UNIVERSIDADE DE SANTIAGO DE COMPOSTELA

Facultade de Química

Departamento de Química Analítica, Nutrición e Bromatoloxía

Estudio de la determinación de ftalatos en muestras de interés clínico y alimentario

Memoria que para optar al grado de Doctora presenta

Cristina Pérez Feás

Santiago de Compostela, noviembre 2011

UNIVERSIDADE DE SANTIAGO DE COMPOSTELA

Facultade de Química

Dpto. de Química Analítica, Nutrición e Bromatoloxía

Dña. Pilar Bermejo Barrera, Catedrática de Química Analítica, y Dña. María del Carmen Barciela Alonso, Profesora Contratada Doctora, ambas del Departamento de Química Analítica, Nutrición y Bromatología de la Facultad de Química de la Universidad de Santiago de Compostela,

INFORMAN,

Que la presente Tesis Doctoral, titulada ***"Estudio de la determinación de ftalatos en muestras de interés clínico y alimentario"*** ha sido realizada bajo nuestra dirección por Cristina Pérez Feás en el Dpto. de Química Analítica, Nutrición y Bromatología de la Universidad de Santiago de Compostela.

Y para que así conste firman la presente en Santiago de Compostela, a 3 de noviembre de 2011

Pilar Bermejo Barrera

María del Carmen Barciela Alonso

Agradecimientos

A lo largo de todos estos años pensé en más de una ocasión como sería esta primera página de mi Tesis, estas líneas en las que se agradece la ayuda prestada y el apoyo recibido durante todo este tiempo y por fin ha llegado este momento y aquí estoy para dedicaros estas líneas.

Quiero dar las gracias en primer lugar a mis directoras de Tesis, Pilar Bermejo Barrera y Mª del Carmen Barciela Alonso, por confiar en mí y por haberme dado la oportunidad de iniciar este trabajo a pesar de que existieran otros proyectos personales. Por su apoyo, comprensión, paciencia y confianza en que este proyecto llegaría a su fin.

Al personal de la Unidad de Masas del CACTUS de Santiago de Compostela y especialmente a Esteban y a Noemi por su colaboración en el desarrollo experimental de una parte de este trabajo.

Al Servicio de Neonatología y al Servicio de Farmacia Hospitalaria del Hospital Clínico Universitario de Santiago de Compostela por su colaboración en el estudio llevado a cabo con las muestras de nutrición parenteral.

Al grupo de investigación de Química Analítica de la Universidad de Córdoba, y especialmente a la profesora Soledad Rubio Bravo por su dedicación y el buen trato recibido durante mi corta estancia en Córdoba y también a Francisco, Amalia y Noelia, por su amistad y porque me hicieron sentir como en casa desde el primer día.

A mi primo Xesús, porque gracias a él conocí a Pilar y sin su ayuda jamás hubiera iniciado este proyecto.

A todas las personas que formaron y forman parte del Grupo de Elementos Traza, Espectroscopía y Especiación del Dpto. de Química Analítica, por crear y mantener el buen ambiente en los ratitos del café que, por cierto, ya hace tiempo que echo de menos.
A los profesores, Devesa, Elena, Chelo, Antonio y Raquel por mostrar su interés en esta Tesis a lo largo de todos estos años.
A Sandra, por su compañía y los buenos momentos que compartimos al inicio de esta Tesis. A Paloma, por su actitud incansable, siempre dispuesta a echarte una mano. A Ebru y a Antía con las que compartí la aventura de analizar ftalatos y sus problemas asociados. A Julia, Jacobo, Patry, Cris Morena, Vane, Nati y Marta por su compañerismo y sus sonrisas en todas las idas y venidas por los pasillos del departamento.

A Raimunda, por los ánimos recibidos cada tarde para seguir adelante.

Y por supuesto, quiero agradecer de forma muy especial, a mi familia, a mis padres, a mi hermana, a mis amigas y a Juan, el apoyo contínuo y la fuerza que me dieron en todas las etapas de este proyecto.

ÍNDICE

ÍNDICE

CAPÍTULO II: Phthalates Determination in Physiological Saline Solutions by HPLC-ES-MS

CAPÍTULO III: Direct LC-ES-MS/MS Determination of Phthalates in Physiological Saline Solutions

CAPÍTULO IV: Phthalates Determination in Pharmaceutical Formulae Used in Parenteral Nutrition by HPLC-ES-MS: Importance in Public Health

CAPÍTULO V: Presence of Phthalates in Contact Lens and Cleaning Solutions

ABREVIATURAS Y ACRÓNIMOS

Abreviaturas y Acrónimos

A

amu	Atomic mass unit
APCI	Atmospheric pressure chemical ionization
API	Atmospheric pressure ionization

B

BBP	Butyl benzyl phthalate

C

CAD	Collision-activated dissociation
CE	Capillary electrophoresis
CE	Collision energy
CF-LPME	Continuous-flow liquid phase microextraction
CFME	Continuous-flow microextraction
cps	Counts per seconds
CV	Coefficient of variation
CW-DVB	Carbowax-divinylbencene
CXP	Collision cell exit potential
CZE	Capillary zone electrophoresis
C18	Octadecyl modified silica phase
C8	Octyl modified silica phase

D

DBP	Dibutyl phthalate
DEP	Diethyl phthalate
DEHP	Diethylhexyl phthalate
DiDP	Diisodecyl phthalate
DiNP	Diisononyl phthalate

DPP	Dipropil phthalate
D-LPME	Dynamic liquid phase microextraction
DMP	Dimethyl phthalate
DP	Declustering potential
DPeP	Dipentyl phthalate
DOP	Dioctyl phthalate

E

EC	European Commission
ECD	Electron capture detection
EP	Enhance potential
EPA	Environmental protection agency
ESI	Electrospray ionization
EU	European Union
EVA	Ethylvinylacetate

F

FDA	Food and Drug Administration
FID	Flame ionization detection
FP	Focusing potential

G

GC	Gas chromatography

H

HPLC	High performance liquid chromatography
HS-LPME	Headspace-liquid phase microextraction

I

i.d.	Internal diameter
IUPAC	International Union of Pure and Applied Chemistry

IS	Internal standard

L

LC	Liquid chromatography
LD	Liquid desorption
LLE	Liquid-liquid extraction
LOD	Limit of detection
LOQ	Limit of quantitation
LPME	Liquid phase microextraction
LVI	Large volume injection

M

m/z	Mass/charge
MAE	Microwave assisted extraction
MEKC	Micellar electrokinetic chromatography
MBP	Monobutyl phthalate
MBzP	Monobenzyl phthalate
MDP	Monodecyl phthalate
MEP	Monoethyl phthalate
MEHP	Monoethylhexyl phthalate
min	Minutes
MMP	Monomethyl phthalate
MNP	Monononyl phthalate
MOP	Monooctyl phthalate
MRM	Multiple reaction monitoring
MS	Mass spectrometry
MS/MS	Tandem mass spectrometry
MWNTs	Multi-walled carbon nanotubes

N

n.d.	Not detected

P

PA	Phthalic acid
PAEs	Phthalic acid esters
PANI	Polyaniline
PDMS	Polydimethylsiloxane
PDMS-DVB	Polydimethylsiloxane- divinylbencene
PE	Polyethylene
PEEK	Poly (ether ether ketone)
PI	Positive ionization
PP	Polypropylene
ppb	Parts-per-billion
PPG	Polypropylenglycole
ppm	Parts-per-million
PS-DVB	Polystyrene-divinylbencene
PS-2	Styrene-divinyl polymer
PTFE	Polytetrafluoroethylene
PVC	Polyvinyl chloride

R

r	Correlation coefficient
rpm	Revolutions per minute
RSD	Relative standard deviation
RT	Retention time

S

SB-LPME	Solvent bar- liquid phase microextraction
SBSE	Stir bar sorptive extraction
SCCNFP	Scientific Comittee on Cosmetic Products and Non Food Products
SD	Standard deviation
SDB-XD	Octadecyl-coated styrene divinylbenzene polymer
SDME	Single drop microextraction
SDS	Sodium dodecyl sulfate
SFE	Supercritical fluid extraction

SIM	Selected ion monitoring
SLE	Solid-liquid extraction
S-LPME	Static-liquid phase microextraction
s/n	Signal-to-noise ratio
SPE	Solid phase extraction
SPME	Solid phase microextraction
SRM	Selective reaction monitoring

T

TIC	Total ion current
t_R	Retention time
TD	Thermal desorption

U

UV	Ultraviolet

V

v/v	Volume/volume
VWD	Variable-wavelength detector

OBJETIVOS

Objetivos

Los ftalatos o ésteres del ácido ftálico son compuestos químicos usados en la industria desde 1930, principalmente como plastificantes en polímeros y también como aditivos en gran variedad de productos. Los efectos de la exposición humana a los ftalatos han sido ampliamente discutidos y en la actualidad están clasificados por la Unión Europea como disruptores endocrinos. Debido a su fuerte impacto ambiental y a sus repercusiones sobre la salud, diversas organizaciones gubernamentales han establecido regulaciones para limitar la exposición humana. Teniendo en cuenta estas consideraciones, el interés en el estudio de este tipo de sustancias químicas ha aumentado enormemente durante los últimos años por lo que se hace necesario seguir investigando en el desarrollo de métodos analíticos selectivos y sensibles que nos permitan analizar estos compuestos a nivel de trazas.

El objetivo común a todos los capítulos experimentales que componen esta Tesis Doctoral es evaluar el nivel de exposición a ftalatos en la población como consecuencia del uso de distintos productos y evidenciar así el riesgo que supone para la salud humana mediante el desarrollo de métodos sensibles y específicos con el menor grado posible de manipulación de la muestra.

La técnica analítica elegida ha sido la cromatografía líquida de alta resolución acoplada a la espectrometría de masas, simple y en tándem (LC-MS y LC-MS/MS). En algunos casos fue necesario desarrollar previamente distintas técnicas de extracción/preconcentración de la muestra.

Los ftalatos utilizados en este estudio han sido elegidos en función de su repercusión sobre la salud pública y medioambiental.

A continuación se enumeran los objetivos perseguidos en cada uno de los capítulos:

Capítulo I:

- Realizar una amplia revisión bibliográfica para abordar el grado de conocimiento científico existente en relación con el análisis de ftalatos en todo tipo de matrices, con el fin de conocer desde los procedimientos de extracción y preconcentración, las técnicas de separación y los sistemas de detección más adecuados hasta los problemas de contaminación en el análisis de estos compuestos y su posible origen.

Capítulo II:

- Desarrollar un método analítico adecuado para la identificación y cuantificación de una mezcla de cuatro diésteres del ácido ftálico (DMP, DEP, BBP y DBP) mediante LC-MS.
- Aplicar el método desarrollado al análisis de muestras de suero salino comercializadas en envases de plástico monodosis con el fin de evidenciar la posible migración de ftalatos desde los envases de plástico a las soluciones salinas.

Capítulo III:

- Adaptar el método analítico desarrollado en el capítulo anterior a la cromatografía líquida de alta resolución con espectrometría de masas en tándem (LC-MS/MS) con el fin de mejorar la sensibilidad del método y conseguir mayor información sobre los niveles de ftalatos liberados desde los envases de plástico monodosis a las muestras de suero salino.

Capítulo IV:

- Ampliar el método analítico al análisis de una mezcla de seis ftalatos, entre los que se incluyen el DMP, DEP, BBP, DBP, DEHP y DOP mediante LC-MS, utilizando el DPeP como estándar interno.
- Desarrollar una técnica de extracción/preconcentración que permita aplicar el método optimizado para la determinación de estos compuestos en las nutriciones parenterales utilizadas en el ámbito hospitalario.

Capítulo V:

- Estudiar la presencia de cuatro ftalatos (DMP, DEP, BBP, DBP) en soluciones comerciales utilizadas en la limpieza de lentes de contacto aplicando el método de LC-MS/MS desarrollado previamente.
- Estudiar la posible migración de estos ftalatos desde las lentes de contacto a una solución de lágrima artificial.

Capítulo VI:

- Desarrollar un método de extracción y preconcentración para un grupo de seis ftalatos (DMP, DEP, BBP, DBP, DEHP y DOP) a partir de muestras de film transparente utilizado en la conservación de alimentos.
- Determinar y cuantificar estos compuestos mediante el método LC-MS desarrollado previamente en muestras de film plástico de distintas marcas comerciales.

CAPÍTULO I

Analytical Methods for Phthalates Determination in Biological and Environmental Samples: A Review

Bermejo Barrera P., Barciela Alonso M.C., Pérez Feás C., Peña Vázquez E., Herbello Hermelo P.

ISBN: 978-1-60741-701-9
Editor: Bradley C. Vaughn, pp.1-28

Resumen

Los compuestos químicos protagonistas en el desarrollo de este trabajo son los ftalatos, químicamente denominados diésteres del ácido ftálico o dialquil o alquil-aril ésteres del ácido 1,2-bencenodicarboxílico. Se trata de un grupo de compuestos químicos ampliamente distribuídos en el ambiente como consecuencia de su uso en la fabricación de una gran variedad de productos, entre los que se incluyen plásticos, cosméticos, lacas de uñas, sprays para el pelo, perfumes, juguetes, dispositivos médicos, material de construcción, material de envasado de alimentos, ropa, pinturas, lubricantes, adhesivos etc.

A *nivel industrial*, el interés de los ftalatos radica en sus propiedades físico-químicas que los convierten en los aditivos idóneos en la fabricación de polímeros por la función plastificante que desempeñan. De la producción mundial de ftalatos (se estima en varios millones de toneladas al año) aproximadamente el 10% se utiliza en la industria textil, en la formulación de pesticidas, en la fabricación de productos para el cuidado personal (lociones, desodorantes, perfumes), tintas, pinturas, etc y el 90% restante es utilizado como plastificante en los procesos de polimerización que tienen lugar en la fabricación de los plásticos.

Los plásticos son estructuras macromoleculares formadas por un componente estructural principal, el polímero, al que se le añaden una serie de aditivos para obtener determinados efectos tecnológicos; entre estos aditivos se encuentran los ftalatos cuya función es aumentar la flexibilidad y maleabilidad en estos productos.

La ausencia de uniones químicas covalentes entre los ftalatos y la matriz polimérica que forma los plásticos facilita la migración de los mismos y su posterior liberación al medio.

El interés de estos compuestos en *Salud Pública* radica en los riesgos para la salud que se derivan de la presencia de estas sustancias en nuestro entorno (suelo, agua, aire, alimentos…) y como consecuencia su aparición en el organismo humano.
Debido a su amplio uso y moderada persistencia son considerados importantes contaminantes ambientales y su regulación abarca todos los aspectos de su producción, transporte, uso y eliminación.
Resultados de estudios toxicológicos revelan que los ftalatos pueden afectar a la salud humana en diversos aspectos, especialmente al sistema reproductivo, endocrino y respiratorio. Algunos ftalatos que han sido objeto de estudio en este trabajo como el BBP, DBP y DEHP han sido clasificados como tóxicos para la reproducción (categoría 2) por la Unión Europea.

Teniendo en cuenta estas consideraciones, el interés en el estudio de este tipo de sustancias químicas ha aumentado enormemente durante los últimos años por lo que se han ido desarrollando numerosos métodos para su determinación en una amplia gama de matrices, incluyendo agua, suelo, aire, artículos para el cuidado personal y alimentos.

En el trabajo que se desarrolla a continuación se exponen las distintas técnicas y métodos analíticos descritos en la literatura para la determinación de ftalatos en diferentes tipos de muestras; desde los tratamientos de extracción y preconcentración realizados sobre las muestras (SPE, LLE, SBSE, etc) hasta las técnicas empleadas para su separación (HPLC, GC, CE) y posterior identificación mediante el uso de distintos detectores (UV, ECD, FID, MS).
En la última parte se hace una mención especial al principal problema relacionado con el análisis de ftalatos, la contaminación, puesto que este riesgo está presente en todo el proceso analítico y puede derivar en resultados falsos positivos o en concentraciones sobre-estimadas de estas sustancias.

Contents

ANALYTICAL METHODS FOR PHTHALATES DETERMINATION IN BIOLOGICAL AND ENVIRONMENTAL SAMPLES: A REVIEW

Bermejo Barrera P., Barciela Alonso M.C., Pérez Feas C., Peña Vázquez E., Herbello Hermelo P.

Department of Analytical Chemistry, Nutrition and Bromatology, Faculty of Chemistry, University of Santiago de Compostela, Avenida das Ciencias, s/n. E-15782, Santiago de Compostela, Spain.

This chapter summarizes and discusses the analytical methods and techniques described in the literature for phthalate determination in different matrix samples (water, soil, sediments, sludge, air and biological samples). Different sample treatments, extraction and preconcentration steps, such as liquid-liquid extraction (LLE), solid phase extraction (SPE), solid phase microextraction (SPME), stir bar sportive extraction (SBSE) and solid/liquid extraction (SLE) have been evaluated. Separation techniques such as gas chromatography (GC) or high performance liquid chromatography (HPLC) coupled with different detectors have been compared in terms of detection limits and practical applications.

Keywords: Phthalates, sample preparation, liquid chromatography, gas chromatography

1. Introduction

Diester of phthalic acid, commonly referred to as phthalates (PAEs), are a group of chemical compounds widely used in industry and commerce due to their large variety of uses. Due to the ability to improve softness and flexibility to plastics, they are used mainly as plasticizers in a wide variety of products including medical devices, children's toys and all types of packaging. Furthermore, phthalates are also used as industrial solvents and lubricants, as an additive in the textile industry and in pesticides, and also in personal care products such as deodorants, lotions and perfumes, to retain colour and fragrance [1-4]. The main drawback of the use of PAEs is that they can migrate from the material to the environment and pollute water, soil, air and food-products. Furthermore, certain phthalate esters and/or their metabolites are suspected to be human carcinogenic agents and endocrine disruptors, [5] which make their trace determination of special importance. In particular, dibutyl phthalate (DBP), butyl benzyl phthalate (BBP), and di-(2-ethylhexyl) phthalate (DEHP) are in the list of the proposed substances suspected of producing endocrine alterations published by European Union (EU) [6].

Section 307 of US Clean Water Act establishes that dimethyl phthalates (DMP), diethyl phthalate (DEP), butyl benzyl phthalate, dibutyl phthalate, di-(2-ethylhexyl) phthalate and dioctyl phthalate (DOP) must be considered priority toxic pollutants [7]. These concerns have been further aggravated by recent analysis of human blood and urine samples, where traces of various phthalates (or their metabolites) have been found [8, 9]. For these reasons, the interest in the study of these types of chemical substances has increased during the last few years, and therefore it is essential to develop reliable and sensitive analytical methods to determine this group of compounds at trace levels.

This review summarizes and discusses the analytical methods and techniques described in the literature for phthalate determination in different matrix samples

(water, soil sediments, sludge, air and biological samples). Different sample treatments, extraction and preconcentration steps, such as liquid-liquid extraction (LLE), solid phase extraction (SPE), and solid phase microextraction (SPME) have been evaluated. Separation techniques such as gas chromatography (GC) or high performance liquid chromatography (HPLC) coupled with different detectors, such as UV detector, flame ionization detection (FID), electron capture detection (ECD) or mass spectrometry (MS) (all types of MS analyzes) have been compared in terms of detection limits and practical applications.

The major problem in phthalate analysis is sample contamination, resulting in false positive results or over-estimated concentrations. Due to the fact that phthalates are widely used, they are present in air, water, and organic solvents and plastics; they are adsorbed onto glass and other materials. Therefore, the risk of contamination is present in the whole analytical scheme, including sampling, sample preparation and analysis by chromatography. The different cleaning methods proposed in the literature, for avoiding contamination from material used in the laboratory, have been reviewed in this work.

2. Sample preparation

In order to detect PAEs at sub ppm levels, a clean up/preconcentration step is necessary before instrumental analysis. Different methods have been developed with this purpose such as liquid-liquid extraction (LLE), liquid-phase microextraction (LPME), single drop microextraction (SDME), solid phase extraction (SPE), solid phase microextraction (SPME), stir bar sorptive extraction (SBSE) and solid/liquid extraction (SLE).

2.1. Liquid-Liquid extraction (LLE)

Various liquid-liquid extraction (LLE) approaches have been used for isolation of PAEs from aqueous samples. In these methods, the extraction is carried out in a funnel, mixing the sample with an organic solvent, such as, hexane, dichloromethane, cyclohexane or ether. After the extraction, the organic phase is dried and concentrated to obtain higher sensitivity.

Different LLE methods have been developed for PAEs and their metabolites determination in different biological matrices. Mortensen et al. [10] used a liquid extraction with a mixture of ethyl acetate and ciclohexane (95:5) for a quantitative determination of PAEs in human milk by LC-MS-MS. On the other hand, Sorensen [11] extracted these compounds from milk and milk products using a mixture of tert-butyl methyl ether and hexane using the same determination technique.

A LLE method for DEHP in serum samples was used by Faouzi et al. [12] using a mixture of 2 mL acetonitrile and 2 mL sodium hydroxide (1N). The sample with the mixture acetonitrile:sodium hydroxide was shaken for 10 min using an alternating agitator and centrifuged at 3000 rpm. The clear supernatant was then injected into the chromatograph for the analysis.

DEHP has been extracted by Kambia et al. [13, 14] from total parenteral nutrition and from plasma. In this case, the sample (1 mL) was treated with 1M sodium hydroxide (1 mL) and hexane (2 mL). The mixture was vortexed (2 min), centrifuged (1620 x g for 5 min) and the separated organic layer (fraction 1) was transferred into a clean conical glass tube. The aqueous phase was extracted again with 2 mL of hexane and the mixture treated as above. The separated organic phase (fraction 2) was combined with the fraction 1 and the total organic phase was evaporated to dryness in a water bath at 40ºC under a nitrogen stream. The residue was dissolved in 100 μL of acetonitrile and 20 μL of this solution was injected in HPLC. These authors applied the same extraction procedure for the determination of DEHP in human plasma samples [15]. Recently, Ji-an Chen et al. [16] analyzed

di-n-buthyl phthalate and other organic pollutants in Chongquing women undergoing parturition. The authors analyzed these compounds in venous blood, umbilical cord blood, breast milk and urine. For PAEs extraction from blood and milk, the samples were first treated with anhydrous sodium sulfate (until saturation). The resulting solution was extracted with 10 mL of hexane by vortexing for 30 min. The top organic layer was collected and the remaining sample was extracted again using the same procedure. The two extracts were combined and the solvent was evaporated under nitrogen flow until 1 mL of solution remained. Each urine sample was extracted twice with 10 mL of hexanol/ethanol (8:1) in a separating funnel. The extracts were combined and the solvent evaporated as above.

Recently, Orsi et al. [17] used a simple and rapid method for the determination of PAEs presents in nail cosmetics. The method is based on ultrasonic extraction of the sample with ethanol-water (90:10 v/v) followed by HPLC separation and UV detection.

LLE procedures have some disadvantages such as, the use of high volumes of solvents. In addition, the process, generally, was off-line, and time consuming. LLE is limited due to the presence of trace levels of phthalates in commercially available solvents, even solvents for trace analysis. Therefore, accurate determinations below 0.1 μg L^{-1} are questionable with this method.

In recent years, studies have been carried out towards miniaturization of liquid-liquid extraction procedures, reducing the amount of organic solvent.

Liquid-phase microextraction (LPME) is a new method for sample preparation, whereas only a few microliters of solvents are used to preconcentrate compounds from aqueous samples [18]. In this technique, a microdrop of organic, water immiscible solvent is suspended from a microsyringe needle, which is then immersed in a stirred aqueous sample solution for a specified period of time [19-23]. After equilibrium, the microdrop of organic solvent loaded with the analyte is

determined. This method has been termed single-drop microextraction (SDME). It can be performed in different modes, including: static liquid-phase microextraction (S-LPME), dynamic liquid-phase microextraction (D-LPME), continuous-flow liquid-phase microextraction (CF-LPME), solvent bar liquid-phase microextraction (SB-LPME) and headspace liquid-phase microextraction (HS-LPME) [24]. The advantages of this technique are: the simplicity, low cost, ease of operation, rapid, small volume of organic extractants, and minimal exposure to toxic organic solvents, being environmental friendly.

Recently, Farahani et al. [25] developed a method for phthalate determination in water samples using a liquid-phase microextration (LPME) prior to the analysis by gas chromatography. In the method proposed by these authors 10.0 mL of aqueous sample was transferred into an 11.0 mL vial. Then, 10.0 μL of 1-dodecanol were delivered to the solution surface using a microsyringe. The vial was sealed and then the magnetic stirrer was turned on. Under the proper stirring conditions, the suspended microdrop remains in the top-center position of the aqueous sample. The microdrop movement was affected by the flow field, which favores the promotion of the mass transfer inside the microdrop [26]. After the desired extraction time, the sample vial was transferred into an ice beaker and the organic solvent was solidified after 4 min. Then, the solidified solvent was transferred into a conical vial and melted immediately. Finally, 1.00 μL of the extractant was injected into the gas chromatograph.

In some cases, S-LPME can present certain disadvantages (depending on the organic solvent and the volume on the organic drop, usually no more than 5 μL) such as instability of microdrop, relatively low reproducibility and sensitivity. Thus, to avoid these problems, Jinrong Yao et al. [24] proposed a modified S-LPME method, for phthalate determinations in landfill leachates. The most attractive feature of this method is the use of a polychloropropene rubber tube (PGR tube) instead of a microsyringe to load the organic solvent. The PGR tube

and the sample vial were placed horizontally, so the selection of the organic solvent was not affected by the density of the extractant. The authors used this method for DMP, DEP and DnBP determination in landfill leachates by liquid chromatography, obtaining good precision and recovery.

A fast and simple method, using HF-SDME, has been developed by R. Batlle et al. [27], to facilitate the identification and quantification of seven dialkil phthalate esters (Diethylhexil phthalate (DEHP), dibutyl phthalate (DBP), diisobutyl phthalate (DiBP), dimethyl phthalate (DMP), diisopropyl phthalate (DiPP) and diethyl phthalate (DEP)) in three aqueous food simulants (distilled water (A), 3% (w/v) acetic acid/water (B) and 15% (v/v) ethanol/water (C)). In this study, better results have been obtained using 7:3:0.5 Dichloromethane:hexane:toluene for food simulants A and C, and 1:9:0.5 Dichloromethane:hexane:toluene for food simulant B as extractant solvent. The method has been shown to be highly practical because of its high reproducibility, convenient dynamic range and detection limits. Therefore, this methodology reduces the amount of solvent necessary for the whole procedure (5 μL), thus eliminating the need for additional cleaning or concentration steps. The same technique was used by Pisillakis et al. [28], for PAE_S determination from water, using toluene as extraction solvent.

Recently, Pie Liang et al. [29] developed the fist method for PAEs determination using continuous-flow microextraction (CFME) combined with liquid chromatography with variable-wavelength detector (VWD). The CFME procedure consists of four steps: (1) the sample solution is continuously pumped and vertically upward at a constant flow rate into the bulb glass extraction chamber (~ 0.2 mL) via the connecting PTFE tube; (2) after the chamber has been filled with the sample solution, the required volume of organic solvent (3.0 μL of tetrachloromethane) is introduced into the extraction chamber by the microsyringe, and forms a drop at the tip of the microsyringe and remains above the PTFE tube outlet in the extraction chamber; (3) as the solvent drop is immersed into the

sample solution, the analytes are extracted into the solvent drop from the sample when the sample is continuously ejected from the PTFE tube into the chamber (flow rate 0.4 $mLmin^{-1}$); (4) after extracting for a prescribed period of time, the solvent drop is retracted into the microsyringe, and the microsyringe is removed from the chamber. Then, the needle tip is cleaned carefully with a tissue to remove possible water contamination, and the extraction solvent with the extracted analyte is injected into the LC system for analysis. The enrichment factors of this method for DMP, DEP and DnBP reached at most 27, 44 and 20 respectively.

2.2. Solid phase extraction (SPE)

SPE appears to be a more suitable technique for PAEs extraction and preconcentration from water or aqueous samples. The advantage of this technique, with respect LLE is that it requires a minimal use of solvents, thus reducing health risk and sample contamination, permiting the simultaneous extraction of multiple samples. Moreover, a large concentration factor can be obtained without solvent concentration, avoiding the concentration of concomitants present in the organic solvent.

SPE is carried out using different sorbents. The most widely used sorbents to extract PAEs from water, urine sample, wine etc., are silica polymers such as silica-based C18 and C8 [30-41]. Other sorbents used are organic polymers such as polyestirene [42], crosslinked polystyrene-divinylbencene (PS-DVB) or hydroxilated PS-DVB [43-45], styrenedivinyl polimer (PS-2), octadesyl-coated styrenedivinylbenzene polymer (SDB-XD) [33] and styrene-divinylbenzene methacrylate copolymer [46-48]. The authors justified the use of these organic polymers because the loading properties of organic carbon are superior to those of silica-based adsorbents. Another alternative proposed by Ya-Qi Cal et al. [49] is the use of polytetrafluoroethylene (PTFE) as sorbent in SPE. The use of this compound as a sorbent is based on the the fact that PTFE shows a very strong

hydrophobic property and that PTFE experimental containers can readily adsorb neutral hydrophobic compounds in their surfaces [50, 51]. In this way, the authors studied the potential of PTFE turnings as the matrix for SPE of trace phthalate esters from aqueous samples. By driving the aqueous sample solution to pass through a PTFE turnings in the column, the analytes were retained on the PTFE turnings packed column. The retained analytes were then eluted with acetonitrile, followed by the HPLC-UV analysis. The method proposed presents acceptable recovery results (92.1-127.5%) for the five phthalates studied (DBP, DCHP, DOP, DNP and DDP). The use of PTFE turnings as SPE sorbent, presents clear advantages such as durability and easy of availability.

Another SPE sorbent used by Ya-Qi Cai et al. [52] is the Multi-walled carbon nanotubes (MWNTs). MWNTs possess many unique electronic, mechanical and chemical properties, high surface area, and excellent strength, being very appealing for a great number of important applications [52]. The authors demonstrated for the first time that MWNTs can be used as effective adsorbent for SPE of four phthalates (DEP, di-n-propyl-phthalate, di-iso-butyl-phthalate and di-cyclohexyl-phthalate) from aqueous samples. Moreover, the authors compared the analytical performance of this method with others using commercial SPE adsorbents such as C18, C8 and PS-DVB. The results showed that multi-walled nanotubes were more effective than, or as effective, as these adsorbents for the SPE of these four analytes.

An alternative method was proposed by Yoshihiro Saito et al. [53] using a miniaturized solid-phase extraction coupled with HPLC-UV. In this case, the authors used a fiber-in-tube capillary for SPE. Fiber-in-tube capillary was prepared by packing Zylon® filaments (11.5 µm i.d. x 100 mm) into a poly(ether ether ketone) (PEEK) tube of 0.25 mm i.d. x 100 mm. These filaments were longitudinally packed into the tube, and the total number of packed filaments was

about 330. With this method the authors obtained limits of quantification for DBP and DEHP in wastewater lower than 0.5 ng mL^{-1}.

A sensitive and selective column adsorption method was proposed by Hatsumata et al. [54] for the off-line preconcentration and determination of PAEs (BBP, DBP, DCHP). The PAEs were preconcentrated on *Saccharomyces cerevisiae* immobilized on silica gel and then determined by HPLC. With the proposed method the preconcentration step takes about 30 min for 50 mL of aqueous sample. The maximum preconcentraction factor was 40 for BBP and DBP, and 80 for DCHP. The recovery of spiked PAEs in a river water sample was in a range 98-101%.

Recently, micelle-like surfactant aggregates adsorbed on solid materials (namely hemi-micelle or admicelle) in SPE have been studied as a good alternative for the preconcentration of a variety of organic pollutants. The sorbents used in SPE are produced by adsorbing ionic surfactants (such as sodium dodecylsulfate or cetyltrimethylammonium bromide) on the metal oxides (such as alumina or silica). The use of this technique in SPE has many advantages, such as high extraction efficiency, high breakthrough volume, easy elution of analytes and high flow rate for sample loading; furthermore, this technique requires no clean-up steps and the adsorbents are easy to regenerate [55]. In this way, Tohru Saitoh et al. [56] studied the Aerosol-OT-ν-alumina admicelles for the concentration of hydrophobic organic compounds in water (including phthalates). The AOT-ν-alumina admicelles were successfully prepared by mixing ν-alumina and di-2-ethylhexylsodium sulfosuccinate (AOT) in a weakly acid aqueous solution. The large sample loading capacity of the AOT- ν-alumina admicelles enables highly an efficient concentration of trace analytes. The authors obtained a concentration factor for different compounds, including DEP, DBP and DEHP, of 500 fold.

F.J. López-Jiménez et al. [57] used sodium dodecyl sulfate (SDS)-alumina hemimicelles for the preconcentration of BBP, DBP and DEHP in environmental

water samples. The authors used cartridge columns filled with 500 mg of alumina. These cartridges were conditioned with 10 mL of a nitric acid solution (pH 2). Then, hemimicelles were formed on the alumina by passing a 25 mL 0.01 M nitric acid solution containing 40 mg of SDS. Recoveries of PAEs above 95% were obtained for all samples studied (raw and treated sewage samples) and a preconcentration factor of 500 can be easily achieved by SPE of 1 L of sample and elution with 2 mL of methanol.

Last year, Jidon Li et al. [55] analyzed five phthalates by HPLC-UV after preconcentration by SPE using ionic liquid mixed hemimicelles. The authors evaluated mixed hemimicelles prepared by adsorbing 1-Hexyl-3-methyllimidazolium bromide ([C_6 min] Br) and 1-Dodecyl-3-methyllimidazolium bromide ([C_{12} min] Br) on silica surface on the phthalate concentrations. In this case, ([C_{12} min] Br)-coated silica as adsorbents was selected for the preconcentration step due to the higher capacity for the analytes and the preconcentration factor obtained was 600 folds.

Table 1 summarizes the applications and different conditions of SPE for phthalates determination preconcentration.

Table 1. *Applications of solid phase extraction (SPE) for phthalate analysis*

Sample	Analyte	Sorbent Material	Sorbent conditioning	Elution	Determination	Ref
Urine	DEHP	HySphere-C18 HD (on-line)	4 mL methanol followed by 4 mL water and 4.0 mL acidified buffer	0.1% acetic acid-acetonitrile (75:25 v/v)	HPLC-MS/MS	3
Urine	MBP, MBP,MEHP	HySphere-C18 HD (on-line)	4 mL methanol followed by 4 mL water and 4 mL acidified buffer	0.1% acetic acid-acetonitrile (75:25 v/v)	HPLC-MS/MS	9
Human milk, comsumer milk and infant formula	mMP, mEP, mBP, mBzP, mEHP, mNP	Oasis HLB, Waters	Acetonitrile and basic buffer	Acetonitrile (2 mL)	LC-MS-MS	10
River Water	MMP, MBP, MEHP, DMP, DBP, DEHP	PS-2 and C18; SDB-XD	Dichlorometane, acetone, methanol and purified water; acetone	Dichlorometane	GC-MS	33
Food, water, tea, coffee	DMP, DEP, DBP, BBP, DEHP	Oasis HLB glass (6 mL; 0.5g)		2 mL methanol	GC-MS	34

***Table 1**. (Continued)*

Sample	Analyte	Sorbent Material	Sorbent conditioning	Elution	Determination	Ref
Textile wastewater	DBP, Bis(2-ethylhexyl) phthalate	C18 and Isolute ENV+ cartridge	7 mL of methanol followed 3 mL of water at 1mLmin^{-1}	2 x 5 mL of methanol/hexane; 10mL of solution 5mM in triethylamine (TEA) and 5mM acetic acid/methanol (1:9) v/v	LC-APCI-MS	35
Water	DMP, DEP, DBP DEHP	C18	Deionized water followed by about 2 mL of methanol	2 mL of methanol in dichloromethane (50:50 v/v)	GC-FID	36
Extracts of sludges	DEP, DBP DEHP	C18	7 mL of methanol and 3 mL of HPLC water	Hexane/methanol 9:1	LC-MS	37
Urban wastewater samples	DMP, DEP, DBP, BBP, DEHP, DOP	LiChrolut RP-18 (C18)	5 mL diethyl ether, 5 mL methanol and 5 mL deionized water	6 mL mixture of diethyl ether-methanol (9:1; v/v)	LC-MS	38
Wastewater	DMP, DEP, DBP, BBP, DEHP, DOP	LiChrolut RP-18 (C18)	5 mL diethyl ether, 5 mL methanol and 5 mL deionized water	6 mL mixture of diethyl ether-methanol (9:1; v/v)	GC-MS	39

***Table 1**. (Continued)*

Sample	Analyte	Sorbent Material	Sorbent conditioning	Elution	Determination	Ref
Drugs	DEHP, MEHP	Oasis HLB extraction column (on-line)		Acetonitrile/Water 90/10 (v/v)	LC-MS/MS	40
Human milk	13 phthalate metabolites	Oasis HLB column	2 mL HPLC grade methanol and 2 mL water	0.5 mL acetonitrile	HPLC-MS/MS	41
Water	BBP, DEHP	Polyestyrene based SPE column	Dichloromethane (6 mL), methanol (6 mL) and water (6-7 mL)	Methanol + dichlorometane (1+1) v/v	HPLC-UV	42
Water, landfill leachates	DMP, DEP, DBP, BBP and DEHP	Hydroxilated polyestirene-divinilbenzene polymer	5 mL of ethyl acetated followed by 5 mL of methanol and conditioned with 5 mL of acidified water	Toluene and ethyl acetate	GC-MS	45
Urine	MMP, MEP, MBP, MCHP, MEHP, MOP,MEOHP, MEHHP	Stirene-divinylbenzene methacrylate copolymer SPE cartridge	Acetonitrile (1 mL) and pH 2 phosphate buffer (1 mL, 0.14M NaH_2PO_4 in 0.85% H_3PO_4)	1 mL of acetonitrile followed by ethyl acetate (1 mL)	HPLC-MS/MS	46

***Table 1**. (Continued)*

Sample	Analyte	Sorbent Material	Sorbent conditioning	Elution	Determination	Ref
Urine	miBP, PA, mCPP, mEP, mBP, MBzP, mEHP, mEHHP and mEOHP	Stirene-divinylbenzene methacrylate copolymer SPE cartridge	Acetonitrile (1 mL) and pH 2 phosphate buffer (1 mL, 0.14M NaH_2PO_4 in 0.85% H_3PO_4)	1 mL of acetonitrile followed by ethyl acetate (1 mL)	HPLC-ESI-MS/MS	47
Wastewater	DBP, DMP DEHP	Stirene-divinylbenzene sorbent Lichrolut EN	7 mL methanol and 3 mL water	2 x 5 mL acetonitrile waiting 5 min between the two aliquots	LC-APCI-MS	48
Water	DBP, DCHP, DOP DNP, DDP	PTFE turnings	5 mL of acetonitrile and 10 mL of Milli-Q purified water	10 mL of acetonitrile	HPLC-UV	49
River and sea water	DEP, di-n-propyl-phthalate, di-iso-butyl-phthalate and di-cyclohexyl-phthalate	Multi-walled carbon nanotubes	Washed with 5 mL of methanol and activated with 5 mL of water.	Acetonitrile (5 mL)	HPLC-UV	52

Table 1. *(Continued)*

Sample	Analyte	Sorbent Material	Sorbent conditioning	Elution	Determination	Ref
River water	BBP, DBP, DCP	*Saccharomyces cerevisiae* immobilized on silica gel	50 mL of HCl (1M) and 50 mL of water.	2.5-7.5 mL of acetone, acetonitrile, ethanol and or methanol	HPLC-UV	54
Environmental samples	DEP, DnPP, DnBP, DcHP, DEHP	($[C_{12}$ min] Br)-coated silica	An extraction cartridge was prepared with 0.5 g of silica. Then 25 mL solution containing 25 mg of [C_{12} min] Br was passed through the cartridge column.	3 mL methanol pH 2	HPLC-UV	55
Water	DEP, DBP, DEHP	AOT-γ-alumina admicelles	Alumina was ultrasonically washed with 1M nitric acid for 3 min and thoroughly rinsed with water. To prepare an admicelle column, 50 mL of 0.01M nitric acid containing AOT was passed through a cartridge filled with alumina.	1 mL acetonitrile	HPLC	56

***Table 1**. (Continued)*

Sample	Analyte	Sorbent Material	Sorbent conditioning	Elution	Determination	Ref
Raw and treated sewage samples	DEHP, BBP, DBP	Hemimicelles of sodium dodecyl sulphate (SDS) produced on alumina	10 mL of a nitric acid (pH=2). Then hemimicelles were formed on the alumina passing 25 mL0.01M nitric acid solution containing 40 mg SDS	2 mL of methanol	LC-MS	57
Water	DMP,DEP, BBP, DBP, DEHP, DOP	ENV+ cartridge	10 mL acetonitrile and 10 mL reagent water	10 mL acetonitrile	LC-APCI-MS	106
Water	DnBP, BBP, DEHP	LiChrolut EN	3 mL methanol and 3 mL Milli-Q water; 5 mL acetonitrile and 5 mL Milli-Q water	On-line 100 µL ethyl acetate; 5 mL acetonitrile	GC-MS; HPLC-ESI-MS	107
Urine	MEP, MBP, MCHP, MBzP, MOP, MEHP, MDP	Oasis HLB, Waters	1 mL acetonitrile followed 2.0 mL of basic buffer	Acetonitrile (2 mL) followed Ethyl acetate (2 mL)	HPLC-APCI-MS	108
Urine	11 urinary phthalate metabolites	Nexus SPE cartridges (Varian)		Acetonitrile (2 mL) followed Ethyl acetate (2 mL)	HPLC-APCI-MS/MS	111

2.3. Solid-phase microextraction (SPME)

Solid-phase microextraction (SPME) in sample preparation became very popular in the late 1990s [58]. In this extraction technique, a fused silica fiber coated with a thin layer of polymer phase is immersed in the aqueous sample, while the sample is stirred. After a certain amount of time (range from minutes to hours) the coated fiber is retracted and transferred in a holder to the GC and desorbed in a hot inlet. The adsorbed compounds are desorbed and injected into the chromatographic column for the analysis. The SPME procedure (sampling, extraction concentration and sample introduction in one step) significantly reduces the risk of contamination and simplifies the overall analytical process. Several studies employing direct SPME for extraction of phthalates from water have been published. These methods used different fibers such as polyacrilate [59, 60], carbowax [61], carbowax-divinilbencene (CW-DVB) [62-64], polyaniline (PANI) [65], polydimethylsiloxane (PDMS) [64, 66] or polydimethylsiloxane-divinilbencene (PDMS-DVB) [63, 64, 67-69]. SPME has a great number of applications in water samples, but in recent years other applications have appeared in complex matrices, such as, vegetable oil or milk samples. For example, Holadová et al. [70] used a headspace-solid-phase microextraction for PAEs determination in vegetable oil. The authors compared the results obtained using different SPME fibers (silica fibers coated with Polydimethylsiloxane (PDMS), poliacrylate, carboxen/polidimethylsiloxane and polydimethylsiloxane-divinilbencene) and different matrix modifiers (hexane, methanol, acetonitrile, dimethylformamide, and water). The results showed that, employing PDMS 100 together with methanol as the matrix modifier, headspace SPME phthalate determination in vegetable oil samples is possible. Some applications and the experimental conditions, using SPME are summarized in the *Table 2*.

