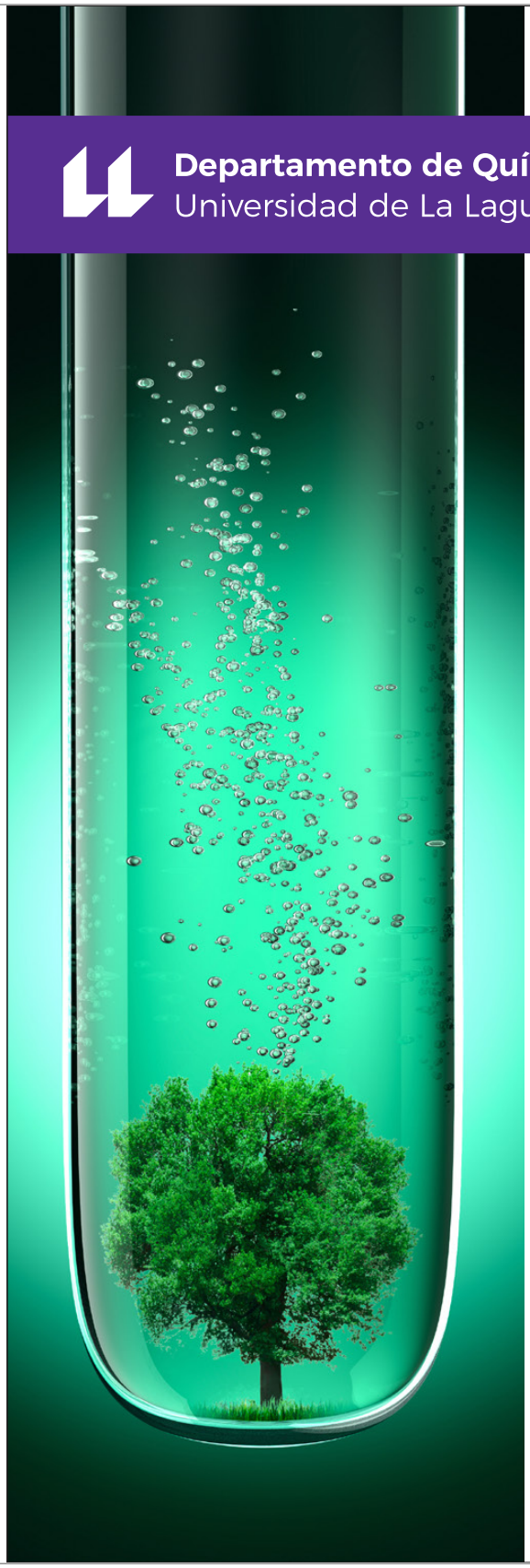


Departamento de Química
Universidad de La Laguna

New extraction protocols for the determination of endocrine disrupting compounds in samples of environmental and agri-food interest

Javier González Sálamo

2018

Supervisor:
Miguel Ángel Rodríguez Delgado

Co-supervisor and tutor:
Javier Hernández Borges

Departamento de Química
Universidad de La Laguna

D. MIGUEL ÁNGEL RODRÍGUEZ DELGADO, CATEDRÁTICO DE QUÍMICA ANALÍTICA, Y D. JAVIER HERNÁNDEZ BORGES, PROFESOR TITULAR DE QUÍMICA ANALÍTICA, DEL DEPARTAMENTO DE QUÍMICA DE LA FACULTAD DE CIENCIAS DE LA UNIVERSIDAD DE LA LAGUNA,

INFORMAN:

Que D. Javier González Sálamo, Graduado en Química por la Universidad de La Laguna, ha realizado bajo nuestra dirección los trabajos conducentes a la realización de su Tesis Doctoral titulada NEW EXTRACTION PROTOCOLS FOR THE DETERMINATION OF ENDOCRINE DISRUPTING COMPOUNDS IN SAMPLES OF ENVIRONMENTAL AND AGRI-FOOD INTEREST.

Revisado el trabajo, autorizamos su presentación, para que se pueda proceder a su lectura y defensa pública, y optar al grado de Doctor en Química con Mención Internacional por esta Universidad.

Y para que así conste, firmamos el presente en San Cristóbal de La Laguna, a 17 de octubre de 2018.

Fdo: Dr. Miguel Ángel Rodríguez Delgado
(Director)

Fdo: Dr. Javier Hernández Borges
(Co-director y tutor)

C/ Padre Herrera s/n
38207 La Laguna
Santa Cruz de Tenerife. España

T: 900 43 25 26

ull.es

FACULTAD DE CIENCIAS

DEPARTAMENTO DE QUÍMICA

NEW EXTRACTION PROTOCOLS FOR THE DETERMINATION OF ENDOCRINE DISRUPTING COMPOUNDS IN SAMPLES OF ENVIRONMENTAL AND AGRI-FOOD INTEREST

MEMORIA PARA LA OBTENCIÓN DEL GRADO DE DOCTOR

JAVIER GONZÁLEZ SÁLAMO

SAN CRISTÓBAL DE LA LAGUNA, TENERIFE

OCTUBRE 2018

Tesis cofinanciada por la Agencia Canaria de Investigación, Innovación y Sociedad de la Información de la Consejería de Economía, Industria, Comercio y Conocimiento y por el Fondo Social Europeo (FSE), Programa Operativo Integrado de Canarias 2014-2020, Eje 3 Tema Prioritario 74 (85%).

Agradecimientos

Ahora que finaliza esta etapa tan importante de mi vida, me vienen un sinfín de buenos recuerdos a la mente. Buenos momentos que, afortunadamente, no he vivido solo, y en los que siempre he estado rodeado de compañeros, familia y amigos. Es por ello por lo que quiero agradecerles el haber compartido conmigo estos años.

En primer lugar, y como no podía ser de otra manera, quiero dar las gracias a las dos personas sin las que esta tesis no hubiera sido posible, los Dres. Miguel Ángel Rodríguez Delgado y Javier Hernández Borges. Miguel Ángel, gracias por haber confiado en mí y haberme permitido integrarme en tu grupo de investigación para trabajar a tu lado, sin ni siquiera conocerme. Gracias por tu apoyo incondicional, por tus consejos y por todo lo que me has enseñado durante estos años. Javi, gracias por estar siempre a mi lado, por tus consejos, por tu dedicación incansable, por transmitirme tu sentido de la responsabilidad y del buen hacer, por darme la confianza y serenidad necesarias en los momentos más difíciles. Porque simplemente saber que estabas ahí, me da la seguridad necesaria para afrontar cada reto. A los dos, GRACIAS.

Quería agradecer también a los demás miembros del grupo AQAIMPA, los profesores Galindo, María Jesús y Tere, por los buenos momentos que he pasado junto a ellos, por sus consejos, su apoyo y, sobre todo, el cariño que me han demostrado durante todos estos años.

Este camino no habría sido el mismo sin mis compañeros de laboratorio, Bárbara, Antonio, Miguel Ángel, María, Giovanni, Diana y Álvaro. Con ustedes he compartido el día a día, los buenos y los malos momentos. Gracias por sus consejos y apoyo. Antonio, gracias por haber sido mi maestro en mis inicios. Eres todo un ejemplo de esfuerzo, dedicación y humildad. Bárbara, gracias por estar ahí. Contigo he compartido todos y cada uno de los momentos que he pasado en el laboratorio. Ha sido todo un placer haber estado a tu lado, porque no podría haber soñado con una compañera mejor que tú. Miguel Ángel, aunque nos conocimos tarde, sin duda, ha merecido la pena. Gracias por todo lo que me has enseñado y por tu apoyo durante estos años.

Quiero dar las gracias también al resto de compañeros del Área de Química Analítica y del Departamento de Química de la Universidad de La Laguna. Ha sido un placer trabajar con ustedes. Me gustaría hacer una mención especial a los miembros del grupo GIN-QAAMA, con quienes he compartido tan buenos momentos.

A mis compañeros desde el primer día que pisé la facultad. Juntos hemos compartido las alegrías y las penas de la vida universitaria. Diego, Provi, Pedro, Adrián, gracias por estar siempre ahí.

I would also like to thank Dr. Christian Neusüß and his research group for helping me during my stay at the University of Aalen. I would like to especially thank Cristina Montealegre for her help and support during my stay in Germany.

Me gustaría agradecer también al SEGAI-ULL, concretamente al Servicio de Técnicas Agroalimentarias, por su colaboración en esta tesis doctoral.

A mis amigos por estar siempre a mi lado. A Caro y a David por su apoyo y preocupación a pesar de la distancia. A los que considero como mis hermanos, Sofía y Luis, gracias por estar siempre ahí, por entenderme y por disculpar mis ausencias en tantos momentos. GRACIAS.

A toda mi familia por preocuparse siempre por mí y apoyarme en cada etapa de mi vida.

A Miguel, Maysa e Isabel, porque con ustedes siempre me he sentido como en casa. Gracias por todo su cariño y por interesarse siempre por mí.

A mis abuelos Manuel y Carmen. Allá donde estén quiero darles las gracias por todo lo que me enseñaron, por sus consejos y por transmitirme unos valores de trabajo constante y humildad que siempre llevaré conmigo. Siempre en mi corazón.

A mis abuelos Santiago y Ana. Con ustedes pasé gran parte de mi infancia y, en parte, por ustedes hoy estoy aquí. Han sido como unos padres para mí. Nunca olvidaré las siestas con "los documentales de la 2" junto a abuelo, o los paseos y las meriendas en la terraza de alguna cafetería con abuela. Sé que ustedes siempre han estado orgullosos de mí, pero quiero que sepan que yo también lo estoy de ustedes. Muchas gracias por todo.

A mi hermano Sergio, por estar siempre a mi lado apoyándome en todo lo que hago, por escucharme, por aconsejarme. Aunque no recuerdo si te lo he dicho alguna vez, aprovecho estas líneas para decirte que siento una gran admiración por ti y que eres todo un ejemplo para mí. No dejes nunca de ser como eres. Gracias hermano.

Ana, quiero que sepas que me siento muy afortunado de poder estar a tu lado. Eres la mejor compañera que podría tener. Gracias por estar a mi lado cada día, incansable, aconsejándome, apoyándome y comprendiendo mis ausencias en tantos momentos. Gracias por celebrar conmigo cada victoria como si fuera tuya, y por animarme en los malos momentos, sacándome siempre una sonrisa. Simplemente, GRACIAS.

Por último, la pieza más importante de todo esto, mis padres, Francisco y Ana. No hay duda de que, si hay alguien responsable de que haya conseguido llegar hasta este momento, esos son ustedes. Gracias por haber estado ahí desde siempre, apoyándome, aconsejándome y ayudándome en todo lo que he hecho. Gracias por la tranquilidad que he sentido siempre al saber que ustedes estaban ahí, y gracias por soportarme en los malos momentos. Me siento muy afortunado por tenerlos a mi lado. Esta tesis también es de ustedes.

A todos, GRACIAS.

Javier González Sálamo

Octubre, 2018

A mis padres
A mi hermano

TABLE OF CONTENTS

TABLE OF CONTENTS

CHAPTER I
INTRODUCTION

I.- INTRODUCTION

I.1.- Endocrine disruptors

Emerging contaminants (ECs) are pollutants which have attracted much attention in the last years for both the scientific community and the society due to the potential adverse effects they can have on the environment and, consequently, in humans' health [1,2]. Despite this fact, ECs are mainly constituted by organic chemical compounds which are not regulated or not fully regulated even in developed countries.

Among the wide variety of ECs which are currently known, especial attention has been paid to those which have demonstrated to own an endocrine disrupting activity [3,4]. This group of chemicals, commonly known as endocrine-disrupting compounds (EDCs), comprises a good number of natural substances (e.g. natural oestrogens or phytoestrogens), as well as a broad spectrum of synthetic products derived from the human activity (pesticides, plasticisers, pharmaceuticals, personal care products, etc.) [5].

Nowadays, EDCs have been defined by the European Commission as "exogenous substances or mixtures that alter function(s) of the endocrine system and, consequently, cause adverse health effects in an intact organism, its progeny or (sub)populations" [6], that is to say, in a simpler way, any chemical substance which alters the normal hormone function. Historically, the term "endocrine disruptor" was coined for the first time in 1991, during the Wingspread Conference focused on EDCs organised by Theodora Colborn (1927-2014, as a fellow of the W. Alton Jones Foundation), and her co-workers in Wisconsin [3,7,8], which met 21 scientists from 15 disciplines to discuss about the evidence that different chemical compounds were affecting the reproduction and growth of animals, as well as humans' health [9,10]. Since then, the number of publications in which the term "endocrine disruptor" has been used has notably increased, as can be seen in Figure I.1. However, and despite there exists the wrong believing that EDCs studies had this conference as starting point, many cases that would now be labelled as caused by EDCs had already been observed and published since the 1940s [8,9]. As an example, in February 1946, Bennetts and co-workers reported a problem in Australian *merino* sheep which manifested with infertility, prolapse of the uterus (normally months after the parturition) and dystocia [11]. Regarding endocrine disruption in humans, one of the first cases were reported in 1949, when pilots of "crop dusters" (agricultural aircrafts) suffered a drop of sperm counts related to the use of dichlorodiphenyltrichloroethane (DDT) [12].

Due to the relatively high number of EDCs which are currently known and their heterogeneity, different classification strategies have been given by some authors under several criteria [3]. Among them, the simplest one classifies EDCs according to their nature, dividing them in two categories, *natural* (which can be found in foods or in humans, such as phytoestrogens or natural oestrogenic hormones) and *synthetic chemicals* (pesticides, fungicides, pharmaceuticals, plasticisers, etc.) [4]. These compounds can also be classified according to their origins, distinguishing among artificial and natural hormones, drugs with proven hormonal side effects, household and industrial chemicals, and industrial and household side products [13]. In this sense, it should be mentioned the classification proposed by Gore et al. in 2014 [14], who categorised EDCs in three groups based on their occurrence: *pesticides*, since some of them such as DDT or chlorpyrifos have proven to produce disorders in the neuroendocrine system; *chemicals in products* (which can be subdivided in children's products and electronics), referring to EDCs which are present in products used every day by humans; and *food contact materials*, since several alterations of the endocrine system have been attributed to this kind of substances.

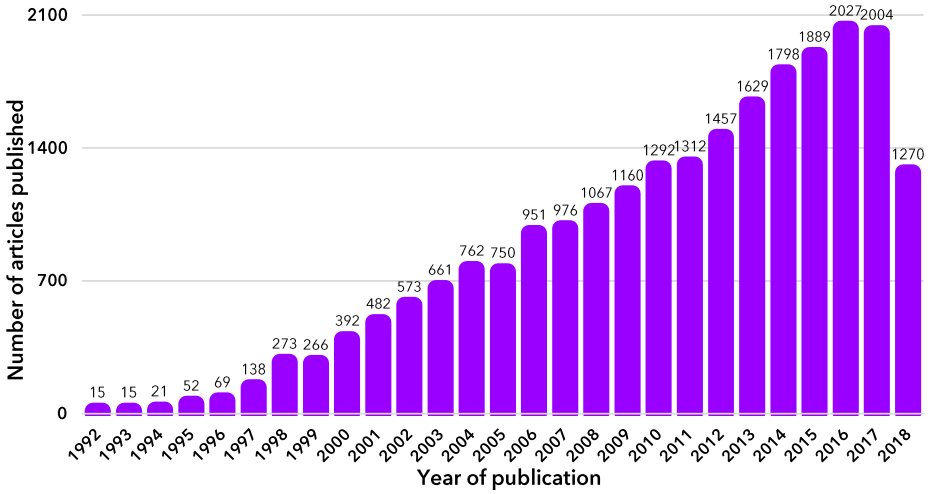


Figure I.1.- Number of scientific articles published per year since 1992 referring to endocrine disruption ("endocrine disrupt*" term was used; * can be any word ending). Data taken from the Web of Science [15].

In order to provide a better understanding of the way in which EDCs affect the endocrine system, it is important to elucidate how it works and the role of natural

hormones. Briefly, the activity of the endocrine system is based on the release of hormones whose three-dimensional structure and chemical composition is unique and complementary to the target cell receptors [14]. Several factors affect the capacity of a hormone to activate the receptor, including the amount of hormone synthesised and released, its transport through the circulatory system or the quantity that reaches the receptor, among others [14]. Thus, any interference in one or more of these steps results in a disruption of the endocrine system. In this sense, it is possible to distinguish between two main classes of EDCs: *mimics* and *blockers* [10]. The first ones base their activity on the interaction with the cell receptors and trigger the mechanisms normally activated by the natural hormone. The second also act binding to the specific receptor, but contrary to the previous ones, they block it so that it cannot be activated by the complementary natural hormone. Moreover, they could also alter the normal synthesis or metabolism processes [10].

Humans are exposed to a great variety of EDCs, such as pesticides commonly used pre- and post-harvest, personal care products, pharmaceutical compounds, etc. which can enter the human organism through multiple routes, which include oral consumption (being food and water the most important sources [16]), contact with skin and/or inhalation, or the biological transfer from the placenta and maternal milk [3], among others. As it will be later discussed, several health disorders and diseases have been attributed to the effect of EDCs in the organism, including the decrease of sperm quality, endometriosis, infertility, thyroid related diseases or neuroendocrine disorders or even several types of cancer [3,17].

I.1.1.- Natural, synthetic and mycoestrogens

Oestrogens, together with androgens, progestogens and corticosteroids, are compounds included in the group of steroid hormones of mammalians, which are synthesised from cholesterol in several endocrine tissues [18]. More specifically, oestrogens are principally synthesised in ovaries, although they are also produced in the adipose tissue of the adrenal glands or even in non-gonadal organs, including brain, heart, skin or liver [19,20]. In general terms, they are present at low concentrations in blood (20-500 ng/L, depending on the menstrual cycle in women) [21] and, due to their lipophilic nature, they are transported by carrier proteins through the bloodstream to the target tissues. Once there, they easily cross the plasma membrane of the cells and bind to the protein receptors present inside [22]. Thus, and despite their low concentrations,

they are involved in very important processes, being responsible for the development of the female secondary sex characters [22]. They also participate in the menstrual cycle regulation, the metabolism of lipids and carbohydrates, prevent bones decalcification, and control blood coagulation as well as the reabsorption of sodium and water by the kidney, among other functions [20,22].

Taking into account the remarkable biological processes in which oestrogens are involved, it is understandable that, among the great variety of EDCs to which humans are exposed, those that mimic the oestrogenic activity have attracted much attention in the last decades. This kind of compounds are characterised by interfering in the normal functions of oestrogens, which can result in important health alterations. At this point, it is important to make a distinction between those oestrogenic compounds which are naturally produced by the organism, commonly called as *endoestrogens*, and another group of compounds with oestrogenic activity with either a synthetic or a natural origin, commonly known as *exoestrogens*, which present similar structural geometries than those of natural hormones [23].

Regarding endoestrogens, the most remarkable are the four free forms estrone (E_1), 17α-estradiol (17α-E_2), 17β-estradiol (17β-E_2) and estriol (E_3). Since they are obtained from cholesterol's enzymatic degradation, they present a common e*strane* structure (C_{18}) with an aromatic ring, a hydroxyl group in C_3 and a methyl group in C_{13}. As can be seen in Figure I.2, different endoestrogenic forms arise from the variability in the cyclopentane ring.

These natural oestrogens have their origin in different tissues and are synthesised by different routes in the organism. Thus, 17β-E_2 and E_1 are mainly produced in ovaries, although it has been found that E_1 can also come from the metabolism of *androstenedione* in the adipose tissue, which is also secreted by the ovaries and suprarenal cortex [10]. It is important to highlight that the synthesis of E_1 in the adipose tissue increases with age, reaching especially important concentrations in postmenopausal and obese women [10,24]. Regarding E_3, it is an oxidation product of 17β-E_2 and E_1, principally originated in the liver and the small intestine [10]. Although these forms are the most common in humans they are not the only ones, and a good number of methoxylated, hydroxylated, sulfonated and glucuronated metabolites can also be found [25,26]. These metabolites are mainly produced in the liver and are principally excreted in the urine as oestrogenically inactive substances. However, some studies have discovered their relationship with some types of cancer diseases [25].

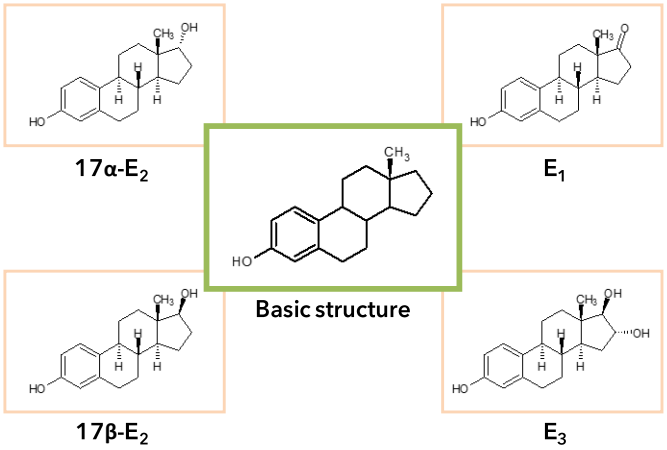


Figure I.2.- Chemical structures of the natural oestrogenic hormones estrone (E_1), 17α-estradiol (17α-E_2), 17β-estradiol (17β-E_2) and estriol (E_3).

In relation to the activity exhibited by endoestrogens, it has been found that 17β-E_2 is the natural oestrogen with the highest oestrogenic activity, which is twelve times larger than that of E_1 and even eighty times higher than that of E_3, being 17α-E_2 the one which presents the lowest effect [10,27,28]. Such activity depends on the affinity of each compound for the nuclear oestrogenic receptors (ERs). In this sense, most studies have identified two subtypes of ERs: ERα type, which is present in adipocytes, in the circulatory, immune and male reproductive systems, and have a dominant role in oestrogen physiology, so a prolonged activation of them may results in an increase of breast and endometrial tissues related cancers; ERβ type, which is mainly present in the ovaries, prostate, vascular system, testis, urethra, bladder and lungs, and they are believed to mediate in the control of gene expression, among other functions [29]. Both of them present very similar three-dimensional structures being only the amino acids sequences and chromosomal location slightly different between them.

In this regard, any chemical compound with a similar structure to natural oestrogens could bind to ERs and, therefore, trigger the mechanisms normally activated by the natural hormone or inhibit them. Traditionally, it has been considered that a chemical compound needs to simulate three main features of the three-dimensional structure of natural oestrogens to have oestrogenic activity (see Figure I.2) [10]: an aromatic ring with the hydroxyl group in C_3; a hydroxyl or ketonic group in C_{17}; and a methyl group located in C_{13}.

Taking this into consideration, several synthetic oestrogenic products have been designed by humans with different purposes. In this sense, ethynylestradiol (EE_2) stands out into this group of exoestrogens because of its wide use as contraceptive, in hormone replacement therapy or even in certain cancer treatments due to its higher resistance to metabolic degradation with respect to some natural oestrogens such as 17β-E_2 [30]. This synthetic oestrogen is structurally similar to the already mentioned endoestrogens, since it presents an *estrane* skeleton with a hydroxyl group in C_3, a methyl group in C_{13} and a hydroxyl group in C_{17}, but with an additional ethynyl group in C_{17}, as it can be seen in Figure I.3.

Besides, there is also a group of non-steroid-based synthetic compounds especially designed to mimic the action of endoestrogens, among which the stilbene derivatives dienestrol (DS), diethylstilbestrol (DES) and hexestrol (HEX) highlight. They present two phenolic rings separated by a hydrocarbon chain, as shown in Figure I.3. Different stilbenes arise from the position of double bonds in the hydrocarbon chain. Such *trans* configuration is similar to 17β-E_2, which seems to be a key aspect for their binding to ERs [10]. These compounds have been used as livestock growth promoters and in clinical therapies [31-33].

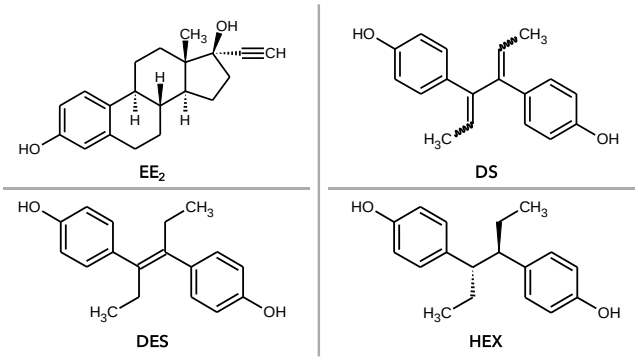


Figure I.3.- Chemical structures of the synthetic oestrogenic compounds ethynylestradiol (EE_2), dienestrol (DS), diethylstilbestrol (DES) and hexestrol (HEX).

In addition to natural oestrogens with an animal origin, which functions are the regulation of growth and sex characters differentiation, there exists a good number of exoestrogens with vegetable and fungus origins known as phytoestrogens and mycoestrogens, respectively, which do not present a steroid structure. In the particular case of mycoestrogens, although their function in the organisms in which they are

produced is not well-defined, it is believed that they act as defences against herbicides and pathogen agents [10]. In this regard, zearalenone (ZEN) and its derivatives zearalanone (ZAN), α-zearalenol (α-ZEL), β-zearalenol (β-ZEL), α-zearalanol (α-ZAL) and β-zearalanol (β-ZAL) are secondary metabolites produced by different *Fusarium* species, which have grabbed much attention due to their proven oestrogenic activity [34]. Among them, ZEN has been the most extensively studied due to its demonstrated immunotoxic, hepatotoxic, teratogenic and carcinogenic effects in several mammalian species, among others [35]. These compounds are resorcycle acid lactones (see Figure I.4) which are present in corn and other cereal grains and, as a consequence, there is a real risk of food contamination by direct contamination of grains and fruits or by their occurrence in animal tissues or animal derived products [36]. Once digested by animals, ZEN is metabolized to α-ZEL and β-ZEL, which can be further metabolized to α-ZAL, β-ZAL and ZAN following different oxidation/reduction processes (see Figure I.4) [37]. In this sense, and despite ZEN has been deeply studied, these conversion reactions are of especial concern, since α-ZEL and α-ZAL have shown much higher affinity for ERs than the rest, showing an oestrogenic capacity comparable to 17β-E_2, the most remarkable endoestrogen [38]. Among them, α-ZAL has been widely used as growth promoter and to reduce stress in livestock, although such practice is currently forbidden in the European Union (EU) [20].

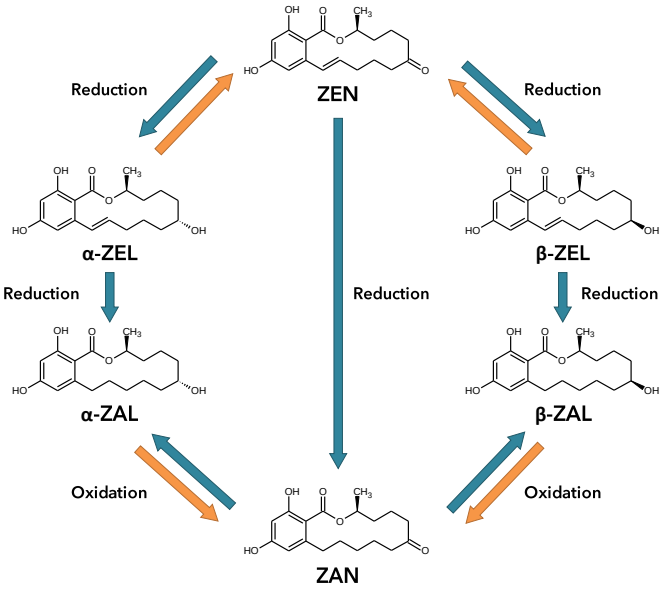


Figure I.4.- Biotransformation of ZEN into its most important derivatives. Redrawn from [35,39].

I.1.1.1.- Importance of the analysis of natural, synthetic and mycoestrogens in water and dairy products

As it has been previously discussed, endoestrogens are involved in a wide variety of important tasks related to the endocrine system in humans [20,22]. All the processes in which they are involved, and which have already been mentioned, can be regulated at the extremely low concentrations at which they are present in the organism. However, an over-exposition to EDCs can bring about an over-activation/blocking of the ERs which can result in multiple health alterations. These compounds can even accumulate in animal and human tissues manifesting their effects only in subsequent generations, which seriously endanger the humans' growth [40]. In this sense, health disorders are normally related to sexual organs disfunctions, such as abnormal sexual differentiation (demasculinising or feminising effects), reduction of sperm quality or early menarche, among others [41,42]. Besides these effects, high levels of oestrogenic compounds in humans are also associated to the development of certain types of cancer, including prostatic and testicular, breast, endometrial and ovaries cancer [43-45]. However, oestrogenic compounds not only affect humans, but also have effects on aquatic wildlife (e.g. feminisation of male fish) or on plants' growth, among others [46].

Considering the serious problems associated with an over-exposition to oestrogenic chemicals, it becomes necessary to identify the sources of contamination to which humans are exposed. Thus, with respect to the endo and exoestrogens already mentioned in the previous section, the ingestion of contaminated food and water constitutes the main exposition route.

In this regard, the main anthropogenic source of water contamination is constituted by urine and human or animal dregs which have been submitted to hormonal treatments or have ingested contaminated food. As a general overview of the dimension of this fact, it is estimated that humans annually discharge 30000 kg of natural oestrogens and other 700 kg of EE_2 from contraceptive pills administration [46]. This data is doubled if it refers to livestock, since high amounts of oestrogenic compounds are administered with different purposes. Such problem becomes worse when animal manure is used in agriculture lands, which is a common practice worldwide [46]. These residues normally end up in waste water treatment plants, so they constitute one of the most important contamination sources of the environment. In fact, EDCs have been detected not only in waste water treatment plants influents, but also in the effluents, since they are not able to

remove them completely. Thus, they pass to sewage slurry and soil, and then to ground and surface water, and finally to drinking water [46-48].

As it was already mentioned, besides water, the diet constitutes the other main via of ingestion of EDCs, through foodstuff of animal (e.g. meat, milk and dairy products, eggs, etc.) and of vegetable or fungal origins, since the last of them may suppose an important source of phytoestrogens or mycoestrogens. Among them, milk and dairy products constitute the most remarkable source of oestrogenic compounds for humans (60-70 % of the total endoestrogens) [49]. Some steroid hormones are directly synthesised in the mammary glands, but others can also reach milk by their transference from blood through the adipose tissue present in the mammary glands thanks to their lipophilic nature. As a consequence, the use of this kind of substances for other purposes not directly related with the production of milk, ends up in affecting this product indirectly [50]. This is evidenced by the massive introduction of *Holstein*-type cattle, which allows maintaining milk production even in gestation periods, when oestrogen levels in milk are increased from 30 to 1000 ng/L. In fact, they produce milk up to 220 of the 280 days that this period lasts [49], so that the levels of oestrogens in this food can far exceed normal values [51]. Moreover, some oestrogenic compounds have been illegally used as growth promoters or to increase the production of milk, which also results in an increment of the EDCs content [20]. Finally, it should be noted that the higher fat content of some products such as cheese, yogurt, cream or butter, favours an increase in the amount of oestrogen they contain [27].

The serious adverse health effects derived from an exposition to oestrogenic compounds, their demonstrated presence in the environment and food chain, and the practices in cattle breeding with fattening purposes [20], have forced several official organisms to establish guidelines to control their occurrence and to prohibit the use of many of them. In this sense, the EU Parliament, in its resolution 2012/2066(INI) of 14 March 2013 on the protection of public health from endocrine disrupters [52], recognises the possible relation between the exposure to EDCs with hormone-related disorders and illnesses in humans, as well as with their interference with the function of the female reproductive system at different levels (e.g. infertility, menstrual cycles, lactation, breast cancer, etc.).

Concerning the water policy of the EU, maximum residue limits (MRLs) have not been established yet for EDCs in the aquatic environment. However, Directive

2013/39/EU [53] regarding priority substances in water, amending Directives 2000/60/EC [54] and 2008/105/EC [55], proposed providing improved data about sources and concentrations of priority pollutants in order to develop strategies of action, and also included for the first time the oestrogens 17β-E_2 and EE_2 in the priority pollutants watch list. Moreover, the later Commission Implementing Decision 2015/495 [56], pursuant to Directive 2008/105/EC [55], also included E_1 in the watch list and provided indicative analytical methods for the determination of compounds included in the list, as well as maximum acceptable limits of detection of the method (LODs), establishing 0.035 ng/L for EE_2 and 0.4 ng/L for 17β-E_2 and E_1.

Regarding the presence of myco, synthetic or endoestrogens in milk or dairy products, no MRLs have been established yet for any of them. However, the EU has set MRLs for oestrogens, among other sexual hormones, in meat since 2010 by Commission Regulation 37/2010 [57], which has been amended in several occasions up to the latest Commission Implementing Regulation 2018/1076 [58]. In this sense, in the Directive 81/602/EEC published in 1981 [59], the EU banned the use of substances with hormonal action with fattening purposes in livestock, including 17β-E_2 and α-ZAL, and prohibited the use and commercialisation of stilbene and its derivatives, as well as their salts and esters and thyrostatic substances, for their administration to animals. Furthermore, Council Directive 93/22/EC published in 1996 [60], which amended Directive 81/602/EEC [59] and its subsequent modifications, extended the prohibition of the use of substances with thyreostatic, oestrogenic, androgenic and gestagenic action to husbandry and aquaculture, and also established that the EU member states must not import meat of treated animals from extra-community countries. Besides, Directive 2003/74/EC [61], which amended Directive 96/22/EC [60], prohibited permanently the use of 17β-E_2 as growth promoter and only authorised its use by veterinarians in certain treatments in which non-effective alternatives to its use are available and in cases in which the animal health is seriously endangered (e.g. foetus maceration or mummification and pyometra in cattle). In one of the last modifications of Directive 96/22/EC (Directive 2008/97/EC [62]), any use of 17β-E_2, including therapeutic applications, since other alternatives were already available, were banned. Finally, and concerning tolerable daily intakes (TDIs) of oestrogenic compounds, it is worthy to mention that the European Food Safety Authority (EFSA) established a TDI of 0.25 μg/kg of body weight for ZEN in 2014.

Taking into consideration the serious health alterations that these substances can produce in humans, wildlife and even plants, and the wide range of sources to which all

of them are daily exposed, as well as the restrictions established by the legislation, it becomes necessary the development and implementation of analytical methodologies which allow the assessment of the occurrence of oestrogenic compounds in the environment and food chain, paying especial attention to water of different origins as well as milk and dairy products.

I.1.1.2.- Analytical methods for the determination of natural, synthetic and mycoestrogens in water and dairy products

As it has been commented, oestrogenic compounds are present at very low concentrations in biological fluids. However, and despite they have also been found at concentrations in the order of ng/L or ng/kg in environmental and food matrices, their presence at such low levels can even have serious effects on human's health and wildlife. For this reason, the development of effective and highly sensitive methodologies for the analysis of this kind of substances in complex matrices has become necessary.

Regarding the determination of these compounds, a good number of analytical techniques have been used with this purpose. In this sense, non-instrumental techniques have been applied, including immunoassays such as enzyme-linked immune sorbent assay (ELISA) [63] and radio immunoassay (RIA) [64], or bioassays such as yeast oestrogen screen (YES) and human breast cancer cell line proliferation [65]. However, since immunoassay techniques have shown cross-binding to certain antibodies [66] they need to be combined with instrumental techniques. This is the main reason why their application is very limited for the analysis of oestrogenic compounds, despite their simplicity, rapidity and low cost [65,67]. Thus, instrumental techniques, including gas chromatography (GC), liquid chromatography (LC) and, although much less used, capillary electrophoresis (CE), coupled all of them to different detection systems, have become the techniques most used for the analysis of oestrogenic compounds, since they allow the separation and individual detection and quantification of the analytes in the studied samples [23]. Finally, it is noteworthy to mention that the use of sensors for the selective and sensitive determination of oestrogenic compounds has also been explored in the last years with success [68,69].

On the one hand, and concerning chromatographic techniques, GC has been less applied for the separation of oestrogenic compounds due to the limited volatility and low stability at high temperatures of these compounds, which make necessary the inclusion

of a derivatisation step prior to GC separation. Thus, although some authors have carried out their determination without derivatisation [70], it is usually preferred their transformation into more volatile derivatives, being the most common the silylation of the hydroxyl group in C_{17}, which reduces the polarity of the molecule, and increase its volatility and thermal stability [71]. In this sense, the derivatisation agents most commonly used have been N-O-bis-(trimethylsilyl)-trifluoroacetamide (BSTFA), N-methyl-N-trimethylsilyltrifluoroacetamide (MSTFA) and N-(tert-butyldimethylsilyl)-N-methyltrifluoroacetamide, leading to the formation of tert-butyldimethylsilyl and trimethylsilyl derivatives [71]. However, the main drawback of using these derivatisation agents is that they can generate multiple derivatives in those compounds with more than one active hydrogen, resulting in less sensitivity and selectivity [71]. To overcome this problem, catalysers are normally used, increasing the efficiency of the silylation reaction and giving the complete silylation of the active hydrogens. In this sense, substances such as trimethylchlorosilane (TMCS) and pentafluorobenzyl bromide have been used in combination with BSTFA [72], and trimethyliodosilane or NH_4I with MSTFA [73]. In this last case, the addition of small amounts dithioerythritol in order to prevent the oxidative degradation of the derivatising agent is very frequent [23,74].

Table I.1 compiles some examples of the application of GC for the determination of the oestrogenic compounds described in previous sections. As can be seen, conventional GC has been the most used, although two-dimensional GC (GCxGC) has also been used in certain applications but for their simultaneous analysis with other steroidal hormones [75]. Regarding detection systems coupled to GC, mass spectrometry (MS) or tandem mass spectrometry (MS/MS) have been the most used [23,65] due to their higher sensitivity and selectivity which offers a reliable identification of the analytes. Concerning ionisation sources, electron ionisation (EI) is used in almost all cases, independently of the analyser used, among which single quadrupole (Q) [76-78], triple quadrupole (QqQ) [79] or time-of-flight (TOF) [75] can be found. In any case, other detectors such as the electron capture detector (ECD) [78] have also allowed the suitable determination of this kind of EDCs. In general, non-polar columns have been used, and BSTFA has been the most extended derivatisation agent.

On the other hand, LC is not limited by the low volatility and thermal stability of these analytes, a fact that has made it the most suitable instrumental technique for their analysis, since it also allows the determination of both conjugated and non-conjugated oestrogens without a previous hydrolysis [74].

Table I.1.- Some examples of the application of GC to the analysis of oestrogenic compounds in water and dairy products.

Analytes[a]	Matrix	Stationary phase	Derivatisation mixture	Analytical technique	Reference
E_1, 17β-E_2, E_3 and EE_2	Waste water	5 % phenyl/95 % poly(methylsiloxane) and 50 % phenyl/50 % poly(methylsiloxane)	BSTFA/TMCS	GCxGC-MS (TOF)	[75]
E_1, E_3, EE_2 and DES	Tap, river and water	5 % phenyl/95 % poly(methylsiloxane)	MSTFA	GC-MS (Q)	[76]
17β-E_2, DS, DES, HEX, ZAN, α-ZAL, β-ZAL, α-ZEL and β-ZEL	Milk	5 % phenyl/95 % poly(methylsiloxane)	MSTFA/NH_4I/DTT (for mycoestrogens) HFBA (for stilbenes and steroid hormones)	GC-MS (Q)	[77]
E_1, 17β-E_2, E_3, EE_2 and DES	River and waste water	5 % diphenyl/95 % poly(dimethylsiloxane) and 100 % poly(dimethylsiloxane)	PFPA/PFPOH	GC-ECD	[78]
E_1, 17α-E_2, 17β-E_2, E_3, EE_2, DS, HEX, ZEN, α-ZAL, β-ZAL, α-ZEL and β-ZEL	Milk and yogurt	5 % diphenyl-arylene/95 % poly(dimethylsiloxane)	BSTFA/TMCS	GC-MS/MS (QqQ)	[79]
E_1, 17β-E_2, E_3 and EE_2	Butter	5 % phenyl/95 % poly(methylsiloxane)	HFBA	GC-MS (Q)	[80]
E_1, 17β-E_2, E_3 and EE_2	Waste water	5 % phenyl/95 % poly(methylsiloxane)	BSTFA/TMCS	GC-MS (Q)	[81]

DTT: DL-dithiothreitol; HFBA: heptafluorobutyric anhydride; PFPA: pentafluoropropionic anhydride; PFPOH: 2,2,3,3,3-pentafluoro-1-propanol
[a] Only the analytes studied in this PhD Thesis are shown.

Some examples of the application of LC to the analysis of the previously mentioned oestrogenic compounds are shown in Table I.2. In this sense, high-performance LC (HPLC) has been the modality selected in most cases [23,65], although in the last years, the use of ultra-high-performance LC (UHPLC) [82,83] has notably increased due to the advantages it presents compared to HPLC (e.g. higher efficiency with better resolution between peaks and reduced analysis time) [84]. Although less usual, miniaturised LC techniques such as, capillary-LC (CLC) [85] and nano-LC (nano-LC)

[86] have also been used, providing lower times of analysis with a reduced volume of sample and solvents. Very few applications of two-dimensional LC (LCxLC) can also be found in the literature [87], though in these cases it is applied for their simultaneous analysis with a high number of analytes of different nature.

Table I.2.- Some examples of the application of LC to the analysis of oestrogenic compounds in water and dairy products.

Analytes[a]	Matrix	Stationary phase	Ionisation source (mode)	Analytical technique	Reference
E_1, 17α-E_2, 17β-E_2, E_3, EE_2, DS, DES, HEX, ZEN, ZAN, α-ZAL, β-ZAL, α-ZEL and β-ZEL	Animal and human milk	C_{18}	ESI (-) (ZAN was detected in positive mode)	UHPLC-MS/MS (QqQ)	[82]
E_1, 17α-E_2, 17β-E_2 and E_3	Yogurt, cheese and butter	C_{18}	ESI (-)	UHPLC-MS/MS (QqQ)	[83]
E_1, 17α-E_2, 17β-E_2, E_3 and EE_2	River and waste water	C_{18}	ESI (+)	CLC-MS/MS (QqQ)	[85]
E_1, 17α-E_2, 17β-E_2, E_3, EE_2, DS, ZEN, α-ZAL, β-ZAL and α-ZEL	Mineral water	Phenyl	ESI (-)	Nano-LC-MS (IT)	[86]
E_1, 17α-E_2, 17β-E_2, E_3, EE_2, DS, DES and HEX	Milk	C_{18}	-	HPLC-DAD/FD	[88]
E_1, 17α-E_2, 17β-E_2, E_3, EE_2, α-ZAL, β-ZAL, α-ZEL and β-ZEL	Ground water, waste water, runoff water and soil	C_{18}	APPI (+)	HPLC-MS/MS (QqQ)	[89]

APCI: atmospheric pressure chemical ionisation; DAD: diode array detector; ESI: electrospray ionization; FD: fluorescence detector; APPI: atmospheric pressure photoionization
[a] Only the analytes studied in this PhD Thesis are shown.

Regarding analytes determination, different detection systems have been coupled to LC devices. In this sense, diode array detectors (DAD) [88] and fluorescence detectors (FD) [88] have been selected in a good number of applications because of their simplicity, ease of use and low cost, as well as the high sensitivity of the second one. However, the coupling with MS detectors has been the most extensively used for the determination of oestrogenic compounds with different analysers, including ion trap (IT) [86], TOF [90], QTOF [91], Q [92] and QqQ [89], being the last one the most used in the last years. In relation to the ionisation sources selected to couple MS detectors to LC systems, electrospray ionisation (ESI) source has been the one preferred for the analysis of oestrogenic compounds, although in a few applications other atmospheric pressure ionisation (API) sources, both atmospheric pressure chemical ionisation (APCI) [93] and atmospheric pressure photoionisation (APPI) [89], have been used. It is important to highlight that, although ESI is more prone to ion suppression phenomena due to the presence of matrix components than the other API sources [74], in general terms, API sources present problems with compounds with low ionisation capacity like oestrogens [94]. This drawback can be solved by chemical derivatisation [74] or using mobile phase additives with moieties with high proton or electron affinity when APCI is used, or with permanently charged or easily protonable or deprotonable moieties when ESI is used [74]. In this sense, the addition of dansyl chloride is the most extended practice with positive ESI-MS detection [27,95]. Besides, the addition of NH_4OH (negative mode) [26,96] or formic acid (positive mode) [27,95] to the mobile phase, which are normally composed by acetonitrile (ACN)/H_2O or methanol (MeOH)/H_2O mixtures, has also proven to result in an enhancement of the sensitivity [23].

Despite the good performance shown by chromatographic techniques for the analysis of oestrogenic compounds, CE has also been applied in certain cases with suitable results since, while it is prone to provide low sensitivity as consequence of the low injection volumes and short optical path length when UV or DAD detection is used [97], it has several advantages with respect to LC and GC: lower solvent consumption and sample volumes, lower times of analysis, higher efficiency and selectivity, etc. [98]. Regarding the CE analysis of oestrogenic compounds described in previous sections (see Table I.3), despite their high pK_a values, which make them to remain neutral in a wide pH range, they have been analysed by capillary zone electrophoresis (CZE), using strong basic background electrolyte (BGE) solutions [99]. However, the modality most used is electrokinetic chromatography (EKC), using micelles (micellar electrokinetic

chromatography – MEKC-) [100,101] or micro-emulsions (micro-emulsion electrokinetic chromatography – MEEKC-) [102] as pseudostationary phases. In MEKC, ionic surfactants such as sodium dodecyl sulphate (SDS) [102,103] or ammonium perfluorooctanoate (APFO) [100,101] have been the most frequently added to the BGE. Finally, it is noteworthy to mention that capillary electrochromatography (CEC) has also been used in a reduced number of applications [86,104,105] using capillary columns packed with C_{18} [104,105] and phenyl particles [86].

Table I.3.- Some examples of the application of CE to the analysis of oestrogenic compounds in water and dairy products.

Analytes[a]	Matrix	BGE	Analytical technique	Reference
E_1, 17β-E_2 and E_3	Tap, river and pond water	60 mM NaOH, pH 12.8	CZE-ED	[99]
E_1, 17α-E_2, 17β-E_2, E_3, EE_2, DS, ZEN, α-ZAL, β-ZAL, α-ZEL and β-ZEL	Mineral, run-off and waste water	45 mM APFO 10 % (v/v) MeOH, pH 9.0	MEKC-ESI(+)-MS (IT)	[100]
E_1, 17α-E_2, 17β-E_2, E_3, EE_2, ZEN, α-ZAL, β-ZAL, α-ZEL and β-ZEL	Milk and yogurt	45 mM APFO 10 % (v/v) MeOH, pH 9.0	MEKC-ESI(+)-MS (IT)	[101]
EE_2 and DES	Waste water	25 mM phosphate pH 2.0, 200 mM SDS, 900 mM butanol, 80 mM heptane, with 20 % propanol	MEEKC-DAD	[102]
E_1, E_3 and DES	Mineral water, tap water, river water and honey	50 mM SDS, 50 mM H_3PO_4, 20 % (v/v) ACN, pH 2.0	MEKC-DAD	[103]

ED: Electrochemical detector
[a] Only the analytes studied in this PhD Thesis are shown.

As can be seen in the table, CE has been coupled to DAD [102,103], electrochemical detectors (ED) [99] as well as MS detectors [100,101] for the analysis of the indicated target analytes. In relation to the last ones, IT [101] has been used as analyser, also using ESI sources, despite the problems derived from the low flows and high voltages applied in this kind of ionisation sources [101]. In fact, ESI sources for

CE-MS hyphenation frequently use an additional flow of an organic or aquo-organic solvent (sheath liquid) which favours the ionisation of the analytes and the electrical contact closure but results in a dilution of the analytes which provides a decrease in the sensitivity [106]. Finally, it should be highlighted that the surfactants used in MEKC should be semi-volatile when MS detectors are used [101] unless a partial filling of the capillary is carried out [106], since conventional surfactants such as SDS can deposit and contaminate the interface, increasing the noise level as well as providing ionic suppression [107].

As it has been mentioned, these oestrogenic compounds are frequently present at extremely low concentrations in the matrices they have been analysed. This fact, together with the high complexity of certain matrices (e.g. food, biological samples, among others) make necessary the development and application of sample pre-treatment techniques which allow the selective extraction and preconcentration of the analytes, as well as an efficient clean-up in order to remove matrix interferences, before their analysis by means of the techniques previously mentioned. A good application/design of such sample pre-treatment strategies is important, since it will result in an increment of the sensitivity of the method.

In this sense, and as previously mentioned, it should be considered that oestrogenic compounds can undergo biological processes of conjugation with sulphate and glucuronic acid resulting in non-biologically active substances [23,74], but they can be transformed into their free active forms [23,74]. For this reason, when biological or food samples are analysed, the determination of both free and conjugated forms is of great concern. Normally, the conjugated fraction is determined by the difference between the total and free oestrogens content [23]. For total oestrogenic compounds content is necessary a hydrolysis step to transform all oestrogens into their free forms before the extraction procedure. The most extended practise consists in the enzymatic hydrolysis, using generally a mixture of β-glucuronidase and arylsulfatase (*Helix pomatia* juice), which is carried out during several hours under certain conditions of temperature and pH which have to be optimised to avoid the formation of side products [20,23,74]. Besides that, the presence of proteins should also be considered when oestrogenic compounds are analysed in food samples, since oestrogens have a great affinity for these macromolecules and proteins can also be irreversibly retained in LC stationary phases reducing their durability [23]. In order to solve these problems, different deproteinization strategies have been developed, principally consisting on the addition of an organic

solvent (MeOH, ACN, acetone, ethyl acetate or mixtures of them) together with salts and acids (acetic acid (AcOH) or perchloric acid) or buffers (acetate buffer) [23].

As already mentioned, such hydrolysis and deproteinization steps, when applied, are generally followed by an extraction procedure. In this sense, different techniques have been applied to the extraction of oestrogenic compounds from environmental and food samples, being liquid-liquid extraction (LLE), solid-liquid extraction (SLE) and, especially, solid-phase extraction (SPE), the most widely used. It should be pointed out that these methodologies are preceded by grinding, freezing-drying and homogenising steps when they are applied to solid samples [20]. However, these methods can result time consuming, labour intensive and also consume large volumes of organic solvents. For these reasons, some modifications of the classical methods have been developed and applied, trying to make the process more effective. In this sense, pressurised liquid extraction (PLE) [108], microwave-assisted extraction (MAE) [109] and ultrasound-assisted extraction (UAE) [110] have been applied, among others. Regarding SPE, the inclusion of new sorbents such as molecularly imprinted polymers (MIPs) [111], carbon nanotubes (CNTs) [112], metal-organic frameworks (MOFs) [113] or modified magnetic nanoparticles (m-NPs) [82] have allowed to create more selective and effective extraction procedures. In relation to liquid-phase extraction (LPE), different miniaturised variants have also been used for the extraction of oestrogenic compounds trying to reduce the organic solvents consumption, including dispersive liquid-liquid microextraction (DLLME) [114] or hollow-fibre liquid-phase microextraction (HF-LPME) [79].

I.1.2.- Phthalic acid esters (PAEs)

As it has been commented, there is a wide diversity of compounds which have been identified as EDCs, among which natural hormones, phytoestrogens and mycoestrogens are of especial concern because of the proven serious adverse effects they can produce not only in humans, but also in the environment and, particularly, in wildlife. However, there also exist a broad spectrum of synthetic substances as a result of the human activity. Among them, plasticisers have awakened great interest in last years because of the extensive use of plastic materials in the current society. Today, humans are living in what could be called the 'plastic era', since classic materials such as glass, wood, paper or metal have been replaced by plastics in many applications.

Plasticisers are constituted by an extensive group of substances used as additives during plastics production in order to modify the physical properties of the material, since

they normally increase the plasticity or increase the viscosity of plastic. A great variety of plasticisers are currently available depending on the plastic material to be modified, among which phthalic acid esters (PAEs), commonly known as phthalates, stand out above any other. PAEs are esters of 1,2-benzenedicarboxylic acid synthesised from the phthalic anhydrite and the corresponding alcohol (see Figure I.5). All of them present the common basic structure shown in the figure, with an ortho-substituted benzene ring where R and R′ are straight or branched chains, or even cycles, which do not normally exceed thirteen carbons. Thus, there is a wide number of PAEs with different structures and, consequently, different physico-chemical properties. In this sense, they are usually classified in two categories: low molecular weight PAEs, referring to those with side chains with up to six carbons; and high molecular weight PAEs with more than six carbon atoms in their side chains [115,116].

The fields in which PAEs are applied as plasticisers depend directly on their properties/structure. In this sense, high molecular weight PAEs such as diisononyl phthalate (DINP) or diisodecyl phthalate (DIDP), among others, are widely used in industry [117,118], since their long side chains introduced in the polymeric matrix increase the flexibility, softness, elongation and durability of rigid plastics like polyvinyl chloride (PVC). Thus, their use is involved in the production of self-adhesive films and labels, wire and cables, coated fabrics, wall coverings, roofing membranes, automotive applications [117,118], and even food wraps and packaging [119]. On the contrary, low molecular weight PAEs, including dibutyl phthalate (DBP), diisobutyl phthalate (DIBP), butylbenzyl phthalate (BBP) or di(2-ethylhexyl) phthalate (DEHP), though also used in certain PVC products, are part of adhesives, inks, paints or medical devices. Finally, dimethyl phthalate (DMP) and diethyl phthalate (DEP), which are the PAEs with the shortest side chains, are not considered as plasticisers because they are not used in plastics production. Instead, they are used as solvents and fragrances fixers, as well as additives in medical devices, household, cosmetics and personal care products [117].

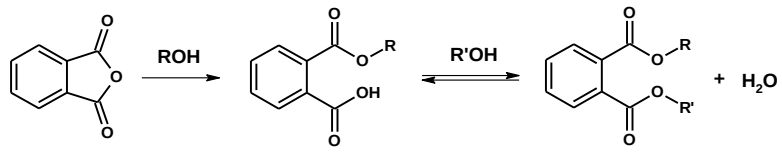


Figure I.5.- Reaction between phthalic anhydride and the corresponding alcohol to produce PAEs. Adapted from Katsikantami et al. [117].

Besides the above-mentioned PAEs, and due to the so extended use of phthalates and their wide range of application as well as the demonstrated adverse effects they can have on humans, different alternative plasticisers have been proposed in the last decades, although their emission, human exposure and health effects data are still limited. In this sense, benzoates, citrates, phosphate esters, vegetable oil derivatives, terephthalates and adipates have been used, among others. In particular, di(2-ethylhexyl) adipate (DEHA) has been one of the most applied and studied. DEHA is a diester synthesised from adipic acid and 2-ethylhexanol (see Figure I.6) [120]. This adipate is among the plasticisers with the highest annual production in the EU, between 10000 and 100000 tonnes per year. DEHA is used in PVC products such as vinyl flooring, wire and cable, coated fabrics, tubing or toys, among other applications, substituting some of the classical long side chain PAEs [120].

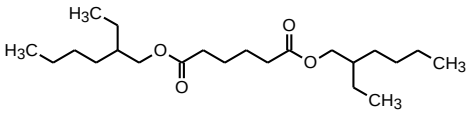


Figure I.6.- Structure of di(2-ethylhexyl) adipate (DEHA).

I.1.2.1.- Importance of the analysis of PAEs in water and beverages

The wide range of application of plastics, which includes a variety of resins (i.e. PVC, polyvinyl acetate (PVA), polyethylene terephthalate (PET), polystyrene (PS) and polyethylene (PE)), have brought with it an important increase in global plastic production which is currently around 335 million tonnes per year [121]. Such amount of plastic materials results in a great plastic waste generation which can accumulate both in terrestrial and aquatic environments when it is not properly managed [122], which is a major risk to the environment itself. Despite this fact, plastic polymers are considered inert structures from a toxicological point of view, since they cannot be normally absorbed by the human organism, therefore their potential risk for humans' health is considerably low. However, the main problem lies in the substances added to plastics during their manufacturing, which also includes PAEs or their substitutives such as DEHA. Phthalates are not chemically bonded to the polymeric matrix and they can easily migrate to the surrounding media [117,123]. This migration depends principally on the features of the migrant, the composition of the plastic material, the environment in contact with it and the surface of contact between them. Besides, there are some external aspects that can also influence the process, including temperature, pH, solar radiation or time of contact

[117,123]. This fact, together with the widespread use of these compounds (e.g. cosmetics, personal care products, paints, pharmaceuticals, food packaging, etc.), have exposed humans and also wildlife to a high number of PAEs contamination sources [115]. As a result, several routes of contamination have been identified, including: the ingestion of plastic packed food or beverages, being especially important in children who are also exposed to plastic teats, toys and mouthing contaminated hands and other objects; inhalation of air and dust in rooms or vehicles built with plastic materials due to the high volatility of some PAEs (DEHA has also detected in these matrices [120]), dermal contact with fragrances, personal care products, clothing or cleaning products, among others; and the use of certain medical devices, since PAEs are used in drug pills polymeric coatings or intravenous injection of contaminated pharmaceuticals (plastic bags used in blood transfusions, extracorporeal membranes used in dialysis, etc.) [115].

Besides, it should not be forgotten that the easy migration capacity shown by these compounds result in their widespread distribution in the environment compartments, which can also constitute an important contamination source for human beings and wildlife. In this sense, and as an example, DEHP, di-n-octyl phthalate (DNOP), DBP, BBP, DMP and DEP have been detected at concentrations between 0.5 and 22.2 ng/m^3 in the atmosphere in the urban area of Paris [124], though it should be highlighted that the concentration levels of PAEs in the atmosphere depend on different factors, being higher in urban areas or in dry seasons. The fact that rainfall transfers them from the atmosphere to surface waters have also resulted in their presence in water at concentrations below 10 μg/L in most cases [125]. Among them, DEHP and DBP have been the most detected in water and sediments sampled in urban areas because they are two of the most used [125]. Concerning their presence in soils, they have been found even at concentrations in the order of mg/kg [126]. That is especially worrisome in agricultural soils, since PAEs may be absorbed by crops and vegetables and incorporated into the food chain [127]. It is also important to mention that all plastics, cosmetics, personal care products and pharmaceuticals waste also contribute to environmental waters and soils contamination with PAEs. Moreover, and although there are different biodegradation processes which lead to PAEs degradation in environmental samples, these normally require several days or even years for long chain PAEs, which makes waste water treatment very difficult to completely remove them from the effluents [125]. Finally, it should not be forgotten that food chain constitutes one of the main sources of contamination for humans, especially for DEHP and DINP [128]. In this sense, the use of plastics in food packaging is a common practice, resulting in an important migration of

PAEs from the container to the food or beverage which is wanted to be preserved. Thus, the current use of plastic bottles is of especial concern, since it is really difficult to find water or soft drinks which are not bottled with plastic materials, being PET one of the most used materials with this goal due to its strength and clarity [129]. Thus, PAEs residues have been found in beverages at concentrations in the order of μg/kg or even at low mg/kg levels [129]. In any case, it should be mentioned the possibility of beverages contamination before or during bottling [130].

Once PAEs have been absorbed by the organism, they are metabolised into their monoesters by hydrolysis and oxidative processes producing more hydrophilic molecules, which are finally excreted in urine, faeces or sweat [117]. Both PAEs and their metabolites have been detected in the circulatory system, saliva, semen, breast milk and amniotic fluid [115,117]. This fact constitutes a serious risk for humans, since PAEs have been demonstrated to mimic the actions of natural hormones in the organism, producing several endocrine system disorders [115]. In this sense, it is important to point out that it is believed that their free monoester metabolites are the compounds which induce such toxicity [117]. For this reason, no distinction is frequently made between PAEs and their metabolites [117]. Regarding the effects that these compounds can have on health, they are directly related to the age of exposure, being necessary to establish three categories: foetus and new-borns, children, and adults.

The first group is the most sensitive to the effects of PAEs, since their metabolic system is not mature, and for them it is really difficult to remove any pollutant from their organism. Moreover, it is important to take into account that their exposition to EDCs during their growth can have irreversible health alterations. As an example, shorter pregnancy periods and abnormalities in new-borns dimensions (head and biparietal diameters, femur length or abdominal circumference), have been detected in male and female new-borns [117]. In this sense, it should be highlighted that the lipophilic nature of PAEs makes them prone to accumulate in breast and, as a consequence, breastfeeding constitutes one of the most important contamination sources for new-borns [117]. Besides, prenatal exposure to DBP or DEHP has been related with neurological related disorders and syndromes, as well as low intelligence quotient scores in ages ranged 6-10 years old [117]. Regarding the second group, as for other EDCs, alterations in sex-development have been related to PAEs exposure, including speed up or delay in prepubertal development. Moreover, they have also been related with other diseases such as obesity, allergies, asthma, and there are some indications that suggest that PAEs

metabolites can be related to autism [115,117]. Finally, adults' exposure to PAEs have shown to impair semen quality and cause infertility in men and have been related to endometriosis and breast cancer in women, among other diseases [115,117].

The serious health disorders that PAEs can produce on humans as a consequence of their interactions with the endocrine system even at very low concentrations, together with the continuous exposition to which humans are submitted, have made several official organisations/administrations to initiate actions to control their use. In this sense, due to the so extended use of plastics in food packaging, the EU has established guidelines of good practices in the production of plastics intended to come in contact with food as well as the materials and objects to come in contact with them by means of the Regulations 1935/2004 [131], 2023/2006 [132] and 10/2011 [133]. Besides, Directive 2007/19/EC [134] limits the use of DBP, DEHP, BBP, DINP and DIDP in food contact materials, establishing the situations in which they can be used and their specific migration limits (SML). It should be mentioned that these SMLs are the maximum permitted amount of PAEs released from a material or article into food or food simulant.

Apart from the EU, other administrations have also established restrictions in PAEs use, as the United States Environmental Protection Agency (US EPA) which also initiated actions to regulate the use of DBP, DIBP, BBP, di-n-pentyl phthalate (DNPP), DEHP, DNOP, DINP and DIDP in plastics manufacturing [135]. Besides, the EFSA has also set a TDI of 0.05 mg/kg of body weight for DEHP and recommends providing improved estimates of exposure [136]. Finally, the World Health Organization (WHO) has also established a TDI for this compound of 25 μg/kg of body weight and recommends not to exceed a concentration of 8 μg/L in drinking-water [137]. In this regard, it should also be remarked, that DEHP has also been included in the watch list given in Directive 2013/38/EU [53] as a priority substance in the field of water policy.

I.1.2.2.- Analytical methods for the determination of PAEs in water and beverages

As it has already been mentioned, the serious health disorders that PAEs can produce in the organism, even at low concentrations, has made the EU to establish low SMLs for some of them in food. For this reason, the development of new methodologies which allow their determination with a high sensitivity has become necessary.

In general, the analysis of plasticisers, and especially the analysis of PAEs, constitutes a challenging task independently of the complexity of the sample matrix,

which is also an important aspect to be considered. That is due to the fact that PAEs are ubiquitous in any analytical laboratory and their migration capacity is an important drawback, especially when they are present in the sample at low levels. That is not only due to the great amount of plastic materials which are normally used in the laboratory, including pipette tips and filters, but also because they are present in solvents, reagents or even the laboratory atmosphere, which makes very difficult their complete removal. However, there are some actions that can be carried out to reduce contamination sources as much as possible, including [138]: the replacement of plastics by glassware, Teflon, aluminium or stainless steel materials; the use of aggressive cleaning processes such as calcination at high temperatures (450-550 °C during several hours) for non-volumetric glassware or the use of strong oxidising agent solutions for volumetric glassware; the use of solvents of high purity; and the utilisation of phthalate-free gloves and pipette tips. Moreover, Milli-Q water should also be analysed before use, since it is obtained by deionisation with ion exchange cartridges in which a great amount of plastic materials is present. Besides, the total contamination degree of the laboratory should be carefully evaluated with the daily analysis of procedural blanks in every batch of samples. Finally, it should also be considered that cosmetics and personal care products can also contain PAEs, so their used must be avoided or minimized by the analyst during sample handling [138].

Regarding the analytical techniques used for the determination of phthalates, chromatographic techniques are the most extended, both LC and GC, although very few applications of CE can also be found. Furthermore, it is noteworthy to mention that several sensors have been developed for the selective detection and quantification of PAEs [139,140].

Among chromatographic techniques, GC is the most commonly used due to the appropriate volatility and thermal stability of these analytes, which allow their GC determination without a previous derivatisation step. Table I.4 compiles some examples of the application of GC to the determination of PAEs. In this sense, conventional GC, with 5 % phenyl/95 % poly(dimethylsiloxane) fused-silica capillary columns, has been the most extensively used for the efficient separation of PAEs, due to their relative low polarity, providing good resolution between peaks. However, some applications of GCxGC [141] or fast-GC [142] have also been published, though, as it frequently occurs, especially in the first case, for the analysis of a high number of compounds of different nature. Regarding the identification and quantification of the analytes, GC systems have been

generally coupled to MS detectors due to their high sensitivity and selectivity, being the most suitable for the determination of PAEs at the low levels at which they are present in the samples. In most applications, EI sources have been used, independently of the analyser. In this sense, the most used has been Q [143,144] operating in the selected ion monitoring (SIM) mode, although IT [145] or QqQ [146,147] analysers have also been used, the last normally working in multiple reaction monitoring (MRM) mode, although in some cases it has been operated in the SIM mode for a higher sensitivity. Despite the so extended use of MS detectors for the determination of PAEs, simpler and more classic detection systems such as flame ionisation detector (FID) [148] and ECD [149] have also been used providing a good sensitivity, especially the last one.

Table I.4.- Some examples of the application of GC to the analysis of PAEs in water and beverages.

Analytes[a]	Matrix	Stationary phase	Analytical technique	Reference
DBP, DEHP and BBP	Plastic bottled water	5 % phenyl/95 % poly(methylsiloxane)	GC-MS (Q)	[143]
DEHP	River, lake and waste water	5 % phenyl/95 % poly(methylsiloxane)	GC-MS (Q)	[144]
DEP, DBP, BBP, DEHP and DNOP	Sediments	Mid-polarity (not specified)	GC-MS (IT)	[145]
DBP, DIBP, BBP and DEHP	Tea	5 % phenyl/95 % poly(dimethylsiloxane)	GC-MS (Q)	[146]
DEP, DIBP, DBP, DMEP, DEEP, DNPP, BBP, DBEP, DCHP, DEHP and DNOP	Chinese spirit	5 % phenyl/95 % poly(methylsiloxane)	GC-MS/MS (QqQ)	[147]
DEP, DBP and DEHP	Plastic bottled water	100 % poly(dimethylsiloxane)	GC-FID	[148]

DBEP: di(2-butoxyethyl) phthalate; DCHP: dicyclohexyl phthalate; DEEP: di(2-ethoxyethyl) phthalate; DMEP: di(2-methoxyethyl) phthalate
[a] Only the analytes studied in this PhD Thesis are shown.

In relation to GC-MS hyphenation employing EI sources, there are some considerations that should be taken into account. The fragmentation pathways of PAEs

with alkyl side chains (except for DMP) are similar, giving the m/z 149 as the most intensive parent ion. This fragment corresponds to the structure shown in Figure I.7, which points out that the fragmentation of PAEs primarily occurs on the aliphatic side chain [150]. As a consequence, and despite the use of MS/MS, the determination of PAEs can result on a very difficult task, also from the instrumental point of view, since good resolution between peaks is necessary in order to not lose sensitivity.

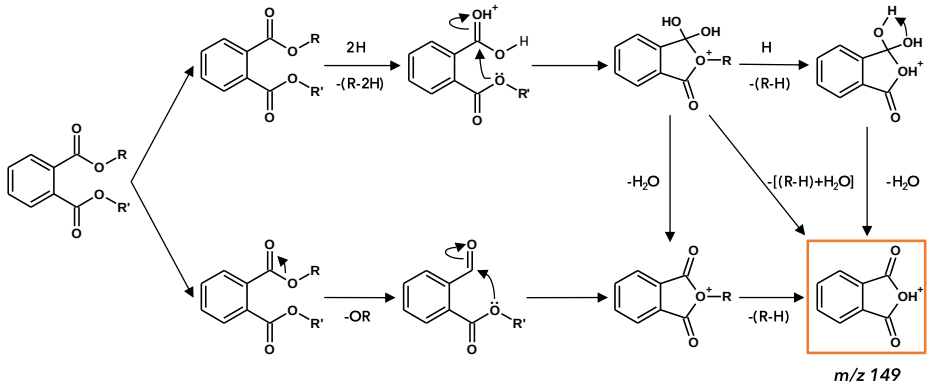


Figure I.7.- Mass spectral fragmentation pathway of alkyl esters to form the protonated phthalic anhydride which corresponds to m/z 149. Adapted from Yin et al. [150].

In spite of the good performance of GC for the separation and determination of PAEs, LC constitutes a reliable alternative, showing better selectivity for the separation of isomeric mixtures and monoester metabolites [151]. In this sense, HPLC has been the modality most used, although the use of UHPLC has increased in the last years since the advantages already mentioned (see Table I.5). In a similar way than GC, non-polar columns have been used for the separation of PAEs, being C_{18} the most common, although shorter chain stationary phases such as C_8 have also been used, since they provide narrower peaks than C_{18} resulting in a better separation of isomers. As mobile phases, the mixtures ACN/H_2O and MeOH/H_2O, containing or not small amounts of organic acids, have been mostly selected. Regarding the detection systems used in combination with LC, DAD [152] and ultraviolet-visible (UV-Vis) [153,154] detectors have been widely applied for the determination of PAEs, despite the advantages derived from the use of MS detection. In any case, MS has also been extensively used, principally employing ESI sources working in positive mode, with QqQ [155,156] as the most used analyser.

Table I.5.- Some examples of the application of LC to the analysis of PAEs in water and beverages.

