraction of the 35 selected analytes (**Table 4.17**) in a single step. The different sorbents were selected according to the different retention mechanisms. The experiments were carried out by processing 50 mL of tap water spiked with 200 ng L^{-1} of each compound. Five mL of methanol followed by 5 mL of acetonitrile were used for elution. The recoveries obtained at pH 7 with the different cartridges tested were similar for some compounds, especially for pesticides (**Figure 4.23**). However, for the metabolites of pesticides and for some pharmaceuticals better recoveries were obtained with HLB. These results can be explained by the ability of HLB cartridge to retain compounds with different acid-base characteristics and therefore HLB sorbent (150 mg) was selected.

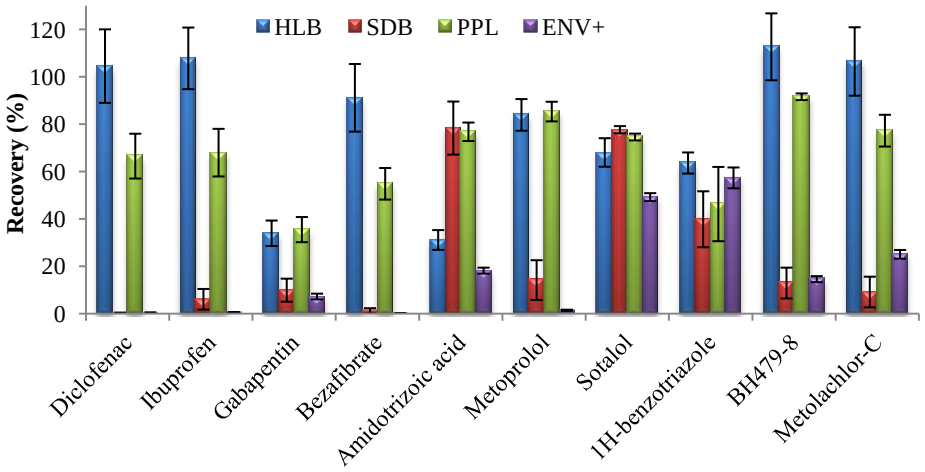


Figure 4.23 Recoveries obtained for selected compounds in tap water using different SPE cartridges (n=3).

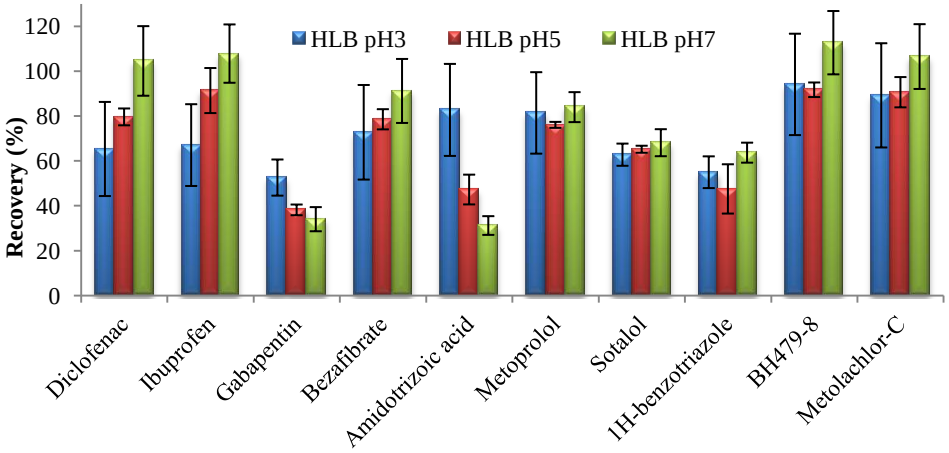


Figure 4.24 Recoveries obtained for selected compounds in tap water with sample pH adjustment for Oasis HLB cartridges (n=3).

Different pHs (3, 5 and 7) were also tested. As a great number of the compounds studied are neutral at pH 7, better recoveries were obtained at this pH using HLB cartridge. However, for gabapentin and amidotrizoic acid, which are acidic compounds, higher recoveries were obtained at pH 3, whereas for hydrochlorothiazide, diclofenac, ibuprofen and sulfamethoxazole, an important reduction on their recoveries were obtained at pH 3. At pH 5, the recoveries of gabapentin and amidotrizoic acid did not increase significantly with respect to those obtained at pH 7 and, in the case of diclofenac and simazine, among others, a

decrease was obtained (**Figure 4.24**). Therefore, as a compromise between the different compounds to be extracted, the pH of the sample was adjusted to pH 7 before solid phase extraction using an HLB cartridge.

Different elution volumes (4, 6, 8 and 10 mL) of a 50:50, MeOH:ACN elution solution were then tested. No significant differences in the recoveries were obtained by using the different elution volumes, except for diclofenac, which recovery increased from 51% to 84% when the elution volume increases from 4 mL to 8 mL. The recoveries of lamotrigine (55%), gabapentin (50%) and amidotrizoic acid (30%) were low and did not depend on the elution volume used. Thus, an elution volume of 8 mL (4 mL of MeOH and 4 mL of ACN) was selected.

4.3.4 Method performance

A ten-point calibration curve was constructed by analysing standard solutions in which the analytes and the surrogate standards were added into tap water before performing the overall analytical procedure. The standards of calibration were injected at the beginning of every sequence of analysis and during the run one calibration standard was also injected as a quality control every ten samples.

For the determination of the method intra-day repeatability six water samples spiked at a concentration level of 25 ng L^{-1} were analysed and relative standard deviation (%) was evaluated. The repeatability experiments were carried out in tap water, surface water, ground water and effluent waste water. The method detection limits (MDL) were calculated according to the German standard method DIN 32645 [131] using a ten-point calibration curve in the range 2 - 40 ng L^{-1}.

Linearity was studied in different concentration ranges, 4 - 800 ng L^{-1}, 4 - 400 ng L^{-1} or 12 - 2000 ng L^{-1}, depending on the compound and in relation with their sensitivity and LOD. The determination coefficients were greater than 0.99, except for carbamazepine, gabapentin and most of the X-ray contrast media (**Table 4.14**). The MDLs ranged from 0.5 ng L^{-1} for carbamazepine to 7.8 ng L^{-1} for amidotrizoic acid showing the multiresidue method developed to be useful for the determination of various pharmaceuticals, corrosion inhibitors and pesticides at low ng L^{-1}. The

precision of the method in the analysis of tap water was in the 2 - 23% range, except for iomeprol (33%) and amidotrizoic acid (34%), in the 1 - 19% range for surface water, in the 2 - 20% range for groundwater and in the 1 - 21% range for wastewater (**Table 4.14**).

Two water samples, tap and surface waters, were spiked at 40 ng L^{-1} with the analytes and SS/IS (for amidotrizoic acid-d6, iopamidol-d8, iohexol-d5 and DMI the concentration was 10 times more) in order to evaluate the whole process efficiency, including the SPE recovery and the matrix effect. The response obtained in the analysis of samples spiked before SPE extraction after subtracted the response of the samples spiked only with SS/IS was divided by that obtained in analysing the standard solutions of the analytes. Experiments were performed in triplicate. The efficiencies of the method were between 79% and 134% in tap water and between 75% and 144% in surface water (**Table 4.14**). The results obtained allow us to conclude that SS/IS correction was satisfactory for most of the compounds, and few differences were observed between efficiencies in tap water and in surface water.

Table 4.14 Method performance parameters.

COMPOUND	LINEAR RANGE (ng L^{-1})	r^2	MDL (ng L^{-1})	MQL (ng L^{-1})	INTRA-DAY PRECISION %RSD (N=6)				METHOD EFFICIENCY (%) (N=3)[A]	
					Tap water	Surface water	Ground water	Effluent WW	Tap water	Surface water
Diclofenac	4 - 800	0.999	2.5	8.5	5	5	4	2	102	104
Ibuprofen	12 - 2000	0.996	1.8	6.1	5	9	3	4	108	75
4-AAA	4 - 400	0.9997	0.7	2.7	4	3	2	2	97	93
4-FAA	4 - 400	0.9997	1.2	4.4	4	4	3	4	100	101
Carbamazepine	4 - 800	0.865	0.5	1.7	4	3	4	1	101	102
10,11-DHC	4 - 800	0.999	0.7	2.7	2	4	4	3	109	106
Lamotrigine	4 - 800	0.996	1.2	4.1	5	4	5	4	93	112
Gabapentin	4 - 400	0.974	1.4	5.0	5	4	6	4	88	84
Bezafibrate	4 - 800	0.999	0.8	2.8	3	1	4	5	93	93
Iopamidol	12 - 2000	0.995	1.8	6.4	7	3	6	6	97	66
Iopromide	12 - 2000	0.988	2.3	7.8	13	8	7	4	90	83
Iomeprol	12 - 2000	0.870	6.3	22.0	33	12	20	10	129	144
Amidotrizoic acid	12 - 2000	0.957	7.8	25.5	34	16	14	5	134	133
Iohexol	12 - 2000	0.976	5.4	17.9	14	19	17	21	n.c.	n.c.
Metoprolol	4 - 800	0.999	1.6	5.6	5	6	5	3	95	97
Sotalol	4 - 400	0.998	0.9	3.3	9	5	8	5	94	95
Sulfamethoxazole	4 - 800	0.999	0.9	3.3	3	5	2	2	n.c.	n.c.
Acetyl-sulfamethoxazole	4 - 800	0.998	0.9	3.2	3	5	3	3	n.c.	n.c.
Hydrochlorothiazide	12 - 2000	0.995	2.8	9.6	7	7	5	2	79[b]	103[b]
1H-benzotriazole	4 - 800	0.999	2.4	8.2	19	2	6	4	104	105
4MBT + 5MBT	4 - 800	0.999	3.3	11.0	13	4	9	4	105	101
Isoproturon	4 - 400	0.9996	0.9	3.4	4	6	5	3	102	101
Chloridazon	4 - 800	0.995	1.0	3.8	7	7	7	3	96	96
Atrazine	4 - 400	0.999	1.3	4.6	6	5	6	2	102	95
Desethylatrazine	4 - 400	0.999	1.1	3.8	3	6	2	1	98	98
Simazine	4 - 800	0.999	0.8	3.1	4	4	4	3	104	104
Terbuthylazine	4 - 400	0.998	1.3	4.8	6	4	8	7	89	83
Metazachlor	4 - 400	0.996	0.9	3.1	4	5	6	4	102	107
BH479-4	4 - 800	0.996	0.6	2.3	12	6	6	2	84	91
BH479-8	4 - 800	0.999	1.5	5.3	5	11	5	7	90	88
Metolachlor	4 - 400	0.998	1.4	5.0	7	5	11	4	n.c.	n.c.
Metolachlor-C	4 - 800	0.998	2.5	8.2	23	19	7	9	92	100
Metolachlor-S	4 - 800	0.999	0.8	3.1	3	4	4	2	90	81
Chlortoluron	4 - 400	0.999	0.9	3.2	3	7	7	3	102	102
Diuron	4 - 800	0.999	1.3	4.8	7	8	8	6	99	100

a: 40 ng L^{-1}

b: n=2

n.c.: not calculated

MDL: method detection limit

MQL: method quantification limit

4.3.5 Analysis of water samples

The multiresidue method developed was applied to determine the target compounds in 56 water samples. Composite samples were collected in WWTP (12 Influents and 12 effluents) in the state of Baden-Wüttemberg, Germany. The other samples were grab samples and were collected in rivers (30) and in a WWTP in Girona, Spain (1 influent and 1 effluent). All the samples were collected during September and November 2012. The most important results are summarized in **Table 4.15**, which contain the minimum and maximum concentration level, the medium concentration detected and the percentage of positives samples detected of the target compounds.