The main drawbacks with SPME are that extraction fibers are expensive and have a limited life [71], sample carry-over between extractions has been reporter for some analytes [72] and for limited types of SPME fibers commercially available.

A modification of the SPME technique is in-tube SPME. This is a microextraction and preconcentration technique using an open tubular fused-silica capillary with an inner surface coating as the SPME device. The advantage of this technique is that it can be coupled on-line with HPLC, allowing complete automation, shortening analysis time and improving accuracy and precision. Mitami et al. [73] developed a method for the determination of nine phthalates in infusion solutions in plastic containers using in-tube SPME-HPLC with limits of detection in the range of 1-10 ng mL^{-1}. In this case, the analytes were extracted from the sample directly into an open-tubular capillary (a Supel-Q Plot capillary column (60 cm x 0.32-mm i.d., 12 µm) by 20 repeated draw/eject cycles of 40 µL of sample solution. The extracted compounds were desorbed using a mobile phase flow (acetonitrile: water). The in-tube SPME method has shown a sensitivity 18-125 times higher than the direct injection method. Cháfer-Pericás et al. [74] used the same technique for DBP and DEHP determination in environmental samples. In this case, the TRB-5 coated capillary was used as an in-tube SPME device and the number of cycles to carry out the extraction was fixed at 7 using 50 µL of sample. The limits of detection obtained were 1 and 2.5 µg L^{-1} for DBP and DEHP, respectively.

***Table 2**. SPME Methods for the phthalate analysis*

Sample	Analyte	Fiber Type	Sample volume and Treatment	Determination	Ref
Water	DnBP, DEHP	65 µm polydimethylsyloxane-divinilbencene 65 µm carbobax-divinilbencene, 85-µm polyacrylate, 75 µm carboxen-divynylbencene and 30 µm polydimethylsyloxane	3.5 mL of sample (pH 6). Extraction time 30 min at 80ºC.	GC-MS	59
Water	DMP, DEP, DnBP, BBP, DEHP, DOP	PDMS, PA	3 mL of water. Extraction during 20 min. Desorption during 5 min at 250ºC.	GC/ECD	60
Water	DBP, DEHP	Carbowax	4 mL of sample. Extraction time 15 h (stirring at 1000 rpm). Desorption with 100 µL of methanol:ethanol (80:20) during 2 min.	HPLC-ESI-MS	61
Water	DMP, DEP, DBP, BBP, DEHP, DOP, DNP	PDMS, PA, PDMS-DVB, DVB-Carboxen-PDMS, CW-DVB	5 mL of sample. Extraction time 45 min at 22ºC. The desorption time was 5 min at 270ºC	GC-MS	62

***Table 2**. (Continued)*

Sample	Analyte	Fiber Type	Sample volume and Treatment	Determination	Ref
Cow milk	DMP, DEP, DBP, BBP, DEHP, DOP	PDMS, PDMS/DVB, CAR/PDMS, DVB/CAR/PDMS, PDMSDVB-StablFlex-65µm, CW/DVB- StablFlex-70µm, Polyacrylate	Five grams of cow milk weighed into a 15 mL SPME vial. A magnetic stirring bar and 2.5 g of sodium chloride were added into the vial. Extraction time 2 min at 90°C. The extraction time 60 min at the same temperature.	GC-MS	63
Drinking water	DMP, DEP, DPP,	PDMS, PDMS/DVB, CW/DVB	5mL of sample. Extraction time 30 min at room temperature (stirring at 1200 rpm). Desorbed into the mass spectrometer during 40 s.	Fiber introduction mass spectrometry (FIMS)	64
Water	DMP, DEP, DAP, DBP, DOP	Polyaniline (PANI)	10 mL of sample. The solution was continuosly stirred during 20 min at 30°C. The desorption temperature were 280°C during 3 min.	GC-FID	65
Water	DEP, BBP, DOP	PDMS	30 mL of water. Extraction time: 15-60 min. Extraction temperature: 30-90°C. Desorption time: 1-5 min	GC-MS	66

***Table 2**. (Continued)*

Sample	Analyte	Fiber Type	Sample volume and Treatment	Determination	Ref
Wastewater	DMP, DEP, DBP, BBP, DEHP, DOP	CW-DVB, PDMS, PDMS-DVB, PA and CAR-PDMS	10 mL of sample. Extraction time 80 min. Desorption time was 5 min at 260ºC.	GC-MS	68
Aqueous media	DEP	PDMS/DVB, CW/TRP (carbowax/templated resin)	The extraction time was 15 min. desorption with 500 µL of the mobile phase (acetonitrile:water 52.5:47.5) or acetonitrile	HPLC-UV	69
Vegetable oil	DMP,DEP, DnBP, BBP, DEHP, DnOP	PDMS	Oil sample modified with 1 mL methanol. The sample was incubated 60 min at 40ºC. The extraction time was 20 min. Desorption temperature was 250ºC	GC/MSD and GC/ECD	70
Environmental water samples	DBP, DEHP	Capillary coated with 95% polydimethilsiloxane and 5% polydiphenylsiloxane (TRB-5)	100 µL of sample (.Flow rate for the extraction procedure 300 µLmin^{-1}(30 extraction cicles). Desoprtion with 100 µL of mobile-phase	HPLC-UV	74

2.4. Stir bar sorptive extraction

Stir bar sorptive extraction (SBSE) was first introduced by Baltussen et al. in 1999 and based on the same principles of SPME [75]. The authors used polydimethylsiloxane (PDMS) (50-300 µL), coated in a stir bar, to preconcentrate different analytes. The amount of PDMS coated in the stir bar is considerably higher than in SPME fiber; thus, the results showed high recoveries, better sensitivity and higher capacity. In the first works using this technique, once the extraction step was over, the stir bar was dried and the analytes thermally desorbed in a desorption unit, usually installed in a gas chromatograph. In this way, Prieto et al. [76] used SBSE for simultaneous preconcentration of a wide variety of organic pollutants (including DMP, DEP, DBP, BBP, DEHP and DOP) from water samples, obtaining, good sensitivity and recovery. Tan et al. [77] used the same technique for trace analysis of selected endocrine disruptors (including DEP, DBP, BBP, DEHP) in water, biosolid and sludge samples. The method has many practical advantages such as small sample volume (10 mL aqueous or < 1g sludge sample) and simplicity of extraction.

An alternative to SBSE with thermal desorption (TD), is liquid desorption (LD). In this case, the analytes are desorbed using a small amount of organic solvent. LD can also be combined with GC and a large volume injection (LVI) when a thermal unit is not available. In this way, Serôdio et al. [78] developed a method for endocrine disrupter chemicals determination (including BBP and octylphthalate) in water using SBSE-LD in combination with LVI and GC coupled to mass spectrometry. In this case, the extraction procedure was performed for 60 min with a stirring speed of 750 rpm at room temperature (20ºC). After sampling the stir bars were removed with a clean tweezers dried with a lint-free tissue and placed into a 2 mL glass vial filled with 100 µL of acetonitrile ensuring the total immersion. Solvent back extraction was performed using ultrasonic treatment for 15 min at a constant temperature (25ºC). Afterward, the stir bars were removed; the acetonitrile

extract was evaporated under gently purified nitrogen and after redissolved in 80 µL of ethyl acetate. The method described presents an excellent linear dynamic range for almost all endocrine disrupter chemicals from waters samples at ultra-trace level (0.025-0.400 μgL^{-1}). Later, the same authors studied a method for phthalates (DMP, DEP, DBP, BBP, DEHP, DOP) determination in drinking water, using the same technique [79]. The only difference is that in this case, methanol was used as a back extraction solvent. With the proposed method, low detections limits were obtained for all phthalates studied (3-40 ngL^{-1}).

Another application of SBSE with LD for phthalate determination was proposed by L. Brossa et al. [80]. The authors studied the extraction procedure for DEP, DNBP, DEHP BBP, DnOP determination by GC-MS. The extraction procedure was carried out with 10 mL of aqueous sample (containing the analytes), 20 gL^{-1} of NaCl and 10% methanol. The stir bar was immersed in the vial containing the aqueous sample for 30 min at 50ºC and 1200 rpm. Then, the stir bar was removed and dried. The analytes were desorbed by placing the stir bar in a vial containing 0.5 mL of isooctane in the stirrer unit (1000 rpm) for 30 min at room temperature. The limits of detection obtained were between 0.02-5 μgL^{-1}.

2.5 Solid/liquid extraction

The analysis of PAEs in solid samples is more complex than in liquid samples due to the difficulty to extract the compounds from the solid matrix. The technique commonly used for PAEs extraction from solid samples is the Soxhlet extraction. Different authors have been reported in the literature about PAEs extraction from solid samples using this technique [81-84]. Different solvents have been used such as ethyl acetate, for the PAEs extraction from packaging films [84], n-hexane or acetone/n-hexane from soil [85], methanol from plant matter [85], n-hexane/methyl ethyl or acetone/methanol from sludge [86], dichlorometane from dump [87] and sludges [88], acetone/hexane (1:1) from soil and biosolids [83] and

hexane/dichloromethane from soil [89]. The methods reported in the literature using this technique present a good recovery, but, the main problem from Soxhlet extraction is the time needed (as higher as 10 h) for total extraction.

A modification of the Soxhlet extraction is the system used by Sablayrolles et al. [90]. These authors used a Soxtec System HT2 (Tecator, France) for PAEs extraction from sludge and vegetables. This is a semi-automated apparatus working on the Soxhlet principle, while allowing extractions which are faster, more economical (better solvent recuperation) and safer (dissociation of the extraction and heating units). The solvent used in this case for the extraction procedure was hexane.

Another alternative for the solid/liquid extraction is the ultrasonic extraction. This technique has been applied to PAEs extraction in different matrices such as suspended matter, soil and liver samples using different solvents such as acetone, acetone-petroleum ether (1:1 v/v), methanol and a mixture of acetonitrile phosphoric acid and sodium chloride [44, 91-94]. The advantage of this technique compared with Soxhlet extraction is the time required. In this case, the extraction can be performed in times less than 1 h (usually 10-15 min). However, for some PAEs in soils this method is less effective than Soxhlet extraction, obtaining bad recoveries [92].

Microwave assisted extraction (MAE) has been applied to the extraction of PAEs from solid samples such as atmospheric particle matter or sediments. MAE consists of heating the sample with the extracting solvent inside the extraction vessel with microwave energy. This technique presents advantages compared with the traditional techniques, such as being less time consuming, using lower volume of organic solvent, offering automated temperature control and capability of processing different samples in the same time. In this way, E. Cortazar et al. [95] developed a method for DEHP determination in sediment samples using this technique. In this case, the authors used 100% methanol as extracting solvent and

the extraction procedure was carried out at 159 kPa for 15 min. L. Bartolomé et al. [96] developed a method for PAEs extraction from sediments using acetone as an extracting solvent using a pressure of 145 kPa during 15 min. O. Alvarez-Avilés et al. [97] determined DEP and BBP in atmospheric particulate matter using MAE-SBSE-TD-GC-MS. The optimized conditions for MAE were 20 mL of acetone at 80ºC for 10 min.

3. Separation techniques

Gas chromatography and liquid chromatography are the techniques usually used for PAEs separation in different matrices, such as environmental samples or biological samples.

3.1. Gas Chromatography

The analysis of phthalic acid esters (PAEs) is mostly performed by gas chromatography (GC). Generally, GC methods present better sensibility than HPLC methods, although this depends on the pre-treatment step, the instrumental conditions and the sample matrix [6]. Phthalates can be detected using electron capture detection (ECD) [60, 70], flame ionization detection (FID) [36, 65, 87, 98, 99] or mass spectrometry (MS). Some official methods (US EPA methods 606 and 8060) describe the use of ECD as a detector for phthalate determination. Although ECD detectors are relatively sensitive for phthalates, the specificity is restricted. The most recommended detector for phthalate analysis is mass spectrometry detection. All types of MS analyzers, including quadrupole analysers, triple quadrupole analyzers, ion traps and magnetic sector instruments have been used for phthalates determination [100-104].

The chromatographic separation was usually performed using capillary columns coated with phenyl methylpolisiloxane or dimethylpolysiloxane as stationary phase. The separations were carried out using different temperature programmes, usually varying the oven temperature from 50ºC to 300ºC. Some applications for PAEs determination using GC coupled with different detectors are shown in T*able3*.

***Table 3**. GC methods for PAEs determination*

Sample	Analyte	Analytical column	Oven temperature programme	Detector	Limit of detection	Ref
Blood, milk and urine	DBP.	Fused silica column phenyl methyl siloxane (30 m x 0.25 mm, 0.25 µm)	No linear gradient from 80ºC to 280ºC.	MS: m/z: 149		16
Water	DMP, DEP, DAP, DnBP, BBP, DCHP, DEHP	DB-5MS-fused silica column (30 m x 0.25 mm, 0.25 µm)	No linear gradient from 60ºC to 285ºC.	MS: m/z: 163/194, 149/177, 149/189, 149/223 149/206, 149/167, 149/279	0.02-0.05 μgL^{-1}	25
Food simulants	DMP, DEP, DiPP, DBP, DiBP, DEHP, DOP	DB-5 fused silica column (30 m x 0.32 mm, 0.25 µm)	No linear gradient from 60ºC to 285ºC.	FID	0.02-0.40 μgL^{-1}	27
Water	DMP, DEP, DBP, BBP, DEHP, DOP	HP-5MS capillary column (30 m x 0.25 mm, 0.25 µm)	No linear gradient from 60ºC to 300ºC.	MS. m/z: 163/194, 149/177, 149/223, 149/206/91, 167/149/279, 149/279	0.003-0.01 μgL^{-1}	28
Wine	DMP, DEP, DBP, BBP, iBP, DEHP	Restek RTX-5MS capillary column (5% diphenyl 95% dimethypolysiloxane) (30 m x 0.25 mm, 0.25 µm)	No linear gradient from 70ºC to 280ºC.	MS. m/z: 163 for DMP, 149 for the other PAEs	0.015-0.018 μgmL^{-1}	32

Table 3. *(Continued)*

Sample	Analyte	Analytical column	Oven temperature programme	Detector	Limit of detection	Ref
River water	MMP, MEP, MiPRP, MPRP, MIBP, MBP, MPEP, MCHP, MEHP, MBZP, MOP	HP-5MS capillary column (30m x 0.25mm, 0.25µm)	No linear gradient from 50ºC to 300ºC.	MS. m/z: 163/167 for quantification of monoesters. 149 for quantification of phthalic acid diesters excep DMP, in which m/z 163 was used.	0.010-0.030µgL^{-1}	33
Egypcians traditional foods and driks	DMP, DEP, DBP, BBP, DEHP	HP-5MS capillary column (5% diphenyl 95% dimethypolysiloxane) (80m x 0.25mm, 0.25µm)	No linear gradient from 60ºC to 230ºC	MS. m/z: 163.770, 149.177, 149.150, 149.910, 149.167, 129.112	40-100 ngL^{-1}	34
Wastewater	DBP, DEHP	DB-17-HT (30m x 0.25mm, 0.1µm)	40ºC increasing to 350ºC at 8ºC/min.	MS		35
Water	DMP, DEP, DBP, DEHP	PTE^{TM}-5 (30 m x 0.25mm, 0.25µm)	No linear gradient from 180ºC to 280ºC.	FID	28.5, 27.2, 42.7, 60.6 ngL^{-1}	36

Table 3. *(Continued)*

Sample	Analyte	Analytical column	Oven temperature programme	Detector	Limit of detection	Ref
Water	DMP, DEP, DnBP, BBP, DEHP, BnOP,	HP-5MS (cross-linked 5% methyl silicone) (30m x 0.25mm, 0.25µm)	60ºC increased to 280ºC at 20ºC/min; hold for 5 min.	MS. m/z: 149 for all phthalate esters except for DMP (163)	2-27 ngL^{-1}	59
Water	DMP, DEP, DnBP, BBP, DEHP, DnOP	DB-35 (35% phenyl 65% polymethylsiloxane) (30m x 0.25mm, 0.15µm)	No linear gradient from 45ºC to 270ºC. Total run time 50 min.	ECD	LOQ: 0.003-0.05 μgL^{-1}	60
Water	DMP, DEP, DBP, BBP, DEHP, DOP, DNP	HP-5MS (5% phenylmethylsiloxane) (30m x 0.20mm, 0.25µm)	60ºC (5 min), increased to 280ºC at 15ºC/min; hold for 5 min.	MS. m/z: 163/194, 149/177, 149/223, 149/206/91, 167/149/279, 149/279, 149/167.	0.005-0.04 μgL^{-1}	62
Cow milk	DMP, DEP, DBP, BBP, DEHP,DOP	DB-5 (30m x 0.25mm, 0.1µm)	No linear gradient from 55ºC to 280ºC.	MS. m/z: 163/77/194, 149/177/104, 149/223/104, 149/91/206, 149/167/279, 149/279/104	0.01-4.7 ngg^{-1}	63

***Table 3**. (Continued)*

Sample	Analyte	Analytical column	Oven temperature programme	Detector	Limit of detection	Ref
Water	DEP, BBP, BBP, DEHP, DOP	HP-5 capillary column (30m x 0.32mm, 0.25µm)	No linear gradient from 70ºC to 280ºC.	MS. m/z:149.177, 149.104, 149.91, 149.167, 149.279	0.07-3.15µgL^{-1}	66
Vegetable oil	DMP, DEP, DnBP, BBP, DEHP, DnOP	DB-35 (35% phenyl 65% polymethylsiloxane) (30m x 0.25mm, 0.15µm)	No linear gradient from 45ºC to 270ºC. Total run time 50 min.	ECD	LOQ: 0.2-0.5 mgkg^{-1}	70
Water	DMP, DEP, DBP, BBP, DEHP, DOP	HP-5MS (30 m x 0.25 mm, 0.25 µm) (5% diphenyl, 95% dimethylpolysiloxane)	No linear gradient from 50ºC to 290ºC during 39.33 min	MS. m/z: 77/163, 149/177, 104/149, 91/ 149, 149/167, 149/ 279	0.1-10 ngL^{-1}	76
Water, biosolid and sludge	DEP, DBP, BBP, DEHP	DB-5MS-fused silica column (30m x 0.25mm, 0.25µm)	No linear gradient from 50ºC to 300ºC during 40 min	MS. m/z: 149/177, 149/223, 149/206, 149/279.	2.0 ngL^{-1} and 0.02 ngg^{-1}	77
Water	BBP, MOP	HP-5MS (30 m x 0.25 mm, 0.25 µm) (5% diphenyl, 95% dimethylpolysiloxane)	No linear gradient from 50ºC to 280ºC during 40 min	MS. m/z: 149 and 91, 149 and 279	<25 ngL^{-1}	78

***Table 3**. (Continued)*

Sample	Analyte	Analytical column	Oven temperature programme	Detector	Limit of detection	Ref
Drinking water	DMP, DEP, DBP, BBP, DEHP, BOP	TRB-5MS (30 m x 0.25 mm, 0.25 µm) (5% diphenyl, 95% dimethylpolysiloxane)	No linear gradient from 70ºC to 280ºC during 24.50 min	MS. m/z: 163/77, 149/177, 149/150, 149/91, 129/112, 149/279	0.15-0.6 μgL^{-1}	79
Water	BBP, DOP	HP-5MS capillary column (30m x 0.25mm, 0.25µm)	No linear gradient from 60ºC to 290ºC. Total run time 30.64 min.	MS.	0.02 μgL^{-1}	80
Soils and biosolids	DMP, DEP, DnBP, DuBeP, DEHP, DnOP	DB-5MS-fused silica column (30m x 0.25mm, 0.25µm)	No linear gradient from 50ºC to 280ºC during 13.73 min	MS. m/z: 163 for DMP and DnOP, and 149 for other PAEs.	0.1-5.0 μgL^{-1}	83
Soil	DMP, DEP, DBP, DEHP,	Col-elite5 (39m x 0.25mm, 0.25µm)	No linear gradient from 180ºC to 280ºC during 13.73 min	FID		87
Sewage sludge	DEHP	TRB-Meta X5 Tracer, Technokroma, (30m x 0.25mm, 0.25µm)	220ºC 12 min hold, 20ºC/min to 300ºC, 7 min hold.	MS: m/z: 149/167		88

***Table 3**. (Continued)*

Sample	Analyte	Analytical column	Oven temperature programme	Detector	Limit of detection	Ref
Sludge end vegetables	DMP, DEP, DBP, BBP, DEHP, DOP	Restek RTX-5MS capillary column (5% diphenyl, 95% dimethylpolysiloxane) (30m x 0.25mm, 0.25µm)	Started at 50ºC for 1 min, followed by an increase of 20ºC/min to 310ºC which is manteined for 6 min.	MS.	0.003 μgmL^{-1}	90
Soil	DMP, DEP, DiBP, DBP, BMEP, BMPP, DAP, DHP, BBP, HEHP, BBEP	DB-5 fused silica column (30m x 0.25mm, 0.25µm)	No linear gradient from 50ºC to 280ºC.	MS. m/z: 163/149 for DMP and 149 for other PAEs.	0.001-0.022 μgg^{-1}	92
Sediments	DMP, DEP, DBP, BBP, DEHP, DOP	HP-5 capillary column (30m x 0.25mm, 0.25µm)	No linear gradient from 800ºC to 280ºC.	MS. m/z:163/77, 149/177, 149/223, 149/91, 149/167, 149/279	<25 $ngkg^{-1}$	95
Sediments	DMP, DEP, DBP, BBP, DEHP, DOP	HP-5 fused silica column (30m x 0.25mm, 0.25µm)	No linear gradient from 60ºC to 290ºC.	MS. m/z: 77/163, 149/177, 104/149, 91/149, 149/167, 149/279	0.5-22 ng	96

Table 3. *(Continued)*

Sample	Analyte	Analytical column	Oven temperature programme	Detector	Limit of detection	Ref
Atmospheric particulate matter	DEP, BBP	DB-5 fused silica column (30m x 0.32mm, 0.25µm)	No linear gradient from 40ºC to 280ºC.	MS	90, 20 ngL^{-1}	97
Food packaging films	DMP, DEHP, DMTP, DPP, DNP, DEP, DnBP, BBP	Supelco column MDN-5 (95% polydimethylsiloxane, 5% polydiphenylsiloxane) (30m x 0.25mm, 0.25µm)	No linear gradient from 50ºC to 310ºC.	FID	13.88-87.63 mgL^{-1}	98
Water	DMP, DEP, DPP, DIBP, DBP, DEHP	HP-5 capillary column (30m x 0.32mm, 0.25µm)	No linear gradient from 100ºC to 270ºC.	FID	0.2-4 μgL^{-1}	99
Olive oil	DMP, DEP, DDBP, BBP, DEHP, DOP	Varian Factor Four 5-ms (95% polydimethylsiloxane, 5% polydiphenylsiloxane) capillary column (30m x 0.25mm, 0.25µm)	No linear gradient from 100ºC to 250ºC.	MS-MS. m/z: 149/135, 149, 121/81, 121/65, 121/65, 121/65	4.6-168 μgkg^{-1}	101
Migration from toys and childcare articles	DINP, DIDP, DEHP	DB-17HT (50% dimethyl-50% diphenyl polysiloxane) (30m x 0.25mm, 0.15µm)	60ºC for 3 min, ramped at 10ºC/min to 290ºC, and finally held for 10 min.	MS. m/z: 149	0.1 μgmL^{-1}	102

Table 3. *(Continued)*

Sample	Analyte	Analytical column	Oven temperature programme	Detector	Limit of detection	Ref
Medical polyvinyl chloride tubing	DEHP	DB-1 capillary column (30m x 0.25mm, 0.1µm)	From 50ºC to 300ºC at 20ºC/min, with an initial isotherm of 3 min and final isotherm of 10 min. Total run time 22.5 min.	MS.		103
Wastewater	DMP, DEP, DBP, BBP, BEHP, DOP	ZB-5 MS Zebron (30m x 0.32mm, 0.25µm)	No linear gradient from 120ºC to 260ºC.	MS. m/z: 194/163/133, 177/149, 223/205/149, 205/149, 279/149, 279/149	6-90 ngL^{-1}	104

3.2. Liquid Chromatography

High-performance liquid chromatography (HPLC) can be used as an alternative technique and is especially useful for analysis of isomeric mixtures and metabolites of phthalates without derivatisation [47]. Ultraviolet detection has been used for phthalate determination in environmental and biological samples [8, 42, 49, 52, 55, 56, 61, 69, 93, 105, 73], but the use of mass spectrometry has increased in recent years, operating with single spectrometer [35, 37, 38, 48, 57, 106-110] or using mass spectrometer in tandem [3, 9, 40, 41, 46, 47, 110-112] with applications in different matrix samples (sludge, urban wastewater, urine, milk and drugs). Although GC-MS offered higher sensitivity for phthalate determination than LC-MS, LC-MS approach offered some advantages compared with GC-MS, such as, higher selectivity, more reliable quantification of PAEs isomeric mixtures, simpler cleanup procedures and shorter analysis time [100].

Separation of phthalates using liquid chromatography is usually performed in reverse phase using C18 or C8 columns, but some applications appear in the literature using other columns such as phenyl columns [10, 105, 113]. Tables *4* and *5* summarized different methods for PAEs determination in different matrices using HPLC as a separation technique coupled with different detectors. The separation conditions, including type of column, mobile phase, flow rate etc., as well as the detectors used and the limits of detection obtained, are also listed in the tables.

Table 4. *HPLC-UV methods for PAEs determination*

Sample	Analyte	Analytical column	Mobile phase	Detector	Limit of detection	Ref
Nail cosmetics	DMP, DEP, DPP, DiBP, DEHP	Zorbax Eclipse XDB C18 (150 mm x 4.6 mm i.d. 3.5 µm particle diameter)	Linear gradient elution with ethanol-water starting from 50 to 95% ethanol in 30 min.	UV. Wavelengt: 254 nm.	0.4-0.6 μgmL^{-1}	8
Parenteral nutrition and plasma	DEHP	Waters Spherisorb C18 column (150 mm x 4.6 mm i.d. 5 µm particle diameter)	Mixture acetonitrile –aqueous buffer (0.08% triethanolamine adjusted to pH 2.8 with 1 M phosphoric acid) mixture (88:12, v/v) at flow-rate of 1.0 $mLmin^{-1}$.	UV. Wavelengt: 202 nm.	LOQ= 20 $ngmL^{-1}$	13
Intravenous parenteral emulsions containing fat	DEHP	Waters Spherisorb C18 column (150 mmx 4.6 mm i.d. 5 µm particle diameter)	Mixture acetonitrile –aqueous buffer (0.08% triethanolamine adjusted to pH 2.8 with 1 M phosphoric acid) mixture (88:12, v/v) at flow-rate of 1.0 $mLmin^{-1}$.	UV. Wavelengt: 202 nm.		14
Blood of haemodialyzed patients	DEHP	Waters Spherisorb C18 column (150 mmx 4.6 mm i.d. 5 µm particle diameter)	Mixture acetonitrile –aqueous buffer (0.08% triethanolamine adjusted to pH 2.8 with 1 M phosphoric acid) mixture (88:12, v/v) at flow-rate of 1.0 $mLmin^{-1}$.	UV. Wavelengt: 202 nm.		15

Table 4. *(Continued)*

Sample	Analyte	Analytical column	Mobile phase	Detector	Limit of detection	Ref
Landfill leachates	DMP. DEP, DnBP	Venusil XBC18 column (250mmx4.6mm, i.d.:5µm).	Mixture methanol water (80:20 v/v). Flow rate 1.0 mLmin^{-1}. Temperature 25ºC	UV. Wavelength: 280 nm	0.0012, 0.0014, 0.0022 mgL^{-1}	24
Water	DMP, DEP, DnBP	Zorbax SB C8 (150 mmx 4.6 mm i.d. 5 µm particle diameter)	Mixture methanol-water (75:25 v/v)at flow rate of 0.8 mL min^{-1}. Temperature: 25ºC.	UV. Wavelength: 280 nm	2.0, 1.0 5.0 ngmL^{-1}	29
Waters	DBP, DCHP, DOP DNP, DDP	Zorbax Eclipse XDB C8 (150 mmx 4.6 mm i.d. 5 µm particle diameter)	Mixture acetonitrile/water (97:3)	UV. Wavelength: 226nm.		49
River an sea water	DEP, DnPP, DiPP DcHP	Agilent Zorbax Eclipse XDB-C8 column (150 mmx 4.6 mm i.d. 5 µm particle diameter)	Mixture acetonitrile/water (67:33) at flow rate of 1 mLmin^{-1}.	UV. Wavelength: 226nm		52
Wastewater	DBP, DEHP	Inertsil ODS 2 column (4.6 mm i.d. x 250 mm, 5 µm particle size) and Speriorex ODS (1.0 mm i.d. x 150 mm, 5 µm particle size)	Mixture methanol/water. Flow rate: 0.5 and 50µ Lmin^{-1} for colums of 4.6 and 1.0 mm i.d. respectively	UV. Wavelength: 254nm.	Limit of quantification : ≤ 0.1 and 0.5 ngmL^{-1}	53

***Table 4**. (Continued)*

Sample	Analyte	Analytical column	Mobile phase	Detector	Limit of detection	Ref
River water	BBP, DBP, DCP	MIGHTYSIL RP-18 GP 150 (150 mmx 4.6 mm i.d.)	Mixture ethanol/water (67:33) at flow rate of 0.7 $mLmin^{-1}$.	UV. Wavelength: 254nm		54
Water samples	DEP, DnPP, DnBP, DcHP, DEHP	Diamonsil-C18 (250 mm x 4.6 mm i.d. 4 µm particle diameter)	Mixture acetonitrile/water (75:25) at flow rate of 1 $mLmin^{-1}$.	UV. Wavelength: 226nm	0.12-0.17 μgL^{-1}	55
Aqueous samples	DEP	ChromCart colum (250 x 3.0 mm i.d.) packedwith Nucleosil C18-50 dp 5 µm. Thermostatized at 30 ºC.	Mixture acetonitrile/water (52.5:47.5) at flow rate of 0.5 $mLmin^{-1}$.	UV. Wavelength: 254nm.	Limit of quantificatio 5 $ngmL^{-1}$	69
Aqueous samples	DEP, DPP, BBP, DBP, DAP, DCHP, DHP, DEHP, DOP	Hypersil ODS (150 mm x 4.6 mm i.d. 5 µm particle diameter)	Linear gradient elution with acetonitrilel-water starting from 65 to 75% at 1.5 $mLmin^{-1}$ for 5 min run, from 75% to 95% at 1.5-2.0 $mLmin^{-1}$ for a 5 min run, and held 95% at 2.0 $mLmin^{-1}$ for 2 min.	UV. Wavelength: 225 nm.		73

***Table 4**. (Continued)*

Sample	Analyte	Analytical column	Mobile phase	Detector	Limit of detection	Ref
Environmental water samples	DBP, DEHP	A Genesis C18 (5cm x 4.6mm i.d. 4 µm particle diameter)	Mixture acetonitrile-water in gradient elution mode at flow rate of 1mLmin^{-1}. The elution program was initiated with 40% of water and it was maintained constant for 1 min. At 5 min the content of acetonitrile was 100%, it was maintained constant until the end of the chromatogram.	UV. Wavelength: 230 nm	1 and 2.5 µgL^{-1}	74
Liver samples	MEHP, DEHP	Altima C18 column (150mm x 4.6mm i.d. 5 µm particle diameter)	A gradient elution range of 60% to 100% acetonitrile with a gradient time of 5 min at a flow rate of 1 mLmin^{-1}, then increased to 2mLmin^{-1} for 3 min while keeping the final solvent composition at 100% acetonitrile. pH 3 with 25 mM $NaH_2PO_4.H_2O$ buffer.	UV. Wavelength: 235 nm.	0.57, 1.37 µgmL^{-1}.	93

***Table 4**. (Continued)*

Sample	Analyte	Analytical column	Mobile phase	Detector	Limit of detection	Ref
Standard solutions	DPP, DIBP, DMP, DEP, DBP, BBP, DCHP, DEHP, DNOP, DAP, DHP	Acquity UPLC BEH phenyl column (Waters) (50mm x 2.1mm 1.7µm particle size thermostatized at 45ºC. Agilent SB-phenyl column (250mm x 4.6mm, 5µm particle size thermostatized at 25ºC.	The mobile phase was a no linear gradient prepared from methanol (component A) and water (component B). From 50% to 100% of A.	UV. Wavelength: 225 nm		105
Physiological saline, distilled water for injection and glucose solution	DEHP	Shodex C18-5A (150mm x 4.6mm i.d.)	Mixture acetonitrile/methanol/water (60:100:25)	UV. Wavelength: 225nm.		109

***Table 5**. HPLC-MS or MS-MS methods for PAEs determination*

Sample	Analyte	Analytical column	Mobile phase	Detector	Limit of detection	Ref
Urine	DEHP, MEHP, DOP, DBP, BBzP, DEP	Luna Phenyl-Hexil column (3µm, 150 mm x 4.6mm i.d.)	Non linear solvent gradient. Mobil phase A: 10% acetonitrile containing 1.0% (v/v) acetic acid. Mobil phase B: 90% acetonitrile containing 1.0% (v/v) acetic acid. Flow rate 1 $mLmin^{-1}$. Mobil phase C: 100% acetonitrile	ESI-MS-MS	0.25-1.0 μgL^{-1}	1
Urine	DEHP metabolites	Inertsil ODS-3 (2.1 x 50mm i.d., particle size 5 µm)	Non linear solvent gradient. Mobil phase A: 0.1 % (v/v) acetic acid aqueous solution. Mobil phase B: acetonitrile.	ESI-MS-MS.	0.7-1.1 $ngmL^{-1}$	3
Urine	MBP, MBzP, MEHP	Inertsil ODS-3 (2.1 x 50 mm i.d., particle size 5 µm)	Non linear solvent gradient. Mobil phase A: 0.1% (v/v) acetic acid aqueous solution. Mobil phase B: acetonitrile.	ESI-MS-MS. Precursor ion → product ion: m/z, 221→71 for MBP, 255→183 for MBzP and 277→134 for MEHP.	1.3, 1.7, 0.7 $ngmL^{-1}$	9

***Table 5**. (Continued)*

Sample	Analyte	Analytical column	Mobile phase	Detector	Limit of detection	Ref
Milk	MMP, MEP, MBP, MBzP MEHP, NNP	Betasil phenyl column n (3µm, 100 mm x 2mm)	Non linear solvent gradient from 100% mobile phase A (0.1% acetic acid in water) to 100% mobile phase B (0.1% acetic acid in acetonitrile)	ESI-MS-MS. ESI in negative ion mode.	0.01-0.5 μgL^{-1}	10
Milk and milk products	DBP, BBP, DEHP, DiNP, DiDP	Luna C5 100A column (5 µm, 50mm x 2.0mm i.d.)	Isocratic mode. Mobile phase: 2.0% v/v water in methanol/ acetonitrile (1+1)	ESI-MS-MS.	9, 4, 6, 5, 5 μgkg^{-1}	11
Sewage sludge	DEP, DBP, DEHP	Lichrospher 100 RP-18 (250 x 4 mm and 5 µm particle size)	Non linear solvent gradient. Mobil phase A: 50% methanol/50% ecetonitrile. Mobil phase B: water. Both with 0.5% of acetic acid.	APCI-MS. ESI in positive ion mode. m/z values: 149.	15, 25, 50 ngg^{-1}	37
Urban wastewater	DMP, DEP, DBP, DEHP, DOP	Gemini C18 (150mm x 4.6 mm i.d., 5 µm particle size)	Non linear solvent gradient. Mobil phase A: 1.0% (v/v) acetic acid aqueous solution. Mobil phase B: acetonitrile.	APCI (or) ESI-MS. m/z values: 149 for all phthalates except DMP, 199, 163 for DMP	LOQ (ngL^{-1}): 57, 12, 25, 19, 41, 26.	38