Analytes[a]	Matrix	Stationary phase	Ionisation source (mode)	Analytical technique	Reference
BBP, DBP, DPP, DCHP and DEHP	Drinking waters in contact with plastic recipients	C_{18}	-	HPLC-DAD	[152]
DEP and DBP	Plastic bottled water	C_{18}	-	HPLC-UV	[153]
DBP, BBP, DEHP and DCHP	Tap water, lake water, drinks, tonic lotion and human serum	C_{18}	-	HPLC-UV-Vis	[154]
DEHP	Tap and surface water	C_{18}	ESI (+)	HPLC-MS/MS (QqQ)	[155]
DEHP	Water, soft drinks and energy drinks	C_8	ESI (-)	HPLC-MS/MS (QqQ)	[156]
DMEP, DEEP, DPP, BBP, DIBP, DBP, DBEP, DIPP, DNPP, DCHP, DNOP, DINP and DIDP	Mineral, pond and waste water	C_{18}	ESI (+)	UHPLC-MS/MS (QqQ)	[157]

DBEP: di(2-butoxyethyl) phthalate; DCHP: dicyclohexyl phthalate; DEEP: di(2-ethoxyethyl) phthalate; DIPP: diisopentyl phthalate; DMEP: di(2-methoxyethyl) phthalate; DPP: dipropyl phthalate
[a] Only the analytes studied in this PhD Thesis are shown.

As can be seen in Table I.6, although less used, CE has also been successfully applied for the determination of PAEs. Since phthalates do not have acid groups in their structures, they remain neutral independently of the pH of the media. For this reason, MEKC [158-161] and, in fewer occasions MEEKC [162,163], have been the modes selected for their separation, using SDS surfactant as pseudo-stationary phase in the BGE. Besides, ACN has been added as BGE modifier in most applications, although other organic solvents such as 2-propanol, or even butanol and n-octane have also been used in order to promote the separation of the analytes. As can be seen in the table, most applications have chosen UV [160-162] and DAD [159] detectors, while MS has been hardly applied [158]. In this last case, ESI sources in positive mode with IT as analysers have been used in most of the few applications which can be found in the literature.

Table I.6.- Some examples of the application of CE to the analysis of PAEs in water and beverages.

Analytes[a]	Matrix	BGE	Analytical technique	Reference
DEP, DBP and DPP	Waste water	25 mM SDS in 10 mM ammonium acetate and 20 % (v/v) ACN, pH 7.0	MEKC-ESI(+)-MS (IT)	[158]
DBP and DEHP	Soft drinks	3.46 % (w/w) SDS, 0.5 % (w/w) n-octane, 7 % (w/w) 1-butanol, 0.25 % (w/w) Pluronic® F-127 and 78.69 % (w/w) of 30 mM NaH_2PO_4, pH 2.5	MEEKC-UV	[162]
BBP, DEP, DBP and DIBP	Chinese white spirit	5 mM β-CD, 50 mM SDS, 25 mM borate buffer, 9 % (v/v) ACN, pH 9.2	MEKC-DAD	[159]
DEP and DEHP	Beverages and urine	50 mM H_3PO_4–NaOH, 160 mM SDS, 15 % (v/v) ACN and 15 % (v/v) 2-propanol, pH 2.0	MEKC-UV	[160]
DEP, DBP and DEHP	Tap water, sea water and beverages	120 mM SDS, 50 mM H_3PO_4, 15 % (v/v) ACN and 15 % (v/v) 2-propanol, pH 2.0	Reverse flow MECK-UV	[161]

β-CD: β-cyclodextrine; DPP: dipropyl phthalate; PDA: photodiode array detector
[a] Only the analytes studied in this PhD Thesis are shown.

As it has been previously discussed, the high complexity of certain matrices and the low concentrations at which PAEs are present make necessary the development of extraction and clean-up methods which allow the preconcentration of the analytes, at the same time that they allow the obtention of cleaner extracts before their determination by the above-mentioned instrumental techniques. At this point, it is important to highlight that this step of the analytical procedure is the most prone to introduce laboratory contamination, since during sample treatment the matrix is exposed to several of the PAEs sources already mentioned (pipette tips, solvents, atmosphere, etc.). For this reason, most efforts should be directed to reduce contamination during this stage.

In relation to the pre-treatment procedures applied to the extraction of PAEs from environmental and beverage samples, different solvent-based extraction techniques have been used, being the most extended for food analysis. Thus, the extraction of water and drinks can be carried out by LLE techniques thanks to their non-fatty nature. In this sense, the QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) method [164] as well as miniaturised techniques such as DLLME [165] and HF-LPME [160] have been used providing a high extraction efficiency. Despite the good performance shown by solvent-based extraction techniques, the use of sorbents in different SPE modes has also been extensively explored for the extraction of PAEs, since the wide number of sorbents available allow to increase the selectivity of the methodology maintaining a lower organic solvent consumption. In this sense, new sorbents such as MIPs [166], CNTs [167], MOFs [168] or coated m-NPs [169], among others, have proven to be excellent alternatives for effective extraction of PAEs from a wide number of matrices.

I.2.- Current trends in sample preparation

In the last decades, trends in Analytical Chemistry have been focused on the development of eco-friendly methods following the 12 principles of Green Analytical Chemistry and trying to apply them to every part of the analytical procedure, from sampling to analyte determination techniques [170]; in some cases, even with the aim of avoiding sample pre-treatment steps, e.g. the application of sensors [171] or the use of direct analysis in real time-MS (DART-MS) [172]. However, in such cases there is a clear limitation in the number of analytes that can be determined at a time, or also the LODs that can be finally achieved. As a consequence, the application of sample preparation steps which allow the effective extraction, preconcentration and clean-up of the sample are usually necessary. In this regard, most efforts are currently aimed at the reduction or elimination of hazardous chemicals (e.g. organic solvents, reagents, etc.), the reduction of analytical wastes and analysis time, the simplification of the procedures, and the possibility of automation, without losing extraction efficiency. For this purpose, different alternatives have been proposed: a) miniaturisation of the extraction techniques, which allows a reduction not only of the chemicals used during the process, but also of the sample amount; b) the use of greener solvents, such as certain ionic liquids (ILs), instead of conventional organic solvents; and c) the use of new selective sorbent materials which allow both extraction and clean-up in the same step. All these strategies have been successfully applied to the analysis of contaminants, among which there also are the compounds with oestrogenic activity already mentioned.

I.2.1.- New extraction materials

Nowadays, material engineering has provided a wide number of new materials with extraordinary properties which have been applied in different fields. Among them, Analytical Chemistry can also be highlighted since it has introduced much of these materials as sorbents in sample preparation approaches, which has allowed the development of novel methodologies which offer extraordinary selectivity and extraction efficiency [173]. As can be seen in Table I.7, new specific materials and combinations of them have been used as sorbents in some miniaturised sorbent-based extraction techniques, including solid-phase microextraction (SPME) [174,175], micro-SPE (μ-SPE) [176], dispersive SPE (dSPE) [112,177,178] and magnetic dSPE (m-dSPE) [82,157,179], which have been applied to the extraction of several analytes, including oestrogenic compounds and PAEs. Among these new sorbents, MIPs [174,177], carbonaceous materials such as CNTs [112,179] or graphene [157,176], MOFs [175,178] and different types of NPs [82,157,179] have been some of the most commonly used. Since MIPs, CNTs, MOFs and m-NPs have been used during the development of this PhD Thesis, they will be described below with more detail; examples of their application will also be given.

Table I.7.- Some examples of the application of new sorbents for the extraction of oestrogenic compounds and PAEs.

Analytes[a]	Matrix	Extraction sorbent	Analytical method	LODs	Reference
E_1, 17α-E_2, 17β-E_2, E_3, EE_2, DS, DES, HEX, ZEN, ZAN, α-ZAL, β-ZAL, α-ZEL and β-ZEL	Milk	Fe_3O_4@pDA	m-dSPE-UHPLC-MS/MS	0.63-11.8 μg/kg[b]	[82]
ZEN, α-ZAL, β-ZAL, α-ZEL, β-ZEL and ZAN	Mineral water, pond water, waste water and powdered infant milk	MWCNTs	dSPE-HPLC-MS/MS	0.01-9.56 μg/L	[112]
DMEP, DEEP, DPP, BBP, DIBP, DBP, DBEP, DIPP, DNPP, DCHP, DNOP, DINP and DIDP	Mineral, pond and waste waters	Fe_3O_4@ Reduced graphene oxide	m-dSPE-UHPLC-MS/MS	6-178 ng/L[b]	[157]

Table I.7.- (Continued).

Analytes[a]	Matrix	Extraction sorbent	Analytical method	LODs	Reference
E_1, 17β-E_2, E_3 and EE_2	Milk powder	MIP	SPME-HPLC-UV	0.83-2.5 µg/L	[174]
EE_2, DES and HEX	Milk	MOF-5	SPME-HPLC-DAD	0.17-0.56 µg/L	[175]
E_1, 17β-E_2, EE_2 and DES	Water	Graphene	µ-SPE-HPLC-UV	0.8-1.7 ng/L	[176]
DBP and DEHP	Water	TMU-6	dSPE-GC-FID	0.2-0.7 µg/L	[178]
DEP, DBP, BBP, DEHP and DNOP	Plastic bottled water and artificial saliva	MIP	dSPE-GC-MS	0.31-0.41 µg/L	[177]
DEP, DBP, BBP, DEHP and DNOP	Carbonated soft drinks	Fe_3O_4/Ag@ MWCNTs	m-dSPE-GC-MS	12-25 ng/L	[179]

DBEP: di(2-butoxyethyl) phthalate; DCHP: dicyclohexyl phthalate; DEEP: di(2-ethoxyethyl) phthalate; DIPP: diisopentyl phthalate; DMEP: di(2-methoxyethyl) phthalate; DPP: dipropyl phthalate; MWCNTs: multi-walled carbon nanotubes; pDA: polydopamine
[a] Only the analytes studied in this PhD Thesis are shown. [b] Limits of quantification (LOQs).

I.2.1.1.- Molecularly imprinted polymers

MIPs have been designed to establish specific interactions with the target analytes or groups of analytes structurally similar, which avoids the retention of interfering species [180]. Although the molecularly imprinting concept was introduced more than 30 years ago [181], the first applications of MIPs as selective sorbents in SPE were introduced in the 90's [182,183]. Since then, SPE based on MIP sorbents has become a very popular option for the selective extraction of different analytes from a wide variety of matrices [184].

MIP synthesis frequently involves three main stages (see Figure I.8): the formation of a complex between the template molecule and the functional monomers by means of non-covalent bonds (most extended practice); the polymerisation of these monomers around the template with a cross-linking agent in the presence of an initiator to fix their three-dimensional positions; and the final removal of the template molecule by intensive

washing steps. This process provides a polymeric matrix with empty cavities which are complementary to the template molecule in shape, size and position of the functional groups [180,184].

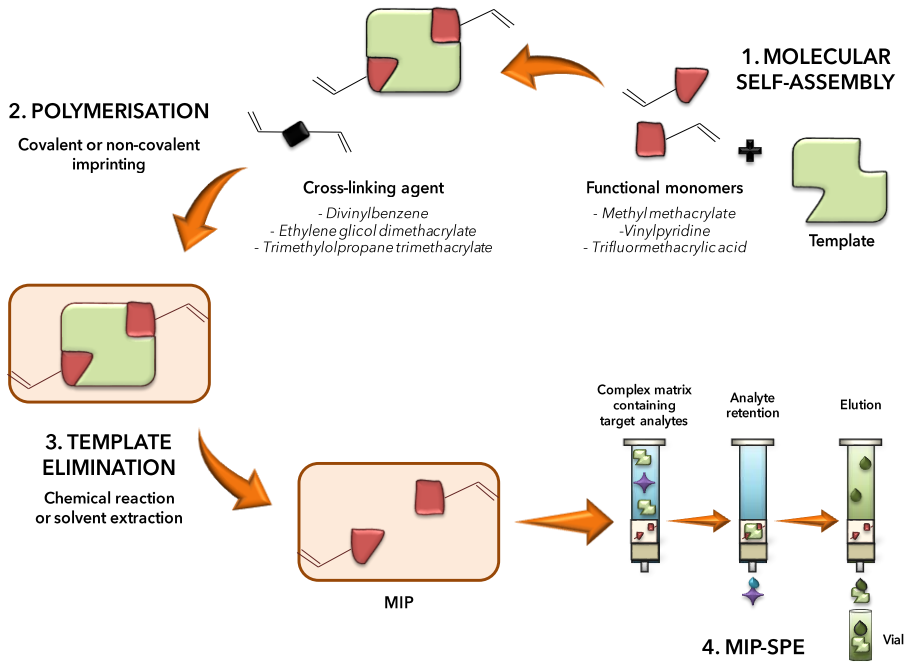


Figure I.8.- Scheme of the MIP synthesis process and MIP-SPE extraction.

Taking this into account, it seems clear that the selectivity of a MIP is given during the synthesis process, being necessary to carry out a good selection of the components involved in its production, paying especial attention to the template molecule, the functional monomers and the cross-linking agent. While a good selection of the template molecule and the cross-linking agent is important, suitable functional monomers with complementary functional groups to those present in the template molecule can be crucial, since it will determine not only the stability of the complex monomer/template during the synthesis, but also the capacity of the MIP to interact selectively with the target molecules [184,185]. Among the most common functional monomers, it can be highlighted the use of 4-vinylpyridine (preferred for acid templates) and methacrylic acid (preferred for basic templates), among others such as 2-vinylpyridine, acrylamide, trifluoromethacrylic acid or 2-hydroxyethyl methacrylate [186]. In any case, it is important

to highlight that both 4-vinylpyridine and methacrylic acid have been used with acid and basic compounds thanks to their capacity to establish hydrogen bonds [184]. Regarding the cross-linking agent, ethylene-glycol dimethacrylate, trimethylolpropane trimethacrylate, N,N-methylenebisacrylamide or divinylbenzene have been some of the most used. Besides, the solvents used during synthesis also play an important role during polymerisation, being low polar and aprotic solvents (e.g. ACN, $CHCl_3$, toluene, etc.) the most frequently used, since they allow the suitable formation of hydrogen bonds between the template and the monomers [186]. Finally, it should be considered that template bleeding during sample treatment is one of the main drawbacks of the use of MIPs when the molecule used as template is the same that will be analysed, since it can lead to an overestimation of the concentration of the target analyte, which is especially important in trace analysis. In order to overcome this problem, it is important to wash consciously the polymer, though the use of 'dummy' templates, which are molecules structurally analogue to the target analytes, have also been proposed [185,187].

It is important to mention that, although MIPs are normally designed for one target analyte, it can usually retain the analyte for which it was created together with other structurally related compounds. This fact is known as 'cross-reactivity' and, although it could be considered as a negative aspect in certain cases due to the loss in selectivity, it can result advantageous when a high affinity for a family of related substances is desired [185]. Current trends focused on multiresidue analysis have taken advantage of this fact up to the point that some MIPs have been synthesised using more than one template for the extraction of several analytes belonging to different families [185,188].

The selectivity and versatility shown by this kind of sorbents, have driven their use as chiral stationary phases in separation techniques [189], for the construction of sensors [180] or as sorbents in sample preparation approaches [190], which constitutes one of the most important uses of these materials. Thus, MIPs have been applied in different sorbent-based extraction techniques, including conventional SPE [191,192], dSPE [193,194], m-dSPE [195,196], SPME [197,198] or stir-bar sorptive extraction (SBSE) [199,200], among others. It is important to highlight that despite the high selectivity of these new materials, they can only be applied for their extraction from biological fluids such as milk, plasma or serum with the application of a previous deproteinization step since, although macromolecules cannot access the selective cavities, they can be retained in the surface of the polymer hindering the retention of the target analytes [201] (if protein removal is not efficient, they could also appear in the final extract which clearly hinders analyte

determination). Concerning the application of MIPs to the analysis of oestrogenic compounds, a good number of works can be found in which these sorbents have been used for the selective extraction of natural [111,202,203] or synthetic [111,203,204] oestrogens, mycoestrogens [195,205] and PAEs [177,206] from different environmental and food matrices. In this sense, conventional SPE [111,195,205], dSPE [177], m-dSPE [204,206] and SPME [203] based on MIPs have also been proposed.

I.2.1.2.- Carbon-based materials: Carbon nanotubes

Carbon-based nanomaterials, include a wide variety of peculiar structures, i.e. CNTs [207], graphene [208], fullerenes [209], nanodiamonds [210] and nanohorns [211], that have also been widely used for extraction purposes. They are characterised by their possibility to be covalently or non-covalently modified, and their capability to establish hydrophobic and π-π interactions, apart from other interesting properties [212]. Among them, CNTs are, by far, the most widely applied and also the ones that have been used during the development of this PhD Thesis.

CNTs are molecular-scale tubes considered as graphene sheet structures rolled up in the shape of a cylinder with open or closed ends, depending on the synthesis method used during their production. This nanomaterial was reported for the first time by Sumio Iijima in 1991 [213], who carried out their synthesis by means of an arc-discharge evaporation method [214]. Nowadays, there are different procedures to obtain this allotropic form of carbon in great amounts, including not only arc-discharge but also chemical deposition [215] and laser ablation [216]. These synthesis methodologies allow the obtention of both single-walled CNTs (SWCNTs) and multi-walled CNTs (MWCNTs), with different torsions (armchair, zig-zag and chiral), and with different diameters (0.4-100 nm) and lengths [217] (see Figure I.9). They have a high length/diameter ratio, tensile strength, thermal conductivity, stability and resilience and an excellent electrical conductivity, among others, which are properties that make them unique [218].

Such extraordinary features have driven their application in different fields, finding a new application area in Analytical Chemistry. In this regard, they have been used for the construction of electrochemical sensors [219], as part of membranes with filtration purposes [220], as matrices in matrix-assisted laser desorption/ionisation (MALDI) [221], in surface-assisted laser desorption/ionisation (SALDI) [222] or as stationary and pseudo-stationary phases in different separation techniques [217]. However, one of the most important uses of CNTs in this field is their application in sorbent-based extraction

techniques, including SPE [223,224], dSPE [225,226], m-dSPE [227,228], SPME [229,230], SBSE [231] and matrix solid-phase dispersion (MSPD) [232].

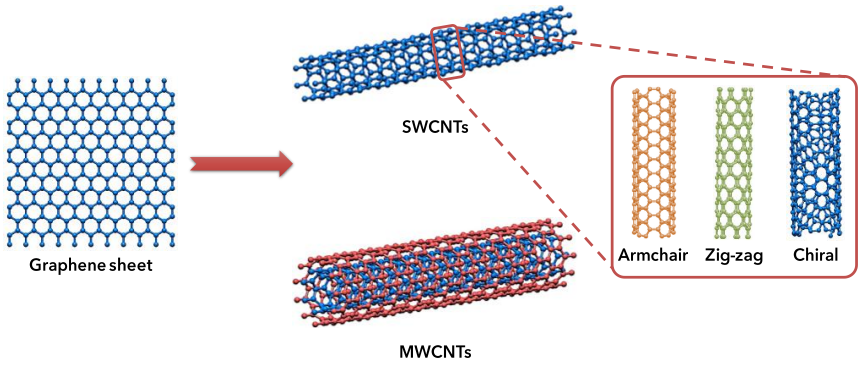


Figure I.9.- SWCNTs and MWCNTs structures and torsions.

It is important to mention that, while CNTs have been used under their pristine form, they have also been functionalised or combined with other materials, normally in order to improve their selectivity or their extraction capacity, although in certain cases it has been carried out to improve their solubility, which is very low in most solvents as a consequence of the strong inter-tube van der Waals interactions [233]. In this sense, the process usually includes an initial oxidative or acidic treatment of pristine CNTs followed by different procedures for their covalent [234] or non-covalent functionalisation [235]. In the first case, it is possible to carry out a direct or an indirect functionalisation by covalent linkage between the functional groups and the CNT skeleton or the carboxylic groups present on its surface, respectively. The second one is based on physical adsorption processes of molecules onto the CNT sidewall by van der Waals, hydrophobic or electrostatic forces, among others [217]. Thus, this versatility has made possible their application for the extraction of a wide variety of organic and inorganic analytes from environmental, agri-food and biological matrices [233].

With respect to the application of CNTs as sorbents for oestrogenic compounds analysis, they have been used not only for the extraction of natural [80,236,237] and synthetic [80,237] oestrogens, but also mycoestrogens [112,238] and phthalates [179,239,240] in several matrices of environmental and agri-food interest. Regarding the techniques in which CNTs have been applied, dSPE [112,236] and m-dSPE [179,238,240] have been the most common, although MSPD [80] and SPME [239] have also been successfully used. In this sense, MWCNTs have been the most applied, especially in

combination with m-NPs [179,237,238], while SWCNTs and oxidised MWCNTs have been less used.

I.2.1.3.- Metal-organic frameworks

MOFs are a new class of microporous materials constituted by the self-assembling of metal ions or metal clusters and bi- or multidentate organic ligands through coordination bonds, shaping hybrid organic-inorganic crystalline structures. The wide variety of organic ligands and metal ions/clusters available and the multiple ways in which they can be combined, have driven almost an infinite number of different MOFs with different pore diameters and cavity sizes. In fact, there exist around 20000 MOFs which have already been characterised (Figure I.10 shows some examples of MOFs structures highly used in SPE). Regarding their production, to date different approaches have been developed with this purpose, including conventional electric heating (solvothermal or hydrothermal methods), and microwave, electrochemical, sonochemical, room temperature and surfactant-assisted synthesis [241].

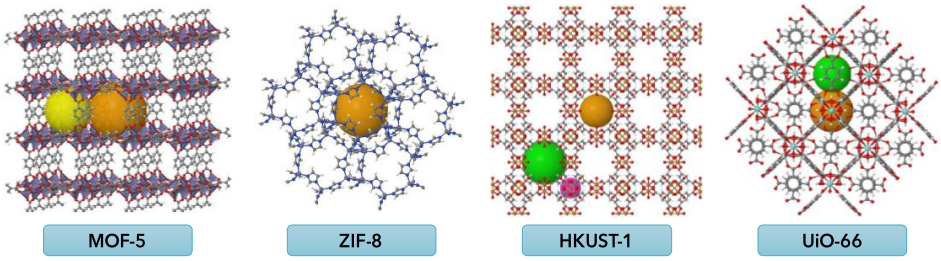


Figure I.10.- Some examples of MOFs structures used as sorbents in SPE methodologies [242].

These structures are characterised by a high porosity, which results in an extremely high surface area (up to ~10000 m^2/g), with great thermal stability (up to 300-600 °C), ultra-low densities (< 0.13 g/cm^3), uniform cavities and specific pore size (up to ~10 nm) and high adsorption affinity [242,243]. As a result of these extraordinary properties, MOFs have been used in different applications, such as heterogenous catalysis, gas storage, or even drug delivery [242,244]. Regarding their use in the Analytical Chemistry field, MOFs have been applied for the construction of sensors [245], as stationary phases in separation techniques [246] and, above all, as sorbents in sample preparation techniques [242]. In this sense, apart from their high surface area and stability, MOFs are easy tuneable and, therefore, their hydrophilic-hydrophobic and polar/non-

polar properties can be modified, which clearly increase their applications in this field [242]. Thus, MOFs have been applied as sorbents mainly in dSPE [247,248] and m-dSPE [249,250] procedures for a wide number of analytes in different environmental, food and biological samples, although some applications of MOFs in SPME [251], SBSE [252] and MSPD [253] can also be found in the literature.

It is important to mention that after the synthesis process, the solvents or the organic linkers remain inside the pores of the MOF. For this reason, it is necessary to develop an activation step before their final use, which allows the removal of guest molecules that hinder the full access of the porous structure of these materials [241]. This process is normally carried out applying mild conditions in order to prevent changes in the structure, since collapse of the pores after removing guest molecules may take place for certain MOFs [247]. In this sense, different activation strategies have been designed, including the use of supercritical CO_2 or freeze drying, being a simple heating and vacuum the most commonly used because of its simplicity [241]. It should be mentioned that the heating/vacuum method could lead to a partial or complete loss of porosity. Thus, the exchange of high boiling point solvents used during the synthesis by others with a lower boiling point which can be removed from the pores by simple vacuum application has been proposed [241].

In relation to the use of MOFs as sorbents for the extraction of compounds with oestrogenic activity, few applications can be found in this sense. Despite this fact, natural [76,113,254] and synthetic [76,113,175,254] oestrogens, and PAEs [168,255] have been successfully extracted from different environmental, food and biological matrices, while no applications have been found for the extraction of mycoestrogens. These materials have been used in different sorbent-based extraction techniques, including dSPE [76,113], m-dSPE [168], SPME [175] or SBSE [254]. Regarding the type of MOF, MIL-53 [113], MOF-5 [175], MIL-101 [76,168,255] or MOF-3 [254] have been some of the MOFs used in this sense.

I.2.1.4.- Magnetic nanoparticles

m-NPs are nano-size structures which, as it has been previously commented for other nanomaterials, present unique characteristics that make them suitable for their application in different fields. In particular, they have been successfully used in catalysis, for the construction of sensors, as stationary phases or in sample preparation, among others [256,257].

These nanomaterials are generally composed of metals (e.g., iron, cobalt, nickel, silver, gold, etc.), alloys (e.g., FePt, $CoPt_3$, etc.) or metallic oxides (e.g., $MnFe_2O_4$, $MgFe_2O_4$, $CoFe_2O_4$, etc.) [258,259], being iron oxides Fe_3O_4 (magnetite) and γ-Fe_2O_3 (maghemite) the most used in sample preparation by far, as a result of their low toxicity, great extraction capacity (high surface-to-volume ratio), ease of manipulation because of their superparamagnetic behaviour at room temperature, the multiple and easy ways of obtaining them, and the fact that they can be easily surface modified thanks to the hydroxyl groups usually present on their surface which are also responsible of their good dispersibility in water [260,261].

Regarding their synthesis, there are several procedures which allow the obtention of particles with different sizes, and that include thermal decomposition, solvothermal/hydrothermal reactions, microemulsion/nanoemulsion based synthesis, flow injection methods, aerosol/vapour processes, chemical co-precipitation or metal reduction, among other alternatives less used [261,262]. However, the synthesis of m-NPs presents two main problems that should be considered before addressing their preparation. Firstly, m-NPs show tendency to form agglomerates in order to reduce the energy associated to their high surface-to-volume ratio [262]. Secondly, naked m-NPs also show a great chemical reactivity and are easily oxidised in air, resulting in a loss of magnetism and dispersibility [262]. In order to overcome these drawbacks, they are usually coated with inorganic (e.g., silica, alumina, etc.) and organic (e.g., polymers, surfactants, etc.) layers which protect them during and after the synthesis, resulting in a good number of cases a core-shell type structure, since their morphologies are not always spherical. In this sense, although it is not strictly necessary, the use of inorganic shells, specially silica, is quite common as supports for further modifications with other materials or functional groups, since it results in abundant silanol groups in the surface which make easier the binding of the external layer (see Figure I.11) [257]. All these coatings are commonly used to improve the extraction selectivity of m-NPs [262,263], existing a very close relationship between the coating used and their subsequent application. Among the coatings that have been proposed in the literature, it is possible to find a good number of polymers [264,265], ILs [266,267] or surfactants [268,269], among others. Besides, their functionalisation with different chemical groups or molecules is also a very extended practice, normally applied for the selective extraction of heavy metal ions [270]. It is important to mention that in the last years, m-NPs have been combined with new materials in a good number of applications, including MIPs [271,272], MOFs [252,273] or CNTs [274,275], among others, creating composites which combine the good features and

selectivity of these materials with the advantages derived from the magnetic properties of such NPs.

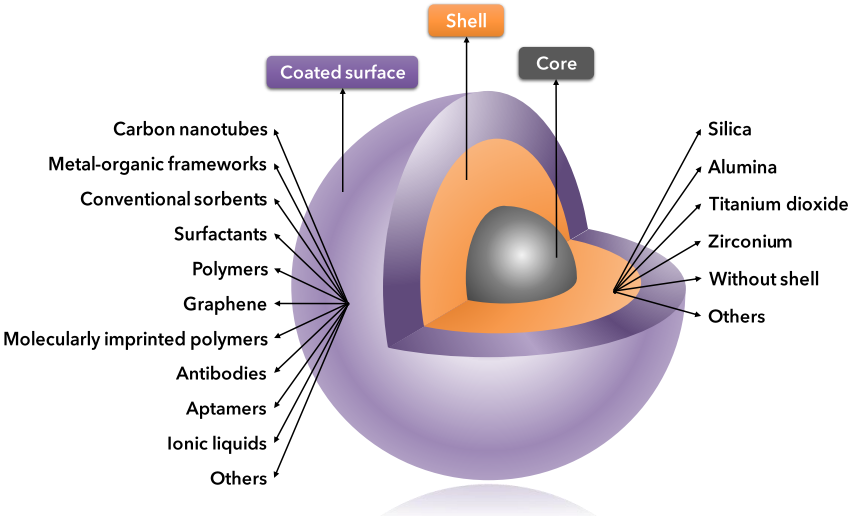


Figure I.11.- Scheme of the core-shell structure and materials most commonly used as shells and coatings with extraction purposes. Adapted from Ríos et al. [257].

Regarding the application of this kind of nanomaterials to the analysis of oestrogenic compounds, they have allowed the efficient and selective extraction of different natural and synthetic oestrogens [82,169,204,276-278], mycoestrogens [82,169,276,279] and PAEs [168,280-283] from a wide variety of samples, including biological, food and environmental matrices, being principally used in m-dSPE procedures [82,168,169,276-282]. In this sense, Fe_3O_4 m-NPs have been the most common, normally combined with other materials such as graphitised carbon black (GCB) [279], surfactants [277,281] or polymers [82,169,276,280], especially MIPs using EE_2 [278], DS [204] or DBP and BBP [283] as templates. It also should be highlighted their combination with other nanomaterials such as CNTs [282] or MOFs [168].

I.2.2.- Miniaturisation in sample preparation

As it has already been mentioned, sample preparation constitutes one of the most important steps involved in any analytical procedure since most analyses are carried out in highly complex samples in which many analytes are present at very low concentrations. However, when needed, the application of classic procedures can result laborious and

time consuming, can have a high cost due to the great amount of organic solvents and reagents used, and can have a negative effect on the environment if the residues generated during the process are not adequately managed. For this reason, current trends in sample preparation are also focused on the simplification of such procedures, the reduction of the solvents and reagents used for extraction, and even the automation of the process [284,285]. In this sense, new miniaturised techniques, in which very low amounts of sorbents (mg or even μg) and solvents (μL) are enough for an effective extraction of the target analytes, have emerged as simple and cost-effective and, in some cases, easily automatable alternatives to conventional SLE and LLE procedures. Thus, a good number of miniaturised techniques have been developed and successfully applied to the extraction and preconcentration of a broad spectrum of analytes from matrices of different nature, also providing a good clean-up performance [284,286].

I.2.2.1.- Sorbent-based procedures

Miniaturisation in sorbent-based extraction is mainly focused on the use of very small amounts of sorbents (< 100 mg) disposed as particles of extremely low size or as extremely thin coatings. Some examples of such miniaturised procedures not only include the well-known SPME [287] and SBSE [288], as well as some variations of the last one like stir-cake sorptive extraction (SCSE) [289], rotating-disc sorptive extraction (RDSE) [290], stir-rod sorptive extraction (SRSE) [291], but also miniaturised versions of classic SPE like μ-SPE [292], μ-dSPE [112] or magnetic μ-dSPE (m-μ-dSPE) [293], among others less used. All these techniques are characterised by their simplicity, cost-effectiveness, time-saving and low waste generation, constituting very interesting alternatives to conventional sample preparation methods [286,294]. Since μ-dSPE and m-μ-dSPE have been used during the development of this PhD Thesis, they will be extensively described below.

Since its first application by Burnham et al. [295] in 1972, SPE has suffered a great evolution, becoming one of the methodologies most extensively used in sample preparation. The wide variety of sorbents and formats currently available has given this extraction technique a great versatility [296], which has allowed its application for the extraction of a broad spectrum of organic and inorganic compounds from matrices with very different origins [297-299], and also for clean-up purposes [300,301]. However, although SPE presents some well-known advantages with respect to other solvent-based procedures, such as simplicity, easy handling and lower solvent consumption with respect to classic LLE, this technique also shows some drawbacks, including, reusability (which is

not normally recommended), cartridge blocking when microparticles are present in the matrix, difficulty to carry out several extractions simultaneously, and the long time required per extraction due to sorbent conditioning and sample-loading steps [302]. Trying to solve these problems, different miniaturised versions of conventional SPE have been proposed in the last decades. In this sense, it is important to highlight that there currently exist several formats in which sorbents can be used in μ-SPE. Thus, regarding alternatives with packed sorbents (see Figure I.12), besides classic cartridges and discs with reduced amounts of sorbent, it is also possible to find more recent miniaturised devices such as well plate SPE (normally containing ninety-six microcolumns), pipette tip SPE or spin column SPE [286]. In this regard, it should also be mentioned that besides offline SPE procedures, online SPE also constitutes an alternative to be considered, since it supposes a total or partial automation of the process, while reduces analyte losses and extraction time [303].

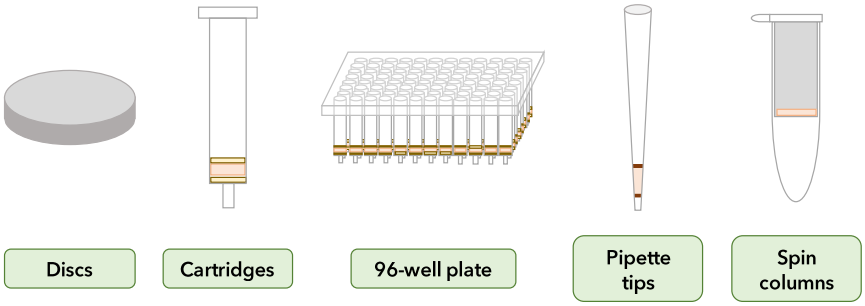


Figure I.12.- Most representative μ-SPE formats with sorbent amounts less than 100 mg.

Besides, among the multiple modifications currently available, the application of SPE under its dispersive mode (dSPE) has currently become one of the most extensively used because of its simplicity and time-saving in comparison with conventional SPE [302]. In this sense, dSPE has resulted a key point in the development of important and extended methodologies such as the QuEChERS method, in which dSPE is used with clean-up purposes, employing a wide variety of sorbents depending on the interferences which are desired to be removed. In fact, the term dSPE was coined with the introduction of the QuEChERS method and it has been adopted and used since then [302,304]. The sorbents most commonly used for QuEChERS extraction are primary secondary amine (PSA, for fatty acids, sugars and pigments removal), GCB (pigments), C_{18} (lipids) or Florisil (polar compounds). However, more recently, alternative sorbents have been specifically designed by commercial companies, among which there can be found CarbonX (United

Science), ChloroFiltr (UCT), EMR-Lipid (Agilent Technologies), NANO (Agela), Oasis-Prime (Waters Chromatography), Phree (Phenomenex), Z-SEP and MIPs (Millipore Sigma) [305].

However, dSPE can also be used with extraction and preconcentration purposes. In this sense, the miniaturised version of this technique, μ-dSPE, has been developed in order to increase the expeditiousness and to reduce the cost of the procedure, since smaller amounts of extraction sorbent (less than 100 mg) are used [302]. Regarding the type of sorbent used, it is important to consider that it should ideally meet certain features, including high porosity and surface area, good surface contact with the sample (good dispersibility), high purity and chemical stability in the conditions given during extraction (components of the sample matrix, pH, solvents, etc.) [302]. In this sense, there is an extensive variety of sorbents which has been used, including C_{18} bonded-silica particles, polymers, MIPs, m-NPs, CNTs, MOFs, graphene, etc. [302,306].

From an operational point of view, there are no differences between dSPE and its miniaturised version. The sorbent is directly dispersed in the liquid sample matrix or extract (especially when solid or semi-solid samples are analysed) containing the target analytes, which avoids the conditioning step (see Figure I.13). Such dispersion, normally assisted by ultrasounds or manual/vortex shaking, allows the rapid and uniform interaction between analytes and sorbent, resulting in a reduction of the extraction time [306]. After analytes retention, the sorbent can be separated from the sample matrix using two different procedures. On the one hand, the sample matrix can be discarded by decantation after a centrifugation of the mixture. Then, the sorbent is re-dispersed in the desorption solvent, centrifuged and the supernatant containing the analytes is collected by decantation (Figure I.13 A). On the other hand, the sorbent can be retained in an empty column containing frits at the bottom, while the sample matrix is discarded. Then, another frit is placed on the top of the sorbent and analytes are eluted similarly to conventional SPE (Figure I.13 B).

As can be seen, the whole procedure results simpler and faster than SPE with packed sorbents, since sorbent conditioning and sample loading steps are avoided. It is also worth mentioning that sorbent dispersion provides an immediate and more effective contact between phases, resulting in a faster retention of the analytes onto the sorbent. Finally, it should be highlighted that this technique is especially indicated for the analysis of samples in which it is frequent the presence of microparticles or microorganisms which can lead to the blockage of the cartridges used in conventional SPE [302].

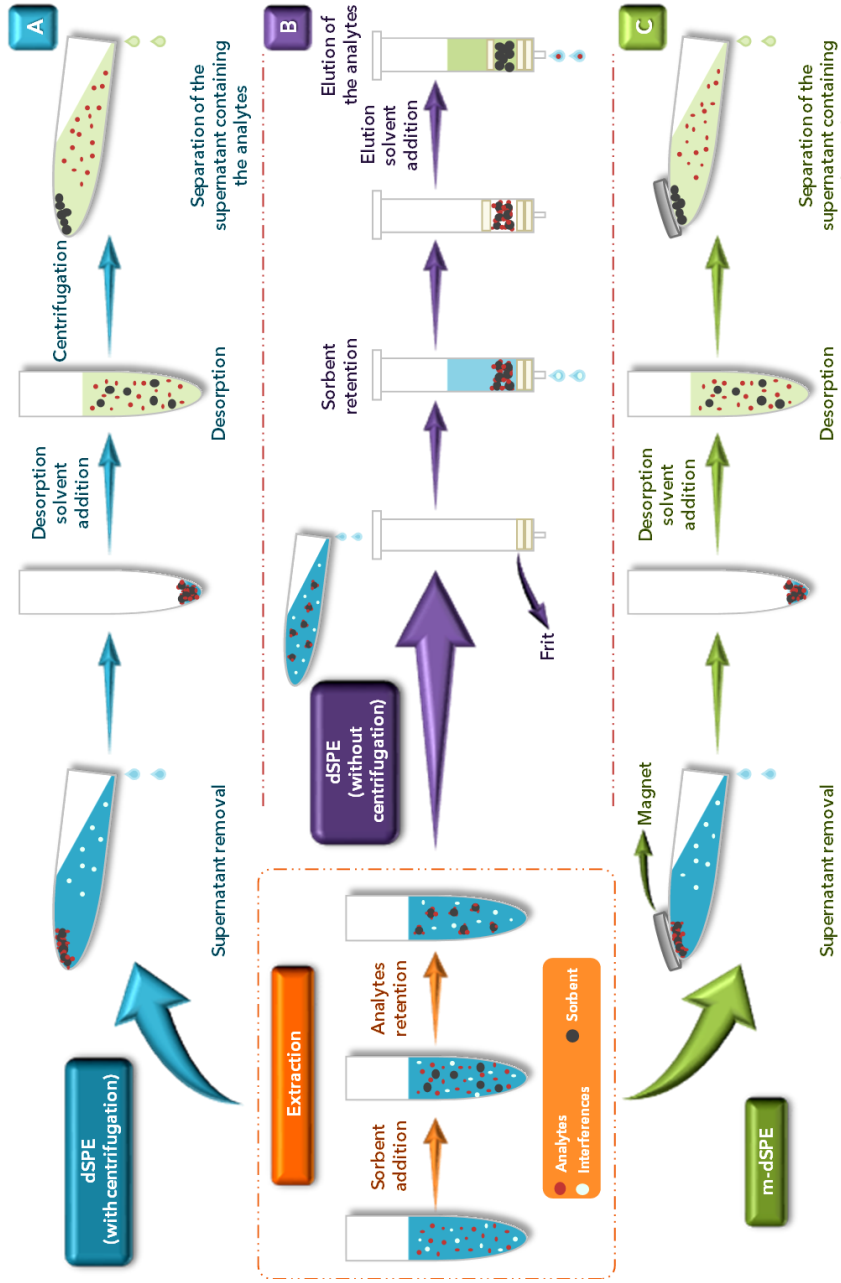


Figure I.13.- Schematic representation of A) dSPE with centrifugation, B) dSPE without centrifugation, and C) m-dSPE procedures.

As it has already been indicated, the introduction of nanomaterials in sorbent-based extraction procedures has supposed a considerable advance on these techniques. They play an important role in miniaturised methodologies, since also small amounts of sorbents can be used as a result of their high surface-to-volume ratio which increases the extraction capacity of the methodology. Concerning μ-dSPE, it is possible to find in the literature applications of CNTs [307], MOFs [248], graphene oxide [308] and different types of NPs [309], among others, as well as combinations of them [310]. In the particular case of the use of m-NPs (as sorbents or as part of them), they have provided an important simplification of the μ-dSPE procedure. In that case, in which the procedure is called m-μ-dSPE, the initial extraction step is common to the non-magnetic version. However, the sorbent is easily retained and isolated from the sample matrix using an external magnetic field, avoiding any centrifugation or sorbent retention, which results in a significant reduction of the extraction time [294,302]. Thus, after analytes retention, a magnet is placed in the outer wall of the recipient to retain the sorbent, which allows removing the supernatant by decantation. Then, the sorbent is re-dispersed in the desorption solvent and isolated again with the magnet to recover the supernatant containing the analytes by decantation (Figure I.13 C). Depending on the strength of the magnet, as well as on the paramagnetic behaviour of the m-NPs, more or less time is necessary before decantation.

The inherent advantages of using dSPE approaches have allowed the application of both μ-dSPE and m-μ-dSPE for the extraction of a wide variety of inorganic and organic compounds from matrices of very different nature [306]. In this sense, these techniques have also been applied for the analysis of oestrogenic compounds, using both magnetic [82,168,169,277] and non-magnetic [311,312] sorbents, in environmental, food and biological samples.

I.2.2.2.- Solvent-based procedures

As it is well-known, classic LLE methods are characterised by long extraction times and the use of large volumes of organic solvents, as well as a low sensitivity in the subsequent determination of the target analytes (as a result of the obtention of complex and dirty extracts or of an unsuitable preconcentration factor). In order to overcome these important drawbacks, different liquid-phase microextraction (LPME) methodologies have emerged in the last decades. Such miniaturised versions of the technique are based on classical LLE but using extremely low volumes of toxic organic solvents (from 1 μL to

several hundreds of µL). Thus, the extraction process takes place by the transference of the target analytes from an aqueous phase (donor phase) to a very low volume of a water-immiscible organic solvent (acceptor phase), allowing the extraction and preconcentration of the analytes in a single step. This fact has made these techniques less-time consuming, simpler, cost effective and environmentally friendly alternatives, while they also provide an increase in the sensitivity [284,313,314]. There currently exist several modes in which LPME techniques can be applied, which are normally grouped in three main categories, single-drop microextraction (SDME) [315], HF-LPME [316] and DLLME [317], which will be described below, paying especial attention to HF-LPME, since it has been used in this PhD Thesis.

The first LPME procedure developed was SDME, introduced in 1996 by Jeannot and Cantwell [318]. It is based on the suspension of a single microdroplet (1-10 µL) of a non-aqueous solvent at the tip of a microsyringe for the extraction of the analytes from an aqueous or a gaseous sample. After a certain time of contact with the sample or with its headspace to warranty the extraction of the analytes, the drop is retracted into the syringe for its analysis by chromatographic or electrophoretic techniques [313]. Depending on the nature of the interaction between the sample matrix and the acceptor phase, it is possible to distinguish several categories: *direct immersion SDME (DI-SDME)* [319], when the drop supported on the syringe needle is directly immersed in the sample; *headspace SDME (HS-SDME)* [320], when the microdroplet is not immersed in the sample but maintained in the headspace; *directly suspended droplet microextraction (DSDME)* [321], when the microdroplet is deposited on the surface of the sample without syringe supporting; *liquid-liquid-liquid microextraction (LLLME)* [322], when the analytes are first extracted by a water-immiscible organic solvent and then simultaneously extracted from this phase by an aqueous microdroplet supported on the tip of a syringe; *continuous flow microextraction (CFME)* [323], when the organic drop is suspended into an extraction chamber through which the sample flows continuously; *drop-to-drop microextraction (DDME)* [324], which is similar to DI-SDME but using very reduced sample volumes; *solid drop LPME (SDLPME)* [325], when the drop is delivered on the sample surface and cooled below the melting point of the organic solvent selected (10-30 °C) after extraction, in order to simplify the collection of the droplet; and *bubble-in-drop SDME (BID-SDME)* [326], when an air bubble is created inside the single drop that increase the contact surface [284,313,327]. Regarding the application of SDME procedures to the extraction of compounds with oestrogenic activity, this technique has been used in a reduced

number of cases for the extraction and preconcentration of natural and synthetic oestrogens [326,328-330] and PAEs [321,324,331] from matrices of different nature.

Despite the inherent advantages of the use of SDME, there are some important drawbacks which limit its application, such as its low extraction capacity due to the low volume of the droplet and, therefore, its reduced surface contact with the sample, as well as irreproducibility due to variations on the volume of the drop during extraction (as a result of its partial dissolution in the sample). Besides, the microdroplet (which is frequently obtained using hexane, xylene, cyclohexane, toluene or even ILs as extraction solvents) can be easily detached from the syringe needle, being necessary to control the extraction conditions, including stirring speed, temperature and extraction time [314].

In order to provide an alternative to solve the instability of the droplet in SDME procedures, Pedersen-Bjergaard and Rasmussen [332] introduced HF-LPME in 1999. In this method, the HF, which is frequently made of PP, is impregnated in the extraction solvent (which is also immiscible in water, e.g. 1-octanol, toluene, dihexyl ether, etc.), forming what is frequently called a supported liquid membrane (SLM). The lumen of the fibre can be filled with the same organic solvent (which is called *two-phase* HF-LPME) [88] or with an acidic or a basic aqueous phase (which is called *three-phase* HF-LPME) [333] (see Figure I.14). Once the HF is introduced in the sample, the analytes pass through the SLM into the acceptor phase of the lumen. After extraction, the acceptor phase can be retracted into the syringe similarly to SDME, or the fibre can be immersed into another solvent to back-extract the analytes from the it. In two-phase LPME, the fibre can be attached to the syringe needle by one of its ends and the other can be closed or not, while in the three phase system, the free end of the fibre must be closed to avoid the loss of the acceptor phase [284,313,314]. Finally, it should be mentioned that this technique can also be performed in a dynamic mode [334], in which the acceptor phase is continuously renewed by connecting both ends of the fibre to a flow system [314]. In relation to the extraction of oestrogenic compounds using HF-LPME, it is possible to find few applications of this technique to the analysis of natural and synthetic oestrogens [79,88,335], mycoestrogens [79] and PAEs [160,336,337] from food, environmental and biological matrices.

Regarding DLLME, it was introduced in 2006 by Rezaee and co-workers [338] as a simpler, faster and more efficient method than SDME and HF-LPME methods, also using less volumes of organic solvents. In this methodology, a mixture of an extraction solvent

(water-immiscible) and a disperser (miscible with both extraction solvent and the sample) is rapidly injected into the aqueous sample, and then the mixture is vigorously shaken, generating a cloudy solution containing tiny droplets of extraction solvent. This leads to a high contact surface between the sample and the extraction solvent favouring the extraction process. Thus, the equilibrium is achieved very quickly, obtaining very high enrichment factors due to the low volume of extractant. Once the extraction is finished, the mixture is centrifuged to separate the extraction solvent containing the analytes, which is taken from the recipient using a microsyringe for its chromatographic or electrophoretic analysis.

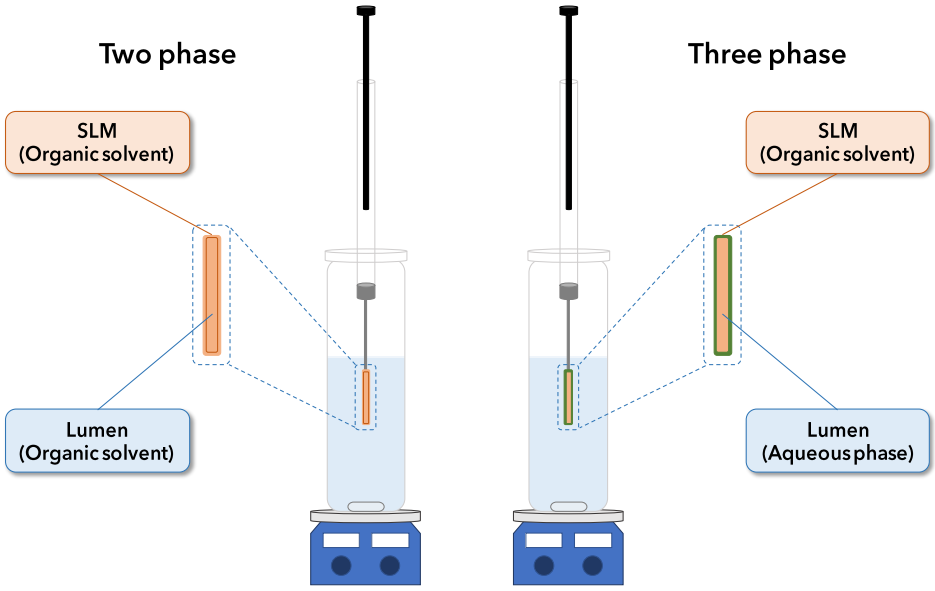


Figure I.14.- Schematic representation of the different HF-LPME modes.

At this point, it is important to mention that depending on the density of the extractant solvent, it is possible to distinguish among the following approaches: *conventional DLLME* [114], when the extractant solvent (e.g. dichloromethane (DCM), chlorobenzene, $CHCl_3$, etc.) has higher density than the sample, being collected at the bottom of the recipient after centrifugation; *floating organic-DLLME (FO-DLLME)* [325], if the final drop is collected at the surface of the sample as consequence of the lower density of the extractant solvent used (e.g. long chain alcohols); and *solidification of a floating organic drop-DLLME (SFO-DLLME)* [340], when the extractant solvent used present a

melting point in the range 10-30 °C and the mixture is cooled after extraction in order to favour phases separation. Then, the droplet is melted by increasing the temperature and the analysis can take place. Regarding the disperser solvent used, normally intermediate polarity solvents are selected, since it should be miscible with both aqueous sample and the extractant solvent, which are highly immiscible between them. The dispersers most commonly used are acetone, ACN, MeOH or ethanol [313,314]. Finally, it is important to mention that these DLLME modes have been successfully applied in some occasions to the analysis of natural and synthetic oestrogens [101,114,341], as well as mycoestrogens [101,114,342] and phthalates [343-345] from different matrices of interest.

CHAPTER II
OBJECTIVES

II.- OBJECTIVES

As it has been previously indicated, humans are highly exposed to EDCs due to the multiple contamination sources and accessible routes to the organism, including oral consumption, skin contact, inhalation, etc. It has been demonstrated that these compounds are responsible for several health disorders and diseases related with the endocrine system which also include some types of cancer. Among the wide variety of EDCs currently known, those with oestrogenic activity have arisen great interest in the scientific community.

Among the existing EDCs, the presence of natural oestrogens, which are steroid hormones produced by mammalians, should be highlighted, since they are involved in very important processes, being responsible for the development of female secondary sex characters, among other functions. Besides, there is also a wide variety of other natural as well as synthetic EDCs that can interact with the ERs by mimicking the activity of oestrogens when such compounds reach the organism. In this sense, some of them are used as growth promoters of livestock and in veterinary treatments, which constitutes the main source of their presence in the environment and in food of animal origin (i.e. meat, milk and dairy products). Furthermore, the so-called mycoestrogens and phytoestrogens also constitute an important source of contamination especially for humans. With respect to PAEs, which also have endocrine disrupting activity, their use as plasticisers in industrial applications stands out from any other. While it is true that plastics are polymers of high molecular weight which do not normally constitute a risk for humans, these additives are not chemically bonded to them and show a great tendency to migrate to the surrounding environment. This fact, together with the notably increase of plastics production during the last decades, which has brought with it a great plastic waste generation and accumulation both in terrestrial and aquatic environments as a result of their improper management, have widely distributed them up to the point that they have been found in environmental waters, food, soils and even in the atmosphere, constituting a really serious risk for humans.

The uncountable sources of contamination to which humans are exposed and the low concentrations at which these EDCs can produce such serious effects on health, make necessary the development of new effective and sensitive analytical methodologies to allow their determination at the low levels at which they appear in environmental and food matrices. In this sense, the use of new sorbents and miniaturised techniques which allow

a high extraction efficiency, selectivity and sensitivity, with low consumption of reagents and organic solvents, have become important alternatives to address their analysis.

Thus, and considering all the above-mentioned, the main objective of this PhD Thesis is the development of novel analytical methodologies based on the use of new materials as highly selective extraction sorbents for the determination of different groups of compounds with oestrogenic activity, including natural and synthetic oestrogens, mycoestrogens and PAEs, in samples of environmental and agri-food interest, using new extraction techniques in combination with chromatographic systems coupled to MS detectors. To achieve this goal, the following specific objectives have been established:

- The use of LC and GC coupled to MS/MS detectors for the suitable separation and quantification of different groups of EDCs, since these techniques are the most appropriated for the determination of this type of compounds in real samples.
- The application of different sorbent-based methods, including conventional SPE, dSPE and m-dSPE, also in their miniaturised form, for the extraction and preconcentration of EDCs in milk, dairy products, beverages and water samples.
- The evaluation of new materials such as MIPs, polymeric coated m-NPs, pristine MWCNTs or MOFs as selective and efficient sorbents.
- The application of HF-LPME for the selective extraction and preconcentration of PAEs from water samples.
- The validation of the developed methodologies in terms of calibration, repeatability, precision, trueness, matrix effects, LODs and limits of quantification (LOQs) of the method in order to demonstrate their effectiveness and reliability to determine the selected EDCs at the low levels at which they can appear in samples.
- The application of such methodologies to the analysis of EDCs in different water samples including Milli-Q, mineral, tap, pond and waste water, different milk and dairy products samples including skimmed, semi-skimmed and whole cow milk and semi-skimmed goat milk as well as unsweetened natural yogurt, and different plastic bottled beverages including flavoured mineral waters and an isotonic drink, with the aim of demonstrating their applicability as well as quantifying the content of such EDCs in samples of different nature.

CHAPTER III
EXPERIMENTAL

III.- EXPERIMENTAL

III.1.- Analytical standards, solvents, reagents and solutions

- Analytical standards of the oestrogenic compounds 17α-E_2 (CAS 57-91-0), 17β-E_2 (CAS 50-28-2), DES (CAS 56-53-1), DS (CAS 84-17-3), E_1 (CAS 53-16-7), EE_2 (CAS 57-63-6), HEX (CAS 84-16-2), ZAN (CAS 5975-78-0), ZEN (CAS 17924-92-4), α-ZAL (CAS 26538-44-3), α-ZEL (CAS 36455-72-8), β-ZAL (CAS 42422-68-4) and β-ZEL (CAS 71030-11-0) were purchased from Sigma-Aldrich Chemie and used without further purification (purity ≥ 95 %). 17β-estradiol-2,4,16,16,17-d_5 (17β-E_2-d_5, CAS 221093-45-4) from Sigma-Aldrich Chemie was used as internal standard (IS) without further purification (purity ≥ 95 %). Table III.1 shows the structures and some properties of the oestrogenic compounds studied in the present PhD Thesis. Stock solutions of each analyte of 100 mg/L were precisely prepared in MeOH and stored in the darkness at -18 °C, except for natural oestrogens, for which concentration was 1000 mg/L. Working analyte mixtures were daily prepared by dilution with the appropriate volume of mobile phase.

- Analytical standards of the PAEs DBP (CAS 84-74-2), dicyclohexyl phthalate (DCHP, CAS 84-61-7), DEHP (CAS 117-81-7), DIDP (CAS 89-16-7), DINP (CAS 20548-62-3), di(2-methoxyethyl) phthalate (DMEP, CAS 117-82-8), DNOP (CAS 117-84-0) and dipropyl phthalate (DPP, CAS 131-16-8) were purchased from Sigma-Aldrich Chemie and used without further purification (purity ≥ 96 %). Analytical standards of the PAEs BBP (CAS 85-68-7), di(2-butoxyethyl) phthalate (DBEP, CAS 117-83-9), di(2-ethoxyethyl) phthalate (DEEP, CAS 605-54-9), DEP (CAS 84-66-2), DIBP (CAS 84-69-5), diisopentyl phthalate (DIPP, CAS 605-50-5) and DNPP (CAS 131-18-0) and the adipate DEHA (CAS 103-23-1) were purchased from Dr. Ehrenstorfer and used without further purification (purity ≥ 98.4 %). Dibutyl phthalate-3,4,5,6-d_4 (DBP-d_4, CAS 93952-11-5) from Sigma Aldrich Chemie and dihexyl phthalate-3,4,5,6-d_4 (DHP-d_4, CAS 1015854-55-3) from Dr. Ehrenstorfer were used as ISs without further purification (purity ≥ 97 %). Table III.2 shows the structures and some properties of the PAEs as well as DEHA studied in the present PhD Thesis. Stock solutions of each analyte at 1000 mg/L were precisely prepared in ACN (when LC analysis was carried out) or cyclohexane (when GC analysis was developed) and stored in the darkness at -18 °C. Working analyte mixtures were daily prepared by dilution with the appropriate volume of mobile phase for LC analysis or cyclohexane for GC analysis.

Table III.1.- Chemical structure and properties of the studied oestrogenic compounds.

Analyte	Structure	Molecular formula	MM (g/mol)	Solubility in water (g/L, 25 °C)	Vapor pressure (mmHg)	Log K_{OW}	Melting point (°C)	pK_a
17α-E_2		$C_{18}H_{24}O_2$	272.4	$3 \cdot 10^{-3}$ (pH 1-9)	$9.82 \cdot 10^{-9}$	4.146 ± 0.256	215-220	10.27 ± 0.60
17β-E_2		$C_{18}H_{24}O_2$	272.4	$3 \cdot 10^{-3}$ (pH 1-9)	$9.82 \cdot 10^{-9}$	4.146 ± 0.256	174-177	10.27 ± 0.60
E_1		$C_{18}H_{22}O_2$	270.4	$5.7 \cdot 10^{-3}$ (pH 1-8)	$1.54 \cdot 10^{-8}$	3.624 ± 0.369	256-258	10.25 ± 0.40
EE_2		$C_{20}H_{24}O_2$	296.4	$3.9 \cdot 10^{-3}$ (pH 1-9)	$3.74 \cdot 10^{-9}$	4.106 ± 0.315	144-146	10.24 ± 0.60
DES		$C_{18}H_{20}O_2$	268.4	$6.7 \cdot 10^{-3}$ (pH 1-8)	$3.29 \cdot 10^{-7}$	5.330 ± 0.300	169-172	10.18 ± 0.26
DS		$C_{18}H_{18}O_2$	266.3	$5.6 \cdot 10^{-3}$ (pH 1-7)	$8.07 \cdot 10^{-7}$	4.920 ± 0.309	227-228	9.21 ± 0.15

Table III.1.- (Continued).

Analyte	Structure	Molecular formula	MM (g/mol)	Solubility in water (g/L, 25 °C)	Vapor pressure (mmHg)	Log K_{ow}	Melting point (°C)	pK_a
HEX		$C_{18}H_{22}O_2$	270.4	$2.0 \cdot 10^{-2}$ (pH 1-8)	$5.92 \cdot 10^{-7}$	5.141 ± 0.223	184-189	9.80 ± 0.26
ZAN		$C_{18}H_{22}O_5$	320.4	-	$6.65 \cdot 10^{-14}$	4.28	191-193	7.83 ± 0.40
ZEN		$C_{18}H_{22}O_5$	318.4	$9.6 \cdot 10^{-1}$ (pH 1-5)	$5.21 \cdot 10^{-15}$	2.765 ± 1.193	162-165	7.58 ± 0.40
α-ZAL		$C_{18}H_{26}O_5$	322.4	$5.5 \cdot 10^{-1}$ (pH 1-6)	$4.16 \cdot 10^{-14}$	4.648 ± 0.508	180-182	8.08 ± 0.60
α-ZEL		$C_{18}H_{24}O_5$	320.4	1.9 (pH 1-5)	$3.40 \cdot 10^{-15}$	3.184 ± 1.175	169-170	7.61 ± 0.60
β-ZAL		$C_{18}H_{26}O_5$	322.4	$5.5 \cdot 10^{-1}$ (pH 1-6)	$4.16 \cdot 10^{-14}$	4.648 ± 0.508	152-158	8.08 ± 0.60
β-ZEL		$C_{18}H_{24}O_5$	320.4	1.9 (pH 1-5)	$3.40 \cdot 10^{-15}$	3.184 ± 1.175	173-174	7.61 ± 0.60

Data taken from SciFinder® database [346]. MM: Molecular mass.

Table III.2.- Chemical structure and properties of the studied PAEs and DEHA.

Analyte	Structure	Molecular formula	MM (g/mol)	Solubility in water (g/L, 25 °C)	Vapor pressure (mmHg, 25 °C)	Log Kow	Melting point (°C)	Boiling point (°C)
BBP		$C_{19}H_{20}O_4$	312.1	2.69	$8.25 \cdot 10^{-6}$	4.73	-35	370
DBEP		$C_{20}H_{30}O_6$	366.2	1.67	$2.17 \cdot 10^{-3}$	4.06	-55	270
DBP		$C_{16}H_{22}O_4$	278.2	0.0112	$2.01 \cdot 10^{-5}$	4.72	-35	340
DCHP		$C_{20}H_{26}O_4$	330.2	4.0[a]	$8.69 \cdot 10^{-7}$	6.20	66	225
DEEP		$C_{16}H_{22}O_6$	310.1	-	$6.34 \cdot 10^{-5}$[b]	2.50[b]	34	345
DEHA		$C_{22}H_{42}O_4$	370.3	0.00078[c]	$8.50 \cdot 10^{-7}$[d]	8.10[b]	-68	214
DEHP		$C_{24}H_{38}O_4$	390.3	0.00027	$1.42 \cdot 10^{-7}$	7.60	-55	230
DEP		$C_{12}H_{14}O_4$	222.1	1.080	$2.10 \cdot 10^{-3}$	2.47	-41	298

Table III.2.- (Continued).

Analyte	Structure	Molecular formula	MM (g/mol)	Solubility in water (g/L, 25 °C)	Vapor pressure (mmHg, 25 °C)	Log Kow	Melting point (°C)	Boiling point (°C)
DIBP		$C_{16}H_{22}O_4$	278.2	0.00062[a]	$4.76 \cdot 10^{-5}$	4.11	-64	297
DIDP		$C_{28}H_{46}O_4$	446.3	0.00028	$5.28 \cdot 10^{-7}$	10.36	-50	423
DINP		$C_{26}H_{42}O_4$	419.3	0.0002[d]	$5.40 \cdot 10^{-7}$	9.37	-48	406
DIPP		$C_{18}H_{26}O_4$	306.2	0.20	$3.54 \cdot 10^{-4}$[c]	5.50[c]	-	336
DMEP		$C_{14}H_{18}O_6$	282.1	8.50[e]	$2.28 \cdot 10^{-4}$	1.11	-45	340
DNOP		$C_{24}H_{38}O_4$	390.3	0.000022	$1.00 \cdot 10^{-7}$	8.10	-25	220
DNPP		$C_{18}H_{26}O_4$	306.2	0.0008	$2.8 \cdot 10^{-5}$[f]	5.62	-55	342
DPP		$C_{14}H_{18}O_4$	250.1	37.9	$3.89 \cdot 10^{-4}$[b]	10.47	-	318

[a] 24 °C. [b] Calculated. [c] 22 °C. [d] 20 °C. [e] Temperature not specified. [f] Predicted value. Data taken from SciFinder® [346] and PubChem [347] databases. MM: Molecular mass.

- ACN, MeOH and acetone of HPLC grade as well as ACN and MeOH of HPLC-MS grade were from VWR International and from Merck, respectively. Cyclohexane of GC-MS grade was from Merck. 1-octanol was from Sigma-Aldrich Chemie. DCM was purchased from Scharlau Chemie.
- AFFINIMIP®SPE Estrogens and AFFINIMIP®SPE Zearalenone cartridges containing 100 mg of the sorbent were from Polyintell.
- Basolite® F300 (iron (III) 1,3,5-benzenetricarboxylate) MOF with a purity ≥ 90 %, a surface area of 1300-1600 m^2/g and a bulk density of 0.16-0.35 g/cm^3 was from Sigma-Aldrich Chemie.
- Buffer solutions of pH 4.00 and pH 7.00 for pH-meter adjustment were purchased from VWR International.
- Crisolyt KCl 3 M solution for the correct maintenance of the pH-meter membrane was provided by Crison.
- Electrospray calibrant solution for IT-MS calibration, containing betaine, trifluoroacetic acid ammonium salt, hexamethoxyphosphazine, hexakis (2,2-difluoroethoxy) phosphazine, hexakis (1H, 1H, 3H-tetrafluoropropoxy) phosphazine, hexakis (1H, 1H, 5H-octafluoropentoxy) phosphazine, hexakis (1H, 1H, 7H-dodecafluoroheptoxy) phosphazine, hexakis (1H, 1H, 9H-perfluorononyloxy) phosphazine, hexakis (1H, 1H, 4H-hexafluorobutyloxy) phosphazine, hexakis (1H, 1H, 6H-decafluorohexyloxy) phosphazine, hexakis (1H, 1H, 8H-tetradecafluorooctyloxy) phosphazine, tris (trifluoromethyl)-1,3,5-triazine and tris (heptafluoropropyl)-1,3,5-triazine in ACN/deionised water 95/5 (v/v), was from Fluka.
- Glycerin was from Sigma-Aldrich Chemie.
- Hydrochloric acid (HCl) 25 % (w/w) was purchased from Merck while sulphuric acid (H_2SO_4) 96 % (w/w) technical grade, formic acid 98 % (w/w) HPLC grade, and AcOH 98 % (w/w) were provided by Panreac Química and Sigma-Aldrich Chemie.
- KCl solution of 147 µS/cm, 1413 µS/cm and 12.88 mS/cm for the conductivity meter adjustment were obtained from Crison.
- Milli-Q water (conductivity of 4.06 µS/cm at 25 °C, organic matter content of 2 µg/L) was obtained from a Milli-Q gradient system A10 from Millipore.

- Nochromix® solution in H_2SO_4 for the cleaning of glassware was purchased from Sigma-Aldrich Chemie.
- Pristine MWCNTs with an average diameter of 110-170 nm and 5-9 μm length were acquired from Sigma-Aldrich Chemie.
- Sodium hydroxide (NaOH) was acquired from Panreac Química. Iron (II) sulphate hydrate ($FeSO_4 \cdot nH_2O$) and dopamine (DA) hydrochloride were purchased from Sigma-Aldrich Chemie with a purity ≥ 86 %. Iron (III) chloride hexahydrate ($FeCl_3 \cdot 6H_2O$) was from Scharlau and sodium dihydrogen phosphate dihydrate ($NaH_2PO_4 \cdot 2H_2O$) and disodium hydrogen phosphate dihydrate ($Na_2HPO_4 \cdot 2H_2O$) were acquired from Merck with a purity ≥ 98 %.

III.2.- Laboratory ware

- Accurel Q3/2 PP HF membrane (600 μm i.d., 200 μm wall thickness and 0.2 μm pore size) was purchased from Membrana GmbH.
- Beakers of 25, 50, 100, 250, 500 and 1000 mL were from Schott Duran.
- BR-5 ms fused silica capillary column 5 % diphenyl/95 % poly(dimethyl arylenesiloxane) (30 m length x 0.25 mm o.d., 0.25 μm film thickness) was from Bruker Daltonik GmbH.
- Corning® Costar® Spin-X® cellulose acetate or nylon membrane PP centrifuge tube filters with a pore size of 0.22 μm were from Sigma-Aldrich Chemie.
- Glass amber vials of 2 mL of capacity with screw caps and PTFE septa were from Supelco.
- Glass amber vials of 22 and 40 mL of capacity with solid caps and PTFE liners were from Supelco.
- Glass bottles of 500 and 1000 mL with PP screw caps were from VWR International.
- Glass crystallizers of 500 mL of capacity were from Duran.
- Glass empty SPE columns of 6 mL of capacity, 7.5 cm length, 1.5 cm o.d. and 1.2 cm i.d. were from Supelco.
- Glass vials of 22 mL of capacity were from Supelco.

- Glass vials of 700 µL of capacity (40 mm length and 8 mm i.d.) with PE caps for HPLC were from Waters Chromatography.
- Graduated cylinders of 25, 100, 250 and 500 mL were from Proton.
- Grinding Erlenmeyer flasks of 50 and 100 mL were from Witeg.
- Syringes of 25 and 100 µL of capacity of borosilicate glass and stainless-steel plunger and syringes of 10 mL capacity of borosilicate glass and a PTFE plunger were from Hamilton.
- HP–5 ms fused silica capillary columns (15 m length × 0.25 mm o.d., 0.25 µm film thickness, 5 % phenyl/95 % poly(methylsiloxane)) were from Agilent Technologies.
- Insert glass conical vials of 0.25 mL of capacity (6 mm × 31 mm × 4.6 mm) were from Supelco.
- Norm-Ject® syringes of 12 and 50 mL of PP and PE plungers were from Henke Sass Wolf.
- Pasteur pipettes were acquired from VWR International.
- Permanent disc magnet composed of Nd-Fe-B of 30 mm × 7 mm with a weight of 38 g and strength of 14 kg coated with Ni-Cu-Ni was acquired from Super-Magnete.
- PET syringe filters with a pore size of 0.45 µm and 25 mm diameter (Chromafil® Xtra PET-45/25) and with a pore size of 0.20 µm and 15 mm diameter (Chromafil® Xtra PET-20/15) for polar and non-polar media were from Macherey-Nagel.
- Polyvinylidene fluoride filter membranes Durapore® with a pore size of 0.22 µm and a diameter of 47 mm were from Millipore.
- PP centrifuge tubes of 15 and 50 mL were from VWR International.
- PP microtubes of 1.5 mL were from Sarstedt.
- PTFE coated stirring bars of different sizes were from VWR International.
- PTFE frits for SPE glass tubes with 20 µm of pore size, diameter of 11.5 mm and thickness of 3 mm were from Supelco.
- SPB-5 fused silica capillary column (30 m length x 0.25 mm o.d., 0.25 µm film thickness, 5 % diphenyl/95 % poly(dimethylsiloxane)) from Supelco.