All the studied pharmaceutical compounds including metabolites and transformation products have been detected in a wide range of concentrations in the water samples analysed, obtaining the highest concentrations in influent and effluents of WW samples. Diclofenac, 4-AAA, 10,11-DHC, lamotrigine, gabapentin, and acetyl-sulfamethoxazole have been detected in the 56 samples analysed, with concentrations in the range 4 - 3900 ng L^{-1}, 12 - 12400 ng L^{-1}, 7 - 5320 ng L^{-1}, 5 - 995 ng L^{-1}, 3 - 3110 ng L^{-1}, and 6 - 4690 ng L^{-1}, respectively. Iopamidol, iomeprol, iopromide and iohexol were also been frequently (90%, 100%, 83% and 80%, respectively) detected in river water, as well as carbamazepine, sotalol, sulfamethoxazole, and hydrochlorothiazide that were frequently detected in the different matrices analysed. In general, it has to be noted a decrease in the median concentration in effluent wastewater sample with respect to influent wastewater concentration.

1H-benzotriazole, a corrosion inhibitor, was found in all the samples analysed and present the highest median concentration (1715 ng L^{-1}, 16800 ng L^{-1} and 8700 ng L^{-1} for river, influent WW and effluent WW, respectively) with respect the rest of the compounds analysed. These concentration values are in agreement with those obtained by Scheurer et al. [244] in analysing wastewater samples of the same area.

With regard to pesticides, at least six of the studied compounds were detected in all the 56 samples. The most frequently detected pesticides in wastewaters were chloridazon, simazine, and metazachlor metabolite BH479-4 (100% of positive

samples in both influent and effluent WW samples). Isoproturon, atrazine, metazachlor and diuron were other highly frequent pesticides detected (100%, 100%, 85% and 92% in effluent samples). In river waters, isoproturon, chloridazon, atrazine, simazine, metazachlor and BH479-4 were detected in all the samples, followed by desethylatrazine, BH479-8, metolachlor and diuron that were highly frequent detected (87% of the samples). In all the cases, the pesticides included in the European directive were found below the maximum allowable concentration established in the environmental quality standard [245].

Table 4.15 Summary of the concentrations calculated for the water samples.

COMPOUND	RIVER WATER (N=30)				INFLUENT WASTE WATER (N=13)				EFFLUENT WASTE WATER (N=13)			
	Median concentration (ng L^{-1})	Minimum level (ng L^{-1})	Maximum level (ng L^{-1})	Positive samples (%)	Median concentration (ng L^{-1})	Minimum level (ng L^{-1})	Maximum level (ng L^{-1})	Positive samples (%)	Median concentration (ng L^{-1})	Minimum level (ng L^{-1})	Maximum level (ng L^{-1})	Positive samples (%)
Diclofenac	154	4	763	100	2440	272	3900	100	1155	490	1925	100
Ibuprofen	<MDL	<MDL	116	37	10700	48[a]	17400	92	<MDL	<MDL	484	23
4-AAA	491	12	891	100	8620	342	12400	100	2270	413	5350	100
4-FAA	281	<MDL	1110	93	2640	1370	18800	100	1930	920	2500	100
Carbamazepine	261	<MDL	697	83	1270	<MDL	3730	85	1895	197	2525	100
10,11-DHC	341	7	1040	100	2160	809	5320	100	1830	555	2935	100
Lamotrigine	125	5	677	100	505	194	912	100	775	284	995	100
Gabapentin	148	3[a]	595	100	1300	568	3110	100	745	209	2185	100
Bezafibrate	21	<MDL	160	87	1230	47	6610	100	160	35	434	100
Iopamidol	180	<MDL	3210	90	<MDL	<MDL	402	38	74	<MDL	1075	69
Iopromide	56	<MDL	379	83	340	<MDL	5720	62	<MDL	<MDL	1690	31
Iomeprol	235	134	1510	100	1540	1340	16100	92	965	670	3365	100
Amidotrizoic acid	20[a]	<MDL	167	57	<MDL	<MDL	517	15	<MDL	<MDL	1930	38
Iohexol	29	<MDL	107	80	193	<MDL	13000	62	111	<MDL	5225	54
Metoprolol	194	<MDL	795	90	3420	<MDL	7980	92	1790	<MDL	2890	92
Sotalol	32	<MDL	128	97	335	59	675	100	239	35	456	100
Sulfamethoxazole	115	<MDL	469	97	423	<MDL	2540	85	183	<MDL	990	92
Acetyl-sulfamethoxazole	22	6	60	100	851	163	4690	100	55	37	169	100
Hydrochlorothiazide	284	<MDL	1860	93	4950	1655	6370	100	4175	1605	6300	100

a: below method quantification limit (<MQL)

MDL: method detection limit

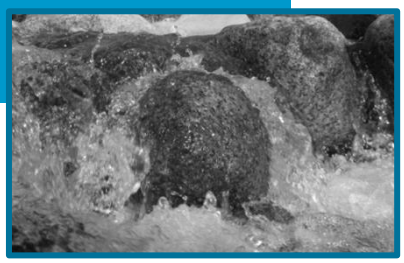
Table 4.15 Summary of the concentrations calculated for the water samples. (continued)

COMPOUND	RIVER WATER (N=30)				INFLUENT WASTE WATER (N=13)				EFFLUENT WASTE WATER (N=13)			
	Median concentration (ng L^{-1})	Minimum level (ng L^{-1})	Maximum level (ng L^{-1})	Positive samples (%)	Median concentration (ng L^{-1})	Minimum level (ng L^{-1})	Maximum level (ng L^{-1})	Positive samples (%)	Median concentration (ng L^{-1})	Minimum level (ng L^{-1})	Maximum level (ng L^{-1})	Positive samples (%)
1H-benzotriazole	1715	45	25300	100	16800	506	57900	100	8700	1338	21500	100
4MBT+5MBT	437	<MDL	12500	97	2980	566	11800	100	1615	890	6150	100
Isoproturon	16	2[a]	257	100	30[a]	<MDL	115	77	38	6[a]	122	100
Chloridazon	4	3[a]	54	100	27[a]	25[a]	117	100	15[a]	13[a]	106	100
Atrazine	3[a]	2[a]	10	100	16[a]	<MDL	25[a]	92	9[a]	7[a]	12[a]	100
Desethylatrazine	4	<MDL	18	87	<MDL	<MDL	27[a]	8	<MDL	<MDL	16[a]	15
Simazine	7	3[a]	16	100	19[a]	16[a]	67	100	11[a]	9[a]	31	100
Terbuthylazine	<MDL	<MDL	60	47	<MDL	<MDL	53	8	<MDL	<MDL	27	8
Metazachlor	5	1[a]	43	100	11[a]	<MDL	43[a]	85	8	<MDL	23	85
BH479-4	9	4	116	100	30	28	37	100	16	14	21	100
BH479-8	8	<MDL	120	87	<MDL	<MDL	43[a]	15	<MDL	<MDL	29	23
Metolachlor	2[a]	<MDL	249	87	26[a]	<MDL	67	85	12	<MDL	54	62
Metolachlor-C	3[a]	<MDL	29	77	<MDL	<MDL	26[a]	8	<MDL	<MDL	15[a]	23
Metolachlor-S	<MDL	<MDL	68	17	<MDL	<MDL	<MDL	0	<MDL	<MDL	<MDL	0
Chlortoluron	<MDL	<MDL	58	47	<MDL	<MDL	22[a]	15	<MDL	<MDL	11[a]	15
Diuron	14	<MDL	552	87	<MDL	<MDL	93	38	28	<MDL	294	92

a: below method quantification limit (<MQL)

MDL: method detection limit

CHAPTER 5. CONCLUSIONS



In this thesis, different analytical methodologies based on chromatographic separation for the determination of nutrients and micropollutants in water samples have been developed.

In this section the main conclusions are summarised:

1. Two methods have been developed for the speciation of phosphorus oxoanions in water samples:

    a. An HPLC-ICP-AES method using an anion exchange column has been applied to the speciation analysis of phosphorus oxoanions in different water matrices. Several parameters affecting the chromatographic separation have been studied and adjusted, as well as parameters related to the ICP-AES detection. The HPLC-ICP-AES method has been applied to the analysis of spiked tap water and effluent wastewater with recoveries ranging from 90.8 to 142.1%. Detection limits in the 1 - 5 mg P L^{-1} range have been obtained.

    b. In order to obtain lower LODs and using the same HPLC configuration as that in place before, the system was coupled to an ICP-MS as a detector. After adjusting the instrumental conditions and using an ORC pressurised with He, a reduction in the limits of detection was achieved (LODs in the 7 - 30 μg P L^{-1} range). This method was successfully applied to the analysis of effluent wastewater samples. Moreover, a single injection calibration approach (SICA) was used and non-significant differences in terms of precision and accuracy were found between the results obtained with SICA and those obtained with conventional calibration.

2. Several methods have been developed for the preconcentration of endocrine disrupting compounds belonging to different chemical families in water samples using PDMS rod extraction:

    a. A method for the determination of sixteen mainly brominated flame retardants, including some new BFRs, in water samples by GC-NCI-

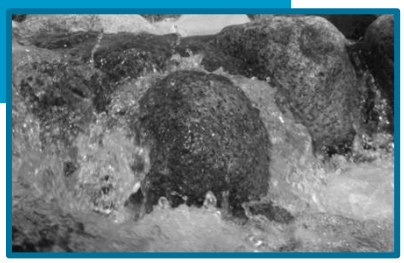
MS has been developed. Several parameters affecting the extraction and the desorption of the compounds have been studied allowing LODs at the low nanogram per litre, ranging from 0.4 to 10 ng L^{-1}, to be achieved. Matrix effects have been corrected by using 3 surrogate/internal standards. The method has been successfully applied to the analysis of different kind of water samples resulting in the detection of BFRs in many samples.

b. Direct and *in-situ* derivatisation methods based on polydimethylsiloxane (PDMS) rod extraction followed by liquid desorption and chromatographic analysis by liquid chromatography and diode array detection (LC-DAD) have been developed for the determination of five phenolic compounds in water samples. For both methods, parameters related to the extraction and the desorption of the compounds have been studied as well as chromatographic and detection conditions. For the direct method, LODs in the 0.6 - 2 μg L^{-1} range were obtained for all compounds except BPA (9.5 μg L^{-1}). With the derivatisation-based method, based on *in-situ* acetylation, lower LODs (0.3 - 0.9 μg L^{-1}) were obtained, especially for BPA. The results demonstrate the feasibility of using these two methods for the determination of BPA, TCP, PCP, NP, and OP in water.