Table 5. *(Continued)*

Sample	Analyte	nalytical column	Mobile phase	Detector	Limit of detection	Ref
Drugs	DEHP, MEHP	Mightysil® RP-18 GP column (5 mm x 2.1mm, 25 µm particle size)	Acetonitrile/water (90/10 v/v) at a flow rate of 0.2 $mL.min^{-1}$	ESI-MS-MS. Precursor ion → product ion: m/z, 391→149 for DEHP, 277→134 for MEHP		40
Human milk	13 Phthalate metabolites	Betasil Phenyl column (3µm, 100mm x 2.1mm)	Non linear solvent gradient. Mobil phase A: water Mobil phase B: acetonitrile. Both with 0.1% of acetic acid.	ESI-MS-MS.	0.2-1.9 $ngmL^{-1}$	41
Urine	16/22 phthalate metabolites	Betasil phenyl column (3µm, 150mm x 2.1mm)	Non linear solvent gradient. Mobil phase A: water containing 0.1% (v/v) acetic acid. Mobil phase B: acetonitrile containing 0.1% (v/v) acetic acid	ESI-MS-MS	0.16-4.30 $ngmL^{-1}$	46 31

***Table 5**. (Continued)*

Sample	Analyte	Analytical column	Mobile phase	Detector	Limit of detection	Ref
Urine	MMP, MEP, MBP, MCHP, MEHP, MOP,MiNP, MiDP, MEOHP	Betasil phenyl column (3µm, 100 mm x 2 mm)	Non linear solvent gradient from 100% mobile phase A (0.1% acetic acid in water) to 100% mobile phase B (0.1% acetic acid in acetonitrile)	ESI-MS-MS. ESI in negative ion mode. Multiple reaction monitoring mode	0.23-1.59 $ngmL^{-1}$	47
Industrial efluents	DBP, DMP, DEHP	Hypersil Green ENV (5µm, 150 mm x 5mm)	Non linear solvent gradient. Mobil phase A: water containing 0.5% (v/v) acetic acid. Mobil phase B: acetonitrile containing 0.5% (v/v) acetic acid. Flow rate 1 $mLmin^{-1}$	APCI-MS. m/z values: 149.	0.06-0.08 μgL^{-1}	48
Raw and treated sewage samples	DEP, DEHP, BBP, DBP	Hypersil C18 (150 mm x 4.6 mm i.d. 5 µm particle diameter)	Mixture methanol/water in gradient mode: (1) 40-90% of methanol in 25 min. (2)10 min with 100% methanol	APCI-MS. m/z values: 313 for BBP, 279 for DBP and 391 for DEHP.	0.07 μgL^{-1} for BBP, 0.01 μgL^{-1} for DBP and DEHP.	57

***Table 5**. (Continued)*

Sample	Analyte	Analytical column	Mobile phase	Detector	Limit of detection	Ref
Sludges and sediment samples	DBP, DEHP	Supelcosil LC-8 column of 25cm x 1.0mm i.d., 5 µm particle size.	Methanol (0.1% acetic acid) at 0.1 $mLmin^{-1}$	ESI-MS. Target ions: 279 (+), 391 (+) for DBP and DEHP respectively.		61
Water and sediment samples	DMP, DEP, BBP, DBP, DEHP, DOP	Kromasil 100 C18 (5µm, 25 mm x 0.46 mm)	Non linear solvent gradient. Mobil phase A: water Mobil phase B: acetonitrile.	APCI (positive mode)-MS.m/z values: 163 for DMP, 177 for DEP, 313 for BBP, 279 and 205 for DBP and 391 and 371 for DEHP and DOP	0.05-1 μgL^{-1}	106
Urine	MEP, MBP, MCHP, MBzP, MEHP, MOP, MNP, MDP	Betasil phenyl column (5µm, 50 mm x 3 mm)	Linear gradient from 100% buffer A (6 mM aqueous ammonium acetate pH 6.5) to 100% buffer B (90% acetonitrile in 6 mM aqueous ammonium acetate, pH 6.5). Flow rate 1.2 $mLmin^{-1}$	APCI-MS-MS	0.5-2 $ngmL^{-1}$	108

Table 5. *(Continued)*

Sample	Analyte	Analytical column	Mobile phase	Detector	Limit of detection	Ref
Urine	mMP, mEP, mBP, mCHP, mBzP, mEHP, mOP, mNP, mDP, mEOHP, mEHHP	Betasil phenyl column (5µm, 50 mm x 2mm)	Non linear solvent gradient. Mobil phase A: water containing 0.1% (v/v) acetic acid. Mobil phase B: acetonitrile containing 0.1% (v/v) acetic acid. Flow rate 0.6 $mLmin^{-1}$	APCI-MS-MS	0.7-1.6 $ngmL^{-1}$	111
Human serum	MEHP and DEHP	Wakosil3 C18, (2.0 x 100 mm, 3 µm)	Acetonitrile/water (15/85 v/v) at 0.2 $mLmin^{-1}$.	ESI-MS-MS. Precursor ion → product ion: m/z, 277→134 for MEHP, 391→149 for DEHP.	5 and 14 $ngmL^{-1}$	112
Urine	Five metabolites of DEHP	Betasil phenyl-hexyl column (3µm, 150mm x4.6mm)	Non linear solvent gradient. Mobil phase water: Water with 1% of acetic acid and acetonitrile.	ESI-MS-MS		113
Urine	MBP, MCHP, MBeP, MEHP, MIDP	Primesphere $5C_{18}HC$ (250 x 3.2 mm)	Non linear solvent gradient. Mobil phase A: 0.05 M ammonium acetate containing 0.1% (v/v) acetic acid. Mobil phase B: methanol containing 0.1% (v/v) acetic acid	APCI (negative mode)-MS.	10-40 $ngmL^{-1}$	118

3.3 Capillary electrophoresis

Capillary electrophoresis (CE) is a separation technique that provides several advantages such as speed, high efficiency and high sensitivity. Although, this technique is not usually used for PAEs determination, in recent years some authors have developed methods for PAEs determination in sediments and urine using this technique. Bao-Yuan Guo et al. [89] developed a method for DMP, DEP, DBP, DEHP and DOP from sediments using micellar electrokinetic chromatography (MEKC). The limits of detection obtained were within in a range of 0.050-0.063 $mgkg^{-1}$. The phthalates contents determined by MEKC were comparables to those obtained by GC-FID. In 2008, Yong-Lai Feng et al. [114] developed a method for MMP, MEP, MBP, MEHP and MEHHP in urine samples, using capillary zone electrophoresis (CZE) coupled with mass spectrometry. The limits of detection obtained were within a range of 0.53-1.3 ngL^{-1}.

4. Contamination problems

The major problem in phthalate analysis is contamination, resulting in false positive results or over-estimated concentrations. The risk of contamination is present in the whole analytical scheme, including sampling, sample preparation and chromatography analysis. Due to the fact that phthalates are widely used, they are present in the air, water, organic solvents, plastic and adsorbed onto glass or other materials [115].

A recent study carried out by Ried et al. [116] shows significant quantities of phthalates from various components commonly found in the environment of the analytical laboratory. Consequently, plastic syringes, pipette tips, plastic filters and all type of plastic material must be avoided, and glass material must be used instead. Once plastic materials containing phthalates are avoided, the main sources

of contamination are phthalates present as vapors or part of the particle matter in air, contaminating all surfaces, particularly glassware, plastic objects and our skin [117].

Due to the fact that the sources of contamination can vary from one laboratory to another and depend on factors such as season, weather and ventilation of the laboratory [117], general recommendation for avoiding contamination are not possible. Franhauser-Noti et al. [117] studied the blank problems in trace analysis of DEHP and DBP by gas chromatography-mass spectrometry. These authors applied a test to identify the sources of system contamination in a systematic manner and described a list of measures to reduce phthalates contamination. The major improvement was obtaining by adding aluminium oxide into the solvents the reservoirs. Another critical factor is the quality of caps for the autosampler vials. These caps can also contain phthalates. As a general precaution, only one injection should be made from each vial.

Different cleaning methods have been proposed to avoid the contamination problems due to the phthalates from the material used in the laboratory. In all of them glass material is rinsed with organic solvents after a rigorous washing [106, 109, 118-121].

C. Pérez Feás et al. [110] cleaned the glassware material prior to analysis according to recommendations specified in EPA method 506 in order to reduce the background contamination. All the material was washed with hot water and soap and rinsed with technical-grade acetone. Then, the glassware was sealed with aluminum foil and stored in a clean environment to avoid adsorption of phthalates from air.

5. Conclusion

The interest for PAEs determination in different matrix samples (water, sediments, sludge, and biological samples) has increased in recent years due to the toxicity of these compounds.

The main problem in PAEs determination is the contamination of the sample, due to the presence of high levels of these compounds in the laboratory environment. A solution to this problem would be the use of methods in which the sample pretreatment is performed out in a closed system or on-line with the detection technique. Both approaches minimize sample preparation.

Different methods have been developed, using a variety of preconcentration and extraction techniques, such as SPE, LLE, SPME, SLE, etc. For aqueous samples SPE and SPME were the most commonly used techniques obtaining good recoveries. For solid samples, Soxhlet extraction was the most usual technique but new methods using ultrasound or microwave energy are nowadays more popular because they shorted the extraction time.

CG and HPLC as separation techniques coupled with different detectors were the main techniques for PAEs determination employed in the literature. Results showed that methods using GC-MS or HPLC-MS-MS presented lower detection limits.

6. References

[1] Koch, H. M., González-Reche, L. M. & Angerer, J. (2003). *J. Chromatogr., B, 784*, 169.

[2] Serodio, P. & Nogueira, J. M. F. (2006). Water. Res., 40, 2572.

[3] Kato, K., Yamauchi, T., Higashimaya, K. & Nakazawa, H. (2003). *J. Liq. Chromatogr. Relat. Technol*., *26*, 2167.

[4] Jones-Lepp, T., Gerlach, C. L. & Cooter, E. J. (2000). *Trends Anal. Chem*., *19*, 286.

[5] Jobling, S., Raynolds, T., White, R., Parker, M. & Sumpter, J. P. A. (1995). *Environ. Health Perspect*., *103*, 582.

[6] Communication from the Comisión to the Council and the European Parliament on the implementation of the community strategy for endocrine disrupters-a range of substances suspected of interferinf with the hormona systems of humans and wildlife, COM (1999) 706; COM (2001) 262 final, Brussels, 2001.

[7] US Environmental Protection Agency (EPA), Protection Agency, *Introduction to Water Policy Standards*, Office of Water, Washinton, DC, 1999.

[8] De Orsi, D., Gagliardi, L., Porrá, R., Berri, S., Climenti, P., Granese, A., Carpani, I. & Tonelli, D. (2006). *Anal. Chim. Acta*, *555*, 238.

[9] Kato, K., Shoda, S., Takahashi, M., Doi, N., Yoshimura, Y. & Nakazawa, H. (2003). *J. Chromatogr*., B, 788, 407.

[10] Mortensen, G. K., Main, K. M., Anderson, A. M., Leffers, H. & Skakkebaek, N. E. (2005). *Anal. Bioanal. Chem*., *382*, 1084.

[11] Soresen, L. K. (2006). Rapad Commu. *Mass spectrom., 20*, 1135.

[12] Faouzi, M. A., Dine, T., Gressier, B., Kambia, K., Luyckx, M., Pagniez, D., Brunet, C., Cazin, M., Babeles, A. & Cazin. J. C. (1999). Inter. *J. Pharma*., *180*, 113.

[13] Kambia, K., Dine, T., Gressier, B., Germe, A. F., Luyckx, M., Brunet, C., Michaud, L., Gottrand, F. (2001). *J. Chromatogr*., B, *755*, 297.

[14] Kambia, K., Dine, T., Gressier, B., Bah, S., Germe, A. F., Luyckx, M., Brunet, C., Michaud, L. & Gottrand, F. (2003). *Inter. J. Pharma*., *262*, 83.

[15] Kambia, K., Dine, T., Azar, R., Gressier, B., Luyckx, M. & Brunet, C. (2001). *Inter. J. Pharma*., *229*, 139.

[16] Ji-an Chen, Huijie Liu, Zhiqun Qiu, Weiqun Shu. (2008).*Environ. Poll*., *156*, 849.

[17] Orssi, D. D., Gagliardi, L., Porrà, R., Berri, S., Climenti, P., Granesse, A., Carpani, I. & Tonelli, D. (2006). *Analytica Chim. Acta*, *555*, 238.

[18] Hou, L. & Lee, H. K. (2004). *J. Chromatogr*., A, *1152*, 37.

[19] Liu, S. & Dasgupta, P. K. (1995). Anal. *Chem*., 67, 2042.

[20] Cardoso, A. A. & Dasgupta, P. K. (1995). *Anal. Chem*., *67*, 2562.

[21] Jeannot, M. A. & Cantwell, F. F. (1996). *Anal. Chem*., *68*, 2236.

[22] Jeannot, M. A. & Cantwell, F. F. (1997). *Anal. Chem*., *69*, 235.

[23] Jeannot, M. A., Cantwell, F. F. (1997). *Anal. Chem*., *69*, 2935.

[24] Jirong Yao, Hui Xu, Lili Lv, Dandan Song, Yangfang Cui, Taozhi Zhan & Yu-Qi FENA. (2008). *Anal. Chim. Acta*, *616*, 42.

[25] Farahni, H. , Reza Ganjali, M., Dinarvand, R. & Norouzi, P. (2008). *Talanta*, *76*, 718.

[26] Khalili Zanjan, M. R., Yamini, Y., Shariati, S. & Jonson, J. A. (2007). *Anal. Chim. Acta*, *585*, 286.

[27] Batlle, R. & Nerín, C. (2004). *J. Chromatogr*., A, *1045*, 29.

[28] Psillakis, E. & Kalogerakis, N. (2003). *J. Chromatogr*., A, *999*, 145.

[29] Pie Liang, Qian Li, Ping Xu, Dan Du, (2008). *Chromatographia*, *68*, 393.

[30] Holadová, H. & Hajslová, J. (1995). Int. J. Environ. *Anal. Chem*., *59*, 43.

[31] Silva, M. J., Preau, J. L., Jr., Needham, L. L., Calafat, A. M. (2008). *J. Chromatogr.*, B, *873*, 180.

[32] del Carlo, M., Pepe, A., Sacchetti, G., Compagnone, D. Mastrocola D. & Cichelli, *A.* (2008). *Food Chemistry*, *111*, 771.

[33] Suzuki, T., Yaguchi, K. & Suzuki, S. (2001). *Environ. Sci. Technol*., *35*, 3757.

[34] Mohamed, M. A. & Ammar, A. S. (2008). *American J. Food and Techn*., 1.

[35] Castillo, M. & Barceló, D. (2001). *Anal. Chim. Acta*, *426*, 253.

[36] Fatoki, O. S. & Noma, A. (2001). *Afr, S. J. Chem*., *54*, 69.

[37] Petrovic, M. & Barceló, D. (2000). *Anal. Chem*., *72*, 4560.

[38] Zafra-Gómez, A., Ballesteros, O., Navalón, A. & Vilches, J. L. (2008). *J. Microchem*., *88*, 87.

[39] Ballesteros, O., Zafra, A., Navalón, A., Vílches, J. L. (2006). *J. Chromatogr. A, 1121*, 154.

[40] Ito, R., Seshimo, F., Miura, N., Kawaguchi, M., Saito, K., Nakazawa, H. (2005). *J. Pharmaceutical and Biomedical Analysis, 39,* 1036.

[41] Calafat, A. M., Slakman, A. R., Silva, M. J., Herbert, A. R., Needham, L. L. (2004). *J. Chromatogr*., B, *805*, 49.

[42] Jara, S., Lysebo, C., Greibrokk, T. & Lundanes, E. (2000). *Anal. Chim. Acta*, *407*, 165.

[43] Davi, M. L., Liboni, M. & Malfatti, G. (1999). Int. *J.Environ. Anal. Chem*., *74*, 155.

[44] Suzuki, T., Yaguchi, K. & Suzuki, S. (2001). *Sci. Technol*., *35*, 3757.

[45] Jonsson, S. & Borén, H. (2002). *J. Chromatogr*., A, *963*, 393.

[46] Kato, K., Silva, M. J., Needham, L. L. & Calafat, A. M. (2005). *J. Chromatogr*., B, *814*, 355.

[47] Silva, M. J., Slakman, A. R., Reidy, J. A., Preau, J. L. Jr., Herbert, A. R., Samandar, E., Needham, L. L. & Calafat, A. M. (2004). *J. Chromatogr. B, 805*, 161.

[48] Castillo, M., Alpendurada, M. F. & Barceló, D. (1997). *J. Mass Spectrometry, 32*, 1110.

[49] Ya-Qi Cai, Guibin Jiang & Jinfu Liu. (2003). *Anal. Scien*., *19*, 1491.

[50] Luna, S. C., Yanagisawa, Y., Ford, T. E. & Spengler, J. D. (2000). *Chemosphere*, *41*, 1857.

[51] Doong, R., Chang, S. & SPNG, Y. (2000). *J. Chromatogr. Sci*., *38*, 528.

[52] Ya-Qi Cai, Gui-Bin Jiang, Jing-Fu Liu &Qing-Xiang Zhou, (2003). *Anal. Chim. Acta*, *494*, 149.

[53] Yoshihiro Saito, Yuji Nakao, Motohiro Imaizumi, Yoriko Morishima, Yoshiaki Kiso & Kiyokatsu Jinno, (2002).Anal. *Bioanal. Chem*., *373*, 81.

[54] katsumata, H., Begum, A., Kaneco, S., Suzuki, T. & Ohta, K. (2004). *Anal. Chim. Acta*, *502*, 167.

[55] Jindon Li, Yaqui Cai, Yali Shi, Shifen Mou, (2008). *Guibin Jian Talanta, 74*, 498.

[56] Sayito, T., Matsushima, S. & Hiraide, M. (2004). *J. Chromtogr. A, 1040*, 185.

[57] López-Jimenez, F. J., Rubio, S. & Pérez-Bendito, D. (2005). *Anal. Chim. Acta*, *551* 142.

[58] Pawliszyn, J. (1999). Applications of solid phase microextraction, *RSC Chromatography monographs.* Royal Society of Chemistry, Letchworth, UK (ISBN 0-85404-525-2).

[59] Peñalver, A., Pocurull, E., Borrull, F. & Marcé, R. M. (2000). *J. Chromatogr. A, 872*, 191.

[60] Prokupková, G., Holandová, K., Poustka, J. & Hajslová, J. (2002). *Anal. Chim. Acta 457*, 211.

[61] Möder, M., Popp, P. & Pawliszyn, J. (1998). *J. Microcolumn Separations, 10(2)*, 225.

[62] Luks-Betlej, K., Popp, P., Janozska, B. & Paschke, H. (2001). *J. Chromatogr*., A *938*, 93.

[63] Feng, Y. L., Zhu, J. & Sensenstein, R. (2005). *Anal. Chim. Acta*, *538*, 41.

[64] Silva, R. C., Meurer, E. C., Eberling, M. N. & Augusto, F. (2005). The Analist, *130*, 188.

[65] Xiang, Li, Ming Zhong, Shifen Xu, Cheng Sun, (2006). *J. Chromatogr. A, 1135*, 101.

[66] Cortazar, E., Zuloaga, O., Sanz, J., Raposo, J. C., Etxebarria, N. & Fernández, L. A. (2002). *J. Chromatogr. A, 978*, 165.

[67] Peñalver, A., Pocurull, E., Borrull, F. & Marcé, R. M. (2001). *J. Chromatogr*. A., 922, 377.

[68] Polo, M., Llompart, M., García-Jares, C., Cela, R. (2005). *J. Chromatogr*. A., *1072*, 63.

[69] Kelly, M. T. & Larroque, M. (1999). *J. Chromatogr*., A, *841*, 177.

[70] Holadová, K., Prokupková, G., Hajslová, J. & *Poustka J.* (2007). *Anal. Chim. Acta*., *528*, 24.

[71] Haberhauer, C., Crnoja, M., Rosenberg, E. & Grasserbauer, M. (2000). *Fresenius J. Anal. Chem*., *366*, 329.

[72] de Fatima, M. (2000). *J. Chromatogr*., A 889, 3.

[73] Mitani, K., Izushi, F. & Kataoka, H. (2004). *J. Anal. Toxicology, 28*, 575.

[74] Cháfer-Pericás, C., Campís-Falcó, P., Prieto-Blanco, M. C. (2008). *Anal. Chim. Acta, 610*, 268.

[75] Baltussen, E., Sandra, P., David, F. & Cramers, C. (1999). *J. Microcol*., Sep, *11*, 737.

[76] Prieto, A., Telleira, O., Etxebarria, N., Fernández, L. A., Usobiaga, A. & Zuloaga, O. (2008). *J. Chromatogr.* A, 1214, 1.

[77] Tan, B. L. L., Hawker, D. W., Müller, J. F., Tremblay, L. A., Chapman., H. F. (2008). *Water Research, 42*, 404.

[78] Serodio, P. & Nogueira, J. M. F. (2004). *Anal. Chim*., Acta, *517*, 21.

[79] Seròdio, P. & Nogueira, J. M. (2006). *Water Research, 40*, 2572.

[80] Brossa, L., Marcé, R. M., Borull, F. & Pocurull, E. (2005). *Chromatographia, 61, 61*.

[81] Aranda, J. M., O´Connor, G. A., Eicement, G. A. (1989). *J. Environ. Qual*., *18*, 45.

[82] Strachan, S. D., Nelson, D. W., Sommers, L. E. (1983). *J. Environ.*, Qual, *12*, 69.

[83] Gibson, R., Min-Jian Wang, E., Padgett, A. & *Beck J.* (2005). *Chemosphere, 61*, 1336.

[84] Bonini, M., Errani, E., Zerbitani, G., Ferri, E. & Girotti, S. (2008). *Michrochem J*., *90*, 31.

[85] Aranda, J. M., O´Connor, G. A. & Eiceman, G. A. (1989). *J. Environ. Qual., 18*, 45.

[86] Strachan, S. D., Nelson, D. W., Sommers, L. E. (1983). *J. Environ. Qual, 12*, 69.

[87] Adeniyi, A., Dayomi, M., Siebe, P. & Okedeyi, O. (2008). *Chem. Central J*., *2*, 1.

[88] Bagó, B., Martín, Y., Mejía, G., Broto-Puig, F., Díaz Ferrero, J. Agut, M. & *Comellas* L., (2005).*Chemosphere, 59*, 1191.

[89] Bao-Yuan Guo, Bei Wen, Xiao-Quan Shan, Shu-Zheng Zhang, Jin-Ming Lin, (2005). *J. Chromatogr. A, 1095*, 189.

[90] Sablayrolles, C., Montréjaud-Vignoles, M., Benanou, D., Patria, L. & Treihou, M. (2005). *J. Chromatogr*., *1072*, 233.

[91] Kirchmann, H., Astrom, H. & Jonsall, G. (1991). *Swedish J. Agric. Res*., *21*, 107.

[92] Ling Ling, Der. Muir, Xue Tong Wang & Xiao Bai Xu, (2005). Int. *J. Environ. Anal. Chem*., *85(2)*, 89.

[93] Debatir Sicar, Sargon J. Albazi, Yousef Atallah & William Pizza, (2008). *J. Chromatogr. Sci*., *46*, 627.

[94] Errekatxo, A., prieto, A., Zuloaga, O., Usobiaga, A., Etxebarria, N. & Fernández, L. A. (2008). *Anal. Bioanal. Chem*., *392*, 1471.

[95] Cortazar, E., Bartolomé, L., Delgado, A., Etxebarria, N., Fernández, L. A., Usobiaga, A. & Zuloaga, O. (2005). *Anal. Chim. Acta*, 534, 247.

[96] Bartolomé, L., Cortazar, E., Raposo, J. C., Usobiaga, A., Zuloaga, O., Etxebarría, N., Fernández, L. A. (2005). *J. Chromatogr*. A, *1068*, 229.

[97] Alvarez-Avilés, O., Cuadra-Rodríguez, L., González-Illán, F., Quiñones-González, J., Rosario, O. (2007). *Anal. Chim. Acta*, *597*, 273-281.

[98] Bonini, M., Errari, E., Zerbinati, G., Ferri, E. & Girotti, S. (2008). *Microchem. J*., *90*, 31.

[99] Kotowska, U., Garbowska, K., Isidorov, V. A. (2006). *Anal. Chim. Acta*, *560*, 110.

[100] David, F., Sandra, P., Tienpont, B., Vanwalleghem, F. (2003). In: C. A., Staples, (Eds.), The Handbook of Environmental Chemistry. Part Q. Phthalate Esters, vol. 3, *Springer*, *Berlin*, 15.

[101] Cavaliere, B., Macchione, B., Sindona, G. & Tagarelli, A. (2008). *J. Chromatogr*., A, *1205*, 137.

[102] Earls, A. O., Axford, I. P. & Braybrook, J. H. (2003). *J. Chromatogr*., A 983, 237.

[103] Inoue, K., Okumura, H., Tae Higuchi, Oka, H., Yoshimura, Y. & Nakazawa, H. (2002).*Clin. Chim. Acta*, *325*, 157.

[104] Ballesteros, O., Zafra, A., Navalón, A. & Vílchez, J. L. (2006). *J. Chromatogr*., *1121*, 154.

[105] Tin Wu, Chao Wang, Xing Wang, Haiqing Xiao, Qiang Ma, Qing Zhang, 2008, *Chromatographia*, *68*, 803.

[106] Gimeno, R. A., Marcé, R. M. & Borrull, F. (2003). *Chromatographia*, *58*, 37.

[107] Brossa, L., Marcé, R. M., Borrull, F. & Pocurull, E. (2005). *Environ. Toxicol. Chem*. *24*, 261.

[108] Blount, B. C., Milgram, K. E., Silva, M. J., Malek, N. A., Reidy, J. A., Needham, L. L. & Broca, J. W. (2000). *Anal. Chem*., *72*, 4127.

[109] Hanawa, T., Muramatsu, E., Asakama, K., Tanaka, M., Kawano, K., Seki, T., Juni, K. & Nakajima, S. (2000). *Int. J. Pharm*., *210*, 109.

[110] Pérez Feás, C., Barciela Alonso, M. C., Peña-Vázquez, E., Herbello Hermelo, P., Bermejo Barrera, P. (2008). *Talanta*, *75*, 1184.

[111] Silva, M. J., Malek, N. A., Hodge, C. C., Reidy, J. A., Kato, K., Barr, D. B., Needham, L. L., Broca, J. W. (2003). *J. Chromatogr*., B, *789*, 393.

[112] Takatori, S., Kitagawa, Y., Kitagawa, M., Nakazawa, H., Hori, S. (2004). *J. Chromatogr*., B, *804*, 397.

[113] Preus, R., Koch, H. M. & Angerer, J. (2005). *J. Chromatogr*. , B, *816*, 269.

[114] Yon-Lai Feng, Jiping Zhu, (2008). *Electrophoresis*, *29*, 1965.

[115] David, F., Sandra, P., Tienpont, B. & Vanwalleghem, F. (2003). In: C. A., Staples, (Eds.), *The Handbook of Environmental Chemistry. Part Q. Phthalate Esters*, *vol 3*, *Springer*, *Berlin*, 15.

[116] Reid, A. M., Brougham, C. A., Fogarty, A. M. & Roche, J. J. (2007). Int. *J. Environ. Anal. Chem*., *87*, 125.

[117] Fankhauser-Noti, A. & Grob, K. (2007). *Anal. Chim. Acta*, *582*, 353.

[118] Anderson, W. A. C., Barnes, K. A., Castle, L., Damant, A. P. & Scotter, M. J. (2002). *Analyst*, *127*, 1193.

[119] Jonson, S., Ejlertsson, J., Ledin, A., Mersiowsky, I. & Svensson, B. H. (2003). *Water Res*., *37*, 609.

[120] Faozi, M. A., Khalfi, F., Dine, T., Luyckx, M., Brunet, C., Gressier, B., Groudaliez, F., Cazin, M., Belabed, A. & Cazin, J. C. (1999). *J. Pharm. Biomed. Anal*., *21*, 923.

[121] Cai, Y. Q., Jiang, G. B., Liu, J. F. & *Zhou* Q.X., (2003).*Anal. Chim*., Acta, *494*, 149.

CAPÍTULO II

Phthalates Determination in Physiological Saline Solutions by HPLC-ES-MS

C. Pérez Feás, M.C. Barciela Alonso, E. Peña-Vázquez, P. Herbello Hermelo, P.Bermejo-Barrera*.

TALANTA 75 (2008) 1184-1189

Resumen

El objetivo en este capítulo consistió, en primer lugar, en el desarrollo de un método analítico adecuado para la identificación y determinación de una mezcla de cuatro diésteres del ácido ftálico (DMP, DEP, BBP y DBP) utilizando como técnica de análisis la cromatografía líquida de alta resolución acoplada a espectrometría de masas (LC-MS).

Se estudiaron las variables que afectan al proceso de separación cromatográfica como son la composición, el pH y el flujo de la fase móvil así como las variables que afectan a su determinación por espectrometría de masas.

Tras la optimización, la separación cromatográfica de los analitos se realiza en un tiempo inferior a 10 minutos mediante una elución en gradiente con acetonitrilo y agua ultrapura, un flujo de fase móvil de 200 $\mu Lmin^{-1}$ y el espectrómetro de masas trabajando en modo de ionización positiva.

A continuación se estudiaron las características analíticas del método de análisis desarrollado, como son la linearidad, los límites de detección (LOD) y cuantificación (LOQ), la precisión y la recuperación. Los resultados obtenidos permiten concluir que el método es rápido, preciso y exacto.

En segundo lugar, el método propuesto se aplicó en el análisis de muestras de suero salino comercializadas en envases de plástico monodosis con el fin de verificar la presencia de ftalatos en disolución debido a la posible migración de los mismos a partir del plástico y determinar su concentración.

Existen diferentes denominaciones para el tipo de muestra que se abarca en este estudio: “solución salina”, “suero fisiológico”, “suero salino”, etc. Todas ellas hacen referencia a una solución acuosa de cloruro sódico al 0.9% cuyas características osmóticas la hacen compatible con el suero sanguíneo. A menudo se emplea por vía intravenosa para reponer líquidos y como vehículo para la administración de medicamentos. Otras aplicaciones son la higiene y limpieza

nasal y ocular de bebés, niños y adultos y para facilitar su uso se comercializa en envases de plástico monodosis (5 mL).

Las muestras fueron adquiridas en lugares de venta al público, tanto en farmacias como en parafarmacias. Se analizaron cuatro muestras de sueros salinos de diferentes marcas comerciales, tres de ellas disponibles en el mercado en envases de plástico monodosis y una cuarta comercializada en envase de vidrio.

El análisis se llevó a cabo mediante inyección directa de las muestras de suero en el cromatógrafo sin necesidad de llevar a cabo ningún tratamiento de las mismas. Se trata de un método sencillo y rápido que permite determinar, directamente y sin extracciones previas, un grupo de ftalatos de gran interés desde el punto de vista de la salud pública.

Los resultados permiten confirmar la presencia de estos compuestos en las muestras comercializadas en envases monodosis, en contraposición con el análisis de una muestra de suero comercializada en envase de vidrio. Su origen podría atribuirse a la migración de dichos compuestos desde el plástico al suero debido a la inexistencia de uniones covalentes entre los ftalatos y la matriz polimérica que constituye el plástico.

Contents

PHTHALATES DETERMINATION IN PHYSIOLOGICAL SALINE SOLUTIONS BY HPLC-ES-MS

C. Pérez Feás, M.C. Barciela Alonso, E. Peña-Vázquez, P. Herbello Hermelo, P.Bermejo-Barrera*.

Department of Analytical Chemistry, Nutrition and Bromatology. University of Santiago de Compostela. Santiago de Compostela, 15782. Spain.

Abstract

Phthalates are a group of chemical compounds with increasing interest from the analytical point of view. The risks for human health associated with some of these compounds have unleashed the necessity to develop analytical methods with great sensitivity that allow us to detect their presence at trace levels in order to assure protection for the population.

A simple and rapid method for determining a group of phthalate esters in aqueous samples was developed. The method was based on high-performance liquid chromatography-(electrospray)-mass spectrometry (HPLC-ES-MS), working in positive ionization (PI) mode. A gradient elution was performed with acetonitrile-ultrapure water starting from 5% to 75% acetonitrile in 5 min followed by isocratic elution during 5 min. Standard calibration curves were linear for all the analytes over the concentration range 10–500 $ngmL^{-1}$. The LOD values found for DMP, DEP, BBP and DBP were 0.8, 3.4, 0.6 and 1.2 $ngmL^{-1}$ respectively. The relative standard deviation ranged from 0.8 to 1.7%, which indicated good method precision.

The proposed analytical method has been applied to the analysis of commercial physiological saline solutions in order to check the presence of phthalates and to determine their concentration.

Keywords: Phthalates; LC-ES-MS; Physiological saline solutions.

*Corresponding author: e-mail address: pbermejo@usc.es (P.Bermejo-Barrera)

1. Introduction

Diesters of phthalic acid, commonly referred to as phthalates, are a group of chemical compounds widely used in industry and commerce due to their large variety of uses. Because of their properties to improve softness and flexibility to the plastics they are used mainly as plasticizers to give products to consumer and industry versatiles, durables and accessibles such as medical devices, children's toys and all kind of packaging. Furthermore, phthalates are also used as industrial solvents and lubricants, as additives in textile industry and pesticides and also in personal care products such as deodorants, lotions and perfumes, to retain the colour and fragrance [1-4].

Approximately 93% of all plasticizers are phthalates, the remaining 7% corresponding to esters and polyesters based on adipate, phosphoric acid, sebacic acid, etc [1]. The world production of these compounds is estimated at several million tonnes per year. Phthalates are not chemically bound in the plastics; therefore, they can be lost from plastic and released to the environment [5].

Consistent toxicological evidence indicates association between several of these phthalate esters and risks for human health and the environment. In particular, dibutyl phthalate (DBP), butyl benzyl phthalate (BBP), and di-(2-ethylhexyl) phthalate (DEHP) are in the list of the proposed substances suspected to produce endocrine alterations published by European Union (EU) [6].

Section 307 of the US Clean Water Act establishes that dimetyl phthalate (DMP), dietyl phthalate (DEP), butyl benzyl phthalate (BBP), dibutyl phthalate (DBP), di-(2-ethylhexyl) phthalate (DEHP) and dioctyl phthalate (DOP) must be considered priority toxic pollutants [7]. These concerns have been further aggravated by recent analysis of human blood and urine samples, where traces of various phthalates (or their metabolites) have been found [8-9]. For these reasons, the interest in the study

of this type of chemical substances has increased during the last few years, and therefore it is essential to develop a reliable and sensible analytical method that allows us to determine and quantify this group of compounds at trace levels.

Several methods have been developed for their determination in different matrices, including water (drinking water, surface water, wastewater), soil, sediment, sludge, dust, air and biota (vegetation, milk, fish, etc) [2, 10-12].

The analysis of phthalic acid esters are mostly performed by gas chromatography (GC) [13-17]. Generally GC methods present better sensibility than HPLC methods, although depend on the pre-treatment step, the instrumental conditions and the sample matrix [6]. High-pressure liquid chromatography (HPLC) can be used as an alternative technique and is especially useful for analysis of isomeric mixtures and metabolites of phthalates without derivatisation [18].

Phthalates can be detected using UV detection [8, 19-21], flame ionisation detection (FID) [22-23], electron capture detection (ECD) [24] or mass spectrometry (MS) [10, 11, 25, 26]. Some official methods (US EPA methods 606 and 8060) describe the use of ECD for the phthalate determination. Although ECD detectors are relative sensitive for phthalates, the specificity is restricted. The most important detector for phthalate analysis is mass spectrometric detection. All types of MS analysers, including quadrupole analysers, triple quadrupole analysers, ion traps and magnetic sector instruments have been used for phthalates determination [27].

The major problem in phthalate analysis is the contamination, resulting in false positive results or over-estimated concentrations. The risk of contamination is present in the whole analytical scheme, including sampling, sample preparation and chromatographic analysis. Due to the fact that phthalates are widely used, they are present in air, water, and organic solvents and plastic and adsorbed on glass or other materials [27].

A recent study carried out by A.M. Reid et al. [28] shows significant quantities of phthalates from various components commonly found in the environmental of analytical laboratory. Consequently, plastic syringes, pipette tips, plastic filters and all type of plastic material must be avoided, and glass material must be used instead. Once plastic materials containing phthalates are avoided, the main source of contamination are phthalates present as vapours or part of the particulate matter in air, contaminating all surfaces, particularly glassware, plastic objects and our skin [29].

As a result of the contribution of all these sources of contamination, the experiments to reduce its produce confusing results because, the sources of contamination vary from one laboratory to another and depend on factors such as season, weather and ventilation of the laboratory [29].

Different cleaning methods have been proposed to avoid the contamination problems due to the phthalates from the material used in the laboratory. In all of them glass material is rinsed with organic solvents after a rigorous washing [11, 30-35].

The aim of this work is to develop a method for phthalates determination presents in trace levels in physiological saline solutions, using HPLC-ES-MS.

2. Experimental

2.1. Reagents and Standards

All reagents used were of analytical reagent-grade. Dimethyl phthalate (DMP) and butyl benzyl phthalate (BBP) were obtained from Supelco (Bellefonte, PA, USA). Diethyl phthalate (DEP) and dibutyl phthtalate (DBP) were obtained from Riedel-de Haën (Seelze, Germany). The purity of these reagents was over 98%.

Lichrosolv gradient grade acetonitrile and methanol were purchased from Merck (Darmstadt, Germany). Technical grade acetone and acetic acid glacial (HPLC) for instrumental analysis were purchased from Panreac (Barcelona, Spain). Ultra-pure (resi-analized) water for environmental inorganic and organic trace analysis was supplied by J.T. Baker (Phillipsburg, NJ, USA).

Individual standard solutions of each phthalate ester at a concentration of 1000 mgL^{-1} were prepared in methanol, preserved of light and stored at 4°C in a Teflon-capped glass vial. From these solutions, a working mixture in methanol was prepared weekly containing all standards of concentration 100 mgL^{-1} each. All the working solutions were prepared daily by diluting this solution.

Special care was taken to avoid the contact of reagents and solvents with plastic materials. In order to reduce background contamination, all glassware was cleaned prior to the analysis according to the recommendations specified in EPA method 506. All material was washed with hot water and soap, rinsed with tap and ultrapure water and finally thorough rinsed with technical-grade acetone. Then, glassware was sealed with aluminium foil and stored in a clean environment to avoid adsorption of phthalates from the air.

2.2. Instrumentation

Phthalates separation and quantification was carried out using liquid chromatography/electrospray ionization-mass spectrometry system.

The HPLC system used was an 1100 Series equipped with an automatic injector (Agilent Technologies, Waldbronn, Germany) that is coupled to an API 150 EX single quadrupole mass spectrometer equipped with a Turboionspray interface (PE Biosystems, Concord, Canada).

The analytical column was a ZORBAX Eclipse XDB-C8 of 50 mm length and 2.1 mm internal diameter (particle size 3.5 μm) supplied by Agilent Technologies.