- Volumetric flasks of 5, 10, 25, 50, 100, 250, 500 and 1000 mL (class A type) were from Afora.
- X-Bridge C_{18} column (100 mm length × 4.6 mm o.d., 3.5 µm particle size) and X-Bridge C_{18} pre-columns (20 mm length × 4.6 mm o.d., 3.5 µm particle size) were from Waters Chromatography.
- *Glassware cleaning:*

  Non-volumetric glassware was washed with tap water and soap, followed by Milli-Q water and dried in an oven at 120 °C for 1 hour. After that, it was calcined at 550 °C for 4-5 hours in a high temperature furnace.

  Volumetric glassware was washed with tap water and soap and let stand with Nochromix® H_2SO_4 solution for 24 hours. Then, the solution was removed, and the material was rinsed ten times with Milli-Q water and three times more with MeOH of high purity. It was dried at room temperature.

III.3.- Equipment

III.3.1.- Instruments

- AW-224 analytical balance with a maximum weighing capacity of 220 g and 0.1 mg of resolution was from Sartorius.
- Manual adjustable Transferpette® S micropipettes with disposable plastic tips with different volume ranges (2-20 µL, 10-100 µL, 20-200 µL, 100-1000 µL and 500-5000 µL) were from Brand.
- GLP 22 pH-meter equipped with a temperature sensor was from Crison.
- CM 35 conductivity meter equipped with cell temperature control was from Crison.
- *HPLC separations:*

  HPLC-IT-MS/MS analyses were performed in a HPLC system equipped with a binary pump (model 1525), an autosampler (model 717 plus) and a column oven using a X-Bridge C_{18} column and a X-Bridge C_{18} pre-column from Waters Chromatography. The HPLC system was hyphenated with an AmaZon SL IT-MS with an ESI source from Bruker Daltonik GmbH.

- *GC separations:*

  GC-FID analyses were carried out in a 3800 GC system equipped with an 8200 CombiPAL autosampler and a FID detector from Varian, using a SPB-5 fused silica capillary column from Supelco.

  GC-QqQ-MS/MS analyses were performed in two different instruments:

  - A 7890B GC from Agilent Technologies equipped with a backflush valve and a CombiPAL autosampler, coupled to a 7000C QqQ MS detector through an EI source from Agilent Technologies, using two identical HP-5 ms fused silica capillary columns from Agilent Technologies.
  - A Scion 436GC equipped with an 8400 autosampler and coupled to a QqQ MS detector through an EI source from Bruker Daltonik GmbH, using a BR-5 ms fused silica capillary column from Bruker Daltonik GmbH.

III.3.2.- Apparatus

- 5415 D centrifuge with time and speed control with a maximum rotation speed of 13200 rpm (16100 rcf) was from Eppendorf.
- 5702 centrifuge with time and speed control with a maximum rotation speed of 4400 rpm (3000 x g) was from Eppendorf.
- Carbolite CWF 11/13 furnace of 13 L of chamber capacity and a maximum temperature of 1100 °C was from Carbolite.
- WTB 7200 heater of 100 L of chamber capacity was from Binder.
- Lab Dancer vortex with a fixed speed 2800 rpm was from VWR International.
- RCT Basic magnetic stirrer with temperature control (0-310 °C) and speed (0-1500 rpm) was from IKA.
- Milli-Q gradient A10 system was from Millipore.
- Rotavapor R-200 equipped with a V-800 vacuum controller and a V-500 vacuum pump was purchased from Büchi Labortechnik.
- Rotavapor RV-10 basic equipped with a thermostatic bath HB-10 from IKA and a CVC 3000 vacuum pump with a vacuum controller was from VWR International.

- 3510E-MT ultrasonic bath (5.7 L capacity) of 40 kHz with time control was from Branson.
- Ultrasons-512 ultrasonic bath (1 L capacity) of 50/60 kHz was from Selecta.
- Visiprep™ DL-SPE vacuum system with a capacity for 12 samples was from Supelco.

III.3.3.- Software

- Bruker MSWS 8 software from Bruker Daltonik GmbH was used for performing control, data acquisition and chromatograms processing of the GC-QqQ-MS/MS system.
- Empower 2 v.6.0 programme from Waters Chromatography was used for performing control of the HPLC system.
- Esquire NT software from Bruker Daltonik GmbH was used for performing control, data acquisition and chromatograms processing of the IT-MS system.
- GCQQQ/Enhanced MassHunter software from Agilent Technologies was used for GC-QqQ-MS/MS system control.
- MassHunter Qualitative Analysis and MassHunter Quantitative Analysis software from Agilent Technologies were used for data acquisition and chromatograms processing of the Agilent GC-QqQ-MS/MS system.
- Microsoft® Office Excel 2007, 2010, 2016 and 365 were used for data processing including calibration curves preparation, recovery data calculations, etc.
- Microsoft® Office Power Point 2007, 2010, 2016 and 365 were used for figures preparation, including chromatograms presentation.

III.4.- Samples

In this PhD Thesis different environmental and food samples were analysed:

- *Section IV.1:*

  Mineral water (pH 6.50 and conductivity of 41 µS/cm at 25 °C) was acquired in a local supermarket of Tenerife. Waste water (pH 8.00 and conductivity of 1420 µS/cm at 25 °C) was collected in a waste water treatment plant of the island of Tenerife. Both

samples were filtered through a 0.20 µm Chromafil® PET-20/25 filter before extraction to remove any solid particle.

- *Section IV.2:*

  Whole and skimmed cow milk, semi-skimmed goat milk and unsweetened natural yogurt, with fat contents in the range 0.3-3.6 g, proteins between 3.0 and 3.4 g and carbohydrates in the range 4.0-4.7 g per 100 mL or 100 g of sample. These values were indicated on the commercial packaging of each product. pH values at 25 °C were between 4.10 and 6.75. All samples were purchased in a local supermarket of Tenerife.

- *Section IV.3 and IV.4:*

  Mineral water (pH 6.50 and conductivity of 21.5 µS/cm at 25 °C) was bought in a local supermarket of Tenerife. Tap water (pH 8.55 and conductivity of 1257 µS/cm at 25 °C) was collected at the laboratory. Pond water (pH 8.53 and conductivity of 300 µS/cm at 25 °C) was collected in a concrete pond used for the irrigation of crops in La Orotava (Tenerife). Waste water (pH 7.47 and conductivity of 1176 µS/cm at 25 °C) was collected in a waste water treatment plant of Tenerife.

- *Section IV.5:*

  Mineral water (pH 6.50 and conductivity of 21.5 µS/cm at 25 °C), lemon-flavoured mineral water (pH 2.65 and conductivity of 1395 µS/cm at 25 °C), apple-flavoured mineral water (pH 2.87 and conductivity of 1237 µS/cm at 25 °C) and an isotonic drink (pH 2.73 and conductivity of 2250 µS/cm at 25 °C) were acquired in a local supermarket of Tenerife. Tap water (pH 8.55 and conductivity of 1300 µS/cm at 25 °C) was collected at the laboratory.

- *Section IV.6:*

  Tap water samples were collected at the laboratory (pH 8.32 and conductivity of 1070 µS/cm at 25 °C), Santa Cruz de Tenerife (pH 8.33 and conductivity of 500 µS/cm at 25 °C) and San Isidro (pH 7.22 and conductivity of 823 µS/cm at 25 °C). Pond water samples were collected from a concrete pond of La Orotava (pH 8.28 and conductivity of 640 µS/cm at 25 °C), a metallic pond of La Guancha (pH 8.38 and conductivity of 1101 µS/cm at 25 °C) and a plastic pond also of La Guancha (pH 10.38 and conductivity of 594 µS/cm at 25 °C), all of them used for the irrigation of crops. Waste water (pH 8.40 and conductivity of 1337 µS/cm at 25 °C) was collected in a treatment plant of Tenerife.

III.5.- Liquid chromatography analysis

III.5.1.- LC-MS/MS analysis (Sections IV.1 and IV.2)

HPLC-MS/MS chromatographic separation was performed at 30 °C following the gradient shown in Table III.3 and using ACN as solvent A and Milli-Q water as solvent B. The flow rate was established at 0.4 mL/min and the injection volume was 20 µL. For analytes MS detection, it was established a capillary voltage of +5500 V (negative mode), an end plate offset voltage of -600 V, a nebulisation gas pressure of 20 psi and a dry gas flow and temperature of 8 L/min and 300 °C, respectively. Regarding the IT parameters, the ion charge control (ICC) was set at 60000, the maximum accumulation time at 200 ms with 10 average scans per experiment and a rolling averaging of 5.

Table III.3.- Gradient programme used for the LC-MS/MS separation of Sections IV.1 and IV.2.

Time (min)	% solvent A	% solvent B	Curve
0	50	50	-
2	90	10	6
6	90	10	6
7	50	50	6

MS/MS experiments were performed by the fragmentation of the deprotonated molecule $[M–H]^-$ which was selected as the precursor ion. In these last experiments, the set mass range was 70–350 m/z, the m/z width was set at 1 and the fragmentation amplitude was varied in the range 0.60–1.2 V.

III.5.2.- GC-MS/MS analysis (Section IV.3)

GC-MS/MS separation was carried out in an Agilent 7890B GC/7000C QqQ MS system with two columns connected by a backflush valve, using helium as carrier gas at flows of 1.5 mL/min for the first column and 1.7 mL/min for the second one, applying the temperature program shown in Table III.4. Two microliters were injected in the splitless mode at 280 °C (after 1.5 min the split was opened at a ratio of 1:50). The MS transfer line and ion source were also set at 280 °C with an electron ionisation energy of −70 eV. The QqQ analyser was operated in the MRM mode, the temperature of the first and second quadrupoles was 180 °C and the collision cell gases were nitrogen (1.5 mL/min) and helium (2.25 mL/min, quenching gas). For each analyte, two different transitions were taken for the quantification and confirmation.

Table III.4.- Temperature programme used for the GC-MS/MS separation of Section IV.3.

Temperature (°C)	Rate (°C/min)	Hold time (min)	Run time (min)
70	-	2	2.00
200	25	-	7.20
260	3	-	27.20
300	30	4	32.53

III.5.3.- GC-FID analysis (Section IV.4)

GC-FID separation was performed in a Varian 3800 GC system using nitrogen as carrier gas at a flow of 1.2 mL/min and applying the temperature program shown in Table III.5. Two microliters were injected in the split/splitless mode (after 1.5 min the split was opened at a ratio of 1:50) at 280 °C. Regarding FID conditions, hydrogen, air and nitrogen (make-up gas) flows were set at 30, 300 and 30 mL/min respectively. Detector temperature was maintained at 300 °C.

Table III.5.- Temperature programme used for the GC-FID separation of Section IV.4.

Temperature (°C)	Rate (°C/min)	Hold time (min)	Run time (min)
70	-	-	0.00
200	30	-	4.33
260	3	-	24.33
300	30	10	35.66

III.5.4.- GC-MS/MS analysis (Section IV.4)

GC-MS/MS separation was carried out in a Scion 436 GC/MS system using helium as carrier gas at a flow of 1.5 mL/min and applying the temperature program shown in Table III.5. The injection volume was 2 µL in the splitless mode at 280 °C (after 1.5 min the split was opened at a ratio of 1:50). The MS transfer line and ion source were also set at 280 °C, with an electron ionisation energy of -70 eV. The QqQ was operated in the MRM mode using argon as collision gas at 1.5 mL/min. For each analyte, two different transitions were taken for the quantification and confirmation.

III.5.5.- LC-MS/MS analysis (Sections IV.5 and IV.6)

HPLC-MS/MS chromatographic separation was performed at 40 °C following the gradient shown in Table III.6 and using ACN containing 0.1 % (v/v) of formic acid as solvent A and Milli-Q water containing 0.1 % (v/v) of formic acid as solvent B. The flow rate

was established at 0.7 mL/min and the injection volume was 20 µL. For analytes detection, it was established a capillary voltage of -4500 V (positive mode), an end plate offset voltage of -500 V, a nebulisation gas pressure of 30 psi and a dry gas flow and temperature of 8 L/min and 350 °C, respectively. Regarding the IT parameters, the ICC was set at 60000, the maximum accumulation time at 200 ms with 10 average scans per experiment and a rolling averaging of 5.

Table III.6.- Gradient programme used for the LC-MS/MS separation of Sections IV.5 and IV.6.

Time (min)	% solvent A	% solvent B	Curve
0	50	50	-
15	95	5	5
20	95	5	6
21	50	50	6

MS/MS experiments were carried out by fragmentation of the protonated molecule $[M-H]^+$, which was selected as the precursor ion. For this purpose, the mass range was set between 90 and 550 m/z, the m/z width was set at 1 and the fragmentation amplitude was varied in the range 0.1-0.6 V.

III.6.-Synthesis of core-shell Fe_3O_4@pDA m-NPs

The synthesis was carried out in two steps as shown in Figure III.1. Firstly, 5.41 g of $FeCl_3 \cdot 6H_2O$ and 2.78 g of $FeSO_4 \cdot nH_2O$ (2:1 molar ratio) were dissolved in 200 mL of HCl 0.5 M solution by magnetic stirring. This solution was then added drop by drop using a burette (50 mL) to 300 mL of NaOH 1.25 M solution under vigorous stirring (850 rpm) at room temperature. After finishing the addition, the black precipitate (Fe_3O_4 m-NPs) was stirred for 30 min more. Then, this mixture (pH 13.3) was neutralised with HCl (25 %, w/w). Secondly, and to generate the polydopamine (pDA) coating, a concentration of 2.75 g/L of the prepared NPs was dispersed in a 15 mM DA solution of phosphate buffered saline (PBS) at pH 8.3 and maintained the polymerisation process for 6 h under stirring (850 rpm) at room temperature. After that, pDA coated NPs were washed with a mixture of ACN/H_2O 50/50 (v/v) 6 times to remove the non-reactive DA, and once more with ACN. Afterwards, they were centrifuged at 4400 rpm for 15 min and dried at 40 °C and 180 mbar in a rotary evaporator. Bare Fe_3O_4 m-NPs were used even after 4 weeks of their synthesis. Regarding pDA coated m-NPs, they were used during one week after their synthesis without observing a decrease in the extraction efficiency.

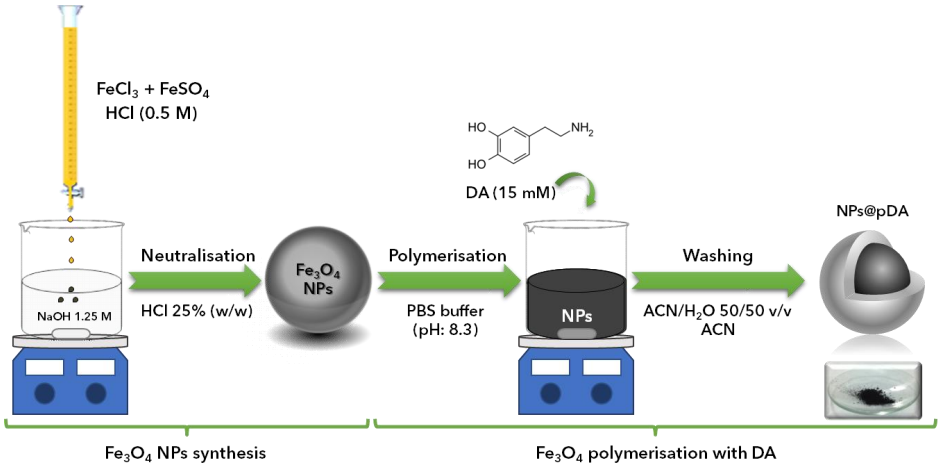


Figure III.1.- Scheme of Fe_3O_4@pDA synthesis.

III.7.- Sample pre-treatment procedures

III.7.1.- Extraction of oestrogens from water samples by MIP-SPE (Section IV.1)

III.7.1.1.- AFFINIMIP®SPE Estrogens cartridge

Before its use, the sorbent was conditioned with 3 mL of ACN and 3 mL of Milli-Q water. Then, 25 mL of Milli-Q water or 100 mL of mineral or waste water at pH 7.0, previously adjusted with 0.1 M NaOH or 0.1 M HCl solutions in each case, were percolated through the MIP-SPE cartridge at a flow rate of 2 mL/min. The sorbent was washed with 3 mL of Milli-Q water and 3 mL of ACN/Milli-Q water 40/60 (v/v). Full vacuum was applied for 20 min to ensure that the polymer was completely dry. Then, elution took place with 5 mL of MeOH, which was later evaporated to dryness at 40 °C and 220 mbar. The dry extract was reconstituted in 250 µL of ACN/Milli-Q water 50/50 (v/v) (initial mobile phase composition), filtered through a 0.20 µm PET syringe filter and injected (20 µL) in the HPLC-MS/MS system (see Figure III.2).

III.7.1.2.- AFFINIMIP®SPE Zearalenone cartridge

Before its use, the sorbent was conditioned with 3 mL of ACN and 3 mL of Milli-Q water. Then, 25 mL of Milli-Q water or 100 mL of mineral or waste water were percolated through the MIP-SPE cartridge at a flow rate of 2 mL/min. The sorbent was washed

with 3 mL of Milli-Q water/ACN/AcOH 58/40/2 (v/v/v). Full vacuum was applied for 20 min to ensure that the polymer was completely dry. Then, elution took place with 5 mL of MeOH/ AcOH 98/2 (v/v), which was later evaporated to dryness at 40 °C and 220 mbar. The dry extract was reconstituted in 250 µL of ACN/Milli-Q water 50/50 (v/v) (initial mobile phase composition), filtered through a 0.20 µm PET syringe filter and injected (20 µL) in the HPLC-MS/MS system (see Figure III.2).

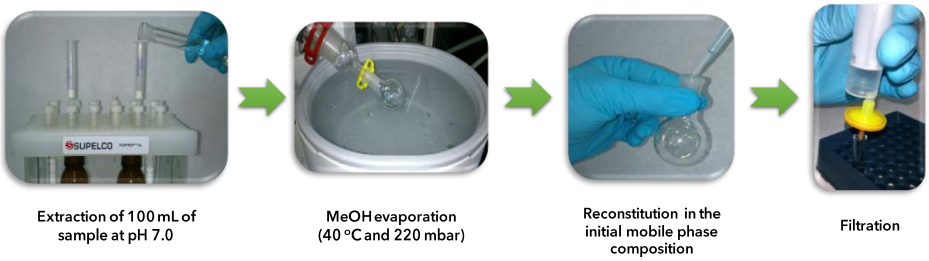


Figure III.2.- Scheme of the SPE of oestrogenic compounds from water samples (Section IV.1).

III.7.2.- Extraction of mycotoxins from milk and yogurt by m-µ-dSPE (Section IV.2)

III.7.2.1.- Milk and yogurt samples deproteinization

Regarding milk samples deproteinization, 1.5 mL of milk were poured into a 50 mL centrifuge tube. Then, 3 mL of ACN and 75 µL of AcOH were added and the samples were vortex-shaken for 1 min. After that, the samples were maintained in the darkness for 15 min and centrifuged at 4400 rpm for 15 min more. The supernatant was evaporated at 40 °C and 180 mbar in a rotary evaporator. The residue (1.5 mL) was re-dissolved in 23.5 mL of Milli-Q water to obtain a total volume of 25 mL and the pH was adjusted to a value of 7.0 with a NaOH 0.1 M solution. Finally, the samples were filtered through a 0.45 µm PET syringe filter, directly onto 80 mg of Fe_3O_4@pDA m-NPs in a 50 mL centrifuge tube.

Concerning yogurt samples, 1.5 g of yogurt were introduced into a 50 mL centrifuge tube. Then, 4.5 mL of ACN and 75 µL of AcOH were added and the mixture was vortex-shaken for 1 min. After that, the sample was maintained in the darkness for 15 min and centrifuged at 4400 rpm for 15 min more. The supernatant was evaporated at 40 °C and 180 mbar. The residue (1.5 mL) was re-dissolved in 23.5 mL of Milli-Q water to obtain a total volume of approximately 25 mL and the pH was adjusted to a value of 7.0 with NaOH 0.1 M. Finally, the samples were filtered through a 0.45 µm PET syringe filter, directly onto 80 mg of Fe_3O_4@pDA m-NPs in a 50 mL centrifuge tube (see Figure III.3).

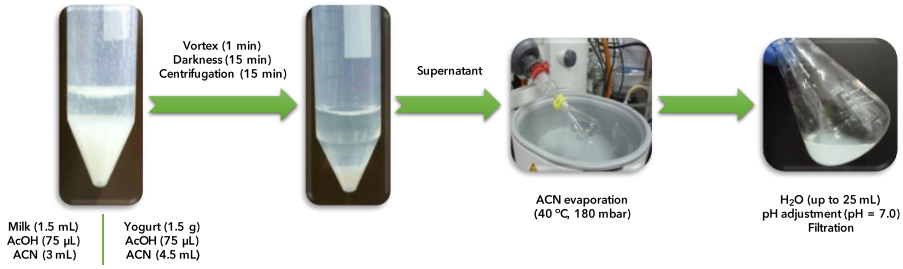


Figure III.3.- Scheme of milk and yogurt samples deproteinization (Section IV.2).

III.7.2.2.- Milk and yogurt samples extraction

After the deproteinization step described in the previous section, the same extraction procedure was applied to both milk and yogurt samples. Briefly, the mixture of the filtered extract and Fe_3O_4@pDA m-NPs was manually shaken for 30 s and then, a permanent magnet was placed for 10 min at the bottom of the tube in order to let the sorbent settle. Subsequently, the liquid sample extract was discarded by decantation, retaining the sorbent in the extraction recipient using the magnet which was always maintained closed to the tube. After drying the Fe_3O_4@pDA m-NPs with a soft current of nitrogen, the analytes were desorbed by the addition of 8 mL of MeOH and a slight manual agitation for 30 s followed by a magnetic deposition period of 5 min, using the permanent magnet again. Finally, the desorption solvent containing the analytes was separated (maintaining again the magnet close to the tube) and evaporated to dryness at 40 °C and 220 mbar. The residue was reconstituted in 500 µL of the initial composition of the mobile phase (ACN/H_2O 50/50, v/v), filtered through a 0.22 µm cellulose acetate membrane PP centrifuge tube filter and injected (20 µL) in the HPLC-MS/MS system (see Figure III.4).

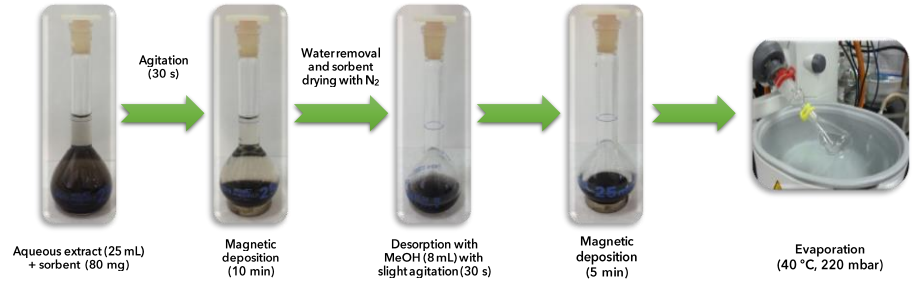


Figure III.4.- Scheme of the m-µ-dSPE procedure used for the extraction of mycotoxins from milk and yogurt samples (Section IV.2).

III.7.3.- Extraction of PAEs and DEHA from water samples by m-μ-dSPE (Section IV.3)

Briefly, 25 mL of a water sample previously adjusted to pH 6.0 with 0.1 M of NaOH or 0.1 M of HCl solutions, were poured onto 60 mg of Fe_3O_4@pDA m-NPs in a volumetric flask and the mixture was manually shaken for 1 min. Then, a permanent disc magnet was placed at the bottom of the flask for 10 min to ensure the complete sorbent settling. After that time, the supernatant sample was discarded by decantation, always maintaining the magnet at the bottom of the flask in order to retain the sorbent in the extraction recipient. Subsequently, the Fe_3O_4@pDA m-NPs were dried with a soft current of nitrogen and the analytes were desorbed by the addition of 6 mL of DCM and a slight manual agitation for 30 s followed by a magnetic sorbent deposition time of 5 min. Then, the desorption solvent containing the target analytes was separated by decantation (maintaining the magnet at the bottom of the flask again) and evaporated to dryness at 40 ºC and room pressure in a rotary evaporator. Finally, the dry extract was reconstituted in 500 μL of cyclohexane and injected (2 μL) in the GC–MS/MS system (see Figure III.5).

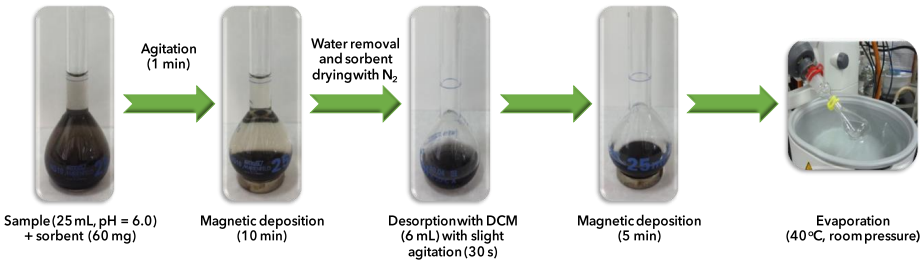


Figure III.5.- Scheme of the Fe_3O_4@pDA-m-μ-dSPE procedure applied for the extraction of PAEs and DEHA from water samples (Section IV.3).

III.7.4.- Extraction of PAEs from water samples by HF-LPME (Section IV.4)

A HF, previously cleaned with 2 mL of cyclohexane for 5 min in an ultrasonic bath and air dried, was used for each extraction. Subsequently, it was inserted into the needle tip of a 25 mL microsyringe and its pores and lumen were filled with 20 mL of 1-octanol in duplicate. The fibre was immersed in 10 mL of water and the extraction was performed for 75 min under stirring of 850 rpm at 60 ºC. After extraction, the fibre was taken out of the vial and introduced into a 300 mL GC micro-vial containing 200 mL of cyclohexane for 10 min in an ultrasonic bath to back-extract the analytes. Then, the solvent was evaporated under a gentle steam of nitrogen, reconstituted in 200 mL of cyclohexane and injected in the GC-MS/MS system (see Figure III.6).

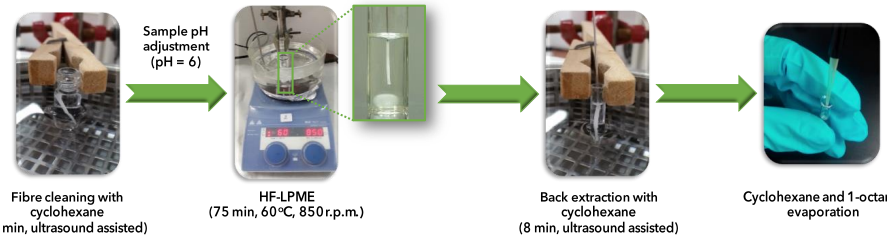


Figure III.6.- Scheme of the HF-LPME procedure applied for the extraction of PAEs from water samples (Section IV.4).

III.7.5.- Extraction of PAEs from beverages by MWCNTs-μ-dSPE (Section IV.5)

Fifty millilitres of each sample (pH was previously adjusted to 6.0 with HCl 0.1 M or NaOH 0.1 M) were introduced in a volumetric flask containing 80 mg of MWCNTs. After agitation for 1 min, the sample was rapidly passed through an empty glass column containing inside three PTFE frits using a vacuum manifold. Then, a new frit was placed onto the retained sorbent to hold it inside the column and vacuum was applied for 30 min to dry the stationary phase. Afterwards, the target analytes were eluted with 25 mL of ACN and the organic solvent was evaporated to dryness at 40 °C and 180 mbar using a rotary evaporator. Finally, the residue was reconstituted in 250 μL of the initial mobile phase (see Figure III.7). The isotonic drink was initially diluted (1/2, v/v) with Milli-Q water.

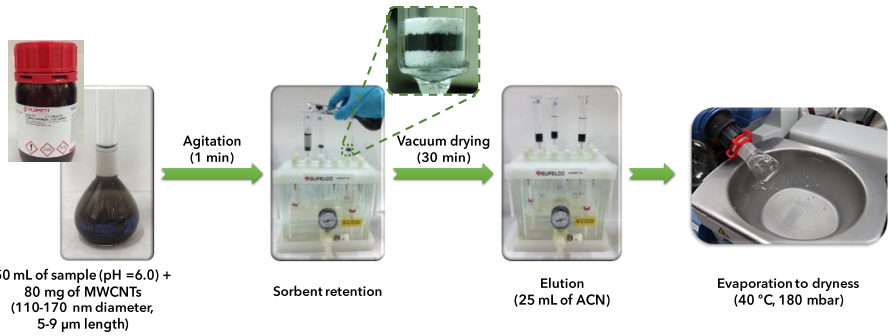


Figure III.7.- Scheme of the MWCNTs μ-dSPE procedure applied for the extraction of PAEs from beverages samples (Section IV.5).

III.7.6.- Extraction of PAEs and DEHA from water samples by MOF-dSPE (Section IV.6)

Fifty millilitres of sample (previously adjusted to pH 6.0) were introduced in a volumetric flask containing 120 mg of the MOF Basolite® F300. After 5 min of manual

agitation, the sample was rapidly passed through an empty glass column containing three PTFE frits, using a vacuum manifold. Once the MOF was retained into the column, a new frit was placed onto the sorbent to hold it inside the column and the sorbent was vacuum dried for 30 min. Afterwards, the target analytes were eluted with 15 mL of ACN and the organic sorbent was evaporated to dryness at 40 °C and 180 mbar. Finally, the residue was reconstituted in 250 µL of the initial mobile phase and injected in the HPLC-MS/MS system (see Figure III.8).

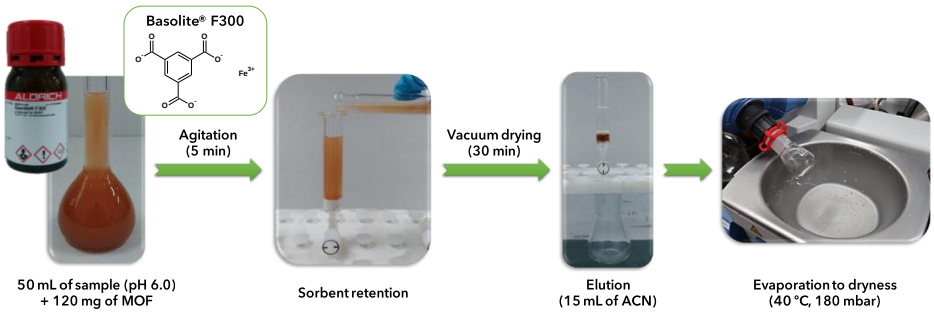


Figure III.8.- Scheme of the MOF-dSPE procedure applied for the extraction of PAEs and DEHA from water samples (Section IV.6).

CHAPTER IV

RESULTS AND DISCUSSION

IV.- RESULTS AND DISCUSSION

IV.1.- Evaluation of two MIPs for the SPE of natural, synthetic and mycoestrogens from environmental water samples before their determination by LC-MS/MS

In this section, the selectivity of two commercial MIP-SPE cartridges (AFFINIMIP®SPE Estrogens and AFFINIMIP®SPE Zearalenone) has been compared for the extraction of twelve oestrogenic compounds of interest (i.e. 17α-E_2, 17β-E_2, E_1, HEX, EE_2, DES, DS, ZEN, α-ZAL, β-ZAL, α-ZEL and β-ZEL) from different water samples (Milli-Q, mineral and waste water). Separation, determination and quantification were achieved by HPLC-IT-MS/MS while MIP-SPE conditions provided by the manufacturer were modified to obtain the best extraction performance. The methodology was validated for each sample by means of recovery and matrix-matched calibration studies.

IV.1.1.- Background

As previously indicated in the Introduction Section, among the different groups of compounds with oestrogenic activity or EDCs that currently exist, of especial interest are the so-called natural oestrogens as E_1, 17β-E_2, 17α-E_2 and E_3, together with different synthetic oestrogenic compounds like EE_2, DS and DES. They can enter the environment through sewage discharge and animal waste disposal [348,349]. In the particular case of 17β-E_2 (usually employed in hormone replacement therapy after menopause) and EE_2 (used as oral contraceptive), both are included in the list of priority substances that should be controlled in surface water [53]. Other compounds with an important oestrogenic activity are the mycotoxins of the family of ZEN and that include the epimers α- and β-ZAL and α- and β-ZEL. Although there is currently special interest in the determination of mycotoxins as natural contaminants in cereals and its derivatives, there is also increasing awareness of the significance of these compounds in water, in which they have also been found [350,351].

The most advanced application area of MIPs is surely their use as SPE sorbents. In fact, a good number of laboratory-made MIP sorbents have been proposed in the literature [352,353] and some of them, though relatively few, have also been commercialised. Most of these MIPs have been designed for one target analyte for which the template has to be carefully selected. However, in many cases, the final MIP is able to develop a selective extraction of a group of structural-analogue compounds (cross-

reactivity) rather than the target analyte alone. That is why a suitable study of the cross-reactivity of the polymer should be developed.

Nowadays, there are different companies that produce and commercialise MIP-SPE cartridges (Sigma-Aldrich, PolyIntell or Biotage). However, regarding the analysis of the oestrogenic compounds previously mentioned, and to the best of our knowledge, only one company sold SPE cartridges devoted to the analysis of natural oestrogens like 17β-E_2 or ZEN and its analogues when this work was developed. This is the case of PolyIntell, which commercialises the cartridges AFFINIMIP®SPE Estrogens and AFFINIMIP®SPE Zearalenone. Regarding the application of the first of them, only two articles had been published when this work was developed [354,355]. This is the case of the work of Lucci et al. [354] who extracted several natural and synthetic oestrogens (E_1, 17β-E_2, 17α-E_2, E_3, EE_2, DS and DES) from river and tap water samples and analysed them by HPLC–APCI-QqQ-MS/MS. Recovery values higher than 82 % (relative standard deviations (RSDs) < 8 %) were obtained for all the compounds except for DES and DS which were higher than 48 % (RSDs < 8 %). Another example is also the work of Matějíček et al. [355] in which E_1, 17β-E_2, E_3, EE_2, octylphenol, nonylphenol and bisphenol-A were determined in river water and sediments. In this case, the content of three MIP-SPE cartridges (AFFINIMIP®SPE Estrogens, AFFINIMIP®SPE Bisphenol-A and AFFINIMIP®SPE Phenolics) were mixed and used for the extraction of the selected compounds from the water samples and the sediment extracts. Chromatographic analysis was carried out by HPLC–IT-MS/MS and the recovery values obtained were higher than 90 % (RSDs < 11.5 %) in all cases. Concerning the use of the second cartridge (AFFINIMIP®SPE Zearalenone), only the work of Lucci et al. [205] was published. In this case, a single compound (ZEN) was extracted from cereal samples and analysed by HPLC-FD. Recovery values above 82 % (RSDs < 6.9 %) were found after the use of the cartridge for the extraction of ACN/water extracts of the cereals.

As a result, the number of applications in which these commercial cartridges were applied was extremely low and little was known about their full potential as SPE sorbents, as well as the possibility of cross-reactivity interactions with other compounds present in the samples.

IV.1.2.- Specific objectives

Considering all the above-mentioned, the following specific objectives were established:

- The development of a HPLC-IT-MS/MS method for the determination of a group of oestrogenic compounds of interest (i.e. 17α-E_2, 17β-E_2, E_1, HEX, EE_2, DES, DS, ZEN, α-ZAL, β-ZAL, α-ZEL and β-ZEL), in order to achieve suitable separation and detection conditions.
- The evaluation of the performance of two commercially available MIP-SPE cartridges (AFFINIMIP®SPE Estrogens and AFFINIMIP®SPE Zearalenone) by means of a comparative study at different levels of concentration in different types of water samples (Milli-Q, mineral and waste water), assessing the cross-reactivity of each MIP.
- The validation of the developed methodologies in the different types of waters for both MIP cartridges in terms of calibration, precision and trueness as well as the obtaining of the LODs and LOQs of the method.

IV.1.3.- Optimisation of the separation and detection conditions

For the optimisation of nebulisation/ionisation conditions the direct infusion of a mixture of the studied analytes in ACN at a concentration of 1 mg/L (flow rate of 20 µL/min) was performed, working in both positive and negative mode. The capillary voltage was varied between 3000 and 6000 V, the end plate offset between -500 and -5500 V, the nebulisation gas pressure (N_2) between 2 and 80 psi, the dry gas flow (N_2) between 0.5 and 12 L/min, and the dry gas temperature between 150 and 350 °C. The highest sensitivity for all analytes was obtained when the negative mode, with a capillary voltage of +5500 V, an end plate offset voltage of -600 V, a nebulisation gas pressure of 20 psi, and a dry gas flow and temperature of 8 L/min and 300 °C, respectively, were applied. It should be remarked that these compounds can be easily ionised under soft conditions in the ESI source, despite the fact that they are generally uncharged in a wide pH range. In fact, some works have previously determined them both in the negative [356,357] and positive mode [358,359].

For the suitable chromatographic separation of the 12 selected compounds, a X-Bridge C_{18} 100 mm x 4.6 mm x 3.5 µm column and a 20 mm x 4.6 mm x 3.5 µm pre-column filled with the same stationary phase, both end-capped, were used. As mobile phase, ACN/Milli-Q water mixtures were tested, containing or not small amounts of ammonia. The best separation was achieved with the application of the gradient elution programme described in Section III.5.1 without ammonia addition, since a decrease in the sensitivity of ZEN and its derivatives was observed in this case. Finally, a slight

enhancement of resolution and efficiency was achieved increasing the temperature of both column and pre-column to 30 °C.

The selection of the parameters that influence MS/MS detection was performed by means of the direct infusion of individual analytes at a concentration of 5 mg/L and applying the nebulisation and detection conditions previously optimised. Table IV.1.1 shows the best fragmentation conditions of the studied analytes, as well as the most intense product ion. MS/MS spectra are also shown in Figure IV.1.1.

Table IV.1.1.- MS/MS fragmentation parameters of the selected oestrogenic compounds.

Analyte	Precursor ion (m/z)	Product ion* (m/z)	Fragmentation amplitude (V)
β-ZAL	321.2	277.1	0.70
β-ZEL	319.2	275.1	0.70
α-ZAL	321.2	277.1	0.70
17β-E_2	271.1	274.1	0.50
α-ZEL	319.2	275.1	0.70
17α-E_2	271.1	253.1	0.70
EE_2	295.1	267.0	0.70
E_1	269.1	272.1	0.70
DES	267.1	238.0	0.70
HEX	269.1	135.0	0.60
ZEN	317.1	274.1	0.60
DS	265.0	236.0	0.60

* The most intense product ion.

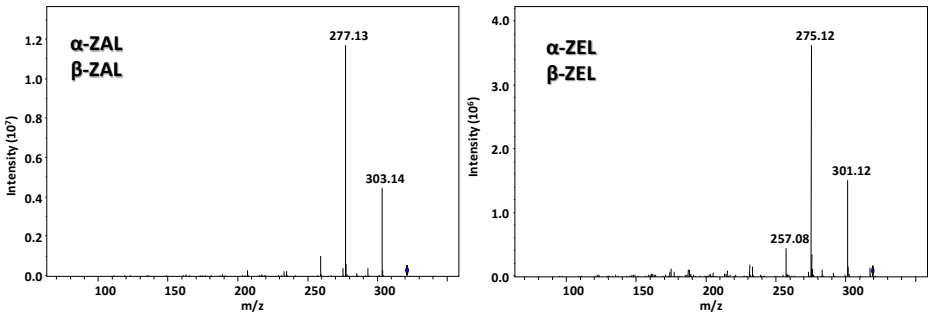


Figure IV.1.1.- MS/MS spectra of the target oestrogenic compounds.

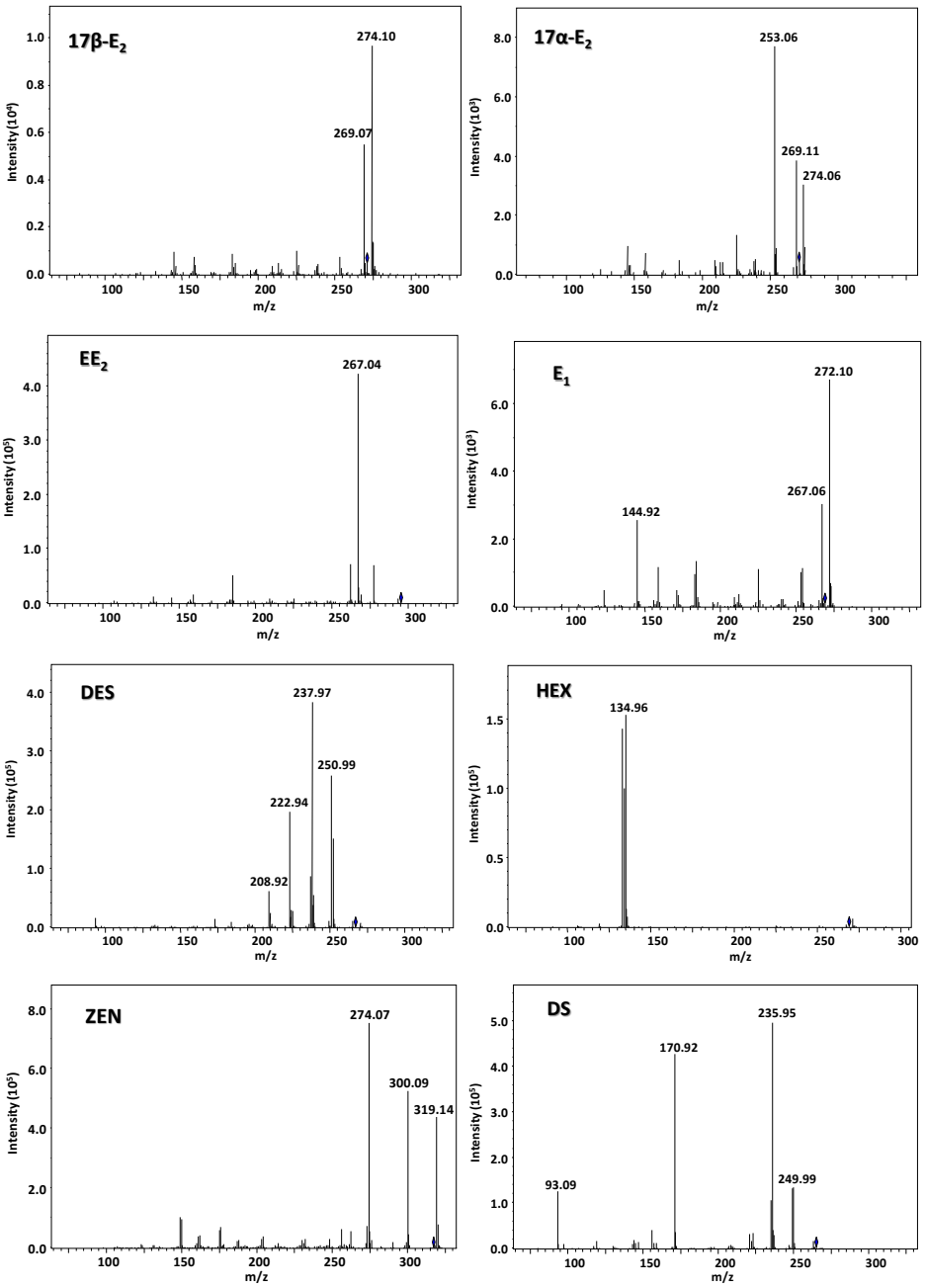


Figure IV.1.1.- (Continued).

IV.1.4.- HPLC–MS intra- and interday precision and calibration studies

A repeatability study consisting of three consecutive injections (n=3) of a mixture of the analytes of interest at two levels of concentration in three different days (n=9) was carried out. Table IV.1.2 shows the obtained results for the intraday precision. As can be seen, a good repeatability for retention times and peak areas was observed, with RSDs lower than 0.3 and 5.2 %, in the same day for both concentration levels. Between days, RSD values below 0.6 and 13.1 % were respectively obtained (data not shown in the table). To prove the linearity of the employed method, instrumental calibration curves based on peak areas were obtained for each compound injecting five concentration levels (n=5) in triplicate, obtaining, for all of them, determination coefficients (R^2) higher than 0.9908. Instrumental LODs and LOQs (Table IV.1.2), calculated as the concentration which provided a S/N ratio of 3 and 10, respectively, were between 0.10 and 4.1 µg/L for the first and between 0.33 and 14 µg/L for the second.

IV.1.5.- MIP-SPE procedure

As a starting point and taking into account that two commercial MIP-SPE cartridges were used, the extraction protocols provided by the manufacturer were studied and adapted (Figure IV.1.2). Preliminary experiments were carried out extracting 25 mL of spiked Milli-Q water containing the target analytes at a concentration of 20 µg/L. Although the manufacturer did not specify that a complete drying of the cartridge was necessary after sample loading (elution solvent evaporation was not even mentioned), to achieve a suitable removal of the water remaining after the washing of the cartridge and a good elution of the analytes as well as the later solvent evaporation, we found that it was necessary to dry both cartridges under vacuum for at least 20 min and also to increase the elution volume from 2–3 mL (2 mL for the "Estrogens cartridge" and 3 mL for the "Zearalenone cartridge") to 5 mL. After evaporation, the dry extract was reconstituted in 250 µL of ACN/Milli-Q water 50/50 (v/v) (initial mobile phase composition). In this way, a good preconcentration factor was achieved. Finally, we found that more repeatable results were achieved if the pH was suitably controlled. That is why a pH value of 7.0 was set after different pH values were tested. Concerning the washing of the cartridge after sample loading, and as previously reported [354], the use of high percentages of ACN, a polar non-protic solvent with a high dielectric constant, is not able to disrupt the specific interactions between the molecules and the functional monomers.

Table IV.1.2.- Intraday precision and calibration data of the selected group of oestrogenic compounds.

Analyte	Intraday precision[a] (n=9, RSD %)		Studied linear range (µg/L)	Regression equation (n=5)		R^2	$S_{y/x}$	$LOD_{instrumental}$[b] (µg/L)	$LOQ_{instrumental}$[c] (µg/L)
	Retention time	Peak area		b ± S_b·$t_{(0.05;3)}$	a ± S_a·$t_{(0.05;3)}$				
β-ZAL	0.3	1.4	20-500	$1.35 \cdot 10^9 \pm 0.06 \cdot 10^9$	$2.41 \cdot 10^7 \pm 1.68 \cdot 10^7$	0.9985	$1.01 \cdot 10^7$	0.22	0.72
β-ZEL	0.1	1.7	20-500	$1.15 \cdot 10^9 \pm 0.05 \cdot 10^9$	$2.13 \cdot 10^7 \pm 1.53 \cdot 10^7$	0.9983	$9.23 \cdot 10^6$	0.28	0.93
α-ZAL	0.0	2.3	20-500	$1.74 \cdot 10^9 \pm 0.12 \cdot 10^9$	$3.91 \cdot 10^7 \pm 3.26 \cdot 10^7$	0.9966	$1.97 \cdot 10^7$	0.51	1.7
17β-E_2	0.2	2.0	60-1500	$5.73 \cdot 10^7 \pm 0.42 \cdot 10^7$	$6.65 \cdot 10^6 \pm 3.60 \cdot 10^6$	0.9959	$2.17 \cdot 10^6$	1.0	3.5
α-ZEL	0.3	0.9	20-500	$1.53 \cdot 10^9 \pm 0.86 \cdot 10^9$	$3.19 \cdot 10^7 \pm 2.42 \cdot 10^7$	0.9976	$1.46 \cdot 10^7$	0.61	2.0
17α-E_2	0.1	1.6	40-1000	$5.65 \cdot 10^7 \pm 0.18 \cdot 10^7$	$2.97 \cdot 10^6 \pm 1.01 \cdot 10^6$	0.9992	$6.08 \cdot 10^5$	4.1	14
EE_2	0.1	0.4	60-1500	$9.23 \cdot 10^7 \pm 0.25 \cdot 10^7$	$5.17 \cdot 10^6 \pm 2.14 \cdot 10^6$	0.9994	$1.29 \cdot 10^6$	0.53	1.8
E_1	0.1	3.0	40-1000	$3.22 \cdot 10^8 \pm 0.12 \cdot 10^8$	$4.79 \cdot 10^6 \pm 6.50 \cdot 10^6$	0.9985	$4.66 \cdot 10^6$	0.56	1.9
DES	0.1	4.9	20-400	$1.45 \cdot 10^9 \pm 0.11 \cdot 10^9$	$1.75 \cdot 10^7 \pm 2.35 \cdot 10^7$	0.9973	$1.21 \cdot 10^7$	0.13	0.44
HEX	0.1	4.9	20-400	$9.26 \cdot 10^8 \pm 1.15 \cdot 10^8$	$2.80 \cdot 10^7 \pm 2.55 \cdot 10^7$	0.9921	$1.32 \cdot 10^7$	0.31	1.0
ZEN	0.0	2.8	20-400	$2.08 \cdot 10^9 \pm 0.16 \cdot 10^9$	$4.58 \cdot 10^7 \pm 3.61 \cdot 10^7$	0.9970	$1.86 \cdot 10^7$	0.10	0.33
DS	0.1	5.2	20-400	$1.16 \cdot 10^9 \pm 0.16 \cdot 10^9$	$3.63 \cdot 10^7 \pm 3.34 \cdot 10^7$	0.9908	$1.94 \cdot 10^7$	0.15	0.49

b: slope; S_b: standard deviation of the slope; a: intercept; S_a: standard deviation of the intercept; $S_{y/x}$: standard deviation of the estimate.
[a] Data obtained for the high concentration level: 0.4-1.2 mg/L. [b] Calculated as the concentration associated to a S/N ratio of 3. [c] Calculated as the concentration associated to a S/N ratio of 10.

AFFINIMIP®SPE Estrogens		AFFINIMIP®SPE Zearalenone
3 mL ACN + 3 mL H_2O	Conditioning	3 mL ACN + 3 mL H_2O
50 mL sample	Retention	12 mL sample
3 mL H_2O + 3 mL ACN/H_2O (40/60)	Washing	3 mL H_2O/ACN/AcOH (58/40/2)
30 s vacuum	Drying	-
3 mL MeOH	Elution	2 mL MeOH/AcOH (98/2)

AFFINIMIP®SPE Estrogens		AFFINIMIP®SPE Zearalenone
3 mL ACN + 3 mL H_2O	Conditioning	3 mL ACN + 3 mL H_2O
100 mL sample	Retention	100 mL sample
3 mL H_2O + 3 mL ACN/H_2O (40/60)	Washing	3 mL H_2O/ACN/AcOH (58/40/2)
20 min vacuum	Drying	20 min vacuum
5 mL MeOH	Elution	5 mL MeOH/AcOH (98/2)

Figure IV.1.2.- SPE protocol of the manufacturer (top) and SPE protocol applied in this work (bottom).

Under these conditions that slightly modified those proposed by the manufacturer, a recovery study was carried out extracting 25 mL of Milli-Q water (n=3) spiked at two concentration levels. The aim was to demonstrate the efficiency and reproducibility of the MIP-SPE procedure for the extraction of the target compounds as well as the possible cross-reactivity of the polymers. Table IV.1.3 shows the recovery values obtained for both cartridges at both levels of concentration. These values were calculated by comparing the peak areas obtained with those of matrix-matched standards. As can be seen, the AFFINIMIP®SPE Estrogens cartridge allowed to obtain recovery values in the range 69–101 %, except for ZEN which recovery values were around 45 %, with RSD values lower than 19 %. Also, AFFINIMIP®SPE Zearalenone cartridge was able to provide recovery values in the range 65–98 % for all cases, with RSD values lower than 15 %. In general, slightly lower recovery percentages were obtained with the AFFINIMIP®SPE Zearalenone cartridge. The enrichment factors obtained ranged between 45 and 100. Thus, it could be concluded that both cartridges allow to obtain

acceptable and repeatable recovery values from Milli-Q water even for those compounds that do not directly belong to the same family for which the MIP was designed. These results suggest that both MIPs show cross-reactivity for the rest of the selected compounds. In fact, and as reported by Lucci et al. for the AFFINIMIP®SPE Estrogens cartridge [354], each target molecule displays at least one hydroxyl group able to interact specifically with the imprinted cavities.

Table IV.1.3.- Mean recovery percentages (n=3), RSD values, analytes concentration and enrichment factors after the application of the MIP-SPE-HPLC-MS procedure to 25 mL of spiked Milli-Q water sample.

Analyte	AFFINIMIP® SPE Estrogens			AFFINIMIP® SPE Zearalenone		
	Level 1[a] Recovery (RSD, %)	Level 2[a] Recovery (RSD, %)	Enrichment factor[b]	Level 1[a] Recovery (RSD, %)	Level 2[a] Recovery (RSD, %)	Enrichment factor[b]
β-ZAL	85 (13)	96 (9)	91	71 (12)	78 (8)	75
β-ZEL	69 (12)	80 (8)	75	**68** (3)	**69** (9)	69
α-ZAL	92 (8)	101 (7)	97	**66** (6)	**68** (6)	67
17β-E_2	84 (3)	94 (4)	89	72 (7)	81 (5)	77
α-ZEL	84 (10)	95 (11)	89	**69** (7)	71 (8)	70
17α-E_2	83 (6)	95 (6)	89	71 (12)	77 (15)	74
EE_2	80 (5)	88 (6)	84	**65** (11)	**67** (11)	66
E_1	86 (14)	93 (7)	90	**66** (6)	70 (7)	68
DES	85 (15)	99 (5)	92	**68** (10)	80 (4)	74
HEX	89 (17)	99 (10)	94	70 (13)	77 (5)	73
ZEN	**47** (4)	**42** (13)	45	76 (10)	79 (10)	77
DS	98 (13)	101 (19)	100	85 (11)	98 (13)	92

[a] Level 1: 2-8 µg/L; level 2: 4-20 µg/L.[b] Theoretical enrichment factor calculated as V_{sample}/V_{final}: 100.

IV.1.6.- Recovery studies in different water samples

To fully compare the ability of both cartridges to extract the selected group of analytes from real samples, a recovery study was carried out extracting two different water samples (mineral and waste water) previously spiked with a mixture of the target analytes at two concentration levels (three extractions were carried out at each level). In this case, the sample volume was increased to 100 mL to improve the enrichment factors and, in consequence, to obtain lower LODs. The previous analysis of non-spiked samples showed that the target analytes were not present in the samples at the LODs of the method.

Table IV.1.4 shows the recovery values obtained when the target analytes were extracted from mineral water samples (Figure IV.1.3) employing both cartridges (intraday precision data). On the one hand, it can be observed that the AFFINIMIP®SPE Estrogens cartridge allowed to obtain recovery values higher than 76 % and RSD values lower than 17 %, except for ZEN and its derivatives for which recovery percentages were lower than 20 % (some of them were even not extracted) and high RSDs were obtained (data not shown). Besides, and although DS showed good recovery values (59–75 %), its RSD value was higher than 20 % at the two concentration levels studied. On the other hand, the AFFINIMIP®SPE Zearalenone cartridge was able to provide recovery percentages higher than 58 % in all cases, with RSD values lower than 20 %. Furthermore, enrichment factors in the range 266–424 (in this case the theoretical enrichment factor was 400) and LODs between 0.010 and 0.44 µg/L were obtained for both cartridges. LODs and LOQs were also calculated taking into account the concentration of the analytes that provided an S/N ratio of 3 and 10, respectively, in a matrix-matched standard.

Table IV.1.4.- Mean recovery percentages (n=3), RSD values, LODs and LOQs after the application of the MIP-SPE-HPLC-MS procedure to a 100 mL of spiked mineral water sample (intraday precision data).

Analyte	AFFINIMIP® SPE Estrogens				AFFINIMIP® SPE Zearalenone			
	Level 1[a] Recovery (RSD, %)	Level 2[a] Recovery (RSD, %)	LOD_m[b] (µg/L)	LOQ_m[c] (µg/L)	Level 1[a] Recovery (RSD, %)	Level 2[a] Recovery (RSD, %)	LOD_m[b] (µg/L)	LOQ_m[c] (µg/L)
β-ZAL	-	-	-	-	85 (20)	101 (7)	0.026	0.085
β-ZEL	-	-	-	-	**65** (14)	73 (11)	0.041	0.14
α-ZAL	-	-	-	-	96 (21)	**67** (4)	0.051	0.17
17β-E_2	90 (13)	105 (1)	0.16	0.54	88 (4)	80 (5)	0.23	0.75
α-ZEL	-	-	-	-	76 (19)	**63** (8)	0.038	0.13
17α-E_2	88 (1)	101 (9)	0.13	0.43	72 (16)	85 (13)	0.20	0.67
EE_2	80 (3)	90 (17)	0.11	0.37	72 (1)	**64** (6)	0.44	1.5
E_1	91 (7)	100 (11)	0.073	0.24	75 (10)	**58** (16)	0.31	1.0
DES	76 (11)	81 (4)	0.010	0.028	**62** (5)	83 (8)	0.038	0.13
HEX	91 (20)	121 (11)	0.048	0.16	81 (8)	84 (11)	0.071	0.24
ZEN	-	-	-	-	83 (12)	78 (3)	0.019	0.063
DS	**59** (**24**)	75 (**52**)	0.010	0.029	**69** (10)	111 (8)	0.075	0.25

[a] Level 1: 0.2-0.8 µg/L; level 2: 1-3 µg/L. [b] LOD of the method. [c] LOQ of the method.

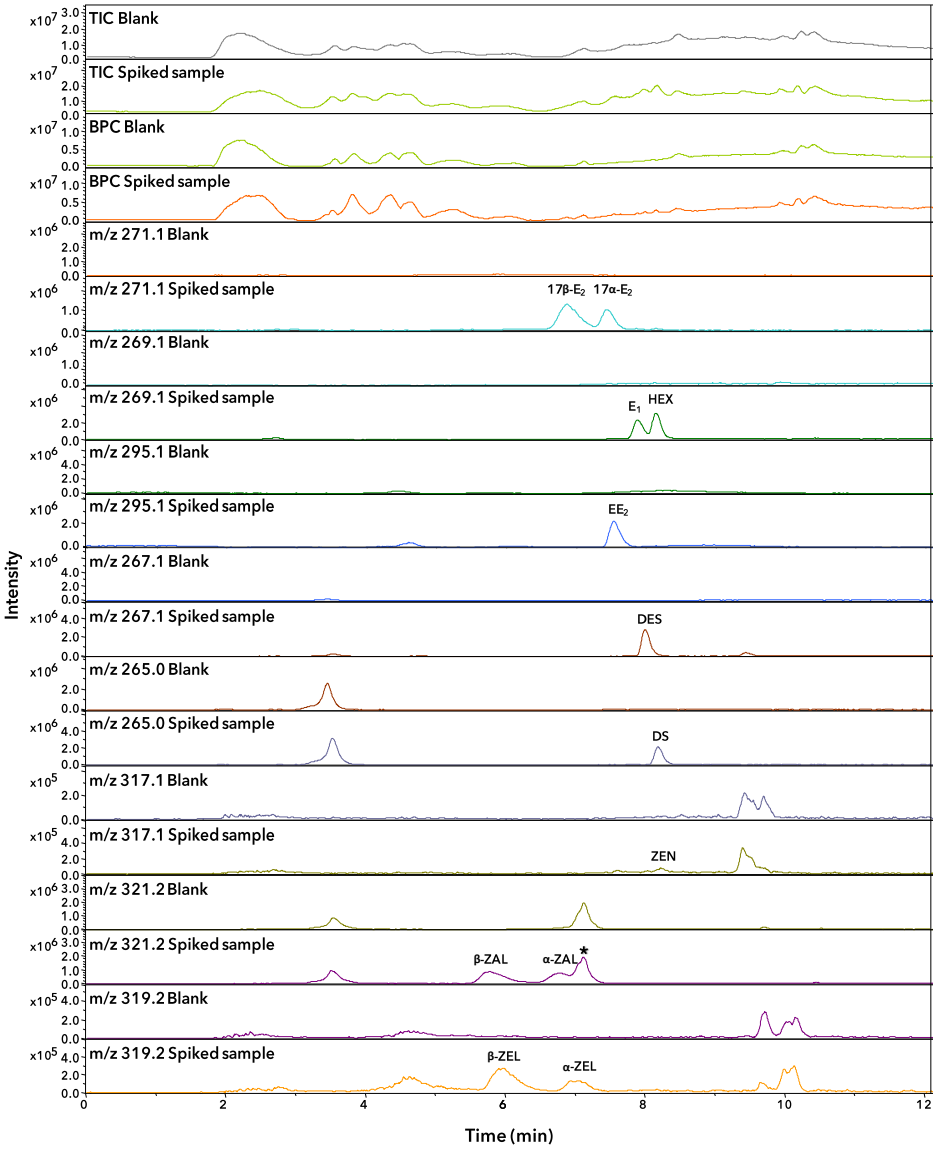


Figure IV.1.3.- Total ion chromatograms (TIC), base peak chromatograms (BPC) and extracted ion chromatograms (EICs) of a spiked and a blank mineral water sample after the application of the MIP-SPE-HPLC-MS method using the AFFINIMIP®SPE Estrogens cartridge. Concentration of the analytes in the sample: 0.1-0.3 mg/L.

Table IV.1.5 shows the recovery percentages obtained from the extraction of a waste water sample using both cartridges (intraday precision data). The recovery values obtained for the AFFINIMIP®SPE Estrogens were higher than 66 % with RSDs lower than 14 %. As also happened for mineral water, neither ZEN nor its derivatives were extracted by this MIP. However, the AFFINIMIP®SPE Zearalenone cartridge allowed to obtain recovery values higher than 63 % with RSD values lower than 16 % in all cases, except for 17α-E_2 and 17β-E_2 for which inconsistent results were obtained, probably due to the higher complexity of the sample. Enrichment factors in the range of 270–398 and LODs between 0.010 and 0.32 μg/L were obtained. These results revealed the better ability of the ZEN cartridge to extract the selected group of oestrogens without any matrix interferences during the re-binding process of the target compounds.

Table IV.1.5.- Mean recovery percentages (n=3), RSD values, LODs and LOQs after the application of the MIP-SPE-HPLC-MS procedure to 100 mL of spiked waste water sample (intraday precision data).

Analyte	AFFINIMIP® SPE Estrogens				AFFINIMIP® SPE Zearalenone			
	Level 1[a] Recovery (RSD, %)	Level 2[a] Recovery (RSD, %)	LOD_m[b] (μg/L)	LOQ_m[c] (μg/L)	Level 1[a] Recovery (RSD, %)	Level 2[a] Recovery (RSD, %)	LOD_m[b] (μg/L)	LOQ_m[c] (μg/L)
β-ZAL	-	-	-	-	80 (3)	86 (10)	0.041	0.14
β-ZEL	-	-	-	-	91 (8)	86 (9)	0.050	0.17
α-ZAL	-	-	-	-	86 (11)	**64** (8)	0.061	0.20
17β-E_2	95 (9)	86 (4)	0.12	0.41	-	-	-	-
α-ZEL	-	-	-	-	**63** (11)	77 (7)	0.10	0.33
17α-E_2	98 (14)	79 (9)	0.061	0.20	-	-	-	-
EE_2	73 (6)	**69** (3)	0.10	0.33	119 (4)	80 (3)	0.32	1.1
E_1	74 (7)	79 (6)	0.054	0.18	97 (13)	84 (11)	0.19	0.62
DES	**69** (8)	**66** (5)	0.010	0.026	**66** (5)	82 (13)	0.037	0.12
HEX	86 (7)	86 (3)	0.024	0.081	86 (16)	102 (9)	0.073	0.24
ZEN	-	-	-	-	93 (5)	88 (6)	0.012	0.041
DS	79 (7)	81 (1)	0.010	0.031	73 (2)	88 (5)	0.12	0.39

[a] Level 1: 0.2-0.8 μg/L; level 2: 1-3 μg/L. [b] LOD of the method. [c] LOQ of the method.

From the previous results, it is clear that the AFFINIMIP®SPE Estrogens cartridge shows less cross-reactivity effect in both samples, which did not happen for Milli-Q water. This may be explained by the fact already described in the literature that the ionic content of a sample may influence the retention capability of some MIPs [360-362].

The results obtained in this work are also in accordance with those previously published in which the same MIP-SPE cartridges have been used. In this sense, the recovery and RSD values obtained with the AFFINIMIP®SPE Estrogens cartridge for E_1, 17β-E_2, 17α-E_2, EE_2, DS and DES, are similar to those obtained by Lucci et al. [354] except for DS for which higher RSD values were obtained in the mineral water samples. Regarding the LODs obtained, our LODs values are slightly higher (though the same sample amount was selected) due to the higher sensitivity of the HPLC–APCI-QqQ-MS/MS system used by Lucci et al. [354]. Regarding the work of Matějíček et al. [355], the recovery values obtained by them for E_1, 17β-E_2, E_3 and EE_2 (the only analytes in common with this work) in water samples, are also similar to the ones reported in this work. However, their LODs are also slightly lower, because of the higher amounts of sample used (250 mL). Even though, it should be remarked that the main aim of this work (which clearly includes a higher number of oestrogenic compounds of interest, with similar structure) was to compare the extraction ability of both MIP cartridges and to demonstrate their cross-reactivity and their suitability for their extraction from different water samples. Finally, and concerning the use of the AFFINIMIP®SPE Zearalenone cartridge, a full comparison cannot be made since the only work that has used it only determined ZEN in cereal samples [205].

IV.1.7.- Conclusions

From the results obtained in this section, the following conclusions can be drawn:

- The performance of the commercial MIP cartridges AFFINIMIP®SPE Estrogens and AFFINIMIP®SPE Zearalenone has been compared for the first time for the extraction of twelve oestrogenic compounds of interest (i.e. 17α-E_2, 17β-E_2, E_1, HEX, EE_2, DES, DS, ZEN, α-ZAL, β-ZAL, α-ZEL and β-ZEL) from different water samples (Milli-Q, mineral and waste water).

- Both separation and detection conditions were optimised and validated by means of repeatability and calibrations studies. The selected conditions allowed the complete separation of the three pairs of epimers (17α-E_2, 17β-E_2, α-ZAL, β-ZAL, α-ZEL and β-ZEL). The instrumental LODs in the range 0.10-4.1 μg/L, R^2 higher than 0.9908 and RSDs between days for retention times (≤ 0.6 %) and peak areas (≤ 13.1 %) obtained, demonstrate the good sensitivity, linearity and repeatability of the HPLC-IT-MS method developed.

- The extraction protocols given by the manufacturer for both cartridges had to be adapted for Milli-Q water (25 mL of sample) to obtain the best extraction performance. A recovery study was also carried out, obtaining recovery percentages between 65 and 101 % for most analytes with RSDs lower than 19 % for both cartridges. These results clearly demonstrate the efficiency and reproducibility of the MIP-SPE procedure.

- The fact that both cartridges allowed obtaining acceptable recovery values for all the target analytes in Milli-Q water, although they do not directly belong to the same family for which the MIP was designed, shows the existence of cross-reactivity for the rest of the selected compounds.

- The developed methodologies were applied and validated in mineral and waste water samples, increasing the sample volume to 100 mL, which allowed quadruplicating the enrichment factors (266-424). AFINIMIP®SPE Estrogens was not able to extract ZEN and its derivatives neither in mineral nor in waste water, while AFFINIMIP®SPE Zearalenone was able to extract all the target analytes with at least a 58 % of recovery, except for 17α-E_2 and 17β-E_2 for which inconsistent results were obtained, probably due to the higher complexity of the sample. This lower cross-reactivity shown in both samples by the AFFINIMIP®SPE Estrogens may be explained by the higher ionic content of these water samples with respect to the Milli-Q water and which has been previously reported in the literature.

- The application of both extraction cartridges to the analysis of mineral and waste water samples, allowed obtaining recovery values of 58-121 % and LOQs of the method between 0.026 and 1.9 μg/L. The results obtained in this work are in accordance with those previously published in the literature in which the same MIP-SPE cartridges have been used, though for the extraction of a reduced number of these analytes.

- This work constitutes one of the very few published applications of these cartridges for the extraction of the target analytes from both types of samples and the first work in which the cross-reactivity of both of them have been studied and compared for the target analytes (which include an important group of natural, synthetic and mycoestrogens) and samples.

IV.2.- Application of core-shell pDA-coated m-NPs (Fe_3O_4@pDA) for the extraction of oestrogenic mycotoxins from milk and yogurt prior to LC-MS/MS analysis

In this section, core-shell pDA-coated m-NPs synthesised in our laboratory have been applied as m-μ-dSPE sorbent for the extraction of a group of six mycotoxins of interest including ZEN, α-ZAL, β-ZAL, α-ZEL, β-ZEL and ZAN, from complex matrices such as milk (whole and skimmed cow milk and semi-skimmed goat milk) and yogurt (an unsweetened natural yogurt) prior to their HPLC–MS/MS analysis. 17β-E_2-d_5 was used as IS in all cases. The procedure included a deproteinization step prior to the extraction. Matrix-matched calibration and a recovery study were carried out in the selected matrices in order to validate the whole methodology.

IV.2.1.- Background

As it was mentioned in the Introduction Section, mycotoxins are toxic secondary metabolites produced by different fungi species, which contaminate agricultural commodities either before harvest or under post-harvest conditions [35]. One of the most relevant groups of mycotoxins found in food includes ZEN and its derivatives α-ZAL, β-ZAL, α-ZEL, β-ZEL and ZAN which, contrary to other mycotoxins, are capable of binding to ERs producing multiple endocrine disorders [363]. These mycotoxins have an important oestrogenic activity and their oestrogenic potential changes from one compound to another. It has been demonstrated that α-ZAL and α-ZEL show a higher oestrogenic potential than β-ZEL or ZEN, since it has shown higher affinity to ERs [38].

The intake of contaminated foodstuffs constitutes the main via of human exposure to such mycotoxins. In this sense, milk and dairy products play a very important role since they represent an important part of the diet of humans all over the world. Besides, this kind of compounds tend to accumulate in fatty tissues due to their lipophilic nature reaching the milk of animals quite easily [88]. Furthermore, it should be taken into account that the contamination of food of animal origin may take place through the intake of contaminated feed or by the intentional administration of these compounds; in particular, α-ZAL has been widely used as growth promoter [364]. As a result, ZEN and its metabolites, including α-ZAL, were banned in the EU in 1996 in order to protect the health of the consumers [60], though MRLs have not been established yet for any of them in milk.