3. A multiresidue method based on the use of an Oasis HLB cartridge has been developed and applied for the simultaneous determination of a group of 35 organic contaminants including pharmaceuticals, pesticides, corrosion inhibitors, metabolites and TPs in different water matrices. The determination was carried out by LC-MS/MS. Matrix effects have been corrected using 20 surrogate/internal standards, and LODs in the 0.2 - 8 ng L^{-1} range have been obtained. The method has been applied to the analysis of 56 water samples including river samples and influent and effluent wastewater samples. All the studied pharmaceuticals including metabolites and transformation products and some pesticides have been detected in a wide range of concentrations in

the water samples analysed. The highest concentrations were obtained in influent and effluents of WW samples.

REFERENCES



[1] E.G. Primel, S.S. Caldas, A.L.V. Escarrone, Multi-residue analytical methods for the determination of pesticides and PPCPs in water by LC-MS/MS: A review, Central European Journal of Chemistry, 10 (2012) 876-899.

[2] Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 establishing a framework for Community action in the field of water policy, Official Journal of the European Communities L 327/1, 22/12/2000.

[3] J. Nurmi, J. Pellinen, Multiresidue method for the analysis of emerging contaminants in wastewater by ultra performance liquid chromatography/time-of-flight mass spectrometry, Journal of Chromatography A, 1218 (2011) 6712-6719.

[4] A.D. Levine, T. Asano, Recovering sustainable water from wastewater, Environmental Science and Technology, 38 (2004) 201A-208A.

[5] T. Oki, S. Kanae, Global hydrological cycles and world water resources, Science, 313 (2006) 1068-1072.

[6] Royal Decree RD 1620/2007 of 7 December 2007 establishing the legal status for the reuse of purified water., Spanish Official Bulletin (BOE) 294 of 8 December 2007, Ministry of Presidence, Government of Spain, pp. 50639, 2007.

[7] S.C. Morton, M. Edwards, Reduced phosphorus compounds in the environment, Critical Reviews in Environmental Science and Technology, 35 (2005) 333-364.

[8] G. Hanrahan, T.M. Salmassi, C.S. Khachikian, K.L. Foster, Reduced inorganic phosphorus in the natural environment: significance, speciation and determination, Talanta, 66 (2005) 435-444.

[9] M.M. McDowell, M.M. Ivey, M.E. Lee, V. Firpo, T.M. Salmassi, C.S. Khachikian, K.L. Foster, Detection of hypophosphite, phosphite, and orthophosphate in natural geothermal water by ion chromatography, Journal of Chromatography A, 1039 (2004) 105-111.

[10] S.C. Morton, D. Glindemann, M.A. Edwards, Phosphates, phosphites, and phosphides in environmental samples, Environmental Science & Technology, 37 (2003) 1169-1174.

[11] J.A. Muñoz, M. López-Mesas, M. Valiente, Minimum handling method for the analysis of phosphorous inhibitors of urolithiasis (pyrophosphate and phytic acid) in urine by SPE-ICP techniques, Analytica Chimica Acta, 658 (2010) 204-208.

[12] V. Ruiz-Calero, M.T. Galceran, Ion chromatographic separations of phosphorus species: a review, Talanta, 66 (2005) 376-410.

[13] M.A. Pasek, Rethinking early Earth phosphorus geochemistry, Proceedings of the National Academy of Sciences of the United States of America, 105 (2008) 853-858.

[14] B. Schink, M. Friedrich, Phosphite oxidation by sulphate reduction, Nature, 406 (2000) 37.

[15] B. Schink, V. Thiemann, H. Laue, M.W. Friedrich, Desulfotignum phosphitoxidans sp. nov., a new marine sulfate reducer that oxidizes phosphite to phosphate, Archives of Microbiology, 177 (2002) 381-391.

[16] J.C. Torres Elguera, E. Yañez Barrientos, K. Wrobel, Monitoring of phosphorus oxide ion for analytical speciation of phosphite and phosphate in transgenic plants by high-performance liquid chromatography-inductively coupled plasma mass spectrometry, Journal of Agricultural and Food Chemistry, 61 (2013) 6622-6628.

[17] W. Yang, G. Tang, L. Song, Y. Hu, R.K.K. Yuen, Effect of rare earth hypophosphite and melamine cyanurate on fire performance of glass-fiber reinforced poly(1,4-butylene terephthalate) composites, Thermochimica Acta, 526 (2011) 185-191.

[18] Y. Sekiguchi, A. Matsunaga, A. Yamamoto, Y. Inoue, Analysis of condensed phosphates in food products by ion chromatography with an on-line hydroxide eluent generator, Journal of Chromatography A, 881 (2000) 639-644.

[19] R.A. Terkeltaub, Inorganic pyrophosphate generation and disposition in pathophysiology, American Journal of Physiology - Cell Physiology, 281 (2001) C1-C11.

[20] F.S. Stover, Capillary electrophoresis of phosphorus oxo anions, Journal of Chromatography A, 834 (1999) 243-256.

[21] Y. Luo, W. Guo, H.H. Ngo, L.D. Nghiem, F.I. Hai, J. Zhang, S. Liang, X.C. Wang, A review on the occurrence of micropollutants in the aquatic environment and their fate and removal during wastewater treatment, Science of the Total Environment, 473-474 (2014) 619-641.

[22] M. Mezcua, M.A. Martínez-Uroz, M.M. Gómez-Ramos, M.J. Gómez, J.M. Navas, A.R. Fernández-Alba, Analysis of synthetic endocrine-disrupting chemicals in food: A review, Talanta, 100 (2012) 90-106.

[23] H.-S. Chang, K.-H. Choo, B. Lee, S.-J. Choi, The methods of identification, analysis, and removal of endocrine disrupting compounds (EDCs) in water, Journal of Hazardous Materials, 172 (2009) 1-12.

[24] J.A. Rogers, L. Metz, V.W. Yong, Review: Endocrine disrupting chemicals and immune responses: A focus on bisphenol-A and its potential mechanisms, Molecular Immunology, 53 (2013) 421-430.

[25] N. Sharma, A. Jain, V.K. Singh, K.K. Verma, Solid-phase extraction combined with headspace single-drop microextraction of chlorophenols as their methyl ethers and analysis by high-performance liquid chromatography-diode array detection, Talanta, 83 (2011) 994-999.

[26] Directive 2008/105/EC of the European Parliament and of the Council on environmental quality standards in the field of water policy, amending and subsequently repealing Council Directives 82/176/EEC, 84/156/EEC, 84/491/EEC, 86/280/EEC and amending Directive 2000/60/EC of the European Parliament and of the Council., Official Journal of the European Union L 348, 24/12/2008.

[27] G. Gatidou, N.S. Thomaidis, A.S. Stasinakis, T.D. Lekkas, Simultaneous determination of the endocrine disrupting compounds nonylphenol, nonylphenol ethoxylates, triclosan and bisphenol A in wastewater and sewage sludge by gas chromatography-mass spectrometry, Journal of Chromatography A, 1138 (2007) 32-41.

[28] Q. Husain, S. Qayyum, Biological and enzymatic treatment of bisphenol A and other endocrine disrupting compounds: A review, Critical Reviews in Biotechnology, 33 (2013) 260-292.

[29] R. Carabias-Martínez, E. Rodríguez-Gonzalo, P. Revilla-Ruiz, Determination of weakly acidic endocrine-disrupting compounds by liquid chromatography-mass spectrometry with post-column base addition, Journal of Chromatography A, 1056 (2004) 131-138.

[30] http://www.chemicalize.org. ChemAxon. (Accessed 22.09.2014)

[31] D. Kim, J. Han, Y. Choi, On-line solid-phase microextraction of triclosan, bisphenol A, chlorophenols, and selected pharmaceuticals in environmental water samples by high-performance liquid chromatography-ultraviolet detection, Analytical and Bioanalytical Chemistry, 405 (2013) 377-387.

[32] E.O. Igbinosa, E.E. Odjadjare, V.N. Chigor, I.H. Igbinosa, A.O. Emoghene, F.O. Ekhaise, N.O. Igiehon, O.G. Idemudia, Toxicological profile of chlorophenols and their derivatives in the environment: The public health perspective, The Scientific World Journal, art. no. 460215 (2013).

[33] P. De Morais, T. Stoichev, M.C.P. Basto, P.N. Carvalho, M.T.S.D. Vasconcelos, A headspace SPME-GC-ECD method suitable for determination of chlorophenols in water samples, Analytical and Bioanalytical Chemistry, 399 (2011) 2531-2538.

[34] WHO, Pentachlorophenol in Drinking-water, WHO/SDE/WSH/03.04/62, 2003, Geneva.

[35] M. Villar-Navarro, M. Ramos-Payán, R. Fernández-Torres, M. Callejón-Mochón, M.T. Bello-López, A novel application of three phase hollow fiber based liquid phase microextraction (HF-LPME) for the HPLC determination of two endocrine disrupting compounds (EDCs), n-octylphenol and n-nonylphenol, in environmental waters, Science of the Total Environment, 443 (2013) 1-6.

[36] F. Ferrara, N. Ademollo, M. Delise, F. Fabietti, E. Funari, Alkylphenols and their ethoxylates in seafood from the Tyrrhenian Sea, Chemosphere, 72 (2008) 1279-1285.

[37] R. Gadzała-Kopciuch, A. Filipiak, B. Buszewski, Isolation, purification and determination of 4-n-nonylphenol and 4-tert-octylphenol in aqueous and biological samples, Talanta, 74 (2008) 655-660.

[38] W. Vetter, N. Rosenfelder, Gas chromatography retention data of environmentally relevant polybrominated compounds, Analytical and Bioanalytical Chemistry, 392 (2008) 489-504.

[39] A. Covaci, S. Harrad, M.A.E. Abdallah, N. Ali, R.J. Law, D. Herzke, C.A. de Wit, Novel brominated flame retardants: A review of their analysis, environmental fate and behaviour, Environment International, 37 (2011) 532-556.

[40] P.L. Andersson, K. Öberg, U. Örn, Chemical characterization of brominated flame retardants and identification of structurally representative compounds, Environmental Toxicology and Chemistry, 25 (2006) 1275-1282.

[41] J.L. Domingo, Human exposure to polybrominated diphenyl ethers through the diet, Journal of Chromatography A, 1054 (2004) 321-326.

[42] C.A. de Wit, D. Herzke, K. Vorkamp, Brominated flame retardants in the Arctic environment - trends and new candidates, Science of the Total Environment, 408 (2010) 2885-2918.

[43] Directive 2003/11/EC of the European Parliament and of the council amending for the 24th time Council Directive 76/769/EEC relating to restrictions on the marketing and use of certain dangerous substances and preparations (pentabromodiphenyl ether, octabromodiphenyl ether), Official Journal of the European Union L 042, 15/02/2003.