2.3. Chromatographic and Mass Spectrometry Conditions

The binary mobile phase consisted of ultra-pure water and acetonitrile, both solvents containing 0.1% (*v/v*) acetic acid. The elution gradient started with 95% of ultra-pure water, which was reduced linearly to 25% in 5 min. Then, this composition was maintained for 5 min before returning to the initial conditions. The column was equilibrated for 10 min.

The flow rate and the injection volume were 200 $\mu L min^{-1}$ and 10 μL, respectively and the chromatographic separation was carried out at room temperature. Under these conditions the separation time was less than 10 min.

Electrospray ionization was performed in positive ion mode. The operational parameters were the same for all of analytes with an ionspray voltage of 5500 V; nitrogen was used as nebulizer and curtain gas at a pressure of 14 psi in both cases; air current at 450ºC and 7000 cc min^{-1} was used as turbo heater gas.

The compound parameters such as declustering potential (DP), focusing potential (FP) and enhance potential (EP) were optimized for each analyte. The optimal conditions are shown in the *Table 1*.

Table 1*- Optimal values of the compound parameters for the four phthalates studied.*

Compound	**Acronym**	**m/z**	**Potentials**		
			DP	**FP**	**EP**
Dimethyl phthalate	DMP	163.25	40.38	73.87	8
Butyl benzyl phthalate	BBP	91.15	25	225	6
Diethyl phthalate Dibutyl phthtalate	DEP, DBP	149.05	25	290	8.5

2.4. Sample preparation

Samples were injected directly in the chromatograph, it wasn´t necessary any sample preparation process.

3. Results and Discussion

3.1. ES-MS Optimization

Four phthalate esters (DMP, DEP, BBP and DBP) were selected for this study.

To evaluate the mass spectral fragmentation pattern of each compound and to optimize the set of parameters used, a standard solution (100 mgL^{-1}) of each compound was analyzed by direct injection in the spectrometer. For these experiments, a KD Scientific, model 100, syringe pump (New Hope, MN, USA) at 15 $\mu Lmin^{-1}$, was used.

Full-scan data acquisition was performed from 80 to 400 *m/z*, with the target mass fixed to the following *m/z* values: 91.15 for BBP, 149.05 for DEP and DBP and 163.25 for DMP. The spectral data provided ions in accordance with previous studies reported in literature [2, 15, 16, 36, 37]. The selected ions were chosen to attain the best response in the SIM mode acquisition. Characteristics as molecular weight, identification ions and retention time corresponding to these compounds are given in *Table 2*.

Table 2- *Molecular weight, selected ions and retention time to the analysis of the target phthalates.*

Phthalate	Molecular weight	SIM ion	Identification ions	RT (min)
Dimethyl phthalate	194	163	149, 163, 181	6.90
Diethyl phthalate	222.24	149	149, 177, 195	7.59
Butyl benzyl phthalate	312.40	91	91, 149, 205, 223, 247	9.18
Dibutyl phthtalate	278.35	149	149, 205, 223	9.44

3.2. Optimization of HPLC separation

After optimizing the detection conditions, the following experiments were conducted to optimize the chromatographic separation of the analytes.

Experiments were carried out using different mobile phases reported in the literature (methanol:water [38], acetonitrile:water [20], acetonitrile (1%methanol):water [21]), working in isocratic mode. The best resolution was obtained using acetonitrile: water as a mobile phase. These results agree with the experiments developed by F.J. López-Jiménez et al. [10]. In order to improve the resolution and to decrease the time of analysis, different experiments were carried out working in gradient mode. The best results were obtained started with 95% of ultrapure water and decreasing this percentage to 25% in 5 min. Then, this composition was maintained for 5 min before returning to the initial conditions. Finally, the column was equilibrated during 10 min before each injection. Other parameters optimized were the percentage of acetic acid and the flow rate of the mobile phase. The optimal conditions were 0.1% (*v/v*) acetic acid and a flow rate of 200 $\mu L min^{-1}$.

The chromatogram obtained for a mixed of these compounds under the optimized conditions is shown in the *Figure 1*.

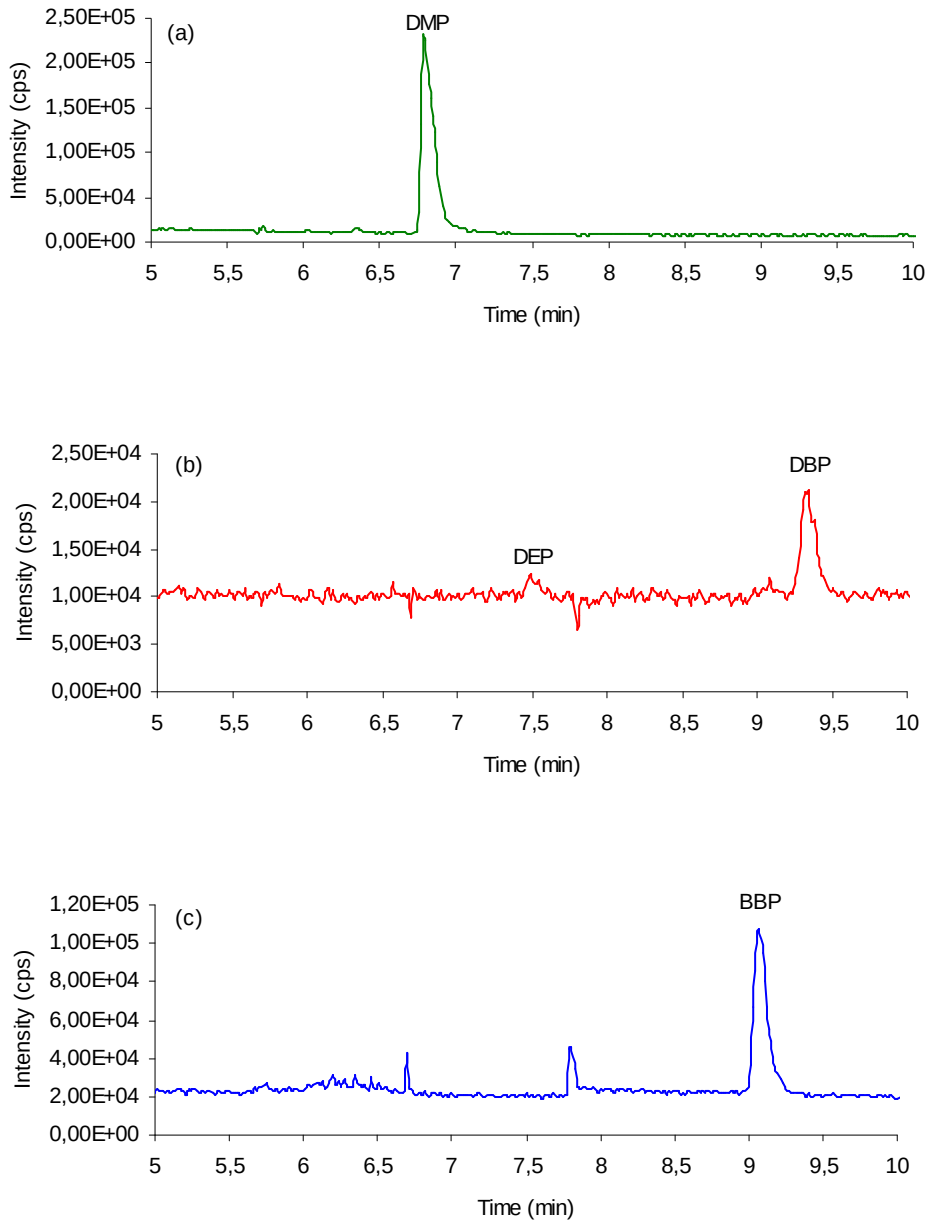


Fig.1- *LC/MS extracted ion chromatogram obtained from a standards solution (100ngmL^{-1}) in a physiological saline sample purchased in a glass bottle to the following m/z values:* ***(a)*** *163.25 for DMP,* ***(b)*** *149.05 for DEP and DBP, and* ***(c)*** *91.15 for BBP.*

3.3. Analytical performance

To evaluate the linearity of the method, a direct calibration curve was realized. Ten μL of standard solutions in ultra-pure water with concentrations ranging from 10 to 500 $ngmL^{-1}$ were injected by triplicate. Detector signals, measured in arbitrary units (peak areas), were plotted versus the amount of analyte injected, expressed in $ngmL^{-1}$ and background levels were subtracted from de results. The equations obtained for each compound were as follows:

DMP:	Q_A= 46399 C + 377217	r = 0.9964
DEP:	Q_A= 1784 C + 16643	r = 0.9987
BBP:	Q_A= 18218 C + 153056	r = 0.9978
DBP:	Q_A= 5166 C + 66474	r = 0.9963

where Q_A is the peak area and C is the concentration in $ngmL^{-1}$.

Standard addition method was applied over the same range of concentrations using a commercial physiological saline solution purchased in a glass bottle. The equations obtained for each compound were as follows:

DMP:	Q_A =12334 C + 12308	r = 0.9998
DEP:	Q_A = 114 C + 1686	r = 0.9979
BBP:	Q_A = 5319 C - 20441	r = 0.9988
DBP:	Q_A = 639 C - 8128	r = 0.9985

To compare slopes of the calibration and addition graphs for the four compounds, the test-t (95% significance levels) [39] was applied and differences were observed for all compounds. This means that the sample matrix had influence in the

sensitivity of the method, so, standard addition graphs had been used to analyze these samples.

The limit of detection (LOD) and limit of quantification (LOQ) for the method were calculated according with the equations:

$$LOD = \frac{3SD}{m} \qquad LOQ = \frac{10SD}{m}$$

where *SD* is the standard deviation of 11 measurements of a blank and *m* is the slope of the addition calibration graph.
The commercial physiological saline solution purchased in a glass bottle was used as a blank. The results obtained for LODs and LOQs are shown in the *Table 3*.

Table 3- *Linear range, correlation coefficients, LODs and LOQs values obtained from the standard addition method in physiological saline solutions.*

Phthalate	**Linear range ($ngmL^{-1}$)**	**Correlation coefficient (r)**	**LODs ($ngmL^{-1}$)**	**LOQs ($ngmL^{-1}$)**
DMP	10-500	0.9998	0.99	3.29
DEP	10-500	0.9979	22.13	73.78
BBP	10-500	0.9988	5.32	17.73
DBP	10-500	0.9985	24.07	80.23

As can be seen in the *Table 3*, LODs are between 0.99 and 24.07 $ngmL^{-1}$ for all compounds, and the highest levels obtained were for DEP and DBP.

To check the precision an interday assay was developed. A physiological saline solution sample purchased in a glass bottle and spiked with three concentration levels (50, 100 and 300 $ngmL^{-1}$) were analyzed during different days (six determinations per concentration each day) for all compounds studied. The results obtained are shown in the *Table 4*. The RSD values were between 1.9 to 10.9% so, the method is precise for all studied compounds.

Table 4*- Relative standard deviation (%) obtained for three concentration levels (based on six determinations) in interday assay.*

Phthalate	**RSD (%)**		
	50 $ngmL^{-1}$	**100 $ngmL^{-1}$**	**300 $ngmL^{-1}$**
DMP	10.9	3.5	3.6
DEP	8.0	4.5	4.1
BBP	5.6	6.2	2.2
DBP	5.1	7.5	1.9

The recovery of the method was evaluated by injection of the physiological saline solution purchased in a glass bottle spiked with three different concentrations of these compounds. The solutions were injected by triplicate and the recovery calculated using the standard addition graph. The results obtained are shown in *Table 5*. The average recoveries were 101.5%, 94.7%, 108.3% and 101.4% for DMP, DEP, BBP and DBP respectively.

***Table 5**- Recovery percentage for physiological saline solutions ± standard deviation.*

Phthalate	**% Recovery**		
	50 ngmL^{-1}	**100 ngmL^{-1}**	**300 ngmL^{-1}**
DMP	103.7 ± 1.4	105.4 ± 1.0	95.0 ± 1.2
DEP	89.7 ± 3.3	99.5 ± 3.5	95.1 ± 3.1
BBP	111.3 ± 9.5	104.2 ± 2.0	109.5 ± 1.5
DBP	105.8 ± 2.7	89.8 ± 2.1	108.7 ± 3.5

3.4. Application to physiological saline solutions

The proposed analytical method has been applied to the analysis of four commercial physiological saline solutions in order to check the presence of these phthalates and to determine their concentration. Samples were injected directly in the chromatograph, it wasn´t necessary any sample preparation process.

The original recipients containing three of these physiological saline solutions were made from plastic material. The other one was in a glass bottle. The phthalate esters are used in the manufacture of the plastic recipients, so the influence of the material on the concentration of the phthalates has been evaluated.

Physiological saline solutions were analyzed in order to verify the presence of different peaks at the same retention time as the compounds studied. Some peaks appeared at the retention times corresponding to DMP, DEP, BBP and DBP. The spectra of these peaks confirmed that they correspond to these four phthalates.

The results obtained for these phthalates in the four samples are given in *Table 6*.

***Table 6**- Concentrations (ngmL^{-1}) ± standard deviation (based on three replicates) found in different physiological saline solutions.*

Physiological saline solutions	DMP	DEP	BBP	DBP
brand A	5 ± 1	335 ± 5	< LOD	50 ± 2
brand B	< LOD	< LOD	< LOD	< LOD
brand C	< LOD	< LOD	5 ± 1	< LOD
brand D	153 ± 2	< LOD	< LOD	< LOD

< LOD: lower than the detection limit.

The levels of these compounds in the brand B, was less than the LODs of the method. The absence of these compounds in this sample can be attributed to that this sample is distributed in a glass bottle. *Figure 2* shows the LC/MS ion chromatograms obtained from brand A of physiological saline solution.

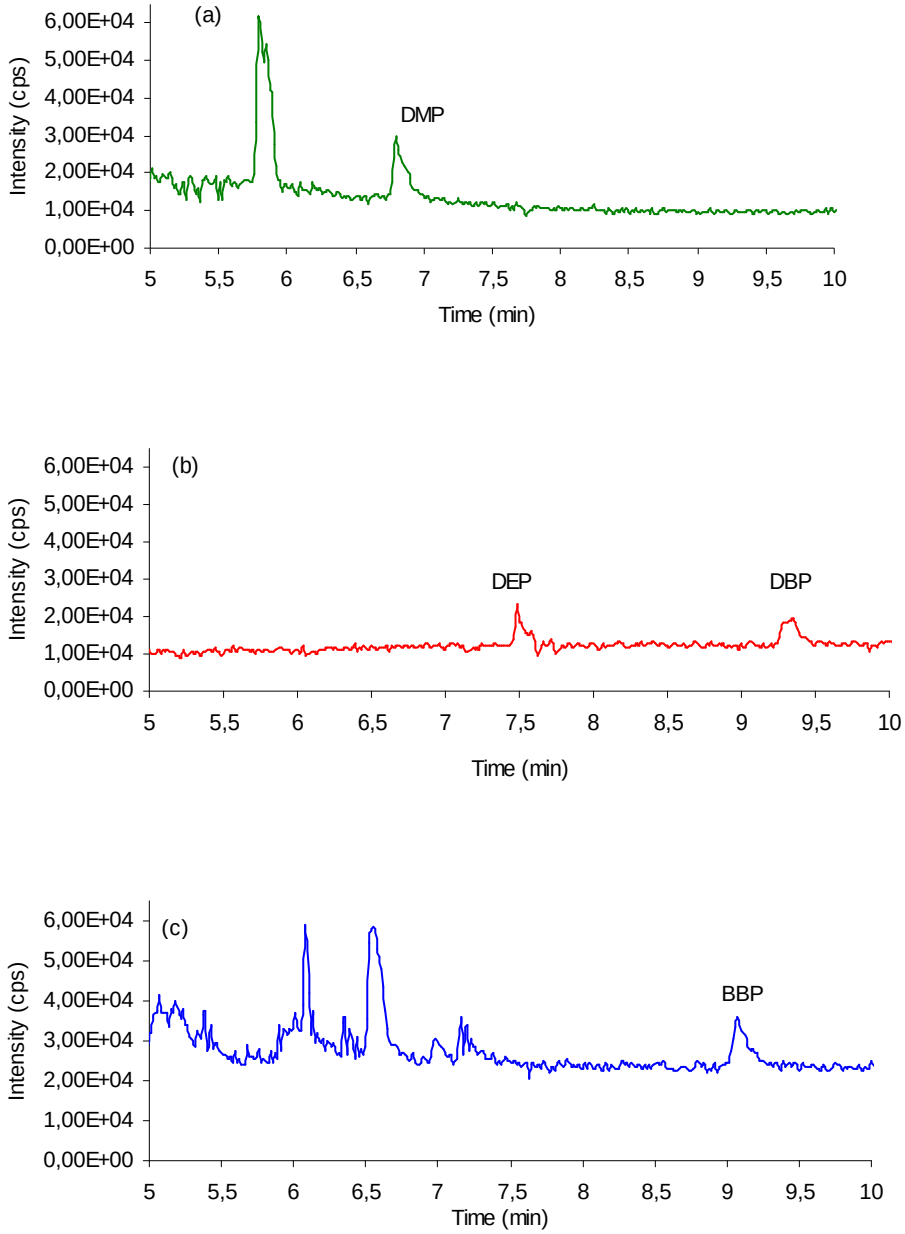


Fig.2- *LC/MS extracted ion chromatogram obtained from brand A physiological saline solution. to the following m/z values:* ***(a)*** *163.25 for DMP,* ***(b)*** *149.05 for DEP and DBP, and* ***(c)*** *91.15 for BBP.*

4. Conclusions

A method for the determination of different phthalates by HPLC-ES-MS was developed. The method is rapid (the separation and determination was realized in less than 10 min), precise and accurate.

Four commercial physiological saline solutions from different brands were analyzed using the proposed method. The results shown, that these compounds are present only in the samples distributed in plastic bottles. In physiological saline solution distributed in glass bottle, these compounds were not detected (<LODs).

Acknowledgements

C. Pérez Feás wishes to thank Ministerio de Educacion y Ciencia for her FPU grant.

5. References

[1] H.M. Koch, L.M. Gonzalez-Reche, J. Angerer, J. Chromatogr. B 784 (2003) 169.

[2] P. Serodio, J.M.F. Nogueira, Water Res. 40 (2006) 2572.

[3] K. Kato, T. Yamauchi, K. Higashimaya, H. Nakazawa, J. Liq. Chromatogr. & Relat. Technol. 26 (2003) 2167.

[4] T. Jones-Lepp, C.L. Gerlach, E.J. Cooter, Trends Anal. Chem. 19 (2000) 286.

[5] A. Gómez-Hens, M.P. Aguilar-Caballos, Trends Anal. Chem. 22 (2003) 847.

[6] Communication from the Commission to the Council and the European Parlament on the implementation of the community strategy for endocrine disrupters -a range of substances suspected of interfering with the hormone systems of humans and wildlife, COM (1999) 706; COM (2001) 262 final, Brussels, 2001.

[7] US Environmental Protection Agency (EPA), Protection Agency, Introduction to Water Policy Standards, Office of Water, Washington, DC, 1999.

[8] D. De Orsi, L. Gagliardi, R. Porrá, S. Berri, P. Chimenti, A. Granese, I. Carpani, D. Tonelli, Anal. Chim. Acta 555 (2006) 238.

[9] K. Kato, S. Shoda, M. Takahashi, N. Doi, Y. Yoshimura, H. Nakazawa. J. Chromatogr. B 788 (2003) 407.

[10] F.J. López-Jiménez, S. Rubio, D. Pérez-Bendito, Anal. Chim. Acta 551 (2005) 142.

[11] R.A. Gimeno, R.M. Marcé, F. Borrull, Chromatographia 58 (2003) 37.

[12] G.K. Mortensen, K.M. Main, A.M. Andersson, H. Leffers, N.E. Skakkebaek, Anal. Bioanal. Chem. 382 (2005) 1084.

[13] A. Peñalver, E. Pocurull, F. Borrull, R.M., Marcé, J. Chromatogr. A 872 (2000) 191.

[14] L. Brossa, R.M. Marcé, F. Borrull, E. Pocurull, Chromatographia 61 (2005) 61.

[15] K. Luks-Betlej, P. Popp, B. Janozska, H. Paschke, J. Chromatogr. A 938 (2001) 93.

[16] M. Polo, M. Llompart, C. García-Jares, R. Cela, J. Chromatogr. A 1072 (2005) 63.

[17] L. Brossa, R.M. Marcé, F. Borrull, E. Pocurull, Environ. Toxicol. Chem. 24 (2005) 261.

[18] M.J. Silva, A.R. Slakman, J.A. Reidy, J.L. Preau Jr., A.R. Herbert, E. Samandar, L.L. Needham, A.M. Calafat, J. Chromatogr. B 805 (2004) 161.

[19] K. Mitani, F. Izushi, H. Kataoka, J. Anal. Toxicol. 28 (7) (2004) 575.

[20] N. Hata, E. Yuwatini, K. Ando, M. Yamada, I. Kasahara, S. Taguchi,. Anal. Sci. 20 (2004) 149.

[21] S. Jara, C. Lysebo, T. Greibrokk, E. Lundanes, Anal. Chim. Acta 407 (2000) 165.

[22] O.S. Fatoki, A. Noma, J. Chem. 54 (2001) 69.

[23] U. Kotowska, K. Garbowska, V.A. Isidorov, Anal. Chim. Acta 560 (2006) 110.

[24] G. Prokupková, K. Holadová, J. Poustka, J. Hajslová, Anal. Chim. Acta 457 (2002) 211.

[25] M. Castillo, A. Oubiña, D. Barceló, Environ. Sci. Technol. 32 (1998) 2180.

[26] K. Kato, M.J. Silva, L.L. Needham, A.M. Calafat, Anal. Chem. 77 (2005) 2985.

[27] F. David, P. Sandra, B. Tienpont, F. Vanwalleghem, in: C.A. Staples (Ed.), The Handbook of Environmental Chemistry. Part.Q. Phthalate Esters, vol. 3, Springer, Berlin, 2003, p. 15.

[28] A.M. Reid, C.A. Brougham, A.M. Fogarty, J.J. Roche, Intern. J. Environ. Anal. Chem. 87 (2007) 125.

[29] A. Fankhauser-Noti, K. Grob, Anal. Chim. Acta 582 (2007) 353.

[30] W.A.C. Anderson, K.A. Barnes, L. Castle, A.P. Damant, M.J. Scotter, Analyst, 127 (2002) 1193.

[31] S. Jonsson, J. Ejlertsson, A. Ledin, I. Mersiowsky, B.H. Svensson, Water Res. 37 (2003) 609.

[32] M.A. Faouzi, F. Khalfi, T. Dine, M. Luyckx, C. Brunet, B. Gressier, F. Goudaliez, M. Cazin, A. Belabed, J. C. Cazin, J. Pharm. and Biomed. Anal. 21 (1999) 923.

[33] S. Takatori, Y. Kitagawa, M. Kitagawa, H. Nakazawa, S. Hori, J. Chromatogr. B 804 (2004) 397.

[34] T. Hanawa, E. Muramatsu, K. Asakama, M. Suzuki, M. Tanaka, K. Kawano, T. Seki, K. Juni, S. Nakajima, Int.. J. Pharm. 210 (2000) 109.

[35] Y.Q. Cai, G.B. Jiang, J.F. Liu, Q.X. Zhou, Anal. Chim. Acta 494 (2003) 149.

[36] R. Kumar. A mass spectral guide for quick identification of phthalate esters. American Laboratory, (1999) 32.

[37] E. Psillakis, N. Kalogerakis, J. Chromatogr. A 999 (2003) 145.

[38] Y. Saito, Y. Nakao, M. Imaizumi, Y. Morishima, Y. Kiso, K. Jinno, Anal. Bioanal. Chem. 373 (2002) 81.

[39] R. Cela (Ed.). Avances en Quimiometría práctica. Universidad de Santiago de Compostela. Santiago de Compostela 1994, p 171

CAPÍTULO III

Direct LC-ES-MS/MS Determination of Phthalates in Physiological Saline Solutions

C. Pérez Feás, M.C. Barciela-Alonso, P. Bermejo-Barrera

JOURNAL OF CHROMATOGRAPHY B, 879 (2011) 231-235

Resumen

El objetivo de este capítulo es hacer uso de la mayor sensibilidad y especificidad que ofrece la espectrometría de masas en tándem para adquirir un mayor conocimiento de los ftalatos presentes en las muestras de solución salina comercializadas en envases de plástico monodosis.

Los ensayos se desarrollaron en los Servicios Generales de la USC con la colaboración del personal de la Unidad de Masas. Se utilizó un cromatógrafo de líquidos acoplado a un espectrómetro de masas con triple cuadrupolo, es decir, dos analizadores cuadrupolo en tándem y entre ellos la célula de colisión. El modo de trabajo elegido fue, como en el caso anterior, el electrospray en modo positivo (ESI+) y los analizadores operando en modo *Multiple Reaction Monitoring* (MRM), lo que hizo posible detectar niveles muy bajos de los analitos.

Para establecer las condiciones de trabajo del MS/MS se inyectó directamente en el detector una solución estándar de cada uno de los ftalatos de estudio (DMP, DEP, BBP y DBP).

La separación cromatográfica de la mezcla de analitos se llevó a cabo reproduciendo las mismas condiciones que las seleccionadas en el capítulo anterior y además en este caso se seleccionaron 40ºC como temperatura óptima del horno que contiene la columna cromatográfica.

El siguiente paso fue el estudio de las características analíticas del método, entre las que se encuentran los límites de detección y cuantificación. En las tablas se puede observar que los LOD y LOQ obtenidos son mucho más bajos que los obtenidos en el capítulo anterior al analizar el mismo tipo de muestras mediante LC-MS.

Tras comprobar la validez del método, en cuanto a linearidad, precisión y exactitud, éste se aplicó al análisis de las muestras de suero salino comercializadas en envases de plástico monodosis.

Los resultados ponen de manifiesto la presencia de DMP en las cuatro muestras analizadas, en 3 muestras se detectaron además niveles de DEP por debajo de los 15 μgL^{-1} y el DBP fue detectado únicamente en una de las muestras. El LOD obtenido para el DBP fue 0.05 μgL^{-1} y esta concentración no fue superada en ninguna de las muestras analizadas.

La principal ventaja del método es que permite detectar los analitos a muy bajas concentraciones sin ningún tratamiento previo de la muestra, disminuyendo además el riesgo de contaminación de la misma durante su procesado, que es uno de los principales problemas en el análisis de ftalatos.

Al igual que en el capítulo anterior, se asoció la presencia de los ftalatos con la liberación de los mismos desde el material de acondicionamiento, se trata, por lo tanto, de una fuente de contaminantes tóxicos que es necesario evitar para proteger la salud de la población. Este trabajo es una aportación más para resaltar la importancia que tienen los materiales de acondicionamiento, tanto de productos sanitarios como de alimentos, en la Salud Pública.

Contents

DIRECT LC-ES-MS/MS DETERMINATION OF PHTHALATES IN PHYSIOLOGICAL SALINE SOLUTIONS

C. Pérez Feás, M.C. Barciela-Alonso, P. Bermejo-Barrera

Department of Analytical Chemistry, Nutrition and Bromatology. University of Santiago de Compostela. Santiago de Compostela 15782, Spain.

Abstract

A method for determining a group of phthalic esters (PAEs) in physiological saline solutions has been developed. The PAEs studied were dimethyl phthalate, diethyl phthalate, butyl benzyl phthalate and dibutyl phthalate. These groups of phthalates were determined by liquid chromatography-electrospray ionization-tandem mass spectrometry, working in positive ion mode. The compounds were separated by liquid chromatography working in gradient mode with acetonitrile-ultrapure water as a mobile phase. The separation was performed starting with 5% of acetonitrile and increasing to 75% in 5 min, followed by isocratic elution for 8 min. The method was precise (with relative standard deviation (RSD) from 1.0 to 6.8%) and sensitive, with LODs of 0.05, 0.38, 0.05, 0.82 $\mu g L^{-1}$ for DMP, DEP, BBP and DBP respectively. The proposed analytical method has been applied to determine these compounds in different physiological saline solutions commercialized in plastic bottles.

Keywords: Phthalates, LC-ES-MS/MS, Physiological saline solutions.

*Corresponding author. Tel.: +34 881 814 266, +34 600 942 346; fax: +34 981 595 012. E-mail address: pilar.bermejo@usc.es (P. Bermejo-Barrera)

1. Introduction

Phthalates (PAEs) are a group of chemical compounds widely used in industry and commerce. Due to the ability to improve the softness and flexibility of plastics, they are widely used as polymer additives in plastics. These compounds are present in a wide variety of consumer products including children toys, cosmetics, personal care products, packaging, etc. [1-3]. Phthalates are not chemically bound to plastic; thus, they can be easily released from the plastic packaging to the contents and the environment [4].

The interest in the study of these types of chemical substances has increased in recent years because some of these compounds, such as dibutyl phthalate (DBP), butyl benzyl phthalate (BBP) and diethyl hexyl phthalate (DEHP), are suspected to be endocrine disruptors and carcinogenic to humans [5, 6]. Therefore, it is essential to develop reliable and sensitive methods for determining this group of compounds at trace levels.

Several methods have been developed for PAEs determination in different matrices such as, biological samples, pharmaceutical drugs and environmental samples. The analysis of PAEs is mostly performed by gas chromatography (GC). Generally, GC methods present better sensitivity than HPLC methods, although these depend on the pre-treatment step, the instrumental conditions and the sample matrix [7]. Phthalates can be detected using electron capture detection (ECD) [8, 9], flame ionization detection (FID) [10-12] and mass spectrometry (MS) [13-15]. HPLC can be used as an alternative technique and is especially useful for analysis of isomeric mixtures and phthalates metabolites without derivatization [16]. HPLC can be used in combination with different detectors such as UV [17-19], mass spectrometry [20-24] and using tandem mass spectrometry [16, 25-28].

In some cases, due to the low levels of these compounds in the samples, a clean up/ preconcentration step is necessary before the instrumental analysis. These sample pre-treatments include liquid-liquid extractions (LLE) [24, 29, 30], liquid-phase microextraction (LPME) [31], single drop microextraction (SDME) [32], solid phase extraction (SPE) [25, 33], solid phase microextraction (SPME) [34, 35], stir bar sorptive extraction (SBSE) [36, 37] and solid-liquid extraction (SLE) [38]. The major problem in phthalate determination is the sample contamination during the sample pre-treatment. Due to the fact that these compounds are widely used, they are present in the environment and can be adsorbed onto the glass and other material. This problem can be diminished by using different methods proposed in the literature to prevent phthalate contamination problems [20, 21, 27] and by reducing the number of steps necessary to prepare the sample.

The aim of this work was to develop a high sensitive method for phthalates determination in physiological saline solution samples by LC-ES-MS/MS without any sample pre-treatment.

2. Experimental

2.1. Reagents and standards

All reagents used were of analytical reagent-grade. Dimethyl phthalate (DMP) and butyl benzyl phthalate (BBP) were obtained from Supelco (Bellefonte, PA, USA). Diethyl phthalate (DEP) and dibutyl phthalate (DBP) were obtained from Riedel-de Haën (Seelze, Germany). The purity of these reagents was over 98%.

Stock standard solutions of each phthalate ester at a concentration of 1000 mgL^{-1} were prepared in methanol, kept in darkness and stored at 4ºC in a Teflon-capped glass vial. From these solutions, a working standard solution in methanol was prepared weekly containing all standards at concentrations of 100 mgL^{-1} each.

Diluted working standard solutions were prepared daily by diluting the working solution.

Lichrosolv gradient grade acetonitrile and methanol were purchased from Merck (Darmstadt, Germany). Technical-grade acetone and glacial acetic acid (HPLC) for instrumental analysis were purchased from Panreac (Barcelona, Spain). Ultrapure (resi-analysed) water for environmental inorganic and organic trace analysis was supplied by J.T. Baker (Phillipsburg, NJ, USA).

Special care was taken to avoid the contact of reagents and solvents with plastic materials. Because of the ubiquity of plasticizers and the tendency of residues to persist, all glassware was cleaned prior to the analysis according to the recommendations specified in U.S. EPA Method 506 [39]. All material was washed with hot water and soap, rinsed with tap and ultrapure water and finally thoroughly rinsed with technical-grade acetone. Glassware was then sealed with aluminium foil and stored in a clean environment to avoid adsorption of phthalates from the air.

2.2. Instrumentation

Phthalates separation and quantification was performed using liquid chromatography/electrospray ionization-tandem mass spectrometry system.

A Series 1100 liquid chromatograph from Agilent Technologies **(**Waldbronn, Germany) was coupled to an API 4000^{TM} Triple Quadrupole Mass spectrometer (Applied Biosystems, Concord, Canada) equipped with a Turbo Ionspray^{TM} ionization source. Mass Spectrometry data were processed with Analyst 1.4.2 software.

A ZORBAX Eclipse XDB-C8 column (2.1 mm x 50 mm, 3.5 µm particle size) supplied by Agilent Technologies was used for the separation of these compounds.

2.3. Chromatographic conditions

Ultrapure water and acetonitrile (both solvents containing 0.1% (*v/v*) acetic acid) were used as a binary mobile phase. Phthalates were separated by LC working in gradient mode with acetonitrile-ultrapure water as a mobile phase. The separation was performed starting with 5% of acetonitrile and increasing to 75% in 5 min, followed by isocratic elution for 8 min.

The flow rate and the injection volume were 200 $\mu L min^{-1}$ and 10 µL respectively, and the chromatographic separation was performed at 40ºC. Under these conditions the separation time was less than 13 min. These optimal conditions are shown in *Table 1*.

Table 1- *Operational conditions for LC-MS/MS*

HPLC (Agilent 1100)	
Column	Zorbax Eclipse XDB-C8 (3.5µm 2.1mm x 50mm)
Mobile phase	Ultrapure water : Acetonitrile (0.1% (v/v) acetic acid)
Mode	Gradient
Flow rate	200 $\mu L min^{-1}$
Oven temperature	40ºC
Injection volume	10 µL
MS/MS (API 4000)	
Ion Spray Voltage	5500 V
Ionization mode	ESI-positive
Curtain gas	25 psi (nitrogren)
GS1 (nebulizer gas)	50 psi
GS2 (auxiliary gas)	60 psi
Ion source temperature	450ºC
CAD (collisionally activated dissociation)	4

2.4. Sample preparation

The samples were injected directly into the chromatograph, without any previous sample preparation process.

3. Results and discussion

3.1. *ES-MS/MS conditions*

The ES-MS/MS conditions for DMP, DEP, BBP and DBP determination by ES-MS/MS were studied. The ion source dependent (turbo ion spray) conditions were the same for all the analytes with an electrospray needle voltage of 5500 V in the positive ion mode. Nitrogen as a nebulizer and turbo heater gas (at 450ºC) was set as a pressure of 50 and 60 psi respectively. The pressure of the curtain gas was also optimized selecting 25 psi as the optima pressure. Ion source collision-activated dissociation (CAD) was studied during the development of the method, selecting 4 V as the optimum condition.

To establish the MS/MS operating conditions used to determine these phthalates by ES-MS/MS, a standard solution (100 mgL^{-1}) of each phthalate were used. These solutions were infused directly into the MS/MS system using the syringe pump system of the API 4000. The phthalates studied were monitored at *m/z* 195, 223, 313 and 279, working in the scan mode, which were assigned to $[M+H]^+$. Moreover, in the product ion MS/MS measurement, the selective reaction monitoring ions (SRM) of DMP, DEP, BBP and DBP were set depending on their precursor ions. The combinations of precursor ion and product, as well the optimum potentials, are shown in *Table 2*.

Table 2- *Optimal values of the compound parameters for the four phthalates studied, m/z transition selected and retention time. (DP: declustering potencial; EP: enhance potential; CE: collision energy; CXP: collision cell exit potential).*

Compound	**Acronym**	***m/z* transition**	**Potentials optimization**				$t_{R\ (min)}$
			DP	**EP**	**CE**	**CXP**	
Dimethyl phthalate	DMP	195→163	31	10	13	14	8.4
Diethyl phthalate	DEP	223→149	36	10	23	12	9.2
Butyl benzyl phthalate	BBP	313→91	41	10	23	6	11
Dibutyl phthtalate	DBP	279→205	50	9	11	10	11.2

3.2 Optimization of LC separation

After optimizing the detection conditions, the following experiments were conducted to optimize the chromatographic separation of the analytes.

Experiments were performed using acetonitrile:water, both solvents containing 0.1% (*v/v*) acetic acid as a mobile phase. This mobile phase was selected based on a previous work developed in our research group for phthalates determination in physiological saline solutions by LC-ES-MS [20]. Experiments were developed using a physiological saline solution spiked with 25 μgL^{-1} of DMP, DEP and BBP, and 100 μgL^{-1} of DBP. The best results were obtained starting the elution with 5% of acetonitrile, which was then increased linearly to 75% in 5 min. This composition was maintained for 8 min before returning to initial conditions. The column was equilibrated for 10 min.

Other parameters optimized were the temperature of the chromatographic column and the flow rate of the mobile phase. The optimum conditions selected were a temperature of 40ºC and a flow rate of 200 $\mu L min^{-1}$.

The chromatogram obtained for the physiological saline solution, spiked with 25 μgL^{-1} of DMP, DEP and BBP, and 100 μgL^{-1} of DBP, under the optimized conditions is shown in *Figure 1*.

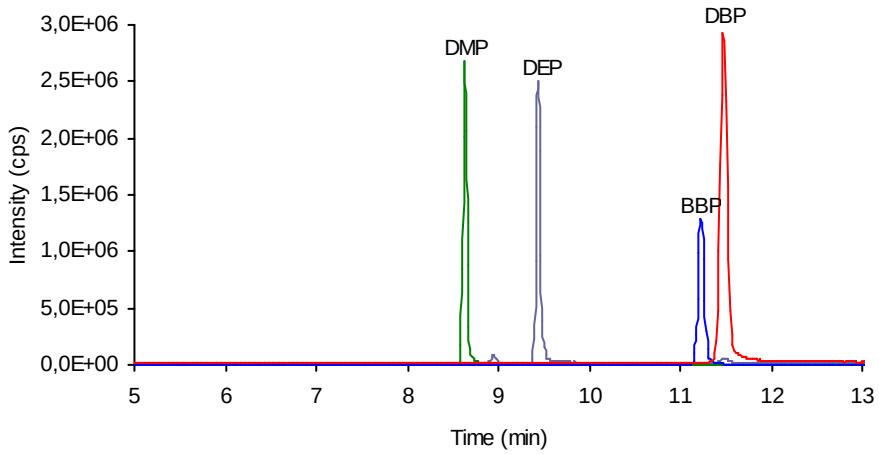


***Figure 1**- LC-MS/MS ion chromatogram obtained from a physiological saline solution spiked with 25 μgL^{-1} of DMP, DEP and BBP, and 100 μgL^{-1} of DBP*

3.3 Analytical performances

After selection of the optimum conditions for LC-ES-MS/MS, the method was evaluated using DMP, DEP, BBP and DBP standard solutions.