Regarding the analysis of ZEN and its metabolites in milk and dairy products, few papers were available in the literature [101,357,365-370]. Due to the complexity of such

samples, a previous extraction step is necessary in order to preconcentrate the target analytes and to remove interferences. Different extraction techniques had been applied to achieve this goal, principally SPE [365-367] using GCB, Oasis HLB (Hydrophilic-Lipophilic Balance) and Oasis WAX (Weak Anion-eXchange) cartridges, though others like DLLME [101] or LLE [368] had also been used. Besides the above-mentioned methods, some works could be found in which several techniques were used in order to improve the removal of the interferences. This was the case of the use of UAE combined with a defatting and a SPE [369] or LLE followed by a SPE procedure with an Oasis HLB cartridges [357]. Finally, it is worthy to mention the work of Zhang et al. [370] who decided not to include any extraction step and to replace it for a dilution and sample filtration.

As previously mentioned, among the current trends in sample preparation, the use of m-dSPE using nanomaterials could be highlighted. Though introduced in 1996 by Towler et al. for the recovery of Ra, Pb and Po from seawater using MnO_2 coated magnetite [371], its use has nowadays attracted much attention principally due to their capacity to be easily isolated from the matrix by an external magnet without retaining residual magnetisation which results in a simple, rapid and efficient procedure. The most common materials used for this purpose are NPs of magnetite (Fe_3O_4), maghemite (γ-Fe_2O_3) and cobalt ferrite ($CoFe_2O_4$) which are superparamagnetic. Such nanomaterial has to be coated with an inorganic or organic layer to increase its stability, protecting it against oxidation and avoiding the formation of agglomerates. In that way, by using specific coatings it is also possible to carry out a further functionalisation of their surface, which may be used to establish successful interactions with the target analytes without losing the magnetic properties. In this sense, DA has attracted wide interest due to its self-polymerisation capacity in aqueous phase under weak alkaline conditions, allowing the formation of a surface adhesive film onto a diversity of organic and inorganic materials [372]. To the best of our knowledge, pDA NPs, also designated as NPs@pDA, have only been applied in m-dSPE in very few occasions [264,276,373-375], that is why there is still the need of demonstrating the full potential of these new materials for the extraction of a wide variety of analytes, specially, from complex food matrices. In particular, they have been successfully applied to the extraction of four aflatoxins from red wine [264], berberine from a Chinese medical plant (*Cortex Phellodendri*) [374], six polycyclic aromatic hydrocarbons (PAHs) from environmental water samples [375] and seven antibiotics, three perfluorinated compounds and benzo(a)pyrene from lake and tap water [373]. Finally, a previous work developed by our research group, applied for the first time

laboratory-made core-shell Fe_3O_4@pDA m-NPs as sorbent for the m-μ-dSPE of a group of twelve compounds with oestrogenic activity from water samples [276]. The synthetic procedure demonstrated to be very easy, simple and with a very low cost.

IV.2.2.- Specific objectives

Taking into account the above, the following specific objectives were established:

- The preparation of core-shell Fe_3O_4@pDA m-NPs and their possible application as sorbent for the m-μ-dSPE of a group of six mycotoxins (ZEN, α-ZAL, β-ZAL, α-ZEL, β-ZEL and ZAN) from complex food matrices, such as milk and yogurt, and their subsequent analysis by HPLC-MS/MS.
- The application of a deproteinization step before the extraction in order to effectively remove milk and yogurt proteins to avoid their irreversible adsorption onto the m-NPs and chromatographic stationary phases.
- The evaluation and application of a m-μ-dSPE procedure for the determination of oestrogenic compounds from water samples to the extraction of these target analytes in such dairy products.
- The validation of the whole methodology both in milk and yogurt samples in terms of linearity, precision and trueness as well as the determination of the LODs and LOQs of the method.

IV.2.3.- m-μ-dSPE procedure

As previously mentioned, the research group in which this PhD Thesis has been developed, proposed and optimised a new method for the determination of different oestrogenic compounds in mineral, tap and waste water samples [276]. Such method involved a m-μ-dSPE step using laboratory made core-shell Fe_3O_4@pDA m-NPs which were synthesised by a chemical co-precipitation procedure and characterised by different surface characterisation techniques (X-ray diffraction, X-ray photoelectron spectroscopy, thermogravimetric analysis, transmission and scanning electron microscopy, infrared and Raman spectroscopy, vibrating sample magnetometry microelectrophoresis and adsorption/desorption isotherms). The selected compounds, which also included ZEN and its metabolites α-ZAL, β-ZAL, α-ZEL, β-ZEL and ZAN, were determined by HPLC-MS/MS. The developed procedure constitutes a simple, fast and environmentally friendly

method that represented the first application of such m-NPs for the extraction of such group of oestrogenic compounds.

Although the validation procedure demonstrated a good performance of the method, it had not been explored yet if it could be applied to the analysis of samples of a higher complexity like milk and dairy products, in which these compounds can appear. That is why it was decided to apply the method to the extraction of mycotoxins from different milk and yogurt samples.

As a consequence of the higher complexity of this kind of samples, the direct application of the methodology previously developed for water samples was not possible. In order to reduce matrix effects and to avoid contamination problems, blockage or damage of the HPLC column, etc., an initial deproteinization step was found necessary to remove milk proteins [88]. Initial experiments were carried out using 3 mL of spiked whole cow milk. After deproteinization with 6 mL of ACN and 150 µL of AcOH, samples were left in the darkness for 15 min and then centrifuged. The supernatant was evaporated to dryness in a rotary evaporator and then reconstituted in Milli-Q water previously adjusted to pH 7.0. Afterwards, the solution was filtered (0.45 µm PET syringe filter) directly onto 60 mg of Fe_3O_4@pDA m-NPs in a 50-mL centrifuge tube.

The filtered solution containing the Fe_3O_4@pDA m-NPs was manually shaken for 30 s and then, a permanent magnet was placed for 10 min at the bottom of the tube. Afterwards, the liquid was discarded retaining the sorbent in the extraction recipient using the magnet. After drying the Fe_3O_4@pDA m-NPs with a soft current of nitrogen, the selected mycotoxins were desorbed by the addition of 6 mL of MeOH and a slight manual agitation for 30 s followed by a magnetic deposition period of 5 min. Finally, the desorption solvent containing the target analytes was separated and evaporated to dryness in a rotavapor and the residue was reconstituted in 500 µL of the initial composition of the mobile phase (ACN/H_2O 50/50, v/v) and filtered through a 0.22 µm PET syringe filter (see Section III.7.2 for further details). Under these conditions, a high background noise which did not allow the correct determination of the selected compounds was observed after HPLC-MS analysis. For this reason, lower volumes of sample (2 and 1.5 mL) were tested in order to reduce such matrix effect. For this purpose, the volume of ACN and AcOH used during the deproteinization step were adjusted maintaining the same sample/ACN/AcOH ratio. The best results were obtained when 1.5 mL of milk, 3 mL of ACN and 75 µL of AcOH were taken. Under these conditions, recovery values of the selected compounds were in the range 60–110 %. In order to

improve these results, it was decided to increase the amount of Fe_3O_4@pDA m-NPs. Thus, 80 and 100 mg were tested, increasing the MeOH volume used for the desorption of the analytes from 6 mL to 8 and 10 mL, respectively. The results showed that it was possible to obtain slightly higher repeatable relative recovery percentages (between 70 and 120 %) when 80 mg of Fe_3O_4@pDA m-NPs and 8 mL of MeOH were used.

Regarding yogurt samples, similar results were also obtained when high amounts of yogurt were taken, that is why 1.5 g of the samples were also found appropriate. However, considering a previous article of our research group [376], the sample/ACN ratio was changed from 1/2 to 1/3 w/v, using the same volume of AcOH. Under these conditions, a more effective deproteinization was obtained when 1.5 g of yogurt, 4.5 mL of ACN and 75 µL of AcOH were taken. Concerning the amount of the extraction sorbent, and as a result of the excellent recovery percentages obtained for milk samples, the same amount of Fe_3O_4@pDA m-NPs used when milk samples were analysed was directly applied. Repeatable relative recovery values between 70 and 120 % were also obtained when 80 mg of Fe_3O_4@pDA m-NPs and 8 mL of MeOH were used.

Figure IV.2.1 shows the total ion chromatograms (TICs) and extracted ion chromatograms (EICs) obtained after the application of the proposed methodology to spiked samples of skimmed cow milk and unsweetened natural yogurt (Table IV.2.1 shows the MS/MS fragmentation parameters applied for analyte confirmation). As it can be seen, suitable separation of all analytes was achieved and, although some intense interferences (labelled with an asterisk) were found at a m/z of 321.2, none of them precluded the correct integration of the analytes' peaks.

IV.2.4.- Validation of the methodology

In order to evaluate the linearity of the methodology, matrix-matched calibration curves based on peak areas were obtained for each analyte injecting nine increasing levels of concentration in triplicate (n=9). Table IV.2.2 shows the results of this study for all the analysed samples. Acceptable R^2 values, higher than 0.9919 in all cases, were obtained which proved the good linearity of the method. The LODs and LOQs were calculated as the concentration which provided a S/N ratio of 3 and 10, respectively. Thus, LODs ranged between 0.29 and 4.8 µg/L for milk samples and between 0.41 and 5.8 µg/kg for yogurt samples. Concerning the LOQs, they ranged between 0.98 and 15 µg/L for milk samples and between 1.4 and 20 µg/kg for yogurt samples.

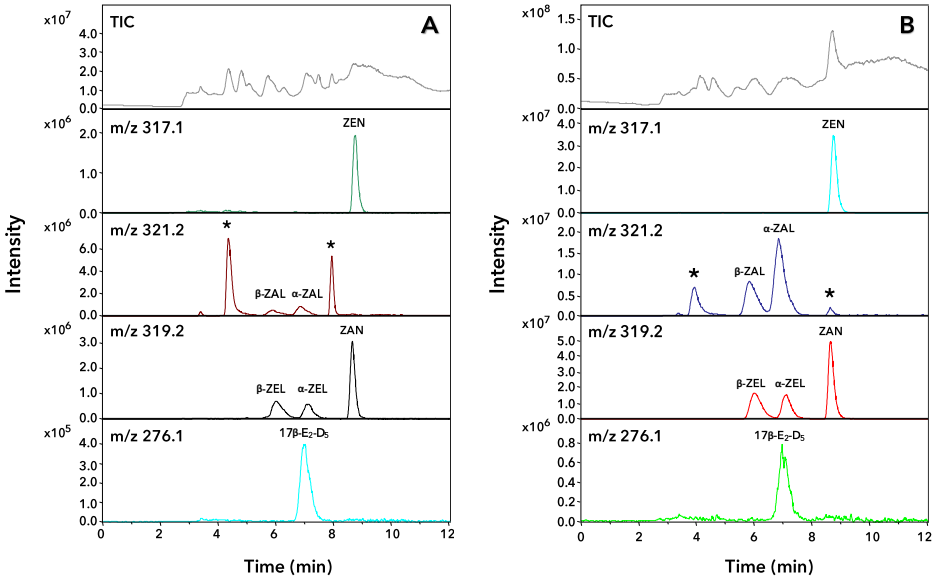


Figure IV.2.1.- A) TIC and EICs of a spiked skimmed cow milk sample (spiking level: 60 µg/L). B) TIC and EICs of a spiked unsweetened natural yogurt sample (spiking level: 400 µg/kg). For experimental conditions see Section III.7.2. (*) Interferences which have not hindered the correct quantitation of the target analytes.

Table IV.2.1.- MS/MS fragmentation parameters of the selected mycotoxins.

Analyte	Precursor ion (m/z)	Product ions* (m/z)	Fragmentation amplitude (V)
ZEN	317.1	274.1	0.60
		300.1	
β-ZAL	321.2	277.1	0.70
		303.1	
α-ZAL	321.2	277.1	0.70
		303.1	
β-ZEL	319.2	275.1	0.70
		301.1	
α-ZEL	319.2	275.1	0.70
		301.1	
ZAN	319.2	275.1	0.70
		301.1	

* Most intense product ions.

Table IV.2.2.- Matrix matched calibration data of the selected group of mycotoxins.

Analyte	Matrix	Studied linear range (µg/L)	Regression equation (n=9)		R^2	$S_{y/x}$	LOD[a,c]	LOQ[b,c]
			b ± S_b·$t_{(0.05;7)}$	a ± S_a·$t_{(0.05;7)}$				
ZEN	Whole cow milk	20-400	43.76 ± 0.85	0.25 ± 0.14	0.9995	0.12	0.65	2.2
	Skimmed cow milk	20-400	45.02 ± 1.20	0.04 ± 0.20	0.9991	0.17	0.34	1.1
	Semi-skimmed goat milk	20-400	37.85 ± 2.40	0.65 ± 0.42	0.9960	0.32	0.96	3.2
	Unsweetened natural yogurt	20-400	36.79 ± 2.45	0.58 ± 0.41	0.9944	0.36	0.65	2.2
β-ZAL	Whole cow milk	20-400	23.31 ± 0.54	0.13 ± 0.09	0.9993	0.08	4.8	16
	Skimmed cow milk	20-400	28.80 ± 1.00	-0.22 ± 0.16	0.9985	0.15	2.6	8.6
	Semi-skimmed goat milk	20-400	24.57 ± 1.73	-0.21 ± 0.30	0.9951	0.23	4.5	15
	Unsweetened natural yogurt	20-400	23.59 ± 0.92	0.18 ± 0.15	0.9981	0.13	5.8	20
α-ZAL	Whole cow milk	20-400	40.77 ± 1.22	-0.05 ± 0.20	0.9989	0.18	2.7	8.9
	Skimmed cow milk	20-400	46.65 ± 1.89	-0.43 ± 0.31	0.9980	0.27	1.5	4.9
	Semi-skimmed goat milk	20-400	44.36 ± 2.67	-0.43 ± 0.47	0.9964	0.36	2.6	8.7
	Unsweetened natural yogurt	20-400	41.93 ± 1.43	-0.08 ± 0.24	0.9986	0.21	3.2	11
β-ZEL	Whole cow milk	20-400	42.34 ± 0.76	-0.01 ± 0.13	0.9996	0.11	2.1	7.1
	Skimmed cow milk	20-400	51.62 ± 2.28	-0.54 ± 0.38	0.9976	0.33	1.1	3.8
	Semi-skimmed goat milk	20-400	43.79 ± 2.35	-0.38 ± 0.41	0.9971	0.31	2.5	8.2
	Unsweetened natural yogurt	20-400	43.09 ± 1.35	-0.15 ± 0.22	0.9988	0.20	2.1	7.0
α-ZEL	Whole cow milk	20-400	28.19 ± 0.39	-0.11 ± 0.06	0.9998	0.57	2.5	8.3
	Skimmed cow milk	20-400	33.76 ± 1.72	-0.41 ± 0.28	0.9967	0.25	1.5	4.9
	Semi-skimmed goat milk	20-400	31.41 ± 1.32	-0.23 ± 0.23	0.9982	0.18	3.0	9.9
	Unsweetened natural yogurt	20-400	31.41 ± 0.97	-0.17 ± 0.16	0.9988	0.14	2.8	9.3
ZAN	Whole cow milk	20-400	61.84 ± 2.18	0.57 ± 0.36	0.9984	0.32	0.47	1.6
	Skimmed cow milk	20-400	64.80 ± 2.69	0.36 ± 0.44	0.9978	0.39	0.29	0.98
	Semi-skimmed goat milk	20-400	55.13 ± 2.96	0.11 ± 0.52	0.9971	0.40	0.51	1.7
	Unsweetened natural yogurt	20-400	52.77 ± 4.27	0.10 ± 0.70	0.9919	0.62	0.41	1.4

b: slope; S_b: standard deviation of the slope; a: intercept; S_a: standard deviation of the intercept; $S_{y/x}$: standard deviation of the estimate.
[a] Calculated as the concentration associated to a S/N ratio of 3. [b] Calculated as the concentration associated to a S/N ratio of 10. [c] LODs and LOQs expressed in µg/L for milk and in µg/kg for yogurt.

Once calibration curves were obtained, a recovery study was carried out extracting three different milk samples (whole cow milk, skimmed cow milk and semi-skimmed goat milk) and a yogurt sample (an unsweetened natural yogurt) previously spiked with a mixture of the analytes of interest at two levels of concentration (five extractions were carried out at each level). In order to check that the matrices used to develop this study were not contaminated, non-spiked samples were submitted to the m-μ-dSPE and injected in the HPLC system. Table IV.2.3 shows the relative recovery values obtained after the extraction of the target analytes from milk and yogurt samples, as well as the LODs and LOQs of the method. The recovery percentages obtained from milk samples were between 77 and 120 %, with RSDs lower than 16 %. Besides, and although the complexity of the yogurt sample is higher than that of milk, good relative recovery values were also obtained for yogurt (between 78 and 102 %), with acceptable RSDs (lower than 16 % in all cases). LODs of the method were in the range 0.21–4.8 μg/L for milk samples and in the range 0.29–4.5 μg/kg for yogurt, while LOQs of the method were comprised between 0.71 and 16 μg/L for milk and between 0.95 and 15 μg/kg for yogurt samples. These limits have been calculated from the instrumental LODs and LOQs, considering the recovery percentages obtained for each analyte and the preconcentration of the sample.

Although certain papers have suggested that this type of m-NPs could be reused [169], because of the simplicity and low cost of the synthesis of the m-NPs, they were only used once, also trying to avoid excessive solvent consumption for their washing as well as possible carry over effects.

As it was mentioned before, up to the date in which this work was developed, few papers could be found in the literature in which the determination of ZEN and its derivatives in milk and yogurt samples had been carried out [101,357,365-370]. It should be highlighted that most papers had determined a reduced number of ZEN-family mycotoxins. In fact, just three of them had studied the same six analytes analysed in this work [365,367,369], while others had only analysed a lower number of them [101,357,366] or even just ZEN and not its derivatives [368,370].

Regarding the LODs of the method, and despite almost all papers had applied a different methodology for the analysis, most of them had obtained values in the range of few μg/L or μg/kg [101,369] which were very similar to the ones obtained in this work, which were between 0.21 and 4.8 μg/L for milk samples and between 0.29 and 4.5 μg/L for yogurt samples. However, other authors had been able to achieve LODs slightly lower

[357,365-367,370], probably due to several factors like the higher volume of sample taken at the beginning of the process and to a higher sensitivity of the detector employed.

Table IV.2.3.- Mean relative recovery percentages (n=5), RSD values, LODs and LOQs of the method after the application of the Fe_3O_4@pDA-m-μ-dSPE procedure to spiked whole cow milk, skimmed cow milk, semi-skimmed goat milk and unsweetened natural yogurt samples.

Analyte	Sample	Relative recovery (RSD, %)		LOD_{method} [a]	LOQ_{method} [a]
		Level 1 (60 μg/L)	Level 2 (400 μg/L)		
ZEN	Whole cow milk	97 (6)	120 (6)	0.49	1.6
	Skimmed cow milk	108 (4)	120 (5)	0.25	0.83
	Semi-skimmed goat milk	120 (9)	112 (8)	0.79	2.6
	Unsweetened natural yogurt	98 (9)	90 (11)	0.45	1.5
β-ZAL	Whole cow milk	82 (10)	84 (7)	4.7	16
	Skimmed cow milk	111 (12)	91 (13)	2.2	7.2
	Semi-skimmed goat milk	97 (15)	82 (3)	4.8	16
	Unsweetened natural yogurt	87 (15)	78 (12)	4.5	15
α-ZAL	Whole cow milk	100 (5)	99 (6)	2.2	7.3
	Skimmed cow milk	102 (9)	97 (9)	1.2	4.2
	Semi-skimmed goat milk	97 (10)	93 (5)	2.6	8.6
	Unsweetened natural yogurt	102 (11)	86 (13)	2.2	7.3
β-ZEL	Whole cow milk	77 (10)	95 (6)	2.0	6.8
	Skimmed cow milk	87 (7)	93 (13)	1.1	3.58
	Semi-skimmed goat milk	96 (5)	93 (4)	2.5	8.2
	Unsweetened natural yogurt	99 (13)	83 (14)	1.5	5.0
α-ZEL	Whole cow milk	89 (10)	107 (4)	2.1	7.0
	Skimmed cow milk	96 (4)	105 (9)	1.3	4.2
	Semi-skimmed goat milk	114 (5)	99 (6)	2.7	8.9
	Unsweetened natural yogurt	99 (14)	90 (12)	1.9	6.5
ZAN	Whole cow milk	99 (6)	120 (6)	0.35	1.2
	Skimmed cow milk	113 (5)	120 (5)	0.21	0.71
	Semi-skimmed goat milk	119 (9)	113 (8)	0.42	1.4
	Unsweetened natural yogurt	96 (11)	93 (11)	0.29	0.95

[a] LODs and LOQs of the method expressed in μg/L for milk and in μg/kg for yogurt.

It should be remarked that the use of m-μ-dSPE has clear advantages over previous SPE procedures used for the extraction of these compounds from milk matrices, including large surface-to-volume ratio, rapid extraction kinetics, high extraction efficiency, and reduction of sample amounts as well as toxic organic solvents, which is in accordance with current analytical trends.

IV.2.5.- Conclusions

From the results obtained in this section, the following conclusions can be drawn:

- A methodology based on a m-μ-dSPE procedure using Fe_3O_4@pDA m-NPs as sorbent followed by HPLC-MS/MS determination has been applied for the extraction of six mycotoxins (ZEN, α-ZAL, β-ZAL, α-ZEL and β-ZEL and ZAN) with oestrogenic activity from different milk (whole and skimmed cow milk and semi-skimmed goat milk) and yogurt (an unsweetened natural yogurt) samples.
- The application of a deproteinization step was necessary before the extraction, since milk and yogurt proteins have to be removed in order to reduce matrix effects and to avoid their strong retention in the HPLC column during mycotoxins determination. Thus, two slightly different deproteinization procedures were optimised and successfully applied to both kind of samples.
- The application of a m-μ-dSPE procedure previously optimised by our group for the extraction of oestrogenic compounds from water samples was directly and successfully applied to the extraction of the selected mycotoxins after the deproteinization process. Validation of the methodology showed that good recovery values (between 77 and 120 %) were obtained, with LOQs in the range of few μg/L and μg/kg in milk and yogurt samples, respectively. These data were in accordance with the other works previously published in the literature in which Fe_3O_4@pDA m-NPs were applied, which demonstrate the good performance of this kind of nanomaterial for the extraction of the selected mycotoxins from milk and dairy products.
- The synthesis of Fe_3O_4@pDA m-NPs was simple and straightforward with a relatively low consumption of reagents and solvents. Since only 80 mg of m-NPs are necessary for each extraction and they can be used even several weeks after their synthesis, a single synthetic batch is enough to develop a high number of extractions.
- This method constitutes a fast, simple and environmentally friendly alternative for the extraction of mycotoxins and represents the first application of Fe_3O_4@pDA m-NPs as sorbents for the extraction of this kind of compounds from milk and yogurt and their determination by HPLC–MS/MS.

IV.3.- Determination of PAEs and DEHA in water samples using core-shell Fe_3O_4@pDA m-NPs and GC-MS/MS

In this section, core-shell Fe_3O_4@pDA m-NPs were applied for the first time as sorbent for the extraction of a group of eleven PAEs of interest (i.e. DEP, DPP, DBP, DIPP, DNPP, BBP, DCHP, DEHP, DNOP, DINP and DIDP) and DEHA from different water samples (Milli-Q, mineral, tap, pond and waste water). Analysis were carried out by GC-QqQ-MS/MS. Parameters that affect the extraction performance were optimised following a step by step approach. The methodology was validated for the five selected water samples by means of matrix-matched calibration, and precision and trueness studies, using DBP-d_4 as IS. Matrix effects were evaluated, and real samples were also analysed.

IV.3.1.- Background

As it was already commented, humanity is living in what it could be called "the plastic era" or "the plastic age", in the sense that plastics have massively replaced classic materials like glass, wood, paper or metal. In fact, it is even said nowadays that if plastics suddenly disappeared, it would take quite a long time to find out a suitable replacement for many of their applications.

From a toxicological point of view, plastics are inert polymeric structures of high molecular weight that cannot be normally absorbed by the human body. However, they contain additives of low molecular weight, catalysers, solvents and trace amounts of free monomers, which are not chemically linked to the polymeric matrix and, therefore, can be easily released to different environmental compartments [377] with their subsequent human exposure. A group of additives with such migration capacity are PAEs, which are widely used as plasticisers to increase the flexibility of plastics and to facilitate their transformation [151,378]. Although many consumer products may also contain them, including household furnishings, cosmetics, personal care products, pharmaceuticals, paints, etc., the fact that PAEs are generally lipophilic clearly influences their leaching and environmental fate and partitioning characteristics [151,379].

It is widely known nowadays that many PAEs have an important endocrine disrupting activity, even at extremely low concentrations [128,380-382]. For this reason, several administrations/organizations have listed certain PAEs as priority compounds to be monitored, as well as TDIs for some of them. This is the case of the US EPA [135] which has initiated actions to address the manufacturing, processing, distribution in commerce,

and/or use of eight PAEs (DBP, DIBP, BBP, DNPP, DEHP, DNOP, DINP and DIDP), or the EFSA which, as a result of the critical effects of DEHP for reproduction, has fixed a TDI of 0.05 mg/kg of body weight for such compound, and recommends that improved estimates of exposure to DEHP from all sources along with their relative importance should be provided [136]. Furthermore, the WHO also established a TDI of 25 µg/kg of body weight for DEHP, which yields a guideline value of 8 µg/L in drinking water [137]. In fact, DEHP is currently in the EU list of priority substances in the field of water policy [53]. Besides, the EU has even restricted the use of several PAEs for certain applications. As an example, DEHP, DBP, BBP, DINP, DIDP and DNOP shall not be used at specific concentrations in plastic used for toys and childcare articles [383] (specifically, DINP, DIDP and DNOP in toys and childcare articles which can be placed in the mouth by children), this also happens for DBP, DEHP, BBP, DINP and DIDP in food contact plastic materials [133]. From all the above, it is clear that there currently exits an important concern of the risks of certain PAEs for human's health, and that there is a necessity of developing or implementing feasible analytical methods able to provide data concerning their presence in different types of samples.

The analysis of PAEs in any analytical laboratory is a challenge, in the sense that plastics are also used during the analytical process and that there exists a certain degree of contamination in the laboratory [119,151,384]. In order to minimise or to correct such contamination, certain precautions should be taken into account [384], among which it is extremely necessary to consider the analysis of procedural blanks on a daily basis. Apart from that, glassware, teflon, aluminium or stainless-steel materials should be used. If volumetric glassware is necessary, it should be cleaned with strong oxidising agents, while if non-volumetric glassware is used, it can be calcined at high temperature. Furthermore, when Milli-Q water is used, it should be first analysed in order to find out if it can be a contamination source or not, since water purification systems are also made of plastics. Besides, the use of high purity solvents and phthalate-free gloves and pipette tips is also compulsory.

As also commented in the previous section, in a preceding work carried out by our research group [276], core-shell Fe_3O_4@pDA m-NPs were prepared and characterised in the laboratory and later successfully applied as sorbents for the m-µ-dSPE of twelve oestrogenic compounds of interest from different water samples. Such m-NPs were also successfully applied in the previous section of this PhD Thesis to the extraction of a group of mycotoxins from milk and yogurt samples. The developed procedure

(including Fe_3O_4@pDA m-NPs synthesis) constituted a simple, fast and environmentally friendly method to determine the selected compounds. To the best of our knowledge, such m-NPs had only been applied in m-SPE in very few occasions [169,264,276,373-375,385,386], most of them for the analysis of oestrogens. In particular, they had been successfully used for the extraction of four aflatoxins from red wine [264], berberine from a Chinese medical plant (*Cortex Phellodendri*) [374], six PAHs from environmental water samples [375], seven antibiotics, three perfluorinated compounds and benzo[a]pyrene from lake and tap water [373], four oestrogenic compounds from different water samples [386], seventeen oestrogenic compounds from river water [169], seven oestrogens and five mycoestrogens from different water samples [276], and, as described in Section IV.2, for the extraction of six mycoestrogens from milk and yogurt samples. As a result, and despite their inherent advantages and the simplicity and rapidity of m-dSPE, Fe_3O_4@pDA m-NPs had not been previously applied as sorbents for the extraction of PAEs.

IV.3.2.- Specific objectives

Considering all the above-mentioned, the following specific objectives were established:

- The preparation of core-shell Fe_3O_4@pDA m-NPs and their possible application as sorbent for the m-μ-dSPE of a group of eleven PAEs (DEP, DPP, DBP, DIPP, DNPP, BBP, DCHP, DEHP, DNOP, DINP and DIDP) and a DEHP commonly used substitutive (DEHA) from five water samples of different origins (Milli-Q, mineral, tap, pond and waste water), as well as their subsequent determination by GC-MS/MS.
- The development of a GC-MS/MS method which allows the suitable separation and determination of the selected group of analytes with a high sensitivity.
- The optimisation of the parameters that affect the m-μ-dSPE performance, including pH of the sample, desorption solvent type, sorbent amount and desorption solvent volume.
- The validation of the whole methodology in each selected sample in terms of linearity, LODs and LOQs of the method, precision and trueness.
- The analysis of several real water samples in which DEHA or the selected PAEs could appear.

IV.3.3.- GC-QqQ-MS/MS analysis

As target analytes a group of 11 PAEs of interest and the adipate DEHA were selected, and DBP-d_4 was used as IS. Among the selected compounds, those PAEs restricted by the EU for toys and childcare articles (DEHP, DBP, BBP, DINP, DIDP and DNOP) were included, in particular, DEHP, which is on the list of priority substances of the UE to be monitored in water samples [53]. Furthermore, seven out of the eight PAEs of high interest for the US EPA (DBP, BBP, DNPP, DEHP, DNOP, DINP, and DIDP) were also considered [135].

As it has already been mentioned, PAEs have enough volatility to be determined by GC without derivatisation [387], though many LC applications have also been proposed [151] as well as few CE methods [161,162,388,389]. In this case, DEHA and the selected group of PAEs were separated by GC–MS/MS using two non-polar columns of 15 m (HP–5 ms), the first of them operated in the backflush mode once the analytes were eluted. The separation and detection conditions selected are shown in Section III.5.2, and MS/MS transitions and collision energies are shown in Table IV.3.1.

Table IV.3.1.- MS/MS transitions and collision energies of DEHA and the target PAEs.

Analyte	Retention time (min)	MW (g/mol)	Quantifier transitions (m/z)	Collision energy (eV)	Qualifier transitions (m/z)	Collision energy (eV)
DEP	7.74	222.09	149.0 → 65.0	25.0	177.0 → 149.0	7.5
DPP	8.91	250.12	149.0 → 65.0	25.0	209.0 → 149.0	5.0
DBP-d_4	10.58	282.37	153.0 → 69.0	25.0	227.0 → 153.0	5.0
DBP	10.60	278.15	149.0 → 65.0	25.0	223.0 → 149.0	7.5
DIPP	11.89	306.18	149.0 → 65.0	27.5	237.0 → 149.0	7.5
DNPP	13.01	306.18	149.0 → 65.0	25.0	237.0 → 149.0	5.0
BBP	16.29	312.14	149.0 → 65.0	25.0	206.0 → 149.0	5.0
DEHA	17.15	370.31	129.0 → 101.0	5.0	241.0 → 111.0	10.0
DCHP	19.46	330.18	149.0 → 65.0	27.5	167.0 → 149.0	5.0
DEHP	20.09	390.28	149.0 → 65.0	27.5	279.0 → 149.0	15.0
DNOP	23.92	390.28	149.0 → 65.0	27.5	279.0 → 149.0	5.0
DINP	26.40	418.31	149.0 → 65.0	27.5	293.0 → 149.0	5.0
DIDP	28.94	446.34	149.0 → 65.0	27.5	307.0 → 149.0	7.5

Concerning MS/MS conditions, one or two precursor ions were selected as well as two product ions as quantifier and qualifier (see Table IV.3.1) as indicated by Commission Decision 2002/657/EC [390].

In order to demonstrate the linearity of the developed GC–MS method, instrumental calibration curves based on analyte peak area/IS peak area ratios were obtained by injecting nine increasing concentration levels (n=9) in triplicate for each analyte, obtaining R^2 values higher than 0.9904 for all of them. Considering the good instrumental sensitivity, the lowest calibration levels (LCLs) were established at 0.5 µg/L (see Table IV.3.2).

Table IV.3.2.- Instrumental calibration data of DEHA and the selected PAEs.

Analyte	Studied linear range (µg/L)	Regression equation (n=9)		$S_{y/x}$	R^2
		$b \pm s_b \cdot t_{(0.05;7)}$	$a \pm s_a \cdot t_{(0.05;7)}$		
DEP	0.5-500	$8.67 \cdot 10^{-3} \pm 6.28 \cdot 10^{-4}$	$3.45 \cdot 10^{-2} \pm 1.14 \cdot 10^{-1}$	$1.25 \cdot 10^{-1}$	0.9935
DPP	0.5-500	$1.16 \cdot 10^{-2} \pm 9.75 \cdot 10^{-4}$	$-1.43 \cdot 10^{-1} \pm 1.71 \cdot 10^{-1}$	$1.87 \cdot 10^{-1}$	0.9913
DBP	0.5-500	$1.29 \cdot 10^{-2} \pm 1.13 \cdot 10^{-3}$	$-1.59 \cdot 10^{-1} \pm 1.99 \cdot 10^{-1}$	$2.18 \cdot 10^{-1}$	0.9904
DIPP	0.5-500	$6.12 \cdot 10^{-3} \pm 4.31 \cdot 10^{-4}$	$-6.90 \cdot 10^{-2} \pm 7.70 \cdot 10^{-2}$	$7.86 \cdot 10^{-2}$	0.9950
DNPP	0.5-500	$8.96 \cdot 10^{-3} \pm 6.97 \cdot 10^{-4}$	$-1.12 \cdot 10^{-1} \pm 1.24 \cdot 10^{-1}$	$1.27 \cdot 10^{-1}$	0.9940
BBP	0.5-500	$9.50 \cdot 10^{-4} \pm 5.97 \cdot 10^{-5}$	$-1.04 \cdot 10^{-2} \pm 1.11 \cdot 10^{-2}$	$1.14 \cdot 10^{-2}$	0.9961
DEHA	0.5-500	$9.91 \cdot 10^{-4} \pm 6.67 \cdot 10^{-5}$	$-1.07 \cdot 10^{-2} \pm 1.19 \cdot 10^{-2}$	$1.22 \cdot 10^{-2}$	0.9955
DCHP	0.5-500	$3.30 \cdot 10^{-3} \pm 2.16 \cdot 10^{-4}$	$-3.55 \cdot 10^{-2} \pm 3.90 \cdot 10^{-2}$	$3.98 \cdot 10^{-2}$	0.9957
DEHP	0.5-500	$3.23 \cdot 10^{-3} \pm 2.04 \cdot 10^{-4}$	$-2.89 \cdot 10^{-2} \pm 3.76 \cdot 10^{-2}$	$3.84 \cdot 10^{-2}$	0.9960
DNOP	0.5-500	$4.13 \cdot 10^{-3} \pm 2.86 \cdot 10^{-4}$	$-5.07 \cdot 10^{-2} \pm 5.21 \cdot 10^{-2}$	$5.32 \cdot 10^{-2}$	0.9952
DINP	0.5-500	$2.74 \cdot 10^{-3} \pm 2.03 \cdot 10^{-4}$	$-3.34 \cdot 10^{-2} \pm 3.57 \cdot 10^{-2}$	$3.65 \cdot 10^{-2}$	0.9946
DIDP	0.5-500	$2.00 \cdot 10^{-3} \pm 1.45 \cdot 10^{-4}$	$-2.43 \cdot 10^{-2} \pm 2.60 \cdot 10^{-2}$	$2.65 \cdot 10^{-2}$	0.9948

b: slope; S_b: standard deviation of the slope; a: intercept; S_a: standard deviation of the intercept.

Figure IV.3.1 shows the TIC obtained under full scan mode as well as the chemical structures of the selected compounds. As can be seen in the figure, all PAEs were completely separated in spite of the use of a MS detector. The main reason for maintaining such a good separation was the fact that, as it has already been described in the Introduction Section [142,391], PAEs share the same parent/precursor ion for the quantification transition (the most intense) and which corresponds to fragment 149 m/z [150], except for the IS (DBP-d_4) and the adipate.

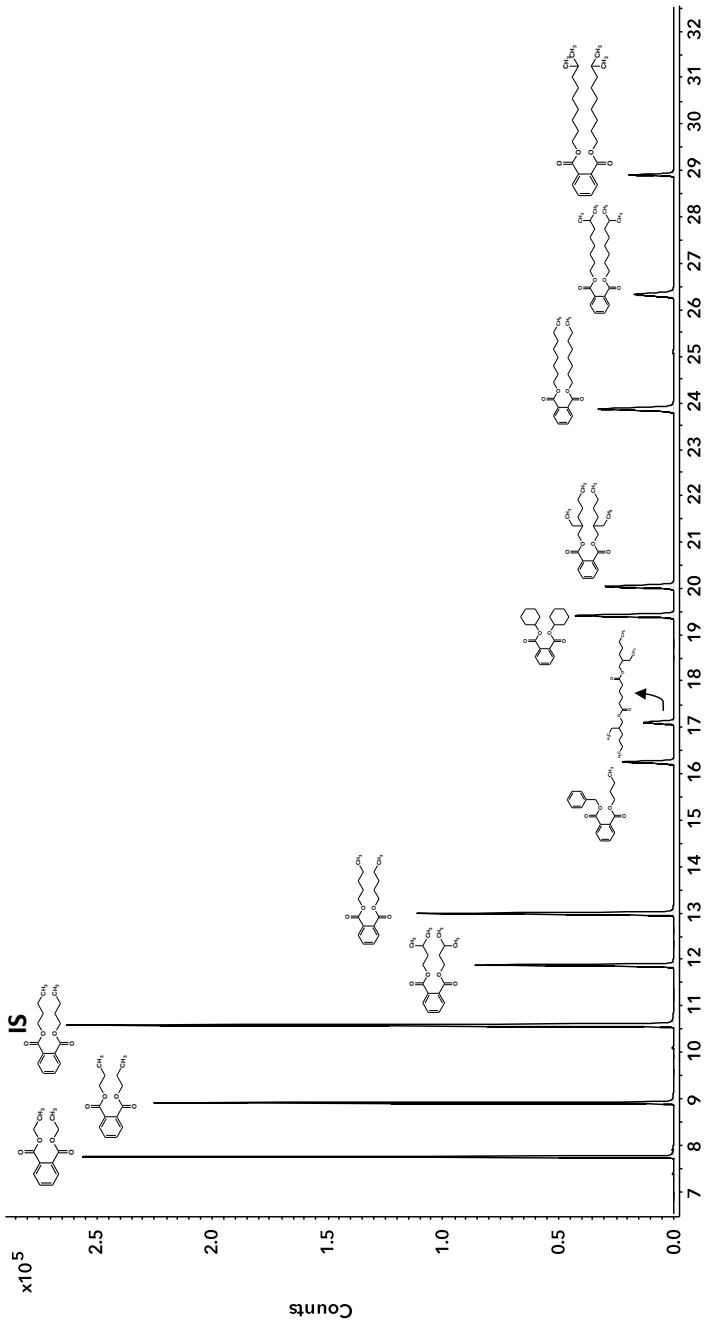


Figure IV.3.1.- GC-MS TIC obtained under full scan mode. Concentration of all the analytes: 250 µg/L.

IV.3.4.- Fe_3O_4@pDA-m-µ-dSPE optimisation

With the aim of extending the application of Fe_3O_4@pDA m-NPs for the extraction of PAEs from water samples, m-µ-dSPE conditions (sample pH, sorbent amount and desorption solvent type and volume) were initially optimised. For this purpose and trying not to introduce important matrix effects and to have a better vision of the influence of each factor in the extraction process, the optimisation of the influencing factors was developed with Milli-Q water (25 mL) prior to the application of the methodology to the samples of study. The influence of each parameter was evaluated considering absolute recovery values obtained in each experiment. For this purpose, samples were spiked with the target analytes and the IS before or after the extraction procedure. Recovery values were calculated dividing the peak areas obtained when samples were spiked before the extraction process by those obtained spiking after the extraction process multiplied by 100. When the content of the target analytes in different samples was evaluated, the IS was added before the extraction process.

Based on a previous study carried out by the group in which the present Thesis was developed [276], 25 mL of Milli-Q water were initially extracted with 60 mg of sorbent and desorbed with 6 mL of MeOH. The solvent containing the target analytes was evaporated at 40 ºC in a rotavapor, reconstituted in 500 µL of cyclohexane and injected in the GC–MS/MS system.

IV.3.4.1.- Sample pH effect

From the previous characterisation study of the synthesised m-NPs [276] it could be concluded that the isoelectric point of Fe_3O_4@pDA m-NPs was 4.3. Consequently, the pDA film is positively charged at pH below 4 and, therefore, it displays some permeability and favourable interactions with anionic species. On the contrary, at higher pH values, the film is permeable to cations and presents repulsions for anionic species. However, and concerning the extraction of PAEs, since these compounds are not ionisable in aqueous solution, the pH of the sample should not have a clear influence on the extraction recovery. In spite of this fact, the effect of the pH of the aqueous sample on the extraction was studied between 2.0 and 10.0 (duplicate extractions were carried out in each case). As expected (see Figure IV.3.2.), it was found out that the pH did not have any influence on the extraction performance except at pH 6.0, at which recovery values slightly increased. Therefore, it was decided to adjust the pH of all the samples to 6.0 for further

analyses which is also a pH value very close to that of a wide variety of environmental water samples.

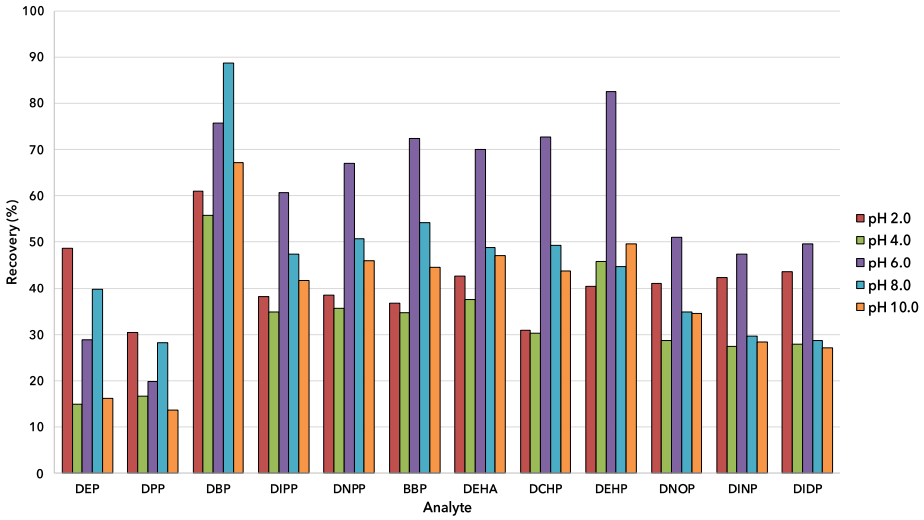


Figure IV.3.2.- Effect of the sample pH on the recovery of DEHA and the selected PAEs after the m-μ-dSPE procedure. Extraction conditions: 25 mL of spiked Milli-Q water at 2.5 μg/L and 60 mg of Fe_3O_4@pDA m-NPs and 6 mL of desorption solvent.

IV.3.4.2.- Desorption solvent type

Regarding the desorption solvent, several experiments were developed in duplicate desorbing with 6 mL of acetone, MeOH, ACN or DCM. In this case, 25 mL of Milli-Q water at pH 6.0 were previously extracted with 60 mg of m-NPs. Results obtained are shown in Figure IV.3.3. As can be seen in the figure, while acetone hardly desorbed the target compounds (recovery values were below 28 %) the highest extraction recovery percentages were obtained with DCM (recovery ranged between 71 and 114 %), especially for DEP and DPP for which an increase of the recovery values up to 89 and 76 %, respectively, were achieved with DCM compared with the rest of the solvents used. As a result, DCM was selected.

IV.3.4.3.- m-NPs amount and desorption solvent volume

Subsequent experiments were developed in order to study the effect of the amount of sorbent together with the desorption solvent volume. For this purpose, 50, 60,

70 and 80 mg of sorbent were tested under previous extraction conditions (25 mL of Milli-Q water at pH 6.0 and desorption with DCM). Results obtained are shown in Figure IV.3.4. It was found out that 60 mg were enough to obtain a quantitative extraction of the selected analytes, since recovery values higher than 70 % were obtained for all the target analytes. At the same time, different volumes of DCM were tested finding that 6 mL were also enough to quantitatively desorb the target analytes (see Figure IV.3.5). For most analytes both 6 and 7 mL provided similar results and therefore, the lowest amount was selected.

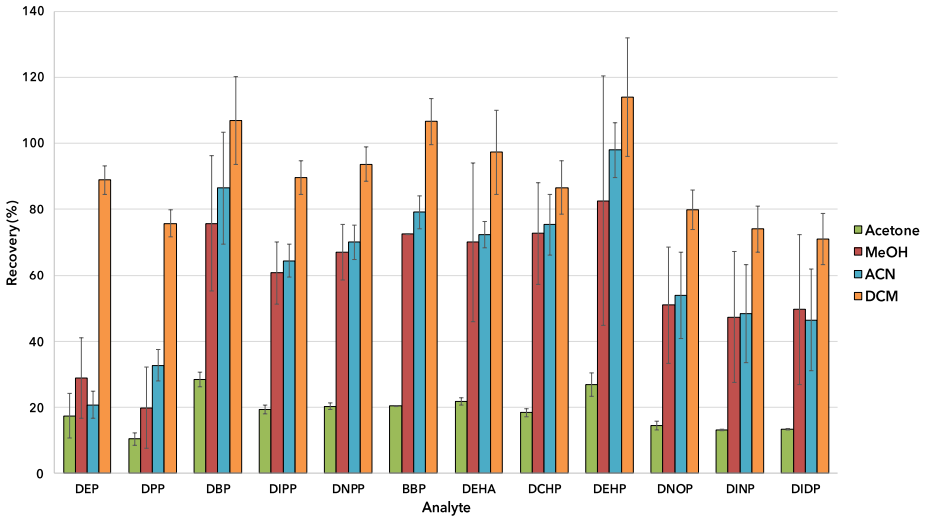


Figure IV.3.3.- Effect of the type of solvent used during the desorption step on the recovery of DEHA and the selected PAEs after the m-μ-dSPE procedure (n=2). Extraction conditions: 25 mL of spiked Milli-Q water at 2.5 μg/L and pH 6.0, 60 mg of Fe_3O_4@pDA m-NPs and 6 mL of desorption solvent.

IV.3.5.- Fe_3O_4@pDA-m-μ-dSPE-GC-QqQ-MS/MS method validation

Once m-μ-dSPE conditions were optimised, the method was applied and validated for the analysis of different types of water samples (Milli-Q, mineral, tap, pond and waste water). For this purpose, and since PAEs may also appear in the studied samples, blank samples were analysed when any of the target analytes were detected. In particular, and as it will be later shown, DEP and DBP were found at higher concentrations. Consequently, and in order to carry out an appropriate validation of the procedure for all the target analytes, the peak areas of these analytes were subtracted when necessary.

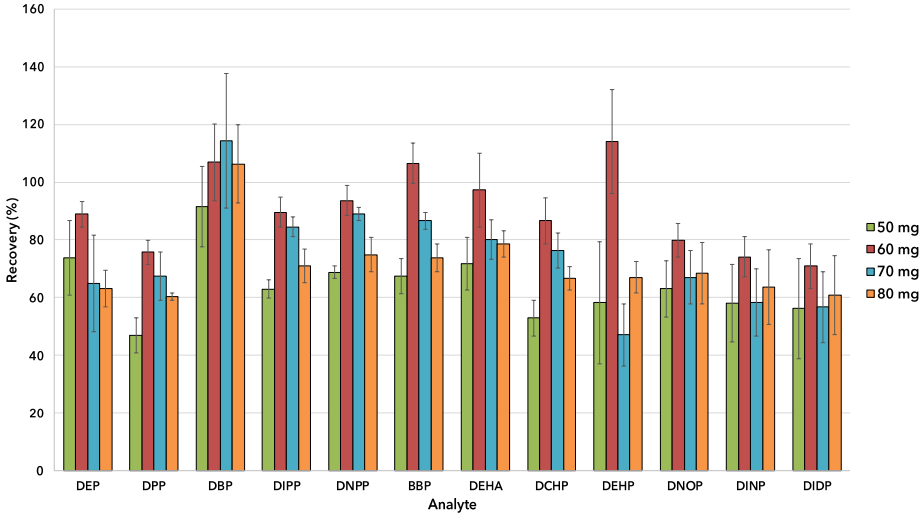


Figure IV.3.4.- Effect of the amount of sorbent used during the extraction step on the recovery of DEHA and the selected PAEs for the m-μ-dSPE procedure (n=2). Extraction conditions: 25 mL of spiked Milli-Q water at 2.5 μg/L and pH 6.0 and 6 mL of DCM as desorption solvent.

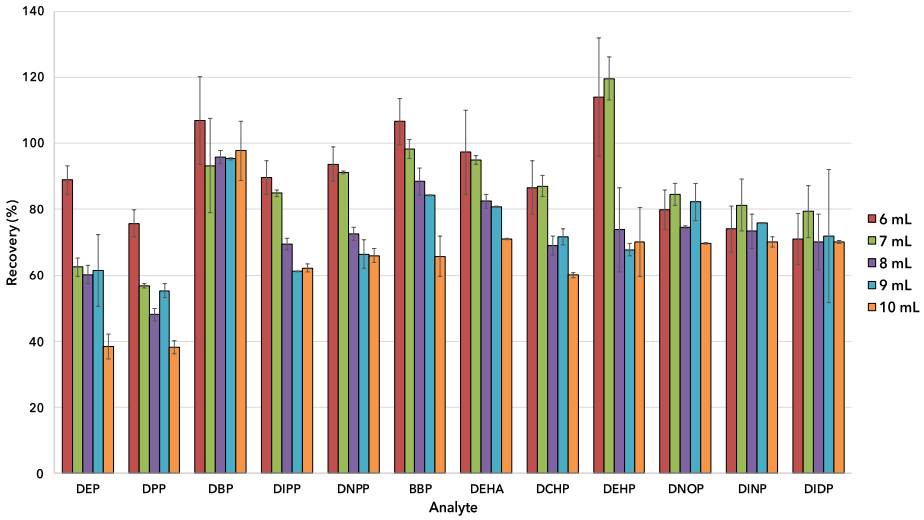


Figure IV.3.5.- Effect of the volume of the desorption solvent on the recovery of DEHA and the selected PAEs after the m-μ-dSPE procedure (n=2). Extraction conditions: 25 mL of spiked Milli-Q water at 2.5 μg/L and pH 6.0, 60 mg of Fe_3O_4@pDA m-NPs and DCM as desorption solvent.

IV.3.5.1.- Matrix effect evaluation and matrix-matched calibration

As it is well-known, the injection of standards in the sample matrix could give biased results by suppressing or by increasing the chromatographic signal [392]. In particular, in GC it is very frequent to obtain an increase in the detector response, which occurs when active surfaces in the system (injector, column, and detector) cause retention and/or degradation of analytes [393]. One of the most commonly used methods to compensate matrix effects in GC is the development of a matrix-matched calibration which equalises the response enhancement for calibration standards and sample extracts as well as the use of ISs.

In this work, matrix-matched calibration curves were obtained by injecting nine different levels of concentrations (n=9) in triplicate. The IS was added to the matrix after the extraction procedure at a concentration of 150 µg/L (concentration in the final extract). Table IV.3.3 shows the calibration data obtained (based on the ratio between analyte and IS peak areas) including the studied linear range, the full calibration curve with the confidence intervals and the R^2 values which were higher than 0.9904 in all cases. For all compounds and matrices, the LCL was 0.5 µg/L except for DINP in tap, pond and waste water which was 1 µg/L. The matrix effect was calculated using the following equation [394]:

$$\text{Matrix effect \%} = \frac{\text{Slope of matrix-matched calibration curve} - \text{Slope of standard in solvent calibration curve}}{\text{Slope of standard in solvent calibration curve}} \cdot 100$$

Matrix effect percentages are indicated in Table IV.3.4. As can be seen in the table, a high signal suppression (< −50 %) was observed for DEP and DPP in Milli-Q water as well as for DIDP and DINP for waste water. On the contrary, a high signal enhancement (> 50 %) was observed for BBP in all types of samples, except in waste water in which a slightly lower signal enhancement (43 %) was observed. For the rest of the analytes and matrices either a moderate (in most cases) or a negligible effect was observed. In general, it was found that the most volatile analytes (DEP, DPP, DBP, DIPP and DNPP) had a similar matrix effect in all water samples. All these results clearly indicated the necessity of taking matrix effects into account for the rest of the study, that is, the development of matrix-matched calibration (Table IV.3.3).

Table IV.3.3.- Matrix-matched calibration data of DEHA and the selected PAEs.

Analyte	Water sample	Studied linear range (µg/L)	Regression equation (n=9)		$S_{y/x}$	R^2
			$b \pm s_b \cdot t_{(0.05;7)}$	$a \pm s_a \cdot t_{(0.05;7)}$		
DEP	Milli-Q	0.5-500	$3.32 \cdot 10^{-3} \pm 2.69 \cdot 10^{-4}$	$5.33 \cdot 10^{-1} \pm 6.36 \cdot 10^{-2}$	$6.36 \cdot 10^{-2}$	0.9911
	Mineral	0.5-500	$4.70 \cdot 10^{-3} \pm 2.32 \cdot 10^{-4}$	$1.97 \cdot 10^{-1} \pm 5.07 \cdot 10^{-2}$	$5.83 \cdot 10^{-2}$	0.9957
	Tap	0.5-500	$4.32 \cdot 10^{-3} \pm 2.17 \cdot 10^{-4}$	$1.08 \cdot 10^{-1} \pm 4.73 \cdot 10^{-2}$	$5.44 \cdot 10^{-2}$	0.9956
	Pond	0.5-500	$5.12 \cdot 10^{-3} \pm 3.79 \cdot 10^{-4}$	$1.65 \cdot 10^{-1} \pm 8.27 \cdot 10^{-2}$	$9.51 \cdot 10^{-2}$	0.9904
	Waste	0.5-500	$4.46 \cdot 10^{-3} \pm 3.06 \cdot 10^{-4}$	$6.27 \cdot 10^{-2} \pm 7.65 \cdot 10^{-2}$	$7.34 \cdot 10^{-2}$	0.9945
DPP	Milli-Q	0.5-500	$5.71 \cdot 10^{-3} \pm 1.88 \cdot 10^{-4}$	$1.43 \cdot 10^{-2} \pm 3.95 \cdot 10^{-2}$	$4.54 \cdot 10^{-2}$	0.9981
	Mineral	0.5-500	$6.75 \cdot 10^{-3} \pm 4.07 \cdot 10^{-5}$	$-2.29 \cdot 10^{-3} \pm 8.56 \cdot 10^{-3}$	$9.83 \cdot 10^{-3}$	0.9999
	Tap	0.5-500	$6.37 \cdot 10^{-3} \pm 5.33 \cdot 10^{-5}$	$-2.11 \cdot 10^{-3} \pm 1.12 \cdot 10^{-2}$	$1.29 \cdot 10^{-2}$	0.9999
	Pond	0.5-500	$7.36 \cdot 10^{-3} \pm 1.94 \cdot 10^{-4}$	$1.11 \cdot 10^{-2} \pm 4.09 \cdot 10^{-2}$	$4.70 \cdot 10^{-2}$	0.9988
	Waste	0.5-500	$6.66 \cdot 10^{-3} \pm 3.39 \cdot 10^{-5}$	$-6.53 \cdot 10^{-3} \pm 7.12 \cdot 10^{-3}$	$8.18 \cdot 10^{-3}$	1.0000
DBP	Milli-Q	0.5-500	$7.20 \cdot 10^{-3} \pm 3.38 \cdot 10^{-4}$	$1.11 \cdot 10^{-1} \pm 7.86 \cdot 10^{-2}$	$7.39 \cdot 10^{-2}$	0.9972
	Mineral	0.5-500	$8.03 \cdot 10^{-3} \pm 3.39 \cdot 10^{-4}$	$2.86 \cdot 10^{-1} \pm 7.14 \cdot 10^{-2}$	$8.20 \cdot 10^{-2}$	0.9969
	Tap	0.5-500	$7.63 \cdot 10^{-3} \pm 1.28 \cdot 10^{-4}$	$1.90 \cdot 10^{-1} \pm 2.69 \cdot 10^{-2}$	$3.10 \cdot 10^{-2}$	0.9995
	Pond	0.5-500	$8.30 \cdot 10^{-3} \pm 3.89 \cdot 10^{-4}$	$3.45 \cdot 10^{-1} \pm 8.19 \cdot 10^{-2}$	$9.41 \cdot 10^{-2}$	0.9962
	Waste	0.5-500	$8.33 \cdot 10^{-3} \pm 2.99 \cdot 10^{-4}$	$3.33 \cdot 10^{-1} \pm 6.30 \cdot 10^{-2}$	$7.24 \cdot 10^{-2}$	0.9977
DIPP	Milli-Q	0.5-500	$4.35 \cdot 10^{-3} \pm 5.57 \cdot 10^{-5}$	$-1.41 \cdot 10^{-2} \pm 1.16 \cdot 10^{-2}$	$1.33 \cdot 10^{-2}$	0.9997
	Mineral	0.5-500	$3.95 \cdot 10^{-3} \pm 1.27 \cdot 10^{-4}$	$-2.92 \cdot 10^{-2} \pm 2.64 \cdot 10^{-2}$	$3.03 \cdot 10^{-2}$	0.9982
	Tap	0.5-500	$4.14 \cdot 10^{-3} \pm 1.24 \cdot 10^{-4}$	$-2.89 \cdot 10^{-2} \pm 2.59 \cdot 10^{-2}$	$2.97 \cdot 10^{-2}$	0.9984
	Pond	0.5-500	$4.15 \cdot 10^{-3} \pm 1.63 \cdot 10^{-4}$	$-2.95 \cdot 10^{-2} \pm 3.38 \cdot 10^{-2}$	$3.89 \cdot 10^{-2}$	0.9973
	Waste	0.5-500	$3.90 \cdot 10^{-3} \pm 1.09 \cdot 10^{-4}$	$-2.81 \cdot 10^{-2} \pm 2.27 \cdot 10^{-2}$	$2.61 \cdot 10^{-2}$	0.9986
DNPP	Milli-Q	0.5-500	$6.53 \cdot 10^{-3} \pm 1.24 \cdot 10^{-4}$	$-3.01 \cdot 10^{-2} \pm 2.57 \cdot 10^{-2}$	$2.95 \cdot 10^{-2}$	0.9994
	Mineral	0.5-500	$6.08 \cdot 10^{-3} \pm 2.92 \cdot 10^{-4}$	$-6.45 \cdot 10^{-2} \pm 6.05 \cdot 10^{-2}$	$6.94 \cdot 10^{-2}$	0.9960
	Tap	0.5-500	$6.03 \cdot 10^{-3} \pm 1.85 \cdot 10^{-4}$	$-4.73 \cdot 10^{-2} \pm 3.83 \cdot 10^{-2}$	$4.40 \cdot 10^{-2}$	0.9983
	Pond	0.5-500	$6.47 \cdot 10^{-3} \pm 2.65 \cdot 10^{-4}$	$-5.03 \cdot 10^{-2} \pm 5.48 \cdot 10^{-2}$	$6.29 \cdot 10^{-2}$	0.9971
	Waste	0.5-500	$6.01 \cdot 10^{-3} \pm 2.17 \cdot 10^{-4}$	$-5.72 \cdot 10^{-2} \pm 4.49 \cdot 10^{-2}$	$5.15 \cdot 10^{-2}$	0.9977
BBP	Milli-Q	0.5-500	$1.82 \cdot 10^{-3} \pm 8.11 \cdot 10^{-5}$	$-1.30 \cdot 10^{-2} \pm 1.76 \cdot 10^{-2}$	$2.02 \cdot 10^{-2}$	0.9965
	Mineral	0.5-500	$1.47 \cdot 10^{-3} \pm 9.78 \cdot 10^{-5}$	$-1.76 \cdot 10^{-2} \pm 2.12 \cdot 10^{-2}$	$2.44 \cdot 10^{-2}$	0.9923
	Tap	0.5-500	$1.64 \cdot 10^{-3} \pm 1.19 \cdot 10^{-4}$	$-1.77 \cdot 10^{-2} \pm 2.57 \cdot 10^{-2}$	$2.96 \cdot 10^{-2}$	0.9909
	Pond	0.5-500	$1.46 \cdot 10^{-3} \pm 5.39 \cdot 10^{-5}$	$-8.68 \cdot 10^{-3} \pm 1.17 \cdot 10^{-2}$	$1.35 \cdot 10^{-2}$	0.9976
	Waste	0.5-500	$1.36 \cdot 10^{-3} \pm 6.84 \cdot 10^{-5}$	$-1.69 \cdot 10^{-2} \pm 1.49 \cdot 10^{-2}$	$1.71 \cdot 10^{-2}$	0.9956
DEHA	Milli-Q	0.5-500	$1.22 \cdot 10^{-3} \pm 9.19 \cdot 10^{-5}$	$-5.13 \cdot 10^{-3} \pm 2.01 \cdot 10^{-2}$	$2.09 \cdot 10^{-2}$	0.9915
	Mineral	0.5-500	$8.54 \cdot 10^{-4} \pm 4.72 \cdot 10^{-5}$	$-8.51 \cdot 10^{-3} \pm 9.62 \cdot 10^{-3}$	$1.10 \cdot 10^{-2}$	0.9952
	Tap	0.5-500	$9.39 \cdot 10^{-4} \pm 6.62 \cdot 10^{-5}$	$-1.09 \cdot 10^{-2} \pm 1.38 \cdot 10^{-2}$	$1.58 \cdot 10^{-2}$	0.9913
	Pond	0.5-500	$1.02 \cdot 10^{-3} \pm 4.69 \cdot 10^{-5}$	$-7.29 \cdot 10^{-3} \pm 9.76 \cdot 10^{-3}$	$1.12 \cdot 10^{-2}$	0.9963
	Waste	0.5-500	$1.12 \cdot 10^{-3} \pm 5.80 \cdot 10^{-5}$	$-1.26 \cdot 10^{-2} \pm 1.21 \cdot 10^{-2}$	$1.39 \cdot 10^{-2}$	0.9953

Table IV.3.3.- (Continued).

Analyte	Water sample	Studied linear range (µg/L)	Regression equation (n=9)		$S_{y/x}$	R^2
			$b \pm s_b \cdot t_{(0.05;7)}$	$a \pm s_a \cdot t_{(0.05;7)}$		
DCHP	Milli-Q	0.5-500	$4.06 \cdot 10^{-3} \pm 1.59 \cdot 10^{-4}$	$-3.29 \cdot 10^{-2} \pm 3.34 \cdot 10^{-2}$	$3.84 \cdot 10^{-2}$	0.9973
	Mineral	0.5-500	$2.90 \cdot 10^{-3} \pm 1.96 \cdot 10^{-4}$	$-3.57 \cdot 10^{-2} \pm 4.11 \cdot 10^{-2}$	$4.72 \cdot 10^{-2}$	0.9921
	Tap	0.5-500	$3.31 \cdot 10^{-3} \pm 1.94 \cdot 10^{-4}$	$-3.64 \cdot 10^{-2} \pm 4.08 \cdot 10^{-2}$	$4.68 \cdot 10^{-2}$	0.9940
	Pond	0.5-500	$3.00 \cdot 10^{-3} \pm 1.08 \cdot 10^{-4}$	$-1.96 \cdot 10^{-2} \pm 2.26 \cdot 10^{-2}$	$2.60 \cdot 10^{-2}$	0.9977
	Waste	0.5-500	$2.72 \cdot 10^{-3} \pm 1.37 \cdot 10^{-4}$	$-3.32 \cdot 10^{-2} \pm 2.88 \cdot 10^{-2}$	$3.31 \cdot 10^{-3}$	0.9955
DEHP	Milli-Q	0.5-500	$3.32 \cdot 10^{-3} \pm 2.69 \cdot 10^{-4}$	$5.33 \cdot 10^{-1} \pm 6.36 \cdot 10^{-2}$	$6.36 \cdot 10^{-2}$	0.9911
	Mineral	0.5-500	$2.82 \cdot 10^{-3} \pm 1.53 \cdot 10^{-4}$	$1.41 \cdot 10^{-2} \pm 3.63 \cdot 10^{-2}$	$3.52 \cdot 10^{-2}$	0.9960
	Tap	0.5-500	$3.07 \cdot 10^{-3} \pm 1.92 \cdot 10^{-4}$	$1.61 \cdot 10^{-2} \pm 4.12 \cdot 10^{-2}$	$4.74 \cdot 10^{-2}$	0.9931
	Pond	0.5-500	$2.96 \cdot 10^{-3} \pm 1.86 \cdot 10^{-4}$	$1.99 \cdot 10^{-2} \pm 3.98 \cdot 10^{-2}$	$4.57 \cdot 10^{-2}$	0.9932
	Waste	0.5-500	$2.51 \cdot 10^{-3} \pm 1.27 \cdot 10^{-4}$	$1.70 \cdot 10^{-2} \pm 2.72 \cdot 10^{-2}$	$3.12 \cdot 10^{-2}$	0.9955
DNOP	Milli-Q	0.5-500	$5.11 \cdot 10^{-3} \pm 3.22 \cdot 10^{-4}$	$-6.76 \cdot 10^{-2} \pm 6.84 \cdot 10^{-2}$	$7.86 \cdot 10^{-2}$	0.9930
	Mineral	0.5-500	$3.37 \cdot 10^{-3} \pm 4.42 \cdot 10^{-4}$	$-4.27 \cdot 10^{-2} \pm 5.13 \cdot 10^{-2}$	$5.89 \cdot 10^{-2}$	0.9910
	Tap	0.5-500	$4.02 \cdot 10^{-3} \pm 2.43 \cdot 10^{-4}$	$-6.10 \cdot 10^{-2} \pm 5.42 \cdot 10^{-2}$	$5.75 \cdot 10^{-2}$	0.9943
	Pond	0.5-500	$3.51 \cdot 10^{-3} \pm 2.53 \cdot 10^{-4}$	$-4.09 \cdot 10^{-2} \pm 5.37 \cdot 10^{-2}$	$6.17 \cdot 10^{-2}$	0.9909
	Waste	0.5-500	$2.44 \cdot 10^{-3} \pm 1.08 \cdot 10^{-4}$	$-2.60 \cdot 10^{-2} \pm 2.30 \cdot 10^{-2}$	$2.64 \cdot 10^{-2}$	0.9965
DINP	Milli-Q	0.5-500	$4.03 \cdot 10^{-3} \pm 2.51 \cdot 10^{-4}$	$-4.96 \cdot 10^{-2} \pm 5.14 \cdot 10^{-2}$	$5.90 \cdot 10^{-2}$	0.9933
	Mineral	0.5-500	$2.19 \cdot 10^{-3} \pm 1.47 \cdot 10^{-4}$	$-2.45 \cdot 10^{-2} \pm 3.02 \cdot 10^{-2}$	$3.47 \cdot 10^{-2}$	0.9921
	Tap	1-500	$2.53 \cdot 10^{-3} \pm 1.31 \cdot 10^{-4}$	$-2.79 \cdot 10^{-2} \pm 2.68 \cdot 10^{-2}$	$3.02 \cdot 10^{-2}$	0.9943
	Pond	1-500	$2.12 \cdot 10^{-3} \pm 1.46 \cdot 10^{-4}$	$-2.07 \cdot 10^{-2} \pm 2.99 \cdot 10^{-2}$	$3.43 \cdot 10^{-2}$	0.9918
	Waste	1-500	$1.33 \cdot 10^{-3} \pm 3.87 \cdot 10^{-5}$	$-1.20 \cdot 10^{-2} \pm 8.33 \cdot 10^{-3}$	$8.66 \cdot 10^{-3}$	0.9987
DIDP	Milli-Q	0.5-500	$3.22 \cdot 10^{-3} \pm 2.30 \cdot 10^{-4}$	$-4.43 \cdot 10^{-2} \pm 4.79 \cdot 10^{-2}$	$5.50 \cdot 10^{-2}$	0.9911
	Mineral	0.5-500	$1.63 \cdot 10^{-3} \pm 9.21 \cdot 10^{-5}$	$-1.48 \cdot 10^{-2} \pm 1.92 \cdot 10^{-2}$	$2.20 \cdot 10^{-2}$	0.9944
	Tap	0.5-500	$1.90 \cdot 10^{-3} \pm 6.73 \cdot 10^{-5}$	$-1.50 \cdot 10^{-2} \pm 1.45 \cdot 10^{-2}$	$1.61 \cdot 10^{-2}$	0.9980
	Pond	0.5-500	$1.31 \cdot 10^{-3} \pm 7.95 \cdot 10^{-5}$	$-9.26 \cdot 10^{-3} \pm 1.65 \cdot 10^{-2}$	$1.90 \cdot 10^{-2}$	0.9936
	Waste	0.5-500	$6.99 \cdot 10^{-4} \pm 2.85 \cdot 10^{-5}$	$-1.67 \cdot 10^{-3} \pm 5.94 \cdot 10^{-3}$	$6.82 \cdot 10^{-3}$	0.9971

b: slope; S_b: standard deviation of the slope; a: intercept; S_a: standard deviation of the intercept; $S_{y/x}$: standard deviation of the estimate.

Table IV.3.4.- Matrix effect (%) for all studied analytes and samples.