[44] European Court of Justice (2008) Cases C-14/06 and C-295/06, Judgement of the Court, 1 April 2008, Directive 2002/95/EC and Commission Decision 2005/717/EC. Official Journal of the European Union, 9/05/2008.

[45] N. Ricklund, A. Kierkegaard, M.S. McLachlan, Levels and Potential Sources of Decabromodiphenyl Ethane (DBDPE) and Decabromodiphenyl Ether (DecaBDE) in Lake and Marine Sediments in Sweden, Environmental Science & Technology, 44 (2010) 1987-1991.

[46] J. Cristale, J. Quintana, R. Chaler, F. Ventura, S. Lacorte, Gas chromatography/mass spectrometry comprehensive analysis of organophosphorus, brominated flame retardants, by-products and formulation intermediates in water, Journal of Chromatography A, 1241 (2012) 1-12.

[47] J.H. Kang, J.C. Kim, G.Z. Jin, H. Park, S.Y. Baek, Y.S. Chang, Detection of Dechlorane Plus in fish from urban-industrial rivers, Chemosphere, 79 (2010) 850-854.

[48] http://scifinder.cas.org. American Chemical Society. 2014. (Accessed 22.09.2014)

[49] S. Mompelat, B. Le Bot, O. Thomas, Occurrence and fate of pharmaceutical products and by-products, from resource to drinking water, Environment International, 35 (2009) 803-814.

[50] L. Patrolecco, N. Ademollo, P. Grenni, A. Tolomei, A. Barra Caracciolo, S. Capri, Simultaneous determination of human pharmaceuticals in water samples by solid phase extraction and HPLC with UV-fluorescence detection, Microchemical Journal, 107 (2013) 165-171.

[51] S. Castiglioni, R. Bagnati, D. Calamari, R. Fanelli, E. Zuccato, A multiresidue analytical method using solid-phase extraction and high-pressure liquid chromatography tandem mass spectrometry to measure pharmaceuticals of different therapeutic classes in urban wastewaters, Journal of Chromatography A, 1092 (2005) 206-215.

[52] S.D. Richardson, T.A. Ternes, Water analysis: Emerging contaminants and current issues, Analytical Chemistry, 83 (2011) 4616-4648.

[53] Y. Yu, L. Wu, Analysis of endocrine disrupting compounds, pharmaceuticals and personal care products in sewage sludge by gas chromatography-mass spectrometry, Talanta, 89 (2012) 258-263.

[54] E. Gracia-Lor, M. Martínez, J.V. Sancho, G. Peñuela, F. Hernández, Multi-class determination of personal care products and pharmaceuticals in environmental and wastewater samples by ultra-high performance liquid-chromatography-tandem mass spectrometry, Talanta, 99 (2012) 1011-1023.

[55] W.C. Li, Occurrence, sources, and fate of pharmaceuticals in aquatic environment and soil, Environmental Pollution, 187 (2014) 193-201.

[56] M.J.M. Bueno, M.J. Gomez, S. Herrera, M.D. Hernando, A. Agüera, A.R. Fernández-Alba, Occurrence and persistence of organic emerging contaminants and priority pollutants in five sewage treatment plants of Spain: Two years pilot survey monitoring, Environmental Pollution, 164 (2012) 267-273.

[57] M.J. Gómez, C. Sirtori, M. Mezcua, A.R. Fernández-Alba, A. Agüera, Photodegradation study of three dipyrone metabolites in various water systems: Identification and toxicity of their photodegradation products, Water Research, 42 (2008) 2698-2706.

[58] M.E. Hoque, F. Cloutier, C. Arcieri, M. McInnes, T. Sultana, C. Murray, P.A. Vanrolleghem, C.D. Metcalfe, Removal of selected pharmaceuticals, personal care products and artificial sweetener in an aerated sewage lagoon, Science of the Total Environment (2013).

[59] I. Ferrer, E.M. Thurman, Analysis of 100 pharmaceuticals and their degradates in water samples by liquid chromatography/quadrupole time-of-flight mass spectrometry, Journal of Chromatography A, 1259 (2012) 148-157.

[60] D.A. Lambropoulou, M.D. Hernando, I.K. Konstantinou, E.M. Thurman, I. Ferrer, T.A. Albanis, A.R. Fernández-Alba, Identification of photocatalytic degradation products of bezafibrate in TiO2 aqueous suspensions by liquid and gas chromatography, Journal of Chromatography A, 1183 (2008) 38-48.

[61] M. Petrovic, B. Skrbic, J. Zivancev, L. Ferrando-Climent, D. Barceló, Determination of 81 pharmaceutical drugs by high performance liquid chromatography coupled to mass spectrometry with hybrid triple quadrupole-linear ion trap in different types of water in Serbia, Science of the Total Environment, 468-469 (2014) 415-428.

[62] S. Idder, L. Ley, P. Mazellier, H. Budzinski, Quantitative on-line preconcentration-liquid chromatography coupled with tandem mass spectrometry method for the determination of pharmaceutical compounds in water, Analytica Chimica Acta, 805 (2013) 107-115.

[63] J.L. Kormos, M. Schulz, T.A. Ternes, Occurrence of Iodinated X-ray Contrast Media and Their Biotransformation Products in the Urban Water Cycle, Environmental Science & Technology, 45 (2011) 8723-8732.

[64] Y. Mu, J. Radjenovic, J.Y. Shen, R.A. Rozendal, K. Rabaey, J. Keller, Dehalogenation of Iodinated X-ray Contrast Media in a Bioelectrochemical System, Environmental Science & Technology, 45 (2011) 782-788.

[65] F. Sacher, B. Raue, H.H. Brauch, Analysis of iodinated X-ray contrast agents in water samples by ion chromatography and inductively-coupled plasma mass spectrometry, Journal of Chromatography A, 1085 (2005) 117-123.

[66] A.C. Alder, C. Schaffner, M. Majewsky, J. Klasmeier, K. Fenner, Fate of β-blocker human pharmaceuticals in surface water: Comparison of measured and simulated concentrations in the Glatt Valley Watershed, Switzerland, Water Research, 44 (2010) 936-948.

[67] M. Gros, S. Rodríguez-Mozaz, D. Barceló, Fast and comprehensive multi-residue analysis of a broad range of human and veterinary pharmaceuticals and some of their metabolites in surface and treated waters by ultra-high-performance liquid chromatography coupled to quadrupole-linear ion trap tandem mass spectrometry, Journal of Chromatography A, 1248 (2012) 104-121.

[68] K. Kümmerer, Antibiotics in the aquatic environment - A review - Part I, Chemosphere, 75 (2009) 417-434.

[69] K. Nödler, T. Licha, K. Bester, M. Sauter, Development of a multi-residue analytical method, based on liquid chromatography-tandem mass spectrometry, for the simultaneous determination of 46 micro-contaminants in aqueous samples, Journal of Chromatography A, 1217 (2010) 6511-6521.

[70] M. Boisvert, P.B. Fayad, S. Sauvé, Development of a new multi-residue laser diode thermal desorption atmospheric pressure chemical ionization tandem mass spectrometry method for the detection and quantification of pesticides and pharmaceuticals in wastewater samples, Analytica Chimica Acta, 754 (2012) 75-82.

[71] I. Michael, L. Rizzo, C.S. McArdell, C.M. Manaia, C. Merlin, T. Schwartz, C. Dagot, D. Fatta-Kassinos, Urban wastewater treatment plants as hotspots for the release of antibiotics in the environment: A review, Water Research, 47 (2013) 957-995.

[72] R. López-Serna, A. Jurado, E. Vázquez-Suñé, J. Carrera, M. Petrovic, D. Barceló, Occurrence of 95 pharmaceuticals and transformation products in urban groundwaters underlying the metropolis of Barcelona, Spain, Environmental Pollution, 174 (2013) 305-315.

[73] C. Baird. Environmental Chemistry. New York (USA): W.H. Freeman and Company; 1995, ISBN 0-7167-2404-9.

[74] A. Masiá, M. Ibáñez, C. Blasco, J.V. Sancho, Y. Picó, F. Hernández, Combined use of liquid chromatography triple quadrupole mass spectrometry and liquid chromatography quadrupole time-of-flight mass spectrometry in systematic screening of pesticides and other contaminants in water samples, Analytica Chimica Acta, 761 (2013) 117-127.

[75] E. Bizkarguenaga, O. Ros, A. Iparraguirre, P. Navarro, A. Vallejo, A. Usobiaga, O. Zuloaga, Solid-phase extraction combined with large volume injection-programmable temperature vaporization-gas chromatography-mass spectrometry for the multiresidue determination of priority and emerging organic pollutants in wastewater, Journal of Chromatography A, 1247 (2012) 104-117.

[76] S. Weiss, J. Jakobs, T. Reemtsma, Discharge of three benzotriazole corrosion inhibitors with municipal wastewater and improvements by membrane bioreactor treatment and ozonation, Environmental Science and Technology, 40 (2006) 7193-7199.

[77] A. Müller, S.C. Weiss, J. Beisswenger, H.G. Leukhardt, W. Schulz, W. Seitz, W.K.L. Ruck, W.H. Weber, Identification of ozonation by-products of 4- and 5-methyl-1H-benzotriazole during the treatment of surface water to drinking water, Water Research, 46 (2012) 679-690.

[78] T. Reemtsma, U. Miehe, U. Duennbier, M. Jekel, Polar pollutants in municipal wastewater and the water cycle: Occurrence and removal of benzotriazoles, Water Research, 44 (2010) 596-604.

[79] H. Wolschke, Z. Xie, A. Möller, R. Sturm, R. Ebinghaus, Occurrence, distribution and fluxes of benzotriazoles along the German large river basins into the North Sea, Water Research, 45 (2011) 6259-6266.

[80] B. Kasprzyk-Hordern, R.M. Dinsdale, A.J. Guwy, The effect of signal suppression and mobile phase composition on the simultaneous analysis of multiple classes of acidic/neutral pharmaceuticals and personal care products in surface water by solid-phase extraction and ultra performance liquid chromatography-negative electrospray tandem mass spectrometry, Talanta, 74 (2008) 1299-1312.

[81] S. Huntscha, H.P. Singer, C.S. McArdell, C.E. Frank, J. Hollender, Multiresidue analysis of 88 polar organic micropollutants in ground, surface and wastewater using online mixed-bed multilayer solid-phase extraction coupled to high performance liquid chromatography-tandem mass spectrometry, Journal of Chromatography A, 1268 (2012) 74-83.

[82] F.F. Mohamed, P. Abdullah, M.R.B. Othman, Z.B. Zakeria, "SPE-LC-Mass spectrometry analysis for basic pharmaceuticals with different therapeutic classes in aquatic environment: A review", Journal of Applied Sciences Research, 8 (2012) 2124-2132.

[83] A. Grossberger, Y. Hadar, T. Borch, B. Chefetz, Biodegradability of pharmaceutical compounds in agricultural soils irrigated with treated wastewater, Environmental Pollution, 185 (2014) 168-177.