The linearity of the response of this method was evaluated using a standard addition method. This addition was performed at seven different concentrations of the standard solution of these phthalates, using a commercial physiological saline solution supplied in a glass bottle. Linear regression was performed by plotting the peak area versus concentration, and was linear over the range of 0-50 μgL^{-1} for DMP, DEP and BBP, and of 0-150 μgL^{-1} for DBP. The equations obtained for each compound were as follows:

DMP:	$Q_A = 273725\ C + 265276$	$r = 0.9996$
DEP:	$Q_A = 325956\ C + 208430$	$r = 0.9978$
BBP:	$Q_A = 255127\ C + 185647$	$r = 0.9986$
DBP:	$Q_A = 129571\ C + 263706$	$r = 0.9956$

Where Q_A is the peak area and C is the concentration in μgL^{-1}.
The limit of detection (LOD) and limit of quantification (LOQ) were calculated based on the IUPAC definition:

$$LOD = \frac{3SD}{m} \qquad LOQ = \frac{10SD}{m}$$

Where *SD* is the standard deviation of ten blank solutions and *m* is the slope of the addition graph. A commercial physiological saline solution supplied in a glass bottle was used as a blank. The results obtained for LODs and LOQs are shown in *Table 3*.

***Table 3**- Linear range, correlation coefficients, LODs and LOQs values obtained from the standard addition method in physiological saline solutions*

Phthalate	Linear range (μgL^{-1})	Correlation coefficient (r)	LOD (μgL^{-1})	LOQ (μgL^{-1})
DMP	0.5 - 50	0.9996	0.05	0.16
DEP	1 - 50	0.9978	0.38	1.27
BBP	1 - 50	0.9986	0.05	0.16
DBP	1 - 150	0.9956	0.82	2.74

The LODs obtained are between 0.05 and 0.82 μgL^{-1}. The highest LOD obtained was for DBP. These LODs are lower than those obtained in a previous study to determine these compounds in the same type of samples by LC-MS [20]. Moreover, the method presents better or comparable sensitivity than other methods proposed in the literature for the determination of these phthalates using GC-MS in water samples. Serodio and Nogueira [2] developed a method for phthalates determination using stir bar sorptive extraction with liquid desorption followed by large volume injection and GC-MS obtaining LODs from 0.15 to 0.60 μgL^{-1}. Peñalver et al. [40] obtained LODs from 15 to 50 μgL^{-1} for these phthalates using GC-MS, and obtained LODs from 0.007 to 0.17 μgL^{-1} using SPME previous to the determination by GC-MS. Koch et al. [1] obtained LODs from 0.25 to 1.0 μgL^{-1} for the determination of these phthalates in urine samples by LC-ESI-MS/MS.

The advantage of the proposed method is that present a good sensitivity when analyzing the sample directly, without any requiring preparation steps (e.g. preconcentration step).

Assays were developed to check intra- and interday precision. For the intraday study, aliquots of a physiological saline solution purchased in a glass bottle were spiked with two concentration levels of all phthalates studied and analysed six

times in the same run. The interday assay was performed in the same way analyzing 12 aliquots of spiked samples in two different days. The results obtained for the intra- and interday assays are shown in *Table 4*.

***Table 4**- Results of intra- and interday assays to validate proposed LC-MS/MS method.*

Phthalate	**Intraday (n=6)**			**Interday (n=12)**		
	Detected average ($ngmL^{-1}$)	**SD**	**RSD (%)**	**Detected average ($ngmL^{-1}$)**	**SD**	**RSD (%)**
DMP	29.86	0.36	1.20	29.88	0.29	0.97
	49.10	2.02	4.12	50.23	1.94	3.86
DEP	24.88	0.31	1.25	26.52	1.81	6.84
	46.24	1.04	2.25	46.95	1.33	2.84
BBP	24.28	0.46	1.89	24.44	0.61	2.51
	48.04	2.41	5.01	49.51	2.28	4.61
DBP	92.06	2.01	2.19	97.88	6.55	6.70
	148.91	6.91	4.64	146.74	5.40	3.68

The RSD values were between 1.2 and 5.0% in the intraday assay and between 1.0 and 6.8% in the interday assay; thus, the method is precise for all the compounds studied.

The analytical recovery of the method was calculated using a blank sample (physiological saline solution commercialized in a glass bottle) spiked with three different concentrations of these compounds (5, 25 and 50 μgL^{-1} for DMP, DEP and BBP and 40, 100 and 150 μgL^{-1} for DBP). The spiked samples were prepared

twice and analysed three times, and the recovery calculated using the standard addition graph. The recovery percentages obtained are shown in *Table 5*. The average analytical recoveries were 106.7, 92.6, 102.9 and 96.4% for DMP, DEP, BBP and DBP, respectively.

Table 5- *Recovery percentage for physiological saline solutions ± standard deviation to validate proposed LC-MS/MS method. (n=3)*

	% Recovery		
Phthalate	***5 μgL^{-1}***	***25 μgL^{-1}***	***50 μgL^{-1}***
DMP	100.3 ± 2.5	118.3 ± 0.8	101.4 ± 3.2
DEP	81.4 ± 1.6	101.4 ± 0.5	94.9 ± 0.6
BBP	111.5 ± 2.8	98.7 ± 0.8	98.6 ± 2.7
	% Recovery		
Phthalate	***40 μgL^{-1}***	***100 μgL^{-1}***	***150 μgL^{-1}***
DBP	92.2 ± 2.2	93.6 ± 1.2	103.4 ± 1.6

3.4 Application to physiological saline solution samples

The proposed analytical method has been applied to the analysis of different physiological saline solution samples, commercialised in plastic bottles, in order to check the presence of these phthalates and determine their concentration. Samples were directly injected into the chromatographic system; and no sample preparation process was necessary.

The results obtained for DMP, DEP, BBP and DBP are given in *Table 6*.

Table 6- *Concentration (μgL^{-1}) ± standard deviation (based on three replicates) found in different physiological saline solutions. < LOD: lower than the detection limit.*

Physiological saline solution	DMP	DEP	BBP	DBP
brand 01	17.4 ± 0.6	14.5 ± 0.4	< LOD	7.7 ± 0.6
brand 02	0.4 ± 0.1	< LOD	< LOD	< LOD
brand 03	19.2 ± 1.5	3.9 ± 0.2	< LOD	< LOD
brand 04	346.8 ± 0.8	2.7 ± 0.1	< LOD	< LOD

The concentration levels obtained for BBP are lower than the LOD for all samples studied, and DBP was only detected in brand 1. The concentration levels varied from 0.4 to 346 μgL^{-1} for DMP and from 0.4 to 14.5 μgL^{-1} for DEP. The brand 2 sample presented the lowest concentration of phthalates, being DMP the only phthalate detected. Phthalate esters are used in the manufacture of plastic containers; thus, the presence of phthalates in the samples can be attributed to the release of these compounds from the plastic containers. As an example, the chromatogram obtained when analyzing the brand 1 sample is shown in *Figure 2*.

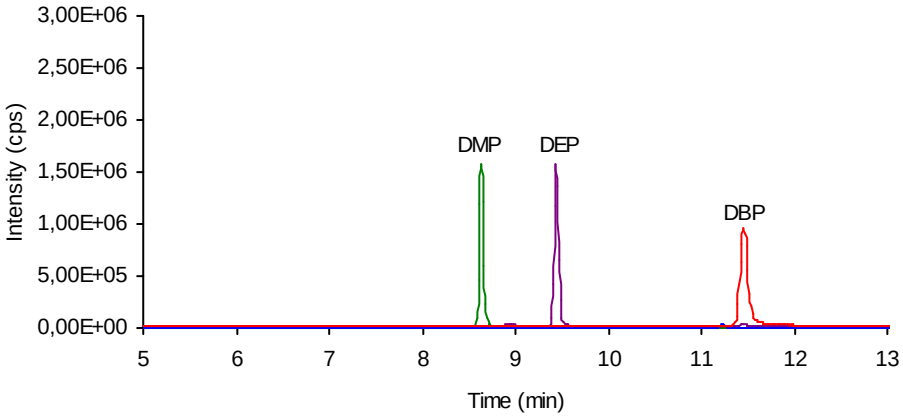


***Figure 2**- LC-ES-MS/MS ion chromatogram obtained from brand 1 physiological saline solution.*

4. Conclusion

A rapid (less than 13 min), sensitive and accurate method for the determination of DMP, DEP, BBP and DBP by LC-ES-MS/MS was developed.

The main advantage of this method, compared with the methods proposed in the literature, is that the compounds can be detected at very low concentration without any sample pre-treatment. Moreover, the limits of detection obtained are comparable with the LODs found in the literature for determining of these phthalates by researches who performed a preconcentration step before the determination by GC-MS.

Another advantage is that the reduction of the number of sample pre-treatment steps decreases the risk of the sample contamination during the analysis, which is a very common problem in the analysis of phthalates.

The method was applied for the determination of these compounds in four physiological saline solutions commercialized in plastic bottles. The presence of these compounds in the samples can be attributed to the different compositions of the plastic containers. Thus, control of material used in the manufacture of the plastic containers is essential to avoid human exposure to these toxic contaminants.

Acknowledgement

The authors wish to thank the Xunta de Galicia (Grupo de Referencia Competitivos 2007 (000047-0) for financial support.

5. References

[1] H.M. Koch, L.M. Gonzalez-Reche, J. Angerer, J. Chromatogr. B 784 (2003) 169.

[2] P. Serodio, J.M.F. Nogueira, Water Res. 40 (2006) 2572.

[3] T. Jones-Lepp, C.L. Gerlach, E.J. Cooter, Trends Anal. Chem. 19 (2000) 286.

[4] A. Gómez-Hens, M.P. Aguilar-Caballos, Trends Anal. Chem. 22(2003)847.

[5] I. Colón, D. Caro, J. Carlos, C.J. Bourdony, O. Rosario, Environ. Health Prespect. 108 (2000) 895.

[6] R.M. Sharpe, Int. J. Androl. 26 (2003) 2.

[7] Communication from the Comision to the Council and the European Parliament on the implementation of the community strategy for endocrine disrupters-a range of sustances suspected of interfering with the hormona systems of humans and wildlife, COM (1999) 706; COM (2001) 262 final, Brussels, 2001.

[8] G. Prokupková, K. Holandová, J. Poustka, J. Hajslová, Anal. Chim. Acta 457 (2002) 211.

[9] K. Holadová, G. Prokupková, J. Hajslová, J. Poustka, Anal. Chim. Acta 528 (2007) 24.

[10] O.S. Fatoki, A. Noma, S. Afr. J. Chem. 54, (2001) 69.

[11] X. Li, M. Zhong, S. Xu, C. Sun, J. Chromatogr. A 1135 (2006) 101.

[12] A. Adeniyi, M. Dayomi, P. Siebe, O. Okedeyi, Chem. Cent. J. 2 (2008) 1.

[13] B. Cavaliere, B. Macchione, G. Sindona, A. Tagarelli, J. Chromatogr. A 1205 (2008) 137.

[14] A.O. Earls, I.P. Oxford, J.H. Braybrook, J. Chromatogr. A 983 (2003) 237.

[15] O. Ballesteros, A. Zafra, A. Navalón, J.L. Vílches, J, Chromatogr. A, 1121 (2006) 154.

[16] M.J. Silva, A.R. Slakman, J.A. Reidy, J.L. Preau Jr., A.R. Herbert, E. Samandar, L.L. Needham, A.M. Calafat, J. Chromatogr. B 805 (2004) 161.

[17] S. Jara, C. Lysebo, T. Greibrok, E. Lundanes, Anal. Chim. Acta 407 (2000) 165.

[18] Y.-Q. Cai, G. Jiang, J. Liu, Anal. Sci. 19 (2003) 1491.

[19] D. Sicar, S.J. Albazi, Y. Atallah, W. Pizza, J. Chromatogr. Sci. 46 (2008) 1471.

[20] C. Pérez Feás, M.C. Barciela Alonso, E. Peña Vázquez, P. Herbello Hermelo, P. Bermejo Barrera, Talanta 75 (2008) 1184.

[21] R.A. Gimeno, R.M. Marcé, F. Borrull, Chromatographia 58(2003) 37.

[22] L. Brossa, R.M. Marcé, F. Borrul, E. Pocurull, Environ. Toxicol. Chem. 24 (2005) 261.

[23] B.C. Blount, K.E. Milgram, M.J. Silva, N.A. Malek, J.A. Reidy, L.L. Needham, J.W. Broca, Anal. Chem. 72 (2000) 4127.

[24] C. Pérez Feás, M.C. Barciela Alonso, A. Sedes-Díaz, P. Bermejo-Barrera, Anal. Bioanal. Chem. 397 (2010) 529.

[25] K. Kato, M.J. Silva, L.L. Needham, A.M. Calafat, J. Chromatogr. B 814 (2005) 355.

[26] M.J. Silva, N.A. Malek, C.C. Hodge, J.A. Reidy, K. Kato, D.B. Barr, L.L.Needham, J.W. Broca, J. Chromatogr. B 789 (2003) 393.

[27] S. Takatori, Y. Kitagawa, M. Kitagawa, H. Nakazawa, S. Hori, J. Chromatogr. B 804 (2004) 397.

[28] R. Ito, F. Seísmo, N. Miura, M. Kawaguchi, K. Saito, H. Nakazawa, J. Pharmaceut. Biomed. Anal. 39(2005) 1036.

[29] K. Kambia, T. Dine, B. Gressier, AF Germe, M. Luyckx C. Brunet, L. Michaud, F. Gottrand. J. Chromatogr. B 755 (2001) 297.

[30] C. Ji-an, L. Huijie, Q. Zhiqun, S. Weiqun Environ. Pollut. 156 (2008) 849.

[31] H. Farahani, M. Reza, R. Dinarvand, P. Norouzi, Talanta 76 (2008) 718.

[32] R. Battle, C. Nerón J. Chromatogr. A 1045 (2004) 29.

[33] M. Del Carlo, A. Pepe, G. Sacchetti, D. Compagnone, D. Mastrocola, A. Cichelli, Food Chem. 111 (2008) 771.

[34] A. Peñalver, E. Pocurull, F. Borull, RM Marcé, J. Chromatogr. A 922 (2001) 377.

[35] K. Holadová, G. Prokupková, J. Hajslová, J. Poustka, Anal. Chim. Acta 582 (2007) 24.

[36] P.Serodio, JMF Nogueira, Anal. Chim. Acta 517 (2004) 21.

[37] A. Prieto, O. Telleira, N. Etxebarria, L.A. Fernández, A. Usobiaga, O. Zuloaga, J. Chromatogr. A 1214 (2008) 1.

[38] M. Bonini, E. Eran, G. Zerbinati, E. Ferri, S. Girotti Michrochem. J. 90 (2008) 31.

[39] USEPA Method 506 revision 1.1. (1995). "Determination of Phthalates and Adipate Esters in Drinking Water by Liqui-Liquid Extraction or Liquid-Solid Extraction and Gas Chromatography with Photoionization Detection. National Exposure Reshearch Laboratory Office of Research and Development U.S. Environmental Protection Agency. Cincinnati, OH 45268.

[40] A. Peñalver, E. Pocurull, F. Borrull, R.M. Marcé, J. Chromatogr. A 872 (2000) 191.

CAPÍTULO IV

Phthalates Determination in Pharmaceutical Formulae Used in Parenteral Nutrition by LC-ES-MS: Importance in Public Health

Cristina Pérez-Feás, María Carmen Barciela-Alonso, Antía Sedes-Díaz,
Pilar Bermejo-Barrera

ANALYTICAL AND BIOANALYTICAL CHEMISTRY (2010) 397: 529-535

Resumen

La nutrición parenteral total (NPT) es una técnica de soporte nutricional artificial cuyo objetivo es mantener el estado nutricional correcto del paciente cuando la vía enteral es inadecuada o insuficiente; se administran por vía endovenosa todos los nutrientes (aminoácidos, carbohidratos, lípidos, electrolitos, vitaminas y elementos traza) en las cantidades y proporciones adecuadas. Los nutrientes alcanzan la circulación venosa directamente, sin atravesar la barrera intestinal.Una NPT puede contener más de 50 componentes diferentes en solución con un alto potencial de interacciones químicas y físico-químicas entre sus ingredientes, sumado a los materiales de acondicionamiento (PVC o EVA) y a los factores externos como el oxígeno, la temperatura, la luz y la humedad.

El material de acondicionamiento juega un papel muy importante en la estabilidad y seguridad del paciente, debido a que sus productos de fabricación interaccionan con los componentes de la nutrición. Se recomienda no utilizar bolsas de PVC para la administración de las nutriciones parenterales debido a que en su fabricación se utilizan ciertos componentes como los ftalatos, que actúan como plastificantes para impartir flexibilidad, que carecen de uniones químicas covalentes con la matriz de PVC y pueden ser arrastrados fácilmente por los componentes lipídicos al seno de la nutrición y, en definitiva, llegar al paciente a través de la circulación sanguínea, pudiendo alcanzarse concentraciones tóxicas. Debido al carácter liposoluble de los ftalatos, las nutriciones parenterales que contengan emulsiones grasas deben almacenarse en bolsas de etilvinilacetato (EVA) y no en bolsas de PVC; sin embargo las conexiones utilizadas entre las bolsas y el catéter siguen conteniendo plastificantes.

El objetivo en este capítulo fue analizar las nutriciones parenterales procedentes de la Unidad de Cuidados Intensivos de neonatos del Hospital Clínico Universitario

de Santiago de Compostela con el fin de detectar y cuantificar la presencia de ftalatos en las mismas a pesar de estar contenidas en bolsas EVA.
El interés radica en que los pacientes ingresados en esta Unidad son los más vulnerables debido a la escasa madurez de sus órganos por lo que resulta de vital importancia conocer el riesgo potencial al que pueden estar sometidos por la migración de ftalatos a través de los dispositivos médicos.
Las muestras del estudio se clasificaron en dos tipos de nutriciones denominadas *tipo A* y *tipo B*; la única diferencia entre ellas es la presencia de una mezcla de oligoelementos, también llamados elementos traza, en la de tipo A y un complejo multivitamínico, que incluye vitaminas liposolubles, en la de tipo B. El resto de los componentes de la nutrición son comunes a ambos tipos de nutriciones.
Las nutriciones tipo A y B se administran a los pacientes en días consecutivos, evitando así la presencia conjunta de elementos traza y vitaminas en la bolsa de nutrición que pueden ocasionar interacciones entre los componentes.

El desarrollo del estudio, desde el punto de vista analítico, fue llevado a cabo utilizando una nutrición parenteral contenida en envase de vidrio constituida por los mismos componentes que las nutriciones parenterales de estudio. Esta muestra "blanco" fue preparada por el Servicio de Farmacia Hospitalaria siguiendo el protocolo de preparación de NPT.

En la primera parte de este capítulo se trabajó sobre el método analítico desarrollado en el capítulo II para adaptar el método de HPLC-MS al análisis de seis ftalatos (DMP, DEP, BBP, DBP, DEHP y DOP) y se utilizó el DPeP como estándar interno.
Por otra parte, la cuantificación de los ftalatos a partir de nutriciones parenterales requirió de un proceso de extracción previo al análisis cromatográfico. Este procedimiento se desarrolló mediante una extracción líquido-líquido (LLE)

empleando pequeños volúmenes de muestra y de disolvente orgánico. Se consiguieron así dos objetivos, la separación de los analitos de la matriz y la preconcentración de los mismos.

Los resultados obtenidos mostraron una clara diferencia en el contenido de ftalatos entre las muestras sin vitaminas y las muestras con vitaminas. Este resultado se atribuyó a que el contenido lipídico de las muestras con vitaminas favorece la liberación de estos compuestos desde las bolsas de infusión a las muestras.

En la última parte de este capítulo se estudió la presencia de ftalatos en el material utilizado en las vías de infusión; para ello se cuantificaron los ftalatos presentes en una muestra de NPT conteniendo vitaminas antes y después de pasar por el tubo de infusión empleado en la administración. Los resultados permitieron confirmar el aumento de DEP y DEHP en la muestra analizada después de pasar por los tubos de infusión.

Estos resultados nos permiten evidenciar la importancia de controlar el material empleado en la fabricación de dispositivos médicos con el fin de evitar la exposición a contaminantes tóxicos, como los ftalatos, que pueden derivar en importantes complicaciones en los pacientes.

Contents

PHTHALATES DETERMINATION IN PHARMACEUTICAL FORMULAE USED IN PARENTERAL NUTRITION BY LC-ES-MS: IMPORTANCE IN PUBLIC HEALTH

Cristina Pérez-Feás, María Carmen Barciela-Alonso, Antía Sedes-Díaz, Pilar Bermejo-Barrera

Department of Analytical chemistry, Nutrition and Bromatology. University of Santiago de Compostela. Santiago de Compostela 15782. Spain.

Abstract

A method for determining a group of phthalate esters in pharmaceutical formulae used in parenteral nutrition samples (with and without vitamins) has been developed. The phthalic acid esters (PAEs) studied were dimethyl phthalate, diethyl phthalate, butyl benzyl phthalate, dibutyl phthalate, di-(2-ethylhexyl) phthalate, and dioctyl phthalate. This group of phthalates was determined by high performance liquid chromatography (HPLC)-electrospray ionization-mass spectrometry, working in positive ion mode. The phthalates analyzed were extracted from the sample using hexane and sodium hydroxide. The hexane was then evaporated, and the compounds were redissolved in acetonitrile. The compounds were separated by HPLC working in a gradient mode with acetonitrile-ultrapure water starting from 5% to 75% acetonitrile in 5 min, followed by isocratic elution for 27 min. Standard calibration curves were linear for all the analytes over the concentration range 10-250 $\mu g L^{-1}$. The method was precise (with RSD from 3.3% to 12.9%) and sensitive. The proposed analytical method has been applied to the analysis of these compounds in different pharmaceutical formulae (with different compositions) for parenteral nutrition samples in order to check the presence of phthalates and determine their concentration.

Keywords: Phthalates, LC-ES-MS, Parenteral nutrition

1. Introduction

Phthalates or phthalic acid esters (PAEs) are a group of chemical compounds widely use in industry and commerce due to their large variety of uses. Due to the ability to increase the softness and flexibility of plastics, they are used mainly as plasticizers in a wide variety of products including medical devices, children´s toys, and all types of packaging. The main drawback of the use of PAEs is that they can migrate from the material to the environment and pollute water, soil and food products. Furthermore, certain phthalate esters and or their metabolites are suspected to be human carcinogenic agents and endocrine disruptors, which make their trace determination particularly important [1, 2]. The interest of the determination of these compounds has increased in recent years due to results obtained in the studies concerning in human blood and urine samples where trace levels of various phthalates (or their metabolites) have been found [3, 4].

Several techniques have been used for PAEs determination in different matrices. In order to detect PAEs at sub ppm levels in different samples, a clean up/preconcentration step is necessary before instrumental analysis. Different methods have been developed with this purpose such as liquid-liquid extraction (LLE) [5-10], liquid-phase microextraction (LPME) [11], single drop microextraction (SDME) [12] solid phase extraction (SPE) [13-17], solid phase microextraction (SPME) [18-20], stir bar sorptive extraction (SBME) [21, 22], and solid/liquid extraction (SLE) [23, 24].

Gas chromatography (GC) and high performance liquid chromatography (HPLC) are the techniques usually used for PAEs separation in different matrices, such as environmental or biological samples. Generally, GC presents higher sensitivity than HPLC methods, although depending on the pre-treatment step, the instrumental conditions, and the sample matrix [1]. HPLC can be used as an

alternative technique and is especially useful for analysis of isomeric mixtures and metabolites of phthalates without derivatization [25]. Ultraviolet detection has been used for phthalate determination in environmental and biological samples [8, 9, 17, 26, 27]. However the use of mass spectrometry has increased in recent years, either operating with a single spectrometer [28, 29] or using a mass spectrometer in tandem [4, 16, 30, 31] with applications in different matrix samples (sludge, urban wastewater, urine, milk, and drugs). Although CG-MS offered higher sensitivity for phthalate determination than LC-MS, LC-MS approach offered some advantages, compared with GC-MS, such as superior selectivity with molecular weight information for the isomeric mixtures, more reliable quantification of PAEs isomeric mixtures, simpler cleanup procedures, and shorter analysis time. Moreover, phthalic acid monoesters can be analyzed without derivatization by HPLC [32].

The major problem in phthalate analysis is contamination, resulting in false positive results or over-estimated concentrations. The risk of contamination is present in the whole analytical scheme, including sampling, sample preparation, and chromatographic analysis. Due to the fact that phthalates are widely used, they are present in air, water, organic solvents and plastic, and adsorbed onto glass or other materials [32].

Different cleaning methods have been proposed to prevent phthalate contamination problems due to the phthalates from material used in the laboratory. In most of these methods, glass material is rinsed with organic solvents after a rigorous washing [17, 31, 33].

The aim of this work was the development of a method for phthalates determination present in parenteral nutrition samples by HPLC-ES-MS.

2. Experimental

2.1. Reagents and Standards

All reagents used were of analytical reagent grade. Dimethyl phthalate (DMP), butyl benzyl phthalate (BBP), dipentyl phthalate (DPeP), and dioctyl phthalate (DOP) were obtained from Supelco (Bellefonte, PA, USA). Diethyl phthalate (DEP) and dibutyl phthtalate (DBP) were obtained from Riedel-de Haën (Seelze, Germany). Diethylhexyl phthalate (DEHP) was obtained from Merck (Darmstad, Germany). The purity of these reagents was over 98%.

Individual standard solutions of each phthalate ester at a concentration of 1000 mgL^{-1} were prepared in methanol, protected from light, and stored at 4°C in a Teflon-capped glass vial. From these solutions, a working mixture in methanol was prepared weekly, containing all standards of concentration 100 mgL^{-1} each. All the working solutions were prepared daily by diluting this solution.

Hexane (PA-ACS-ISO) (Panreac, Barcelona, Spain) and sodium hydroxide (Merck, Darmstadt, Germany) were used in the liquid-liquid extraction.

Lichrosolv gradient grade acetonitrile and methanol were purchased from Merck (Darmstadt, Germany). Technical-grade acetone and acetic acid glacial (HPLC) for instrumental analysis were purchased from Panreac (Barcelona, Spain). Ultrapure (resi-analized) water for environmental inorganic and organic trace analysis was supplied by J.T. Baker (Phillipsburg, NJ, USA).

Special care was taken to avoid contact of reagents and solvents with plastic materials. In order to reduce background contamination, all glassware was cleaned prior to the analysis according to the recommendations specified in EPA method 506. All material was washed with hot water and soap, rinsed with tap and ultrapure water and thoroughly rinsed with technical-grade acetone. Glassware was then sealed with aluminium foil and stored in a clean environment to prevent adsorption of phthalates from air.

2.2. Instrumentation

Phthalates separation and quantification were carried out using a liquid chromatography/electrospray ionization mass spectrometry system.

The HPLC system used was a 1100 Series equipped with an automatic injector (Agilent Technologies, Waldbronn, Germany) that is coupled to an API 150 EX single quadrupole mass spectrometer equipped with a Turboionspray interface (PE Biosystems, Concord, Canada).

The analytical column was a ZORBAX Eclipse XDB-C_8 of 50 mm length and 2.1 mm internal diameter (particle size 3.5 μm) supplied by Agilent Technologies.

A centrifuge Selecta (Barcelona, Spain) working at 3500 rpm was used in the liquid-liquid extraction procedure.

2.3. Chromatographic and Mass Spectrometry Conditions

The binary mobile phase consisted of ultrapure water and acetonitrile, both solvents containing 0.1% (*v/v*) acetic acid. The elution gradient started with 5% of acetonitrile, which was increased linearly to 75% in 5 min. This composition was maintained for 27 min before returning to the initial conditions. The column was then equilibrated for 10 min.

The flow rate and the injection volume were 200 $\mu L min^{-1}$ and 10 μL, respectively, and the chromatographic separation was carried out at room temperature. Under these conditions, the separation time was less than 30 min.

Electrospray ionization was performed in positive ion mode using the operational parameters shown in *Table 1*.

The compound parameters such as declustering potential (DP), focusing potential (FP), and enhance potential (EP) were optimized for each analyte. The optimal conditions are displayed in *Table 1*.

***Table 1**: ES-MS parameters*

Compound	*m/z*	DP	FP	EP
DMP	163.25	40.38	73.87	8
BBP	91.15	25	225	6
DEP, DBP, DPeP, DEHP, DOP	149.05	25	290	8.5

Nebulizer and Curtain gas (N_2): 14 psi; Heater gas: 7000 cc/min; ES temperature: 450ºC; Ionspray voltage: 5500 V; mode: positive. DP: Declustering potential; FP: Focussing Potential; EP: Enhance Potential

2.4. Sample preparation

The six phthalate esters studied in this work were extracted from the sample using a liquid-liquid extraction procedure. Thus, a volume of 1 mL of parenteral nutrition and 1 mL of NaOH 0.1M were introduced into a conical glass tube. The mixture was vortexed for 2 min, and then 2 mL of hexane was added. The solution was shaken for 3 min in samples without vitamins and 5 min in samples with vitamins. In samples with vitamins, a centrifugation step at 3500 rpm for 10 min was used to improve phase separation. The organic layer (fraction 1) was separated and transferred into another clean conical glass tube. The aqueous phase was extracted again with 2 mL of hexane, and the mixture was treated as above. The separated organic phase (fraction 2) was combined with fraction 1, and the total organic phase was evaporated to dryness using a hot water bath under argon stream. The residue was reconstituted with 500 µL of acetonitrile containing 250 $\mu g L^{-1}$ of DPeP (internal standard (IS)) and shaken for 1 min; finally, 10 µL of solution was injected in the HPLC-ES-MS system.

3. Results and Discussion

3.1. ES-MS Optimization

Six phthalate esters (DMP, DEP, BBP, DBP, DEHP, and DOP) were selected for this study. DPeP was used as an internal standard.

To evaluate the mass spectral fragmentation pattern of each compound and to optimize the set of parameters used, a standard solution (100 $\mu g L^{-1}$) of each compound was analyzed by direct injection in the spectrometer. For these experiments, a KD Scientific, model 100, syringe pump (New Hope, MN, USA) at 15 $\mu L min^{-1}$, was used.

Full-scan data acquisition was performed from 800 to 400 *m/z*, with the target mass fixed to the following *m/z* values: 91.15 for BBP, 149.05 for DEP, DBP, DPeP, DEHP, and DOP and 163.25 for DMP. The spectral data provided ions in accordance with previous studies reported in the literature [18, 34-37]. The selected ions were chosen to attain the best response in the SIM mode acquisition.

3.2. Optimization of HPLC separation

After optimizing the detection conditions, the following experiments were conducted to optimize the chromatographic separation of the analytes.

Experiments were carried out using different mobile phases reported in the literature (methanol/water [38], acetonitrile/water [39], acetonitrile (1% methanol)/water [40]), working in isocratic mode. The best resolution was obtained using acetonitrile/water as a mobile phase. These results agree with the experiments developed by López-Jimenez et al. [41]. In order to improve the resolution and to decrease the time of analysis, different experiments were carried out working in gradient mode. The best results were obtained starting with 5% of acetonitrile and increasing this percentage to 75% in 5 min. Then this composition was maintained for 27 min before returning to the initial conditions. Finally, the

column was equilibrated during 10 min before each injection. Other parameters optimized were the percentage of acetic acid and the flow rate of the mobile phase. The optimal conditions were 0.1% (*v/v*) acetic acid and a flow rate of 200 µLmin^{-1}. The chromatogram obtained for a mixed of these compounds under the optimized conditions is shown in *Figure. 1*.

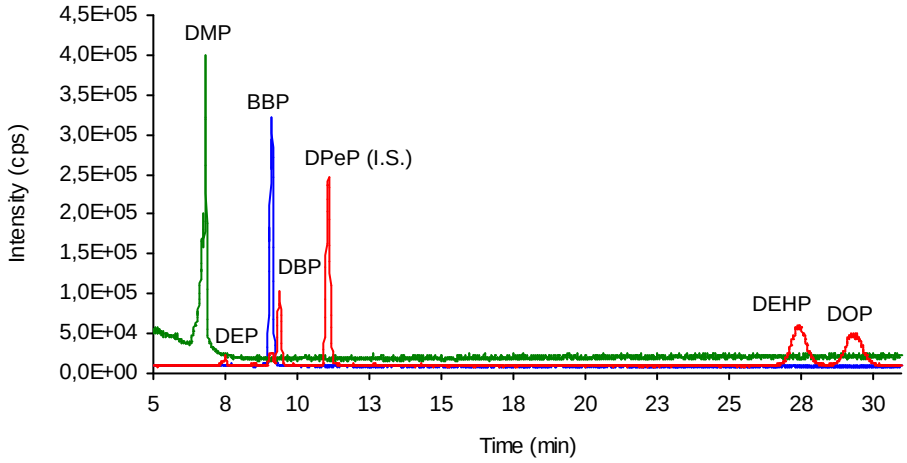


Figure 1- *LC/MS extracted ion chromatogram obtained from a standards solution (100 µgL^{-1}) in acetonitrile. Retention times (RT:min): DMP 6.72, DEP 7.43, BBP 9.02, DBP 9.29, DPeP 10.94, DEHP 26.71, DOP 28.62*

3.3. Phthalate separation by Liquid-Liquid Extraction

The phthalate esters studied were separated using a LLE procedure. The sample was treated with sodium hydroxide, to digest the fat contained in the sample and to

favor the migration of phthalates toward the extracting agent, and hexane was used as an organic phase.

The initial conditions used to develop this study were selected based on of the results obtained by Kambia et al. [8]. Thus, 1 mL of parenteral nutrition and 1 mL of 1M sodium hydroxide were introduced into a conical glass tube. The mixture was vortexed for 2 min, and then 2 mL of hexane was added. The solution was shaken for 5 min and centrifuged for 10 min at 3500 rpm. The organic layer (fraction 1) was separated and transferred into another clean conical glass tube. The aqueous phase was extracted again with 2 mL of hexane, and the mixture was treated as above. The separated organic phase (fraction 2) was combined with fraction 1, and the total organic phase was evaporated to dryness using a hot water bath under argon stream. The residue was reconstituted with 500 µL of acetonitrile containing 250 μgL^{-1} of I.S. and shaken for 1 min; finally, 10 µL of solution was injected in the HPLC-ES-MS system. The parameters studied in this work were sodium hydroxide concentration, volume of hexane, and agitation time. The experiments were developed with two types of parenteral nutrition containing aminoacids, glucose, and electrolytes. The only difference was the presence or absence of vitamins, which confer the lipophilic character to the sample.

The first parameter studied was the volume of hexane. Experiments were carried out using a blank sample of parenteral nutrition (with and without vitamins) spiked with 100 μgL^{-1} of all phthalates studied and varying the volume of hexane between 1 and 3 mL in each extraction (extraction by duplicate). In these experiments, the other parameters were fixed at 1 mL of 1M NaOH and agitation time of 5 min. The results obtained show that the signals remain practically constant with the volume of hexane, for compounds such as DEP, but in general there is an improvement using 2 mL of hexane in each extraction. The results were similar in the two types of samples studied; thus, 2 mL of hexane was selected to develop this study.

Another parameter studied was the sodium hydroxide concentration. For this purpose, experiments were carried out varying the NaOH concentration from 0 to 1.5M. The results obtained show that, in general, the extraction procedure is improved by increasing the NaOH concentration until 0.1M in both types of samples; concentrations higher than 0.1M decreased the percentage of extraction for all compounds studied. Therefore, 0.1M sodium hydroxide was selected for this study. The result obtained disagrees with the result obtained by Kambia et al., which used 1M sodium hydroxide in the extraction procedure. This different result may be attributed to the different composition of the samples analyzed.

The following parameter studied was the agitation time during the extraction procedure. The experiment was carried out varying the agitation time between 1 and 7 min. The best results were obtained using agitation times of 3 min for all compounds in parenteral nutrition samples without vitamins and 5 min for all compounds in parenteral nutrition samples with vitamins.

3.4. Analytical performance

To evaluate the linearity of the method, a direct calibration was performed. Ten microliters of standard solutions in acetonitrile with concentrations ranging from 10 to 250 μgL^{-1} was injected by triplicate. Relative areas (analyte peak area / IS peak area), were plotted versus the amount of analyte injected, expressed in μgL^{-1}, and the background levels were subtracted from the results. The results obtained show good correlation coefficients ($r^2 > 0.9964$) for all the studied compounds.

The standard addition method was applied over the same range of concentrations using a parenteral nutrition sample (with and without vitamins), obtaining good correlation coefficients ($r^2 > 0.9910$) for all the studied compounds.

To compare slopes of direct calibration and addition graphs for the six compounds, the *t* test (95% significance level) [42] was applied. Results for samples without vitamins have shown statistically differences for DMP, whereas statistically

differences were observed for all compounds for samples with vitamins. This means that the sample matrix had influence in the sensitivity of the method; thus, standard addition graphs have been used to analyze the samples in all cases.

The limit of detection (LOD) and limit of quantification (LOQ) for the method were calculated using 11 measurements of an acetonitrile extract of a blank sample. This blank sample was prepared with the same compounds that as the parenteral nutrition and stored in a glass bottle to avoid phthalates contamination and was then treated with the liquid-liquid extraction procedure described in the *"Sample preparation"* section. Limits of detection of target compounds in the parenteral nutrition samples were calculated from the instrumental detection limit, taking into account the amount of sample extracted, the volume of the organic phase used, and the recovery of the method. The results obtained for LODs and LOQs for the different samples (with and without vitamins) are shown in the *Table 2*. The limits of detection are between 0.1 and 10.8 μgL^{-1}, and the highest levels obtained were for DEP and DBP. The LOQ obtained for DEHP is lower than those obtained by Kambia et al. [8] (20 $ngmL^{-1}$) for the determination of this compound in this type of sample using HPLC-UV.

Table 2. *LODs and LOQs obtained for the six phthalates in parenteral nutrition samples (without and with vitamins).*

Nutrition without vitamins	**DMP**	**DEP**	**BBP**	**DBP**	**DEHP**	**DOP**
LOD (μgL^{-1})	1.1	7.0	1.3	7.4	5.0	1.1
LOQ (μgL^{-1})	3.6	23.5	4.5	24.8	16.8	3.7
Nutrition with vitamins	**DMP**	**DEP**	**BBP**	**DBP**	**DEHP**	**DOP**
LOD (μgL^{-1})	0.1	13.2	0.9	10.8	2.5	1.0
LOQ (μgL^{-1})	0.5	44.1	2.9	35.9	8.3	3.3

To check, the intra- and interday precision assays were developed. For the intraday study, six aliquots of a parenteral nutrition sample with vitamins and another six without vitamins, spiked with 100 μgL^{-1} of all compounds studied, were subjected to the extraction procedure described above. The extracts were analyzed in the same day for all compounds studied, and the relative standard deviation was calculated. The interday assay was carried out in the same way by subjecting 12 aliquots of spiked samples (with and without vitamins) to the extraction procedure in two different days. The RSD values were between 2.4% to 9.4% in the intraday assay and between 3.3% and 12.9% in the interday assay; thus, the method is precise for all studied compounds.

The recovery of the method was calculated using a blank sample (with and without vitamins) spiked with three different concentrations of these compounds (50, 100, and 200 μgL^{-1}). The extractions were carried out by duplicate and analyzed by triplicate and the recovery calculated using the standard addition graphs. The average analytical recoveries were 56.0%, 93.0%, 96.6%, 94.1%, 74.4%, and 74.3% for DMP, DEP, BBP, DBP, DEHP and DOP respectively, in nutrition

samples without vitamins, and 78.6%, 56.3 %, 97.6%, 99.2%, 67.8%, and 105.2% for DMP, DEP, BBP, DBP, DEHP and DOP, respectively, in nutrition samples with vitamins.

3.5. Application to parenteral nutrition samples

The proposed analytical method has been applied to the analysis of different parenteral nutrition samples, with and without vitamins, used in the public health system, in order to check the presence of these phthalates and to determine their concentrations.