	Matrix effect (%)				
	Milli-Q water	**Mineral water**	**Tap water**	**Pond water**	**Waste water**
DEP	-62	-46	-50	-41	-49
DPP	-51	-42	-45	-37	-43
DBP	-44	-38	-41	-35	-35
DIPP	-29	-35	-32	-32	-36
DNPP	-27	-32	-33	-28	-33
BBP	92	55	73	53	43
DEHA	23	-14	-5	3	12
DCHP	23	-12	1	-9	-17
DEHP	3	-13	-5	-8	-22
DNOP	24	-19	-3	-15	-41
DINP	47	-20	-8	-23	-51
DIDP	61	-19	-5	-34	-65

IV.3.5.2.- Trueness

In order to check the trueness of the proposed methodology for the analysis of the different types of water samples, a recovery study was carried out by spiking the samples at two different concentration levels (0.5 and 5 µg/L). Relative recovery values were calculated by comparing relative peak areas of spiked water samples with relative peak areas of standards of the same concentration of PAEs. The samples were also spiked with the IS before the extraction at a concentration of 3 µg/L, while standards were spiked after the extraction procedure at a concentration of 150 µg/L in the final extract. Recovery values between 71 and 120 %, with satisfactory RSD values, below 19 %, were obtained (see Table IV.3.5). The LOQs of the method, which were determined taking into account the recovery values and the LCL, ranged between 9 and 20 ng/L and were experimentally checked.

Table IV.3.5.- Results of the recovery study (n=5) of the m-μ-dSPE-GC-MS/MS method for DEHA and the selected PAEs in the different water samples at two levels of concentration.

Analyte	Water sample	Level 1[a] (n=5)	Level 2[b] (n=5)	LOQ_{method}[c] (ng/L)
		Recovery % (RSD %)	Recovery % (RSD %)	
DEP	Milli-Q	95 (19)	73 (19)	12
	Mineral	95 (13)	71 (16)	13
	Tap	86 (11)	108 (15)	11
	Pond	106 (3)	71 (16)	12
	Waste	74 (18)	86 (14)	13
DPP	Milli-Q	82 (8)	80 (3)	12
	Mineral	81 (10)	104 (16)	11
	Tap	88 (10)	81 (5)	12
	Pond	91 (7)	87 (5)	11
	Waste	85 (5)	89 (1)	12
DBP	Milli-Q	119 (6)	115 (12)	8.6
	Mineral	102 (7)	118 (16)	9.2
	Tap	102 (10)	107 (5)	9.6
	Pond	102 (18)	117 (8)	9.2
	Waste	109 (12)	108 (3)	9.3
DIPP	Milli-Q	110 (9)	99 (4)	9.5
	Mineral	95 (7)	115 (15)	9.5
	Tap	97 (10)	99 (6)	10
	Pond	105 (4)	98 (3)	9.8
	Waste	101 (2)	101 (4)	9.8
DNPP	Milli-Q	117 (12)	112 (5)	8.6
	Mineral	103 (17)	110 (11)	9.3
	Tap	100 (9)	101 (5)	9.9
	Pond	105 (5)	101 (4)	9.6
	Waste	108 (4)	106 (4)	9.2
BBP	Milli-Q	120 (13)	110 (5)	9.0
	Mineral	89 (9)	104 (9)	11
	Tap	94 (12)	100 (5)	11
	Pond	103 (3)	96 (9)	10
	Waste	117 (8)	108 (5)	9.3
DEHA	Milli-Q	113 (10)	115 (9)	8.7
	Mineral	101 (9)	107 (9)	10
	Tap	93 (10)	113 (9)	9.7
	Pond	104 (4)	100 (12)	9.8
	Waste	111 (6)	106 (7)	9.2

Table IV.3.5.- (Continued).

Analyte	Water sample	Level 1[a] (n=5)	Level 2[b] (n=5)	LOQ_{method}[c] (ng/L)
		Recovery % (RSD %)	Recovery % (RSD %)	
DCHP	Milli-Q	103 (11)	102 (4)	9.8
	Mineral	95 (18)	105 (14)	10
	Tap	90 (11)	92 (6)	11
	Pond	98 (4)	88 (11)	11
	Waste	96 (3)	95 (3)	11
DEHP	Milli-Q	101 (19)	98 (12)	10
	Mineral	94 (8)	84 (14)	11
	Tap	96 (11)	103 (10)	10
	Pond	96 (11)	96 (5)	11
	Waste	108 (17)	102 (9)	9.8
DNOP	Milli-Q	95 (7)	97 (16)	11
	Mineral	103 (17)	112 (7)	9.5
	Tap	89 (11)	103 (11)	11
	Pond	94 (9)	97 (8)	11
	Waste	100 (8)	101 (8)	10
DINP	Milli-Q	93 (8)	102 (16)	10
	Mineral	103 (18)	112 (8)	9.1
	Tap	87 (12)	106 (14)	20
	Pond	95 (11)	101 (7)	10
	Waste	98 (10)	97 (11)	20
DIDP	Milli-Q	94 (8)	102 (15)	10
	Mineral	105 (16)	111 (7)	9.2
	Tap	86 (13)	108 (14)	10
	Pond	95 (12)	104 (8)	10
	Waste	100 (15)	92 (16)	10

[a] Concentration of the analytes in the sample (level 1): 1 µg/L; [b] Concentration of the analytes in the sample (level 2): 10 µg/L; [c] Calculated from the LCL considering the recovery obtained for every analyte.

IV.3.6.- Real samples analysis

In order to demonstrate the applicability of the method, one sample of each type was analysed. Results are shown in Table IV.3.6 in which it can be clearly observed that DEP, DCHP, DBP, DEHP and BBP were detected in most of the analysed samples. In particular, DEP and DBP were found at concentrations above 0.36 µg/L in mineral, tap and waste water (see Figure IV.3.6), being DEP the one with the highest content (between 2.46 and 4.20 µg/L in such samples). Both of them had been previously found in similar matrices [384,387]. The reason for finding higher amounts of DBP together with DEP is

probably the fact that they are quite common components in plastic bottles and also in personal care and pharmaceutical products [395]. In this sense, it should also be remarked that, as previously reported, these compounds are, after DMP, two of the most volatile ones, so they are usually present in the atmosphere, and rains carry these compounds to lakes, rivers and soils, making them two of the most widespread PAEs around the world [125]. As an example of the above-mentioned, DBP had been previously found in bottled water even at levels between 1.20 and 1.60 µg/L [387], while DEP had been previously found at low µg/L levels (4.5 µg/L) [396] in the same matrix. Both compounds have also been found in drinking and tap water in different countries at ng/L levels [397], and in waste water at few µg/L [398].

Table IV.3.6.- Concentration of the target analytes found in real water samples.

Water samples	Concentration found (µg/L)				
	DEP	DCHP	DBP	DEHP	BBP
Milli-Q	< LOQ	n.d.	< LOQ	< LOQ	< LOQ
Mineral (PET bottle)	2.46 ± 0.72	n.d.	0.36 ± 0.46	< LOQ	< LOQ
Tap	2.81 ± 0.48	< LOQ	1.23 ± 0.15	< LOQ	< LOQ
Pond	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
Waste	4.20 ± 0.52	< LOQ	0.83 ± 0.18	< LOQ	< LOQ

n.d. Not detected

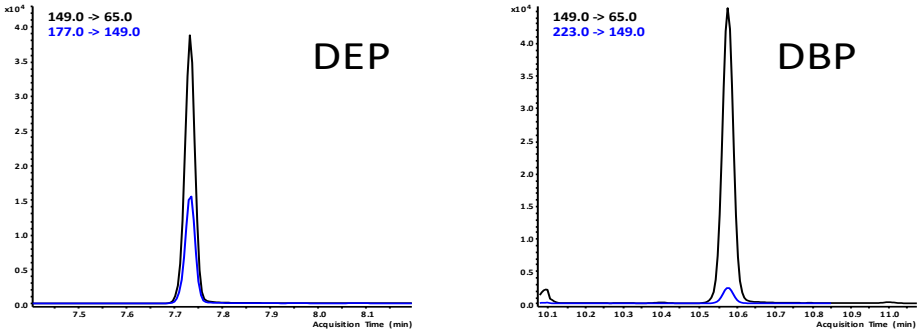


Figure IV.3.6.- GC-MS/MS chromatograms obtained working in the MRM mode for the analytes found in the waste water samples (DEP and DBP).

IV.3.7.- Comparison with other methodologies

As previously indicated, this type of m-NPs had only been used for the extraction of oestrogenic compounds from different water samples in few occasions [169,264,276,373-375,385,386], therefore, the comparison with previous works in which such sorbent had been applied for the extraction of PAEs is not possible. However, and concerning previous applications of NPs as sorbents for the extraction of PAEs from water samples, it should be remarked that only Fe_3O_4 m-NPs had been applied, using different polymers as coatings such as chitosan [399], Ba^{2+}-alginate [400], polythiophene [401] and polypyrrole [280], though SDS [281] had also been successfully applied. Most of these articles had focused on the analysis of three or four PAEs [281,399-401], including DPP, DBP, DCHP, DEHP and DNOP, which were also evaluated in this work. Thus, and to the best of our knowledge, the article of Zhao et al. [280] constituted the only application of polymer-coated Fe_3O_4 m-NPs in which a comparable number of analytes had been studied, though in that case, the methodology was validated just in tap and lake water and the LODs were higher than the ones obtained in this work. Regarding validation parameters, similar recovery values were obtained (70–120 %) and LODs in the ng/L level were generally achieved, except in two cases [280,401], which agreed with the results obtained in this study.

Apart from coated m-NPs, other sorbents based on nanomaterials had also been applied in a good number of occasions for the extraction of PAEs from water samples. As examples, MWCNTs [167,239], graphene [402] or MOFs [255] had been used for the extraction of reduced groups of PAEs (between four and seven), in different extraction modes, including conventional SPE [167], SPME [239], m-dSPE [402] and μ-SPE [255]. In all these methods, the recovery values obtained (70–120 %) were comparable to the ones achieved in this work, though LODs in the μg/L range [167,255] had been obtained in some of these articles, which were higher than the ones obtained in this work.

IV.3.8.- Conclusions

From the results obtained in this section, the following conclusions can be drawn:

- Fe_3O_4@pDA m-NPs were successfully synthesised and applied for the m-μ-dSPE of eleven PAEs (DEP, DPP, DBP, DIPP, DNPP, BBP, DCHP, DEHP, DNOP, DINP and DIDP) and a DEHP commonly used substitutive (DEHA) from five water samples with different origins (Milli-Q, mineral, tap, pond and waste water).

- The GC-MS/MS method developed for the separation and detection of the selected compounds was validated in terms of linearity and sensitivity, obtaining R^2 values higher than 0.9904 and LCLs of 0.5 µg/L for all analytes.
- The m-µ-dSPE procedure was suitably optimised, taking water at pH 6.0, extraction with 60 mg of Fe_3O_4@pDA m-NPs and the desorption of the analytes with 6 mL of DCM as the conditions which provided the best extraction performance. Such conditions could also be successfully applied to the extraction of mineral, tap, pond and waste water.
- The evaluation of the matrix effects revealed an important signal suppression for DEP and DPP in Milli-Q water and for DIDP and DINP in waste water, while a high signal enhancement was found for BBP in all the matrices studied, except in waste water. In general terms, most volatile analytes (DEP, DPP, DBP, DIPP and DNPP) presented similar matrix effects in all water samples. Such presence of matrix effects makes necessary the development of matrix-matched calibration curves for all the analytes in all the matrices studied in this work, for which R^2 values higher than 0.9904 were obtained. LCLs of 0.5 µg/L were also achieved in all cases, except for DINP in tap, pond and waste water, which was 1 µg/L.
- The evaluation of the trueness of the methodology showed that satisfactory recovery values were achieved: 71-120 % with RSDs lower than 19 %. With these results, the LOQs of the method, which were calculated from the LCLs, were extremely low, between 9 and 20 ng/L for all the target analytes studied in this work. This data was in accordance with that obtained in previously published articles.
- The analysis of real samples of each type revealed the presence of DEP, DCHP, DBP, DEHP and BBP at concentrations below the LOQs of the method in most of the analysed matrices. However, DEP and DBP were found at concentrations above 0.36 µg/L in mineral, tap and waste water.
- The proposed methodology demonstrates that Fe_3O_4@pDA m-NPs can be effectively used as sorbent for the extraction of PAEs from water samples, proving to be a very simple, fast and reliable method, with low consumption of reagents and a high sensitivity.

IV.4.- Determination of PAEs in water samples by HF-LPME prior to GC-MS/MS

A new HF-LPME method was developed for the extraction of a group of PAEs of interest from different water samples prior to GC-MS/MS. HF-LPME was carried out using 1-octanol as acceptor phase followed by a back-extraction step with cyclohexane. The different parameters that affect HF-LPME such as sample pH, ionic strength, extraction time, stirring rate, extraction temperature and back-extraction conditions were investigated. The method was validated by means of calibration and recovery studies using DBP-d_4 as IS. The developed methodology was successfully applied to the analysis of the selected PAEs in mineral, tap, pond and waste water samples.

IV.4.1.- Background

As commented in the previous sections, the analysis of PAEs in the different environmental compartment is of high interest nowadays as a result of the potential and already detected health problems for humans, which also include endocrine disrupting effects [403-406]. The low concentration at which PAEs appear in environmental samples [125,407], as a result of their slow migration from plastics as well as their relative dilution in waters, make necessary the application of a suitable sample preparation and preconcentration step before their chromatographic or electrophoretic analysis. In this sense, different approaches such as LLE [408], UAE [409] or SPE [177] have been traditionally and widely used to extract PAEs from aqueous samples, but most of them need large volumes of hazardous organic solvents. Moreover, given the high sensitivity of modern instruments, in many cases, and depending on the type of sample, it is desirable to use a sample preparation method which, in addition to enriching the target analytes, should also perform a simultaneous clean-up step. All of the above should be accompanied by a low consumption of solvents and materials as well as an easy implementation.

Compared with traditional sample preparation methods, LPME is a relatively recent and promising set of simplified and miniaturised techniques that require only several microliters of extraction solvents [314]. Among the different LPME modes that have been proposed, HF-LPME enables to carry out extraction and clean-up in a single step with high selectivity, high enrichment factors, low cost, simplicity of operation and minimal waste [332,410]. In earlier studies, some PAEs had been successfully extracted by HF-LPME but only in five occasions [160,336,337,411,412]. In this sense, Chao et al. [336] carried out the extraction of 3 PAEs (DMP, DEP and BBP) from river water samples

by means of a heat-assisted push/pull perfusion three phase HF-LPME procedure using n-tetradecane and ACN as SLM and acceptor solvent, respectively. In another work of the same research group, Chao and co-workers [411] developed an ultrasound-assisted push/pull perfusion three phase HF-LPME procedure for the extraction of DMP, DEP and BBP from plastic bottled beverages, using the same solvents as SLM and acceptor phase. In the work of Jiang et al. [160], the authors carried out the extraction of three PAEs (DMP, DEP and DEHP) from milk, juice, carbonated drinks, mineral water and urine samples applying a surfactant-free reinforced HF-LPME using a mixture of toluene, isopropanol and water to create the microemulsion. Besides, Mtibe and co-workers [337] developed a three phase HF-LPME procedure using toluene as SLM for the extraction of DBP, BBP and DEHP from waste water samples. Finally, Psillakis et al. [412] carried out the extraction of six PAEs (DMP, DEP, DBP, BBP, DEHP and DNOP) from potable water samples using a HF-LPME procedure with toluene as extraction solvent. As can be seen, all these works were focused on a relatively low number of PAEs (no more than 3 or 6) and were applied to a single type of water sample. As a result, and despite its advantages, the application of the technique had not been fully explored.

The selection of the target PAEs was carried out taking into account their classification as priority pollutants as well as their applicability in products intended to be in contact with water and food and, therefore, be susceptible to being ingested by humans. As examples, among the PAEs commonly used in the industry DIBP, DBP and BBP stand out since they have been detected in leachate samples at average concentrations of 26.27, 14.20 and 5.52 µg/L, respectively [413]. Concentrations of these PAEs have also been routinely reported in water and sediments [125,414]. For example, DBP has been quantified in the ranges 1.69-11.8 µg/L [415], 2.8-122 µg/L [416] and 1.00-13.5 µg/L [417] in river water samples. Regarding sediments, DIBP was found in the range 77.7-147.2 µg/kg and DEEP and DBEP at average concentrations of 8.3 and 75.1 µg/kg, respectively, in lake water samples [418].

IV.4.2.- Specific objectives

Considering all the above-mentioned, the following specific goals were established:

- The development of a new analytical methodology based on a HF-LPME approach for the extraction of nine PAEs of interest (DPP, DIBP, DBP, DIPP, DEEP, DNPP, BBP, DBEP

and DCHP) from mineral, tap, pond and waste water samples and their subsequent determination by GC-MS/MS.

- The evaluation of the influence of the parameters affecting the HF-LPME procedure (i.e. pH and ionic strength (NaCl percentage) of the aqueous phase, extraction time and temperature, stirring speed, type of solvent used during the back-extraction step and back-extraction time) using a step by step approach to achieve the best extraction performance.
- The validation of the whole methodology in terms of linearity, precision and trueness as well as the determination of the LODs and LOQs of the method.
- The study of the applicability of the developed methodology to the analysis of mineral, tap, pond and waste water.

IV.4.3.- GC-MS/MS conditions

As previously commented, PAEs have enough volatility and thermostability to be analysed by GC without derivatisation using non-polar columns [419,420]. In this work, a total of 9 targeted PAEs were initially separated and detected by GC-FID for HF-LPME optimisation purposes. Once the optimum HF-LPME procedure was selected, GC-MS/MS separation and detection was carried out and the methodology was fully validated. Both separation and detection conditions as well as the temperature gradients are indicated in Sections III.5.3 and III.5.4. In this last case, in which DBP-d_4 was used as IS, the MS system was operated in the MRM mode using 1 or 2 precursors and 2 product ions as well as the retention time as identification points, with maximum permitted tolerances of the relative ion intensities of ±20 % [390]. Those peaks which did not meet such requirements were not considered as target analytes. MRM transitions as well as the collision energy values are shown in Table IV.4.1, in which it can clearly be seen that most PAEs have common characteristic transitions (149.0 → 121.0 and 149.0 → 93.0) as already indicated in the literature [142,391].

Once the GC-MS/MS conditions were optimised, instrumental calibration curves based on PAEs relative peak areas (IS was added at a concentration of 1.75 mg/L) were obtained. For this purpose, seven increasing concentration levels (n=7, in the range 0.050-12.5 mg/L) were injected three times, obtaining R^2 values above 0.9980 for all the analytes.

Table IV.4.1.- Retention times, quantifier and qualifier transitions in GC-MS/MS analyses of the selected PAEs.

Analyte	Retention time (min)	Quantifier transition (m/z)	Colision energy (eV)	Qualifier transition (m/z)	Colision energy (eV)
DPP	9.02	149.0 → 121.0	10.0	191.0 → 149.0	15.0
DIBP	9.84	149.0 → 93.0	12.5	149.0 → 121.0	17.5
DBP-d_4 (IS)	10.84	153.0 → 125.0	12.5	153.0 → 97.0	17.5
DBP	10.86	149.0 → 121.0	12.5	149.0 → 93.0	15.0
DIPP	12.33	149.0 → 121.0	15.0	149.0 → 93.0	17.5
DEEP	12.84	149.0 → 121.0	10.0	149.0 → 93.0	15.0
DNPP	13.49	149.0 → 121.0	15.0	149.0 → 93.0	17.5
BBP	16.98	149.0 → 121.0	10.0	149.0 → 93.0	15.0
DBEP	19.40	149.0 → 121.0	10.0	149.0 → 93.0	10.0
DCHP	20.27	149.0 → 121.0	10.0	167.0 → 149.0	15.0

IV.4.4.- HF-LPME optimisation

In order to obtain the best conditions for the analysis of the studied analytes, the parameters that affect the extraction (type of extraction solvent, pH, ionic strength, extraction time, agitation, back-extraction time and temperature) were studied using 10 mL of spiked Milli-Q water with the selected PAEs at a concentration of 1.5 mg/L. In this sense, two-phase HF-LPME using 1-octanol as the acceptor phase was applied, since it has been the approach most widely used to extract different groups of analytes, obtaining satisfactory results due to its low volatility and immiscibility in aqueous solution as well as its high extraction capacity [314,410]. Therefore, a PP HF of 2.0 cm long was employed for all experiments. For this purpose, the HF was previously washed with 2 mL of cyclohexane in an ultrasonic bath and air dried to remove impurities and PAEs residues. Afterwards, it was inserted into the needle tip of a 25 µL Hamilton micro-syringe pre-filled with 1-octanol. Then, 20 µL of the extraction solvent was slowly introduced into the fibre, allowing its correct distribution through the pores and lumen. Subsequently, the syringe was filled again with 20 µL of 1-octanol and the procedure was repeated to ensure the

correct impregnation of the HF without bubbles formation and thus to avoid irreproducibility problems during extraction. Moreover, after extraction, a 8 min back-extraction procedure in an ultrasonic bath based on the use of 200 µL of solvent (sufficient to completely immerse the fibre in a micro-vial of 300 µL) and subsequent evaporation under a gentle steam of nitrogen was employed (without such back-extraction procedure, peak areas were much lower and some of the PAEs could not even be detected). Finally, the dry extract was reconstituted in 200 µL of cyclohexane for its final injection in the GC system.

As previously commented, it is also necessary to take into account the possible existence of contamination by PAEs from the impurities of the reagents, the laboratory material and also the particles suspended in the air. For this purpose, blank samples were also frequently analysed. However, and probably due to the low sensitivity of the FID, no PAEs were detected.

IV.4.4.1.- Effect of the type of solvent used during the back-extraction procedure

After a HF-LPME process, the development of a back-extraction step has shown to provide better results than the conventional retraction of the acceptor phase [410,421,422]. That is why in all cases the analytes were back-extracted from the fibre by immersing it in a micro-vial containing 200 µL of solvent. In this sense, four organic solvents of different polarity were initially tested, including ACN, acetone, MeOH and cyclohexane, maintaining the rest of the parameters as follows: a 2-cm HF impregnated with 1-octanol, 10 mL of spiked Milli-Q water without pH adjustment, extraction time of 25 min at ambient temperature, a stirring speed of 1000 rpm and a back-extraction with 200 µL of solvent for 8 min under ultrasounds. As can be seen in Figure IV.4.1, the results obtained showed that the best extraction efficiency was obtained with acetone and cyclohexane for all analytes. However, due to the high volatility of acetone, which could lead to repeatability problems [88], and to the fact that cyclohexane provided slightly higher results for 5 of the 9 studied compounds, this was selected. In the case of DEEP, it could not be initially detected in the final extract, probably due to the early conditions selected that did not favour the extraction of this analyte.

IV.4.4.2.- Effect of pH of the aqueous phase

The influence of the pH of the donor phase on the extraction efficiency over the range of 2.0-9.0 (including Milli-Q water without pH adjustment) was studied using 10 mL

of Milli-Q water under the conditions previously indicated, including a back-extraction procedure with 200 µL of cyclohexane for 8 min. In general, and, as expected, owing to the fact that these analytes are non-ionisable in aqueous solution, pH did not significantly affect the extraction of PAEs. Therefore, it was decided not to adjust it in future experiments as it has been suggested several times in the literature [336,411], unless a certain irreproducibility was observed in the final application of the procedure which, as can be seen in the following section, did not happen.

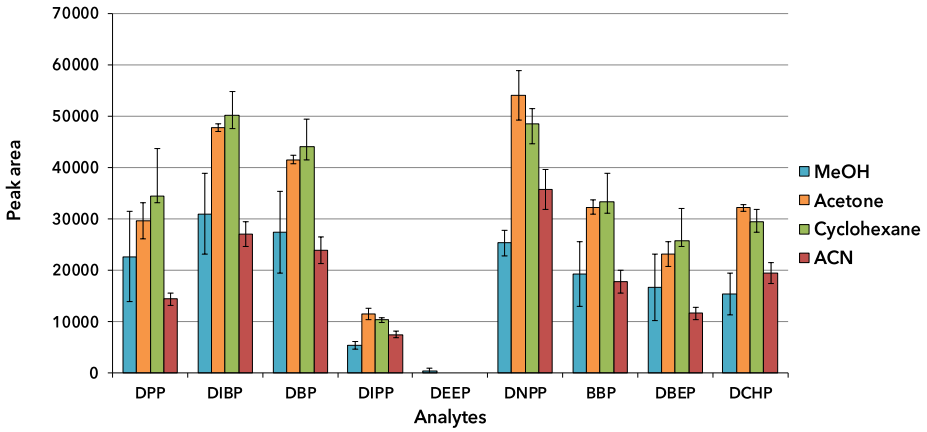


Figure IV.4.1.- Effect of the type of solvent used for the back-extraction on the peak areas of the selected PAEs after the HF-LPME procedure. Extraction conditions: a 2-cm HF impregnated with 1-octanol, 10 mL of spiked Milli-Q water at 1.5 mg/L without pH adjustment nor salt addition, 25 min of extraction at 1000 rpm and ambient temperature and back-extraction with 200 µL of each solvent for 8 min assisted by ultrasounds.

IV.4.4.3.- Effect of the ionic strength (NaCl addition) of the aqueous phase

Under the previously described conditions, the effect of the addition of NaCl at concentrations varying from 0 to 15 % (w/v) was studied. As can be seen in Figure IV.4.2, and especially for compounds such as DIBP, DIPP, DNPP, BBP, DBEP and DCHP, a clear decreasing trend of the peak areas was observed by increasing the percentage of NaCl added. This effect is quite significant for DBP for which extraction efficiency decreased drastically when NaCl is added. In the case of DEEP, the addition of NaCl slightly improved the extraction but not significantly. Therefore, it could be concluded that the addition of NaCl negatively affect the extraction of 8 of the 9 analytes studied, as some authors have previously suggested for a reduced number of PAEs [336,337]. This is

because the addition of salts may exert a certain influence on the physicochemical conditions of the diffusion layer, to the point of hindering the transport of the analytes through it and towards the organic phase [423,424].

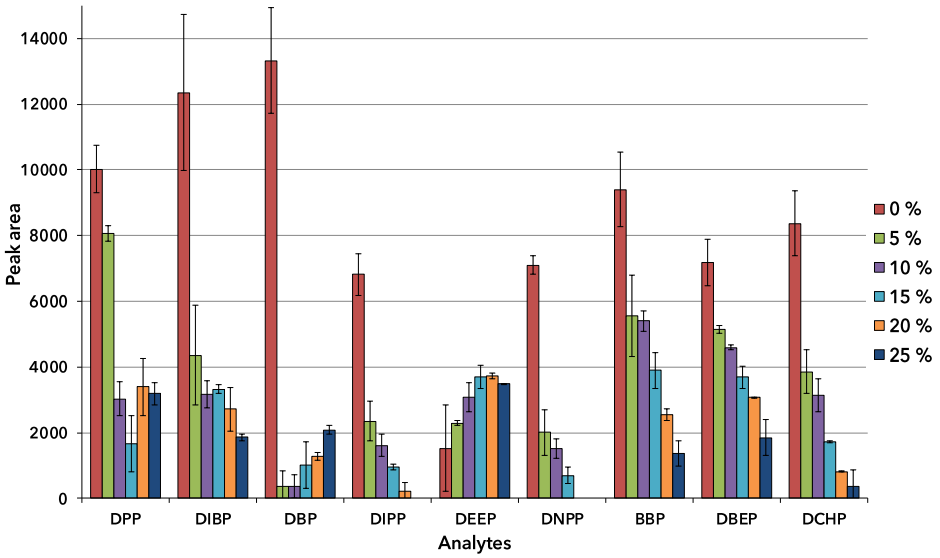


Figure IV.4.2.- Effect of the percentage (w/v) of NaCl on the peak areas of the selected PAEs after the HF-LPME procedure. Extraction conditions: a 2-cm HF impregnated with 1-octanol, 10 mL of spiked Milli-Q water at 1.5 mg/L without pH adjustment, 25 min of extraction at 1000 rpm at ambient temperature and back-extraction with 200 µL of cyclohexane for 8 min assisted by ultrasounds.

IV.4.4.4.- Effect of the extraction time

Mass transfer is a process that depends on the extraction time, as long as there is no saturation of the analytes in the extraction solvent; in such case the equilibrium condition is reached. Taking into account that HF-LPME is a technique in which extraction is usually carried out under non-equilibrium conditions, the influence of extraction time up to 75 min was initially studied. In the obtained data shown in Figure IV.4.3, it could be observed an increasing trend of the extraction over time for the majority of the analytes. In the case of DPP, DBP and DEEP, this increase was not significant. However, and because of the improvement of the extraction efficiency for most of the studied PAEs at high extraction times, it was decided to carry out the extraction for 75 min. It is possible that the longer the extraction time, the better results might be obtained for some of the

analytes; however, it was decided not to increase the time since it is not functional to employ a long extraction if a good/suitable performance is obtained at shorter times.

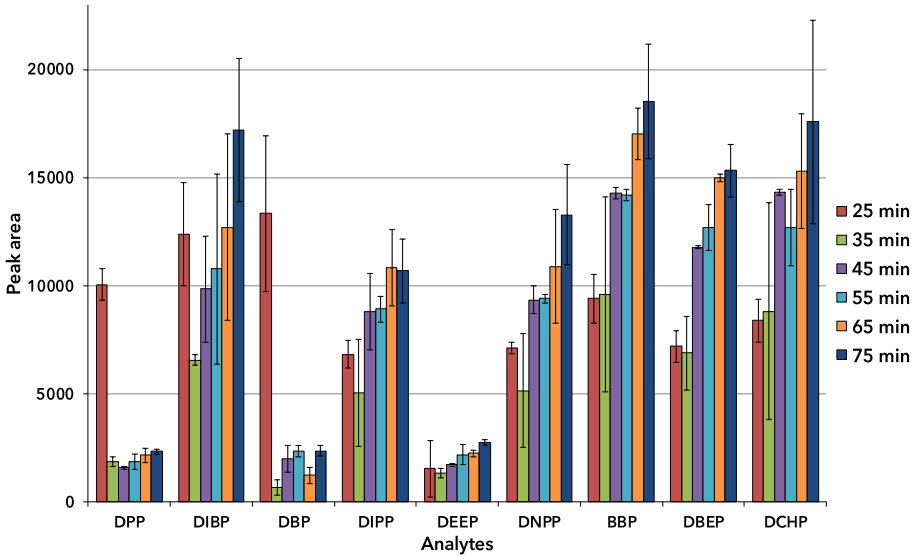


Figure IV.4.3.- Effect of extraction time on the peak areas of the selected PAEs after the HF-LPME procedure. Extraction conditions: a 2-cm HF impregnated with 1-octanol, 10 mL of spiked Milli-Q water at 1.5 mg/L without pH adjustment nor salt addition, extraction at 1000 rpm at ambient temperature and back-extraction with 200 µL of cyclohexane for 8 min assisted by ultrasounds.

IV.4.4.5.- Effect of the stirring speed

The stirring rate is another parameter of great importance in a technique such as HF-LPME since it will inevitably affect the movement of the analytes in the sample and, consequently, the mass transfer. That is why the stirring speed was varied between 500 and 1000 rpm maintaining the rest of the conditions constant: a 2-cm HF impregnated with 1-octanol, 10 mL of Milli-Q water, without pH adjustment, without addition of salt, extraction time of 75 min at ambient temperature and 200 µL of cyclohexane for 8 min under ultrasounds. The results obtained are shown in Figure IV.4.4. As can be seen, further agitation leads to a better extraction of the analytes. In the case of DPP and DBP the effect is not sufficiently clear, as a result of the low extraction of these analytes. However, although the increment is progressive, it was possible to verify experimentally that at maximum speed, the magnetic bar could not provide a homogenous agitation thus reducing the efficiency of the extraction at 1000 rpm. It is important to take into account

that excessive agitation may negatively influence the extraction, since in these cases the movement prevents the analytes from remaining long enough on the fibre surface to be able to penetrate through diffusion. Based on these results, it was decided to select a stirring speed of 850 rpm.

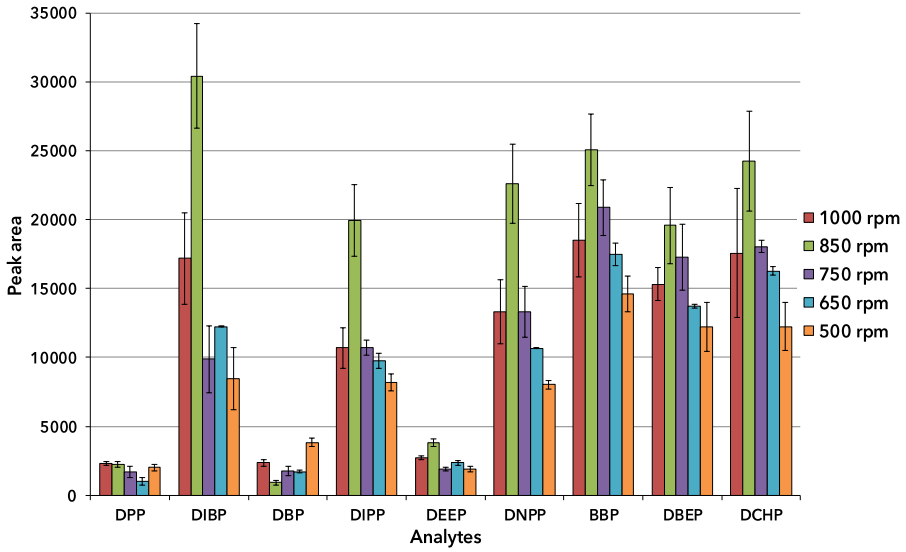


Figure IV.4.4.- Effect of stirring speed on the peak areas of the selected PAEs after the HF-LPME procedure. Extraction conditions: a 2-cm HF impregnated with 1-octanol, 10 mL of spiked Milli-Q water at 1.5 mg/L without pH adjustment nor salt addition, 75 min of extraction at ambient temperature and back-extraction with 200 µL of cyclohexane for 8 min assisted by ultrasounds.

IV.4.4.6.- Effect of the back-extraction time

As already indicated, the back-extraction procedure consists on putting in contact the fibre (after extraction) with a solvent, in this case cyclohexane, under ultrasounds. The back-extraction time is also a variable to be optimised and it was carried out by modifying the time between 5 and 20 min (see Figure IV.4.5). The results showed that the extraction was higher at 10 min, except for DPP and DEEP for which there were hardly any variations. Hence, 10 min was selected for future experiments. At higher times the efficiency decreased, suggesting that the analytes return to the fibre. It is important to emphasise that it was not decided to carry out experiments between 8 and 10 min or between 10 and 15 min, given the few differences between the areas obtained in both cases (at 8 and 15 min).

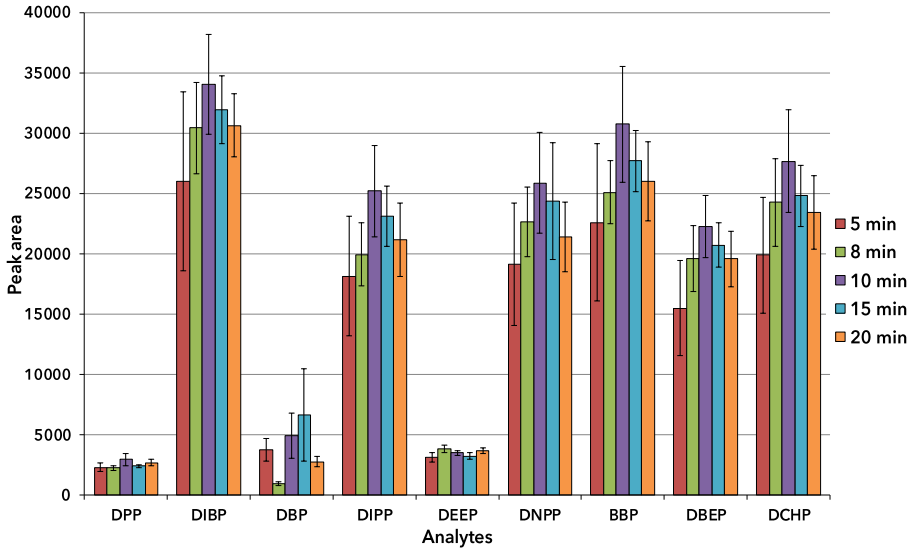


Figure IV.4.5.- Effect of back-extraction time on the peak areas of the selected PAEs after the HF-LPME procedure. Extraction conditions: a 2-cm HF impregnated with 1-octanol, 10 mL of spiked Milli-Q water at 1.5 mg/L without pH adjustment nor salt addition, 75 min of extraction at 850 rpm at ambient temperature and back-extraction with 200 µL of cyclohexane assisted by ultrasounds.

IV.4.4.7.- Effect of the extraction temperature

Temperature may also influence the efficiency of the extraction through two opposed contributions. On the one hand, and as it is known, an increase in temperature favours the mass transfer and, therefore, the displacement of the analytes towards the acceptor phase [284,423]. But on the other hand, an increase causes a decrease in the K_{OW} of the analytes [284,423] which decreases the affinity for that phase. In this case, the effect of the temperature was studied by developing extractions at 30, 40, 60, 70 °C and at room temperature, under the previous conditions: a 2-cm HF impregnated with 1-octanol, 10 mL of Milli-Q water, without pH adjustment, without addition of salt, extraction time of 75 min, stirring speed of 850 rpm and 200 µL of cyclohexane for 10 min under ultrasounds. In all cases, the vial containing the aqueous sample was immersed in a glycerine bath on a heated stirring plate and a temperature probe was immersed therein. The results obtained are shown in Figure IV.4.6. As can be seen, an increase in temperature produces an enhancement of the extraction efficiency for most of the selected analytes up to a maximum of 60 °C, which has already been described in the literature for some of these analytes [412], and falling drastically at temperatures above

60 °C. Based on the results obtained, it was decided to select 60 °C as the appropriate extraction temperature.

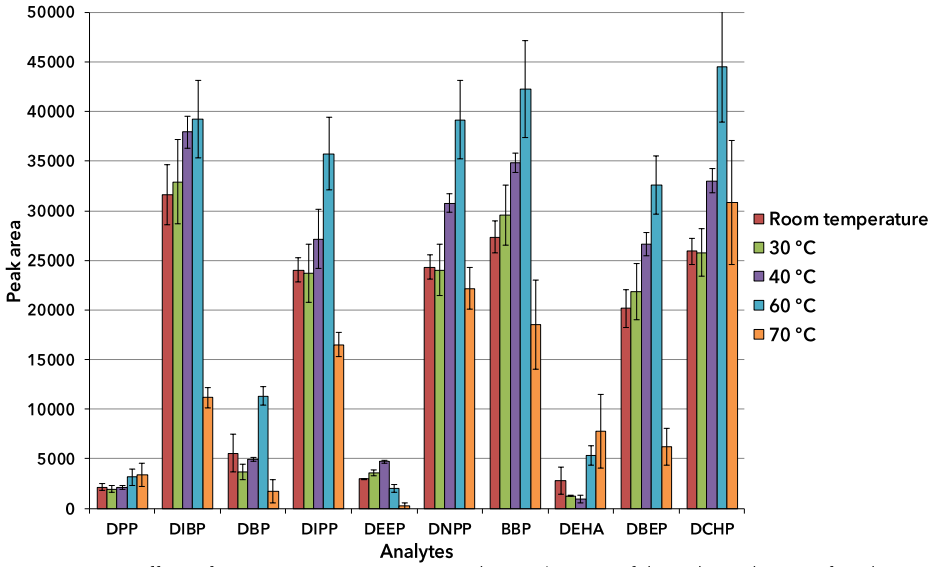


Figure IV.4.6.- Effect of extraction temperature on the peak areas of the selected PAEs after the HF-LPME procedure. Extraction conditions: a 2-cm HF impregnated with 1-octanol, 10 mL of spiked Milli-Q water at 1.5 mg/L without pH adjustment nor salt addition, 75 min of extraction at 850 rpm and back-extraction with 200 µL of cyclohexane for 10 min assisted by ultrasounds.

IV.4.5.- Validation of the HF-LPME-GC-MS/MS method in water samples

To validate the optimised HF-LPME-GC-MS/MS method, calibration and recovery studies were carried out after blank sample analysis. In order to assure the quality of the experiments performed in terms of trueness, laboratory blank sample analyses were first carried out by the direct back-extraction of the HF impregnated with 1-octanol without performing the previous extraction step. The results showed the absence of the PAEs studied at least at concentrations equal to or higher than the LCL set at 1 µg/L.

Method calibration was evaluated by spiking mineral, tap, pond and waste water at seven concentration levels (n=7) before extraction and injecting each level in triplicate using DBP-d_4 as IS in all cases at a concentration of 35 µg/L in the sample. As can be seen in Table IV.4.2, calibration curves were linear in the range studied (1.0-100 mg/L) with R^2 values higher than 0.9901 in all cases.

Table IV.4.2- Method calibration data of the selected PAEs in water samples after the HF-LPME-GC-MS/MS method.

Analyte	Water sample	Studied linear range (µg/L)	Regression equation (n=7)		$S_{y/x}$	R^2
			$b \pm s_b \cdot t_{(0.05;5)}$	$a \pm s_a \cdot t_{(0.05;5)}$		
DPP	Mineral	1-100	$2.53 \cdot 10^{-2} \pm 9.22 \cdot 10^{-4}$	$-3.87 \cdot 10^{-2} \pm 4.06 \cdot 10^{-2}$	$3.10 \cdot 10^{-2}$	0.9990
	Tap	1-100	$2.42 \cdot 10^{-2} \pm 2.78 \cdot 10^{-3}$	$-1.23 \cdot 10^{-1} \pm 1.22 \cdot 10^{-1}$	$9.32 \cdot 10^{-2}$	0.9901
	Pond	1-100	$2.41 \cdot 10^{-2} \pm 1.32 \cdot 10^{-3}$	$-6.04 \cdot 10^{-2} \pm 5.64 \cdot 10^{-2}$	$4.27 \cdot 10^{-2}$	0.9982
	Waste	1-100	$2.37 \cdot 10^{-2} \pm 1.54 \cdot 10^{-3}$	$-7.49 \cdot 10^{-2} \pm 6.79 \cdot 10^{-2}$	$5.18 \cdot 10^{-2}$	0.9968
DIBP	Mineral	1-100	$3.74 \cdot 10^{-2} \pm 1.26 \cdot 10^{-3}$	$-6.09 \cdot 10^{-2} \pm 5.52 \cdot 10^{-2}$	$4.21 \cdot 10^{-2}$	0.9991
	Tap	1-100	$3.46 \cdot 10^{-2} \pm 2.02 \cdot 10^{-3}$	$-6.59 \cdot 10^{-2} \pm 8.81 \cdot 10^{-2}$	$6.72 \cdot 10^{-2}$	0.9974
	Pond	1-100	$3.68 \cdot 10^{-2} \pm 2.96 \cdot 10^{-3}$	$-1.07 \cdot 10^{-1} \pm 1.29 \cdot 10^{-1}$	$9.84 \cdot 10^{-2}$	0.9951
	Waste	1-100	$3.38 \cdot 10^{-2} \pm 2.61 \cdot 10^{-3}$	$-8.59 \cdot 10^{-2} \pm 1.14 \cdot 10^{-1}$	$8.68 \cdot 10^{-2}$	0.9955
DBP	Mineral	1-100	$5.27 \cdot 10^{-2} \pm 1.86 \cdot 10^{-3}$	$-3.92 \cdot 10^{-2} \pm 8.53 \cdot 10^{-2}$	$6.51 \cdot 10^{-2}$	0.9991
	Tap	1-100	$5.02 \cdot 10^{-2} \pm 4.56 \cdot 10^{-3}$	$2.98 \cdot 10^{-2} \pm 2.09 \cdot 10^{-1}$	$1.59 \cdot 10^{-1}$	0.9938
	Pond	1-100	$5.22 \cdot 10^{-2} \pm 1.09 \cdot 10^{-3}$	$-2.01 \cdot 10^{-2} \pm 4.98 \cdot 10^{-2}$	$3.80 \cdot 10^{-2}$	0.9997
	Waste	1-100	$5.06 \cdot 10^{-2} \pm 9.55 \cdot 10^{-4}$	$-4.54 \cdot 10^{-2} \pm 4.37 \cdot 10^{-2}$	$3.34 \cdot 10^{-2}$	0.9997
DIPP	Mineral	1-100	$2.45 \cdot 10^{-2} \pm 1.32 \cdot 10^{-3}$	$-5.90 \cdot 10^{-2} \pm 5.78 \cdot 10^{-2}$	$4.42 \cdot 10^{-2}$	0.9978
	Tap	1-100	$2.14 \cdot 10^{-2} \pm 4.38 \cdot 10^{-4}$	$2.66 \cdot 10^{-3} \pm 1.91 \cdot 10^{-2}$	$1.46 \cdot 10^{-2}$	0.9997
	Pond	1-100	$2.35 \cdot 10^{-2} \pm 7.98 \cdot 10^{-4}$	$-4.56 \cdot 10^{-2} \pm 3.49 \cdot 10^{-2}$	$2.66 \cdot 10^{-2}$	0.9991
	Waste	1-100	$2.15 \cdot 10^{-2} \pm 1.97 \cdot 10^{-3}$	$-7.53 \cdot 10^{-2} \pm 8.62 \cdot 10^{-2}$	$6.58 \cdot 10^{-2}$	0.9937
DEEP	Mineral	1-100	$8.91 \cdot 10^{-4} \pm 7.90 \cdot 10^{-5}$	$-2.53 \cdot 10^{-3} \pm 3.46 \cdot 10^{-3}$	$2.62 \cdot 10^{-3}$	0.9953
	Tap	1-100	$1.05 \cdot 10^{-3} \pm 9.19 \cdot 10^{-5}$	$-3.47 \cdot 10^{-3} \pm 3,13 \cdot 10^{-3}$	$3.16 \cdot 10^{-3}$	0.9942
	Pond	1-100	$8.55 \cdot 10^{-4} \pm 7.31 \cdot 10^{-5}$	$4.90 \cdot 10^{-4} \pm 2.29 \cdot 10^{-3}$	$2.51 \cdot 10^{-3}$	0.9945
	Waste	1-100	$1.03 \cdot 10^{-3} \pm 1.04 \cdot 10^{-4}$	$-3.24 \cdot 10^{-3} \pm 4.68 \cdot 10^{-3}$	$3.57 \cdot 10^{-3}$	0.9924

Table IV.4.2- (Continued).

Analyte	Water sample	Studied linear range (µg/L)	Regression equation (n=7)		$S_{y/x}$	R^2
			$b \pm s_b \cdot t_{(0.05;5)}$	$a \pm s_a \cdot t_{(0.05;5)}$		
DNPP	Mineral	1-100	$3.95 \cdot 10^{-2} \pm 2.04 \cdot 10^{-3}$	$-9.39 \cdot 10^{-2} \pm 8.76 \cdot 10^{-2}$	$6.69 \cdot 10^{-2}$	0.9980
	Tap	1-100	$3.90 \cdot 10^{-2} \pm 2.32 \cdot 10^{-3}$	$-4.89 \cdot 10^{-2} \pm 1.00 \cdot 10^{-1}$	$7.63 \cdot 10^{-2}$	0.9973
	Pond	1-100	$3.83 \cdot 10^{-2} \pm 1.56 \cdot 10^{-3}$	$-5.49 \cdot 10^{-2} \pm 6.72 \cdot 10^{-2}$	$5.13 \cdot 10^{-2}$	0.9987
	Waste	1-100	$3.68 \cdot 10^{-2} \pm 3.02 \cdot 10^{-3}$	$-1.17 \cdot 10^{-1} \pm 1.30 \cdot 10^{-1}$	$9.92 \cdot 10^{-2}$	0.9949
BBP	Mineral	1-100	$1.78 \cdot 10^{-2} \pm 1.53 \cdot 10^{-3}$	$-5.65 \cdot 10^{-2} \pm 6.65 \cdot 10^{-2}$	$5.08 \cdot 10^{-2}$	0.9945
	Tap	1-100	$2.30 \cdot 10^{-2} \pm 1.98 \cdot 10^{-3}$	$-1.46 \cdot 10^{-1} \pm 1.92 \cdot 10^{-1}$	$1.71 \cdot 10^{-1}$	0.9933
	Pond	1-100	$2.38 \cdot 10^{-2} \pm 2.04 \cdot 10^{-3}$	$-1.46 \cdot 10^{-1} \pm 1.98 \cdot 10^{-1}$	$1.76 \cdot 10^{-1}$	0.9934
	Waste	1-100	$2.42 \cdot 10^{-2} \pm 1.80 \cdot 10^{-3}$	$-1.58 \cdot 10^{-1} \pm 1.74 \cdot 10^{-1}$	$1.56 \cdot 10^{-1}$	0.9950
DBEP	Mineral	1-100	$5.08 \cdot 10^{-3} \pm 4.28 \cdot 10^{-4}$	$-1.51 \cdot 10^{-2} \pm 1.91 \cdot 10^{-2}$	$1.45 \cdot 10^{-2}$	0.9947
	Tap	1-100	$5.16 \cdot 10^{-3} \pm 4.60 \cdot 10^{-4}$	$-6.96 \cdot 10^{-3} \pm 2.05 \cdot 10^{-2}$	$1.56 \cdot 10^{-2}$	0.9940
	Pond	1-100	$5.51 \cdot 10^{-3} \pm 2.95 \cdot 10^{-4}$	$-1.01 \cdot 10^{-2} \pm 1.31 \cdot 10^{-2}$	$1.00 \cdot 10^{-2}$	0.9978
	Waste	1-100	$6.29 \cdot 10^{-3} \pm 7.11 \cdot 10^{-4}$	$-3.06 \cdot 10^{-2} \pm 3.17 \cdot 10^{-2}$	$2.42 \cdot 10^{-2}$	0.9904
DCHP	Mineral	1-100	$3.17 \cdot 10^{-2} \pm 2.05 \cdot 10^{-3}$	$-7.84 \cdot 10^{-2} \pm 9.05 \cdot 10^{-2}$	$6.91 \cdot 10^{-2}$	0.9969
	Tap	1-100	$3.43 \cdot 10^{-2} \pm 9.59 \cdot 10^{-4}$	$-3.34 \cdot 10^{-2} \pm 4.13 \cdot 10^{-2}$	$3.12 \cdot 10^{-2}$	0.9995
	Pond	1-100	$3.26 \cdot 10^{-2} \pm 1.50 \cdot 10^{-3}$	$-3.77 \cdot 10^{-2} \pm 6.61 \cdot 10^{-2}$	$5.05 \cdot 10^{-2}$	0.9984
	Waste	1-100	$3.40 \cdot 10^{-2} \pm 2.66 \cdot 10^{-3}$	$-9.33 \cdot 10^{-2} \pm 1.17 \cdot 10^{-1}$	$8.96 \cdot 10^{-2}$	0.9954

b: slope; S_b: standard deviation of the slope; a: intercept; S_a: standard deviation of the intercept; $S_{y/x}$: standard deviation of the estimate.

Recovery of the overall method was studied spiking mineral, tap, pond and waste water before the extraction procedure at three concentration levels (IS was added at 35 µg/L in the sample), performing five consecutive replicates (n=5) at each level (see Table IV.4.3) and comparing the concentrations found at the end of the HF-LPME procedure with those added to the matrix. It can be observed that a good level of agreement was obtained between the concentration calculated at the end of the extraction process and the spiked one. In particular, recovery percentages ranged between 74 and 120 % with RSD values lower than 20 %. This analytical performance shows the feasibility of the procedure for the analysis of this group of PAEs from mineral, tap, pond and waste water with suitable sensitivity.

Table IV.4.3.- Results of the recovery study (n=5) of the HF-LPME-GC-MS/MS method for the selected PAEs in different water samples at three levels of concentration.

Analyte	Water sample	Level 1[a]	Level 2[b]	Level 3[c]
		Recovery % (RSD %)	Recovery % (RSD %)	Recovery % (RSD %)
DPP	Mineral	94 (9)	91 (10)	78 (9)
	Tap	110 (2)	95 (15)	80 (3)
	Pond	92 (8)	93 (6)	89 (10)
	Waste	95 (6)	88 (12)	80 (8)
DIBP	Mineral	95 (7)	102 (8)	87 (6)
	Tap	105 (5)	99 (10)	87 (1)
	Pond	120 (12)	95 (6)	90 (5)
	Waste	90 (7)	92 (7)	88 (6)
DBP	Mineral	108 (5)	103 (5)	87 (4)
	Tap	106 (4)	108 (4)	90 (2)
	Pond	104 (3)	102 (3)	96 (2)
	Waste	99 (6)	103 (2)	91 (3)
DIPP	Mineral	104 (5)	95 (13)	84 (4)
	Tap	100 (11)	104 (5)	98 (6)
	Pond	99 (5)	96 (3)	95 (7)
	Waste	94 (6)	94 (7)	96 (8)
DEEP	Mineral	109 (12)	84 (11)	74 (18)
	Tap	116 (9)	85 (19)	79 (18)
	Pond	95 (19)	95 (6)	104 (20)
	Waste	83 (20)	93 (19)	88 (20)
DNPP	Mineral	102 (4)	96 (11)	90 (7)
	Tap	98 (8)	98 (3)	99 (5)
	Pond	102 (4)	96 (4)	102 (5)
	Waste	92 (4)	92 (4)	99 (6)

Table IV.4.3.- (Continued).

Analyte	Water sample	Level 1[a]	Level 2[b]	Level 3[c]
		Recovery % (RSD %)	Recovery % (RSD %)	Recovery % (RSD %)
BBP	Mineral	116 (5)	100 (6)	104 (12)
	Tap	110 (7)	98 (5)	109 (7)
	Pond	108 (7)	97 (5)	115 (5)
	Waste	120 (2)	87 (9)	90 (5)
DBEP	Mineral	109 (4)	99 (5)	106 (14)
	Tap	109 (9)	96 (6)	120 (7)
	Pond	119 (13)	100 (5)	120 (3)
	Waste	100 (4)	92 (8)	102 (7)
DCHP	Mineral	109 (5)	103 (6)	92 (15)
	Tap	108 (10)	103 (9)	103 (2)
	Pond	110 (7)	99 (7)	112 (7)
	Waste	96 (4)	96 (10)	98 (4)

[a,b,c] Concentration of the analytes in the sample. [a] Level 1: 10 µg/L; [b] Level 2: 50 µg/L; [c] Level 3: 100 µg/L.

Finally, the method was applied to the analysis of the studied real samples, by carrying out a duplicate analysis in each case. Results are shown in Table IV.4.4 in which it can be seen that only DIBP and DBP were determined above the LCL of the method in mineral and waste water, respectively, though other compounds could also be detected depending on the sample. Both, DIBP and DBP have been previously found in similar samples [425,426]. Figure IV.4.7 shows a GC-MS/MS chromatogram of DIBP in mineral water sample and of DBP in waste water sample.

Concerning previous applications of HF-LPME for the extraction of the selected analytes in water samples, among the PAEs selected in this work, only BBP and DBP had been previously evaluated [336,337,411,412] when this work was developed. In those publications, only three compounds were determined, except in one case in which six different analytes, including BBP and DBP were extracted from bottled mineral water [412]. The LOQs obtained for both analytes in such works were slightly lower than the ones achieved in this work, although much more complex procedures were used in two of these applications in which push/pull flow approaches [336,411] were necessary to assist the HF-LPME procedure. Concerning the work developed by Mtibe et al. [337], no validation of the developed methodology was carried out, and, consequently, no comparison could be made since data regarding the quality parameters of the method were not provided.

Table IV.4.4.- Concentration of the studied PAEs found in real water samples.

Analyte	Water sample	Concentration found (µg/L)	Analyte	Water sample	Concentration found (µg/L)
DPP	Mineral	n.d.	DNPP	Mineral	n.d.
	Tap	n.d.		Tap	< LCL
	Pond	n.d.		Pond	< LCL
	Waste	< LCL		Waste	< LCL
DIBP	Mineral	2.56 ± 2.23	BBP	Mineral	n.d.
	Tap	< LCL		Tap	n.d.
	Pond	< LCL		Pond	n.d.
	Waste	< LCL		Waste	< LCL
DBP	Mineral	< LCL	DBEP	Mineral	n.d.
	Tap	< LCL		Tap	n.d.
	Pond	< LCL		Pond	n.d.
	Waste	2.46 ± 1.31		Waste	< LCL
DIPP	Mineral	n.d.	DCHP	Mineral	n.d.
	Tap	n.d.		Tap	n.d.
	Pond	< LCL		Pond	n.d.
	Waste	< LCL		Waste	< LCL
DEEP	Mineral	n.d.			
	Tap	n.d.			
	Pond	n.d.			
	Waste	n.d.			

n.d.: not detected

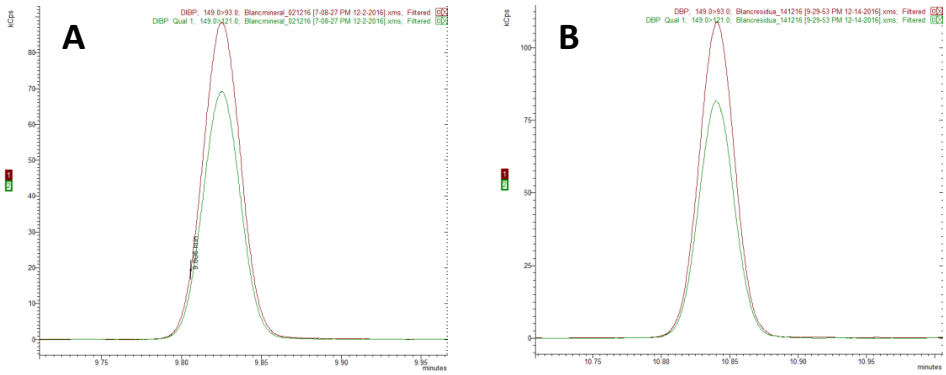


Figure IV.4.7.- GC-MS/MS chromatogram obtained from the analysis of real samples working in the MRM mode. A) For DIBP in a mineral water sample. B) For DBP in a waste water sample.

In general, it should be highlighted that the procedure proposed in this work is very simple and constitutes the first application in which this group of PAEs has been simultaneously analysed in such variety of water samples (i.e. mineral, waste, tap and pond water) and the first time in which the HF-LPME is applied for the evaluation of this kind of compounds in tap and pond water.

IV.4.6.- Conclusions

From the results obtained in this section, the following conclusions can be drawn:

- A methodology based on a HF-LPME procedure followed by the determination by GC-MS/MS has been successfully proposed for the analysis of nine PAEs of interest (DPP, DIBP, DBP, DIPP, DEEP, DNPP, BBP, DBEP and DCHP) in mineral, tap, pond and waste water samples.
- The optimisation of the HF-LPME procedure allowed obtaining the highest recovery of the target analytes and their ulterior application to more complex samples. The application of a back-extraction procedure clearly increased the recovery compared to the conventional retraction of the fibre. The best extraction performance was obtained using 2 cm of a PP HF and 1-octanol as acceptor phase for the extraction of 10 mL of aqueous sample (without pH adjustment and salt addition). The extraction was carried out for 75 min with a stirring agitation of 850 rpm at 60 °C, while the back-extraction was carried out immersing the fibre in 200 µL of cyclohexane and ultrasounds for 10 min.
- The validation of the methodology in terms of linearity, precision and trueness in every kind of water selected for this work (i.e. mineral, tap, pond and waste water) demonstrated that it could be perfectly applied to the analysis of all the target analytes in the selected samples. Calibration curves of the method were developed in the linear range 1-100 µg/L for each analyte in each selected matrix using DBP-d_4 as IS, obtaining R^2 values higher than 0.9901, while precision and trueness were evaluated by means of a recovery study, obtaining values between 74 and 120 % with RSDs lower than 20 % for all analytes in all the matrices evaluated.
- The application of the methodology to the analysis of mineral, tap, pond and waste water from different points of the island of Tenerife revealed the presence of certain PAEs below the LCL, as well as DIBP and DBP in mineral and waste water, respectively,

both at concentrations higher than 2 µg/L, which is in accordance to previous works in which these PAEs have been found in similar matrices.

- The procedure proposed in this work is very simple and constitutes the first application in which this group of PAEs has been simultaneously analysed in such variety of water samples (i.e. mineral, waste, tap and pond water) and the first time in which the HF-LPME is applied for the evaluation of this kind of compounds in tap and pond water.

IV.5.- Determination of phthalates in beverages using MWCNTs-μ-dSPE prior to HPLC-MS/MS

A μ-dSPE method was developed using MWCNTs of 110–170 nm diameter and 5–9 μm length for the extraction of a group of nine PAEs (i.e., DMEP, DEEP, DPP, BBP, DBEP, DIPP, DNPP, DCHP and DNOP) from tap water as well as from different beverages commercialised in plastic bottles (mineral water, lemon and apple flavoured mineral water, and an isotonic drink). Determination was carried out by HPLC-MS/MS. Parameters affecting the performance of the extraction procedure such as sample pH, elution solvent type, MWCNTs amount, elution solvent volume and sample volume were studied and optimised following a step by step approach. The whole methodology was validated in different matrices and the applicability of the developed method was tested in different beverages.

IV.5.1.- Background

Over recent years, a good number of publications have shown the excellent extraction capacity of CNTs for a wide variety of analytes and matrices [217,218,427-430]. These molecular-scale tubes of graphitic carbon, which can be considered as a graphene sheet in the shape of a cylinder, have unique properties for their use as SPE sorbents: high surface area, ability to establish π–π interactions, high chemical and mechanical stability, insolubility in water and organic solvents, among others [217,218]. If necessary, they can be solubilised by adding surfactants or by their functionalisation, which can also increase their reactivity [217,218,427-430].

CNTs (SWCNTs or MWCNTs) can be directly used in SPE, especially for the extraction of non-polar compounds, though several works have even reported their covalent immobilisation onto solid supports or their use as part of composites [259,431]. In general, small amounts of CNTs (lower than those of conventional SPE cartridges) can be used as SPE materials, which clearly contribute to the miniaturisation of the process and to the consumption of lower amounts of organic solvents. However, one of the main drawbacks of their use is probably the fact that no commercial CNTs cartridges are available and that they are normally more expensive than conventional cartridges (especially when functionalised), though some applications of economical CNTs have also been reported [112,432]. Despite all these facts, new applications are highly welcome to

evaluate the utility of these materials and to extend and consolidate their application, which may also depend on the dimensions of the nanotubes.

As previously indicated, the analysis of PAEs in food matrices has attracted much attention in recent years [115,119]. In particular, their analysis in drinking water or beverages, especially those commercialised in plastic bottles, is of importance as a result of the high human consumption and their already mentioned negative effects for health [433]. In this sense, and as far as we know, pristine CNTs of different dimensions had been used for the extraction of a relatively reduced number of them in few occasions when this work was developed. In particular, for the extraction of carbonated drinks [179,434], mineral water [434,435], juice [434], and tap water [167], by either conventional SPE [167] or m-dSPE [179,434,435], though other applications had also used them for similar purposes as composites with other materials [240] or as SPME coatings [207,436]. Concerning the quantities and dimensions of the pristine CNTs applied for the extraction of PAEs, Moazzen et al. used 10 mg of MWCNTs of 30–60 nm diameter × 5.0–30 mm length for the extraction of 10 mL of carbonated drinks [179]; Jiao et al. used 20 mg of MWCNTs of < 8 nm × 0.5–2 μm for the extraction of 200 mL of mineral water [435]; Luo et al. used 4 mg of MWCNTs of 10–20 nm × 5.0–15 μm for the extraction of 10 mL of juice, carbonated drinks, and mineral water [434]; and Cai et al. used 500 mg of MWCNTs of 30–60 nm for the extraction of different water samples [167].

IV.5.2.- Specific objectives

Considering all the above-mentioned, the following specific objectives were established:

- The development of a new analytical methodology based on the use of pristine MWCNTs as sorbent for the μ-dSPE of nine PAEs of interest (i.e. DMEP, DEEP, DPP, BBP, DBEP, DIPP, DNPP, DCHP and DNOP) from different types of plastic bottled water and beverages, and their subsequent determination by HPLC-IT-MS/MS.
- The evaluation of the influence of the parameters affecting the μ-dSPE procedure (i.e. sample pH, elution solvent type, MWCNTs amount, elution solvent volume and sample volume) using a step by step approach to achieve the best extraction efficiency.
- The validation of the whole methodology in terms of linearity, precision and trueness as well as the obtention of the corresponding LODs and LOQs of the method.

- The study of the applicability of the developed methodology to the analysis of plastic bottled water and beverages.

IV.5.3.- Optimisation of separation and detection conditions

In this work, a suitable chromatographic separation of the nine selected PAEs was achieved by HPLC using a X-Bridge C_{18} column (100 mm × 4.6 mm × 3.5 µm) and a precolumn (20 mm × 4.6 mm) with the same stationary phase. A mobile phase composed of a mixture of ACN and Milli-Q water was tested, containing or not small amounts of formic acid. The elution gradient described in Section III.5.5 allowed achieving the best separation with the addition of 0.1 % (v/v) of formic acid, because an increase in the sensitivity was observed. Finally, a slight enhancement of the resolution and efficiency was achieved by rising the temperature of the column and precolumn to 40 °C.

Regarding nebulisation/ionisation conditions, direct infusion of individual solutions of the target compounds in ACN at a concentration of 1 mg/L was performed, working in both positive and negative mode. The nebulisation gas (N_2) pressure was modified between 2.0 and 80.0 psi, and the dry gas (N_2) flow and temperature were modified between 0.5 and 12.0 L/min and between 150 and 350 °C, respectively. The capillary voltage was tested between 3000 and 6000 V and the end plate offset between –500 and –5500 V. The highest sensitivity for all analytes was achieved working in the positive mode, with a capillary voltage of –4500 V, an end plate offset of –500 V, a nebulisation gas pressure of 30.0 psi, and a dry gas flow and temperature of 8.0 L/min and 350 °C.

Finally, fragmentation energies for MS/MS detection were optimised by means of the direct infusion of individual solutions of each analyte in ACN at a concentration of 1 mg/L and by applying the nebulisation and detection conditions previously mentioned. Table IV.5.1 shows the best fragmentation conditions for the selected PAEs, as well as the most intense product ions.

To prove the linearity of the HPLC–MS method, instrumental calibration curves based on analyte peak areas were obtained for each compound by injecting nine increasing concentration levels (n=9) in triplicate. Table IV.5.2 shows the linear range and the full calibration curve equations (including the standard deviations of the slopes and intercepts), as well as the R^2 values, which were higher than 0.9921 in all cases.

Table IV.5.1.- MS/MS fragmentation parameters of the selected PAEs.

Analyte	Precursor ion (m/z)	Product ions* (m/z)	Fragmentation amplitude (V)
DMEP	283.0	206.9	0.35
		-	
DEEP	311.0	220.9	0.35
		-	
DPP	190.9	149.0	0.25
		-	
BBP	311.0	149.0	0.40
		205.0	
DBEP	367.1	249.0	0.34
		-	
DIPP	307.0	219.0	0.37
		145.0	
DNPP	307.0	145.0	0.37
		219.0	
DCHP	331.0	149.0	0.40
		249.0	
DNOP	391.3	149.0	0.27
		261.0	

* Most intense product ions.

Table IV.5.2.- Instrumental calibration data for the selected PAEs.

Analyte	Studied linear range (µg/L)*	Regression equation (n=9)		$S_{y/x}$	R^2
		$b \pm s_b \cdot t_{(0,05;7)}$	$a \pm s_a \cdot t_{(0,05;7)}$		
DMEP	10-500	$8.14 \cdot 10^5 \pm 6.50 \cdot 10^4$	$1.63 \cdot 10^7 \pm 1.56 \cdot 10^7$	$1.32 \cdot 10^7$	0.9921
DEEP	10-500	$1.18 \cdot 10^6 \pm 6.61 \cdot 10^4$	$2.00 \cdot 10^7 \pm 1.59 \cdot 10^7$	$1.35 \cdot 10^7$	0.9961
DPP	10-500	$5.24 \cdot 10^5 \pm 9.71 \cdot 10^3$	$4.69 \cdot 10^5 \pm 2.33 \cdot 10^6$	$1.97 \cdot 10^6$	0.9996
BBP	10-500	$7.42 \cdot 10^5 \pm 4.41 \cdot 10^4$	$9.80 \cdot 10^6 \pm 1.06 \cdot 10^7$	$8.97 \cdot 10^6$	0.9956
DBEP	10-500	$3.53 \cdot 10^6 \pm 2.21 \cdot 10^5$	$2.15 \cdot 10^7 \pm 5.30 \cdot 10^7$	$1.32 \cdot 10^7$	0.9921
DIPP	10-500	$1.02 \cdot 10^6 \pm 3.70 \cdot 10^4$	$6.03 \cdot 10^6 \pm 8.88 \cdot 10^6$	$7.53 \cdot 10^6$	0.9983
DNPP	10-500	$9.07 \cdot 10^5 \pm 4.92 \cdot 10^4$	$4.13 \cdot 10^6 \pm 1.18 \cdot 10^7$	$1.00 \cdot 10^7$	0.9963
DCHP	10-500	$2.48 \cdot 10^6 \pm 1.33 \cdot 10^5$	$1.09 \cdot 10^7 \pm 3.19 \cdot 10^7$	$2.70 \cdot 10^7$	0.9964
DNOP	10-500	$2.39 \cdot 10^6 \pm 1.50 \cdot 10^5$	$3.55 \cdot 10^7 \pm 3.59 \cdot 10^7$	$2.80 \cdot 10^7$	0.9959

* LCL: 10 µg/L.

IV.5.4.- MWCNTs-μ-dSPE optimisation

With the aim of exploring the applicability of the selected MWCNTs for the extraction of the target PAEs from tap water and different beverages commercialised in plastic bottles, the parameters influencing their extraction (sample pH, sorbent amount, elution solvent type and volume) were studied and optimised. To have a better vision of the influence of each factor in the extraction process, the optimisation of the aforementioned parameters was carried out in Milli-Q water (25 mL), trying not to introduce important matrix effects. The influence of each parameter was evaluated by means of absolute recovery values in each experiment, calculated by dividing the peak areas obtained when samples were spiked before the extraction process by those obtained by spiking the final extract, that is, at the end of the extraction process (matrix-matched standard), multiplied by 100.