[84] Sigma-Aldrich, Basics in Solid Phase Extraction (SPE), Euroanalysis, Belgrade, 2011.

[85] F.R.a.A. Rouessac. Chemical Analysis: Modern instrumentation methods and thecniques (English edition). Chichester (England): John Wiley & Sons; 2000, ISBN 0-471-98137-0.

[86] P. López, S.A. Brandsma, P.E.G. Leonards, J. De Boer, Methods for the determination of phenolic brominated flame retardants, and by-products, formulation intermediates and decomposition products of brominated flame retardants in water, Journal of Chromatography A, 1216 (2009) 334-345.

[87] X. Jin, G. Jiang, G. Huang, J. Liu, Q. Zhou, Determination of 4-tert-octylphenol, 4-nonylphenol and bisphenol A in surface waters from the Haihe River in Tianjin by gas chromatography-mass spectrometry with selected ion monitoring, Chemosphere, 56 (2004) 1113-1119.

[88] M. Kawaguchi, R. Ito, N. Endo, N. Okanouchi, N. Sakui, K. Saito, H. Nakazawa, Liquid phase microextraction with in situ derivatization for measurement of bisphenol A in river water sample by gas chromatography-mass spectrometry, Journal of Chromatography A, 1110 (2006) 1-5.

[89] X. Wang, C.P. Diao, R.S. Zhao, Rapid determination of bisphenol A in drinking water using dispersive liquid-phase microextraction with in situ derivatization prior to GC-MS, Journal of Separation Science, 32 (2009) 154-159.

[90] J.R. Dean. Extraction methods for environmental analysis. Chichester (England): John Wiley & Sons; 1998, ISBN 0-471-98287-3.

[91] Sigma-Aldrich, Guide to Solid phase extraction, Bulletin 910, 1998.

[92] F. Augusto, E. Carasek, R.G.C. Silva, S.R. Rivellino, A.D. Batista, E. Martendal, New sorbents for extraction and microextraction techniques, Journal of Chromatography A, 1217 (2010) 2533-2542.

[93] H. Bagheri, A. Mohammadi, A. Salemi, On-line trace enrichment of phenolic compounds from water using a pyrrole-based polymer as the solid-phase extraction sorbent coupled with high-performance liquid chromatography, Analytica Chimica Acta, 513 (2004) 445-449.

[94] L. Elci, N. Kolbe, S.G. Elci, J.T. Anderson, Solid phase extractive preconcentration coupled to gas chromatography-atomic emission detection for the determination of chlorophenols in water samples, Talanta, 85 (2011) 551-555.

[95] H. Bagheri, M. Saraji, New polymeric sorbent for the solid-phase extraction of chlorophenols from water samples followed by gas chromatography-electron-capture detection, Journal of Chromatography A, 910 (2001) 87-93.

[96] J.A. Padilla-Sánchez, P. Plaza-Bolaños, R. Romero-González, N. Barco-Bonilla, J.L. Martínez-Vidal, A. Garrido-Frenich, Simultaneous analysis of chlorophenols, alkylphenols, nitrophenols and cresols in wastewater effluents, using solid phase extraction and further determination by gas chromatography-tandem mass spectrometry, Talanta, 85 (2011) 2397-2404.

[97] B. Kowalski, M. Mazur, The simultaneous determination of six flame retardants in water samples using SPE pre-concentration and UHPLC-UV method, Water, Air, and Soil Pollution, 225 (2014).

[98] P. Labadie, K. Tlili, F. Alliot, C. Bourges, A. Desportes, M. Chevreuil, Development of analytical procedures for trace-level determination of polybrominated diphenyl ethers and tetrabromobisphenol A in river water and sediment, Analytical and Bioanalytical Chemistry, 396 (2010) 865-875.

[99] I. Fulara, M. Czaplicka, Methods for determination of polybrominated diphenyl ethers in environmental samples - Review, Journal of Separation Science, 35 (2012) 2075-2087.

[100] F. Bono-Blay, A. Guart, B. de la Fuente, M. Pedemonte, M.C. Pastor, A. Borrell, S. Lacorte, Survey of phthalates, alkylphenols, bisphenol A and herbicides in Spanish source waters intended for bottling, Environmental Science and Pollution Research, 19 (2012) 3339-3349.

[101] E. Psillakis, N. Kalogerakis, Developments in liquid-phase microextraction, TRAC Trends in Analytical Chemistry, 22 (2003) 565-574.

[102] A. Sarafraz-Yazdi, A. Amiri, Liquid-phase microextraction, TRAC Trends in Analytical Chemistry, 29 (2010) 1-14.

[103] M. Polo, G. Gómez-Noya, J.B. Quintana, M. Llompart, C. García-Jares, R. Cela, Development of a Solid-Phase Microextraction Gas Chromatography/Tandem

Mass Spectrometry Method for Polybrominated Diphenyl Ethers and Polybrominated Biphenyls in Water Samples, Analytical Chemistry, 76 (2004) 1054-1062.

[104] J.X. Wang, D.Q. Jiang, Z.Y. Gu, X.P. Yan, Multiwalled carbon nanotubes coated fibers for solid-phase microextraction of polybrominated diphenyl ethers in water and milk samples before gas chromatography with electron-capture detection, Journal of Chromatography A, 1137 (2006) 8-14.

[105] F. Hernandez, J. Beltran, F.J. Lopez, J.V. Gaspar, Use of solid-phase microextraction for the quantitative determination of herbicides in soil and water samples, Analytical Chemistry, 72 (2000) 2313-2322.

[106] C.V. Antoniou, E.E. Koukouraki, E. Diamadopoulos, Analysis of selected pharmaceutical compounds and endocrine disruptors in municipal wastewater using solid-phase microextraction and gas chromatography, Water Environment Research, 81 (2009) 664-669.

[107] http://www.sigmaaldrich.com/technical-documents/articles/reporter-us/bioanalysis-with-spme.html. Sigma-Aldrich Co. 2014. (Accessed 22.09.2014)

[108] http://www.sigmaaldrich.com/analytical-chromatography/sample-preparation/spme/selecting-spme-fiber.html. Sigma-Aldrich Co. 2014. (Accessed 22.09.2014)

[109] http://www.gerstel.com/en/twister-stir-bar-sorptive-extraction.htm. Gerstel GmbH & Co. (Accessed 22.09.2014)

[110] N. Gilart, R.M. Marcé, F. Borrull, N. Fontanals, New coatings for stir-bar sorptive extraction of polar emerging organic contaminants, TRAC Trends in Analytical Chemistry, 54 (2014) 11-23.

[111] C. Margoum, C. Guillemain, X. Yang, M. Coquery, Stir bar sorptive extraction coupled to liquid chromatography-tandem mass spectrometry for the determination of pesticides in water samples: Method validation and measurement uncertainty, Talanta, 116 (2013) 1-7.

[112] A. Prieto, O. Basauri, R. Rodil, A. Usobiaga, L.A. Fernández, N. Etxebarria, O. Zuloaga, Stir-bar sorptive extraction: A view on method optimisation, novel applications, limitations and potential solutions, Journal of Chromatography A, 1217 (2010) 2642-2666.

[113] J.B. Quintana, R. Rodil, S. Muniategui-Lorenzo, P. López-Mahía, D. Prada-Rodríguez, Multiresidue analysis of acidic and polar organic contaminants in water samples by stir-bar sorptive extraction-liquid desorption-gas chromatography-mass spectrometry, Journal of Chromatography A, 1174 (2007) 27-39.

[114] P. Serôdio, M.S. Cabral, J.M.F. Nogueira, Use of experimental design in the optimization of stir bar sorptive extraction for the determination of polybrominated diphenyl ethers in environmental matrices, Journal of Chromatography A, 1141 (2007) 259-270.

[115] B.L.L. Tan, D.W. Hawker, J.F. Müller, L.A. Tremblay, H.F. Chapman, Stir bar sorptive extraction and trace analysis of selected endocrine disruptors in water, biosolids and sludge samples by thermal desorption with gas chromatography-mass spectrometry, Water Research, 42 (2008) 404-412.

[116] J.M.F. Nogueira, Novel sorption-based methodologies for static microextraction analysis: A review on SBSE and related techniques, Analytica Chimica Acta, 757 (2012) 1-10.

[117] E. Villaverde-de-Sáa, I. Racamonde, J.B. Quintana, R. Rodil, R. Cela, Ion-pair sorptive extraction of perfluorinated compounds from water with low-cost polymeric materials: Polyethersulfone vs polydimethylsiloxane, Analytica Chimica Acta, 740 (2012) 50-57.

[118] A. Prieto, R. Rodil, J.B. Quintana, I. Rodríguez, R. Cela, M. Möder, Evaluation of low-cost disposable polymeric materials for sorptive extraction of organic pollutants in water samples, Analytica Chimica Acta, 716 (2012) 119-127.

[119] A. Delgado, O. Posada-Ureta, M. Olivares, A. Vallejo, N. Etxebarria, Silicone rod extraction followed by liquid desorption-large volume injection-programmable temperature vaporiser-gas chromatography-mass spectrometry for trace analysis of

priority organic pollutants in environmental water samples, Talanta, 117 (2013) 471-482.

[120] M. Popp, S. Hann, G. Koellensperger, Environmental application of elemental speciation analysis based on liquid or gas chromatography hyphenated to inductively coupled plasma mass spectrometry-A review, Analytica Chimica Acta, 668 (2010) 114-129.

[121] M. van Pinxteren, A. Paschke, P. Popp, Silicone rod and silicone tube sorptive extraction, Journal of Chromatography A, 1217 (2010) 2589-2598.

[122] R. Montes, I. Rodríguez, E. Rubí, R. Cela, Suitability of polydimethylsiloxane rods for the headspace sorptive extraction of polybrominated diphenyl ethers from water samples, Journal of Chromatography A, 1143 (2007) 41-47.

[123] A. Giordano, P. Richter, K. Leiva, L. Ascar, Determination of sunscreen compounds in water samples by silicone rod extraction, Journal of the Chilean Chemical Society, 58 (2013) 1741-1743.

[124] A. Assoumani, C. Margoum, Y. Lassalle, B. Herbreteau, K. Faure, M. Coquery, J. Randon, Polydimethylsiloxane rods for the passive sampling of pesticides in surface waters, Water (Switzerland), 5 (2013) 1366-1379.

[125] J. Nácher, Doctoral Thesis, Potencial del acoplamiento GC-MS para la determinación de contaminantes orgánicos en muestras de matriz compleja, 2011, Universitat Jaume I

[126] A. Gonzalez-Gago, S.H. Brandsma, P.E.G. Leonards, J. de Boer, J.M. Marchante-Gayon, J.I.G. Alonso, Determination of ultra-trace levels of priority PBDEs in water samples by isotope dilution GC(ECNI)MS using (81)Br-labelled standards, Analytical and Bioanalytical Chemistry, 401 (2011) 2639-2649.