All samples studied were prepared by the Hospital Clínico Universitario de Santiago de Compostela Pharmacy Department. These samples were prepared for neonates admitted to the intensive care unit and stored in ethyl vinyl acetate at -4ºC until analysis. The analytes were extracted from the sample using the extraction procedure described in the "*Sample preparation*" section.

The results obtained for these phthalates in the samples (with and without vitamins) analyzed are shown in *Table 3. Figure 2* shows the chromatograms obtained for two parenteral nutrition samples studied.

Table 3. *Concentration ($\mu g L^{-1}$) ± standard deviation (base on three replicates) found in different parenteral nutrition samples (without and with vitamins).*

Samples without vitamins	**DMP**	**DEP**	**BBP**	**DBP**	**DEHP**	**DOP**
Sample 1	nd	nd	1.3 ± 0.1	nd	nd	nd
Sample 2	nd	nd	nd	13.3 ± 3.7	7.0 ± 0.5	nd
Sample 3	2.40 ± 0.9	nd	nd	nd	9.3 ± 3.2	nd
Sample 4	nd	nd	11.6 ± 0.2	12.9 ± 5.4	22.2 ± 1.8	nd
Sample 5	nd	nd	nd	nd	19.5 ± 7.0	nd
Sample 6	nd	nd	nd	nd	17.2 ± 0.5	nd
Sample 7	nd	nd	nd	21.6 ± 4.4	36.5 ± 0.8	nd
Samples with vitamins	**DMP**	**DEP**	**BBP**	**DBP**	**DEHP**	**DOP**
Sample 1	6.8 ± 0.1	nd	6.1 ± 0.3	35.3 ± 1.5	215.1 ± 3.9	nd
Sample 2	7.1 ± 0.1	nd	5.9 ± 0.1	42.4 ± 4.7	993.7 ± 4.2	nd

As can be seen in *Table 3*, the phthalates were not detected in samples without vitamins or were detected at very low concentrations.

The results obtained for parenteral nutrition samples with vitamins were very different. All compounds studied, except DEP and DOP, were detected in the four samples studied. The highest concentrations were obtained for DEHP in all samples studied. This demonstrates that the lipid content of the parenteral nutrition increases the release of phthalates from the packaging to the sample.

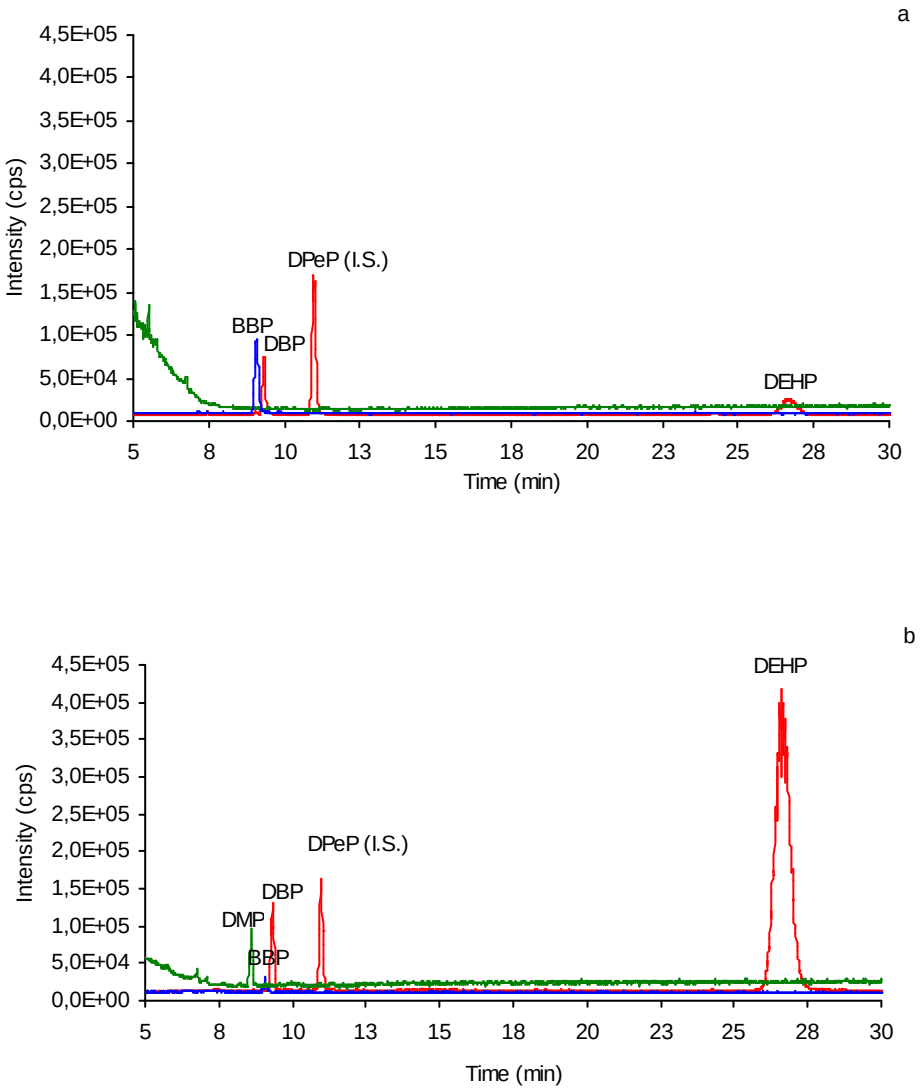


***Figure 2**- LC/MS extracted ion chromatogram obtained from parenteral nutrition sample without vitamins (a) and with vitamins (b).*

Table 4 shows the results corresponding to the same sample with vitamins before (sample A) and after (sample B) passing through the administration tube. In this case, an increment in the concentration of DEP and DEHP was observed. This means that these types of components are employed in the manufacture of infusion lines. As reported, these compounds are usually present in plastics to improve their flexibility, and this can entail a risk to the health of the patients.

Table 4. *Concentration ($\mu g L^{-1}$) ± standard deviation (base on three replicates) found in a sample with vitamins before (sample A) and after (sample B) to pass through the administration tube.*

Samples	DMP	DEP	BBP	DBP	DEHP	DOP
Sample A	6.7 ± 0.1	35.0 ± 7.9	5.3 ± 0.1	29.6 ± 1.5	1605.6 ± 9.1	nd
Sample B	7.8 ± 0.1	120.2 ± 1.9	5.3 ± 0.1	17.8 ± 2.2	1910.8 ± 12.0	nd

4. Conclusion

A sensitive and precise method to separate and determinate six phthalates in parenteral nutrition samples by HPLC-ES-MS was optimized. An LLE method to separate and preconcentrate these compounds in the samples was studied using sodium hydroxide and hexane as an organic phase. The proposed method was applied to the determination of these compounds in parenteral nutrition samples with different compositions. The only difference of these groups of samples is the lipid content (samples with and without vitamins). The results obtained show that the presence of vitamins in the sample increases the release of these compounds from the infusion bags to the sample. This is due to the fact that the lipid content

(some vitamins) favors the release of these compounds from the bag because phthalates are lipid soluble and are not chemically bound to plastics. Moreover, an increase of the DEHP and DEP was observed in the sample passed through the administration tube used to supply the nutrition to the patient. The results confirm previous findings [43-45] and show that infusion lines leach plasticizers in substantial amounts. This large amount of phthalates (especially DEHP) is a cause of worry because it may affect the most vulnerable patients.

Control of material used in the manufacture of medical devices is important to avoid the exposure to toxic contaminants, like phthalates, that may produce several complications in patients.

Acknowledgments

The authors wish to thank the Xunta de Galicia (Grupo de Referencia Competitivos 2007 (000047-0) for financial support. We are also greatful to the Neonatology Service and the Pharmacy Department from Complejo Hospitalario Universitario de Santiago, CHUS, for supplying the parenteral nutrition.

Cristina Pérez Feás wishes to thank Ministerio de Educación y Ciencia for her FPU grant.

5. References

[1] Communication from the Commission to the Council and The European Parliament on the implementation of the community strategy for endocrine disrupters-a range of substances suspected of interfering with the hormone system of humans and wildlife, COM (1999) 706; COM (2001) 262 final, Brussels, 2001

[2] US Environmental Protection Agency (EPA), Protection Agency. Introduction to Water Policy Standards, Office of Water, Washinton, DC, 1999

[3] De OD, Gagliardi L., Porrá R., Berri S., Chimenti P., Granese A., Carpani I., Tonelli D., (2006) Anal. Chim. Acta 555: 238-241

[4] Kato K., Shoda S., Takahashi M., Doi N., Yoshimura Y., Nakazawa H., (2003), J. Chromatogr. B 788: 407-411

[5] Mortensen G.K., MainK M., Anderson A.M., Leffer H., Skakkeback, N.E., (2005) Anal. Bioanal. Chem. 382: 1084-1092

[6] Soresen L.K., (2006) Rapid Commu. Mass Spectrom. 20: 1135-1143

[7] Faouzi M.A., Dine T., Gressier B., Kambia K., Luyckx M., Pagniez D., Brunet C., Cazin M., Babeles A., Cazin J.C., (1999), Inter. J. Pharma., 180: 113-121

[8] Kambia K., Dine T., Gressier B., Germe A.F., Luyckx M., Brunet C., Michaud L. Gottrand F., (2001) J. of Chromatogr. 755: 297-303

[9] Kambia K., Dine T., Gressier B., Germe A.F., Luyckx M., Brunet C., Michaud L., Gottrand F., (2003) Inter. J. Pharma., 262 : 83-91

[10] Ji-an C, Huijie L, Zhiqun Q, Weiqun S, (2008) Environ. Poll. 156: 849-853

[11] Farahani H., Reza M., Dinarvand R., Norouzi P., (2008) Talanta 76: 718-723

[12] Batlle R., Nerin C., (2004) J. Chromatogr. A, 1045: 29-35

[13] Silva M.J., Preau Jr J.L., Needham L.L., Calafat A.M., (2008) J. Chromatogr. B 873: 180-186

[14] Del Carlo M., Pepe A., Sacchetti G., Compagnone D., Mastrocola D., Cichelli A., (2008) Food Chem 111: 771-777

[15] Zafra-Gómez A., Ballesteros O., Navalón A., Vilches J.L., (2008) Michrochem. J., 88: 87-94

[16] Kato K., Silva M.J., Needham L.L., Calafat A.M., (2005) J. Chromatogr. B., 814: 355-360

[17] Cai Ya-Qi, Jiang G-B, Liu Ping-Fu, Zhou P-X, (2003) Anal. Chim. Acta 494, 149-156

[18] Luks-Betlej K., Popp P., Janozska B., Paschke H., (2001) J. Chromatogr. A 938: 93-101

[19] Peñalver A., Pocurull E., Borrull F., Marcé R.M., (2001), J. Chromatogr. A, 922: 377-384

[20] Holadová K., Prokupková G., Hajslová J., Poustka J., (2007) Anal. Chim. Acta 582, 24-33

[21] Serodio P., Nogueira J.M.F., (2004) Anal. Chim. Acta 517: 21-32

[22] Prieto A., Telleira O., Etxebarria N., Fernández L.A., Usobiaga A., Zuloaga O., (2008) J. Chromatogr. A, 1214: 1-10

[23] Gibson R., Wang M-J, Padgett E., Beck A.J., (2005), Chemosphere, 61: 1336-1344

[24] Bonini M., Errani E., Zerbinati G., Ferri E., Girotti S., (2008) Michrochem J. 90: 31-36

[25] Silva M.J., Slakman A.R., Reidy J.A., Preau J.L., Herbert A.R., Samandar E., Needham L.L., Calafat A.M., (2004), J. Chromatogr. B, 805: 161-167

[26] Li J., Cai Y., Shi Y., Mou S., Jian G., (2008), Talanta 74: 498-504

[27] Sahitoh T., Matsushima S., Hiraide M., (2004), J. Chromatogr. A, 1040: 185-191

[28] Pérez Feás C., Barciela Alonso M.C., Peña Vázquez E., Herbello Hermelo P, Bermejo Barrera P (2008) Talanta, 75: 1184-1189

[29] Hanawa T., Muramatsu E., Asakama K., Tanaka M., Kawano K., Seki T., Juni K., Nakajima S., (2000) Int. J. Pharm, 210: 109-115

[30] Silva M.J., Malek N.A., Hodge C.C., Reidy J.A., Kato K., Barr D.B., Needman L.L., Broca J.W., (2003), J. Chromtogr. B, 789: 393-404

[31] Takatori S., Kitagawa Y., Kitagawa M., Nakazawa H., Hori S., (2004) J. Chromatogr. B., 804: 397-401

[32] David F., Sandra P., Tienpont B., Vanwalleghem, in C.A., Staples (ED.), The Handbook of Environmental Chemistry. Part Q. Phthalate Esters, vol. 3, Springer, Berlin, 2003, p.15

[33] Gimeno R.A., Marcé R.M., Borrull F., (2003) Chromatographia 58: 37-41

[34] Serodio P., Nogueira J.M.F. (2006) Water Res. 40: 2572-2582

[35] Polo M., Llompart M., García-Jares C., Cela R., (2005) J. Chromatogr. A., 1072: 63-72

[36] Kumar R., A mass Spectral Guide for Quick Identification of Phthalates Esters, American Laboratory, 1999, p.32

[37] Psillakis E., Kalogerakis N., (2003) J. Chromatogr. A 999: 145-153

[38] Saito Y., Nakao Y., Imaizumi M., Morishima Y., Kiso Y., Jinno K., (2002) Anal. Bioanal. Chem. 373: 81-86

[39] Hata N., Yuwatini E., Ando K., Yamada M., Kasahara I., Taguchi S., (2004) Anal. Sci. 20: 149-152

[40] Jara S., Lysebo C., Greinbrokk T., Lundanes E., (2000) Anal. Chim. Acta 407: 165-171

[41] López-Jimenez F.J., Rubio S., Pérez-Bendito D., (2005) Anal. Chim. Acta 551: 142-149

[42] Cela R. (ed) (1994) Avances en Quimiometría práctica. Universidad de Santiago de Compostela, Santiago de Compostela, Spain, p 171

[43] Loff S., Kabs F., UIT K., Sartoris J., Mandi B., Niessen K.H., and Waag K.L., (2000), J. Pediatric Surgery, 12: 1775-1781

[44] Loff S., Subotic U., Reinicke F., Wischmann, BJ., (2004) J. Pediatr Gastroenterol Nutr, 39:341-345

[45] Subotic U., Animan T., Kiss M., Brade J., Breitkopf K., Loff S., (2007) J. Pediatr Gastroenterol Nutr, 44:71-76

CAPÍTULO V

Presence of Phthalates in Contact Lens and Cleaning Solutions

C. Pérez-Feás, M.C. Barciela-Alonso, P. Bermejo-Barrera

MICROCHEMICAL JOURNAL 99 (2011) 108-113

Resumen

En nuestra vida diaria estamos en contacto continuo con gran cantidad de objetos que contienen ftalatos, desde todo tipo de materiales plásticos hasta gran cantidad de artículos utilizados en el cuidado personal. El riesgo para la salud aumenta cuando los ftalatos, algunos de ellos clasificados como carcinógenos de categoría 2, atraviesan nuestras barreras y entran en el organismo. El acceso de estos compuestos al organismo puede tener lugar por distintas vías: por vía respiratoria (por ejemplo a través del aire contaminado en procesos de producción de plásticos), por vía dérmica (mediante el uso de determinados cosméticos y fragancias), por vía digestiva (mediante la ingesta de alimentos contaminados con ftalatos), por vía intravenosa (por liberación de ftalatos a partir de los dispositivos médicos) e incluso por vía ocular. Se trata, esta última, de una posible vía de entrada de los ftalatos en el organismo hasta ahora desconocida en la literatura científica.

En este capítulo se evaluó la presencia de ftalatos en las lentes de contacto y en sus soluciones de limpieza. Se abarca aquí un tipo de muestras utilizadas por gran parte de la población y que hasta el momento no habían sido objeto de estudio en el análisis de ftalatos.

Las lentes de contacto estudiadas están clasificadas por la FDA como lentes de contacto no iónicas cuyo contenido en agua está comprendido entre el 51 y 80%. Como consecuencia de su uso, la lente permanece en contacto con los ojos durante tiempos prolongados, lo que facilitaría la liberación de ftalatos en el entorno ocular y su consiguiente riesgo para la salud si estos compuestos son utilizados en los procesos de fabricación de estos productos.

En la primera parte de este trabajo se estudió la presencia de ftalatos en distintas soluciones utilizadas en la limpieza de las lentes de contacto comercializadas en envases de plástico multidosis y monodosis.

Posteriormente se estudió la posible migración de ftalatos desde las lentes de contacto blandas a una solución de lágrima artificial. Se realizó un estudio de migración que simuló en lo posible el entorno del ojo humano en cuanto a temperatura y humedad. Cada lente se sumergió en 1 mL de una solución comercial de lágrima artificial y se mantuvo a una temperatura constante de 37ºC y leve agitación a 130 rpm durante 24 h.

La técnica de análisis utilizada en este trabajo fue la cromatografía líquida de alta resolución acoplada a la espectrometría de masas en tándem. Aunque el método desarrollado previamente permite el análisis conjunto de seis ftalatos, en este trabajo se estudiaron únicamente cuatro, el DMP, DEP, BBP y DBP. El DEHP y DOP se excluyeron del estudio por problemas de solubilidad de estos analitos en muestras acuosas. Se trata de un método rápido y sencillo, cuya sensibilidad permite la inyección directa de este tipo de muestras en el sistema HPLC-MS/MS sin necesidad de realizar ningún tratamiento previo.

Del análisis de los resultados obtenidos se concluye que el DEP, el BBP y el DBP fueron detectados y cuantificados en las soluciones de limpieza contenidas en envases monodosis mientras que ninguno de los ftalatos estudiados se detectó en las soluciones de limpieza comercializadas en envases multidosis. Por otra parte, los resultados del estudio de migración confirmaron la liberación de algunos ftalatos a partir de las lentes de contacto.

Como consecuencia de los numerosos estudios toxicológicos que revelan una asociación entre diversos ésteres de ftalatos y riesgos para la salud humana sería necesario ampliar el estudio para conocer con más detalle el modo de liberación y la cantidad máxima de ftalatos que puede ser liberada a partir de estos productos.

Contents

PRESENCE OF PHTHALATES IN CONTACT LENS AND CLEANING SOLUTIONS

C. Pérez-Feás, M.C. Barciela-Alonso, P. Bermejo-Barrera

Department of Analytical Chemistry, Nutrition and Bromatology. University of Santiago de Compostela. Santiago de Compostela 15782, Spain.

Abstract

A fast and simple method using liquid chromatography–tandem mass spectrometry (LC-MS/MS) has been applied to identify and quantify four phthalic acid esters (PAEs) in different contact lens cleaning solutions. A migration study of these compounds from contact lenses has also been performed. The PAEs studied were dimethyl phthalate, diethyl phthalate, butyl benzyl phthalate and dibutyl phthalate. The migration of PAEs from contact lenses was performed by suspending each contact lens in an artificial tear solution at 37ºC and shaking it at 130 rpm for 24 h. The purpose of this study was to determine a possible migration of these compounds to the eyes as a result of the use of contact lenses and their cleaning solutions. The method was precise (with relative standard deviation (RSD) from 2.2 to 11.9%). It was also sensitive, with LODs of 0.03, 0.19, 0.31 and 2.62 μgL^{-1} for DMP, DEP, BBP and DBP respectively. The results obtained confirm the presence of these substances in some types of contact lens cleaning solutions. Furthermore, DBP and BBP were liberated from the contact lenses during the migration study.

Keywords: Phthalates, Contact lenses, Cleaning solutions, LC-MS/MS.

*** Corresponding author**: Pilar Bermejo-Barrera, email: pilar.bermejo@usc.es

1. Introduction

World wide production of phthalates (PAEs) and their frequent application in different products for daily use has resulted in their widespread presence in all parts of the environment. They are used to make plastics more flexible and resilient, and are often referred to as plasticizers.

Plastics are made of monomers and other starting substances which are chemically reacted to a macromolecular structure, the polymer, forming the main structural component of the plastics. Different additives are added to the polymer to achieve defined technological effects. Potential health risks may occur from non- or incompletely reacted monomers and other starting substances and from low molecular weight additives which are transferred via migration from the plastic [1].

PAEs increase the flexibility of plastics only through weak secondary molecular interactions with polymer chains. These compounds are not covalently bound to the vinyl polymer matrix, and can thus be released fairly easily from these products. These plasticizers are found in products such as construction materials, medical devices, toys, and food packaging. Some of these compounds are also used in cosmetics, fragrances and personal care products [2].

A large number of these compounds have been identified as priority hazardous substances by the European Union (EU), the US Environmental Protection Agency (EPA) and by several international organizations [3-5]. Plasticizers can affect several aspects of human health especially the reproductive, endocrine and respiratory systems and can also produce dermatological problems [6-11]. Some phthalates such as benzyl butyl phthalate (BBP), dibutyl phthalate (DBP), di-n-pentyl phthalate (DnPeP) and di-(2-ethylhexyl) phthalate (DEHP) are classified as toxic to reproduction (category 2) by the European Union [12]. The results of toxicological studies have led to the prohibition in 1999 of the addition of

phthalates to prepare plastics intended for toys [13, 14]. Recently, the European Commission has published a new regulation on plastic materials and articles which will come into contact with food (Commission Regulation (EU) Nº 10/2011 of 14 January 2011). This Regulation substitute Commission Directive 2002/72/EC and establishes the specific rules for plastic materials and articles to be applied for their safe use. This Regulation includes the Union list which contains substances authorised to be used in the manufacture of plastics which will come into contact with foods [1].

The determination of phthalates is not an easy task, in fact their widespread presence in laboratory environment, including air, glassware and reagents can produce false positive outputs [15-17]. Therefore, the risk of contamination is present in the whole analytical scheme, including sampling, sample preparation and analysis.

Several methods for PAEs determination at very low concentrations in different matrices are found in the literature (water [16, 18-20], food [21-23], sediments [24], soils [25, 26], biological samples [27-29], toys [30-32], cosmetics [33], etc). Different sample treatments, extraction and preconcentration steps such as liquid-liquid extraction (LLE) [34-37], solid phase extraction (SPE) [38-41], solid phase microextraction (SPME) [42-44], stir bar sorptive extraction (SBSE) [45, 46], and solid/liquid extraction (SLE) [47-49] have been used before the instrumental analysis to determine these compounds in these types of samples.

GC and HPLC as separation techniques coupled with different detectors are the main techniques used in the literature for PAEs determination. The coupling of these separation techniques with mass spectrometry and tandem mass spectrometry increase the sensitivity of the method. The results found in the literature indicate that GC-MS or HPLC-MS/MS present the lowest limits of detection [50].

In recent years, some authors have also focused their research to study the migration of phthalates from different matrices. Earls et al. [30] study the migration

of some phthalates from toys and childcare articles to saliva simulants. The authors determined the phthalates in saliva by GC-MS. Bonini et al. also studied the migration of this type of compounds from food packaging films by GC-FID [49]. The objective of the present work is to evaluate the level of exposition to phthalates and the risks to human health by studying the presence of these compounds in contact lens cleaning solutions at sub ppm level, and by performing a migration study of these compounds from contact lenses to artificial tear solutions.

2. Experimental

2.1. Reagents and standards

All reagents used were of analytical reagent-grade. Dimethyl phthalate (DMP) and Butyl benzyl phthalate (BBP) were obtained from Supelco (Bellefonte, PA, USA). Diethyl phthalate (DEP) and Dibutyl phthalate (DBP) were obtained from Riedel-de Haën (Seelze, Germany). The purity of these reagents was over 98%.

Lichrosolv gradient grade acetonitrile and methanol were purchased from Merck (Darmstadt, Germany). Technical-grade acetone and glacial acetic acid (HPLC) for instrumental analysis were purchased from Panreac (Barcelona, Spain). Ultrapure (resi-analyzed) water for environmental inorganic and organic trace analysis was supplied by J.T. Baker (Phillipsburg, NJ, USA).

2.2. Instrumentation

A Series 1100 liquid chromatograph from Agilent Technologies (Waldbronn, Germany) was coupled to an API 4000™ Triple Quadrupole Mass spectrometer (Applied Biosystems, Concord, Canada) equipped with a Turbo Ionspray™ ionization source. A Zorbax Eclipse XDB-C_8 column (3.5 µm, 2.1 mm x 50 mm)

from Agilent Technologies was used for the separation. Data acquisition and processing were performed using Analyst Software 1.4.2 (Applied Biosystems).

A Boxcult incubator situated on a Rotabit orbital-rocking platform shaker (J.P. Selecta, Barcelona, Spain) was used to perform the migration test from the contact lenses.

2.3. Glassware cleaning

Special care was taken to avoid the contact of reagents and solvents with plastic materials. Because of the ubiquity of plasticizers and the tendency of residues to persist, all glassware was cleaned prior to the analysis according to the recommendations specified in U.S. EPA Method 506 [51].

All material was washed with hot water and soap, rinsed with tap and ultrapure water and finally thoroughly rinsed with technical-grade acetone. Glassware was then sealed with aluminium foil and stored in a clean environment to avoid adsorption of phthalates from the air.

2.4. Standards preparation

Stock standard solutions of each phthalate ester at a concentration of 1000 mgL^{-1} were prepared in methanol, kept in darkness, and stored at 4ºC in a Teflon-capped amber glass bottles until use. From these solutions, a working standard solution in methanol was prepared weekly containing all standards at concentrations of 100 mgL^{-1} each. Diluted working standard solutions were prepared daily.

2.5. Sample preparation

Two types of samples were studied: contact lens cleaning solutions and artificial tear solutions. All samples were purchased in pharmacies.

The contact lens cleaning solutions were injected directly into the liquid chromatography–tandem mass spectrometry system without any pre-treatment step.

The artificial tear solution was used in the migration study of these compounds from contact lenses, using the procedure described in *Section 2.6.* The artificial tear solution used in this study was also injected directly into the LC-MS/MS system.

2.6. Migration test

The objective of this work was to check the presence of these phthalates in contact lenses and to study their migration from contact lenses to artificial tear solutions. The method is based on orbital-horizontal shaking of the contact lenses with artificial tear solution, under strictly controlled conditions of temperature, mode of mechanical agitation, contact time and volume of artificial tear solution. The method is aimed at representing the human eye environment as far as it is possible in the laboratory.

The contact lenses were put into the Teflon-capped amber glass vials with 1 mL of artificial tear solution. The vials were incubated at 37ºC with orbital-horizontal shaking at 130 rpm for 24 h. The solutions were then transferred to another glass vial and directly injected into LC–MS/MS system. No sample preparation process was necessary.

2.7. LC-MS/MS conditions

The LC-MS/MS conditions for DMP, DEP, BBP and DBP determination in aqueous samples were studied in a previous work developed by our research group [52].

Ultrapure water and acetonitrile, both solvents containing 0.1% (*v/v*) acetic acid, were used as a binary mobile phase. Phthalates were separated by LC working in gradient mode. The separation was performed starting with 5% of acetonitrile and increasing to 75% in 5 min, which was increased linearly to 75% in 5 min. This composition was maintained for 8 min before returning to initial conditions. The column was equilibrated for 10 min. Ten microliters of each sample were injected

using the HPLC autosampler configured with syringe washes between injections to eliminate carryover. The flow rate was 200 $\mu L min^{-1}$ and the column oven was maintained at 40ºC. Under these conditions the separation time was less than 13 min. These optimal conditions are shown in *Table 1*.

***Table 1**- Operational conditions for LC-MS/MS*

HPLC (Agilent 1100)	
Column	Zorbax Eclipse XDB-C8 (3.5µm 2.1mm x 50mm)
Mobile phase	Ultrapure water : acetonitrile (0.1% (*v/v*) acetic acid)
Mode	Gradient
Flow rate	200 $\mu L min^{-1}$
Oven temperature	40ºC
Injection volume	10 µL
MS/MS (API 4000)	
Ion Spray Voltage	5500 V
Ionization mode	ESI-positive
Curtain gas	25 psi (nitrogren)
GS1 (nebulizer gas)	50 psi
GS2 (auxiliary gas)	60 psi
Ion source temperature	450ºC
CAD (collisionally activated dissociation)	4

ESI in the positive ion mode was used to form the positively charged analyte ions at the interface under fixed instrument settings (*Table 1*). The combinations of precursor ion and product ions were as follows: DMP (precursor ion → product ion, *m/z* 195 → 163), DEP (*m/z* 223 → 177), BBP (*m/z* 313 → 91) and DBP (*m/z* 279 → 149).

3. Results and discussion

Phthalates (DMP, DEP, BBP and DBP) were determined in contact lens cleaning solutions and artificial tear solutions by LC-ESI-MS/MS. The instrumental conditions used in this work were optimized in a previously work [52]. The working conditions are described in *Section 2.7* and summarized conditions are in *Tables 1* and *2*.

Table 2- *Phthalates and their precursor and product ion transitions, potentials optimization (DP: declustering potential; EP: enhance potential; CE: collision energy; CXP: collision cell exit potential) and retention times (RT)*

Analyte	**Acronym**	**Precursor / product ions (*m/z*)**	**Potentials Optimization**				**RT (min)**
			DP	**EP**	**CE**	**CXP**	
Dimethyl phthalate	DMP	195/163	31	10	13	14	8.6
Diethyl phthalate	DEP	223/177	36	10	23	12	9.6
Butyl benzyl phthalate	BBP	313/91	41	10	23	6	11.6
Dibutyl phthalate	DBP	279/149	50	9	11	10	11.9

One of the main problems involved in the determination of phthalates is laboratory contamination [15]. It was not possible to obtain zero method blanks for the phthalates analysed. However, the contamination level was reduced to a low and rather constant level by using high quality solvents combined with thorough rinsing of all glassware with ultrapure water and technical-grade acetone. The blank results were always subtracted to correct experimental values.

3.1. Analytical performances

Before the determination of these phthalates in contact lens cleaning solutions and artificial tear solutions, the analytical characteristics were studied.

The calibration was performed by the standard addition method, using an artificial tear solution and working in a concentration range from 0.5 to 100 μgL^{-1} for DMP and DEP, from 5 to 100 μgL^{-1} for BBP and from 40 to 250 μgL^{-1} for DBP.

All the standard solutions were analyzed in triplicate. Linear regression was performed by plotting the peak area versus concentration. The coefficients of correlation (r) obtained were higher than 0.9985, indicating adequate linearity. The equations obtained for each compound are shown in *Table 3*.

Table 3- *Linear range, correlation coefficients, LODs and LOQs values obtained from the standard addition method in artificial tear solution*

Analyte	**Linear range (μgL^{-1})**	**Correlation coefficient (r)**	**LOD (μgL^{-1})**	**LOQ (μgL^{-1})**
DMP	0.5-100	0.9993	0.03	0.09
DEP	0.5-100	0.9992	0.19	0.63
BBP	5-100	0.9988	0.31	1.03
DBP	40-250	0.9985	2.62	8.74

The sensitivity of the method was determined by calculating the limit of detection (LOD) and the limit of quantitation (LOQ). LOD and LOQ were assessed based on the IUPAC definition:

$$LOD = \frac{3SD}{m} \qquad LOQ = \frac{10SD}{m}$$

Where *SD* is the standard deviation of ten blank solutions and *m* is the slope of the addition graph. A commercial artificial tear solution was used as a blank. The results obtained for LODs and LOQs are shown in *Table 3*. The LODs obtained are between 0.03 and 2.62 μgL^{-1}. The LODs and LOQs obtained in the present work were compared with values found in the literature. To the best of our knowledge, there are no published papers for the determination of these phthalates in contact lens cleaning solutions and artificial tear solutions; thus, the values obtained can be compared with the results obtained in saline or water samples. Koch et al. [53] determined these phthalates in urine samples by LC-MS/MS obtaining LODs from 0.25 to 1.0 μgL^{-1}. Gimeno et al. [54] obtained LODs from 0.01 to 1 μgL^{-1} for these phthalates in water samples using solid phase extraction previous to the determination by LC-MS. In general, the LODs obtained in this work are comparable or in some cases better than the values found in the literature. The advantage of the proposed method is its simplicity and speed because no preconcentration step is necessary. As sample manipulation is minimized, the contamination problems are greatly reduced, allowing phthalate determination at ppb levels.

The within-run precision was studied using an artificial tear solution spiked with four concentrations of each phthalate (10, 25, 50 and 75 μgL^{-1} for DMP, DEP and BBP and 60, 100, 150 and 200 μgL^{-1} for DBP). Each solution was analyzed six times in the same run. The results obtained are shown in *Table 4*.

The relative standard deviations (RSD) were between 2.2 and 11.9%, thus, the method is precise for all the compounds studied.

Table 4- *Within-run precision assays*

Analyte	**% RSD**			
	10 μgL^{-1}	***25 μgL^{-1}***	***50 μgL^{-1}***	***75 μgL^{-1}***
DMP	8.6	5.1	3.2	6.4
DEP	11.9	7.4	4.1	10.7
BBP	2.5	4.1	2.4	3.7
	60 μgL^{-1}	***100 μgL^{-1}***	***150 μgL^{-1}***	***200 μgL^{-1}***
DBP	8.5	4.7	2.2	5.4

The analytical recovery of the method was determined using an artificial tear solution spiked with three different concentrations of these compounds (10, 25 and 50 μgL^{-1} for DMP, DEP and BBP and 60, 100 and 150 μgL^{-1} for DBP). The spiked samples were analyzed three times, and the recovery calculated using the standard addition graph. The recovery percentages obtained are shown in *Table 5*. The average analytical recoveries were 101.0, 100.1, 102.5 and 103.9% for DMP, DEP, BBP and DBP, respectively.

Table 5- *Recovery percentage ± standard deviation (n=3)*

Analyte	**% Recovery**		
	10 μgL^{-1}	***25 μgL^{-1}***	***50 μgL^{-1}***
DMP	98.9 ± 4.9	100.4 ± 5.2	103.8 ± 3.0
DEP	106.4 ± 0.5	93.7 ± 7.7	100.4 ± 4.4
BBP	108.7 ± 1.7	97.2 ± 2.8	101.5 ± 3.0
	60 μgL^{-1}	***100 μgL^{-1}***	***150 μgL^{-1}***
DBP	98.5 ± 0.9	99.5 ± 0.6	113.8 ± 3.4

The advantage of the proposed method is that it presents good sensitivity and precision, allowing us to detect trace levels of these phthalates with reduced analysis time. Moreover, the simplicity of the method avoids contamination problems, very frequent in phthalates determinations.

3.2. Application of the proposed method to contact lenses cleaning solution

The proposed analytical method has been applied to the analysis of different cleaning solutions commercialised in plastic bottles to check the presence of these phthalates and determine their concentrations.

Samples analyzed in the study are available in two different plastic packages, single-dose (*samples 4 and 5*) and multidose (*samples 1, 2 and 3*). All samples studied are all-in-one solutions that clean, rinse, disinfect, store, remove proteins and lubricate soft contact lenses.

The samples were directly injected into the chromatographic system; no sample preparation process was necessary. Results obtained are given in *Table 6*.

Table 6- *Concentration (μgL^{-1}) ± standard deviation (based on three replicates) found in different contact lens cleaning solutions.*

Cleaning Solutions	DMP	DEP	BBP	DBP
Sample 1	n.d.	n.d.	n.d.	n.d.
Sample 2	n.d.	n.d.	n.d.	n.d.
Sample 3	n.d.	<LOD	n.d.	n.d.
Sample 4	n.d.	9.10±0.47	n.d.	19.66±2.60
Sample 5	n.d.	0.92±0.13	2.62±0.01	n.d.

< LOD: lower than the detection limit. n.d.: not detected.

Analysis of the results shows that the phthalates studied were not detected in the cleaning solutions purchased in multidose containers (60-120 mL). These results can be atributted to the fact that the multi-dose containers are rigid and they do not require the use of plasticizers in their manufacture.

However, in samples purchased in single-dose containers (10 mL) DEP, BBP and DBP were detected and quantified. DMP was not detected in any of the cleaning solutions studied. DEP levels of 9.1 and 0.9 $\mu g\ L^{-1}$ were detected in *sample 4* and *sample 5* respectively. *Sample 4* also presented DBP at concentrations of 19.7 μgL^{-1}. BBP was only detected in *sample 5* in a concentration of 2.6 μgL^{-1}.

The only differences between *sample 1* and *sample 4* are the packaging (multidose or single-dose) and the wetting agent used (sodium hyaluronate and polyvinylpyrrolidone, respectively). Both samples are the same brand, but *sample 1* was purchased in plastic bottle of 60 mL, and *sample 4* was purchased in a single-dose unit of 10 mL. The difference in the results obtained can be attributed to the

release of DEP and DBP, used in the manufacturing process to provide elasticity to the packaging of *sample 4*.

Figure 1 and *Figure 2* show the chromatograms obtained when analyzing both samples; significant differences were found.