As a starting point, 25 mL of Milli-Q water was initially extracted with 100 mg of MWCNTs and eluted with 30 mL of ACN. The eluate was evaporated to dryness at 40 ºC in a rotary evaporator and reconstituted in 500 μL of the initial mobile phase and injected in the HPLC–MS system.

IV.5.4.1.- Sample pH

Although PAEs are not ionisable in aqueous solution and, as previously commented, the pH of the sample should not have a clear influence on the extraction recovery, the effect of this parameter on the extraction efficiency was studied between 4.0 and 12.0 (duplicate extractions were carried out in each case). As expected, the pH did not have a relevant influence on the extraction performance, obtaining similar results independently of its value except at pH 12.0 for DEEP and DPP for which recovery values were slightly lower. Such slight decrease, though not significant, could be caused by a possible hydrolysis of the ester at such high pH. In any case, it was decided to adjust the pH of all samples to 6.0 for further analyses to establish a common pH value, which is an intermediate pH of the analysed beverages and also of water samples, for further validation and analyses in more complex matrices. The main reason for such pH adjustment was to minimise the irreproducibility between extractions. In these initial experiments, excellent recovery values, above 80 %, were obtained for all analytes except for DPP that ranged between 58 and 72 %.

IV.5.4.2.- Elution solvent type

Although ACN provided excellent recovery values for all analytes under the initial selected conditions (25 mL of Milli-Q water at pH 6.0, 100 mg of sorbent, and 30 mL of elution solvent), other solvents, such as MeOH, acetone, and DCM were also studied. Results are shown in Figure IV.5.1. In general, all of them provided lower recovery values for all the selected analytes. In particular, DCM and acetone provided recovery percentages around 10 and 20 % less, respectively, while MeOH provided much lower recovery values (less than 50 %) except for DBEP and DNPP that were hardly recovered. Therefore, ACN was selected for further experiments.

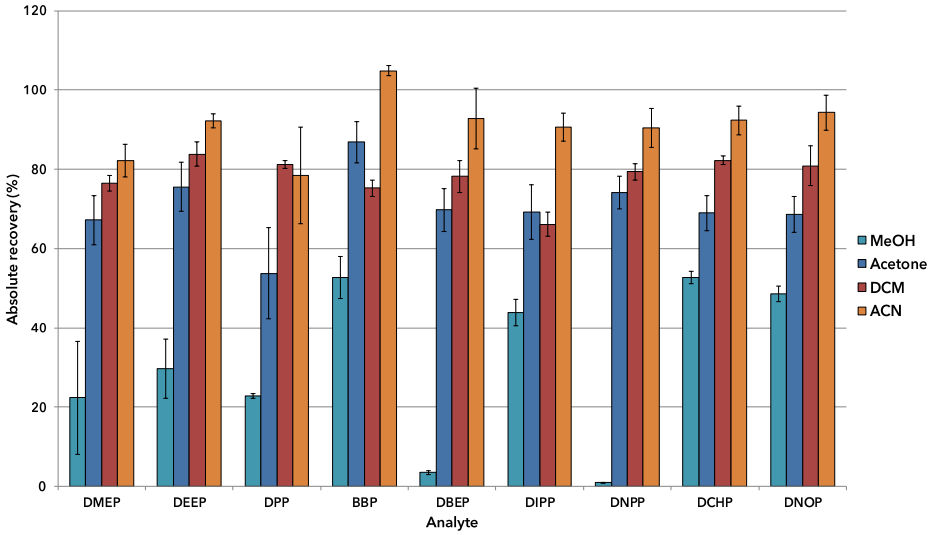


Figure IV.5.1.- Effect of the μ-dSPE elution solvent type on the recovery values of the selected PAEs. Extraction conditions: 25 mL of Milli-Q water at pH 6.0, 100 mg of MWCNTs and 30 mL of elution solvent.

IV.5.4.3.- MWCNTs amount

Regarding the amount of sorbent, 20, 40, 60, and 80 mg were also tested under the above-mentioned extraction conditions (25 mL of Milli-Q water at pH 6.0 and elution with 30 mL of ACN). Results obtained are shown in Figure IV.5.2. Although 60 mg of sorbent provided a slightly higher recovery for DEEP, 40 mg of sorbent was enough for the quantitative extraction of most of the selected PAEs, with recovery values higher than 80 % for all of them.

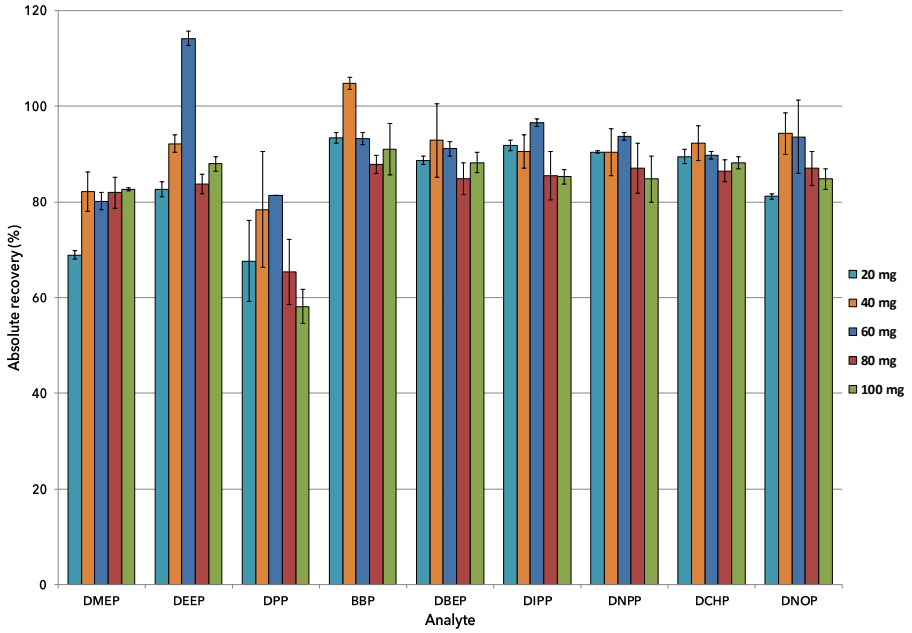


Figure IV.5.2.- Effect of the μ-dSPE sorbent amount on the recovery values of the selected PAEs. Extraction conditions: 25 mL of Milli-Q water at pH 6.0 and elution with 30 mL of ACN.

IV.5.4.4.- Elution solvent volume

Subsequent studies were carried out to assess the effect of elution solvent volume. In this sense, different volumes of ACN were tested (5, 10, 12.5, 15, 20, and 30 mL) to consume the lowest amount of organic solvent possible, finding that 12.5 mL was more than enough to provide a quantitative extraction of the selected analytes. As can be seen in Figure IV.5.3, recovery values ranged between 80 and 103 % for all PAEs, except for DMEP and DPP that were 74 and 73 %, respectively.

IV.5.4.5.- Volume of the sample

To reduce the LODs of the method, the sample volume was increased up to 50 mL. For this reason, the amount of sorbent and elution solvent volume were also duplicated (80 mg of MWCNTs and 25 mL of ACN), obtaining comparable results to when 25 mL of Milli-Q water at pH 6.0, 40 mg of MWCNTs, and 12.5 mL of ACN were used. In any case, some tests were also carried out to try to reduce the elution volume, but lower recovery values were obtained for all the target analytes (between 64 and 96 %).

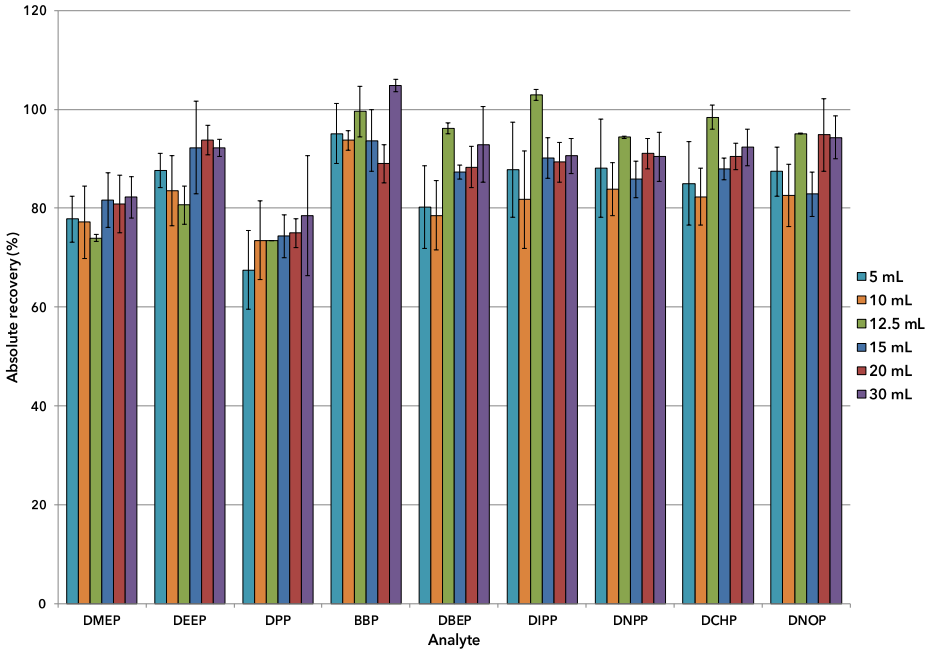


Figure IV.5.3.- Effect of the μ-dSPE elution solvent volume on the recovery values of the selected PAEs. Extraction conditions: 25 mL of Milli-Q water at pH 6.0, 40 mg of MWCNTs and elution with ACN.

IV.5.5.- MWCNTs-μ-dSPE-HPLC–MS/MS method validation

Once the best conditions for the μ-dSPE procedure were selected, the method was applied and validated for the analysis of the target PAEs in tap water and different plastic bottled drinks (mineral water, lemon and apple flavoured mineral waters, as well as an isotonic drink). Taking into account that PAEs may also appear in the studied matrices, blank samples were first analysed. In particular, and as it will be later discussed, BBP was found in all the samples at concentrations lower than the LOQ of the method except for lemon flavoured mineral water in which it was not found.

When the methodology was applied for the analysis of the target PAEs in tap water, mineral, and lemon and apple flavoured mineral water, absolute recovery values obtained were similar to the ones previously achieved for Milli-Q water, as it will be later shown (see Section IV.5.5.2). However, the isotonic drink had to be diluted (1/2) to

maintain such recovery values. Without such dilution, recovery was in the range 8–98 %.

IV.5.5.1.- Matrix-matched calibration and matrix effect evaluation

It is well known that the injection of the target analytes in the sample matrix could result in an effect of suppression or enhancement of the detector signal, which would give biased results [392]. In the particular case of LC-MS, it is quite frequent to find an ion suppression, associated with an inefficient ionisation of the target analytes as a result of the presence of matrix components that difficult such ionisation process [437]. For this reason, one of the most commonly used methods to compensate matrix effects is to carry out matrix-matched calibrations that equalise the response for calibration standards and sample extracts.

In this work, matrix-matched calibration curves were developed by injecting nine different levels of concentrations (n=9) in triplicate. Table IV.5.3 shows the calibration data obtained (based on peak areas), including the studied linear range, the full calibration curve with the confidence intervals, and R^2 values that were higher than 0.9906 in all cases. The LCL was 35 µg/L for all analytes, except for DMEP in the isotonic drink and DNOP in all samples, for which the LCL was set at 75 µg/L.

Table IV.5.3 shows the matrix effect percentages obtained for each analyte and matrix, calculated following the equation used by Matuszewski et al. [438], which consists on comparing the area obtained for a matrix-matched standard with that obtained for a standard in solvent by means of the application of the following equation:

$$\text{Matrix effect \%} = \frac{\text{Area of a matrix-matched standard}}{\text{Area of a standard in solvent}} \cdot 100$$

As can be seen in the table, a clear signal suppression (< 80%) was observed for DNPP, DCHP, and DNOP in the isotonic drink. On the contrary, a signal enhancement (> 120 %) was observed for DEEP, BBP, and DNPP in mineral water, for DMEP, DEEP, DPP, BBP, and DIPP in tap water; for DMEP and DEEP in lemon flavoured mineral water, and for DEEP, BBP, DBEP, DIPP, and DNPP in apple flavoured mineral water. Judging by these results, it is clear the necessity of taking matrix effects into consideration for the rest of the study by developing matrix-matched calibration.

Table IV.5.3.- Matrix-matched calibration data of the selected PAEs and matrix effect percentage.

Analyte	Sample	Studied linear range (µg/L)	Regression equation (n=9)		$S_{y/x}$	R^2	ME % (RSD %)[a]
			$b \pm s_b \cdot t_{(0.05;7)}$	$a \pm s_a \cdot t_{(0.05;7)}$			
DMEP	Mineral water	50-500	$8.79 \cdot 10^5 \pm 5.35 \cdot 10^4$	$2.92 \cdot 10^7 \pm 1.29 \cdot 10^7$	$1.05 \cdot 10^7$	0.9954	116 (16)
	Tap water	50-500	$1.06 \cdot 10^6 \pm 1.18 \cdot 10^5$	$3.12 \cdot 10^7 \pm 2.81 \cdot 10^7$	$1.69 \cdot 10^7$	0.9936	144 (12)
	Lemon flavoured water	50-500	$9.18 \cdot 10^5 \pm 8.31 \cdot 10^4$	$2.61 \cdot 10^7 \pm 1.99 \cdot 10^7$	$1.55 \cdot 10^7$	0.9919	124 (6)
	Apple flavoured water	50-500	$1.04 \cdot 10^6 \pm 5.34 \cdot 10^4$	$1.99 \cdot 10^7 \pm 1.16 \cdot 10^7$	$9.07 \cdot 10^6$	0.9974	117 (6)
	Isotonic drink	75-500	$8.44 \cdot 10^5 \pm 6.18 \cdot 10^4$	$4.16 \cdot 10^7 \pm 1.81 \cdot 10^7$	$1.25 \cdot 10^7$	0.9922	117 (17)
DEEP	Mineral water	35-500	$1.33 \cdot 10^6 \pm 1.00 \cdot 10^5$	$5.38 \cdot 10^7 \pm 2.42 \cdot 10^7$	$1.96 \cdot 10^7$	0.9929	130 (14)
	Tap water	35-500	$1.68 \cdot 10^6 \pm 1.40 \cdot 10^5$	$3.49 \cdot 10^7 \pm 3.38 \cdot 10^7$	$2.74 \cdot 10^7$	0.9914	147 (9)
	Lemon flavoured water	35-500	$1.50 \cdot 10^6 \pm 1.18 \cdot 10^5$	$4.65 \cdot 10^7 \pm 2.51 \cdot 10^7$	$1.85 \cdot 10^7$	0.9954	142 (7)
	Apple flavoured water	35-500	$1.52 \cdot 10^6 \pm 8.00 \cdot 10^4$	$4.98 \cdot 10^7 \pm 1.93 \cdot 10^7$	$1.56 \cdot 10^7$	0.9965	137 (8)
	Isotonic drink	35-500	$1.14 \cdot 10^6 \pm 7.98 \cdot 10^4$	$3.50 \cdot 10^7 \pm 1.70 \cdot 10^7$	$1.25 \cdot 10^7$	0.9963	117 (7)
DPP	Mineral water	35-500	$5.78 \cdot 10^5 \pm 2.41 \cdot 10^4$	$3.90 \cdot 10^6 \pm 5.79 \cdot 10^6$	$4.70 \cdot 10^6$	0.9978	115 (11)
	Tap water	35-500	$5.68 \cdot 10^5 \pm 4.67 \cdot 10^4$	$1.11 \cdot 10^7 \pm 1.12 \cdot 10^7$	$9.12 \cdot 10^6$	0.9916	128 (5)
	Lemon flavoured water	35-500	$5.21 \cdot 10^5 \pm 2.06 \cdot 10^4$	$7.09 \cdot 10^6 \pm 4.97 \cdot 10^6$	$4.03 \cdot 10^6$	0.9980	109 (12)
	Apple flavoured water	35-500	$5.18 \cdot 10^5 \pm 3.54 \cdot 10^4$	$1.04 \cdot 10^7 \pm 8.52 \cdot 10^6$	$6.92 \cdot 10^6$	0.9942	119 (13)
	Isotonic drink	35-500	$5.05 \cdot 10^5 \pm 1.88 \cdot 10^4$	$5.48 \cdot 10^6 \pm 4.53 \cdot 10^6$	$3.68 \cdot 10^6$	0.9983	105 (7)
BBP	Mineral water	35-500	$8.92 \cdot 10^5 \pm 4.54 \cdot 10^4$	$1.43 \cdot 10^7 \pm 1.09 \cdot 10^7$	$8.87 \cdot 10^6$	0.9968	129 (10)
	Tap water	35-500	$9.98 \cdot 10^5 \pm 6.34 \cdot 10^4$	$9.02 \cdot 10^6 \pm 1.53 \cdot 10^7$	$1.24 \cdot 10^7$	0.9950	139 (12)
	Lemon flavoured water	35-500	$8.67 \cdot 10^5 \pm 3.45 \cdot 10^4$	$1.41 \cdot 10^7 \pm 8.31 \cdot 10^6$	$6.75 \cdot 10^6$	0.9980	117 (17)
	Apple flavoured water	35-500	$8.77 \cdot 10^5 \pm 3.45 \cdot 10^4$	$2.63 \cdot 10^7 \pm 8.31 \cdot 10^6$	$6.75 \cdot 10^6$	0.9981	122 (7)
	Isotonic drink	35-500	$7.12 \cdot 10^5 \pm 4.40 \cdot 10^4$	$-3.26 \cdot 10^6 \pm 1.06 \cdot 10^7$	$8.60 \cdot 10^6$	0.9952	120 (17)
DBEP	Mineral water	35-500	$3.48 \cdot 10^6 \pm 8.63 \cdot 10^4$	$6.47 \cdot 10^7 \pm 2.08 \cdot 10^7$	$1.05 \cdot 10^7$	0.9954	105 (9)
	Tap water	35-500	$3.93 \cdot 10^6 \pm 2.94 \cdot 10^5$	$8.88 \cdot 10^7 \pm 7.09 \cdot 10^7$	$1.69 \cdot 10^7$	0.9936	120 (11)
	Lemon flavoured water	35-500	$3.51 \cdot 10^6 \pm 2.23 \cdot 10^5$	$5.88 \cdot 10^7 \pm 5.36 \cdot 10^7$	$1.55 \cdot 10^7$	0.9919	103 (5)
	Apple flavoured water	35-500	$4.02 \cdot 10^6 \pm 1.61 \cdot 10^5$	$1.30 \cdot 10^8 \pm 3.89 \cdot 10^7$	$9.07 \cdot 10^6$	0.9974	134 (16)
	Isotonic drink	35-500	$2.91 \cdot 10^6 \pm 2.34 \cdot 10^5$	$7.23 \cdot 10^7 \pm 5.88 \cdot 10^7$	$1.25 \cdot 10^7$	0.9922	99 (16)

Table IV.5.3.- (Continued).

Analyte	Sample	Studied linear range (µg/L)	Regression equation (n=9)		$S_{y/x}$	R^2	ME % (RSD %)[a]
			$b \pm s_b \cdot t_{(0.05;7)}$	$a \pm s_a \cdot t_{(0.05;7)}$			
DIPP	Mineral water	35-500	$1.18 \cdot 10^6 \pm 5.56 \cdot 10^4$	$1.20 \cdot 10^7 \pm 1.34 \cdot 10^7$	$1.09 \cdot 10^7$	0.9972	112 (6)
	Tap water	35-500	$1.14 \cdot 10^6 \pm 8.62 \cdot 10^4$	$2.27 \cdot 10^7 \pm 2.08 \cdot 10^7$	$1.69 \cdot 10^7$	0.9928	121 (7)
	Lemon flavoured water	35-500	$1.12 \cdot 10^6 \pm 3.70 \cdot 10^4$	$1.20 \cdot 10^7 \pm 8.92 \cdot 10^6$	$7.24 \cdot 10^6$	0.9986	106 (6)
	Apple flavoured water	35-500	$1.23 \cdot 10^6 \pm 4.58 \cdot 10^4$	$1.39 \cdot 10^7 \pm 1.10 \cdot 10^7$	$8.95 \cdot 10^6$	0.9983	125 (6)
	Isotonic drink	35-500	$8.65 \cdot 10^5 \pm 3.55 \cdot 10^4$	$1.85 \cdot 10^7 \pm 8.56 \cdot 10^6$	$6.94 \cdot 10^6$	0.9979	100 (19)
DNPP	Mineral water	35-500	$1.05 \cdot 10^6 \pm 3.12 \cdot 10^4$	$2.14 \cdot 10^7 \pm 7.51 \cdot 10^6$	$6.10 \cdot 10^6$	0.9989	123 (20)
	Tap water	35-500	$1.03 \cdot 10^6 \pm 6.66 \cdot 10^4$	$1.75 \cdot 10^7 \pm 1.60 \cdot 10^7$	$1.30 \cdot 10^7$	0.9948	120 (16)
	Lemon flavoured water	35-500	$9.90 \cdot 10^5 \pm 2.81 \cdot 10^4$	$1.73 \cdot 10^7 \pm 6.78 \cdot 10^6$	$5.50 \cdot 10^6$	0.9990	118 (17)
	Apple flavoured water	35-500	$1.10 \cdot 10^6 \pm 3.59 \cdot 10^4$	$1.14 \cdot 10^7 \pm 8.65 \cdot 10^6$	$7.02 \cdot 10^6$	0.9987	131 (15)
	Isotonic drink	35-500	$7.20 \cdot 10^5 \pm 5.82 \cdot 10^4$	$1.95 \cdot 10^7 \pm 1.40 \cdot 10^7$	$1.14 \cdot 10^7$	0.9919	78 (10)
DCHP	Mineral water	35-500	$2.71 \cdot 10^6 \pm 9.40 \cdot 10^4$	$2.79 \cdot 10^7 \pm 2.26 \cdot 10^7$	$1.84 \cdot 10^7$	0.9985	112 (12)
	Tap water	35-500	$2.62 \cdot 10^6 \pm 1.89 \cdot 10^5$	$4.26 \cdot 10^7 \pm 4.55 \cdot 10^7$	$3.70 \cdot 10^7$	0.9935	110 (9)
	Lemon flavoured water	35-500	$2.58 \cdot 10^6 \pm 4.08 \cdot 10^4$	$1.78 \cdot 10^7 \pm 9.83 \cdot 10^6$	$7.98 \cdot 10^6$	0.9997	103 (7)
	Apple flavoured water	35-500	$2.85 \cdot 10^6 \pm 1.46 \cdot 10^5$	$4.04 \cdot 10^7 \pm 3.50 \cdot 10^7$	$2.84 \cdot 10^7$	0.9967	120 (8)
	Isotonic drink	35-500	$1.96 \cdot 10^6 \pm 9.79 \cdot 10^4$	$3.58 \cdot 10^7 \pm 2.36 \cdot 10^7$	$1.91 \cdot 10^7$	0.9969	79 (2)
DNOP	Mineral water	75-500	$2.22 \cdot 10^6 \pm 3.07 \cdot 10^5$	$6.71 \cdot 10^7 \pm 9.48 \cdot 10^7$	$3.58 \cdot 10^7$	0.9937	98 (8)
	Tap water	75-500	$2.39 \cdot 10^6 \pm 1.39 \cdot 10^5$	$7.93 \cdot 10^6 \pm 4.07 \cdot 10^7$	$2.11 \cdot 10^7$	0.9974	94 (12)
	Lemon flavoured water	75-500	$2.17 \cdot 10^6 \pm 9.70 \cdot 10^4$	$7.70 \cdot 10^7 \pm 2.84 \cdot 10^7$	$2.18 \cdot 10^7$	0.9969	95 (11)
	Apple flavoured water	75-500	$2.30 \cdot 10^6 \pm 2.00 \cdot 10^5$	$3.83 \cdot 10^7 \pm 5.85 \cdot 10^7$	$3.13 \cdot 10^7$	0.9942	114 (14)
	Isotonic drink	75-500	$5.02 \cdot 10^5 \pm 7.16 \cdot 10^4$	$7.37 \cdot 10^6 \pm 2.15 \cdot 10^7$	$8.95 \cdot 10^6$	0.9907	17 (15)

b: slope; S_b: standard deviation of the slope; a: intercept; S_a: standard deviation of the intercept; $S_{y/x}$: standard deviation of the estimate.
[a] Matrix effect calculated following the equation used by Matuszewski et al. [438].

IV.5.5.2.- Trueness

The trueness of the present method was evaluated carrying out a recovery study by spiking the samples at two different levels of concentration (0.37 and 1.2 µg/L). Absolute recovery values were calculated by comparing peak areas of the spiked drink samples with peak areas of standards (in the matrix) of the same concentration of PAEs. Recovery percentages between 70 and 117 % were obtained with satisfactory RSD values, which were below 17 % (see Table IV.5.4). The LOQs of the method were determined from the LCL and taking into account the recovery obtained in each case, obtaining values ranging between 0.17 and 1.5 µg/L, which were experimentally checked. Figure IV.5.4 shows an EIC of a lemon flavoured water sample that was spiked with the target analytes at 0.37 µg/L before the extraction process.

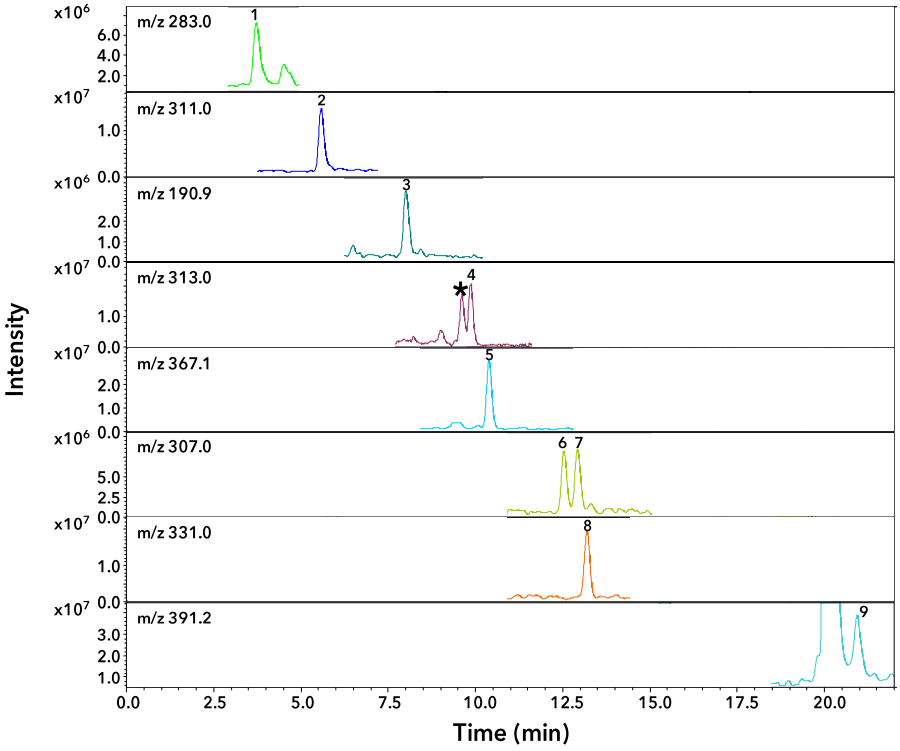


Figure IV.5.4.- EICs of a lemon flavoured mineral water sample spiked with the target analytes at 0.37 µg/L before the extraction process. Extraction conditions: 50 mL of sample at pH 6.0, 80 mg of MWCNTs and 25 mL of ACN. (1) DMEP, (2) DEEP, (3) DPP, (4) BBP, (5) DBEP, (6) DIPP, (7) DNPP, (8) DCHP, (9) DNOP. * Interference

Table IV.5.4.- Results of the recovery study (n=5) of the μ-dSPE-HPLC-MS/MS method from different bottled drink samples at two levels of concentration.

Analyte	Sample	Level 1	Level 2	LOQ_{method} (μg/L)
		Recovery % (RSD %)	Recovery % (RSD %)	
DMEP	Mineral water	76 (17)	80 (7)	0.22
	Tap water	70 (17)	101 (3)	0.20
	Lemon flavoured mineral water	79 (13)	74 (8)	0.23
	Apple flavoured mineral water	71 (12)	72 (9)	0.24
	Isotonic drink	80 (7)	75 (3)	1.5
DEEP	Mineral water	70 (7)	90 (12)	0.22
	Tap water	71 (7)	92 (8)	0.21
	Lemon flavoured mineral water	78 (6)	81 (9)	0.22
	Apple flavoured mineral water	87 (3)	75 (3)	0.22
	Isotonic drink	71 (10)	77 (14)	0.71
DPP	Mineral water	71 (12)	79 (6)	0.23
	Tap water	73 (17)	79 (14)	0.23
	Lemon flavoured mineral water	71 (3)	82 (6)	0.23
	Apple flavoured mineral water	74 (15)	99 (11)	0.20
	Isotonic drink	80 (14)	75 (4)	0.68
BBP	Mineral water	76 (12)	110 (15)	0.19
	Tap water	79 (16)	92 (12)	0.20
	Lemon flavoured mineral water	91 (3)	99 (5)	0.18
	Apple flavoured mineral water	104 (4)	83 (2)	0.19
	Isotonic drink	103 (14)	85 (15)	0.56
DBEP	Mineral water	77 (13)	98 (12)	0.20
	Tap water	88 (5)	99 (2)	0.19
	Lemon flavoured mineral water	92 (3)	90 (6)	0.19
	Apple flavoured mineral water	103 (6)	86 (1)	0.19
	Isotonic drink	79 (3)	84 (10)	0.64
DIPP	Mineral water	97 (5)	87 (7)	0.19
	Tap water	96 (9)	94 (3)	0.18
	Lemon flavoured mineral water	96 (7)	90 (7)	0.19
	Apple flavoured mineral water	94 (7)	82 (17)	0.20
	Isotonic drink	87 (6)	89 (14)	0.60

Table IV.5.4.- (Continued).

Analyte	Sample	Level 1	Level 2	LOQ_{method} (µg/L)
		Recovery % (RSD %)	Recovery % (RSD %)	
DNPP	Mineral water	117 (11)	85 (3)	0.17
	Tap water	94 (9)	92 (3)	0.19
	Lemon flavoured mineral water	90 (8)	91 (5)	0.19
	Apple flavoured mineral water	92 (6)	85 (11)	0.20
	Isotonic drink	96 (8)	91 (12)	0.56
DCHP	Mineral water	96 (5)	91 (4)	0.19
	Tap water	100 (6)	95 (2)	0.18
	Lemon flavoured mineral water	99 (3)	95 (3)	0.18
	Apple flavoured mineral water	107 (4)	90 (2)	0.18
	Isotonic drink	83 (8)	89 (14)	0.61
DNOP	Mineral water	93 (11)	74 (6)	0.45
	Tap water	93 (11)	102 (11)	0.38
	Lemon flavoured mineral water	75 (18)	72 (6)	0.51
	Apple flavoured mineral water	92 (13)	89 (4)	0.41
	Isotonic drink	85 (8)	84 (17)	1.3

[a] Concentration of the analytes in the sample (level 1): 0.37 µg/L.
[b] Concentration of the analytes in the sample (level 2): 1.2 µg/L.
[c] Calculated from the LCL considering the recovery obtained for every analyte.

IV.5.6.- Evaluation of the background contamination of the laboratory and real sample analysis

As already commented, an important issue concerning the analysis of PAEs in an analytical laboratory is the determination of the background contamination, considering the possible presence of some of the studied compounds. Despite the fact that all the precautions indicated in the experimental section were taken (non-volumetric glassware was calcined, volumetric glassware was cleaned with strong oxidising agents, high-purity solvents, phthalate-free pipette tips and globes were used, etc.), a certain degree of contamination may exist and should be evaluated before determining the content of PAEs of any sample. In our case, the analysis of procedural blanks (the application of the whole procedure without any type of water) revealed no PAEs contamination in the injection vial. Therefore, no influence of the background contamination of the target PAEs took place.

Finally, to prove the applicability of the developed methodology, one sample of each type was analysed. In all cases, BBP was detected, but at concentrations below the LOQ of the method, except for apple flavoured mineral water, for which no PAEs were found.

IV.5.7.- Comparison with other methodologies

As previously indicated, pristine CNTs of different dimensions had been previously used when this work was developed for the SPE of PAEs from water or beverages in few occasions [167,179,434,435]. Concerning the analysis of tap water, the only matrix of this work that has been previously analysed using other pristine CNTs as extraction sorbents, Cai et al. [167] analysed four PAEs in tap water (river and sea water were also analysed). Recovery values and LOQs achieved are comparable to the ones obtained in this work, though in our case a higher number of PAEs were analysed, of which DCHP and DPP were the only PAEs in common. However, Jiao et al. [435] achieved lower LOQs when they analysed 13 PAEs (including DPP, DNPP, BBP, DCHP, and DNOP) in mineral water, probably because 200 mL of water were extracted (four times higher volumes that the one of this work). Finally, Luo et al. [434] developed a m-dSPE for the extraction of 15 PAEs (including DMEP, DEEP, BBP, DBEP, DCHP, DIPP, and DNOP, the ones in common with this work) with recovery values similar to the ones of this work and LOQs between 10 and 130 ng/L, which can probably be justified by the use of GC-QqQ-MS/MS. Even though, and as previously indicated, not all the selected PAEs of this work had been previously extracted by CNTs and also from the rest of the studied samples.

It should also be remarked that one of the main advantages of the MWCNTs used in this method is the fact that they are relatively cheap. As an example, in Spain, 10 g of the selected MWCNTs (110–170 nm diameter and 5–9 μm length) cost approximately 353 €, being possible to develop up to 250 extractions with a cost of approximately 1.4 € per extraction. This cost is much lower than that of conventional SPE cartridges, which is clearly an important advantage of the use of these extraction materials. Contrary to the use of other MWCNTs (highly expensive), the ones proposed in this work are highly recommended for their use in routine laboratory analysis. Moreover, it is important to highlight that though dSPE may be considered more labour intensive than conventional SPE, the fact that these MWCNTs can be easily weighted, no sorbent conditioning is necessary and only 1 min was enough to complete the extraction of the target analytes, which is a clear advantage over the application of commercial cartridges.

IV.5.8.- Conclusions

From the results obtained in this section, the following conclusions can be drawn:

- A methodology based on a μ-dSPE procedure using pristine MWCNTs as sorbent followed by the determination by HPLC-MS/MS allowed the analysis of nine PAEs of interest (i.e. DMEP, DEEP, DPP, BBP, DBEP, DIPP, DNPP, DCHP and DNOP) in different plastic bottled water and beverages.
- The HPLC-MS/MS method developed for the separation and detection of the selected analytes was validated in terms of linearity and sensitivity, obtaining R^2 values higher than 0.9921 and LCLs of 10 μg/L.
- The optimisation of the μ-dSPE procedure showed that the highest extraction of the selected analytes could be achieved with 50 mL of water at pH 6.0, 80 mg of MWCNTs and 25 mL of ACN. In the case of the isotonic drink, it had to be previously diluted with Milli-Q water (1/2, v/v) to obtain the same extraction efficiency.
- The evaluation of the matrix effects showed a clear signal suppression for DNPP, DCHP and DNOP in the isotonic drink, while a signal enhancement was found for DEEP, BBP and DNPP in mineral water, for DMEP, DEEP, DPP, BBP and DIPP in tap water, for DMEP and DEEP in lemon flavoured mineral water and for DEEP, BBP, DBEP, DIPP and DNPP in apple flavoured mineral water. This data made necessary the development of matrix-matched calibration in all the matrices studied, obtaining R^2 values higher than 0.9906 with a LCL of 35 μg/L for all analytes, except for DMEP in the isotonic drink and DNOP in all matrices (75 μg/L).
- The study of the trueness of the method at two levels of concentration showed that mean recovery values were in the range 70-117 % with RSD percentages below 17 %. The LOQs of the method, which were obtained from the LCL and considering the recovery obtained for each analyte, ranged between 0.17 and 1.5 μg/L. These data are in accordance with those shown in previously published articles. However, it is important to mention that not all the PAEs selected have been previously extracted using MWCNTs, being the first time that CNTs are used for PAEs analysis in the beverages studied in this work, except for mineral water.
- The analysis of mineral, tap, lemon flavoured and apple flavoured water samples, and an isotonic drink demonstrated the applicability of the developed methodology. In all

the analysed samples, only BBP was detected at concentrations below the LOQ of the method, except in apple flavoured mineral water, in which no PAEs were found.

- The μ-dSPE-HPLC-MS/MS methodology developed in this work constitutes a simple, cost-effective and reliable alternative for the determination of PAEs in plastic bottled drinks.

IV.6.- Use of Basolite® F300 MOF for the dSPE of PAEs and DEHA from water samples prior to HPLC-MS/MS determination

In this section, a commercial MOF (Basolite® F300) was applied for the first time as dSPE sorbent of a group of PAEs (BBP, DBEP, DIPP, DNPP, DCHP, DEHP, DNOP and DINP) and DEHA. Milli-Q, pond, tap and waste water were selected as samples. DHP-d_4 was used as procedural IS. HPLC-IT-MS/MS was used for the determination of the target compounds. Extraction conditions were optimised following a step by step approach. The whole methodology was validated in Milli-Q, tap, pond and waste water in terms of linearity, precision and trueness. Matrix effects were also evaluated, and samples of tap, pond and waste water were analysed.

IV.6.1.- Background

During the last years, trends in sample preparation have been focused on the use of new materials with a high extraction capacity and selectivity towards the target analytes. In this sense, nanomaterials have shown to provide excellent results due to their high surface-to-volume ratio and to the feasible incorporation of a great variety of functional groups and molecules, which can clearly improve their selectivity and extraction capacity [259]. Among the wide number of nanomaterials that could be applied in this regard, MOFs have aroused great interest [242,259], principally due to their versatility. As it is well known, MOFs are materials with an extremely high porosity obtained by the self-assembling of metal cations (clusters or secondary building units) and organic ligands (linkers) via coordinative bonds [259], whose combination give place to the frameworks with the highest surface area known [242]. Besides that, the large variety of organic electron donor ligands and metallic ions and clusters available, and the uncountable ways in which they can be combined, make possible to create almost an infinite number of MOFs [242,259], which also provide an extraordinary control of the pore diameter and dimensions of the cavities inside the structure. Moreover, it is noteworthy to mention the possibility of functionalising the internal surface of the cavities and the chemical and thermal stability of MOFs, which have made them sorbents of special interest to be used in analytical applications [259]. However, and despite the obvious advantages derived from their use, MOFs have barely been selected for the extraction of PAEs from environmental water samples. As far as we know, only three works had been published in this regard [168,178,255]. In these articles, laboratory synthesised MIL-101 (chromium(III) terephthalate) [168,255] and TMU-6 ([Zn(oxybisbenzoate)(N^1,N^4-bis((pyridin-4-yl)

methylene)-benzene-1,4diamine)$_{0.5}$]$_n$ (dimethylformamide)$_z$) [178] MOFs had been applied for the extraction of 3-6 PAEs from plasma, tap and well water [168] and from different bottled water samples [178,255]. In all cases, good results were obtained, with recovery values ranging between 70 and 120 % and LODs in the low µg/L range.

As previously mentioned, such MOFs were synthesised in the laboratory following several methodologies. However, nowadays, there is a good number of MOFs which are commercially available with a reasonable price which can be considered for their application as extraction sorbents. One of those commercial MOFs is the iron trimesate Basolite® F300 (MIL-100 (Fe)) which is a crystalline solid made of iron (III) with 1,3,5-benzenetricarboxylic acid as binding ligand (see Figure IV.6.1). However, and to the best of our knowledge, its use had not been explored yet for the extraction of PAEs. In fact, it had been applied for the removal of certain compounds from water samples in one occasion [439] but not for their analysis.

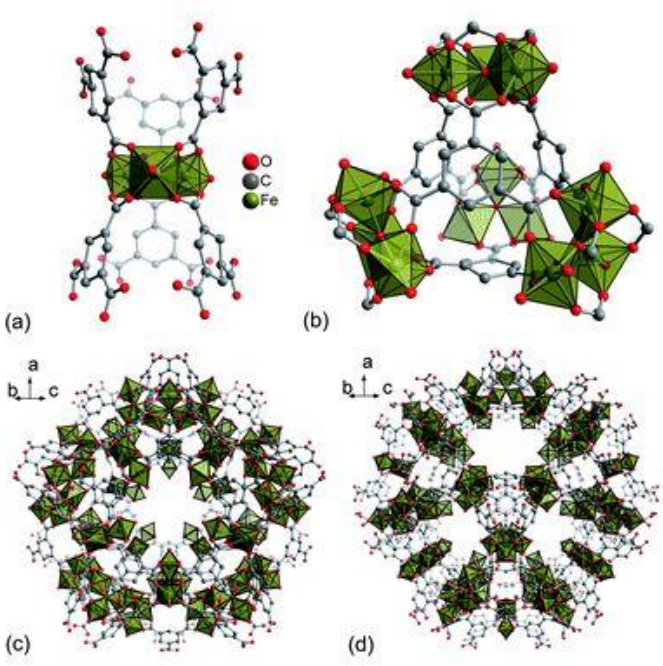


Figure IV.6.1.- a) Secondary building unit, b) supertetrahedra, c) small S cage, and d) large L cage of Basolite® F300 (MIL-100 (Fe)). Reprinted from Jeremias et al. [440] with permission of the Royal Society of Chemistry.

IV.6.2.- Specific objectives

In view of the foregoing, the following specific goals were established:

- The development of a new analytical methodology based on the use of the MOF Basolite® F300 as sorbent for the dSPE of eight PAEs (i.e. BBP, DBEP, DIPP, DNPP, DCHP, DEHP, DNOP and DINP) and an adipate (DEHA) from different water samples and their subsequent determination by HPLC-IT-MS/MS.
- The evaluation of the influence of the parameters affecting the dSPE procedure (i.e. pH of the sample, elution solvent nature, sorbent amount and elution solvent volume) using a step by step approach to obtain the best extraction efficiency.
- The validation of the whole methodology in terms of linearity, precision and trueness as well as the obtention of the corresponding LODs and LOQs of the method.
- The study of the applicability of the developed methodology to the analysis of water samples with different origins such as tap, pond and waste water.

IV.6.3.- HPLC-MS/MS conditions

In this work, eight PAEs and the adipate DEHA, as well as DHP-d_4 which was used as IS, were suitably determined by HPLC-MS/MS. Since some of the analytes studied in this work were also determined in the previous one, the same instrumental conditions were taken as starting point, resulting the best conditions for the determination of the selected group of PAEs. Thus, the best separation of the analytes was achieved with the elution gradient described in Section III.5.5, using an ACN/Milli-Q water mixture, both containing a 0.1 % (v/v) of formic acid, as mobile phase. Best separation resolution and efficiency were obtained when the temperature was increased up to 40 °C. Regarding MS conditions, an end plate offset of -500 V, a capillary voltage of -4500 V, a dry gas flow and temperature of 8 L/min and 350 °C, respectively, and a nebulisation gas pressure of 30.0 psi, provided the highest sensitivity of all analytes working in the positive mode. Concerning the best fragmentation conditions and the most intense product ion, they are indicated in Table IV.6.1.

Instrumental calibration curves based on PAEs relative peak areas (IS was added at a concentration of 200 µg/L) were obtained. For this purpose, nine increasing concentration levels (n=9, in the range 10-500 µg/L) were injected three times, obtaining R^2 values above 0.9977 (see Table IV.6.2).

Table IV.6.1.- MS/MS fragmentation parameters of the selected PAEs.

Analyte	Precursor ion (m/z)	Product ion (m/z)	Fragmentation amplitude (V)
BBP	311.0	149.0	0.40
		205.0	
DBEP	367.1	249.0	0.34
		-	
DIPP	307.0	218.9	0.37
		149.0	
DNPP	307.0	149.0	0.37
		219.0	
DCHP	331.0	149.0	0.40
		249.0	
DEHP	391.1	149.0	0.30
		167.0	
DEHA	371.	129.1	0.40
		259.1	
DNOP	391.3	149.0	0.27
		261.0	
DINP	419.3	149.0	0.29
		275.0	

Table IV.6.2.- Instrumental calibration data of the selected PAEs.

Analyte	Studied linear range (µg/L)*	Regression equation (n=9)		$S_{y/x}$	R^2
		$b \pm s_b \cdot t_{(0.05;7)}$	$a \pm s_a \cdot t_{(0.05;7)}$		
BBP	10-500	$1.30 \cdot 10^{-3} \pm 4.15 \cdot 10^{-5}$	$2.13 \cdot 10^{-2} \pm 9.70 \cdot 10^{-3}$	$8.14 \cdot 10^{-3}$	0.9989
DBEP	10-500	$5.71 \cdot 10^{-3} \pm 1.94 \cdot 10^{-4}$	$1{,}12 \cdot 10^{-1} \pm 4.54 \cdot 10^{-2}$	$3.81 \cdot 10^{-2}$	0.9988
DIPP	10-500	$1.81 \cdot 10^{-3} \pm 6.72 \cdot 10^{-5}$	$1.02 \cdot 10^{-2} \pm 1.57 \cdot 10^{-2}$	$1.32 \cdot 10^{-2}$	0.9985
DNPP	10-500	$1.56 \cdot 10^{-3} \pm 4.05 \cdot 10^{-5}$	$1.50 \cdot 10^{-2} \pm 9.47 \cdot 10^{-3}$	$7.95 \cdot 10^{-3}$	0.9993
DCHP	10-500	$4.19 \cdot 10^{-3} \pm 4.86 \cdot 10^{-5}$	$3.92 \cdot 10^{-2} \pm 1.14 \cdot 10^{-2}$	$9.54 \cdot 10^{-3}$	0.9999
DEHP	10-500	$4.48 \cdot 10^{-3} \pm 1.12 \cdot 10^{-4}$	$1.24 \cdot 10^{-1} \pm 2.61 \cdot 10^{-2}$	$2.19 \cdot 10^{-2}$	0.9993
DEHA	10-500	$3.83 \cdot 10^{-3} \pm 1.74 \cdot 10^{-4}$	$1.37 \cdot 10^{-1} \pm 4.07 \cdot 10^{-2}$	$3.42 \cdot 10^{-2}$	0.9978
DNOP	10-500	$3.85 \cdot 10^{-3} \pm 1.04 \cdot 10^{-4}$	$6.29 \cdot 10^{-2} \pm 2.43 \cdot 10^{-2}$	$2.04 \cdot 10^{-2}$	0.9992
DINP	10-500	$4.52 \cdot 10^{-3} \pm 1.56 \cdot 10^{-4}$	$4.95 \cdot 10^{-2} \pm 3.65 \cdot 10^{-2}$	$3.07 \cdot 10^{-2}$	0.9987

b: slope; S_b: standard deviation of the slope; a: intercept; S_a: standard deviation of the intercept.
* LCL: 10 µg/L.

IV.6.4.- MOF-dSPE optimisation

In order to explore the applicability of Basolite® F300 as sorbent for the dSPE of the target analytes from tap, pond and waste water, parameters like the pH of the sample, sorbent amount and elution solvent type and volume were varied and optimised. To better appreciate the influence of the previously mentioned factors, 50 mL of Milli-Q water were spiked with the target analytes.

As starting conditions, Milli-Q water without pH adjustment was spiked and extracted during 5 min with 120 mg of MOF. Thirty mL of ACN were initially used for analytes elution. The eluate was dried at 40 °C and 180 mbar and reconstituted in 250 µL of the mobile phase for its injection in the HPLC-MS/MS system. Before use, the MOF was dried in an oven for 12 hours at 120 °C and cooled at room temperature in a desiccator.

IV.6.4.1.- pH of the sample

As commented in previous sections, the pH of the sample should not affect PAEs recovery. Even though, its effect was studied by varying it between 2.0 and 8.0 (two extractions were developed at each pH value), finding that it did not practically affected the extraction performance since similar results were obtained in the studied pH range (recovery values above 60 %) as also found in previous works [441,442]. However, since pH 6.0 provided slightly higher recovery percentages for those PAEs with shorter chains (BBP, DBEP, DIPP, DNPP and DCHP) it was decided to fix such value for further analyses. Besides, this pH adjustment allowed to obtain a higher reproducibility between extractions and samples, since it was an intermediate value respect to the pH of the real samples.

IV.6.4.2.- Elution solvent nature

In spite of the excellent results obtained for all analytes under the conditions previously tested (50 mL of Milli-Q water at pH 6.0, 120 mg of the MOF and 30 mL of ACN), elution solvents like DCM, acetone, cyclohexane and MeOH were also tested (see Figure IV.6.2). In general, the highest recovery was obtained with ACN, except for DEHA which was highly recovered with acetone (it should be mentioned that this is the only adipate of the group of analytes) and for DEHP with DCM and cyclohexane. In particular, MeOH provided recovery percentages lower than 60 % in all cases. Therefore, ACN was

selected for further experiments since, under the selected conditions it provided recovery values between 67 and 92 %.

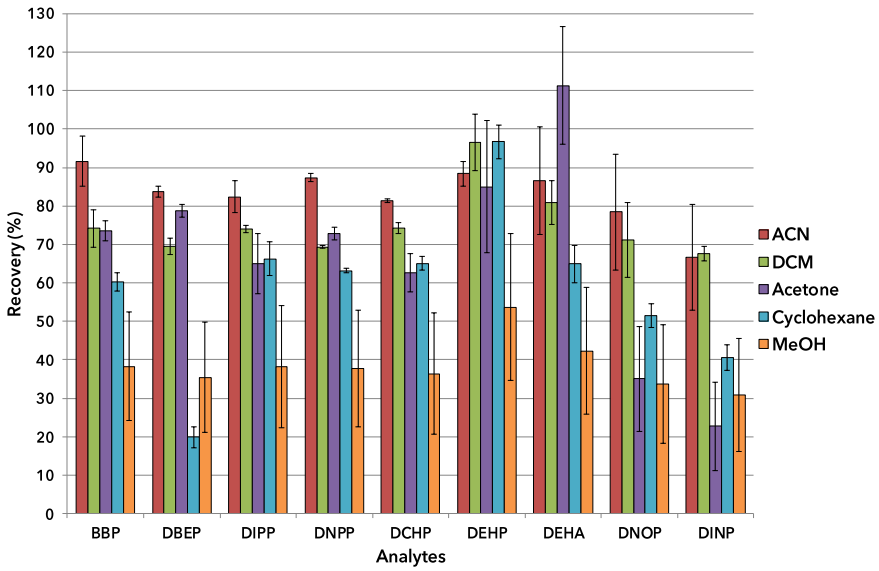


Figure IV.6.2.- Elution solvent type effect on the recovery of the selected analytes. Conditions: 50 mL of Milli-Q water (pH 6.0), 120 mg of Basolite® F300 and elution with 30 mL of each solvent.

IV.6.4.3.- Sorbent amount

In relation to the amount of MOF used, 80, 100, 140, 160 and 180 mg were also evaluated with the above-mentioned extraction conditions. As can be seen in Figure IV.6.3, a decrease in the recovery values of around 10-20 % was observed for all analytes (except for DEHP) when amounts lower than 120 mg of sorbent were used, while the application of higher amounts generally provided similar results to the ones obtained with 120 mg of the MOF. Thus, 120 mg of sorbent were selected for further experiments, since recovery percentages were above 75 % for all analytes, except for DINP (recovery was 67 %).

IV.6.4.4.- Elution solvent volume

To evaluate the influence of the volume of the elution solvent in the method performance and also to reduce the amount of organic solvent used, lower volumes of ACN (10, 15, 20 and 25 mL) were also tested (see Figure IV.6.4). Among the different

volumes tested, 15 mL provided a quantitative extraction of the target compounds, since a slight decrease of the recovery were observed for some analytes (DIPP, DNPP, DEHA and DNOP) when lower volumes were used. These conditions allowed obtaining highly acceptable recovery values (76-91 % for all analytes).

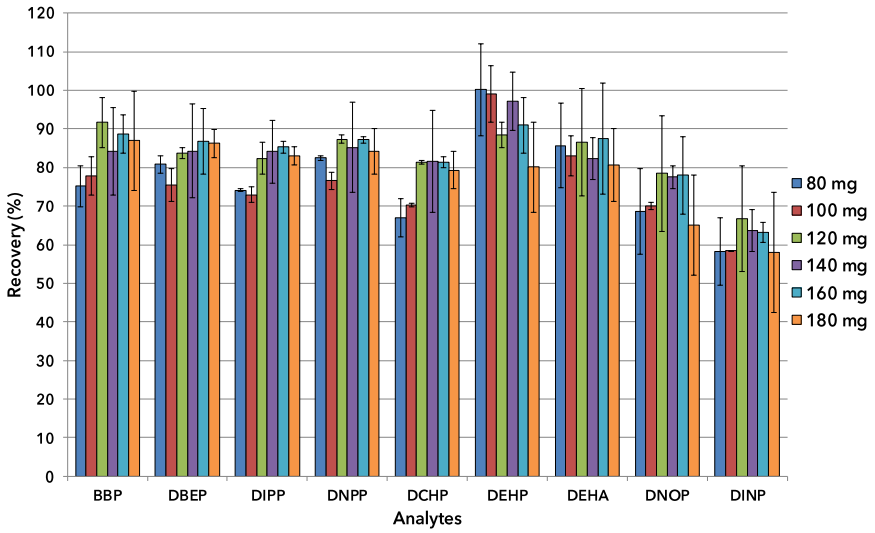


Figure IV.6.3.- Amount of Basolite® F300 effect on the recovery of the selected analytes. Conditions: 50 mL of Milli-Q water (pH 6.0) and elution with 30 mL of ACN.

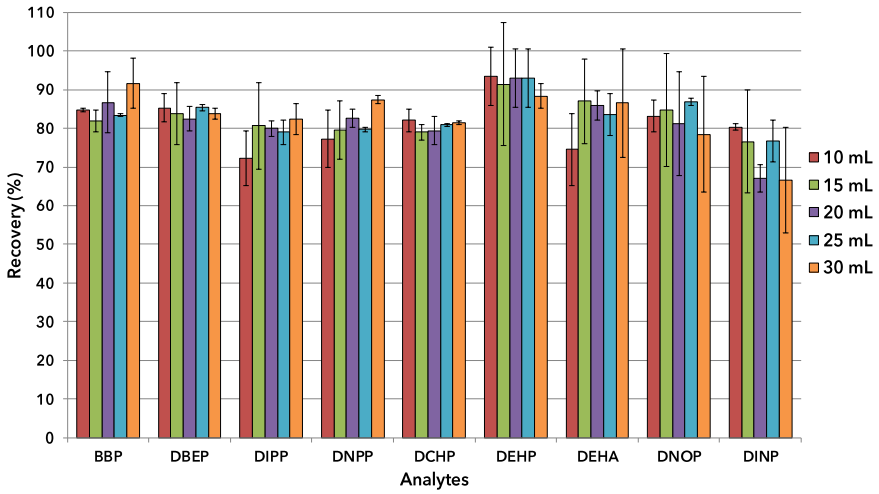


Figure IV.6.4.- Elution solvent volume effect on the recovery of the selected analytes. Conditions: 50 mL of Milli-Q water (pH 6.0), 120 mg of Basolite® F300 and elution with ACN.

IV.6.5.- Validation of the method

After the optimisation of the MOF-dSPE-HPLC-MS/MS method, calibration and recovery studies were developed in Milli-Q water in addition to tap and two environmental water samples (pond and waste water) in order to validate the methodology in these matrices. Blank samples were first analysed to check the presence of PAEs in them. Thus, when any of the target analytes were detected, peak areas were subtracted. This only happened for DEHP, as it will be later explained.

IV.6.5.1.- Matrix-matched calibration data and matrix effect evaluation

As it is widely known, the presence of the components of the matrix during the injection of the target analytes could lead to biased results as a consequence of the inhibition or enhancement of the signal of the detector [392]. In this sense, matrix-matched calibration is one of the most usual methods to compensate this effect, which equalise the signal for calibration standards in pure solvent and that obtained in sample extracts.

To correct and to evaluate such matrix effects, matrix-matched calibration curves were obtained at nine different concentrations levels (n=9). Each level was injected in triplicate. With this aim, nine samples were fortified with the IS and the analytes after the final drying of the extract in the rotary evaporator. In this case, the IS was added at a concentration of 200 µg/L. Table IV.6.3 compiles the regression data of matrix-matched calibration curves. As can be seen, R^2 values were above 0.9905.

Table IV.6.3 also compiles the matrix effect percentages obtained for each target analyte and water samples, which was calculated using the equation proposed by Matuszewski et al. [438], as indicated in the previous section. From the data shown, it is clear that a small signal enhancement (> 120 %) was detected for DEHP in all analysed matrices and also for DEHA in Milli-Q and tap water.

IV.6.5.2.- Trueness evaluation

A recovery study was also carried out to assess the trueness of the developed method, by fortifying the different matrices at 0.37 µg/L and 1.9 µg/L. The IS was added at a concentration of 1 µg/L in the sample and 200 µg/L in the final extract when the matrix-matched standards were injected to calculate the recovery values (see Figure IV.6.5). As it can be seen in Table IV.6.4, relative recovery values were in the range 70-118 % with

RSD percentages below 20 %. Regarding the LOQs of the method (which were between 22 and 69 ng/L), they were calculated from the LCL, considering the recovery value obtained in each case. This demonstrates the high sensitivity of the methodology, which is also in accordance with the LOQs obtained in other methods in which PAEs were analysed in similar samples [115,443].

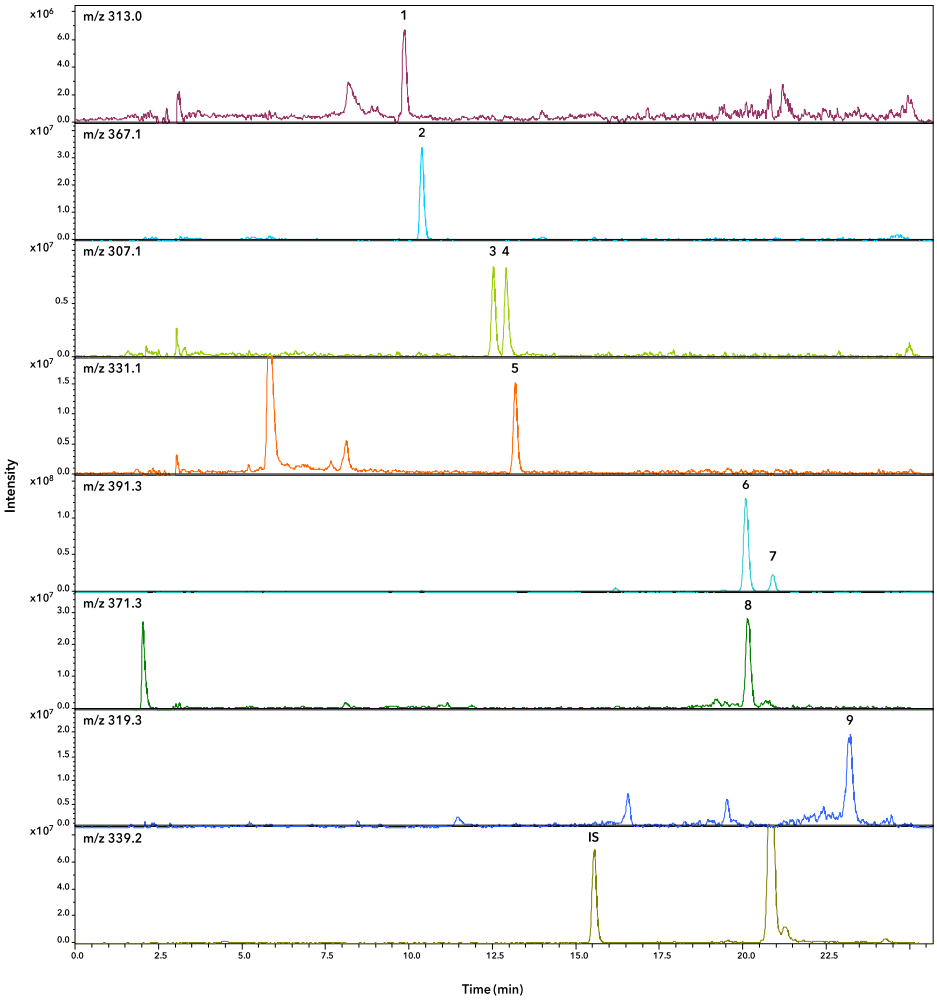


Figure IV.6.5.- EIC of a pond water sample spiked with the selected analytes at 0.37 µg/L at the beginning of the dSPE process. Conditions: 50 mL of sample (pH 6.0), 120 mg of MOF and 15 mL of ACN. (1) BBP, (2) DBEP, (3) DIPP, (4) DNPP, (5) DCHP, (6) DEHP, (7) DEHA, (8) DNOP, (9) DINP. * Interference.

Table IV.6.3.- Matrix-matched calibration data of the target analytes and matrix effect percentage.

Analyte	Water sample	Studied linear range (µg/L)	Regression equation (n=9)		$S_{y/x}$	R^2	ME (%)[a]
			$b \pm s_b \cdot t_{(0.05;7)}$	$a \pm s_a \cdot t_{(0.05;7)}$			
BBP	Milli-Q	10-500	$1.18 \cdot 10^{-3} \pm 1.07 \cdot 10^{-4}$	$3.78 \cdot 10^{-2} \pm 2.49 \cdot 10^{-2}$	$2.02 \cdot 10^{-2}$	0.9920	106 (7)
	Tap	5-500	$1.20 \cdot 10^{-3} \pm 4.35 \cdot 10^{-5}$	$1.33 \cdot 10^{-2} \pm 9.58 \cdot 10^{-3}$	$8.98 \cdot 10^{-3}$	0.9984	107 (6)
	Pond	10-500	$1.15 \cdot 10^{-3} \pm 6.32 \cdot 10^{-5}$	$2.35 \cdot 10^{-2} \pm 1.48 \cdot 10^{-2}$	$1.20 \cdot 10^{-2}$	0.9970	104 (9)
	Waste	5-500	$1.26 \cdot 10^{-3} \pm 8.12 \cdot 10^{-5}$	$2.62 \cdot 10^{-2} \pm 1.79 \cdot 10^{-2}$	$1.68 \cdot 10^{-2}$	0.9948	104 (9)
DBEP	Milli-Q	10-500	$5.63 \cdot 10^{-3} \pm 5.93 \cdot 10^{-4}$	$1.59 \cdot 10^{-1} \pm 1.48 \cdot 10^{-1}$	$1.03 \cdot 10^{-1}$	0.9917	118 (9)
	Tap	5-500	$5.35 \cdot 10^{-3} \pm 1.74 \cdot 10^{-4}$	$4.76 \cdot 10^{-2} \pm 3.83 \cdot 10^{-2}$	$3.59 \cdot 10^{-2}$	0.9987	105 (6)
	Pond	10-500	$5.70 \cdot 10^{-3} \pm 5.25 \cdot 10^{-4}$	$1.04 \cdot 10^{-1} \pm 1.11 \cdot 10^{-1}$	$8.58 \cdot 10^{-2}$	0.9936	110 (6)
	Waste	5-500	$5.87 \cdot 10^{-3} \pm 4.24 \cdot 10^{-4}$	$8.00 \cdot 10^{-2} \pm 9.35 \cdot 10^{-2}$	$8.76 \cdot 10^{-2}$	0.9935	102 (7)
DIPP	Milli-Q	10-500	$1.63 \cdot 10^{-3} \pm 1.14 \cdot 10^{-4}$	$3.37 \cdot 10^{-2} \pm 2.66 \cdot 10^{-2}$	$2.16 \cdot 10^{-2}$	0.9952	107 (10)
	Tap	5-500	$1.74 \cdot 10^{-3} \pm 2.18 \cdot 10^{-5}$	$4.87 \cdot 10^{-3} \pm 4.79 \cdot 10^{-3}$	$4.49 \cdot 10^{-3}$	0.9998	112 (7)
	Pond	10-500	$1.79 \cdot 10^{-3} \pm 5.12 \cdot 10^{-5}$	$8.22 \cdot 10^{-3} \pm 1.20 \cdot 10^{-2}$	$9.71 \cdot 10^{-3}$	0.9992	106 (6)
	Waste	5-500	$1.74 \cdot 10^{-3} \pm 4.17 \cdot 10^{-5}$	$1.20 \cdot 10^{-2} \pm 9.18 \cdot 10^{-3}$	$8.61 \cdot 10^{-3}$	0.9993	96 (12)
DNPP	Milli-Q	10-500	$1.47 \cdot 10^{-3} \pm 8.98 \cdot 10^{-5}$	$2.65 \cdot 10^{-2} \pm 2.10 \cdot 10^{-2}$	$1.70 \cdot 10^{-2}$	0.9963	106 (5)
	Tap	5-500	$1.56 \cdot 10^{-3} \pm 3.18 \cdot 10^{-5}$	$8.91 \cdot 10^{-3} \pm 7.00 \cdot 10^{-3}$	$6.56 \cdot 10^{-3}$	0.9995	114 (4)
	Pond	10-500	$1.57 \cdot 10^{-3} \pm 5.09 \cdot 10^{-5}$	$8.48 \cdot 10^{-3} \pm 1.19 \cdot 10^{-2}$	$9.65 \cdot 10^{-3}$	0.9989	108 (4)
	Waste	5-500	$1.53 \cdot 10^{-3} \pm 3.34 \cdot 10^{-5}$	$1.39 \cdot 10^{-2} \pm 7.68 \cdot 10^{-3}$	$6.66 \cdot 10^{-3}$	0.9995	97 (11)
DCHP	Milli-Q	10-500	$4.05 \cdot 10^{-3} \pm 2.28 \cdot 10^{-4}$	$6.87 \cdot 10^{-2} \pm 5.33 \cdot 10^{-2}$	$4.33 \cdot 10^{-2}$	0.9968	114 (10)
	Tap	5-500	$3.98 \cdot 10^{-3} \pm 8.72 \cdot 10^{-5}$	$2.24 \cdot 10^{-2} \pm 1.92 \cdot 10^{-2}$	$1.80 \cdot 10^{-2}$	0.9994	113 (8)
	Pond	10-500	$4.10 \cdot 10^{-3} \pm 2.18 \cdot 10^{-4}$	$3.53 \cdot 10^{-2} \pm 5.09 \cdot 10^{-2}$	$4.13 \cdot 10^{-2}$	0.9972	107 (8)
	Waste	5-500	$4.24 \cdot 10^{-3} \pm 3.46 \cdot 10^{-4}$	$4.40 \cdot 10^{-2} \pm 7.62 \cdot 10^{-2}$	$7.14 \cdot 10^{-2}$	0.9917	101 (10)

Table IV.6.3.- (Continued).

Analyte	Water sample	Studied linear range (µg/L)	Regression equation (n=9)		$S_{y/x}$	R^2	ME (%)[a]
			$b \pm s_b \cdot t_{(0.05;7)}$	$a \pm s_a \cdot t_{(0.05;7)}$			
DEHP	Milli-Q	10-500	$6.18 \cdot 10^{-3} \pm 6.51 \cdot 10^{-4}$	$6.75 \cdot 10^{-2} \pm 1.62 \cdot 10^{-1}$	$1.13 \cdot 10^{-1}$	0.9917	196 (10)
	Tap	5-500	$3.17 \cdot 10^{-3} \pm 1.99 \cdot 10^{-4}$	$1.69 \cdot 10^{-1} \pm 4.38 \cdot 10^{-2}$	$4.11 \cdot 10^{-2}$	0.9951	187 (4)
	Pond	10-500	$2.48 \cdot 10^{-3} \pm 1.95 \cdot 10^{-4}$	$1.02 \cdot 10^{-1} \pm 4.86 \cdot 10^{-2}$	$3.40 \cdot 10^{-2}$	0.9953	136 (4)
	Waste	5-500	$3.07 \cdot 10^{-3} \pm 1.22 \cdot 10^{-4}$	$2.87 \cdot 10^{-1} \pm 2.72 \cdot 10^{-2}$	$2.42 \cdot 10^{-2}$	0.9984	126 (6)
DEHA	Milli-Q	10-500	$3.92 \cdot 10^{-3} \pm 3.74 \cdot 10^{-4}$	$5.73 \cdot 10^{-2} \pm 9.31 \cdot 10^{-2}$	$6.50 \cdot 10^{-2}$	0.9932	131 (18)
	Tap	5-500	$3.35 \cdot 10^{-3} \pm 2.72 \cdot 10^{-4}$	$7.55 \cdot 10^{-2} \pm 5.98 \cdot 10^{-2}$	$5.61 \cdot 10^{-2}$	0.9918	126 (19)
	Pond	10-500	$3.38 \cdot 10^{-3} \pm 3.67 \cdot 10^{-4}$	$2.61 \cdot 10^{-2} \pm 9.13 \cdot 10^{-2}$	$6.38 \cdot 10^{-2}$	0.9912	114 (11)
	Waste	5-500	$3.61 \cdot 10^{-3} \pm 2.39 \cdot 10^{-4}$	$5.58 \cdot 10^{-2} \pm 5.26 \cdot 10^{-2}$	$4.93 \cdot 10^{-2}$	0.9946	94 (11)
DNOP	Milli-Q	10-500	$3.49 \cdot 10^{-3} \pm 2.53 \cdot 10^{-4}$	$4.39 \cdot 10^{-2} \pm 5.90 \cdot 10^{-2}$	$4.79 \cdot 10^{-2}$	0.9948	94 (18)
	Tap	5-500	$3.40 \cdot 10^{-3} \pm 1.94 \cdot 10^{-4}$	$5.50 \cdot 10^{-2} \pm 4.28 \cdot 10^{-2}$	$4.01 \cdot 10^{-2}$	0.9959	112 (18)
	Pond	10-500	$3.00 \cdot 10^{-3} \pm 2.16 \cdot 10^{-4}$	$5.53 \cdot 10^{-2} \pm 5.04 \cdot 10^{-2}$	$4.09 \cdot 10^{-2}$	0.9948	94 (14)
	Waste	5-500	$3.55 \cdot 10^{-3} \pm 2.14 \cdot 10^{-4}$	$4.24 \cdot 10^{-2} \pm 4.71 \cdot 10^{-2}$	$4.42 \cdot 10^{-2}$	0.9955	85 (15)
DINP	Milli-Q	10-500	$4.15 \cdot 10^{-3} \pm 4.05 \cdot 10^{-4}$	$1.48 \cdot 10^{-2} \pm 9.46 \cdot 10^{-2}$	$7.68 \cdot 10^{-2}$	0.9905	85 (10)
	Tap	5-500	$4.18 \cdot 10^{-3} \pm 2.10 \cdot 10^{-4}$	$4.46 \cdot 10^{-2} \pm 4.62 \cdot 10^{-2}$	$4.33 \cdot 10^{-2}$	0.9969	110 (8)
	Pond	10-500	$3.29 \cdot 10^{-3} \pm 2.76 \cdot 10^{-4}$	$3.25 \cdot 10^{-2} \pm 6.45 \cdot 10^{-2}$	$5.23 \cdot 10^{-2}$	0.9930	87 (8)
	Waste	5-500	$4.01 \cdot 10^{-3} \pm 1.59 \cdot 10^{-4}$	$2.17 \cdot 10^{-2} \pm 3.51 \cdot 10^{-2}$	$3.29 \cdot 10^{-2}$	0.9980	82 (6)

a: intercept; S_a: standard deviation of the intercept; b: slope; S_b: standard deviation of the slope; $S_{y/x}$: standard deviation of the estimate.
[a] Matrix effect calculated following the equation used by Matuszewski et al. [438].