[127] M.J. He, X.J. Luo, M.Y. Chen, Y.X. Sun, S.J. Chen, B.X. Mai, Bioaccumulation of polybrominated diphenyl ethers and decabromodiphenyl ethane in fish from a river system in a highly industrialized area, South China, Science of the Total Environment, 419 (2012) 109-115.

[128] V. Yusa, A. Pastor, M. de la Guardia, Microwave-assisted extraction of polybrominated diphenyl ethers and polychlorinated naphthalenes concentrated on semipermeable membrane devices, Analytica Chimica Acta, 565 (2006) 103-111.

[129] J.B. Quintana, I. Rodríguez, Strategies for the microextraction of polar organic contaminants in water samples, Analytical and Bioanalytical Chemistry, 384 (2006) 1447-1461.

[130] M. Kawaguchi, K. Inoue, M. Yoshimura, N. Sakui, N. Okanouchi, R. Ito, Y. Yoshimura, H. Nakazawa, Trace analysis of phenolic xenoestrogens in water samples by stir bar sorptive extraction with in situ derivatization and thermal desorption-gas chromatography-mass spectrometry, Journal of Chromatography A, 1041 (2004) 19-26.

[131] F. Sacher, F.T. Lang, H.J. Brauch, I. Blankenhorn, Pharmaceuticals in groundwaters - Analytical methods and results of a monitoring program in Baden-Wurttemberg, Germany, Journal of Chromatography A, 938 (2001) 199-210.

[132] G. Shanmugam, S. Sampath, K.K. Selvaraj, D.G.J. Larsson, B.R. Ramaswamy, Non-steroidal anti-inflammatory drugs in Indian rivers, Environmental Science and Pollution Research, 21 (2014) 921-931.

[133] L. Cherta, J. Beltran, T. Portolés, F. Hernández, Multiclass determination of 66 organic micropollutants in environmental water samples by fast gas chromatography-mass spectrometry, Analytical and Bioanalytical Chemistry, 402 (2012) 2301-2314.

[134] J. Robles-Molina, B. Gilbert-López, J.F. García-Reyes, A. Molina-Díaz, Monitoring of selected priority and emerging contaminants in the Guadalquivir River and other related surface waters in the province of Jaén, South East Spain, Science of the Total Environment, 479-480 (2014) 247-257.

[135] A. Naccarato, E. Gionfriddo, G. Sindona, A. Tagarelli, Simultaneous determination of benzothiazoles, benzotriazoles and benzosulfonamides by solid phase microextraction-gas chromatography-triple quadrupole mass spectrometry in environmental aqueous matrices and human urine, Journal of Chromatography A, 1338 (2014) 164-173.

[136] D. Profrock, P. Leonhard, S. Wilbur, A. Prange, Sensitive, simultaneous determination of P, S, Cl, Br and I containing pesticides in environmental samples by GC hyphenated with collision-cell ICP-MS, Journal of Analytical Atomic Spectrometry, 19 (2004) 623-631.

[137] A.P. Vonderheide, J. Meija, M. Montes-Bayón, J.A. Caruso, Use of optional gas and collision cell for enhanced sensitivity of the organophosphorus pesticides by GC-ICP-MS, Journal of Analytical Atomic Spectrometry, 18 (2003) 1097-1102.

[138] M.J. Gómez, M. Petrovic, A.R. Fernández-Alba, D. Barceló, Determination of pharmaceuticals of various therapeutic classes by solid-phase extraction and liquid chromatography-tandem mass spectrometry analysis in hospital effluent wastewaters, Journal of Chromatography A, 1114 (2006) 224-233.

[139] H. Pech, A. Henry, C.S. Khachikian, T.M. Salmassi, G. Hanrahan, K.L. Foster, Detection of Geothermal Phosphite Using High-Performance Liquid Chromatography, Environmental Science & Technology, 43 (2009) 7671-7675.

[140] M.M. Ivey, K.L. Foster, Detection of phosphorus oxyanions in synthetic geothermal water using ion chromatography-mass spectrometry techniques, Journal of Chromatography A, 1098 (2005) 95-103.

[141] Z.L. Chen, W.X. He, M. Beer, M. Megharaj, R. Naidu, Speciation of glyphosate, phosphate and aminomethylphosphonic acid in soil extracts by ion chromatography with inductively coupled plasma mass spectrometry with an octopole reaction system, Talanta, 78 (2009) 852-856.

[142] B.B.M. Sadi, A.P. Vonderheide, J.A. Caruso, Analysis of phosphorus herbicides by ion-pairing reversed-phase liquid chromatography coupled to inductively coupled plasma mass spectrometry with octapole reaction cell, Journal of Chromatography A, 1050 (2004) 95-101.

[143] M. Rezaee, Y. Yamini, S. Shariati, A. Esrafili, M. Shamsipur, Dispersive liquid-liquid microextraction combined with high-performance liquid chromatography-UV detection as a very simple, rapid and sensitive method for the determination of bisphenol A in water samples, Journal of Chromatography A, 1216 (2009) 1511-1514.

[144] R.S. Zhao, X. Wang, J.P. Yuan, L.L. Zhang, Solid-phase extraction of bisphenol A, nonylphenol and 4-octylphenol from environmental water samples using microporous bamboo charcoal, and their determination by HPLC, Microchimica Acta, 165 (2009) 443-447.

[145] T. Vega-Morales, Z. Sosa-Ferrera, J.J. Santana-Rodríguez, Development and optimisation of an on-line solid phase extraction coupled to ultra-high-performance liquid chromatography-tandem mass spectrometry methodology for the simultaneous determination of endocrine disrupting compounds in wastewater samples, Journal of Chromatography A, 1230 (2012) 66-76.

[146] E. Gracia-Lor, J.V. Sancho, R. Serrano, F. Hernández, Occurrence and removal of pharmaceuticals in wastewater treatment plants at the Spanish Mediterranean area of Valencia, Chemosphere, 87 (2012) 453-462.

[147] A. Putschew, S. Schittko, M. Jekel, Quantification of triiodinated benzene derivatives and X-ray contrast media in water samples by liquid chromatography-electrospray tandem mass spectrometry, Journal of Chromatography A, 930 (2001) 127-134.

[148] W. Seitz, W.H. Weber, J.Q. Jiang, B.J. Lloyd, M. Maier, D. Maier, W. Schulz, Monitoring of iodinated X-ray contrast media in surface water, Chemosphere, 64 (2006) 1318-1324.

[149] T. Reemtsma, L. Alder, U. Banasiak, A multimethod for the determination of 150 pesticide metabolites in surface water and groundwater using direct injection liquid chromatography-mass spectrometry, Journal of Chromatography A, 1271 (2013) 95-104.

[150] C. Erger, P. Balsaa, F. Werres, T.C. Schmidt, Multi-component trace analysis of organic xenobiotics in surface water containing suspended particular matter by solid phase extraction/gas chromatography-mass spectrometry, Journal of Chromatography A, 1249 (2012) 181-189.

[151] S.C. Morton, D. Glindemann, X. Wang, X. Niu, M. Edwards, Analysis of reduced phosphorus in samples of environmental interest, Environmental Science and Technology, 39 (2005) 4369-4376.

[152] H. De Brabandere, N. Forsgard, L. Israelsson, J. Petterson, E. Rydin, M. Waldeback, P.J.R. Sjoberg, Screening for organic phosphorus compounds in aquatic sediments by liquid chromatography coupled to ICP-AES and ESI-MS/MS, Analytical Chemistry, 80 (2008) 6689-6697.

[153] Z.X. Guo, Q.T. Cai, Z.G. Yang, Determination of glyphosate and phosphate in water by ion chromatography - inductively coupled plasma mass spectrometry detection, Journal of Chromatography A, 1100 (2005) 160-167.

[154] Z.X. Guo, Q.T. Cai, Z. Yang, Ion chromatography/inductively coupled plasma mass spectrometry for simultaneous determination of glyphosate, glufosinate, fosamine and ethephon at nanogram levels in water, Rapid Communications in Mass Spectrometry, 21 (2007) 1606-1612.

[155] B. Divjak, M. Novic, W. Goessler, Determination of bromide, bromate and other anions with ion chromatography and an inductively coupled plasma mass spectrometer as element-specific detector, Journal of Chromatography A, 862 (1999) 39-47.

[156] C.L. Camp, B.L. Sharp, H.J. Reid, J. Entwisle, H. Goenaga-Infante, Analysis of mono-phosphate nucleotides as a potential method for quantification of DNA using high performance liquid chromatography-inductively coupled plasma-mass spectrometry, Analytical and Bioanalytical Chemistry, 402 (2012) 367-372.

[157] L. Winkel, J. Feldmann, A.A. Meharg, Quantitative and Qualitative Trapping of Volatile Methylated Selenium Species Entrained through Nitric Acid, Environmental Science & Technology, 44 (2009) 382-387.

[158] C. Van Moorleghem, L. Six, F. Degryse, E. Smolders, R. Merckx, Effect of Organic P Forms and P Present in Inorganic Colloids on the Determination of Dissolved P in Environmental Samples by the Diffusive Gradient in Thin Films Technique, Ion Chromatography, and Colorimetry, Analytical Chemistry, 83 (2011) 5317-5323.

[159] N. Gros, B. Gorenc, Performance of ion chromatography in the determination of anions and cations in various natural waters with elevated mineralization, Journal of Chromatography A, 770 (1997) 119-124.

[160] I. Dahllof, O. Svensson, C. Torstensson, Optimising the determination of nitrate and phosphate in sea water with ion chromatography using experimental design, Journal of Chromatography A, 771 (1997) 163-168.

[161] C.K. Zacharis, P.D. Tzanavaras, Determination of bisphosphonate active pharmaceutical ingredients in pharmaceuticals and biological material: A review of analytical methods, Journal of Pharmaceutical and Biomedical Analysis, 48 (2008) 483-496.

[162] A. Seubert, On-line coupling of ion chromatography with ICP-AES and ICP-MS, Trends in Analytical Chemistry, 20 (2001) 274-287.

[163] S.C. Morton, D. Glindemann, X.R. Wang, X.J. Niu, M. Edwards, Analysis of reduced phosphorus in samples of environmental interest, Environmental Science & Technology, 39 (2005) 4369-4376.

[164] D.F.T.D.F. Thompson, F. Michopoulos, C.J. Smith, C.J. Duckett, R.W. Wilkinson, P. Jarvis, I.D. Wilson, Profiling biological samples using ultra performance liquid chromatography-inductively coupled plasma-mass spectrometry (UPLC-ICP-MS) for the determination of phosphorus and sulfur-containing metabolites, Molecular Biosystems, 7 (2011) 1149-1157.

[165] D.D. Richardson, B.B.M. Sadi, J.A. Caruso, Reversed phase ion-pairing HPLC-ICP-MS for analysis of organophosphorus chemical warfare agent degradation products, Journal of Analytical Atomic Spectrometry, 21 (2006) 396-403.

[166] M. Popp, S. Hann, A. Mentler, M. Fuerhacker, G. Stingeder, G. Koellensperger, Determination of glyphosate and AMPA in surface and waste water using high-performance ion chromatography coupled to inductively coupled plasma dynamic reaction cell mass spectrometry (HPIC-ICP-DRC-MS), Analytical and Bioanalytical Chemistry, 391 (2008) 695-699.