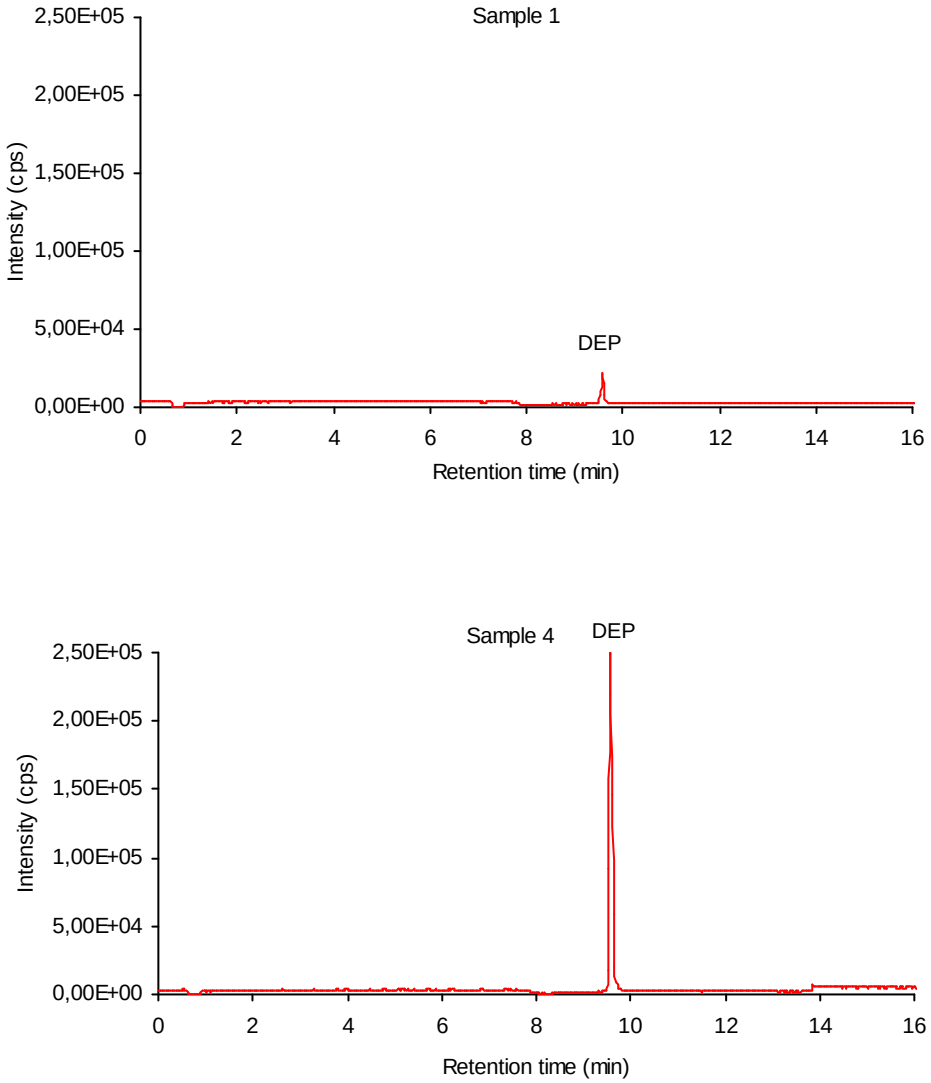


Figure 1- *LC-ES-MS/MS ion chromatogram obtained for DEP in sample 1 (cleaning solution multidose) and sample 4 (cleaning solution single-dose)*

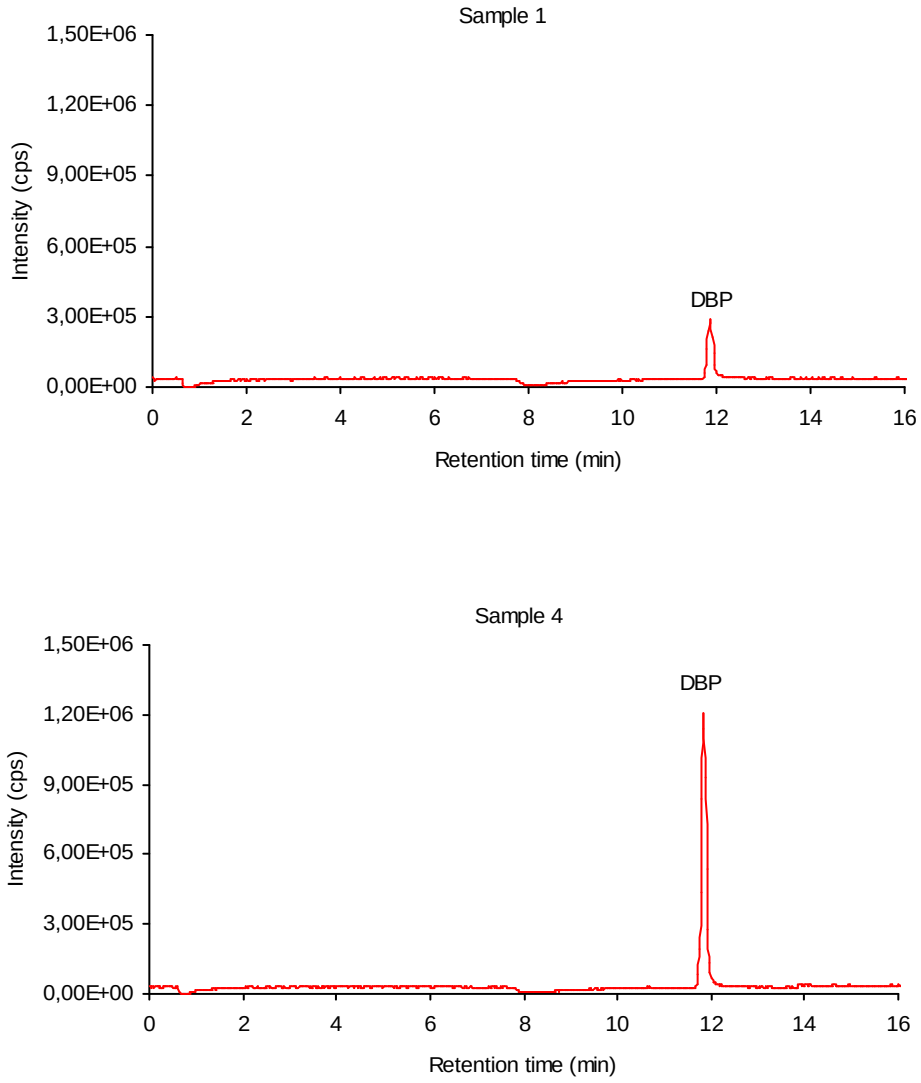


Figure 2- *LC-ES-MS/MS ion chromatogram obtained for DBP in sample 1 (cleaning solution multidose) and sample 4 (cleaning solution single-dose)*

3.3. Migration study of phthalates from contact lenses to artificial tear solution

In the second part of the study soft contact lenses were subjected to a migration test. The aim was to evaluate the possible release of phthalates from the contact lenses to artificial tear solution and determine their concentrations.

The method is based on orbital-horizontal shaking of the contact lenses with artificial tear solution, under strictly controlled conditions of temperature, mode of mechanical agitation, contact time and volume of artificial tear solution. The method is aimed at representing the human eye environment as far as it is possible in the laboratory.

Soft contact lenses classified by the FDA as non-ionic contact lenses containing between 51 and 80% of water were studied. The main component of this type of lens is the hydrogel, which consists of a solid phase (polymer) dispersed in an aqueous phase.

These contact lenses are disposable; each manufacturer sets the period of use, which ranges between 1 and 30 days. *Sample A* was Omafilcon A contact lens which contains 59% of water and its main monomer is oxietilfosforilcolina methacrylate. *Sample B* was Hioxifilcon A contact lens (55% of water) that is made of a copolymer of GMA (glycerol monomethacrylate) with HEMA (hydroxyethyl methacrylate).

Each contact lens was introduced into the Teflon-capped amber glass vial with 1 mL of artificial tear solution. Another vial with artificial tear solution but without a contact lens was used as a blank. The vials were incubated at 37ºC with orbital-horizontal shaking at 130 rpm for 24 h. The solutions were then transferred to another glass vial and directly injected into LC–ESI-MS/MS system. No sample preparation process was necessary before the LC-ESI-MS/MS determination. The results obtained are given in *Table 7*.

Table 7- *Concentration (μgL^{-1}) ± standard deviation (based on three replicates) found in artificial tear solution after applying the migration test to the contact lenses.*

Cleaning Solutions	DMP	DEP	BBP	DBP
Artificial tear solution	n.d.	n.d.	n.d.	n.d.
Sample A	n.d.	<LOD	3.13±0.05	169.85±2.61
Sample B	n.d.	0.27±0.04	3.95±0.04	184.19±1.94

< LOD: lower than the detection limit. n.d.: not detected.

Results show a significant release of some of the phthalates studied. DBP levels above 165 μgL^{-1} were detected in both cases. BBP was also detected. The concentrations found were 3.1 and 3.9 μgL^{-1} for *sample A* and *B* respectively. DEP was only detected in *sample B* at concentrations near the LOD. DMP was not detected in any samples studied.

The results obtained in this migration study indicate that the daily use of these contact lenses can be an important source of exposure to these compounds. Research should be developed to minimize the release of these compounds from the contact lenses, or to manufacture contact lenses with materials free of phthalates.

4. Conclusion

To the best of our knowledge, this is the first time that the presence of phthalates was studied in contact lenses and their cleaning solutions. These products are used every day by many consumers who are not aware of the impact of phthalates on human health.

A fast and simple method using LC-ESI-MS/MS has been applied. Results demonstrate the presence of different phthalates in contact lens cleaning solutions purchased in single-dose units. No phthalates were detected in cleaning solutions packaged in semi-rigid or rigid containers. These results are attributed to the plasticizers used in the manufacture of single-dose packaging to make them softer.

Moreover, the migration test confirms the release of some phthalates from soft contact lenses. The phthalate released in the highest concentration was DBP with concentrations above 165 μgL^{-1} in contact lenses *A* and *B*. BBP was also detected in the two contact lenses and the levels found were below 5 μgL^{-1}. DEP was only detected in *sample B* at levels near the LOD.

The results obtained in this study indicate that the use of certain soft contact lenses might be of concern. Toxicological evidence indicates an association between several of these phthalate esters and risks to human health; therefore, more research studies are needed to know in great detail the release mode and the maximum amount of phthalates that can be released from these products.

Acknowledgement

The authors wish to thank the Xunta de Galicia (Grupo de Referencia Competitivos 2007 (000047-0)) for financial support.

5. References

[1] 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. L. 12 (2011) 1–89

[2] A. Gómez-Hens, M.P Aguilar-Caballos, Social and economic interest in the control of phthalic acid esters, TrAC Trends Anal. Chem. 22 (2003) 847-857

[3] W. Brack, HJC. Klamer, M. Lopez de Alda, D. Barcelo. Effect-directed analysis of key toxicants in European river basins. A review. Environ. Sci. Pollut. Res. Int. 14 (2007) 30-38

[4] M. Coquery, A. Morin, A. Becue, B. Lepot. Priority substances of the European Water Framework Directive: analytical challenges in monitoring water quality, TrAC Trends Anal. Chem. 24 (2005) 117-127

[5] P. Roose, U.A.Th. Brinkman. Monitoring organic microcontaminants in the marine environment: principles, programmes and progress, TrAC Trends Anal. Chem. 24 (2005) 897-926

[6] B.G Lake, R.J Price, M.E. Cunnigham, D.G Walters, Comparison of the effects of di(2-ethylhexyl)adipate on hepatic peroxisome proliferation and cell replication in the rat and mouse, Toxicology 123 (1997) 217-226

[7] EC-CSTEE, Opinion on the toxicological characteristics and risks of certain citrates and adipates used as substitute for phthalates as plasticizers in certain soft PVC products, Scientific Committee on Toxicity, Ecotoxicity and the Environment (CSTEE), Brussels, 1999

[8] K.W. Seo, K.B. Kim, Y.J. Kim, J.Y. Choi, K.T. Lee, K.S. Choi, Comparison of oxidative stress and changes of xenobiotic metabolizing enzymes induced by phthalates in rats, Food Chem. Toxicol. 42 (2004) 107-114

[9] M. Dalgaard, U. Hass, A.M. Vinggaard, K. Jarkfelt, H.R. Lam, I.K. Sorensen, H.M. Sommer, O. Ladefoged, Di(2-ethylhexyl)adipate (DEHA) induced developmental toxicity but non antiandrogenic effects in pre- and postnatally exposed Wistar rats, Reprod. Toxicol. 17 (2003) 163-170

[10] M. Wormuth, M. Scheringer, M. Vollenweider, K. Ungerbühler, What are the sources of exposure to eight frequently used phthalic acid esters in Europeans? Risk Anal. 26 (2006) 803-824

[11] K. Miyata, K. Shiraishi, S. Houshuyama, N. Imatanaka, T. Umano, Y. Minobe, K. Yamasaki, Subacute oral toxicity study of di(2-ethylhexyl)adipate based on the draft protocol for the "Enhanced OECD test guideline no. 407", Arch. Toxicol. 80 (2006) 181-186

[12] 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, Off. J. Eur. Union. L. 396 (2006) 1–849 Appendix 6

[13] Commission Decision 1999/815/EC of 7 December 1999 adopting measures prohibiting the placing on the market of toys and childcare articles intended to be placed in the mouth by children under three years of age made of soft PVC containing one or more of the substances di-iso-nonyl phthalate (DINP), di(2-ethylhexyl) phthalate (DEHP), dibutyl phthalate (DBP), di-iso-decyl phthalate (DIDP), di-n-octyl phthalate (DNOP), and butylbenzyl phthalate (BBP), Off. J. Eur. Union. L. 315 (1999) 46–49

[14] Directive 2005/84/EC of the European Parliament and of the Council of 14 December 2005 amending for the 22nd time Council Directive 76/769/EEC on the approximation of the laws, regulations and administrative provisions of the Member States relating to restrictions on the marketing and use of certain dangerous substances and preparations (phthalates in toys and childcare articles), Off. J. Eur. Union. L. 344 (2005) 40–43

[15] A Fankhauser-Noti, K Grob, Blank problems in trace analysis of diethylhexyl and dibutyl phthalate: Investigation of the sources, tips and tricks, Anal. Chim. Acta 582 (2007) 353-360

[16] G Prokupková, K. Holadová, J. Poustka, J. Hajslová, Development of a solid-phase microextraction method for the determination of phthalic acid esters in water, Anal. Chim. Acta 457 (2002) 211-223

[17] A.M. Reid, C.A. Brougham, A.M. Fogarty, J.J. Roche, An investigation into possible sources of phthalate contamination in the environmental analytical laboratory, Int. J. Environ. Anal. Chem. 87 (2007) 125-133

[18] O.S. Fatoki, A. Noma, Determination of phthalate esters in the aquatic environment, S. Afr. J. Chem. 54 (2001) 1-15

[19] L. Brossa, R.M. Marcé, F. Borrull, E. Pocurull, Occurrence of twenty-six endocrine disrupting compounds in environmental water samples from Catalonia, Spain, Environ. Toxicol. Chem. 24 (2005) 261-267

[20] K. Luks-Betlej, P. Popp, B. Janozska, H. Paschke, Solid phase microextraction of phthalates from water, J. Chromatogr. A 938 (2001) 93-101

[21] Y-L. Feng, J. Zhu, R. Sensenstein, Development of a headspace solid-phase microextraction method combined with gas chromatography mass spectrometry for the determination of phthalate esters in cow milk, Anal. Chim. Acta 538 (2005) 41-48

[22] K Kambia, T Dine, B Gressier, A.F. Germe, M. Luyckx, C. Brunet, L. Michaud, F. Gottrand, High-performance liquid chromatographic method for the determination of di(2-ethylhexyl) phthalate in total parenteral nutrition and in plasma, J. Chromatogr. B 755 (2001) 297-303

[23] B. Cavaliere, B. Macchione, G. Sindona, A. Tagarelli, Tandem mass spectrometry in food safety assessment: The determination of phthalates in olive oil, J. Chromatogr. A 1205 (2008) 137-143

[24] E Cortazar, L. Bartolomé, A. Delgado, N. Etxebarria, L.A. Fernández, A. Usobiaga, O. Zuloaga, Optimization of microwave-assisted extraction for the determination of nonylphenols and phthalate esters in sediment samples and comparison with pressurized solvent extraction, Anal. Chim. Acta 534 (2005) 247-254

[25] Guo Bao-Yuan, B. Wen, Shan Xiao-Quan, Zhang Shu-Zheng, Lin Jin-Ming, Separation and determination of phthalates by micelar electrokinetic chromatography, J. Chromatogr. A 1095 (2005) 189-192

[26] A. Adeniyi, M. Dayomi, P. Siebe, O. Okedeyi, An assessment of the levels of phthalate esters and metals in the Muledane open dump, Thohoyandou, Limpopo province, South Africa, Chem. Central J. 2 (2008) 9-17

[27] K. Kato, S. Shoda, M. Takahashi, N. Doi, Y. Yoshimura, H. Nakazawa, Determination of three phthalate metabolites in human urine using on-line solid-phase extraction-liquid chromatography-tandem mass spectrometry, J. Chromatogr. B 788 (2003) 407-411

[28] K. Kato, M.J. Silva, L.L. Needham, A.M. Calafat, Determination of total phthalates in urine by isotope-dilution liquid chromatography-tandem mass spectrometry, J. Chromatogr. B 814 (2005) 355-360

[29] S Takatori, Y. Kitagawa, M. Kitagawa, H. Nakazawa, S. Hori, Determination of di(2-ethylhexyl)phthalate and mono(2-ethylhexyl)phthalate in human serum using liquid chromatography-tandem mass spectrometry, J. Chromatogr. B 804 (2004) 397-401

[30] A.O. Earls, I.P. Axford, J.H. Braybrook, Gas chromatography-mass spectrometry determination of the migration of phthalate plasticisers from polyvinyl chloride toys and childcare articles, J. Chromatogr. A 983 (2003) 237-246

[31] T. Yuzawa, Ch. Watanabe, R.R. Freeman, S. Tsuge, Rapid and simple determination of phthalates in plastic toys by a thermal desorption-GC/MS method, Anal. Sci. 25(9) (2009) 1057-1058

[32] T. Rothenbacher, W. Schwack. Rapid and nondestructive analysis of phthalic acid esters in toys made of poly(vinyl chloride) by direct analysis in real time single-quadrupole mass spectrometry, Rapid Commun. Mass Spectrom. 23(17) (2009) 2829-2835

[33] D. De Orsi, L. Gagliardi, R. Porra, S. Berri, P. Chimenti, A. Granese, I. Carpani, D. Tonelli, A environmentally friendly reversed-phase liquid chromatography method for phthalates determination in nail cosmetics, Anal. Chim. Acta 555 (2006) 238-241

[34] C. Pérez-Feás, M.C. Barciela-Alonso, A. Sedes-Díaz, P. Bermejo-Barrera, Phthalates determination in pharmaceutical formulae used in parenteral nutrition by LC-ES-MS: importance in public health, Anal. Bioanal. Chem. 397 (2010) 529–535

[35] G.K. Mortensen, K.M. Main, A.M. Andersson, H. Leffers, N.E. Skakkebaek, Determination of phthalate monoesters in human milk, consumer milk, and infant formula by tandem mass spectrometry (LC-MS/MS), Anal. Bioanal. Chem. 382 (2005) 1084-1092

[36] L.K. Soerensen, Determination of phthalates in milk and milk products by liquid chromatography/tandem mass spectrometry, Rapid Commun. Mass Spectrom. 20 (2006) 1135-1143

[37] M.A. Faouzi, T. Dine, B. Gressier, K. Kambia, M. Luyckx, D. Pagniez, C. Brunet, M. Cazin, A. Belabed, J.C. Cazin Exposure of hemodialysis patients to di-2-ethylhexyl phthalate, Int. J. Pharm. 180 (1999) 113-121

[38] M.J. Silva, J.L. Jr.Preau, L.L. Needham, A.M. Calafat, Cross validation and ruggedness testing of analytical methods used for the quantification of urinary phthalate metabolites, J. Chromatogr. B 873 (2008) 180-186

[39] M Del Carlo, A. Pepe, G. Sacchetti, D. Compagnone, D. Mastrocola, A. Cichelli, Determination of phthalate esters in wine using solid-phase extraction and gas chromatography-mass spectrometry, Food Chem. 111 (2008) 771-777

[40] M.A. Mohamed, A.S. Ammar, Quantitative analysis of phthalates plasticizers in traditional Egyptian foods (Koushary and Foul Medams), Black tea, instant coffee and bottled waters by solid phase extraction-capillary gas chromatography-mass spectroscopy. Am. J. Food Technol. 3 (2008) 341-346

[41] A. Zafra-Gómez, O. Ballesteros, A. Navalón, J.L. Vilches, Determination of some endocrine disrupter chemicals in urban wastewater samples using liquid chromatography-mass spectrometry, Microchem. J. 88 (2008) 87-94

[42] K. Holadová, G. Prokupková, J. Hajslová, J. Poustka, Headspace solid-phase microextraction of phthalic acid esters from vegetable oil employing solvent based matrix modification, Anal. Chim. Acta 582 (2007) 24-33

[43] M.T. Kelly, M. Larroque, Trace determination of diethyl phthalate in aqueous media by solid-phase microextraction-liquid chromatography, J. Chromatogr. A 841 (1999) 177-185

[44] C. Cháfer-Pericás, P. Campíns-Falcó, M.C. Prieto-Blanco, Automatic in-tube SPME and fast liquid chromatography: A cost-effective method for the estimation of dibutyl and di-2-ethylhexyl phthalates in environmental water simples, Anal. Chim. Acta 610 (2008) 268-273

[45] A. Prieto, O. Telleria, N. Etxebarria, L.A. Fernández, A. Usobiaga, O. Zuloaga, Simultaneous preconcentration of a wide variety of organic pollutants in water samples: Comparison of stir bar sorptive extraction and membrane-assisted solvent extraction, J. Chromatogr. A 1214 (2008) 1-10

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

[47] R. Gibson, M.J. Wang, E. Padgett, A.J. Beck, Analysis of 4-nonylphenols, phthalates, and polychlorinated biphenyls in soils and biosolids, Chemosphere 61 (2005) 1336-1344

[48] J.M. Aranda, G.A. O´Connor, G.A. Eiceman, Effects of sewage sludge on bis(2-ethylhexyl) phthalate uptake by plants. J. Environ. Qual. 18 (1989) 45-50

[49] M. Bonini, E. Errani, G. Zerbinati, E. Ferri, S. Girotti, Extraction and gas chromatographic evaluation of plasticizers content in food packaging films, Microchem. J. 90 (2008) 31-36

[50] P. Bermejo Barrera, M.C. Barciela Alonso, C. Pérez Feas, E. Peña Vázquez, P. Herbello Hermelo, Bisphenol A and Phthalates: Uses, Health Effects and Environmental Risks. Chapter 2. ISBN 978-1-60741-701-9, (2011)

[51] USEPA Method 506 revision 1.1. (1995) Determination of Phthalates and Adipate Esters in Drinking Water by Liqui-Liquid Extraction or Liquid-Solid Extraction and Gas Chromatography with Photoionization Detection. National Exposure Reshearch Laboratory Office of Research and Development U.S. Environmental Protection Agency, Cincinnati, Ohio 45268, 1995

[52] C. Pérez-Feás, M.C. Barciela-Alonso, P. Bermejo-Barrera, Direct LC-ES-MS/MS determination of phthalates in physiological saline solutions, J. Chromatogr. B 879 (2011) 231–235

[53] H.M Koch, L.M. Gonzalez-Reche, J. Angerer, On-line clean-up by multidimensional liquid chromatography-electrospray ionization tandem mass spectrometry for high throughput quantification of primary and secondary phthalate metabolites in human urine, J. Chromatogr. B 784 (2003) 169-182

[54] R.A. Gimeno, R.M. Marcé, F. Borrull Determination of plasticizers by high-performance liquid chromatography and atmospheric pressure chemical ionization mass spectrometry in water and sediment samples, Chromatographia 58 (2003) 37-41

CAPÍTULO VI

Phthalates Determination in Plastic Wrapping Films by High Performance Liquid Chromatography Coupled with Electrospray Ionization-Mass Spectrometry

Cristina Pérez-Feás, María Carmen Barciela-Alonso and Pilar Bermejo-Barrera

Resumen

En el último capítulo de esta Tesis la investigación se orientó a la determinación y cuantificación de ftalatos en un tipo de muestras de uso diario en muchos hogares, los *films*, utilizados para proteger y conservar alimentos. La flexibilidad y extensibilidad de estos productos se obtiene mediante la adición de distintos agentes plastificantes durante el proceso de fabricación.

En este caso coexisten dos factores importantes que favorecen la migración de los ftalatos desde el plástico al alimento; por un lado, la falta de uniones covalentes entre los ftalatos y la matriz polimérica facilita el desplazamiento de los mismos a través del film y por otro, debido al carácter lipofílico que presentan su migración se ve favorecida cuando el film entra en contacto con alimentos con un alto contenido en grasas.

En relación a los ftalatos y su contacto con alimentos, recientemente la Comisión Europea publicó el Reglamento (UE) Nº 10/2011 que deroga la Directiva 2002/72/EC y que establece reglas específicas sobre los materiales plásticos y artículos destinados a entrar en contacto con alimentos. En su anexo I establece la lista de sustancias autorizadas en la producción de polímeros y sus limitaciones, entre ellas se establecen limitaciones para el uso de DiMP, DBP, DEHP y BBP.

El objetivo en este estudio se centró en el análisis cualitativo y cuantitativo de los ftalatos contenidos en los films. En primer lugar se desarrolló un procedimiento de extracción sólido-líquido; se compararon el hexano y la acetona como disolventes de extracción, y se estudió el tiempo de ultrasonidos y el volumen de extractante. Las condiciones óptimas de la extracción se llevaron a cabo poniendo 0.5 g de muestra en contacto con 10 mL de acetona bajo la acción de ultrasonidos durante 15 min. La fase orgánica se llevó a sequedad con ayuda de una corriente de argon y el extracto obtenido se redisolvió con 1 mL de una solución de 200 μgL^{-1} de DPeP en acetonitrilo. Por último esta disolución se analizó mediante cromatografía

líquida de alta resolución acoplada a espectrometría de masas utilizando las condiciones de trabajo optimizadas en los capítulos anteriores.

El método optimizado se aplicó al análisis de seis ftalatos (DMP, DEP, BBP, DBP, DEHP y DOP) en distintas marcas comerciales de film transparente, detectándose DEP, BBP, DBP y DEHP en las cinco muestras analizadas.

En este capítulo se pone de manifiesto la presencia de ftalatos en los films. Debido a su conocida toxicidad y peligro para la salud sería interesante realizar un estudio de migración de los ftalatos detectados en estas muestras para conocer el riesgo de exposición que se deriva del uso de estos productos en contacto con alimentos y el grado de cumplimiento de la legislación vigente.

Contents

PHTHALATES DETERMINATION IN PLASTIC WRAPPING FILMS BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY COUPLED WITH ELECTROSPRAY IONIZATION-MASS SPECTROMETRY

Cristina Pérez-Feás, María Carmen Barciela-Alonso and Pilar Bermejo-Barrera*

Department of Analytical Chemistry, Nutrition and Bromatology.

University of Santiago de Compostela. Santiago de Compostela 15782. Spain.

Abstract

A method for determining dimethyl phthalate, diethyl phthalate, dibutyl phthalate, butyl benzyl phthalate, dioctyl phthalate and diethylhexyl phthalate in plastic wrapping films by High Performance Liquid Chromatography coupled with Electrospray Ionization-Mass Spectrometry has been developed in this work. These compounds were extracted from the sample using a solid-liquid extraction procedure. A comparison of acetone and hexane as extracting agents was performed, and acetone was selected. After the extraction procedure, the extracts of acetone were evaporated and the compounds were then redissolved in acetonitrile. The compounds were separated by HPLC working in a gradient mode with acetonitrile and ultrapure water as a mobile phase. The method was precise (with RSD from 1.9 to 12.2%) and sensitive (LODs from1.6 to 39.4 μgKg^{-1}. The proposed analytical method has been applied to check the presence of these compounds in different plastic wrapping film samples and to determine their concentration.

Keywords: Phthalates, HPLC-ES-MS, plastic wrapping film.

* Corresponding author: Tel: +34 881814266 ext 14266; fax: +34 981595012. E-mail: pilar.bermejo@usc.es

1. Introduction

Phthalates (PAEs) are a group of chemical compounds widely used in industry, mainly as plasticizers, due to the ability to improve the flexibility and softness of plastics. The plastic wrapping films are usually made with polyvinylidene chloride (PVDC), polyvinylchloride (PVC) or polyethylene (PE). The flexibility and extensibility of these films are obtained adding different plasticizers including phthalates [1, 2]. Phthalates are not chemically bound to plastic; thus, they can migrate from the plastic packaging to the contents and the environment [3]. Moreover, the lipophilic nature of these compounds favours their migration from the plastic to high fat content food [4, 5]. The problem associated with the use of these compounds is that certain phthalate esters and/or their metabolites are suspected to be human carcinogenic agents and endocrine disruptors, which make their trace determination particularly important [6, 7]. Recently, the European Commission has published a new regulation on plastic materials and articles which come into contact with food (Commission Regulation (EU) Nº 10/2011 of 14 January 2011). This Regulation includes the Union list which contain substances authorized to be used in the manufacture of plastic which come into contact with food [8].

Gas chromatography (GC) [9-13] and high performance liquid chromatography (HPLC) [14-18] coupled with different detectors are the common techniques used for phthalate determination in different matrices. In some cases, phthalate determination using these techniques was preceded by a clean up/preconcentration step. These sample pre-treatments include, liquid-liquid extraction (LLE) [19-21], solid phase microextraction (SPME) [12, 22], solid phase extraction (SPE) [23, 24] and solid-liquid extraction (SLE) [25, 26], among others.

In recent years, different studies have reported the presence of phthalates in plastic products for food use, including packaging bags, wrapping film, container boxes for use in microwave ovens, etc. For example, Hao-Yu Shen et al. [27] determined eight phthalates (DEP, DBP, DIBP, DBP, BBP, DCHP, DEHP and DOP) in 25 of these plastic products by gas chromatography in combination with mass spectrometry (GC-MS). These compounds were extracted from the plastic by sonication-assisted extraction with hexane. With this method it is possible to detect these compounds at the level of 10 $\mu g Kg^{-1}$. Bonini et al. [25] proposed a method for evaluation of plasticizers content in food packaging films by gas chromatography with flame ionization detector (FID). The extraction of the plasticizers studied from the plastic films was performed by a Soxhlet extraction with ethyl acetate. Ultrasonic solvent extraction combined with solid-phase microextraction (SPME) was used by Xiujuan et al [28] to extract phthalates from different types of plastics such as blood bags, transfusion tubing, food packaging bags and mineral water bottles. The extraction procedure was performed using methanol as an extracting solvent and shaking for 30 minutes by ultrasonic agitation at room temperature.

In others studies developed in recent years, the migration of phthalates from different matrices were studied. Bonini et al. [25] studied the migration of this type of compounds from food packaging films by GC-FID. Pérez-Feás et al. [29] studied the migration of these compounds from contact lenses to artificial tear solution by HPLC-MS-MS. Gärtner et al. [30] studied the migration of phthalates in infant food packed in recycled paperboard using GC-MS.

The objective of the present work was the development a method for phthalate determination in plastic wrapping film samples by HPLC-ES-MS. The developed method was used to study the presence of these compounds in different plastic film samples commercialized by various manufactures.

2. Experimental

2.1. Reagents and Standards

All reagents used were of analytical reagent-grade. Dimethyl phthalate (DMP), Butyl benzyl phthalate (BBP), Dipentyl phthalate (DPeP) and Dioctyl phthalate (DOP) were obtained from Supelco (Bellefonte, PA, USA). Diethyl phthalate (DEP) and Dibutyl phthtalate (DBP) were obtained from Riedel-de Haën (Seelze, Germany). Diethylhexyl phthalate (DEHP) was obtained from Merck (Darmstad, Germany). The purity of these reagents was above 98%.

Individual standard solutions of each phthalate ester at a concentration of 1000 mgL^{-1} were prepared in methanol, kept in darkness, and stored at 4°C in a Teflon-capped glass vial. From these solutions, a working standard solution in methanol was prepared weekly containing all standards at a concentration of 100 mgL^{-1} each. Diluted working standard solutions were prepared daily.

Hexane (PA-ACS-ISO) and acetone (Panreac, Barcelona, Spain) were used in the liquid-liquid extraction.

Lichrosolv gradient grade acetonitrile and methanol were purchased from Merck. Technical grade acetone and glacial acetic acid (HPLC) for instrumental analysis were from Panreac. Ultra-pure (resi-analized) water for environmental inorganic and organic trace analysis was from J.T. Baker (Phillipsburg, NJ, USA).

2.2. Instrumentation

Phthalates separation and quantification was performed using a liquid chromatography/electrospray ionization mass spectrometry system.

The HPLC system used was a 1100 Series equipped with an automatic injector (Agilent Technologies, Waldbronn, Germany) coupled to an API 150 EX single quadrupole mass spectrometer equipped with a Turboionspray interface (PE Biosystems, Concord, Canada).

The analytical column was a ZORBAX Eclipse XDB-C_8 of 50 mm length and 2.1 mm internal diameter (particle size 3.5 μm) from Agilent Technologies.
An ultrasonic bath (VWR International, Radnor, PA, USA) was used in the extraction procedure.

2.3. Chromatographic and mass spectrometry conditions

The LC-MS conditions for DMP, BBP, DBP, DEP, DEHP and DPeP determination were studied in a previous work developed by our research group [19].
Ultrapure water and acetonitrile, both solvents containing 0.1% (v/v) acetic acid, were used as a binary mobile phase. Phthalates were separated by LC working in a gradient mode. The separation was performed starting with 5% of acetonitrile and increasing to 75% in 5 min. The composition was maintained for 27 min before returning to the initial conditions. The column was then equilibrated for 10 min. The flow rate and the injection volume were 200 $\mu L min^{-1}$ and 10 μL respectively. The chromatographic separation was performed at room temperature. Under these conditions, the retention times obtained were 7.04, 7.88, 9.66, 9.91, 11.60, 26.23 and 27.96 minutes for DMP, DEP, BBP, DBP, DPeP, DEHP, DOP, respectively.
Electrospray ionization was performed in positive ion mode using the operational parameters shown in *Table 1*.

***Table 1**: ES-MS parameters*

Compound	*m/z*	DP	FP	EP
DMP	163.25	40.38	73.87	8
BBP	91.15	25	225	6
DEP, DBP, DPeP, DEHP, DOP	149.05	25	290	8.5

Nebulizer and Curtain gas (N_2): 14 psi; Heater gas: 7000 cc/min; ES temperature: 450ºC; Ionspray voltage: 5500 V; mode: positive. DP: Declustering Potential; FP: Focussing Potential; EP: Enhance Potential

2.4. Sample preparation

Phthalates were analysed in different samples of plastic wrapping films. The phthalates studied were extracted from the plastic film (previously cut and homogenised) using a solid-liquid extraction procedure. Thus, 0.5 g of sample and 10 mL of acetone were introduced into a conical glass tube. This tube was then sonicated, using an ultrasonic bath, during 15 min. The liquid phase was separated and transferred into another conical glass tube and was then evaporated to dryness using an argon stream. The residue was then reconstituted with 1 mL of acetonitrile containing 200 μgL^{-1} of DPeP (internal standard (IS)) and shaking for 1 min; finally, 10 µL of solution was injected in the HPLC-ESI-MS system.

2.5. Glassware cleaning

Special care was taken to avoid contact of reagents and solvents with plastic materials. Because of the ubiquity of plasticizers and the tendency of residues to persist, all glassware was cleaned prior to the analysis according to the recommendations specified in U.S. EPA method 506 [31].

All material was washed with hot water and soap, rinsed with tap and ultrapure water, and finally thoroughly rinsed with technical-grade acetone. Glassware was

then sealed with aluminium foil and stored in a clean environment to avoid adsorption of phthalates from air.

3. Results and discussion

Phthalates (DMP, DEP, BBP, DBP, DEHP and DOP) were extracted from plastic wrapping films using a solid-liquid extraction procedure. The extracted compounds were separated and quantified by HPLC-ESI-MS.

The instrumental conditions of HPLC-ESI-MS used in this work were optimized in a previous study developed in our research group [19]. The working conditions are described in *Section 2.3* and summarised in *Table 1*.

3.1. Phthalate separation by solid-liquid extraction

The phthalate esters studied in this work were extracted from the transparent films using a solid-liquid extraction procedure. Experiments were performed to select the optimum conditions for the extraction procedure. The effect of two different extracting agents (acetone and hexane) was studied in this work. These extracting agents were selected based on previous studies found in the literature [19, 27].

The solid-liquid extraction procedure was performed using 0.5 g of a plastic film pool sample and using either acetone or hexane as extracting agents. The variables studied were the volume of the extracting agent and sonication time.

The first experiments were performed to select the optimum volumes of the extracting agent. Thus, 0.5 g of sample and different volumes of acetone or hexane (between 5 and 25 mL) were introduced into a conical glass tube. This mixture was sonicated during 30 min in an ultrasonic bath. The liquid phase was then separated, transferred into another conical glass tube and evaporated to dryness under an argon stream. The residue was then reconstituted with 1 mL of acetonitrile

containing 200 μgL^{-1} of DPeP (internal standard (IS)) and shaking for 1 min; finally, 10 µL of the solution was injected into the HPLC-ESI-MS system. The extraction procedure was performed in duplicate for all volumes studied, and each extract was analysed in triplicate.

The results obtained show that only four phthalates (DEP, BBP, DBP and DEHP) were detected in all the extract obtained. This indicates that DMP and DOP are not present in the film pool sample used for developing this study, or they are present at very low concentrations. Therefore, the variables affecting the extraction procedure were optimized based on the results obtained for these four phthalates. *Figure 1* and *Figure 2* show the variation of the relative peak area when the volume of hexane and acetone are varied.

The relative peak area increases slightly with the volume of hexane until approximately 15 mL and then remains practically constant or decreases, for DBP, BBP and DEHP. On the contrary, for DEP, the relative peak area decreases when using higher volumes of hexane until 10 mL and then remains practically constant. Taking into account the results obtained for the four phthalates studied, 15 mL was selected as the optimum volume of hexane to develop this study.

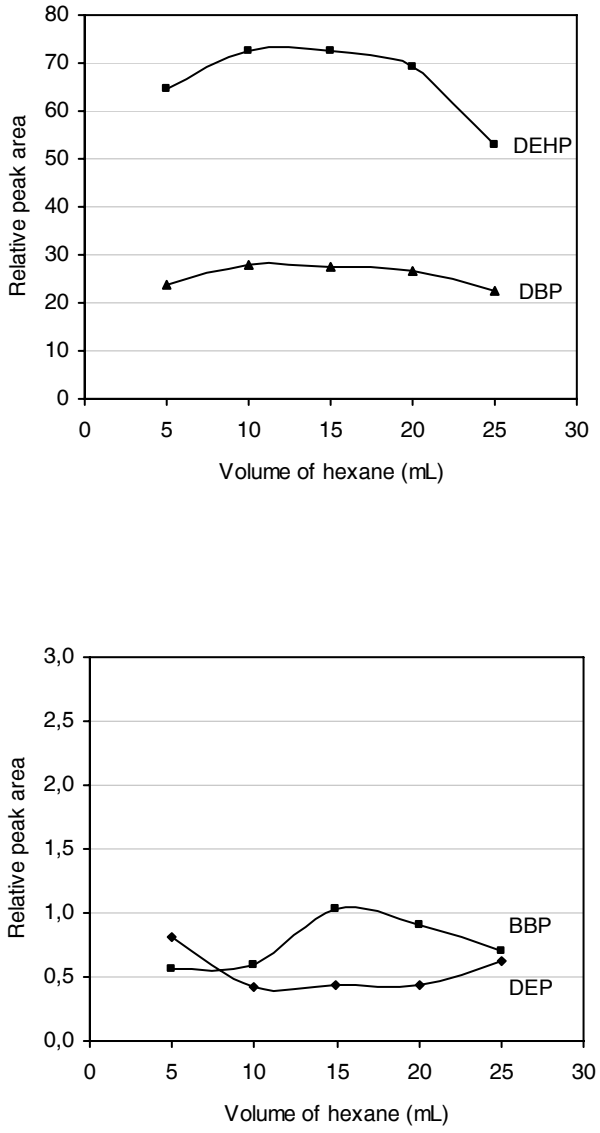


Figure 1. *Influence of volume of hexane in the solid-liquid extraction procedure*

The results obtained using acetone as extracting agent (*Figure 2*) show that the relative peak area remains practically constant for BBP and DEP for all volumes studied, while, the relative peak area increases slightly for DBP and DEHP until 10 mL of acetone and then remains practically constant. Thus, 10 mL was selected as the optimum volume of acetone to develop this work.

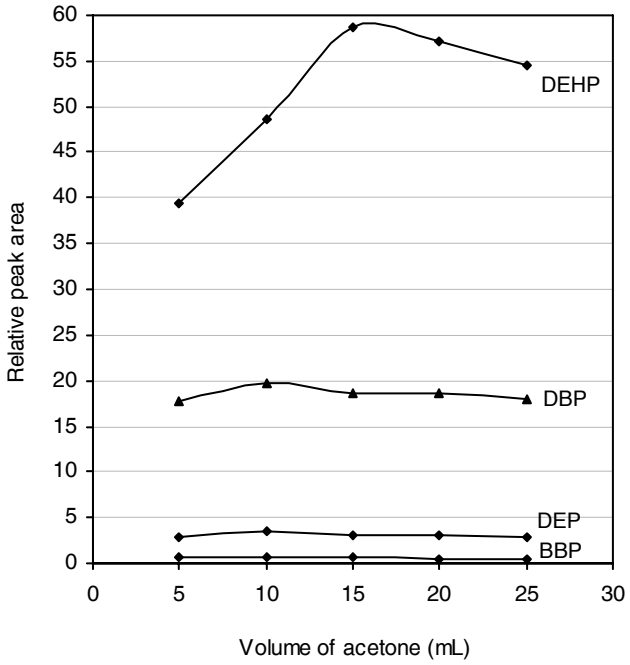


Figure 2. *Influence of volume of acetone in the solid-liquid extraction procedure*

The next parameter studied was the sonication time. Experiments were developed following the extraction procedure described above and varying the sonication time between 15 to 120 min. These experiments were performed using either hexane or acetone as extracting solvents. The results obtained indicated that there was no

significant difference between the times studied. Thus, 15 minutes was selected as the optimum sonication time.