Table IV.6.4.- Recovery data (n=5) of the dSPE-HPLC-MS/MS method.

Analyte	Sample	Spiked level 1[a]	Spiked level 2[b]	LOQ_{method} (ng/L)
		Recovery % (RSD %)	Recovery % (RSD %)	
BBP	Milli-Q water	86 (11)	91 (3)	56
	Tap water	86 (6)	80 (4)	30
	Pond water	96 (1)	88 (2)	54
	Waste water	94 (9)	86 (6)	28
DBEP	Milli-Q water	89 (11)	97 (4)	54
	Tap water	81 (10)	91 (1)	29
	Pond water	93 (6)	91 (3)	54
	Waste water	82 (11)	90 (5)	29
DIPP	Milli-Q water	88 (7)	95 (4)	55
	Tap water	88 (8)	81 (4)	30
	Pond water	87 (5)	78 (5)	61
	Waste water	85 (11)	80 (6)	30
DNPP	Milli-Q water	95 (8)	92 (4)	53
	Tap water	87 (14)	92 (3)	28
	Pond water	90 (4)	86 (3)	57
	Waste water	83 (11)	89 (6)	29
DCHP	Milli-Q water	86 (11)	88 (4)	57
	Tap water	75 (16)	70 (10)	34
	Pond water	75 (4)	70 (6)	69
	Waste water	74 (12)	73 (10)	34
DEHP	Milli-Q water	104 (5)	100 (14)	49
	Tap water	90 (16)	118 (4)	24
	Pond water	106 (8)	97 (4)	49
	Waste water	117 (17)	107 (9)	22
DEHA	Milli-Q water	110 (5)	100 (4)	48
	Tap water	112 (14)	109 (9)	23
	Pond water	101 (8)	94 (3)	51
	Waste water	108 (15)	101 (2)	24
DNOP	Milli-Q water	81 (8)	92 (4)	58
	Tap water	96 (10)	89 (14)	27
	Pond water	92 (13)	96 (2)	53
	Waste water	101 (7)	95 (11)	26
DINP	Milli-Q water	76 (19)	85 (7)	62
	Tap water	98 (8)	90 (19)	27
	Pond water	84 (17)	82 (4)	60
	Waste water	113 (14)	84 (12)	25

[a] Level 1: 0.375 µg/L in the sample. [b] Level 2: 1.9 µg/L in the sample. [c] Calculated from the LCL considering the recovery obtained for each analyte.

IV.6.6.- Background contamination and real sample analysis

To evaluate the background contamination of the laboratory, procedural blanks were analysed (the entire procedure was applied without using/adding any kind of water) finding the presence of DEHP. Consequently, the peak area of this analyte was subtracted when necessary to correctly determine its concentration in the real samples analysed.

The applicability of this methodology was proven by the analysis of one Milli-Q water sample, three tap water samples taken from different locations of Tenerife, three pond waters contained in different recipients and one waste water sample. In all the analysed samples, DEHP was detected (concentrations in the range 0.21-4.04 μg/L) and confirmed by means of MS/MS experiments. Table IV.6.5 shows the concentrations of DEHP found in each sample. As can be seen in the table, DEHP was found at concentrations higher than 1 μg/L in tap water. This fact is in accordance with previous studies already published in which DEHP has been detected at different concentrations [397,444]. In any case, it is important to mention that, as it is well-known, levels of contamination depend on the location as well as the contact time and conditions between the material and the sample. Regarding the analysis of pond water, the concentrations of DEHP are slightly higher than those found in tap water. This fact was expected, since the water with which these ponds are filled comes from similar suppliers and they are exposed to rain [125]. Besides, there was one pond in which the concentration was higher than the others (4.04 ± 0.23 μg/L) probably because there is a clear contribution of the material used to build the pond which was made of plastic. Finally, the analysis of waste water samples revealed the lowest concentrations of DEHP. Even though it might be expected the opposite, there are several studies in which DEHP has been found in waste water samples and has also been demonstrated that the concentrations of DEHP in the effluents of waste water treatment plants is lower than the ones found in the influents [445,446], which is in accordance with the results obtained in the present work.

Table IV.6.5.- Concentrations of DEHP found in the analysed samples (n=2 in each case).

Sample	Concentration found (μg/L)
Milli-Q water	0.21 ± 0.26
Tap water (laboratory)	1.78 ± 0.12
Tap water (Santa Cruz de Tenerife)	1.35 ± 0.11
Tap water (San Isidro)	1.03 ± 0.11
Pond water (concrete pond)	1.97 ± 0.14
Pond water (metallic pond)	2.24 ± 0.15
Pond water (plastic pond)	4.04 ± 0.23
Waste water	0.81 ± 0.07

IV.6.7.- Comparison with previous works

As already indicated, MOFs had scarcely been used as sorbents for the SPE of PAEs from water [168,178,255] when this work was developed. Regarding tap water, which is the only sample of this work that had been formerly analysed using another MOF, Dargahi et al. [168] determined five PAEs from tap and well water (human plasma samples were also analysed) using MIL-101 in combination with Fe_3O_4 NPs for their m-dSPE before their GC-MS determination. Recovery obtained was in the range 90-107 % while LOQs were between 0.08 and 0.15 µg/L. However, since none of such PAEs were analysed in this work, no comparison can be suitably made, though, in general, the LOQs are in the same order of magnitude. In the work by Tahmasebi and co-workers [178], authors developed a method based on the dSPE of two PAEs (including DEHP, the only one in common with this work) and one adipate from bottled mineral water and boiling water kept in plastic containers, using TMU-6 as sorbent, obtaining comparable recovery percentages for DEHP but higher LOQs than the ones achieved in this work. Finally, Wang et al. [255] achieved the extraction of 6 PAEs (including BBP, DEHP and DNOP) from bottled water by packing MIL-101 into a PP membrane sheet creating a µ-SPE device, which allowed obtaining comparable results with LOQs slightly lower than those obtained in this work.

IV.6.8.- Conclusions

From the data obtained in this section, the following conclusions can be drawn:

- The commercial MOF Basolite® F300 has been applied for the first time as sorbent for the dSPE of eight PAEs (i.e. BBP, DBEP, DIPP, DNPP, DCHP, DEHP, DNOP and DINP) and an adipate (DEHA) from different water samples (Milli-Q, tap, pond and waste water).
- The HPLC-MS/MS method developed for the separation and detection of the selected analytes was validated in terms of linearity and sensitivity, obtaining R^2 values higher than 0.9977 and LCLs of 10 µg/L.
- The optimised dSPE allowed the quantitative extraction of the selected analytes. Sample pH value, the type and volume of the elution solvent and the amount of sorbent were studied, obtaining that water at pH 6.0, 120 mg of MOF and 15 mL of ACN provided the highest extraction efficiency.

- The evaluation of the matrix effects showed a clear enhancement of the signal for DEHP in all matrices and for DEHA in Milli-Q and tap water. This fact made necessary the development of matrix-matched calibration in all the matrices studied in this work, obtaining R^2 values higher than 0.9905 with LCLs between 5 and 10 µg/L.

- The assessment of the trueness of the method by means of a recovery study, showed that recovery percentages were in the range 70-118 % with RSD values below 20 %. Considering this data, the LOQs of the method, which were calculated from the LCLs, ranged between 22 and 69 ng/L for all PAEs. These data are in accordance with those previously published articles, although in such works, different MOFs were used and different PAEs were analysed, which makes difficult the establishment of a suitable comparison between data obtained.

- The analysis of Milli-Q, tap, pond and waste water samples collected in different points of the island of Tenerife was also carried out in order to demonstrate the applicability of the developed methodology. Such analysis revealed that only DEHP was present in all the samples at concentrations in the range 0.21-4.04 µg/L. This PAE was found at concentration around 1 µg/L in tap water, which is in accordance with previous studies. Concentrations found in pond waters were slightly higher than those found in tap water which is normal considering the water comes from similar suppliers and they are also exposed to atmospheric conditions (clear contribution of rain), which can also contribute to an increase in the concentration. The highest concentration was found in the water collected in a plastic pond, which points out to a clear contribution of the material used to build the pond. Finally, the lowest concentrations of DEHP were found in waste water which, although it can result contradictory, it can be explained because waste water treatment plants remove most of PAEs from water, but low concentrations of these compounds remain in the effluents of such plants.

CHAPTER V
GENERAL CONCLUSIONS

V.- GENERAL CONCLUSIONS

Regarding the results derived from this PhD Thesis, and in order to provide a general overview of the developed work, the following general conclusions can be drawn:

- The MIPs applied for the SPE of the oestrogenic compounds selected in this PhD Thesis have shown not only a good extraction capacity but also a great selectivity for the compounds they have been designed for. However, the cross-reactivity observed suggests the necessity of evaluating this phenomenon when this kind of sorbents are used. Such effect can be clearly advantageous when structurally similar compounds want to be simultaneously and selectively determined.

- The use of Fe_3O_4@pDA m-NPs constitutes a simple, fast and reliable alternative for the extraction of the different families of oestrogenic compounds selected in this PhD Thesis from food and environmental matrices. This sorbent has shown an extraordinary extraction capacity which, in combination with its magnetic properties, results in an important simplification of the sample preparation procedure. All these aspects, together with the simple and cost-effective process developed for their synthesis, make this nanocomposite a very attractive sorbent for its application in this field.

- Two phase HF-LPME using 1-octanol as acceptor phase, has proven to be very effective for the extraction of PAEs from water samples of different origin and complexity. This technique has allowed the selective extraction and preconcentration of the analytes in one step, resulting in a significant reduction of global extraction time. Moreover, the low volumes of sample and organic solvents used, make this LPME approach a cheap and environmentally friendly alternative.

- The μ-dSPE procedure developed in this PhD Thesis using MWCNTs of 110–170 nm diameter and 5–9 μm length as sorbent, constitutes a very simple, cost-effective and reliable alternative for the efficient extraction of PAEs from plastic bottled beverages, providing high recovery percentages and clean final extracts.

- The commercial MOF Basolite® F300 which was evaluated for the dSPE of a group of PAEs from water samples has shown an adequate extraction capacity of the target analytes. Such dSPE procedure allowed a simple and fast extraction of the analytes, maintaining a low consumption of organic solvents.

- Different actions have been taken in this PhD Thesis in order to develop an appropriate validation of the methodology and to avoid an overestimation of the PAEs content,

which included the reduction of the use of plasticware, calcination of non-volumetric glassware at high temperatures, the use of strong oxidising agents for volumetric glassware cleaning, or the use high purity solvents, among others. However, and despite the precautions taken before and during analysis, blank analyses revealed the presence of some of the studied PAEs, showing that all the precautions taken may not be enough, being necessary the analysis of procedural blanks with every batch of samples to assess the contamination degree of the laboratory and to consider it in the final result.

- The application of the developed methodologies to the analysis of PAEs in real samples revealed the presence of some of the selected compounds at concentrations above the LOQ of the methods, which was in accordance with the data shown in the literature. Despite these compounds were found at the μg/L level, such concentrations can be enough to produce serious health alterations. This finding should be a matter of concern for the society and the environment, and more efforts should continue to be focused on establishing stricter SMLs for this type of compounds in food and environmental matrices, and to minimize such migration.

- To sum up, the application of miniaturised extraction techniques using different new extraction materials for the analysis of oestrogenic compounds in food and environmental samples, has demonstrated their extraordinary versatility and efficiency for the analysis of different families of compounds from highly complex matrices. In particular, the introduction of magnetic sorbents has proven to provide an important simplification of the sample preparation step, reducing the time of analysis and allowing a cost reduction of the whole procedure.

- Judging by the good performance shown by miniaturised techniques, and especially those proposed in this PhD Thesis in which new highly porous and selective materials have been used with extraction purposes, as well as the great number of publications to date in this sense, it seems clear that miniaturisation combined with the application of nanomaterials, which are in agreement with Green Analytical Chemistry principles, will play a key role in the development of more efficient and selective analytical methodologies.

GLOSSARY

GLOSSARY

μ-dSPE	Micro-dispersive solid-phase extraction
μ-SPE	Micro solid-phase extraction
17α-E_2	17α-estradiol
17β-E_2	17β-estradiol
17β-E_2-d_5	17β-estradiol-2,4,16,16,17-d_5
ACN	Acetonitrile
AcOH	Acetic acid
APCI	Atmospheric pressure chemical ionisation
APFO	Ammonium perfluorooctanoate
API	Atmospheric pressure ionisation
APPI	Atmospheric pressure photoionisation
BBP	Butylbenzyl phthalate
BGE	Background electrolite
BID-SDME	Bubble-in-drop single-drop microextraction
BPC	Base peak chromatogram
BSTFA	N-O-bis-(trimethylsilyl)-trifluoroacetamide
C_{18}	Octadecylsilane
CAS	Chemical Abstract Service
CE	Capillary electrophoresis
CEC	Capillary electrochromatography
CFME	Continuous flow microextraction
CLC	Capillary-liquid chromatography
CNT	Carbon nanotube
CZE	Capillary zone electrophoresis
DA	Dopamine
DAD	Diode array detector
DART	Direct analysis in real time
DBEP	Di(2-butoxyethyl) phthalate
DBP	Dibutyl phthalate

DBP-d_4	Dibutyl phthalate-3,4,5,6-d_4
DCHP	Dicyclohexyl phthalate
DCM	Dichloromethane
DDME	Drop-to-drop microextraction
DDT	Dichlorodiphenyltrichloroethane
DEEP	Di(2-ethoxyethyl) phthalate
DEHA	Di(2-ethylhexyl) adipate
DEHP	Di(2-ethylhexyl) phthalate
DEP	Diethyl phthalate
DES	Diethylstibestrol
DHP-d_4	Dihexyl phthalate-3,4,5,6-d_4
DI	Direct immersion
DIBP	Diisobutyl phthalate
DIDP	Diisodecyl phthalate
DINP	Diisononyl phthalate
DIPP	Diisopentyl phthalate
DLLME	Dispersive liquid-liquid microextraction
DMEP	Di(2-methoxyethyl) phthalate
DMP	Dimethyl phthalate
DNOP	Di-n-octyl phthalate
DNPP	Di-n-pentyl phthalate
DPP	Dipropyl phthalate
DS	Dienestrol
DSDME	Directly suspended droplet microextraction
dSPE	Dispersive solid-phase extraction
E_1	Estrone
ECD	Electron capture detector
ECs	Emerging contaminants
ED	Electrochemical detector
EDCs	Endocrine-disrupting compounds

EE_2	17α-ethynylestradiol
EFSA	European Food Safety Authority
EI	Electron ionisation
EIC	Extracted ion chromatogram
EKC	Electrokinetic chromatography
ELISA	Enzyme-linked immune sorbent assay
EPA	Environmental Protection Agency
ER	Oestrogenic receptor
ESI	Electrospray ionisation
EU	European Union
FD	Fluorescence detector
FID	Flame ionisation detector
FO-DLLME	Floating organic dispersive liquid-liquid microextraction
GC	Gas chromatography
GCB	Graphitised carbon black
GCxGC	Two-dimensional gas chromatography
HEX	Hexestrol
HF	Hollow-fibre
HFBA	Heptafluorobutyric anhydride
HLB	Hydrophilic-lipophilic balance
HPLC	High-performance liquid chromatography
HS	Headspace
ICC	Ion charge control
IL	Ionic liquid
IS	Internal standard
IT	Ion trap
LC	Liquid chromatography
LCL	Lowest calibration level
LCxLC	Two-dimensional liquid chromatography
LLE	Liquid-liquid extraction

LLLME	Liquid-liquid-liquid microextraction
LOD	Limit of detection
LOQ	Limit of quantification
LPE	Liquid-phase extraction
LPME	Liquid-phase microextraction
m-μ-dSPE	Magnetic-micro dispersive solid-phase extraction
MAE	Microwave-assisted extraction
MALDI	Matrix-assisted laser desorption/ionisation
m-dSPE	Magnetic-dispersive solid-phase extraction
MEEKC	Micro-emulsion electrokinetic chromatography
MEKC	Micellar electrokinetic chromatography
MeOH	Methanol
MIL	Material Institute Lavoisier
MIP	Molecularly imprinted polymer
MM	Molecular Mass
m-NP	Magnetic nanoparticle
MOF	Metal-organic framework
MRL	Maximum residue limit
MRM	Multiple reaction monitoring
MS	Mass spectrometry
MS/MS	Tandem mass spectrometry
MSPD	Matrix solid-phase dispersion
MSTFA	N-methyl-N-trimethylsilyltrifluoroacetamide
MWCNT	Multi-walled carbon nanotube
NP	Nanoparticle
PAE	Phthalic acid ester
PAH	Polycyclic aromatic hydrocarbon
PBS	Phosphate buffered saline
PDA	Photodiode array detector
pDA	Polydopamine

PE	Polyethylene
PET	Polyethylene terephthalate
PFPA	Pentafluoropropionic anhydride
PFPOH	2,2,3,3,3-pentafluoro-1-propanol
PhD	Philosophiae doctor
PLE	Pressurised liquid extraction
PP	Polypropylene
PS	Polystyrene
PSA	Primary secondary amine
PTFE	Polytetrafluoroethylene
PVA	Polyvinyl acetate
PVC	Polyvinyl chloride
Q	Quadrupole
QqQ	Triple quadrupole
QTOF	Quadrupole time-of-flight
QuEChERS	Quick, Easy, Cheap, Effective, Rugged and Safe
R^2	Determination coefficient
rcf	Relative centrifugal force
RDSE	Rotating-disc sorptive extraction
RIA	Radio immunoassay
rpm	Revolutions per minute
RSD	Relative standard deviation
SALDI	Surface-assisted laser desorption/ionisation
SBSE	Stir-bar sorptive extraction
SCSE	Stir-cake sorptive extraction
SDLPME	Solid drop liquid-phase microextraction
SDME	Single-drop microextraction
SDS	Sodium dodecyl sulphate
SFO-DLLME	Solidification of a floating organic drop dispersive liquid-liquid microextraction

SIM	Selected ion monitoring
SLE	Solid-liquid extraction
SLM	Supported liquid membrane
SML	Specific migration limit
SPE	Solid-phase extraction
SPME	Solid-phase microextraction
SRSE	Stir-rod sorptive extraction
SWCNT	Single-walled carbon nanotube
TDI	Tolerable daily intake
TIC	Total ion chromatogram
TMCS	Trimethylchlorosilane
TMU	Tarbiat Modares University
TOF	Time-of-flight
UAE	Ultrasound-assisted extraction
UHPLC	Ultra-high-performance liquid chromatography
US	Unites States
UV	Ultraviolet
Vis	Visible
WAX	Weak Anion-eXchange
WHO	World Health Organization
YES	Yeast oestrogen screen
ZAN	Zearalanone
ZEN	Zearalenone
α-ZAL	α-zearalanol
α-ZEL	α-zearalenol
β-ZAL	β-zearalanol
β-ZEL	β-zearalenol

REFERENCES

REFERENCES

[1] M. Taheran, M. Naghdi, S.K. Brar, M. Verma, R.Y. Surampalli, Emerging contaminants: Here today, there tomorrow! *Environmental Nanotechnology, Monitoring & Management* 10 (2018) 122-126.

[2] C. Sophia, E.C. Lima, Removal of emerging contaminants from the environment by adsorption. *Ecotox. Environ. Safe* 150 (2018) 1-17.

[3] E.R. Kabir, M.S. Rahman, I. Rahman, A review on endocrine disruptors and their possible impacts on human health. *Environ. Toxicol. Pharmacol.* 40 (2015) 241-258.

[4] E. Diamanti-Kandarakis, J.-P. Bourguignon, L.C. Giudice, R. Hauser, G.S. Prins, A.M. Soto, R.T. Zoeller, A.C. Gore, Endocrine-disrupting chemicals: An endocrine society scientific statement. *Endocr. Rev.* 30 (2009) 293-342.

[5] R. Hampl, J. Kubátová, L. Stárka, Steroids and endocrine disruptors-History, recent state of art and open questions. *J. Steroid Biochem. Mol. Biol.* 155 (2016) 217-223.

[6] Commission of the European Communities. Communication from de Commission to the Council and the European Parliament. Community Strategy for Endocrine Disrupters: A range of substances suspected of interfering with the hormone systems of humans and wildlife. Brussels, 1999.

[7] T. Colborn, C. Clement, (Ed.). *Chemically-induced alterations in sexual and functional development: The wildlife/human connection*. Princeton. Princeton Scientific Pub. Co., 1991.

[8] P. Matthiessen, Historical perspective on endocrine disruption in wildlife. *Pure Appl. Chem.* 75 (2003) 2197-2206.

[9] Endocrine Society. Endocrine Society webpage. Available at: https://bit.ly/2Pqx9DD. Accessed July 2018.

[10] N.O. Serrano, M.F. Fernández Cabrera, P. Martín Olmedo, Endocrine disrupters. The case of estrogen xenobiotics. *Rev. Salud Ambient.* 1 (2001) 6-11.

[11] H.W. Bennetts, E.J. Uuderwood, F.L. Shier, A specific breeding problem of sheep on subterranean clover pastures in Western Australia. *Aust. Vet. J.* 22 (1946) 2-12.

[12] P.L. Singer, Queries and minor notes - Occupational oligospermia. *JAMA-J. Am. Med. Assoc.* 140 (1949) 1249-1250.

[13] F.A. Caliman, M. Gavrilescu, Pharmaceuticals, personal care products and endocrine disrupting agents in the environment - A review. *Clean-Soil Air Water* 37 (2009) 277-303.

[14] A.C. Gore, D. Crews, L.L. Doan, M. La Merrill, H. Patisaul, A. Zota, Endocrine Society. Available at: https://bit.ly/1RN8wun. Accessed July 2018.

[15] Web of Science. Available at: https://bit.ly/2RHYSRw. Accessed August 2018.

References

[16] C. Barrios-Estrada, M. de Jesús Rostro-Alanis, B.D. Muñoz-Gutiérrez, H.M.N. Iqbal, S. Kannan, R. Parra-Saldívar, Emergent contaminants: Endocrine disruptors and their laccase-assisted degradation - A review. *Sci. Tot. Environ.* 612 (2018) 1516-1531.

[17] J.J. Amaral Mendes, The endocrine disrupters: A major medical challenge. *Food Chem. Toxicol.* 40 (2002) 781-788.

[18] W.L. Miller, Steroid hormone synthesis in mitochondria. *Mol. Cell. Endocrinol.* 379 (2013) 62-73.

[19] J. Cui, Y. Shen, R. Li, Estrogen synthesis and signaling pathways during ageing: From periphery to brain. *Trends Mol. Med.* 19 (2013) 197-209.

[20] H. Noppe, B. Le Bizec, K. Verheyden, H.F. De Brabander, Novel analytical methods for the determination of steroid hormones in edible matrices. *Anal. Chim. Acta* 611 (2008) 1-16.

[21] E. Carmina, R.A. Lobo, *Evaluation of hormonal status. Reproductive endocrinology. Physiology, pathophysiology and clinical management.* J.F. Strauss, R.L. Barbieri, (Ed.). Philadelphia, Elsevier Health Sciences, 2009.

[22] D.L. Nelson, M.M. Cox, *Lehninger Principles of Biochemistry*, sixth edition. W.H. Freeman, (Ed.). New York, MacMillan, 2013.

[23] B. Socas-Rodríguez, M. Asensio-Ramos, J. Hernández-Borges, A.V. Herrera-Herrera, M.Á. Rodríguez-Delgado, Chromatograhic analysis of natural and synthetic estrogens in milk and dairy products. *TrAC-Trend. Anal. Chem.* 44 (2013) 58-77.

[24] A. Alsayari, L. Kopel, M.S. Ahmed, A. Pay, T. Carlson, F.T. Halaweish, Design, synthesis, and biological evaluation of steroidal analogs as estrogenic/anti-estrogenic agents. *Steroids* 118 (2017) 32-40.

[25] H. Zhao, Y. Jiang, Y. Liu, C. Yun, L. Li, Endogenous estrogen metabolites as biomarkers for endometrial cancer via a novel method of liquid chromatography-mass spectrometry with hollow-fiber liquid-phase microextraction. *Horm. Metab. Res.* 47 (2015) 158-164.

[26] J. Tso, D.S. Aga, A systematic investigation to optimize simultaneous extraction and liquid chromatography tandem mass spectrometry analysis of estrogens and their conjugated metabolites in milk. *J. Chromatogr. A* 1217 (2010) 4784-4795.

[27] H. Malekinejad, P. Scherpenisse, A. Bergwerff, Naturally occurring estrogens in processed milk and in raw milk (from gestated cows). *J. Agric. Food Chem.* 54 (2006) 9785-9791.

[28] S. Fritsche, H. Steinhart, Occurrence of hormonally active compounds in food: A review. *Eur. Food Res. Technol.* 209 (1999) 153-179.

[29] D.E. Bronowicka-Kłys, M. Lianeri, P.P. Jagodziński, The role and impact of estrogens and xenoestrogen on the development of cervical cancer. *Biomed. Pharmacother.* 84 (2016) 1945-1953.

[30] D. Ren, B. Huang, B. Yang, X. Pan, D.D. Dionysiou, Mitigating 17α-ethynylestradiol water contamination through binding and photosensitization by dissolved humic substances. *J. Hazard. Mater.* 327 (2017) 197-205.

[31] M. Liu, M. Li, B. Qiu, X. Chen, G. Chen, Synthesis and applications of diethylstilbestrol-based molecularly imprinted polymer-coated hollow fiber tube. *Anal. Chim. Acta* 663 (2010) 33-38.

[32] B. Shao, R. Zhao, J. Meng, Y. Xue, G. Wu, J. Hu, X. Tu, Simultaneous determination of residual hormonal chemicals in meat, kidney, liver tissues and milk by liquid chromatography-tandem mass spectrometry. *Anal. Chim. Acta* 548 (2005) 41-50.

[33] L.O. Reis, E.L. Zani, H.A. García-Perdomo, Estrogen therapy in patients with prostate cancer: A contemporary systematic review. *Int. Urol. Nephrol.* 50 (2018) 993-1003.

[34] H. Demaegdt, B. Daminet, A. Evrard, M.-L. Scippo, M. Muller, L. Pussemier, A. Callebaut, K. Vandermeiren, Endocrine activity of mycotoxins and mycotoxin mixtures. *Food Chem. Toxicol.* 96 (2016) 107-116.

[35] H. Belhassen, I. Jiménez-Díaz, J.P. Arrebola, R. Ghali, H. Ghorbel, N. Olea, A. Hedili, Zearalenone and its metabolites in urine and breast cancer risk: A case-control study in Tunisia. *Chemosphere* 128 (2015) 1-6.

[36] M. Kleinova, P. Zöllner, H. Kahlbacher, W. Hochsteiner, W. Lindner, Metabolic profiles of the mycotoxin zearalenone and of the growth promoter zeranol in urine, liver, and muscle of heifers. *J. Agric. Food Chem.* 50 (2002) 4769-4776.

[37] B. Jarošová, J. Javůrek, O. Adamovský, K. Hilscherová, Phytoestrogens and mycoestrogens in surface waters - Their sources, occurrence, and potential contribution to estrogenic activity. *Environ. Int.* 81 (2015) 26-44.

[38] S. Marin, A.J. Ramos, G. Cano-Sancho, V. Sanchis, Mycotoxins: Occurrence, toxicology, and exposure assessment. *Food Chem. Toxicol.* 60 (2013) 218-237.

[39] A.F. Erasmuson, B.G. Scahill, D.M. West, Natural zeranol (α-zearalanol) in the urine of pasture-fed animals. *J. Agric. Food Chem.* 42 (1994) 2721-2725.

[40] R.T. Zoeller, Endocrine disruptors: Do family lines carry an epigenetic record of previous generations' exposures? *Endocrinology* 147 (2006) 5513-5514.

[41] J. Toppari, Environmental endocrine disrupters and disorders of sexual differentiation. *Semin. Reprod. Med.* 20 (2002) 305-312.

[42] D.P. de Almeida Ferreira Braga, E. Borges Jr., *Environmental factors, food intake, and social habits in male patients and its relationship to intracytoplasmic sperm injection outcomes. Handbook of fertility: Nutrition, diet, lifestyle and reproductive health.* R.R. Watson, (Ed.). San Diego, Elsevier, 2015, p. 355-367.

References

[43] X. Xu, T.D. Veenstra, S.D. Fox, J.M. Roman, H.J. Isaaq, R. Falk, J.E. Saavedra, L.K. Keefer, R.G. Ziegler, Measuring fifteen endogenous estrogens simultaneously in human urine by high-performance liquid chromatography-mass spectrometry. *Anal. Chem.* 77 (2005) 6646-6654.

[44] D. Ganmaa, A. Sato, The possible role of female sex hormones in milk from pregnant cows in the development of breast, ovarian and corpus uteri cancers. *Med. Hypotheses* 65 (2005) 1028-1037.

[45] A. Bouskine, M. Nebout, B. Mograbi, F. Brücker-Davis, C. Roger, P. Fenichel, Estrogens promote human testicular germ cell cancer through a membrane-mediated activation of extracellular regulated kinase and protein kinase A. *Endocrinology* 149 (2008) 565-573.

[46] M. Adeel, X. Song, Y. Wang, D. Francis, Y. Yang, Environmental impact of estrogens on human, animal and plant life: A critical review. *Environ. Int.* 99 (2017) 107-119.

[47] C.M. Maragos, Zearalenone occurrence in surface waters in central Illinois, USA. *Food Addit. Contam. B* 5 (2012) 55-64.

[48] Q. Xu, M. Wang, S.Q. Yu, Q. Tao, M. Tang, Trace analysis of diethylstilbestrol, dienestrol and hexestrol in environmental water by Nylon 6 nanofibers mat-based solid-phase extraction coupled with liquid chromatography-mass spectrometry. *Analyst* 136 (2011) 5030-5037.

[49] D. Ganmaa, P.Y. Wang, L.Q. Qin, K. Hoshi, A. Sato, Is milk responsible for male reproductive disorders? *Med. Hypotheses* 57 (2001) 510-514.

[50] P.-N. Jouan, Y. Pouliot, S.F. Gauthier, J.-P. Laforest, Hormones in bovine milk and milk products: A survey. *Int. Dairy J.* 16 (2006) 1408-1414.

[51] L. Xu, L. Zhang, Y. Zhang, Q. Sheng, A. Zhao, Qualitative and quantitative comparison of hormone contents between bovine and human colostrums. *Int. Fairy J.* 21 (2011) 54-57.

[52] European Parliament Resolution (2012/2066(INI)) of 14 March 2013 on the protection of public health from endocrine disrupters. *Off. J. Eur. Commun.* C036 (2016) 85-91.

[53] Directive 2013/39/EU of the European Parliament and of the Council of 12 August 2013 amending Directives 2000/60/EC and 2008/105/EC as regards priority substances in the field of water policy. *Off. J. Eur. Union* L226 (2013) 1-17.

[54] 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. *Off. J. Eur. Commun.* L327 (2000) 1-72.

[55] 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. *Off. J. Eur. Union* L348 (2008) 84-97.

[56] Commission Implementing Decision (EU) 2015/495 of 20 March 2015 establishing a watch list of substances for Union-wide monitoring in the field of water policy pursuant to Directive 2008/105/EC of the European Parliament and of the Council. *Off. J. Eur. Union* L78 (2015) 40-42.

[57] Commission Regulation (EU) No 37/2010 of 22 December 2009 on pharmacologycally active substances and their classification regarding maximum residue limits in foodstuffs of animal origin. *Off. J. Eur. Union* L15 (2010) 1-72.

[58] Commission Implementing Regulation (EU) 2018/1076 of 30 July 2018 amending Regulation (EU) No 37/2010 to classify the substance isoflurane as regards its maximum residue limit. *Off. J. Eur. Union* L194 (2018) 41-43.

[59] Council Directive 81/602/EEC of 31 July 1981 concerning the prohibition of certain substances having a hormonal action and of any sybstances having a thyrostathic action. *Off. J. Eur. Union* L222 (1981) 32-33.

[60] Council Directive 96/22/EC of 29 April 1996 concerning the prohibition on the use in stockfarming of certain substances having a hormonal or thyrostatic action and of beta-agonists, and repealing Directives 81/602/EEC, 88/146/EEC and 88/299/EEC. *Off. J. Eur. Commun.* L125 (1996) 3-9.

[61] Directive 2003/74/EC of the European Parliament and of the Council of 22 September 2003 amending Council Directive 96/22/EC concerning the prohibition on the use in stockfarming of certain substances having a hormonal or thyrostatic action and of beta-agonists. *Off. J. Eur. Union* L262 (2003) 17-21.

[62] Directive 2008/97/EC of the European Parliament and of the Council of 19 November 2008 amending Council Directive 96/22/EC concerning the prohibition on the use in stockfarming of certain substances having a hormonal or thyrostatic action and of beta-agonists. *Off. J. Eur. Union* L318 (2008) 9-11.

[63] S. Wang, W. Huang, G. Fang, Y. Zhang, H. Qiao, Analysis of steroidal estrogen residues in food and environmental samples. *Int. J. Environ. Anal. Chem.* 88 (2008) 1-25.

[64] P.E. Lønning, Estradiol measurement in translational studies of breast cancer. *Steroids* 99 (2015) 26-31.

[65] T.Y. Fang, S.M. Praveena, C. de Burbure, A.Z. Aris, S.N.S. Ismail, I. Rasdi, Analytical techniques for steroid estrogens in water samples - A review. *Chemosphere* 165 (2016) 358-368.

[66] J. Tate, G. Ward, Interferences in immunoassay. *Clin. Biochem. Rev.* 25 (2004) 105-120.

[67] H. Tomšíková, J. Aufartová, P. Solich, Z. Sosa-Ferrera, J.J. Santana-Rodríguez, L. Nováková, High-sensitivity analysis of female-steroid hormones in environmental samples. *TrAC-Trend. Anal. Chem.* 34 (2012) 35-58.

[68] A. Nezami, R. Nosrati, B. Golichenari, R. Rezaee, G.I. Chatzidakis, A.M. Tsatsakis, G. Karimi, Nanomaterial-based aptasensors and bioaffinity sensors for quantitative detection of 17β-estradiol. *TrAC-Trend. Anal. Chem.* 94 (2017) 95-105.

[69] L. Xu, Z. Zhang, Q. Zhang, P. Li, Mycotoxin determination in foods using advanced sensors based on antibodies or aptamers. *Toxins* 8 (2016) 239-255.

[70] B. Yilmaz, Y. Kadioglu, Determination of 17β-estradiol in rabbit plasma by gas chromatography with flame ionization detection. *Indian J. Pharm. Sci.* 74 (2012) 248-253.

[71] J.M. Halket, V.G. Zaikin, Derivatization in mass spectrometry-1. Silylation. *Eur. J. Mass Spectrom.* 9 (2003) 1-21.

[72] A. Azzouz, B. Jurado-Sánchez, B. Souhai, E. Ballesteros, Simultaneous determination of 20 pharmacologically active substances in cow's milk, goat's milk, and human breast milk by gas chromatography-mass spectrometry. *J. Agric. Food Chem.* 59 (2011) 5125-5132.

[73] M.H. Choi, K.R. Kim, J.K. Hong, S.J. Park, B.C. Chung, Determination of non-steroidal estrogens in breast milk, plasma, urine and hair by gas chromatography/mass spectrometry. *Rapid Commun. Mass Spectrom.* 16 (2002) 2221-2228.

[74] A.L. Capriotti, C. Cavaliere, V. Colapicchioni, S. Piovesana, R. Samperi, A. Laganà, Analytical strategies based on chromatography-mass spectrometry for the determination of estrogen-mimicking compounds in food. *J. Chromatogr. A* 1313 (2013) 62-77.

[75] M. Kopperi, J. Ruiz-Jiménez, J.I. Hukkinen, M.-L. Riekkola, New way to quantify multiple steroidal compounds in wastewater by comprehensive two-dimensional gas chromatography-time-of-flight mass spectrometry. *Anal. Chim. Acta* 761 (2013) 217-226.

[76] Z. Huang, H.K. Lee, Performance of metal-organic framework MIL-101 after surfactant modification in the extraction of endocrine disrupting chemicals from environmental water samples. *Talanta* 143 (2015) 366-373.

[77] I. Matraszek-Zuchowska, B. Wozniak, A. Posyniak, Determination of hormones residues in milk by gas chromatography-mass spectrometry. *Food Anal. Method.* 10 (2017) 727-739.

[78] N. Migowska, M. Caban, P. Stepnowski, J. Kumirska, Simultaneous analysis of non-steroidal anti-inflammatory drugs and estrogenic hormones in water and wastewater samples using gas chromatography-mass spectrometry and gas chromatography with electron capture detection. *Sci. Tot. Environ.* 441 (2012) 77-88.

[79] G. D'Orazio, J. Hernández-Borges, A.V. Herrera-Herrera, S. Fanali, M.Á. Rodríguez-Delgado, Determination of estrogenic compounds in milk and yogurt samples by hollow-fibre liquid-phase microextraction-gas chromatography-triple quadrupole mass spectrometry. *Anal. Bioanal. Chem.* 408 (2016) 7447-7459.

[80] R. Su, X. Wang, X. Xu, Z. Wang, D. Li, X. Zhao, X. Li, H. Zhang, A. Yu, Application of multiwall carbon nanotubes-based matrix solid phase dispersion extraction for determination of

hormones in butter by gas chromatography mass spectrometry. *J. Chromatogr. A* 1218 (2011) 5047-5054.

[81] A. González, J. Avivar, V. Cerdà, Estrogens determination in wastewater samples by automatic in-syringe dispersive liquid-liquid microextraction prior silylation and gas chromatography. *J. Chromatogr. A* 1413 (2015) 1-8.

[82] B. Socas-Rodríguez, J. Hernández-Borges, A.V. Herrera-Herrera, M.Á. Rodríguez-Delgado, Multiresidue analysis of oestrogenic compounds in cow, goat, sheep and human milk using core-shell polydopamine coated magnetic nanoparticles as extraction sorbent in micro-dispersive solid-phase extraction followed by ultra-high-performance liquid chromatography tandem mass spectrometry. *Anal. Bioanal. Chem.* 410 (2018) 2031-2042.

[83] C. Cavaliere, A.L. Capriotti, P. Foglia, S. Piovesana, R. Samperi, S. Ventura, A. Laganà, Natural estrogens in dairy products: Determination of free and conjugated forms by ultra high performance liquid chromatography with tandem mass spectrometry. *J. Sep. Sci.* 38 (2015) 3599-3606.

[84] S. Fekete, J. Schappler, J.-L. Veuthey, D. Guillarme, Current and future trends in UHPLC. *TrAC-Trend. Anal. Chem.* 63 (2014) 2-13.

[85] P. Kozlík, Z. Bosáková, E. Tesařova, P. Coufal, R. Čabala, Development of a solid-phase extraction with capillary liquid chromatography tandem mass spectrometry for analysis of estrogens in environmental water samples. *J. Chromatogr. A* 1218 (2011) 2127-2132.

[86] G. D'Orazio, J. Hernández-Borges, M. Asensio-Ramos, M.Á. Rodríguez-Delgado, S. Fanali, Capillary electrochromatography and nano-liquid chromatography coupled to nano-electrospray ionization interface for the separation and identification of estrogenic compounds. *Electrophoresis* 37 (2016) 356-362.

[87] H.P. Nguyen, L. Li, J.W. Gatson, D. Maass, J.G. Wigginton, J.W. Simpkins, K.A. Schug, Simultaneous quantification of four native estrogen hormones at trace levels in human cerebrospinal fluid using liquid chromatography-tandem mass spectrometry. *J. Pharmaceut. Biomed.* 54 (2011) 830-837.

[88] B. Socas-Rodríguez, M. Asensio-Ramos, J. Hernández-Borges, M.Á. Rodríguez-Delgado, Hollow-fiber liquid-phase microextraction for the determination of natural and synthetic estrogens in milk samples. *J. Chromatogr. A* 1313 (2013) 175-184.

[89] D.D. Snow, T. Damon-Powell, S. Onanong, D.A. Cassada, Sensitive and simplified analysis of natural and synthetic steroids in water and solids using on-line solid-phase extraction and microwave-assisted solvent extraction coupled to liquid chromatography tandem mass spectrometry atmospheric pressure photoionization. *Anal. Bioanal. Chem.* 405 (2013) 1759-1771.

[90] R. López-Roldán, M. López de Alda, M. Gros, M. Petrovic, J. Martín-Alonso, D. Barceló,

Advanced monitoring of pharmaceuticals and estrogens in the Llobregat River basin (Spain) by liquid chromatography–triple quadrupole-tandem mass spectrometry in combination with ultra performance liquid chromatography–time of flight-mass spectrometry. *Chemosphere* 80 (2010) 1337-1344.

[91] J.V. Bussche, A. Decloedt, L. Van Meulebroek, N. De Clercq, S. Lock, J. Stahl-Zeng, L. Vanhaecke, A novel approach to the quantitative detection of anabolic steroids in bovine muscle tissue by means of a hybrid quadrupole time-of-flight-mass spectrometry instrument. *J. Chromatogr. A* 1360 (2014) 229-239.

[92] W. Yan, Y. Li, L. Zhao, J.-M. Lin, Determination of estrogens and bisphenol A in bovine milk by automated on-line C_{30} solid-phase extraction coupled with high-performance liquid chromatography–mass spectrometry. *J. Chromatogr. A* 1216 (2009) 7539-7545.

[93] G.-W. Lie, C.-Y. Chen, G.-S. Wang, Comparison of electrospray ionization, atmospheric pressure chemical ionization and atmospheric pressure photoionization for determining estrogenic chemicals in water by liquid chromatography tandem mass spectrometry with chemical derivatizations. *J. Chromatogr. A* 1216 (2009) 956-966.

[94] I. Athanasiadou, Y.S. Angelis, E. Lyris, C. Georgakopoulos, Chemical derivatization to enhance ionization of anabolic steroids in LC-MS for doping-control analysis. *TrAC-Trend. Anal. Chem.* 42 (2013) 137-156.

[95] D.W. Farlow, X. Xu, T.D. Veenstra, Quantitative measurement of endogenous estrogen metabolites, risk-factors for development of breast cancer, in commercial milk products by LC–MS/MS. *J. Chromatogr. B* 877 (2009) 1327-1334.

[96] C. Farke, E. Rattenberger, S.U. Roiger, H.H.D. Meyer, Bovine colostrum: Determination of naturally occurring steroid hormones by liquid chromatography-tandem mass spectrometry (LC-MS/MS). *J. Agric. Food Chem.* 59 (2011) 1423-1427.

[97] C. Cruces-Blanco, L. Gámiz-Gracia, A.M. García-Campaña, Applications of capillary electrophoresis in forensic analytical chemistry. *TrAC-Trend. Anal. Chem.* 26 (2007) 215-226.

[98] M. Koel, M. Kaljurand, Application of the principles of green chemistry in analytical chemistry. *Pure Appl. Chem.* 78 (2006) 1993-2002.

[99] P. Li, W. Zhao, X. Wang, G. Shi, T. Zhou, Stacking and simultaneous determination of estrogens in water samples by CE with electrochemical detection. *J. Sep. Sci.* 36 (2013) 334-340.

[100] G. D'Orazio, M. Asensio-Ramos, J. Hernández-Borges, S. Fanali, M.Á. Rodríguez-Delgado, Estrogenic compounds determination in water samples by dispersive liquid–liquid microextraction and micellar electrokinetic chromatography coupled to mass spectrometry. *J. Chromatogr. A* 1344 (2014) 109-121.

[101] G. D'Orazio, M. Asensio-Ramos, J. Hernández-Borges, M.Á. Rodríguez-Delgado, S. Fanali,

Evaluation of the combination of a dispersive liquid–liquid microextraction method with micellar electrokinetic chromatography coupled to mass spectrometry for the determination of estrogenic compounds in milk and yogurt. *Electrophoresis* 36 (2015) 615-625.

[102] B. Fogarty, E. Dempsey, F. Regan, Potential of microemulsion electrokinetic chromatography for the separation of priority endocrine disrupting compounds. *J. Chromatogr. A* 1014 (2003) 129-139.

[103] Y. Guan, C. Jiang, C. Hu, L. Jia, Preparation of multi-walled carbon nanotubes functionalized magnetic particles by sol-gel technology and its application in extraction of estrogens. *Talanta* 83 (2010) 337-343.

[104] S. Liu, X. Wu, Z. Xie, X. Lin, L. Guo, C. Yan, G. Chen, On-line coupling of pressurized capillary electrochromatography with end-column amperometric detection for analysis of estrogens. *Electrophoresis* 26 (2005) 2342-2350.

[105] S. Liu, Z. Xie, X. Wu, X. Lin, L. Guo, G. Chen, Separation of structurally related estrogens using isocratic elution pressurized capillary electrochromatography. *J. Chromatogr. A* 1092 (2005) 258-262.

[106] G.W. Somsen, R. Mol, G.J. de Jong, On-line coupling of electrokinetic chromatography and mass spectrometry. *J. Chromatogr. A* 1217 (2010) 3978-3991.

[107] L. Yang, C.S. Lee, Micellar electrokinetic chromatography-mass spectrometry. *J. Chromatogr. A* 780 (1997) 207-218.

[108] Z. Zhang, S.M. Rhind, C. Kerr, M. Osprey, C.E. Kyle, Selective pressurized liquid extraction of estrogenic compounds in soil and analysis by gas chromatography–mass spectrometry. *Anal. Chim. Acta* 685 (2011) 29-35.

[109] Y. Yang, H. Li, J. Zhang, N. Sun, H. Sun, Analysis of infant formula for steroid hormones by gas chromatography-tandem mass spectrometry using microwave-assisted extraction and gel permeation chromatography clean up. *Food Anal. Method.* 7 (2014) 798-805.

[110] C. Cavaliere, A.L. Capriotti, F. Ferraris, P. Foglia, R. Samperi, S. Ventura, A. Laganà, Multiresidue analysis of endocrine-disrupting compounds and perfluorinated sulfates and carboxylic acids in sediments by ultra-high-performance liquid chromatography–tandem mass spectrometry. *J. Chromatogr. A* 1438 (2016) 133-142.

[111] D. Zacs, I. Perkons, V. Bartkevics, Determination of steroidal oestrogens in tap water samples using solid-phase extraction on a molecularly imprinted polymer sorbent and quantification with gas chromatography-mass spectrometry (GC-MS). *Environ. Monit. Assess.* 188 (2016) 433-445.

[112] B. Socas-Rodríguez, J. González-Sálamo, J. Hernández-Borges, M.Á. Rodríguez-Delgado, Application of multiwalled carbon nanotubes as sorbents for the extraction of mycotoxins in

water samples and infant milk formula prior to high performance liquid chromatography mass spectrometry analysis. *Electrophoresis* 37 (2017) 1359-1366.

[113] G. Gao, S. Li, S. Li, Y. Wang, P. Zhao, X. Zhang, X. Hou, A combination of computational–experimental study on metal-organic framework MIL-53(Al) as sorbent for sumultaneous determination of estrogens and glucocorticoids in water and urine samples by dispersive micro-solid-phase extraction coupled to UPLC-MS/MS. *Talanta* 180 (2018) 358-367.

[114] B. Socas-Rodríguez, J. Hernández-Borges, M. Asensio-Ramos, A.V. Herrera-Herrera, J.A. Palenzuela, M.Á. Rodríguez-Delgado, Determination of estrogens in environmental water samples using 1,3-dipentylimidazolium hexafluorophosphate ionic liquid as extraction solvent in dispersive liquid–liquid microextraction. *Electrophoresis* 35 (2014) 2479-2487.

[115] N.Z.I.H. Harunarashid, L.H. Lim, M.H. Harunsani, Phthalate sample preparation methods and analysis in food and food packaging: A review. *Food Anal. Method.* 10 (2017) 3790-3814.

[116] D. Kashyap, T. Agarwal, Concentration and factors affecting the distribution of phthalates in the air and dust: A global scenario. *Sci. Tot. Environ.* 635 (2018) 817-827.

[117] I. Katsikantami, S. Sifakis, M.N. Tzatzarakis, E. Vakonaki, O.-I. Kalantzi, A.M. Tsatsakis, A.K. Rizos, A global assessment of phthalates burden and related links to health effects. *Environ. Int.* 97 (2016) 212-236.

[118] American Chemistry Council. Phthalates. High phthalates uses and applications. Available at: https://bit.ly/2NA6pyO. Accessed August 2018.

[119] J. Yang, Y. Li, Y. Wang, J. Ruan, J. Zhang, C. Sun, Recent advances in analysis of phthalate esters in foods. *TrAC-Trend. Anal. Chem.* 72 (2015) 10-26.

[120] T.T. Bui, G. Giovanoulis, A.P. Cousins, J. Magnér, I.T. Cousins, C.A. de Wuit, Human exposure, hazard and risk of alternative plasticizers to phthalate esters. *Sci. Tot. Environ.* 541 (2016) 451-467.

[121] Plastics Europe. Plastics - the Facts 2017. An analysis of European plastics production, demand and waste data. Available at: https://bit.ly/2GvymZS. Accessed August 2018.

[122] S.L. Wright, F.J. Kelly, Plastic and human health: A micro issue? *Environ. Sci. Technol.* 51 (2017) 6634-6647.

[123] E. Fasano, T. Cirillo, F. Esposito, S. Lacorte, Migration of monomers and plasticizers from packed foods and heated microwave foods using QuEChERS sample preparation and gas chromatography/mass spectrometry. *LWT-Food Sci. Technol.* 64 (2015) 1015-1021.

[124] M.J. Teil, M. Blanchard, M. Chevreuil, Atmospheric fate of phthalate esters in an urban area (Paris-France). *Sci. Tot. Environ.* 354 (2006) 212-223.

[125] D.-W. Gao, Z.-D. Wen, Phthalate esters in the environment: A critical review of their

occurrence, biodegradation, and removal during wastewater treatment processes. *Sci. Tot. Environ.* 541 (2016) 986-1001.

[126] D. Gao, Z. Li, H. Wang, H. Liang, An overview of phthalate acid ester pollution in China over the last decade: Environmental occurrence and human exposure. *Sci. Tot. Environ.* 645 (2018) 1400-1409.

[127] J. Sun, X. Wu, J. Gan, Uptake and metabolism of phthalate esters by edible plants. *Environ. Sci. Technol.* 49 (2015) 8471-8478.

[128] S. Benjamin, E. Masai, N. Kamimura, K. Tagahashi, R.C. Anderson, P.A. Faisal, Phthalates impact human health: Epidemiological evidences and plausible mechanism of action. *J. Hazard. Materials* 340 (2017) 360-383.

[129] X.-L. Cao, Phthalate esters in foods: Sources, occurrence, and analytical methods. *Compr. Rev. Food Sci. F.* 9 (2010) 21-43.

[130] I. Al-Saleh, N. Shinwari, A. Alsabbaheen, Phthalates residues in plastic bottled waters. *J. Toxicol. Sci.* 36 (2011) 469-478.

[131] Regulation (EC) Nº 1935/2004 of the European Parliament and of the Council of 27 October 2004 on materials and articles intended to come into contact with food and repealling Directives 80/590/EEC and 89/109/EEC. *Off. J. Eur. Union* L338 (2004) 4-17.

[132] Commission Regulation (EC) No 2023/2006 of 22 December 2006 on good manufacturing practice for materials intended to come into contact with food. *Off. J. Eur. Union* L384 (2006) 75-78.

[133] Commission Regulation (EU) No 10/2011 of 14 January 2011 on plastic materials and articles intended to come into contact with food. *Off. J. Eur. Union* L12 (2011) 1-89.

[134] Commission Directive 2007/19/EC of 20 March 2007 amending Directive 2002/72/EC relating to plastic materials and articles intended to come into contact with food and Council Directive 85/572/EEC laying down the list of simulants to be used for testing migration of constituents of plastic materials and articles intended to come into contact with foodstuffs. *Off. J. Eur. Union* L91 (2007) 17-36.

[135] US Environment Protection Agency. Phthalates Action Plan (2012). https://bit.ly/2qlWxkl. Accessed August 2018.

[136] Opinion of the scientific panel on food additives, flavourings processing aids and materials in contact with food (AFC) on a request from the commission related to bis(2-ethylhexyl) phthalate (DEHP) for use in food contact materials. *EFSA J.* 243 (2005) 1-20.

[137] World Health Organization. Di(2-ethylhexyl) phthalate in drinking water (2003). https://bit.ly/2pLleF2. Accessed August 2018.

[138] N. Szczepańska, M. Rutkowska, K. Owczarek, J. Płotka-Wasylka, J. Namieśnik, Main complications connected with detection, identification and determination of trace organic

cconstituents in complex matrix samples. *TrAC-Trend. Anal. Chem.* 105 (2018) 173-184.

[139] Y.-R. Liang, Z.-M. Zhang, Z.-J. Liu, K. Wang, X.-Y. Wu, K. Zeng, H. Meng, Z. Zhang, A highly sensitive signal-amplified gold nanoparticle-based electrochemical immunosensor for dibutyl phthalate detection. *Biosens. Bioelectron.* 91 (2017) 199-202.

[140] X. Li, X. Wang, L. Li, H. Duan, C. Luo, Electrochemical sensor based on magnetic graphene oxide@gold nanoparticles-molecular imprinted polymers for determination of dibutyl phthalate. *Talanta* 131 (2015) 354-360.

[141] P.P. de Souza, Z. de L. Cardeal, R. Augusti, P. Morrison, P.J. Marriott, Determination of volatile compounds in Brazilian distilled cachaça by using comprehensive two-dimensional gas chromatography and effects of production pathways. *J. Chromatogr. A* 1216 (2009) 2881-2890.

[142] L. Barp, G. Purcaro, F.A. Franchina, M. Zoccali, D. Sciarrone, P.Q. Tranchida, L. Mondello, Determination of phthalate esters in vege oils using direct immersion solid-phase microextraction and fast gas chromatography coupled with triple quadrupole mass spectrometry. *Anal. Chim. Acta* 887 (2015) 237-244.

[143] M.Z. Jeddi, N. Rastkari, R. Ahmadkhaniha, M. Yunesian, Endocrine disruptor phthalates in bottled water: Daily exposure and health risk assessment in pregnant and lactating women. *Environ. Monit. Assess.* 188 (2016) 534-550.

[144] C. Li, X. Ma, X. Zhang, R. Wang, X. Li, Q. Liu, Preparation of magnetic molecularly imprinted polymer nanoparticles by surface imprinting by a sol-gel process for the selective and rapid removal of di-(2-ethylhexyl) phthalate from aqueous solution. *J. Sep. Sci.* 40 (2017) 1429-1630.

[145] V. Fernández-González, C. Moscoso-Pérez, S. Muniategui-Lorenzo, P. López-Mahía, D. Prada-Rodríguez, Reliable, rapid and simple method for the anaalysis of phthalates in sediments by ultrasonic solvent extraction followed by head space-solid phase microextraction gas chromatography mass spectrometry determination. *Talanta* 162 (2017) 648-653.

[146] W. Liang, J. Wang, X. Zang, C. Wang, Z. Wang, A porous carbon derived from amino-functionalized material of Institut Lavoisier as a solid-phase microextraction fiber coating for the extraction of phthalate esters from tea. *J. Sep. Sci.* 39 (2016) 1331-1338.

[147] J. Wang, X. Li, Q. Zhang, J. Xiong, H. Li, Determination of phthalate esters in Chinese spirits using isotope dilution gas chromatography with tandem mass spectrometry. *J. Sep. Sci.* 38 (2015) 1700-1710.

[148] H. Hashemi-Moghaddam, S. Maddah, Coating of optical fiber with a smart thermosensitive polymer for the separation of phthalate esters by solid-phase microextraction. *J. Sep. Sci.* 41 (2018) 886-892.

[149] K. Holadová, G. Prokůpková, J. Hajšlová, J. Poustka, Headspace solid-phase microextraction of phthalic acid esters from vegetable oil empolying solvent based matrix modification. *Anal. Chim. Acta* 582 (2007) 24-33.

[150] P. Yin, H. Chen, X. Liu, Q. Wang, Y. Jiang, R. Pan, Mass spectral fragmentation pathways of phthalate esters by gas chromatography-tandem mass spectrometry. *Anal. Lett.* 47 (2014) 1579-1588.

[151] C.A. Staples, (Ed.). *Phthalate esters.* Berlin, Springer-Verlag, 2003.

[152] C. Li, J. Xu, D. Chen, Y. Xiao, Detection of phthalates migration from disposable tablewares to drinking water using hexafluoroisopropanol-induced catanionic surfactant coacervate extraction. *J. Pharmaceut. Anal.* 6 (2016) 292-299.

[153] D. Salazar-Beltrán, L. Hinojosa-Reyes, E. Ruiz-Ruiz, A. Hernández-Ramírez, J.L. Guzmán-Mar, Determination of phthalates in bottled water by automated on-line solid phase extraction coupled to liquid chromatography with UV detection. *Talanta* 168 (2017) 291-297.

[154] S. Zhou, N. Song, X. Lv, Q. Jia, Preparation of carboxylatocalix[4]arene functionalized magnetic polyionic liquid hybrid material for the pre-concentration of phthalate esters. *J. Chromatogr. A* 1565 (2018) 19-28.

[155] I. Aparicio, J. Martín, J.L. Santos, J.L. Malvar, E. Alonso, Stir bar sorptive extraction and liquid chromatography-tandem mass spectrometry determination of polar and non-polar emerging and priority pollutants in environmental waters. *J. Chromatogr. A* 1500 (2017) 43-52.

[156] A. Khedr, Optimized extraction method for LC–MS determination of bisphenol A, melamine and di(2-ethylhexyl) phthalate in selected soft drinks, syringes, and milk powder. *J. Chromatogr. B* 930 (2013) 98-103.

[157] Á. Santana-Mayor, B. Socas-Rodríguez, M.M. Afonso, J.A. Palenzuela-López, M.Á. Rodríguez-Delgado, Reduced graphene oxide-coated magnetic-nanoparticles as sorbent for the determination of phthalates in environmental samples by micro-dispersive solid-phase extraction followed by ultra-high-performance liquid chromatography tandem mass spectrometry. *J. Chromatogr. A* 1565 (2018) 36-47.

[158] J.P. Quirino, Sweeping of neutral analytes in partial-filling micellar electrokinetic chromatography with electrospray ionization mass spectrometry. *Electrophoresis* 32 (2011) 665-668.

[159] J. Sun, H. He, S. Liu, Determination of phthalic acid esters in Chinese white spirit using dispersive liquid–liquid microextraction coupled with sweeping β-cyclodextrin-modified micellar electrokinetic chromatography. *J. Sep. Sci.* 37 (2014) 1679-1686.

[160] T.-F. Jiang, T.-T. Liang, M.-E. Yue, Y.-H. Wang, Z.-H. Lv, Surfactant-free microemulsion reinforced hollow-fiber liquid-phase microextraction combined with micellar electrokinetic

capillary chromatography for detection of phthalic acid esters in beverage and urine. *Food Anal. Method.* 9 (2016) 7-15.

[161] M.E. Yue, Q. Lin, Q. Li, J. Xu, T.F. Jiang, Determination of PAEs by integrative coupling method of headspace in-tube microextraction and reverse-flow micellar electrokinetic capillary chromatography. *Food Anal. Method.* 10 (2017) 3565-3571.

[162] S.-Y. Hsieh, C.-C. Wang, S.-M. Wu, Microemulsion electrokinetic chromatography for analysis of phthalates in soft drinks. *Food Chem.* 141 (2013) 3486-3491.

[163] H.-C. Chao, H.-W. Liao, C.-H. Kuo, Using water plug-assisted analyte focusing by micelle collapse in combination with microemulsion electrokinetic chromatography for analyzing phthalate esters. *J. Chromatogr. A* 1445 (2016) 149-157.

[164] S. Yadav, S. Rai, A.K. Srivastava, S. Panchal, D.K. Patel, V.P. Sharma, S. Jain, L.P. Srivastava, Determination of pesticide and phthalate residues in tea by QuEChERS method and their fate in processing. *Environ. Sci. Pollut. Res.* 24 (2017) 3074-3083.

[165] G. Leng, W. Chen, Y. Wang, Simple field-based automated dispersive liquid-liquid microextraction of trace level phthalate esters in natural waters with gas chromatography and mass spectrometric analysis. *J. Sep. Sci.* 39 (2016) 3392-3399.

[166] H. Shaikh, N. Memon, H. Khan, M.I. Bhanger, S.M. Nizamani, Preparation and characterization of molecularly imprinted polymer for di(2-ethylhexyl) phthalate: Application to sample clean-up prior to gas chromatographic determination. *J. Chromatogr. A* 1247 (2012) 125-133.

[167] Y.-Q. Cai, G.-B. Jiang, J.-F. Liu, Q.-X. Zhou, Multi-walled carbon nanotubes packed cartridge for the solid-phase extraction of several phthalate esters from water samples and their determination by high performance liquid chromatography. *Anal. Chim. Acta* 494 (2003) 149-156.

[168] R. Dargahi, H. Ebrahimzadeh, A.A. Asgharinezhad, A. Hashemzadeh, M.M. Amini, Dispersive magnetic solid-phase extraction of phthalate esters from water samples and human plasma based on a nanosorbent composed of MIL-101(Cr) metal-organic framework and magnetite nanoparticles before their determination by GC-MS. *J. Sep. Sci.* 41 (2018) 948-957.

[169] A.L. Capriotti, C. Cavaliere, G. La Barbera, S. Piovesana, R. Samperi, R. Zenezini Chiozzi, A. Laganá, Polydopamine-coated magnetic nanoparticles for isolation and enrichment of estrogenic compounds from surface water samples followed by liquid chromatography-tandem mass spectrometry determination. *Anal. Bioanal. Chem.* 408 (2016) 4011-4020.

[170] A. Gałuszka, Z. Migaszewski, J. Namieśnik, The 12 principles of green analytical chemistry and the SIGNIFICANCE mnemonic of green analytical practices. *TrAC-Trend. Anal. Chem.* 50 (2013) 78-84.

[171] S. Ansari, Combination of molecularly imprinted polymers and carbon nanomaterials as a

versatile biosensing tool in sample analysis: Recent applications and challenges. *TrAC-Trend. Anal. Chem.* 93 (2017) 134-151.

[172] C.W. Klampfl, M. Himmelsbach, Direct ionization methods in mass spectrometry: An overview. *Anal. Chim. Acta* 890 (2015) 44-59.

[173] E.V.S. Maciel, A.L. de Toffoli, F.M. Lanças, Recent trends in sorption-based sample preparation and liquid chromatography techniques for food analysis. *Electrophoresis* 39 (2018) 1582-1596.

[174] X. Wu, J. Wu, S. Wang, R. Wang, Y. Li, T. Wen, D. Zhong, J. Hong, Q. Hu, X. Zhou, An analytical method for estrogens in milk powder by pseudo template imprinted polymer coated fiber coupled with HPLC. *Anal. Methods* 4 (2012) 3300-3306.

[175] H. Lan, D. Pan, Y. Sun, Y. Guo, Z. Wu, Thin metal organic frameworks coatings by cathodic electrodeposition for solid-phase microextraction and analysis of trace exogenous estrogens in milk. *Anal. Chim. Acta* 937 (2016) 53-60.

[176] N.N. Naing, S.F.Y. Li, H.K. Lee, Evaluation of graphene-based sorbent in the determination of polar environmental contaminants in water by micro-solid phase extraction-high performance liquid chromatography. *J. Chromatogr. A* 1427 (2016) 29-36.

[177] E. Özer, B. Osman, T. Yazıcı, Dummy molecularly imprinted microbeads as solid-phase extraction material for selective determination of phthalate esters in water. *J. Chromatogr. A* 1500 (2017) 53-60.

[178] E. Tahmasebi, M.Y. Masoomi, Y. Yamini, A. Morsali, Application of a Zn(II) based metal-organic framework as an efficient solid-phase extraction sorbent for preconcentration of plasticizer compounds. *RSC Adv.* 6 (2016) 40211-40218.

[179] M. Moazzen, A.H. Mahvi, N. Shariatifar, G.J. Khaniki, S. Nazmara, M. Alimohammadi, R. Ahmadkhaniha, N. Rastkari, M. Ahmadloo, A. Akbarzadeh, S. Dobaradaran, A.N. Baghani, Determination of phthalate acid esters (PAEs) in carbonated soft drinks with MSPE/GC-MS method. *Toxin. Rev.* (2018), in press.

[180] J. Ashley, M.-A. Shahbazi, K. Kant, V.A. Chidambara, A. Wolff, D.D. Bang, Y. Sun, Molecularly imprinted polymers for sample preparation and biosensing in food analysis: Progress and perspectives. *Biosens. Bioelectron.* 91 (2017) 606-615.

[181] R. Arshady, K. Mosbach, Synthesis of substrate-selective polymers by host-guest polymerization. *Makromol. Chem.* 182 (1981) 687-692.

[182] B. Sellergren, Direct drug determination by selective sample enrichment on an imprinted polymer. *Anal. Chem.* 66 (1994) 1578-1582.

[183] L.I. Andersson, A. Paprica, T. Arvidsson, A highly selective solid phase extraction sorbent for preconcentration of sameridine made by molecular imprinting. *Chromatographia* 46 (1997) 57-62.

[184] A. Beltran, F. Borrull, P.A.G. Cormack, R.M. Marcé, Molecularly-imprinted polymers: Useful sorbents for selective extractions. *TrAC-Trend. Anal. Chem.* 29 (2010) 1363-1375.

[185] N. Gilart, F. Borrull, N. Fontanals, R.M. Marcé, Selective materials for solid-phase extraction in environmental analysis. *Trend. Environ. Anal. Chem.* 1 (2014) e8-e18.

[186] L. Chen, S. Xu, J. Li, Recent advances in molecular imprinting technology: Current status, challenges and highlighted applications. *Chem. Soc. Rev.* 40 (2011) 2922-2942.

[187] M. Lopez-Nogueroles, S. Lordel-Madeleine, A. Chisvert, A. Salvador, V. Pichon, Development of a selective solid phase extraction method for nitro musk compounds in environmental waters using a molecularly imprinted sorbent. *Talanta* 110 (2013) 128-134.

[188] Y.-P. Duan, C.-M. Dai, Y.-L. Zhang, Ling-Chen, Selective trace enrichment of acidic pharmaceuticals in real water and sediment samples based on solid-phase extraction using multi-templates molecularly imprinted polymers. *Anal. Chim. Acta* 758 (2013) 93-100.

[189] M. Rutkowska, J. Płotka-Wasylka, C. Morrison, P.P. Wieczorek, J. Namieśnik, M. Marć, Application of molecularly imprinted polymers in analytical chiral separations and analysis. *TrAC-Trend. Anal. Chem.* 102 (2018) 91-102.

[190] A. Speltini, A. Scalabrini, F. Maraschi, M. Sturini, A. Profumo, Newest applications of molecularly imprinted polymers for extraction of contaminants from environmental and food matrices: A review. *Anal. Chim. Acta* 974 (2017) 1-26.

[191] D. Moreno-González, F.J. Lara, N. Jurgovská, L. Gámiz-Gracia, A.M. García-Campaña, Determination of aminoglycosides in honey by capillary electrophoresis tandem mass spectrometry and extraction with molecularly imprinted polymers. *Anal. Chim. Acta* 891 (2015) 321-328.

[192] X. Hu, X. Wu, F. Yang, Q. Wang, C. He, S. Liu, Novel surface dummy molecularly imprinted silica as sorbent for solid-phase extraction of bisphenol A from water samples. *Talanta* 148 (2016) 29-36.

[193] W. Lu, W. Ming, X. Zhang, L. Chen, Molecularly imprinted polymers for dispersive solid-phase extraction of phenolic compounds in aqueous samples coupled with capillary electrophoresis. *Electrophoresis* 37 (2016) 2487-2495.

[194] A. Nezhadali, Z. Es'haghi, A. Khatibi, Selective extraction of progesterone hormones from environmental and biological samples using a polypyrrole molecularly imprinted polymer and determination by gas chromatography. *Anal. Methods* 8 (2016) 1813-1827.

[195] J.L. Urraca, J.F. Huertas-Pérez, G.A. Cazorla, J. Gracia-Mora, A.M. García-Campaña, M.C. Moreno-Bondi, Development of magnetic molecularly imprinted polymers for selective extraction: Determination of citrinin in rice samples by liquid chromatography with UV diode array detection. *Anal. Bioanal. Chem.* 408 (2016) 3033-3042.

[196] O.A. Attallah, M.A. Al-Ghobashy, A.T. Ayoub, M. Nebsen, Magnetic molecularly imprinted

polymer nanoparticles for simultaneous extraction and determination of 6-mercaptopurine and its active metabolite thioguanine in human plasma. *J. Chromatogr. A* 1561 (2018) 28-38.

[197] J.-W. Li, Y.-L. Wang, S. Yan, X.-J. Li, S.-Y. Pan, Molecularly imprinted calixarene fiber for solid-phase microextraction of four organophosphorous pesticides in fruits. *Food Chem.* 192 (2016) 260-267.