[167] K. Blaabjerg, J. Hansen-Moller, H.D. Poulsen, High-performance ion chromatography method for separation and quantification of inositol phosphates in diets and digesta, Journal of Chromatography B, 878 (2010) 347-354.

[168] M. Garcia-Lopez, I. Rodriguez, R. Cela, K.K. Kroening, J.A. Caruso, Determination of organophosphate flame retardants and plasticizers in sediment samples using microwave-assisted extraction and gas chromatography with inductively coupled plasma mass spectrometry, Talanta, 79 (2009) 824-829.

[169] M. Shah, J.A. Caruso, Inductively coupled plasma mass spectrometry in separation techniques: Recent trends in phosphorus speciation, Journal of Separation Science, 28 (2005) 1969-1984.

[170] D.R. Bandura, V.I. Baranev, S.D. Tanner, Detection of ultratrace phosphorus and sulfur by quadrupole ICPMS with dynamic reaction cell, Analytical Chemistry, 74 (2002) 1497-1502.

[171] C.J. Smith, I.D. Wilson, L. Weidolf, F. Abou-Shakra, M. Thomsen, Enhanced detection of sulphur and phosphorous containing compounds in HPLC-inductively coupled plasma mass spectrometry using chemical resolution via hexapole-based reaction with oxygen, Chromatographia, 59 (2004) S165-S170.

[172] P. Allain, L. Jaunault, Y. Mauras, J.M. Mermet, T. Delaporte, Signal enhancement of elements due to the presence of carbon-containing compounds in inductively coupled plasma mass-spectrometry, Analytical Chemistry, 63 (1991) 1497-1498.

[173] Z.C. Hu, S.H. Hu, S. Gao, Y.S. Liu, S.L. Lin, Volatile organic solvent-induced signal enhancements in inductively coupled plasma-mass spectrometry: a case study of methanol and acetone, Spectrochimica Acta Part B-Atomic Spectroscopy, 59 (2004) 1463-1470.

[174] M. Kovačevič, W. Goessler, N. Mikac, M. Veber, Matrix effects during phosphorus determination with quadrupole inductively coupled plasma mass spectrometry, Analytical and Bioanalytical Chemistry, 383 (2005) 145-151.

[175] I. Rodushkin, P. Nordlund, E. Engström, D.C. Baxter, Improved multi-elemental analyses by inductively coupled plasma-sector field mass spectrometry through methane addition to the plasmat, Journal of Analytical Atomic Spectrometry, 20 (2005) 1250-1255.

[176] J.E. Carr, K. Kwok, G.K. Webster, J.W. Carnahan, Effects of liquid chromatography mobile phases and buffer salts on phosphorus inductively coupled plasma atomic emission and mass spectrometries utilizing ultrasonic nebulization and membrane desolvation, Journal of Pharmaceutical and Biomedical Analysis, 40 (2006) 42-50.

[177] E. Paredes, S.E. Maestre, M.S. Prats, J.L. Todoli, Single-injection calibration approach for high-performance liquid chromatography, Journal of Chromatography A, 1185 (2008) 178-184.

[178] E. Paredes, S.E. Maestre, S. Prats, J.L. Todolí, Simultaneous determination of carbohydrates, carboxylic acids, alcohols, and metals in foods by high-performance liquid chromatography inductively coupled plasma atomic emission spectrometry, Analytical Chemistry, 78 (2006) 6774-6782.

[179] T.M. McBeath, E. Lombi, M.J. McLaughlin, E.K. Bunemann, Polyphosphate speciation for soil and fertilizer analysis, Communications in Soil Science and Plant Analysis, 38 (2007) 2445-2460.

[180] G. Grindlay, L. Gras, J. Mora, M.T.C. de Loos-Vollebregt, Carbon-related matrix effects in inductively coupled plasma atomic emission spectrometry, Spectrochimica Acta Part B - Atomic Spectroscopy, 63 (2008) 234-243.

[181] J. Machat, V. Otruba, V. Kanicky, Spectral and non-spectral interferences in the determination of selenium by inductively coupled plasma atomic emission spectrometry, Journal of Analytical Atomic Spectrometry, 17 (2002) 1096-1102.

[182] S.E. Maestre, J. Mora, V. Hernandis, J.L. Todoli, A system for the direct determination of the nonvolatile organic carbon, dissolved organic carbon, and inorganic carbon in water samples through inductively coupled plasma atomic emission spectrometry, Analytical Chemistry, 75 (2003) 111-117.

[183] E. Hernandez-Nataren, A. Sahuquillo, R. Rubio, J.F. Lopez-Sanchez, Solid-phase extraction (SPE) assays to ascertain the mechanisms of retention of antimony species in several stationary phases, Microchemical Journal, 97 (2011) 74-77.

[184] G. Raber, R. Raml, W. Goessler, K.A. Francesconi, Quantitative speciation of arsenic compounds when using organic solvent gradients in HPLC-ICPMS, Journal of Analytical Atomic Spectrometry, 25 (2010) 570-576.

[185] S.N. Walford, Applications of ion chromatography in cane sugar research and process problems, Journal of Chromatography A, 956 (2002) 187-199.

[186] J. Vial, A. Jardy, Experimental comparison of the different approaches to estimate LOD and LOQ of an HPLC method, Analytical Chemistry, 71 (1999) 2672-2677.

[187] R.S. Houk, Mass spectrometry of inductively coupled plasmas, Analytical Chemistry, 58 (1986) 97 A-105 A.

[188] C. Agatemor, D. Beauchemin, Matrix effects in inductively coupled plasma mass spectrometry: A review, Analytica Chimica Acta, 706 (2011) 66-83.

[189] M. Colon, M. Hidalgo, M. Iglesias, Arsenic determination by ICP-QMS with octopole collision/reaction cell. Overcome of matrix effects under vented and pressurized cell conditions, Talanta, 85 (2011) 1941-1947.

[190] C. Han, J. Geng, X. Xie, X. Wang, H. Ren, S. Gao, Determination of phosphite in a eutrophic freshwater lake by suppressed conductivity ion chromatography, Environmental Science and Technology, 46 (2012) 10667-10674.

[191] J. Carter, L. Ebdon, E.H. Evans, Speciation of silicon and phosphorous using liquid chromatography coupled with sector field high resolution ICP-MS, Microchemical Journal, 76 (2004) 35-41.

[192] S.i. Fujii, K. Inagaki, A. Takatsu, T. Yarita, K. Chiba, Determination of phosphorus using capillary electrophoresis and micro-high-performance liquid chromatography hyphenated with inductively coupled plasma mass spectrometry for the quantification of nucleotides, Journal of Chromatography A, 1216 (2009) 7488-7492.

[193] http://www.ccbgi.org/sanejament_detall.php?id_municipi=3&any=2011. Consorci de la Costa Brava. 2014. (Accessed 22.09.2014)

[194] http://www.ccbgi.org/sanejament_detall.php?id_municipi=7&any=2011. Consorci de la Costa Brava. 2014. (Accessed 22.09.2014)

[195] M.F. Miller, S.M. Chernyak, S.E. Domino, S.A. Batterman, R. Loch-Caruso, Concentrations and speciation of polybrominated diphenyl ethers in human amniotic fluid, Science of the Total Environment, 417-418 (2012) 294-298.

[196] S.N. Zhou, E.J. Reiner, C. Marvin, T. Kolic, N. Riddell, P. Helm, F. Dorman, M. Misselwitz, I.D. Brindle, Liquid chromatography-atmospheric pressure photoionization tandem mass spectrometry for analysis of 36 halogenated flame retardants in fish, Journal of Chromatography A, 1217 (2010) 633-641.

[197] M. Karlsson, A. Julander, B. van Bavel, L. Hardell, Levels of brominated flame retardants in blood in relation to levels in household air and dust, Environment International, 33 (2007) 62-69.

[198] H. Frederiksen, L. Aksglaede, K. Sorensen, O. Nielsen, K.M. Main, N.E. Skakkebaek, A. Juul, A.-M. Andersson, Bisphenol A and other phenols in urine from Danish children and adolescents analyzed by isotope diluted TurboFlow-LC-MS/MS, International Journal of Hygiene and Environmental Health, 216 (2013) 710-720.

[199] R. Ito, M. Kawaguchi, H. Honda, Y. Koganei, N. Okanouchi, N. Sakui, K. Saito, H. Nakazawa, Hollow-fiber-supported liquid phase microextraction with in situ derivatization and gas chromatography-mass spectrometry for determination of chlorophenols in human urine samples, Journal of Chromatography B, 872 (2008) 63-67.

[200] C.A. De Wit, J.A. Björklund, K. Thuresson, Tri-decabrominated diphenyl ethers and hexabromocyclododecane in indoor air and dust from Stockholm microenvironments 2: Indoor sources and human exposure, Environment International, 39 (2012) 141-147.

[201] H.M. Stapleton, J.G. Allen, S.M. Kelly, A. Konstantinov, S. Klosterhaus, D. Watkins, M.D. McClean, T.F. Webster, Alternate and new brominated flame retardants

detected in U.S. house dust, Environmental Science & Technology, 42 (2008) 6910-6916.

[202] Z.Y. Xie, A. Moller, L. Ahrens, R. Sturm, R. Ebinghaus, Brominated Flame Retardants in Seawater and Atmosphere of the Atlantic and the Southern Ocean, Environmental Science & Technology, 45 (2011) 1820-1826.

[203] P. López, S.A. Brandsma, P.E.G. Leonards, J. De Boer, Optimization and development of analytical methods for the determination of new brominated flame retardants and polybrominated diphenyl ethers in sediments and suspended particulate matter, Analytical and Bioanalytical Chemistry, 400 (2011) 871-883.

[204] A. Kierkegaard, J. Björklund, U. Fridén, Identification of the flame retardant decabromodiphenyl ethane in the environment, Environmental Science & Technology, 38 (2004) 3247-3253.

[205] C. Yang, X.Z. Meng, L. Chen, S.Q. Xia, Polybrominated diphenyl ethers in sewage sludge from Shanghai, China: Possible ecological risk applied to agricultural land, Chemosphere, 85 (2011) 418-423.

[206] J. Xu, L. Wu, W. Chen, A.C. Chang, Simultaneous determination of pharmaceuticals, endocrine disrupting compounds and hormone in soils by gas chromatography-mass spectrometry, Journal of Chromatography A, 1202 (2008) 189-195.

[207] V. Andreu, E. Ferrer, J.L. Rubio, G. Font, Y. Pico, Quantitative determination of octylphenol, nonylphenol, alkylphenol ethoxylates and alcohol ethoxylates by pressurized liquid extraction and liquid chromatography-mass spectrometry in soils treated with sewage sludges, Science of the Total Environment, 378 (2007) 124-129.