[198] J.-K. Ma, X.-C. Huang, S.-L. Wei, Preparation and application of chlorpyrifos molecularly imprinted solid-phase microextraction probes for the residual determination of organophosphorous presticides in fresh and dry foods. *J. Sep. Sci.* 41 (2018) 3152-3162.

[199] X. Li, X. Mei, L. Xu, X. Shen, W. Zhu, J. Hong, X. Zhou, Development and application of novel clonazepam molecularly imprinted coatings for stir bar sorptive extraction. *J. Colloid. Interf. Sci.* 468 (2016) 183-191.

[200] J. Peng, D. Liu, T. Shi, H. Tian, X. Hui, H. He, Molecularly imprinted polymers based stir bar sorptive extraction for determination of cefaclor and cefalexin in environmental water. *Anal. Bioanal. Chem.* 409 (2017) 4157-4166.

[201] H.D. de Faria, L.C.D.C. Abrão, M.G. Santos, A.F. Barbosa, E.C. Figueiredo, New advances in restricted access materials for sample preparation: A review. *Anal. Chim. Acta* 959 (2017) 43-65.

[202] H. Lu, S. Xu, Hollow mesoporous structured molecularly imprinted polymers for highly sensitive and selective detection of estrogens from food samples. *J. Chromatogr. A* 1501 (2017) 10-17.

[203] H. Lan, N. Gan, D. Pan, F. Hu, T. Li, N. Long, L. Qiao, An automated solid-phase microextraction method based on magnetic molecularly imprinted polymer as fiber coating for detection of trace estrogens in milk powder. *J. Chromatogr. A* 1331 (2014) 10-18.

[204] X.-P. He, Z.-R. Lian, L.-J. Tan, J.-T. Wang, Preparation and characterization of magnetic molecularly imprinted polymers for selective trace extraction of dienestrol in seawater. *J. Chromatogr. A* 1469 (2016) 8-16.

[205] P. Lucci, D. Derrien, F. Alix, C. Pérollier, S. Bayoudh, Molecularly imprinted polymer solid-phase extraction for detection of zearalenone in cereal sample extracts. *Anal. Chim. Acta* 672 (2010) 15-19.

[206] J. Qiao, M. Wang, H. Yan, G. Yang, Dispersive solid-phase extraction based on magnetic dummy molecularly imprinted microspheres for selective screening of phthalates in plastic bottled beverages. *J. Agric. Food Chem.* 62 (2014) 2782-2789.

[207] J. Li, Q. Su, K.-Y. Li, C.-F. Sun, W.-B. Zhang, Rapid analysis of phthalates in beverage and alcoholic samples by multi-walled carbon nanotubes/silica reinforced hollow fibre-solid phase microextraction. *Food Chem.* 141 (2013) 3714-3720.

[208] X. Wang, Y. Wang, Y. Qin, L. Ding, Y. Chen, F. Xie, Sensitive and selective determination of polycyclic aromatic hydrocarbons in mainstream cigarette smoke using a graphene-coated solid-phase microextraction fiber prior to GC/MS. *Talanta* 140 (2015) 102-108.

[209] M. Fischnaller, R. Bakry, R.M. Vallant, L.A. Huber, G.K. Bonn, C_{60}-fullerene bound silica for the preconcentration and the fractionation of multiphosphorylated peptides. *Anal. Chim. Acta* 761 (2013) 92-101.

[210] C.-C. Hsieh, J.Y. Guo, S.-U. Hung, R. Chen, Z. Nie, H.-C. Chang, C.-C. Wu, Quantitative analysis of oligosaccharides derived from sulfated glycosaminoglycans by nanodiamond-based affinity purification and matrix-assisted laser desorption/ionization mass spectrometry. *Anal. Chem.* 85 (2013) 4342-4349.

[211] M. Roldán-Pijuán, R. Lucena, S. Cárdenas, M. Valcárcel, Micro-solid phase extraction based on oxidized single-walled carbon nanohorns immobilized on a stir borosilicate disk: Application to the preconcentration of the endocrine disruptor benzophenone-3. *Microchem. J.* 115 (2014) 87-94.

[212] B. Socas-Rodríguez, J. González-Sálamo, J. Hernández-Borges, *Carbon nanomaterials in sample preparation. Carbon-based nanomaterials in Analytical Chemistry*. C.D. García, A.G. Crevillén, A. Escarpa, (Ed.). The Royal Society of Chemistry, 2019, p. 37-68.

[213] S. Iijima, Helical microtubules of graphitic carbon. *Nature* 354 (1991) 56-58.

[214] M. Keidar, Factors affecting synthesis of single wall carbon nanotubes in arc discharge. *J. Phys. D: Appl. Phys.* 40 (2007) 2388-2393.

[215] Y. Kobayashi, H. Nakashima, D. Takagi, Y. Homma, CVD growth of single-walled carbon nanotubes using size-controlled nanoparticle catalyst. *Thin Solid Films* 464-465 (2004) 286-289.

[216] M. Kusaba, Y. Tsunawaki, Production of single-wall carbon nanotubes by a XeCl excimer laser ablation. *Thin Solid Films* 506-507 (2006) 255-258.

[217] L.M. Ravelo-Pérez, A.V. Herrera-Herrera, J. Hernández-Borges, M.Á. Rodríguez-Delgado, Carbon nanotubes: Solid-phase extraction. *J. Chromatogr. A* 1217 (2010) 2618-2641.

[218] A.V. Herrera-Herrera, M.Á. González-Curbelo, J. Hernández-Borges, M.Á. Rodríguez-Delgado, A.V. Herrera-Herrera, Carbon nanotubes applications in separation science: A review. *Anal. Chim. Acta* 734 (2012) 1-30.

[219] I.V. Zaporotskova, N.P. Boroznina, Y.N. Parkhomenko, L.V. Kozhitov, Carbon nanotubes: Sensor properties. A review. *Modern Electronic Materials* 2 (2016) 95-105.

[220] E. Celik, H. Park, H. Choi, H. Choi, Carbon nanotube blended polyethersulfone membranes for fouling control in water treatment. *Water Res.* 45 (2011) 274-282.

[221] X.-S. Li, J.-H. Wu, L.-D. Xu, Q. Zhao, Y.-B. Luo, B.-F. Yuan, Y.-Q. Feng, A magnetite/oxidized

carbon nanotube composite used as an adsorbent and a matrix of MALDI-TOF-MS for the determination of benzo[a]pyrene. *Chem. Commun.* 47 (2011) 9816-9818.

[222] Y.-K. Kim, H.-K. Na, S.-J. Kwack, S.-R. Ryoo, Y. Lee, S. Hong, S. Hong, Y. Jeong, D.-H. Min, Synergistic effect of graphene oxide/MWCNT films in laser desorption/ionization mass spectrometry of small molecules and tissue imaging. *ACS Nano* 5 (2011) 4550-4561.

[223] S.G. Ozcan, N. Satiroglu, M. Soylak, Column solid phase extraction of iron(III), copper(II), manganese(II) and lead(II) ions food and water samples on multi-walled carbon nanotubes. *Food Chem. Toxicol.* 48 (2010) 2401-2406.

[224] Z.-G. Yu, W.-M. Ding, Y.-H. Chen, J. You, Y.-Y. Liu, H. Wang, Z.-D. Yang, Oxidized multi-walled carbon nanotubes as SPE sorbent for determination of mefenacet and its degradation products residues in water samples by RRLC-UV-ESI-MS. *Chromatographia* 72 (2010) 33-38.

[225] G. Lasarte-Aragonés, R. Lucena, S. Cárdenas, M. Valcárcel, Effervescence-assisted carbon nanotubes dispersion for the micro-solid-phase extraction of triazine herbicides from environmental waters. *Anal. Bioanal. Chem.* 405 (2013) 3269-3277.

[226] M.Á. González-Curbelo, A.V. Herrera-Herrera, J. Hernández-Borges, M.Á. Rodríguez-Delgado, Analysis of pesticides residues in environmental water samples using multiwalled carbon nanotubes dispersive solid-phase extraction. *J. Sep. Sci.* 36 (2013) 556-563.

[227] D. Huang, C. Fu, Z. Li, C. Deng, Development of magnetic multiwalled carbon nanotubes as solid-phase extraction technique for the determination of p-hydroxybenzoates in beverage. *J. Sep. Sci.* 35 (2012) 1667-1674.

[228] Q. Zhao, F. Wei, Y.-B. Luo, J. Ding, N. Xiao, Y.-Q. Feng, Rapid magnetic solid-phase extraction based on magnetic multiwalled carbon nanotubes for the determination of polycyclic aromatic hydrocarbons in edible oils. *J. Agric. Food Chem.* 59 (2011) 12794-12800.

[229] A. Sarafraz-Yazdi, M.S. Ardaki, A. Amiri, Determination of monocyclic aromatic amines using headspace solid-phase microextraction based on sol–gel technique prior to GC. *J. Sep. Sci.* 36 (2013) 1629-1635.

[230] F. Wu, W. Lu, J. Chen, W. Liu, L. Zhang, Single-walled carbon nanotubes coated fibers for solid-phase microextraction and gas chromatography–mass spectrometric determination of pesticides in tea samples. *Talanta* 82 (2010) 1038-1043.

[231] Z. Es'haghi, H.A. Hoseini, S. Mohammadi-Nokhandani, J. Ebrahimi, Pseudo-stir bar hollow fiber solid/liquid phase microextraction combined with anodic stripping voltammetry for determination of lead and cadmium in water samples. *J. Adv. Res.* 5 (2014) 685-693.

[232] S.-X. Guan, Z.-G. Yu, H.-N. Yu, C.-H. Song, Z.-Q. Song, Z. Qin, Multi-walled carbon nanotubes as matrix solid-phase dispersion extraction adsorbent for simultaneous analysis of residues

of nine organophosphorus pesticides in fruit and vegetables by rapid resolution LC-MS-MS. *Chromatographia* 73 (2011) 33-41.

[233] B. Socas-Rodríguez, A.V. Herrera-Herrera, M. Asension-Ramos, J. Hernández-Borges, Recent applications of carbon nanotube sorbents in analytical chemistry. *J. Chromatogr. A* 1357 (2014) 110-146.

[234] L. Meng, C. Fu, Q. Lu, Advanced technology for functionalization of carbon nanotubes. *Prog. Nat. Sci.* 19 (2009) 801-810.

[235] C.-Y. Hu, Y.-J. Xu, S.-W. Duo, R.-F. Zhang, M.-S. Li, Non-covalent functionalization of carbon nanotubes wtih surfactants and polymers. *J. Chin. Chem. Soc.* 56 (2009) 234-239.

[236] H. Zhu, W. Chen, Z. Li, J. He, X. Tang, C. Wang, Extraction of natural estrogens in environmental waters by dispersive multiwalled carbon nanotube-based agitation-assisted adsorption and ultrasound-assisted desorption. *Anal. Methods* 6 (2014) 1235-1241.

[237] J. Ding, Q. Gao, X.-S. Li, W. Huang, Z.-G. Shi, Y.-Q. Feng, Magnetic solid-phase extraction based on magnetic carbon nanotube for the determination of estrogens in milk. *J. Sep. Sci.* 34 (2011) 2498-2504.

[238] Z. Han, K. Jiang, Z. Fan, J.D. Di Mavungu, M. Dong, W. Guo, K. Fan, K. Campbell, Z. Zhao, Y. Wu, Multi-walled carbon nanotubes-based magnetic solid-phase extraction for the determination of zearalenone and its derivatives in maize by ultra-high performance liquid chromatography-tandem mass spectrometry. *Food Control* 79 (2017) 177-184.

[239] X.-L. Song, Y. Chen, J.-P. Yuan, Y.-J. Qin, R.-S. Zhao, X. Wang, Carbon nanotube composite microspheres as a highly efficient solid-phase microextraction coating for sensitive determination of phthalate acid esters in water samples. *J. Chromatogr. A* 1468 (2016) 17-22.

[240] M.Z. Jeddi, R. Ahmadkhaniha, M. Yunesian, N. Rastkari, Magnetic solid-phase extraction based on modified magnetic nanoparticles for the determination of phthalate diesters in water samples. *J. Chromatogr. Sci.* 53 (2015) 385-391.

[241] V.F. Cheong, P.Y. Moh, Recent advancement in metal-organic framework: Synthesis, activation, functionalisation, and bulk production. *Mater. Sci. Tech.* 34 (2018), in press.

[242] P. Rocío-Bautista, P. González-Hernández, V. Pino, J. Pasán, A.M. Afonso, Metal-organic frameworks as novel sorbents in dispersive-based microextraction approaches. *TrAC-Trend. Anal. Chem.* 90 (2017) 114-134.

[243] F. Maya, C.P. Cabello, R.M. Frizzarin, J.M. Estela, G.T. Palomino, V. Cerdà, Magnetic solid-phase extraction using metal-organic frameworks (MOFs) and their derived carbons. *TrAC-Trend. Anal. Chem.* 90 (2017) 142-152.

[244] Y. Wang, M. Rui, G. Lu, Recent applications of metal-organic frameworks in sample pretreatment. *J. Sep. Sci.* 41 (2018) 180-194.

[245] X. Fang, B. Zong, S. Mao, Metal-organic framework-based sensors for environmental contaminant sensing. *Nano-Micro Lett.* 10 (2018) 64-83.

[246] J. Zhang, Z. Chen, Metal-organic frameworks as stationary phase for application in chromatographic separation. *J. Chromatogr. A* 1530 (2017) 1-18.

[247] P. Rocío-Bautista, C. Martínez-Benito, V. Pino, J. Pasán, J.H. Ayala, C. Ruiz-Pérez, A.M. Afonso, The metal-organic framework HKUST-1 as efficient sorbent in a vortex-assisted dispersive micro solid-phase extraction of parabens from environmental waters, cosmetic creams, and human urine. *Talanta* 139 (2015) 13-20.

[248] N. Li, L. Wu, L. Nian, Y. Song, L. Lei, X. Yang, K. Wang, Z. Wang, L. Zhang, H. Zhang, A. Yu, Z. Zhang, Dynamic microwave assisted extraction coupled with dispersive micro-solid-phase extraction of herbicides in soybeans. *Talanta* 142 (2015) 43-50.

[249] S.-H. Huo, X.-P. Yan, Facile magnetization of metal-organic framework MIL-101 for magnetic solid-phase extraction of polycyclic aromatic hydrocarbons in environmental water samples. *Analyst* 137 (2012) 3445-3451.

[250] X. Liu, C. Wang, Q. Wu, Z. Wang, Metal-organic framework-templated synthesis of magnetic nanoporous carbon as an efficient absorbent for enrichment of phenylurea herbicides. *Anal. Chim. Acta* 870 (2015) 67-74.

[251] S. Lin, N. Gan, J. Zhang, L. Qiao, Y. Chen, Y. Cao, Aptamer-functionalized stir bar sorptive extraction coupled with gas chromatography–mass spectrometry for selective enrichment and determination of polychlorinated biphenyls in fish samples. *Talanta* 149 (2016) 266-274.

[252] S. Lin, N. Gan, L. Qiao, J. Zhang, Y. Cao, Y. Chen, Magnetic metal-organic frameworks coated stir bar sorptive extraction coupled with GC–MS for determination of polychlorinated biphenyls in fish samples. *Talanta* 144 (2015) 1139-1145.

[253] R. dos A. de Jesus, L.F.S. Santos, S. Navickiene, M.E. de Mesquita, Evaluation of metal-organic framework as low-cost adsorbent material in the determination of pesticide residues in soursop exotic fruit (*Annona muricata*) by liquid chromatography. *Food Anal. Method.* 8 (2015) 446-451.

[254] C. Hu, M. He, B. Chen, C. Zhong, B. Hu, Polydimethylsiloxane/metal-organic frameworks coated stir bar sorptive extraction coupled to high performance liquid chromatography-ultraviolet detector for the determination of estrogens in environmental water samples. *J. Chromatogr. A* 1310 (2013) 21-30.

[255] T. Wang, J. Wang, C. Zhang, Z. Yang, X. Dai, M. Cheng, X. Hou, Metal-organic framework MIL-101(Cr) as a sorbent of porous membrane-protected micro-solid-phase extraction for the analysis of six phthalate esters from drinking water: A combination of experimental and computational study. *Analyst* 140 (2015) 5308-5316.

[256] X.-S. Li, G.-T. Zhu, Y.-B. Luo, B.-F. Yuan, Y.-Q. Feng, Synthesis and applications of

functionalized magnetic materials in sample preparation. *TrAC-Trend. Anal. Chem.* 45 (2013) 233-247.

[257] Á. Ríos, M. Zougagh, Recent advances in magnetic nanomaterials for improving analytical processes. *TrAC-Trend. Anal. Chem.* 84 (2016) 72-83.

[258] U. Jeong, X. Teng, Y. Wang, H. Yang, Y. Xia, Superparamagnetic colloids: Controlled synthesis and niche applications. *Adv. Mater.* 19 (2007) 33-60.

[259] J. Tian, J. Xu, F. Zhu, T. Lu, C. Su, G. Ouyang, Application of nanomaterials in sample preparation. *J. Chromatogr. A* 1300 (2013) 2-16.

[260] G. Giakisikli, A.N. Anthemidis, Magnetic materials as sorbents for metal/metalloid preconcentration and/or separation. A review. *Anal. Chim. Acta* 789 (2013) 1-16.

[261] J. He, M. Huang, D. Wang, Z. Zhang, G. Li, Magnetic separation techniques in sample preparation for biological analysis: A review. *J. Pharmaceut. Biomed. Anal.* 101 (2014) 84-101.

[262] A.-H. Lu, E.L. Salabas, F. Schüth, Magnetic nanoparticles: Synthesis, protection, functionalization, and application. *Angew. Chem. Int. Ed.* 46 (2007) 1222-1244.

[263] L. Xie, R. Jiang, F. Zhu, H. Liu, G. Ouyang, Application of functionalized magnetic nanoparticles in samples preparation. *Anal. Bioanal. Chem.* 406 (2014) 377-399.

[264] C. McCullum, P. Tchounwou, L.-S. Ding, X. Liao, Y.-M. Liu, Extraction of aflatoxins from liquid foodstuff samples with polydopamine-coated superparamagnetic nanoparticles for HPLC-MS/MS analysis. *J. Agric. Food Chem.* 62 (2014) 4261-4267.

[265] Q. Zhao, Q. Lu, Y.-Q. Feng, Dispersive microextraction based on magnetic polypyrrole nanowires for the fast determination of pesticide residues in beverage and environmental water samples. *Anal. Bioanal. Chem.* 405 (2013) 4765-4776.

[266] J. Chen, X. Zhu, Magnetic solid phase extraction using ionic liquid-coated core–shell magnetic nanoparticles followed by high-performance liquid chromatography for determination of Rhodamine B in food samples. *Food Chem.* 200 (2016) 10-15.

[267] M. Bouri, M. Gurau, R. Salghi, I. Cretescu, M. Zougagh, Á. Rios, Ionic liquids supported on magnetic nanoparticles as a sorbent preconcentration material for sulfonylurea herbicides prior to their determination by capillary liquid chromatography. *Anal. Bioanal. Chem.* 404 (2012) 1529-1538.

[268] S.R. Yazdinezhad, A. Ballesteros-Gómez, L. Lunar, S. Rubio, Single-step extraction and cleanup of bisphenol A in soft drinks by hemimicellar magnetic solid phase extraction prior to liquid chromatography/tandem mass spectrometry. *Anal. Chim. Acta* 778 (2013) 31-37.

[269] A. Beiraghi, K. Pourghazi, M. Amoli-Diva, A. Razmara, Magnetic solid phase extraction of mefenamic acid from biological samples based on the formation of mixed hemimicelle

aggregates on Fe_3O_4 nanoparticles prior to its HPLC-UV detection. *J. Chromatogr. B* 945-946 (2014) 46-52.

[270] A.A. Asgharinezhad, H. Ebrahimzadeh, M. Rezvani, N. Shekari, M. Loni, A novel 4-(2-pyridylazo) resorcinol functionalised magnetic nanosorbent for selective extraction of Cu(II) and Pb(II) ions from food and water samples. *Food Addit. Contam.* 31 (2014) 1196-1204.

[271] M. Karimi, F. Aboufazeli, H.R.L.Z. Zhad, O. Sadeghi, E. Najafi, Determination of sulfonamides in chicken meat by magnetic molecularly imprinted polymer coupled to HPLC-UV. *Food Anal. Method.* 7 (2014) 73-80.

[272] Y.-K. Lv, C.-X. Zhao, P. Li, Y.-D. He, Z.-R. Yang, H.-W. Sun, Preparation of doxycycline-imprinted magnetic microspheres by inverse-emulsion suspension polymerization for magnetic dispersion extraction of tetracyclines from milk samples. *J. Sep. Sci.* 36 (2013) 2656-2663.

[273] M.R. Sohrabi, Z. Matbouie, A.A. Asgharinezhad, A. Dehghani, Solid phase extraction of Cd(II) and Pb(II) using a magnetic metal-organic framework, and their determination by FAAS. *Microchim. Acta* 180 (2013) 589-597.

[274] G.D. Tarigh, F. Shemirani, Simultaneous in situ derivatization and ultrasound-assisted dispersive magnetic solid phase extraction for thiamine determination by spectrofluorimetry. *Talanta* 123 (2014) 71-77.

[275] N. Rastkari, R. Ahmadkhaniha, Magnetic solid-phase extraction based on magnetic multi-walled carbon nanotubes for the determination of phthalate monoesters in urine samples. *J. Chromatogr. A* 1286 (2013) 22-28.

[276] B. Socas-Rodríguez, J. Hernández-Borges, P. Salazar, M. Martín, M.Á. Rodríguez-Delgado, Core–shell polydopamine magnetic nanoparticles as sorbent in micro-dispersive solid-phase extraction for the determination of estrogenic compounds in water samples prior to high-performance liquid chromatography–mass spectrometry analysis. *J. Chromatogr. A* 1397 (2015) 1-10.

[277] J. Wang, Z. Chen, Z. Li, Y. Yang, Magnetic nanoparticles based dispersive micro-solid-phase extraction as a novel technique for the determination of estrogens in pork samples. *Food Chem.* 204 (2016) 135-140.

[278] S. Wang, R. Wang, X. Wu, Y. Wang, C. Xue, J. Wu, J. Hong, J. Liu, X. Zhou, Magnetic molecularly imprinted nanoparticles based on dendritic-grafting modification for determination of estrogens in plasma samples. *J. Chromatogr. B* 905 (2012) 105-112.

[279] A.L. Capriotti, C. Cavaliere, P. Foglia, G. La Barbera, R. Samperi, S. Ventura, A. Laganà, Mycoestrogen determination in cow milk: Magnetic solid-phase extraction followed by liquid chromatography and tandem mass spectrometry analysis. *J. Sep. Sci.* 39 (2016) 4794-4804.

[280] H. Zhao, M. Huang, J. Wu, L. Wang, H. He, Preparation of Fe_3O_4@PPy magnetic nanoparticles as solid-phase extraction sorbents for preconcentration and separation of phthalic acid esters in water by gas chromatography-mass spectrometry. *J. Chromatogr. B* 1011 (2016) 33-44.

[281] X. Zhao, S. Liu, P. Wang, Z. Tang, H. Niu, Y. Cai, F. Wu, H. Wang, W. Meng, J.P. Giesy, Surfactant-modified flowerlike layered double hydroxide-coated magnetic nanoparticles for preconcentration of phthalate esters from environmental water samples. *J. Chromatogr. A* 1414 (2015) 22-30.

[282] F. Makkliang, P. Kanatharana, P. Thavarungkul, C. Thammakhet, Development of magnetic micro-solid phase extraction for analysis of phthalate esters in packaged food. *Food Chem.* 166 (2015) 275-282.

[283] S.-L. Wei, W.-T. Liu, X.-C. Huang, J.-K. Ma, Preparation and application of a magnetic plasticizer as a molecularly imprinted polymer adsorbing material for the determination of phthalic acid esters in aqueous samples. *J. Sep. Sci.* (2018), in press.

[284] J.M. Kokosa, A. Przyjazny, M.A. Jeannot, *Solvent microextraction: Theory and practice*. New Jersey, John Wiley & Sons, 2009.

[285] F. Pena-Pereira, *Miniaturization in sample preparation*. F. Pena-Pereira, (Ed.). De Gruyter, 2014.

[286] J.L. Anderson, A. Berthod, V. Pino, A.M. Stalcup, (Ed.). *Analytical separation science. Sample preparation, method validation, and analytical applications*. Wiley-VCH Verlag, 2015.

[287] A. Gutiérrez-Serpa, P.I. Napolitano-Tabares, V. Pino, F. Jiménez-Moreno, A.I. Jiménez-Abizanda, Silver nanoparticles supported onto a stainless steel wire for direct-immersion solid-phase microextraction of polycyclic aromatic hydrocarbons prior to their determination by GC-FID. *Microchim. Acta* 185 (2018) 341-351.

[288] W. Zhang, Z. Zhang, J. Zhang, J. Meng, T. Bao, Z. Chen, Covalent immobilization of graphene onto stainless steel wire for jacket-free stir bar sorptive extraction. *J. Chromatogr. A* 1351 (2014) 12-20.

[289] M. Mei, X. Huang, Determination of fluoroquinolones in environmental water and milk samples treated with stir cake sorptive extraction based on a boron-rich monolith. *J. Sep. Sci.* 39 (2016) 1908-1918.

[290] P. Richter, A. Cañas, C. Muñoz, C. Leiva, I. Ahumada, Rotating disk sorbent extraction for pre-concentration of chromogenic organic compounds and direct determination by solid phase spectrophotometry. *Anal. Chim. Acta* 695 (2011) 73-76.

[291] Y.-B. Luo, Q. Ma, Y.-Q. Feng, Stir rod sorptive extraction with monolithic polymer as coating and its application to the analysis of fluoroquinolones in honey sample. *J. Chromatogr. A* 1217 (2010) 3583-3589.

[292] R.A. Teixeira, D.H.Â. Flores, R.C.S. da Silva, F.V.A. Dutra, K.B. Borges, Pipette-tip solid-phase extraction using poly(1-vinylimidazole-co-trimethylolpropane trimethacrylate) as a new molecularly imprinted polymer in the determination of avermectins and milbemycins in fruit juice and water samples. *Food Chem.* 262 (2018) 86-93.

[293] X. Du, S. Lin, N. Gan, X. Chen, Y. Cao, T. Li, P. Zhan, Multi-walled carbon nanotube modified dummy-template magnetic molecularly imprinted microspheres as solid-phase extraction material for the determination of polychlorinated biphenyls in fish. *J. Sep. Sci.* 37 (2014) 1591-1600.

[294] J. Płotka-Wasylka, N. Szczepańska, M. de la Guardia, J. Namieśnik, Miniaturized solid-phase extraction techniques. *TrAC-Trend. Anal. Chem.* 73 (2015) 19-38.

[295] A.K. Burnham, G.V. Calder, J.S. Fritz, G.A. Junk, H.J. Svec, R. Willis, Identification and estimation of neutral organic contaminants in potable water. *Anal. Chem.* 44 (1972) 139-142.

[296] A. Andrade-Eiroa, M. Canle, V. Leroy-Cancellieri, V. Cerdà, Solid-phase extraction of organic compounds: A critical review (Part I). *TrAC-Trend. Anal. Chem.* 80 (2016) 641-654.

[297] B. Hashemi, P. Zohrabi, M. Shamsipur, Recent developments and applications of different sorbents for SPE and SPME from biological samples. *Talanta* 187 (2018) 337-347.

[298] C. Kim, H.-D. Ryu, E.G. Chung, Y. Kim, J.-K. Lee, A review of analytical procedures for the simultaneous determination of medically important veterinary antibiotics in environmental water: Sample preparation, liquid chromatography, and mass spectrometry. *J. Environ. Manage.* 217 (2018) 629-645.

[299] A. Andrade-Eiroa, M. Canle, V. Leroy-Cancellieri, V. Cerdà, Solid-phase extraction of organic compounds: A critical review. Part ii. *TrAC-Trend. Anal. Chem.* 80 (2016) 655-667.

[300] C. Yu, Y. Li, Q. Zhang, N. Zou, K. Gu, X. Li, C. Pan, Decrease of pirimiphos-methyl and deltamethrin residues in stores rice with post-harvest treatment. *Int. J. Environ. Res. Public Health* 11 (2014) 5372-5381.

[301] Y. Qin, P. Zhao, S. Fan, Y. Han, Y. Li, N. Zou, S. Song, Y. Zhang, F. Li, X. Li, C. Pan, The comparison of dispersive solid phase extraction and multi-plug filtration cleanup method based on multi-walled carbon nanotubes for pesticides multi-residue analysis by liquid chromatography tandem mass spectrometry. *J. Chromatogr. A* 1385 (2015) 1-11.

[302] B. Socas-Rodríguez, A.V. Herrera-Herrera, M. Asensio-Ramos, J. Hernández-Borges, *Dispersive solid-phase extraction. Analytical separation science. Sample preparation, method validation, and analytical applications.* J.L. Anderson, A. Berthod, V. Pino, A.M. Stalcup, (Ed.). Weinheim, Germany, Wiley-VCH Verlag, 2015, p. 1525-1569.

[303] N. Ozbek, A. Baysal, S. Akman, M. Dogan, *Solid-phase extraction. Analytical separation science. Sample preparation, method validation, and analytical applications.* J.L. Anderson, A. Berthod, V. Pino, A.M. Stalcup, (Ed.). Weinheim, Germany, Wiley-VCH Verlag, 2015, p.

1571-1594.

[304] M. Anastassiades, S.J. Lehotay, D. Štajnbaher, F.J. Schenck, Fast and easy multiresidue method employing acetonitrile extraction/partitioning and "dispersive solid-phase extraction" for the determination of pesticide residues in produce. *J. AOAC Int.* 86 (2003) 412-431.

[305] M.Á. González-Curbelo, B. Socas-Rodríguez, A.V. Herrera-Herrera, J. González-Sálamo, J. Hernández-Borges, M.Á. Rodríguez-Delgado, Evolution and applications of the QuEChERS method. *TrAC-Trend. Anal. Chem.* 71 (2015) 169-185.

[306] T. Khezeli, A. Daneshfar, Development of dispersive micro-solid phase extraction based on micro and nano sorbents. *TrAC-Trend. Anal. Chem.* 89 (2017) 99-118.

[307] X. Xu, N. Long, J. Lv, L. Wang, M. Zhang, X. Qi, L. Zhang, Functionalized multiwalled carbon nanotube as dispersive solid-phase extraction materials combined with high-performance liquid chromatography for thiabendazole analysis in environmental and food samples. *Food Anal. Method.* 9 (2016) 30-37.

[308] L. Yu, P. Li, Q. Zhang, W. Zhang, X. Ding, X. Wang, Graphene oxide: An adsorbent for the extraction and quantification of aflatoxins in peanuts by high-performance liquid chromatography. *J. Chromatogr. A* 1318 (2013) 27-34.

[309] S. Hassanpoor, G. Khayatian, A.R.J. Azar, Ultra-trace determination of arsenic species in environmental waters, food and biological samples using a modified aluminum oxide nanoparticle sorbent and AAS detection after multivariate optimization. *Microchim. Acta* 182 (2015) 1957-1965.

[310] Y. Wang, Y. Wu, H. Ge, H. Chen, G. Ye, X. Hu, Fabrication of metal-organic frameworks and graphite oxide hybrid composites for solid-phase extraction and preconcentration of luteolin. *Talanta* 122 (2014) 91-96.

[311] E.M. Reyes-Gallardo, R. Lucena, S. Cárdenas, Silica nanoparticles-nylon 6 composites: Synthesis, characterization and potential use as sorbent. *RSC Adv.* 7 (2017) 2308-2314.

[312] L. Cheng, S. Pan, C. Ding, J. He, C. Wang, Dispersive solid-phase microextraction with graphene oxide based molecularly imprinted polymers for determining bis(2-ethylhexyl) phthalate in environmental water. *J. Chromatogr. A* 1511 (2017) 85-91.

[313] M.R. Ganjali, M. Rezapour, P. Norouzi, F. Faridbod, *Liquid-phase microextraction. Analytical separation science. Sample preparation, method validation, and analytical applications.* J.L. Anderson, A. Berthod, V. Pino, A.M. Stalcup, (Ed.). Weinheim, Germany, Wiley-VCH Verlag, 2015, p. 1625-1657.

[314] M. Asensio-Ramos, L.M. Ravelo-Pérez, M.Á. González-Curbelo, J. Hernández-Borges, Liquid phase microextraction applications in food analysis. *J. Chromatogr. A* 1218 (2011) 7415-7437.

[315] M.A. Jeannot, A. Przyjazny, J.M. Kokosa, Single drop microextraction–Development, applications and future trends. *J. Chromatogr. A* 1217 (2010) 2326-2336.

[316] S. Pedersen-Bjergaard, K.E. Rasmussen, Liquid-phase microextraction with porous hollow fibers, a miniaturized and highly flexible format for liquid–liquid extraction. *J. Chromatogr. A* 1184 (2008) 132-142.

[317] M. Rezaee, Y. Yamini, M. Faraji, Evolution of dispersive liquid–liquid microextraction method. *J. Chromatogr. A* 1217 (2010) 2342-2357.

[318] M.A. Jeannot, F.F. Cantwell, Solvent microextraction into a single drop. *Anal. Chem.* 68 (1996) 2236-2240.

[319] N.S. Pano-Farias, S.G. Ceballos-Magaña, R. Muñiz-Valencia, J.M. Jurado, Á. Alcázar, I.A. Aguayo-Villareal, Direct immersion single drop micro-extraction method for multi-class pesticides analysis in mango using GC–MS. *Food Chem.* 237 (2017) 30-38.

[320] E.G. Amvrazi, M.A. Martini, N.G. Tsiropoulos, Headspace sigle-drop microextraction of common pesticide contaminants in honey-method develpment and comparison with other extraction methods. *Int. J. Environ. Anal. Chem.* 92 (2012) 450-465.

[321] D. Liu, S. Min, H. Ping, X. Song, The application of directly suspended droplet microextraction for the evaluation of phthalic acid esters in cow's milk by gas chromatography mass spectrometry. *J. Chromatogr. A* 1443 (2016) 66-74.

[322] M. Ma, F.F. Cantwell, Solvent microextraction with simultaneous back-extraction for sample cleanup and preconcentration: Preconcentration into a single microdrop. *Anal. Chem.* 71 (1999) 388-393.

[323] Y. Li, L. Zhang, L. Wu, S. Sun, H. Shan, Z. Wang, Purification and enrichment of polycyclic aromatic hydrocarbons in environmental water samples by column clean-up coupled with continuous flow single drop microextraction. *J. Chromatogr. A* 1567 (2018) 81-89.

[324] M. Zhang, J. Huang, J. Zeng, J. Yu, Ultrasonic-assisted drop-to-drop solvent microextraction in a capillary tube coupled with GC-FID for trace analysis of phthalate esters. *J. Chromatogr. Sci.* 52 (2014) 739-744.

[325] M.R.K. Zanjani, Y. Yamini, S. Shariati, J.Å. Jönsson, A new liquid-phase microextraction method based on solidification of floating organic drop. *Anal. Chim. Acta* 585 (2007) 286-293.

[326] M.J. George, Application of the mixed-solvent BID-SDME technique for determination of some stilbene hormones in water downstream of a cattle slaughterhouse, using gas chromatography and mass spectrometry. *Int. J. Environ. Anal. Chem.* 96 (2016) 247-256.

[327] D.B.G. Williams, M.J. George, R. Meyer, L. Marjanovic, Bubbles in solvent microextraction: The influence of intentionally introduced bubbles on extraction efficiency. *Anal. Chem.* 83 (2011) 6713-6716.

[328] Y. Jiang, X. Zhang, T. Tang, T. Zhou, G. Shi, Determination of endocrine disruptors in environmental water by single-drop microextraction and high-performance liquid chromatography. *Anal. Lett.* 48 (2015) 710-725.

[329] X. Xiao, Y. Yin, Y. Hu, G. Li, Microwave-assisted extraction coupled with single drop microextraction and high-performance column liquid chromatography for the determination of trace estrogen adulterants in soybean isoflavone dietary supplements. *J. AOAC Int.* 93 (2010) 849-854.

[330] M.J. George, L. Marjanovic, D.B.G. Williams, Picogram-level quantification of some growth hormones in bovine urine using mixed-solvent bubble-in-drop single drop micro-extraction. *Talanta* 144 (2015) 445-450.

[331] R. Batlle, C. Nerín, Application of single-drop microextraction to the determination of dialkyl phthalate esters in food simulants. *J. Chromatogr. A* 1045 (2004) 29-35.

[332] S. Pedersen-Bjergaard, K.E. Rasmussen, Liquid−liquid−liquid microextraction for sample preparation of biological fluids prior to capillary electrophoresis. *Anal. Chem.* 71 (1999) 2650-2656.

[333] M. Liu, B. Qiu, X. Jin, L. Zhang, X. Chen, G. Chen, Determination of estrogens in wastewater using three-phase hollow fiber-mediated liquid-phase microextraction followed by HPLC. *J. Sep. Sci.* 31 (2008) 622-628.

[334] S.-P. Huang, S.-D. Huang, Dynamic hollow fiber protected liquid phase microextraction and quantification using gas chromatography combined with electron capture detection of organochlorine pesticides in green tea leaves and ready-to-drink tea. *J. Chromatogr. A* 1135 (2006) 6-11.

[335] H.Y. Kim, S.H. Yoon, T.-Y. Jeong, S. Yu, S.D. Kim, Determination of conjugated estrogens in human urine using carrier-mediated hollow-fiber liquid phase microextraction and LC-MS/MS. *Desalin. Water Treat.* 57 (2016) 16024-16033.

[336] Y.-Y. Chao, T.-Y. Chien, T.-H. Kuo, Y.-A. Lu, Y.-H. Shih, S.-Y. Chen, Y.-L. Hunag, On-line heat-assisted push/pull perfusion hollow fiber liquid–liquid–liquid microextraction for the rapid determination of phthalate esters in river water samples. *Anal. Methods* 5 (2013) 5602-5608.

[337] A. Mtibe, T.A.M. Msagati, A.K. Mishra, B.B. Mamba, Determination of phthalate ester plasticizers in the aquatic environment using hollow fibre supported liquid membranes. *Phys. Chem. Earth* 50-52 (2012) 239-242.

[338] M. Rezaee, Y. Assadi, M.-R.M. Hosseini, E. Aghaee, F. Ahmadi, S. Berijani, Determination of organic compounds in water using dispersive liquid–liquid microextraction. *J. Chromatogr. A* 1116 (2006) 1-9.

[339] P. Wang, X. Qiu, Y. Yang, Determination of estrogens in human urine by vortex-assisted dispersive liquid-liquid microextraction based on floating organic acid droplet combined with high-performance liquid chromatography-fluorescence detection. *J. Liq. Chromatogr.*

Relat. Technol. 38 (2015) 640-646.

[340] H. Farahani, M.R. Ganjali, R. Dinarvand, P. Norouzi, Screening method for phthalate esters in water using liquid-phase microextraction based on the solidification of a floating organic microdrop combined with gas chromatography–mass spectrometry. *Talanta* 76 (2008) 718-723.

[341] N. Wei, Z. Zheng, Y. Wang, Y. Tao, Y. Shao, S. Zhu, J. You, X.-E. Zhao, Rapid and sensitive determination of multiple endocrine-disrupting chemicals by ultrasound-assisted in situ derivatization dispersive liquid–liquid microextraction coupled with ultra-high-performance liquid chromatography/tandem mass spectrometry. *Rapid Commun. Mass Spectrom.* 31 (2017) 937-950.

[342] Y. Rodríguez-Carrasco, J.C. Moltó, J. Mañes, H. Berrada, Development of microextraction techniques in combination with GC-MS/MS for the determination of mycotoxins and metabolites in human urine. *J. Sep. Sci.* 40 (2017) 1572-1582.

[343] K. Ebrahim, P. Poursafa, M.M. Amin, Development of a simple and valid method for the trace determination of phthalate esters in human plasma using dispersive liquid-liquid microextraction coupled with gas chromatography-mass spectrometry. *J. Sep. Sci.* 40 (2017) 4403-4410.

[344] G. Montevecchi, F. Masino, L. Zanasi, A. Antonelli, Determination of phthalate esters in distillates by ultrasound-vortex-assisted dispersive liquid-liquid micro-extraction (USVADLLME) coupled with gas chromatography/mass spectrometry. *Food Chem.* 221 (2017) 1354-1360.

[345] P. Viñas, N. Campillo, M. Pastor-Belda, A. Oller, M. Hernández-Córdoba, Determination of phthalate esters in cleaning and personal care products by dispersive liquid–liquid microextraction and liquid chromatography–tandem mass spectrometry. *J. Chromatogr. A* 1376 (2015) 18-25.

[346] SciFinder® database. Available at: https://bit.ly/1dXYcMZ. Accessed July 2018.

[347] PubChem webpage. Available at: https://bit.ly/1t5jyUe. Accessed July 2018.

[348] Y. Shi, D.-D. Peng, C.-H. Shi, X. Zhang, Y.-T. Xie, B. Lu, Selective determination of trace 17β-estradiol in dairy and meat samples by molecularly imprinted solid-phase extraction and HPLC. *Food Chem.* 126 (2011) 1916-1925.

[349] D.W. Kolpin, J. Schenzel, M.T. Meyer, P.J. Phillips, L.E. Hubbard, T.-M. Scott, T.D. Bucheli, Mycotoxins: Diffuse and point source contributions of natural contaminants of emerging concern to streams. *Sci. Total Environ.* 470-471 (2014) 669-676.

[350] K. Gromadzka, A. Waśkiewicz, P. Goliński, J. Świetlik, Occurrence of estrogenic mycotoxin - Zearalenone in aqueous environmental samples with various NOM content. *Water Res.* 43 (2009) 1051-1059.

[351] A.L. Capriotti, G. Caruso, C. Cavaliere, P. Foglia, R. Samperi, Multiclass mycotoxin analysis in food, environmental and biological matrices with chromatography/mass spectrometry. *Mass Spectrom. Rev.* 31 (2012) 466-503.

[352] Y. Hu, J. Pan, K. Zhang, H. Lian, G. Li, Novel applications of molecularly-imprinted polymers in sample preparation. *TrAC-Trend. Anal. Chem.* 43 (2013) 37-52.

[353] A. Martín-Esteban, Molecularly-imprinted polymers as a versatile, highly selective tool in sample preparation. *TrAC-Trend. Anal. Chem.* 45 (2013) 169-181.

[354] P. Lucci, O. Núñez, M.T. Galcerán, Solid-phase extraction using molecularly imprinted polymer for selective extraction of natural and synthetic estrogens from aqueous samples. *J. Chromatogr. A* 1218 (2011) 4828-4833.

[355] D. Matějíček, A. Grycová, J. Vičeк, The use of molecularly imprinted polymers for the multicomponent determination of endocrine-disrupting compounds in water and sediment. *J. Sep. Sci.* 36 (2013) 1097-1103.

[356] Q.-L. Wang, A.-Z. Zhang, X. Pan, L.-R. Chen, Simultaneous determination of sex hormones in egg products by $ZnCl_2$ depositing lipid, solid-phase extraction and ultra performance liquid chromatography/electrospray ionization tandem mass spectrometry. *Anal. Chim. Acta* 678 (2010) 108-116.

[357] L.K. Sørensen, T.H. Elbæk, Determination of mycotoxins in bovine milk by liquid chromatography tandem mass spectrometry. *J. Chromatogr. B* 820 (2005) 183-196.

[358] M.-M. Kushnir, A.-L. Rockwood, B. Yue, A.W. Meikle, *High sensitivity measurement of estrone and estradiol in serum and plasma using LC-MS/MS. Methods in Molecular Biology*. U. Garg, C. Hammett-Stabler, (Ed.). Springer, 2010, p. 219-228.

[359] J.D. Di Mavungu, S. Monbaliu, M.-L. Scippo, G. Maghuin-Rogister, Y.-J. Schneider, Y. Larondelle, A. Callebaut, J. Robbens, C. Van Peteghem, S. De Saeger, LC-MS/MS multi-analyte method for mycotoxin determination in food supplements. *Food Addit. Contam.* 26 (2009) 885-895.

[360] K. Puzio, B. Claude, L. Amalric, C. Berho, E. Grellet, S. Bayoudh, R. Nehmé, Ph. Morin, Molecularly imprinted polymer dedicated to the extraction of glyphosate in natural waters. *J. Chromatogr. A* 1361 (2014) 1-8.

[361] K. Möller, U. Nilsson, C. Crescenzi, Investigation of matrix effects of urine on a molecularly imprinted solid-phase extraction. *J. Chromatogr. B* 811 (2004) 171-176.

[362] L. Yang, X. Zhao, J. Zhou, Selective enrichment and determination of nicosulfuron in water and soil by a stir bar based on molecularly imprinted polymer coatings. *Anal. Chim. Acta* 670 (2010) 72-77.

[363] A. Zinedine, J.M. Soriano, J.C. Moltó, J. Mañes, Review on the toxicity, occurrence,

metabolism, detoxification, regulations and intake of zearalenone: An oestrogenic mycotoxin. *Food Chem. Toxicol.* 45 (2007) 1-18.

[364] M.E. Flores-Flores, E. Lizarraga, A. López de Cerain, E. González-Peñas, Presence of mycotoxins in animal milk: A review. *Food Control* 53 (2015) 163-176.

[365] A.L. Capriotti, C. Cavaliere, S. Piovesana, S. Stampachiacchiere, R. Samperi, S. Ventura, A. Laganá, Simultaneous determination of naturally occurring estrogens and mycoestrogens in milk by ultra high-performance liquid chromatography - tandem mass spectrometry analysis. *J. Agric. Food Chem.* 63 (2015) 8940-8946.

[366] L.C. Huang, N. Zheng, B.Q. Zheng, F. Wen, J.B. Cheng, R.W. Han, X.M. Xu, S.L. Li, J.Q. Wang, Simultaneous determination of aflatoxin M_1, ochratoxin A, zearalenone and α-zearalenol in milk by UHPLC–MS/MS. *Anal. Methods* 146 (2014) 242-249.

[367] X. Xia, X. Li, S. Ding, S. Zhang, H. Jiang, J. Li, J. Shen, Ultra-high-pressure liquid chromatography–tandem mass spectrometry for the analysis of six resorcylic acid lactones in bovine milk. *J. Chromatogr. A* 1216 (2009) 2587-2591.

[368] E. Tsiplakou, C. Anagnostopoulos, K. Liapis, S.A. Haroutounian, G. Zervas, Determination of mycotoxins in feedstuffs and ruminant's milk using an easy and simple LC–MS/MS multiresidue method. *Talanta* 130 (2014) 8-19.

[369] D. Chen, X. Cao, Y. Tao, Q. Wu, Y. Pan, D. Peng, Z. Liu, L. Huang, Y. Wang, X. Wang, Z. Yuan, Development of a liquid chromatography–tandem mass spectrometry with ultrasound-assisted extraction and auto solid-phase clean-up method for the determination of Fusarium toxins in animal derived foods. *J. Chromatogr. A* 1311 (2013) 21-29.

[370] K. Zhang, J.W. Wong, D.G. Hayward, M. Vaclavikova, C.-D. Liao, M.W. Trucksess, Determination of mycotoxins in milk-based products and infant formula using stable isotope dilution assay and liquid chromatography tandem mass spectrometry. *J. Agric. Food Chem.* 61 (2013) 6265-6273.

[371] P.H. Towler, J.D. Smith, D.R. Dixon, Magnetic recovery of radium, lead and polonium from seawater samples after preconcentration on a magnetic adsorbent of manganese dioxide coated magnetite. *Anal. Chim. Acta* 328 (1996) 53-59.

[372] H. Lee, S.M. Dellatore, W.M. Miller, P.B. Messersmith, Mussel-inspired surface chemistry for multifunctional coatings. *Science* 318 (2007) 426-430.

[373] Y.-R. Ma, X.-L Zhang, T. Zeng, D. Cao, Z. Zhou, W.-H. Li, H. Niu, Y.-Q. Cai, Polydopamine-coated magnetic nanoparticles for enrichment and direct detecction of small molecule pollutants coupled with MALDI-TOF-MS. *ACS Appl. Mater. Interfaces* 5 (2013) 1024-1030.

[374] H.-L. Shi, S.-L. Peng, J. Sun, Y.-M. Liu, Y.-T. Zhu, L.-S. Qing, X. Liao, Selective extraction of berberine from *Cortex Phellodendri* using polydopamine-coated magnetic nanoparticles. *J. Sep. Sci.* 37 (2014) 704-710.

[375] Y. Wang, S. Wang, H. Niu, Y. Ma, T. Zeng, Y. Cai, Z. Meng, Preparation of polydopamine coated Fe_3O_4 nanoparticles and their application for enrichment of polycyclic aromatic hydrocarbons from environmental water samples. *J. Chromatogr. A* 1283 (2013) 20-26.

[376] B. Socas-Rodríguez, M. Asensio-Ramos, J. Hernández-Borges, M.Á. Rodríguez-Delgado, Analysis of oestrogenic compounds in dairy products by hollow-fibre liquid-phase microextraction coupled to liquid chromatography. *Food Chem.* 149 (2014) 319-325.

[377] S. Al-Malaika, F. Axtell, R. Rothon, M. Gilbert, (Ed.). *Additives for plastics. Brydson's plastics materials*. Elsevier, 2017, p. 127-168.

[378] M. Rahman, C. Brazel, The plasticizer market: An assessment of traditional plasticizers and research trends to meet new challenges. *Prog. Polym. Sci.* 29 (2004) 1223-1248.

[379] I.T. Cousin, D. Mackay, T.F. Parkerton, (Ed.). *Physical-chemical properties and evaluative fate modelling of phthalate ester. The Handbook of Environmental Chemistry*. Springer, 2003, p. 57-84.

[380] M. Giulivo, M. Lopez de Alda, E. Capri, D. Barceló, Human exposure to endocrine disrupting compounds: Their role in reproductive systems, metabolic syndrome and breast cancer. A review. *Environ. Res.* 151 (2016) 251-264.

[381] T. Schettler, Human exposure to phthalates via consumer products. *Int. J. Endrol.* 29 (2006) 134-139.

[382] T. Lovekamp-Swan, B. Davis, Mechanisms of phthalate ester toxicity in the female reproductive system. *Environ. Health Perspect.* 111 (2003) 139-145.

[383] Regulation (EC) No 1907/2006 of the European Parliament and of the Council of 18 December 2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH), establishing a European Chemicals Agency, amending Directive 1999/45/EC and repealing Council Regulation (EEC) No 793/93 and Commission Regulation (EC) No 1488/94 as well as Council Directive 76/769/EEC and Commission Directives 91/155/EEC, 93/67/EEC, 93/105/EC and 2000/21/EC. Available at: https://bit.ly/2ySgxAJ. Accessed August 2018.

[384] Y. Guo, K. Kannan, Challenges encountered in the analysis of phthalate esters in foodstuffs and other biological matrices. *Anal. Bioanal. Chem.* 404 (2012) 2539-2554.

[385] J. González-Sálamo, B. Socas-Rodríguez, J. Hernández-Borges, M.Á. Rodríguez-Delgado, Core-shell poly(dopamine) magnetic nanoparticles for the extraction of estrogenic mycotoxins from milk and yogurt prior to LC-MS analysis. *Food Chem.* 215 (2017) 362-368.

[386] Z. Huang, H.K. Lee, Study and comparison of polydopamine and its derived carbon decorated nanoparticles in the magnetic solid-phase extraction of estrogens. *J. Chromatogr. A* 1414 (2015) 41-50.

[387] P. Otero, S.K. Saha, S. Moane, J. Barron, G. Clancy, P. Murray, Improved method for rapid

detection of phthalates in bottled water by gas chromatography-mass spectrometry. *J. Chromatogr. B* 997 (2015) 229-235.

[388] M.-E. Yue, J. Xu, W.-G. Hou, Determination of five phthalate esters in running water and milk by micellar electrokinetic capillary chromatography. *J. Anal. Chem.* 70 (2015) 1147-1152.

[389] B.-Y. Guo, B. Wen, X.-Q. Shan, S.-Z. Zhang, J.-M. Lin, Separation and determination of phthalates by micellar electrokinetic chromatography. *J. Chromatogr. A* 1095 (2005) 189-192.

[390] Commission Decision 2002/657/EC of 12 August 2002 implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results. *Off. J. Eur. Commun.* L221 (2002) 8-36.

[391] C. Liao, P. Yang, Z. Xie, Y. Zhao, X. Cheng, Y. Zhang, Z. Ren, Z. Guo, J. Liao, Application of GC-triple quadrupole MS in the quantitative confirmation of polycyclic aromatic hydrocarbons and phthalic acid esters in soil. *J. Chromatogr. Sci.* 48 (2010) 161-166.

[392] M.M. Rahman, A. El-Aty, J.H. Shim, Matrix enhancement effect: A blessing or a curse for gas chromatography?–A review. *Anal. Chim. Acta* 801 (2013) 14-21.

[393] D.R. Erney, A.M. Gillespie, D.M. Gilvydis, C.F. Poole, Explanation of the matrix-induced chromatographic response enhancement of organophosphorus pesticides during open tubular column gas chromatography with splitless or hot on-column injection and flame photometric detection. *J. Chromatogr. A* 638 (1993) 57-63.

[394] H. Kwon, S.J. Lehotay, L. Geis-Asteggiante, Variability of matrix effects in liquid and gas chromatography-mass spectrometry analysis of pesticide residues after QuEChERS sample preparation of different food crops. *J. Chromatogr. A* 1270 (2012) 235-245.

[395] B.O. Clarke, S.R. Smith, Review of 'emerging' organic contaminants in biosolids and assessment of international research priorities for the agricultural use of biosolids. *Environ. Int.* 37 (2011) 226-247.

[396] M.C. Barciela-Alonso, N. Otero-Lavandeira, P. Bermejo-Barrera, Solid phase extraction using molecular imprinted polymers for phthalate determination in water and wine samples by HPLC-ESI–MS. *Microchem. J.* 132 (2017) 233-237.

[397] N. Domínguez-Morueco, S. González-Alonso, Y. Valcárcel, Phthalate occurrence in rivers and tap water from central Spain. *Sci. Tot. Environ.* 500-501 (2014) 139-146.

[398] B.C. Tran, M.J. Teil, M. Blanchard, F. Alliot, M. Chevreuil, BPA and phthalate fate in a sewage network and an elementary river of France. Influence of hydroclimatic conditions. *Chemosphere* 119 (2015) 43-51.

[399] X.L. Zhang, H.Y. Niu, S.X. Zhang, Y.Q. Cai, Preparation of a chitosan-coated C_{18}-functionalized magnetite nanoparticle sorbent for extraction of phthalate ester compounds from environmental water samples. *Anal. Bioanal. Chem.* 397 (2010) 791-798.

[400] S. Zhang, H. Niu, Y. Cai, Y. Shi, Barium alginate caged Fe_3O_4@C_{18} magnetic nanoparticles for the pre-concentration of polycyclic aromatic hydrocarbons and phthalate esters from environmental water samples. *Anal. Chim. Acta* 665 (2010) 167-175.

[401] E. Tahmasebi, Y. Yamini, M. Moradi, A. Esrafili, Polythiophene-coated Fe_3O_4 superparamagnetic nanocomposite: Synthesis and application as a new sorbent for solid-phase extraction. *Anal. Chim. Acta* 770 (2013) 68-74.

[402] Q. Ye, L. Liu, Z. Chen, I. Hong, Analysis of phthalate acid esters in environmental water by magnetic graphene solid phase extraction coupled with gas chromatography-mass spectrometry. *J. Chromatogr. A* 1329 (2014) 24-29.

[403] V.R. Kay, M.S. Bloom, W.G. Foster, Reproductive and developmental effects of phthalate diesters in males. *Crit. Rev. Toxicol.* 44 (2014) 467-498.

[404] N.G. Posnack, The adverse cardiac effects of di(2-ethylhexyl)phthalate and bisphenol A. *Cardiovasc. Toxicol.* 14 (2014) 339-357.

[405] L. Trasande, S. Sathyanarayana, A.J. Spanier, H. Trachtman, T.M. Attina, E.M. Urbina, Urinary phthalates are associated with higher blood pressure in childhood. *J. Pediatr.* 163 (2013) 747-753.

[406] J. Weuve, R. Hauser, A.M. Calafat, S.A. Missmer, L.A. Wise, Association of exposure to phthalates with endometriosis and uterine leiomyomata: Findings from NHANES, 1999–2004. *Environ. Health Perspect.* 118 (2010) 825-832.

[407] M.M. Abdel daiem, J. Rivera-Utrilla, R. Ocampo-Pérez, J.D. Méndez-Díaz, M. Sánchez-Polo, Environmental impact of phthalic acid esters and their removal from water and sediments by different technologies – A review. *J. Environ. Manag.* 109 (2012) 164-178.

[408] M.A. Farajzadeh, S. Sheykhizadeh, P. Khorram, Salting-out homogeneous liquid–liquid extraction in narrow-bore tube: Extraction and preconcentration of phthalate esters from water. *J. Sep. Sci.* 36 (2013) 939-946.

[409] L.L. Ma, S.G. Chu, X.B. Xu, Organic contamination in the greenhouse soils from Beijing suburbs, China. *J. Environ. Monit.* 5 (2003) 786-790.

[410] M.Á. González-Curbelo, J. Hernández-Borges, T.M. Borges-Miquel, M.Á. Rodríguez-Delgado, Determination of organophosphorus pesticides and metabolites in cereal-based baby foods and wheat flour by means of ultrasound-assisted extraction and hollow-fiber liquid-phase microextraction prior to gas chromatography with nitrogen phosphorus detection. *J. Chromatogr. A* 1313 (2013) 166-174.

[411] Y.-Y. Chao, Z.-X. Jian, Y.-M. Tu, Y.-L. Huang, Rapid on-line microextraction of neutral analytes in plastic-bottled beverages through ultrasound-assisted push/pull perfusion hollow-fiber liquid–liquid–liquid microextraction. *Microchem. J.* 110 (2013) 386-394.

[412] E. Psillakis, N. Kalogerakis, Hollow-fibre liquid-phase microextraction of phthalate esters from water. *J. Chromatogr. A* 999 (2003) 145-153.

[413] C. Zhang, Y. Wang, Removal of dissolved organic matter and phthalic acid esters from landfill leachate through a complexation–flocculation process. *Waste Manag.* 29 (2009) 110-116.

[414] F. Zeng, K. Cui, Z. Xie, M. Liu, Y. Li, Y. Lin, Z. Zeng, F. Li, Occurrence of phthalate esters in water and sediment of urban lakes in a subtropical city, Guangzhou, South China. *Environ. Int.* 34 (2008) 372-380.

[415] D. Gao, Z. Li, Z. Wen, N. Ren, Occurrence and fate of phthalate esters in full-scale domestic wastewater treatment plants and their impact on receiving waters along the Songhua River in China. *Chemosphere* 95 (2014) 24-32.

[416] O.S. Fatoki, A. Noma, Solid phase extraction method for selective determination of phthalate esters in the aquatic environment. *Water Air Soil Pollut.* 140 (2002) 85-98.

[417] S.Y. Yuan, C. Liu, C.S. Liao, B.V. Chang, Occurrence and microbial degradation of phthalate esters in Taiwan river sediments. *Chemosphere* 49 (2002) 1295-1299.

[418] X. Zheng, B.-T. Zhang, Y. Teng, Distribution of phthalate acid esters in lakes of Beijing and its relationship with anthropogenic activities. *Sci. Total Environ.* 476-477 (2014) 107-113.

[419] Y. Fan, H. Chen, H. Liu, F. Wang, S. Ma, A. Latipa, S. Wang, C. Wang, Analysis of phthalate esters in dairy products-A brief review. *Anal. Methods* 9 (2017) 370-380.

[420] M.S. Qureshi, A.R.B.M. Yosuff, M.D.H. Wirzal, Sirajuddin, J. Barek, H.I. Afridi, Z. Üstündag, Methods for the determination of endocrine-disrupting phthalate esters. *Crit. Rev. Anal. Chem.* 46 (2016) 146-159.

[421] C. Wang, Q. Wu, C. Wu, Z. Wang, Determination of some organophosphorus pesticides in water and watermelon samples by microextraction prior to high-performance liquid chromatography. *J. Sep. Sci.* 34 (2011) 3231-3239.

[422] S. Zorita, T. Barri, L. Mathiasson, A novel hollow-fibre microporous membrane liquid–liquid extraction for determination of free 4-isobutylacetophenone concentration at ultra trace level in environmental aqueous samples. *J. Chromatogr. A* 1157 (2007) 30-37.

[423] J. Xiong, B. Hu, Comparison of hollow fiber liquid phase microextraction and dispersive liquid–liquid microextraction for the determination of organosulfur pesticides in environmental and beverage samples by gas chromatography with flame photometric detection. *J. Chromatogr. A* 1193 (2008) 7-18.

[424] M. Palit, D. Pardasani, A.K. Gupta, D.K. Dubey, Application of single drop microextraction for analysis of chemical warfare agents and related compounds in water by gas chromatography/mass spectrometry. *Anal. Chem.* 77 (2005) 711-717.

[425] Q. Wu, J.C.W. Lam, K.Y. Kwok, M.M.P. Tsui, P.K.S. Lam, Occurrence and fate of endogenous steroid hormones, alkylphenol ethoxylates, bisphenol A and phthalates in municipal sewage treatment systems. *J. Environ. Sci.* 61 (2017) 49-58.

[426] S. Keresztes, E. Tatár, Z. Czégény, G. Záray, V.G. Milhucz, Study on the leaching of phthalates from polyethylene terephthalate bottles into mineral water. *Sci. Total Environ.* 458-460 (2013) 451-458.

[427] X. Liang, S. Liu, S. Wang, Y. Guo, S. Jiang, Carbon-based sorbents: Carbon nanotubes. *J. Chromatogr. A* 1357 (2014) 53-67.

[428] M. Ahmadi, H. Elmongy, T. Madrakian, M. Abdel-Rehim, Nanomaterials as sorbents for sample preparation in bioanalysis: A review. *Anal. Chim. Acta* 958 (2017) 1-21.

[429] A. Taghani, N. Goudarzi, G. Bagherian, Application of multiwalled carbon nanotubes for the preconcentration and determination of organochlorine pesticides in water samples by gas chromatography with mass spectrometry. *J. Sep. Sci.* 39 (2016) 4219-4226.

[430] A. Abdar, A. Sarafraz-Yazdi, A. Amiri, N. Bagheri, Magnetic solid-phase extraction of polycyclic aromatic hydrocarbons in water samples by Fe_3O_4@polypyrrole/carbon nanotubes. *J. Sep. Sci.* 39 (2016) 2746-2753.

[431] B. Socas-Rodríguez, J. González-Sálamo, J. Hernández-Borges, M.Á. Rodríguez-Delgado, Recent applications of nanomaterials in food safety. *TrAC-Trend. Anal. Chem.* 96 (2017) 172-200.

[432] M. Asensio-Ramos, J. Hernández-Borges, L.M. Ravelo-Pérez, M.Á. Rodríguez-Delgado, Simultaneous determination of seven pesticides in waters using multiwalled carbon nanotube SPE and NACE. *Electrophoresis* 29 (2008) 4412-4421.

[433] S. Net, R. Sempéré, A. Delmont, A. Paluselli, B. Ouddane, Occurrence, fate, behavior and ecotoxicological state of phthalates in different environmental matrices. *Environ. Sci. Technol.* 49 (2015) 4019-4035.

[434] Y.-B. Luo, Q.-W. Yu, B.-F. Yuan, Y.-Q. Feng, Fast microextraction of phthalate acid esters from beverage, environmental water and perfume samples by magnetic multi-walled carbon nanotubes. *Talanta* 90 (2012) 123-131.

[435] Y. Jiao, S. Fu, L. Ding, Q. Gong, S. Zhu, L. Wang, H. Li, Determination of trace leaching phthalate esters in water by magnetic solid phase extraction based on magnetic multi-walled carbon nanotubes followed by GC-MS/MS. *Anal. Methods* 9 (2012) 2729-2734.

[436] H. Asadollahzadeh, E. Noroozian, Sh. Maghsoudi, Solid-phase microextraction of phthalate esters from aqueous media by electrochemically deposited carbon nanotube/polypyrrole composite on a stainless steel fiber. *Anal. Chim. Acta* 669 (2010) 32-38.

[437] D.A. Volmer, L.L. Jessome, Ion suppression: A major concern in mass spectrometry. *LC-GC North America* 24 (2006) 498-510.

[438] B.K. Matuszewski, M.I. Constanzer, C.M. Chavez-Eng, Strategies for the assessment of matrix effect in quantitative bioanalytical methods based on HPLC-MS/MS. *Anal. Chem.* 75 (2003) 3019-3030.

[439] J.E. Efome, D. Rana, T. Matsuura, C.Q. Lan, Metal-organic frameworks supported on nanofibers to remove heavy metals. *J. Mater. Chem. A* 6 (2018) 4550-4555.

[440] F. Jeremias, A. Khutia, S.K. Henninger, C. Janiak, MIL-100(Al,Fe) as water adsorbents for heat transformation purposes-a promising application. *J. Mater. Chem.* 22 (2012) 10148-10151.

[441] J. González-Sálamo, B. Socas-Rodríguez, J. Hernández-Borges, M.Á. Rodríguez-Delgado, Determination of phthalic acid esters in water samples using core-shell poly(dopamine) magnetic nanoparticles and gas chromatography tandem mass spectrometry. *J. Chromatogr. A* 1530 (2017) 35-44.

[442] J. González-Sálamo, M.Á. González-Curbelo, B. Socas-Rodríguez, J. Hernández-Borges, M.Á. Rodríguez-Delgado, Determination of phthalic acid esters in water samples by hollow fiber liquid-phase microextraction prior to gas chromatography tandem mass spectrometry. *Chemosphere* 201 (2018) 254-261.

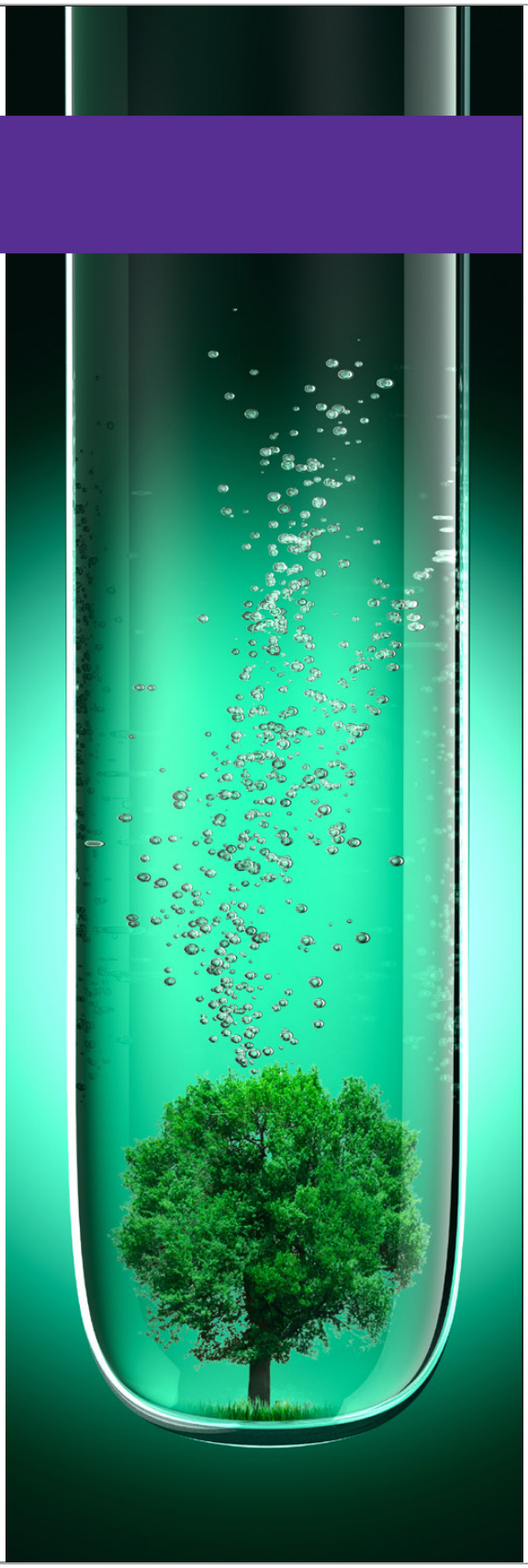
[443] M.V. Russo, P. Avino, L. Perugini, I. Notardonato, Extraction and GC-MS analysis of phthalate esters in food matrices: a review. *RSC Adv.* 5 (2015) 37023-37043.

[444] W. Li, J. Duan, Detection of phthalates in water using ultra performance liquid chromatography-electrospray ionization tandem mass spectrometry MRM mode-'ghost peaks' and measurement methodology. *Anal. Methods* 3 (2011) 314-321.

[445] O.O. Olujimi, O.A. Aroyeun, T.F. Akinhanmi, T.A. Arowolo, Occurrence, removal and health risk assessment of phthalate esters in the process streams of two different wastewater treatment plants in Lagos and Ogun States, Nigeria. *Environ. Monit. Assess.* 189 (2017) 345-361.

[446] T. Salaudeen, O. Okoh, F. Agunbiade, A. Okoh, Phthalate removal efficiency in different wastewater treatment technology in the Eastern Cape, South Africa. *Environ. Monit. Assess.* 190 (2018) 299-313.

New extraction protocols for the determination of endocrine disrupting compounds in samples of environmental and agri-food interest



SUMMARY

In this PhD Thesis, new effective and sensitive analytical methodologies have been developed for the determination of a wide group of endocrine disrupting compounds (natural, synthetic and myco-oestrogens as well as phthalic acid esters) in matrices of environmental and agri-food interest. Selective extraction techniques such as hollow-fibre liquid-phase microextraction, solid-phase extraction using molecularly imprinted polymers, and magnetic and non-magnetic micro-dispersive solid-phase extraction using different nanomaterials as sorbents (polydopamine magnetic nanoparticles, carbon nanotubes and metal-organic frameworks), have been applied to achieve this goal. All these procedures have been combined with high-performance liquid chromatography and gas chromatography mass spectrometry for the suitable separation and determination of the target analytes.

Universidad de La Laguna