[208] W.L. Chen, G.S. Wang, J.C. Gwo, C.Y. Chen, Ultra-high performance liquid chromatography/tandem mass spectrometry determination of feminizing chemicals in river water, sediment and tissue pretreated using disk-type solid-phase extraction and matrix solid-phase dispersion, Talanta, 89 (2012) 237-245.

[209] H.Q. Liu, M.H. Zhang, X.D. Wang, Y.J. Zou, W.W. Wang, M.P. Ma, Y.Y. Li, H.L. Wang, Extraction and determination of polybrominated diphenyl ethers in water

and urine samples using solidified floating organic drop microextraction along with high performance liquid chromatography, Microchimica Acta, 176 (2012) 303-309.

[210] X. Huang, Y. Wang, D. Yuan, X. Li, S. Nong, New monolithic stir-cake-sorptive extraction for the determination of polar phenols by HPLC, Analytical and Bioanalytical Chemistry, 405 (2013) 2185-2193.

[211] S. Nakamura, S. Daishima, Simultaneous determination of alkylphenols and bisphenol a in river water by stir bar sorptive extraction with in situ acetylation and thermal desorption-gas chromatography-mass spectrometry, Journal of Chromatography A, 1038 (2004) 291-294.

[212] J.I. Cacho, N. Campillo, P. Viñas, M. Hernández-Córdoba, Stir bar sorptive extraction coupled to gas chromatography-mass spectrometry for the determination of bisphenols in canned beverages and filling liquids of canned vegetables, Journal of Chromatography A, 1247 (2012) 146-153.

[213] L. Montero, S. Conradi, H. Weiss, P. Popp, Determination of phenols in lake and ground water samples by stir bar sorptive extraction-thermal desorption-gas chromatography-mass spectrometry, Journal of Chromatography A, 1071 (2005) 163-169.

[214] A. Prieto, O. Zuloaga, A. Usobiaga, N. Etxebarria, L.A. Fernández, Use of experimental design in the optimisation of stir bar sorptive extraction followed by thermal desorption for the determination of brominated flame retardants in water samples, Analytical and Bioanalytical Chemistry, 390 (2008) 739-748.

[215] A. Salamova, R.A. Hites, Discontinued and Alternative Brominated Flame Retardants in the Atmosphere and Precipitation from the Great Lakes Basin, Environmental Science & Technology, 45 (2011) 8698-8706.

[216] J.F. Leal, V.I. Esteves, E.B.H. Santos, BDE-209: Kinetic studies and effect of humic substances on photodegradation in water, Environmental Science and Technology, 47 (2013) 14010-14017.

[217] L. Debrauwer, A. Riu, M. Jouahri, E. Rathahao, I. Jouanin, J.P. Antignac, R. Cariou, B. Le Bizec, D. Zalko, Probing new approaches using atmospheric pressure

photo ionization for the analysis of brominated flame retardants and their related degradation products by liquid chromatography-mass spectrometry, Journal of Chromatography A, 1082 (2005) 98-109.

[218] A.R. Fontana, M.M. de Toro, J.C. Altamirano, One-Step Derivatization and Preconcentration Microextraction Technique for Determination of Bisphenol A in Beverage Samples by Gas Chromatography-Mass Spectrometry, Journal of Agricultural and Food Chemistry, 59 (2011) 3559-3565.

[219] A. Peñalver, E. Pocurull, F. Borrull, R.M. Marcé, Solid-phase microextraction coupled to high-performance liquid chromatography to determine phenolic compounds in water samples, Journal of Chromatography A, 953 (2002) 79-87.

[220] C. Hao, X. Zhao, P. Yang, GC-MS and HPLC-MS analysis of bioactive pharmaceuticals and personal-care products in environmental matrices, TRAC Trends in Analytical Chemistry, 26 (2007) 569-580.

[221] V.M. León, B. Álvarez, M.A. Cobollo, S. Muñoz, I. Valor, Analysis of 35 priority semivolatile compounds in water by stir bar sorptive extraction-thermal desorption-gas chromatography-mass spectrometry: I. Method optimisation, Journal of Chromatography A, 999 (2003) 91-101.

[222] International Organization for Standardization, ISO Standard 220302:2006; Water quality - determination of selected polybrominated diphenyl ethers in sediment and sewage sludge - Method using extraction and gas chromatography/mass spectrometry, ISO, Geneva, Switzerland, 2006.

[223] X. Liu, J. Li, Z. Zhao, W. Zhang, K. Lin, C. Huang, X. Wang, Solid-phase extraction combined with dispersive liquidâ€“liquid microextraction for the determination for polybrominated diphenyl ethers in different environmental matrices, Journal of Chromatography A, 1216 (2009) 2220-2226.

[224] J. Llorca-Porcel, G. Martínez-Sánchez, B. Álvarez, M.A. Cobollo, I. Valor, Analysis of nine polybrominated diphenyl ethers in water samples by means of stir bar sorptive extraction-thermal desorption-gas chromatography-mass spectrometry, Analytica Chimica Acta, 569 (2006) 113-118.

[225] S.N. Zhou, E.J. Reiner, C. Marvin, P. Helm, N. Riddell, F. Dorman, M. Misselwitz, L. Shen, P. Crozier, K. MacPherson, I.D. Brindle, Development of liquid chromatography atmospheric pressure chemical ionization tandem mass spectrometry for analysis of halogenated flame retardants in wastewater, Analytical and Bioanalytical Chemistry, 396 (2010) 1311-1320.

[226] J.I. Cacho, N. Campillo, P. Viñas, M. Hernández-Córdoba, Stir bar sorptive extraction with EG-Silicone coating for bisphenols determination in personal care products by GC-MS, Journal of Pharmaceutical and Biomedical Analysis, 78-79 (2013) 255-260.

[227] A. Hubaux, G. Vos, Decision and detection limits for linear calibration curves, Analytical Chemistry, 42 (1970) 849-855.

[228] P. Campíns-Falcó, J. Verdú-Andrés, A. Sevillano-Cabeza, R. Herráez-Hernández, C. Molins-Legua, Y. Moliner-Martinez, In-tube solid-phase microextraction coupled by in valve mode to capillary LC-DAD: Improving detectability to multiresidue organic pollutants analysis in several whole waters, Journal of Chromatography A, 1217 (2010) 2695-2702.

[229] C. Hu, B. Chen, M. He, B. Hu, Amino modified multi-walled carbon nanotubes/polydimethylsiloxane coated stir bar sorptive extraction coupled to high performance liquid chromatography-ultraviolet detection for the determination of phenols in environmental samples, Journal of Chromatography A, 1300 (2013) 165-172.

[230] N. Sheng, F. Wei, W. Zhan, Z. Cai, S. Du, X. Zhou, F. Li, Q. Hu, Dummy molecularly imprinted polymers as the coating of stir bar for sorptive extraction of bisphenol A in tap water, Journal of Separation Science, 35 (2012) 707-712.

[231] H. Shaaban, T. Górecki, Green ultra-fast high-performance liquid chromatographic method using a short narrow-bore column packed with fully porous sub-2 μm particles for the simultaneous determination of selected pharmaceuticals as surface water and wastewater pollutants, Journal of Separation Science, 36 (2013) 252-261.

[232] M. Gros, S. Rodríguez-Mozaz, D. Barceló, Rapid analysis of multiclass antibiotic residues and some of their metabolites in hospital, urban wastewater and river water by ultra-high-performance liquid chromatography coupled to quadrupole-linear ion trap tandem mass spectrometry, Journal of Chromatography A, 1292 (2013) 173-188.

[233] S. Perez, D. Barcelo, Fate and occurrence of X-ray contrast media in the environment, Analytical and Bioanalytical Chemistry, 387 (2007) 1235-1246.

[234] F. Busetti, K.L. Linge, J.W. Blythe, A. Heitz, Rapid analysis of iodinated X-ray contrast media in secondary and tertiary treated wastewater by direct injection liquid chromatography-tandem mass spectrometry, Journal of Chromatography A, 1213 (2008) 200-208.

[235] S. Echeverría, P. Herrero, F. Borrull, N. Fontanals, E. Pocurull, Performance of zwitterionic hydrophilic interaction LC for the determination of iodinated X-ray contrast agents, Journal of Separation Science, 36 (2013) 3688-3695.

[236] W. Ens, F. Senner, B. Gygax, G. Schlotterbeck, Development, validation, and application of a novel LC-MS/MS trace analysis method for the simultaneous quantification of seven iodinated X-ray contrast media and three artificial sweeteners in surface, ground, and drinking water, Analytical and Bioanalytical Chemistry, 406 (2014) 2789-2798.

[237] M. Tankiewicz, C. Morrison, M. Biziuk, Multi-residue method for the determination of 16 recently used pesticides from various chemical groups in aqueous samples by using DI-SPME coupled with GC-MS, Talanta, 107 (2013) 1-10.

[238] F. Wode, C. Reilich, P. van Baar, U. Dünnbier, M. Jekel, T. Reemtsma, Multiresidue analytical method for the simultaneous determination of 72 micropollutants in aqueous samples with ultra high performance liquid chromatography-high resolution mass spectrometry, Journal of Chromatography A, 1270 (2012) 118-126.

[239] M. Oosterhuis, F. Sacher, T.L. ter Laak, Prediction of concentration levels of metformin and other high consumption pharmaceuticals in wastewater and regional

surface water based on sales data, Science of the Total Environment, 442 (2013) 380-388.

[240] M. Pedrouzo, F. Borrull, R.M. Marce, E. Pocurull, Ultra-high-performance liquid chromatography-tandem mass spectrometry for determining the presence of eleven personal care products in surface and wastewaters, Journal of Chromatography A, 1216 (2009) 6994-7000.

[241] J. Quintana, I. Martí, F. Ventura, Monitoring of pesticides in drinking and related waters in NE Spain with a multiresidue SPE-GC-MS method including an estimation of the uncertainty of the analytical results, Journal of Chromatography A, 938 (2001) 3-13.

[242] R. Grabic, J. Fick, R.H. Lindberg, G. Fedorova, M. Tysklind, Multi-residue method for trace level determination of pharmaceuticals in environmental samples using liquid chromatography coupled to triple quadrupole mass spectrometry, Talanta, 100 (2012) 183-195.

[243] L. Maldaner, I.C.S.F. Jardim, Determination of some organic contaminants in water samples by solid-phase extraction and liquid chromatography-tandem mass spectrometry, Talanta, 100 (2012) 38-44.

[244] M. Scheurer, F.R. Storck, C. Graf, H.J. Brauch, W. Ruck, O. Lev, F.T. Lange, Correlation of six anthropogenic markers in wastewater, surface water, bank filtrate, and soil aquifer treatment, Journal of Environmental Monitoring, 13 (2011) 966-973.

[245] Directive 2008/105//EC of the European Parliament and of the Council of 16 December 2008 on environmental quality standards in the field of water policy, amending and subsequently repealing Council Directives 82/176/EEC, 83/513/EEC, 84/156/EEC, 84/491/EEC, 86/280/EEC and amending Directive 2000/60/EC of the European Parliament and of the Council, Official Journal of the European Union L 348/84, 24/12/2008.