After the selection of the volume of extracting solution and the sonication time, experiments were performed to select the best extracting solution. The study was developed taking 0.5 g of a plastic film pool sample and applying the solid-liquid extraction procedure using either hexane or acetone with the conditions optimized in this work (15 mL of hexane or 10 mL of acetone and sonication time of 15 min). As can be seen in *Figure 3*, no differences were observed in the relative peak areas obtained for DEP and BBP using hexane or acetone as extracting solvents. On the contrary, the relative peak area increased for DBP and DEHP when acetone was used. Taking into account these results, acetone was selected as extracting solvent for further studies.

The chromatogram obtained for an extract spiked with 200 μgL^{-1} of all phthalates studied is shown in the *Figure 4*.

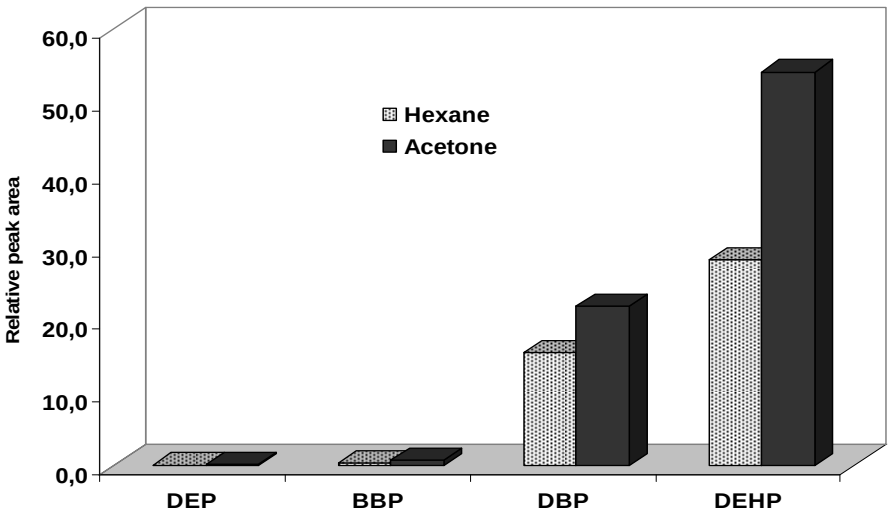


***Figure 3**. Comparison of hexane and acetone as extracting agent*

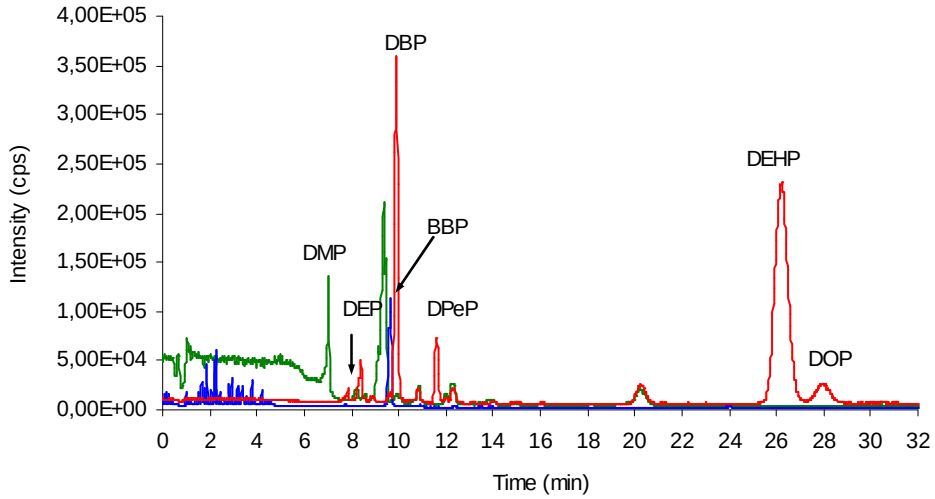


***Figure 4.** HPLC-ESI-MS ion chromatogram obtained from an extract spiked with 200µgL^{-1} of DMP, DEP, BBP, DBP, DPeP, DEHP and DOP.*

3.2. Analytical performances

Before the determination of these phthalates in the extract of sample, the analytical performances were studied.

To evaluate the linearity of the method, a direct calibration was performed. Ten microliters of standard solution in acetonitrile with concentrations ranging from 20 to 1000 μgL^{-1} was injected in triplicate. Relative areas (analyte peak area/IS peak area) were plotted versus the amount of analyte injected, expressed in μgL^{-1}, and the background levels were subtracted from the results. The results obtained (*Table 2*) show good correlation coefficients (r>0.9976) for all studied compounds.

The standard addition method was applied over the same range of concentrations using an extract of the sample, obtaining good correlation coefficients (r>0.9962) (*Table 2*).

Table 2. *Correlation coefficients and slopes for calibration and addition graphs, LOD and LOQ values obtained from standard addition methods for phthalate determination in transparent film.*

Analyte	**Calibration**		**Addition**			
	Correlation coefficient	**Slope**	**Correlation coefficient**	**Slope**	**LOD (μgKg^{-1})**	**LOQ (μgKg^{-1})**
DMP	0.9998	0.0097	0.9978	0.0146	4.9	16.4
DEP	0.9996	0.0005	0.9988	0.0008	35.0	116.7
BBP	0.9976	0.0074	0.9984	0.0094	1.6	5.3
DBP	0.9985	0.0025	0.9939	0.0018	39.4	131.5
DEHP	0.9993	0.0073	0.9991	0.0097	7.2	24.1
DOP	0.9997	0.0071	0.9962	0.0101	2.0	6.6

To compare slopes of direct calibration and addition graphs for the six compounds, the t-test (95% confidence level) [32] was applied. Statistically significant differences were observed for all compounds studied. This means that the sample matrix influences the sensitivity of the method; thus, standard addition graphs have been used to analyze these phthalates in these types of samples.

The sensitivity of the method was determined by calculating the limit of detection (LOD) and the limit of quantitation (LOQ). The LOD and LOQ for the method were calculated using 11 measurements of a blank. This blank was obtained following the extraction procedure proposed in the "sample preparation" section, but in this case without the addition of the sample. Limits of detection of the target compounds in the plastic wrapping film samples were calculated from the instrumental detection limit, taking into account the amount of sample used in the extraction procedure and the volume of organic phase. The values of LOD and LOQ obtained are shown in *Table 2*. The results obtained were between 1.6 and 39.4 μgKg^{-1}. The LODs obtained in this work were compared with values found in the literature. The LODs obtained for BBP, DEHP and DOP are lower than those obtained by Gätner et al. [30] (14, 95 and 21 μgKg^{-1}, respectively) for phthalate determination in paperboard by GC-MS. On the other hand, Shen et al [27] obtained LOD of 10 μgKg^{-1} for DEP, DBP, DEHP and DOP determination in plastic products by GC-MS, being these LODs higher than those obtained in our work for DEHP and DOP (7.2 and 2.0 μgKg^{-1} respectively).

The within-run precision was studied using a pool of different plastic wrapping film samples. The phthalates present in the sample were extracted using the method proposed in this work (described in the *section 2.4*) and then analyzed six times in the same run. Only four phthalates were present in the pool of samples used in this experiment and the relative standard deviations (RSD) were 2.5, 1.9, 2.1, and 2.4% for DEP, BBP, DBP and DEHP, respectively.

To check the intra- and interday precision assays were developed using a pool of plastic wrapping films samples. For the intraday study, six portions of a pool of transparent films samples were subjected to the extraction procedure described above and each extract was analysed in triplicate. The RSD (%) obtained were 9.3, 10.5, 5.8 and 9.9% for DEP, BBP, DBP and DEHP, respectively. The interday assay was performed in the same way by subjecting 12 portions of plastic wrapping film pool sample to the extraction procedure in two different days. The RSD values were 12.2, 11.1, 6.7 and 9.8% for DEP, BBP, DBP and DEHP, respectively. The RSD values obtained in the intra- and interday assay indicate that the method is precise for all phthalates present in the samples studied.

The recovery of the method was calculated using a plastic wrapping film pool sample spiked with four different concentrations of these compounds (5, 40, 60 and 100 $\mu g L^{-1}$). The extraction procedures were performed in duplicate and analyzed in triplicate and the recovery calculated using the standard addition graphs. The average analytical recoveries were 92.3 ± 4.9, 100.4 ± 3.5, 104.6 ± 7.0, 103.8 ± 1.9, and 101.7 ± 1.3 and 100.5 ± 1.6% for DMP, DEP, BBP, DBP, DEHP and DOP, respectively.

3.3. Application to plastic wrapping film samples

The proposed analytical method was applied to the determination of these phthalates in five different plastic wrapping films samples. These five samples were of different brands.

The extraction procedure was performed in duplicate to each sample and the extract was analysed in triplicate. The results obtained are given in *Table 3*.

***Table 3**: Concentration ($\mu g g^{-1}$) ± standard deviation (n=6) found in different plastic wrapping film samples.*

Sample	DMP	DEP	BBP	DBP	DEHP	DOP
A	n.d.	2.1	0.08	6.1	9.6	n.d.
B	0.03	1.9	0.12	34.8	7.2	n.d.
C	n.d.	1.07	0.07	7.8	5.9	n.d.
D	n.d.	0.4	0.06	6.6	2.9	n.d.
E	n.d.	0.2	0.02	2.1	0.84	n.d.

n.d.: DMP < 0.0049 $\mu g g^{-1}$; DOP < 0.002 $\mu g g^{-1}$

The concentration levels obtained varied from 0.02 to 34.85 $\mu g g^{-1}$. DBP was the phthalate found in highest concentration, whereas DOP was not detected in the samples. DMP was only detected in sample B at very low concentration; nevertheless, the other four phthalates were detected in all samples studied. DBP and DEHP were the phthalates found in highest concentrations and shown similar concentrations in the samples studied, except for sample B in which the level of DBP is higher than DEHP (34.85 $\mu g g^{-1}$ of DBP and 7.25 $\mu g g^{-1}$ of DEHP). The chromatogram obtained for sample D is shown in *Figure 5*.

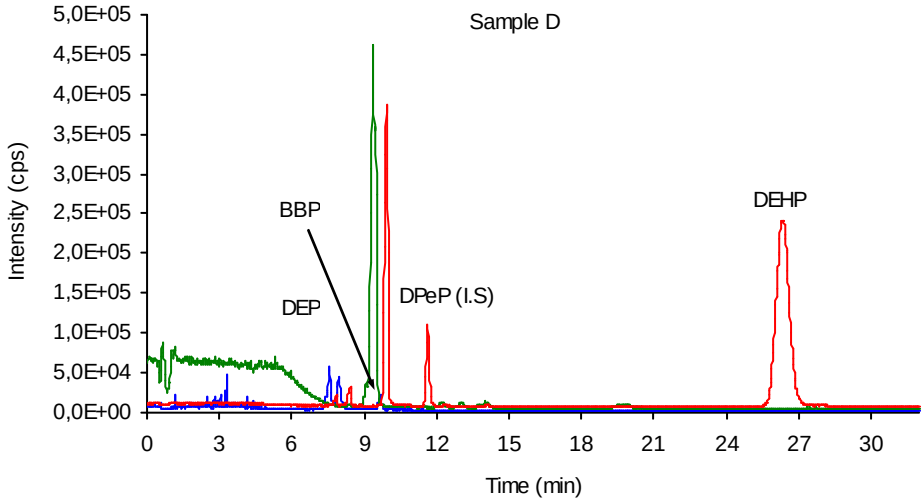


Figure 5. *HPLC-ESI-MS ion chromatogram obtained from sample D.*

The results obtained in this study confirm the presence of these phthalates in plastic wrapping film. The presence of these compounds in these types of films can produce contamination problems due to the migration of these compounds from the plastic film to the food which comes into contact with then. Higher lipid content food increase the migration of phthalates because they are lipid soluble and are not chemical bound to plastic. Therefore, the use of these types of plastic wrapping films increases human exposure to these compounds and implies a health risk.

4. Conclusion

A sensitive, precise and accurate method for the determination of DMP, DEP, BBP, DBP, DEHP and DOP in plastic wrapping film samples by solid-liquid extraction and HPLC-ESI-MS has been developed. Hexane and acetone were evaluated as extracting solvent for the solid-liquid extraction. Acetone was selected as the best extracting agent.

The proposed analytical method was applied for the determination of these phthalates in five samples of plastic wrapping films. DOP was not detected in the samples studied. DMP was only detected in one sample; whereas, DEP, BBP, DBP and DEHP were detected in all the samples studied. DBP and DEHP were the phthalates found in the highest concentrations. The presence of these compounds in these types of films can produce contamination problems due to the migration of these compounds from the plastic film to the food which comes into contact with then, increasing the human exposure to these compounds. The extract control of materials and processes used in the manufacture of these plastic films is essential to avoid the exposure to these toxic compounds.

Acknowledgment

The authors wish to thank the Xunta de Galicia (Grupo de Referencia Competitivos 2007 (000047-0)) for financial support.

5. References

[1] F. Zeng, K. Cui, J. Fu, G. Sheng, H. Yang, Water Air Soil Pollut. 140 (2002) 297.

[2] K. Luks-Betlej, P. Popp, B. Janoska, H. Paschke, J. Chromatogr. A 938 (2001) 93.

[3] A. Gómez-Hens, M.P. Aguilar-Caballos, Trends Anal. Chem. 22 (2003) 847.

[4] O.W.Lau, S.K. Wong, J. Chromatogr. 882 (2000) 255.

[5] M. Hamdani, A. Feigenbaum, Food Addit. Contam. 13 (1996) 717.

[6] Communication from Commision to the Council and The European Parliament on the implementation of the community strategy for endocrine disrupters-a range of substances suspected of interfering with the hormone system of humans and wildlife, COM (1999) 706; COM (2001) 262 final, Brussels, 2001.

[7] US Environmental Protection Agency (EPA) (1999) Protection agency. Introduction to water policy standards. Office of Water, Washintong, DC.

[8] Commission Regulation (EU) No 10/2011 of 14 January 2011 on plastic materials and articles intended to come in contact with food, Off. J. Eur. Union. L. 12(2011) 1.

[9] M. Castillo, D. Barcel, A.S. Pereira, F.R. Aquino Neto, Trends. Anal.Chem., 18 (1999) 26.

[10] B. Cavaliere, B. Macchione, G. Sindona, A. Tagarelli, J. Chromatogr. A 1205(2008) 137.

[11] X.Li, M. Zhong, S. Xu, C. Sun, J. Chromatogr. A 1135 (2006) 1.

[12] K. Holadová, G. Prokupková, J. Hajslová, J. Poustka, Anal. Chim. Acta 582 (2007) 24.

[13] O. Ballesteros, A. Zafra, A. Navalón, J.L. Vilches, J. Chromatogr. A 1121 (2006) 154.

[14] S. Jara, C. Lysebo, T. Greibrok, E. Lundanes, Anal. Chim. Acta 407 (2000)165.

[15] C. Pérez Feás, M.C. Barciela Alonso, E. Peña Vázquez, P. Herbello Hermelo, P. Bermejo Barrera, Talanta 75 (2008) 1184.

[16] L. Brossa, R.M. Marcé, F. Borrul, E. Pocurull, Environ. Toxicol. Chem. 24 (2005) 261.

[17] B.C. Blount, K.E. Milgram, M.J. Silva, N.A. Malek, J.A. Reidy, L.L. Needham, J.W. Broca., Anal. Chem. 72 (2000) 4127.

[18] R. Ito, F. Seísmo, N. Miura, M. Kawaguchi, K. Saito, H. Nakazawa, J. Pharmaceut. Biomed. Anal. 39(2005) 1036.

[19] C. Pérez-Feás, M.C. Barciela-Alonso, A. Sedes-Díaz and P. Bermejo-Barrera, Anal Bioanal Chem (2010) 397:529-535.

[20] G.K. Mortensen, K.M. Main, A.M. Anderson, H.Leffers, N.E. Skakkebaek, Anal. Bioanal. Chem. 382 (2005) 1084-1092.

[21] L.K. Soeresen, Rapid Commun. Mass Spectrom. 20 (2006) 1135.

[22] C. Cháfer-Pericás, P. Campíns-Falcó, M.C. Prieto-Blanco, Anal. Chim. Acta 610 (2008) 268.

[23] M. Del Carlo, A. Pepe, G. Sacchetti, D. Campagnone, D. Mastrocola, A. Cichelli, Food Chem. 111 (2008) 771.

[24] A. Zafra-Gómez, O. Ballesteros, A. Navalón, J.L. Vilches, Michrochem. J. 88 (2008)87.

[25] M. Bonini, E. Errati, G. Zerbinati, E. Ferri, S. Girotti, Microchemical J., 90 (2008) 31.

[26] R. Gibson, M.J. Wang, E. Padgett, A.J. Beck, Chemosphere 61 (2005) 1336.

[27] Hao-Yu Shen, Talanta 66 (2005) 734.

[28] Xiujuan Li, Zhaorui Zeng, Ying Chen, Ying Xu, Talanta 63 (2004) 1013.

[29] C. Pérez Feás, M.C. Barciela Aonso, P. Bermejo Barrera, Microchem. J. 99 (2011) 108.

[30] S. Gärtner, M. Balski, M. Koch, I. Nehls, J. Agr. Chem 57 (2009) 10675.

[31] USEPA Method 506 revision 1.1, Determination of Phthalates and Adiphate Esters in Drinking Water by Liquid-Liquid Extraction or Liquid-Solid Extraction and Gas Chromatography with Phothoionization Detection, national Exposure Research Laboratory Office of Research and Development U.S. Environmental Protection Agency, Cincinnati, Ohio 45268, 1995.

[32] R. Cela (ed) (1994) Avances en Quimiometría práctica. Universidad de Santiago de Compostela, santiago de Compostela, Spain, p 171.

CONCLUSIONES

Conclusiones

1. Se profundizó en el conocimiento de la técnica cromatografía líquida acoplada a espectrometría de masas simple y en tándem y se aplicó al análisis de ftalatos en muestras líquidas y sólidas a nivel de trazas.

2. Se desarrolló y validó un método que permite la determinación de DMP, DEP, BBP y DBP en muestras acuosas mediante LC-MS sin necesidad de realizar un tratamiento previo de la muestra. El método se aplicó al análisis de muestras de suero salino y se detectaron ftalatos únicamente en las muestras contenidas en envases de plástico monodosis.

3. Se adaptó el método anterior, que permite la determinación de DMP, DEP, BBP y DBP en muestras acuosas, a la técnica LC-MS/MS, consiguiendo LOD comprendidos entre 0.05 y 0.82 μgL^{-1}, que permitieron detectar niveles de ftalatos más bajos en las muestras de suero salino.

4. Se desarrolló y validó un método que permite la determinación de DMP, DEP, BBP, DBP, DEHP y DOP en muestras de nutrición parenteral mediante LLE-LC-MS. Los resultados mostraron que la presencia de vitaminas liposolubles en las muestras facilita la liberación de los ftalatos desde las bolsas de infusión a las nutriciones.

5. Se llevó a cabo un estudio de migración de ftalatos a partir de lentes de contacto blandas. Para el análisis de ftalatos se utilizó el método de determinación de LC-MS/MS desarrollado con anterioridad y se aplicó también en el análisis directo de las soluciones utilizadas en la limpieza de las lentillas. El test de migración

confirmó la liberación de algunos ftalatos a partir de las lentes de contacto, siendo el DBP el que se liberó en mayor cantidad. Se detectaron algunos ftalatos en las soluciones de limpieza comercializadas en envases de plástico monodosis, mientras que no se detectó ningún ftalato en las soluciones contenidas en envases de plástico rígido o semi-rígido.

6. Se desarrolló un procedimiento de extracción sólido-líquido para analizar seis ftalatos (DMP, DEP, BBP, DBP, DEHP y DOP) mediante LC-MS en muestras de film transparente utilizado en la conservación de alimentos. La extracción se llevó a cabo sometiendo 0.5 g de muestra junto con 10 mL de acetona a ultrasonidos durante sólo 15 min. DEP, BBP, DBP y DEHP fueron detectados en las cinco muestras analizadas. El DMP solo se detectó en una de ellas, mientras que en ninguna de las muestras se detectó el DOP.

A pesar de los ya conocidos efectos tóxicos que presentan los ftalatos en la salud humana, en esta Tesis se pone de manifiesto la presencia de algunos de ellos en distintos tipos de muestras. En todos los casos, los ftalatos analizados provienen de la liberación de los mismos desde diferentes plásticos (envases monodosis, bolsas de infusión, lentes de contacto y films transparentes) donde desarrollan su función como plastificantes.

Aunque existe un interés creciente en el estudio de tecnologías alternativas al uso de ftalatos para disminuir de esta forma la cantidad de los mismos en los materiales empleados en contacto con alimentos, y recurrir a plastificantes y polímeros biodegradables, todavía queda mucho por hacer para minimizar la presencia de estos compuestos en el medioambiente y en la cadena alimentaria.

ANEXOS

Nova Biomedical

Bradley C. Vaughn
Editor

Bisphenol A and Phthalates

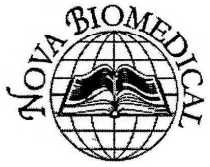
Uses, Health Effects and Environmental Risks

Chemical Engineering Methods and Technology

CHEMICAL ENGINEERING METHODS AND TECHNOLOGY

BISPHENOL A AND PHTHALATES: USES, HEALTH EFFECTS AND ENVIRONMENTAL RISKS

BRADLEY C. VAUGHN
EDITOR



Nova Science Publishers, Inc.
New York

CHEMICAL ENGINEERING METHODS AND TECHNOLOGY

BISPHENOL A AND PHTHALATES: USES, HEALTH EFFECTS AND ENVIRONMENTAL RISKS

CONTENTS

In: Bisphenol A and Phthalates: Uses, Health Effects… ISBN: 978-1-60741-701-9
Editor: Bradley C. Vaughn

Chapter 2

ANALYTICAL METHODS FOR PHTHALATES DETERMINATION IN BIOLOGICAL AND ENVIRONMENTAL SAMPLES: A REVIEW

P. Bermejo Barrera, M. C. Barciela Alonso, C. Pérez Feas, E. Peña Vázquez and P. Herbello Hermelo
Department of Analytical Chemistry, Nutrition and Bromatology, Faculty of Chemistry, University of Santiago de Compostela, Avenida das Ciencias, s/n. E-15782, Santiago de Compostela, Spain

This chapter summarizes and discusses the analytical methods and techniques described in the literature for phthalate determination in different matrix samples (water, soil, sediments, sludge, air and biological samples). Different sample treatments, extraction and preconcentration steps, such as liquid-liquid extraction (LLE), solid phase extraction (SPE), solid phase microextraction (SPME), stir bar sportive extraction (SBSE) and solid/liquid extraction (SLE) have been evaluated. Separation techniques such as gas chromatography (GC) or high performance liquid chromatography (HPLC) coupled with different detectors have been compared in terms of detection limits and practical applications.

Keywords: Phthalates, sample preparation, liquid chromatography, gas chromatography

1. INTRODUCTION

Diester of phthalic acid, commonly referred to as phthalates (PAEs), are a group of chemical compounds widely used in industry and commerce due to their large variety of uses. Due to the ability to improve softness and flexibility to plastics, they are used mainly as plasticizers in a wide variety of products including medical devices, children's toys and all types of packaging. Furthermore, phthalates are also used as industrial solvents and lubricants, as an additive in the textile industry and in pesticides, and also in personal care

products such as deodorants, lotions and perfumes, to retain colour and fragrance.[1, 2, 3, 4] The main drawback of the use of PAEs is that they can migrate from the material to the environment and pollute water, soil, air and food-products. Furthermore, certain phthalate esters and/or their metabolites are suspected to be human carcinogenic agents and endocrine disruptors,[5] which make their trace determination of special importance. In particular, dibutyl phthalate (DBP), butyl benzyl phthalate (BBP), and di-(2-ethylhexyl) phthalate (DEHP) are in the list of the proposed substances suspected of producing endocrine alterations published by European Union (EU).[6]

Section 307 of US Clean Water Act establishes that dimethyl phthalates (DMP), diethyl phthalate (DEP), butyl benzyl phthalate, dibutyl phthalate, di-(2-ethylhexyl) phthalate and dioctyl phthalate (DOP) must be considered priority toxic pollutants.[7] These concerns have been further aggravated by recent analysis of human blood and urine samples, where traces of various phthalates (or their metabolites) have been found.[8,9] For these reasons, the interest in the study of these types of chemical substances has increased during the last few years, and therefore it is essential to develop reliable and sensitive analytical methods to determine this group of compounds at trace levels.

This review summarizes and discusses the analytical methods and techniques described in the literature for phthalate determination in different matrix samples (water, soil sediments, sludge, air and biological samples). Different sample treatments, extraction and preconcentration steps, such as liquid-liquid extraction (LLE), solid phase extraction (SPE), and solid phase microextraction (SPME) have been evaluated. Separation techniques such as gas chromatography (GC) or high performance liquid chromatography (HPLC) coupled with different detectors, such as UV detector, flame ionization detection (FID), electron capture detection (ECD) or mass spectrometry (MS) (all types of MS analyzes) have been compared in terms of detection limits and practical applications.

The major problem in phthalate analysis is sample contamination, resulting in false positive results or over-estimated concentrations. Due to the fact that phthalates are widely used, they are present in air, water, and organic solvents and plastics; they are adsorbed onto glass and other materials. Therefore, the risk of contamination is present in the whole analytical scheme, including sampling, sample preparation and analysis by chromatography. The different cleaning methods proposed in the literature, for avoiding contamination from material used in the laboratory, have been reviewed in this work.

2. SAMPLE PREPARATION

In order to detect PAEs at sub ppm levels, a clean up/preconcentration step is necessary before instrumental analysis. Different methods have been developed with this purpose such as liquid-liquid extraction (LLE), liquid-phase microextraction (LPME), single drop microextraction (SDME), solid phase extraction (SPE), solid phase microextraction (SPME), stir bar sorptive extraction (SBSE) and solid/liquid extraction (SLE).

2.1. Liquid-Liquid Extraction (LLE)

Various liquid-liquid extraction (LLE) approaches have been used for isolation of PAEs from aqueous samples. In these methods, the extraction is carried out in a funnel, mixing the sample with an organic solvent, such as, hexane, dichloromethane, cyclohexene or ether. After the extraction, the organic phase is dried and concentrated to obtain higher sensitivity.

Different LLE methods have been developed for PAEs and their metabolites determination in different biological matrices. Mortensen et al.[10] used a liquid extraction with a mixture of ethyl acetate and ciclohexane (95:5) for a quantitative determination of PAEs in human milk by LC-MS-MS. On the other hand, Sorensen[11] extracted these compounds from milk and milk products using a mixture of tert-butyl methyl ether and hexane using the same determination technique.

An LLE method for DEHP in serum samples was used by Faouzi et al.[12] using a mixture of 2 ml acetonitrile and 2 ml sodium hydroxide (1N). The sample with the mixture acetonitrile:sodium hydroxide was shaken for 10 min using an alternating agitator and centrifuged at 3000 rpm. The clear supernatant was then injected into the chromatograph for the analysis.

DEHP has been extracted by Kambia et al.[13, 14] from total parenteral nutrition and fromplasma. In this case, the sample (1 ml) was treated with 1 M sodium hydroxide (1 ml) and hexane (2 ml). The mixture was vortexed (2 min), centrifuged (1620 x g for 5 min) and the separated organic layer (fraction 1) was transferred into a clean conical glass tube. The aqueous phase was extracted again with 2 ml of hexane and the mixture treated as above. The separated organic phase (fraction 2) was combined with the fraction 1 and the total organic phase was evaporated to dryness in a water bath at 40ºC under a nitrogen stream. The residue was dissolved in 100 µl of acetonitrile and 20 µl of this solution was injected in HPLC. These authors applied the same extraction procedure for the determination of DEHP in human plasma samples.[15] Recently, Ji-an Chen et al.[16] analyzed di-n-buthyl phthalate and other organic pollutants in Chongquing women undergoing parturition. The authors analyzed these compounds in venous blood, umbilical cord blood, breast milk and urine. For PAEs extraction from blood and milk, the samples were first treated with anhydrous sodium sulfate (until saturation). The resulting solution was extracted with 10 ml of hexane by vortexing for 30 min. The top organic layer was collected and the remaining sample was extracted again using the same procedure. The two extracts were combined and the solvent was evaporated under nitrogen flow until 1 ml of solution remained. Each urine sample was extracted twice with 10 ml of hexanol/ethanol (8:1) in a separating funnel. The extracts were combined and the solvent evaporated as above.

Recently, Orsi et al.[17] used a simple and rapid method for the determination of PAEs presents in nail cosmetics. The method is based on ultrasonic extraction of the sample with ethanol-water (90:10 v/v) followed by HPLC separation and UV detection.

LLE procedures have some disadvantages such as, the use of high volumes of solvents. In addition, the process, generally, was off-line, and time consuming. LLE is limited due to the presence of trace levels of phthalates in commercially available solvents, even solvents for trace analysis. Therefore, accurate determinations below 0.1 $\mu g\ l^{-1}$ are questionable with this method.

In recent years, studies have been carried out towards miniaturization of liquid-liquid extraction procedures, reducing the amount of organic solvent

Liquid-phase microextraction (LPME) is a new method for sample preparation, whereas only a few microliters of solvents are used to preconcentrate compounds from aqueous samples.[18] In this technique, a microdrop of organic, water immiscible solvent is suspended from a microsyringe needle, which is then immersed in a stirred aqueous sample solution for a specified period of time.[19, 20, 21, 22, 23] After equilibrium, the microdrop of organic solvent loaded with the analyte is determined. This method has been termed single-drop microextraction (SDME). It can be performed in different modes, including: static liquid-phase microextraction (S-LPME), dynamic liquid-phase microextraction (D-LPME), continuous-flow liquid-phase microextraction (CF-LPME), solvent bar liquid-phase microextraction (SB-LPME) and headspace liquid-phase microextraction (HS-LPME).[24] The advantages of this technique are: the simplicity, low cost, ease of operation, rapid, small volume of organic extractants, and minimal exposure to toxic organic solvents, being environmental friendly.

Recently, Farahani et al.[25] developed a method for phthalate determination in water samples using a liquid-phase microextration (LPME) prior to the analysis by gas chromatography. In the method proposed by these authors 10.0 ml of aqueous sample was transferred into an 11.0 ml vial. Then, 10.0 µl of 1-dodecanol were delivered to the solution surface using a microsyringe. The vial was sealed and then the magnetic stirrer was turned on. Under the proper stirring conditions, the suspended microdrop remains in the top-center position of the aqueous sample. The microdrop movement was affected by the flow field, which favores the promotion of the mass transfer inside the microdrop.[26] After the desired extraction time, the sample vial was transferred into an ice beaker and the organic solvent was solidified after 4 min. Then, the solidified solvent was transferred into a conical vial and melted immediately. Finally, 1.00 µl of the extractant was injected into the gas chromatograph.

In some cases, S-LPME can present certain disadvantages (depending on the organic solvent and the volume on the organic drop, usually no more than 5 µl) such as instability of microdrop, relatively low reproducibility and sensitivity. Thus, to avoid these problems, Jinrong Yao et al.[24] proposed a modified S-LPME method, for phthalate determinations in landfill leachates. The most attractive feature of this method is the use of a polychloropropene rubber tube (PGR tube) instead of a microsyringe to load the organic solvent. The PGR tube and the sample vial were placed horizontally, so the selection of the organic solvent was not affected by the density of the extractant. The authors used this method for DMP, DEP and DnBP determination in landfill leachates by liquid chromatography, obtaining good precision and recovery.

A fast and simple method, using HF-SDME, has been developed by R. Batlle et al.[27], to facilitate the identification and quantification of seven dialkil phthalate esters (Diethylhexil phthalate (DEHP), dibutyl phthalate (DBP), diisobutyl phthalate (DiBP), dimethyl phthalate (DMP), diisopropyl phthalate (DiPP) and diethyl phthalate (DEP)) in three aqueous food simulants (distilled water (A), 3% (w/v) acetic acid/water (B) and 15% (v/v) ethanol/water (C)). In this study, better results have been obtained using 7:3:0.5 Dichloromethane:hexane:toluene for food simulants A and C, and 1:9:0.5 Dichloromethane: hexane:toluene for food simulant B as extractant solvent. The method has been shown to be highly practical because of its high reproducibility, convenient dynamic range and detection limits. Therefore, this methodology reduces the amount of solvent necessary for the whole procedure (5 µl), thus eliminating the need for additional cleaning or concentration steps. The

same technique was used by Pisillakis et al.[28], for PAE_S determination from water, using toluene as extraction solvent.

Recently, Pie Liang et al.[29] developed the fist method for PAEs determination using continuous-flow microextraction (CFME) combined with liquid chromatography with variable-wavelength detector (VWD). The CFME procedure consists of four steps: (1) The sample solution is continuously pumped and vertically upward at a constant flow rate into the bulb glass extraction chamber (~ 0.2 ml) via the connecting PTFE tube; (2) After the chamber has been filled with the sample solution, the required volume of organic solvent (3.0 µl of tetrachloromethane) is introduced into the extraction chamber by the microsyringe, and forms a drop at the tip of the microsyringe and remains above the PTFE tube outlet in the extraction chamber; (3) As the solvent drop is immersed into the sample solution, the analytes are extracted into the solvent drop from the sample when the sample is continuously ejected from the PTFE tube into the chamber (flow rate 0.4 mL min^{-1}); (4) After extracting for a prescribed period of time, the solvent drop is retracted into the microsyringe, and the microsyringe is removed from the chamber. Then, the needle tip is cleaned carefully with a tissue to remove possible water contamination, and the extraction solvent with the extracted analyte is injected into the LC system for analysis. The enrichment factors of this method for DMP, DEP and DnBP reached at most 27, 44 and 20 respectively.

2.2. Solid Phase Extraction (SPE)

SPE appears to be a more suitable technique for PAEs extraction and preconcentration from water or aqueous samples. The advantage of this technique, with respect LLE is that it requires a minimal use of solvents, thus reducing health risk and sample contamination, permiting the simultaneous extraction of multiple samples. Moreover, a large concentration factor can be obtained without solvent concentration, avoiding the concentration of concomitants present in the organic solvent.

SPE is carried out using different sorbents. The most widely used sorbents to extract PAEs from water, urine sample, wine etc., are silica polymers such as silica-based C18 and C8.[30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41] Other sorbents used are organic polymers such as polyestirene[42], crosslinked polystyrene-divinylbencene (PS-DVB) or hydroxilated PS-DVB[43,44,45], styrenedivinyl polimer (PS-2), octadesyl-coated styrenedivinylbenzene polymer (SDB-XD)[33] and Stirene-divinylbenzene methacrylate copolymer.[46, 47, 48] The authors justified the use of these organic polymers because the loading properties of organic carbon are superior to those of silica-based adsorbents. Another alternative proposed by Ya-Qi Cal et al.[49] is the use of polytetrafluoroethylene (PTFE) as sorbent in SPE. The use of this compound as a sorbent is based on the the fact that PTFE shows a very strong hydrophobic property and that PTFE experimental containers can readily adsorb neutral hydrophobic compounds in their surfaces.[50, 51] In this way, the authors studied the potential of PTFE turnings as the matrix for SPE of trace phthalate esters from aqueous samples. By driving the aqueous sample solution to pass through a PTFE turnings in the column, the analytes were retained on the PTFE turnings packed column. The retained analytes were then eluted with acetonitrile, followed by the HPLC-UV analysis. The method proposed presents acceptable recovery results (92.1-127.5%) for the five phthalates studied

(DBP, DCHP, DOP, DNP and DDP). The use of PTFE turnings as SPE sorbent, presents clear advantages such as durability and easy of availability.

Another SPE sorbent used by Ya-Qi Cai et al.[52] is the Multi-walled carbon nanotubes (MWNTs). MWNTs possess many unique electronic, mechanical and chemical properties, high surface area, and excellent strength, being very appealing for a great number of important applications.[52] The authors demonstrated for the first time that MWNTs can be used as effective adsorbent for SPE of four phthalates (DEP, di-n-propyl-phthalate, di-iso-butyl-phthalate and di-cyclohexyl-phthalate) from aqueous samples. Moreover, the authors compared the analytical performance of this method with others using commercial SPE adsorbents such as C18, C8 and PS-DVB. The results showed that multi-walled nanotubes were more effective than, or as effective, as these adsorbents for the SPE of these four analytes.

An alternative method was proposed by Yoshihiro Saito et al.[53] using a miniaturized solid-phase extraction coupled with HPLC-UV. In this case, the authors used a fiber-in-tube capillary for SPE. Fiber-in-tube capillary was prepared by packing Zylon® filaments (11.5 µm i.d. x 100 mm) into a poly(ether ether ketone) (PEEK) tube of 0.25 mm i.d. x 100 mm. These filaments were longitudinally packed into the tube, and the total number of packed filaments was about 330. With this method the authors obtained limits of quantification for DBP and DEHP in wastewater lower than 0.5 ng ml^{-1}.

A sensitive and selective column adsorption method was proposed by Hatsumata et al.[54] for the off-line preconcentration and determination of PAEs (BBP, DBP, DCHP). The PAEs were preconcentrated on *Saccharomyces cerevisiae* immobilized on silica gel and then determined by HPLC. With the proposed method the preconcentration step takes about 30 min for 50 ml of aqueous sample. The maximum preconcentraction factor was 40 for BBP and DBP, and 80 for DCHP. The recovery of spiked PAEs in a river water sample was in a range 98-101%.

Recently, micelle-like surfactant aggregates adsorbed on solid materials (namely hemi-micelle or admicelle) in SPE have been studied as a good alternative for the preconcentration of a variety of organic pollutants. The sorbents used in SPE are produced by adsorbing ionic surfactants (such as sodium dodecylsulfate or cetyltrimethylammonium bromide) on the metal oxides (such as alumina or silica). The use of this technique in SPE has many advantages, such as high extraction efficiency, high breakthrough volume, easy elution of analytes and high flow rate for sample loading; furthermore, this technique requires no clean-up steps and the adsorbents are easy to regenerate.[55] In this way, Tohru Saitoh et al.[56] studied the Aerosol-OT-?-alumina admicelles for the concentration of hydrophobic organic compounds in water (including phthalates). The AOT-?-alumina admicelles were successfully prepared by mixing ?alumina and di-2-ethylhexylsodium sulfosuccinate (AOT) in a weakly acid aqueous solution. The large sample loading capacity of the AOT- ?-alumina admicelles enables highly an efficient concentration of trace analytes. The authors obtained a concentration factor for different compounds, including DEP, DBP and DEHP, of 500 fold.

F.J. López-Jiménez et al.[57] used sodium dodecyl sulfate (SDS)-alumina hemimicelles for the preconcentration of BBP, DBP and DEHP in environmental water samples. The authors used cartridge columns filled with 500 mg of alumina. These cartridges were conditioned with 10 mL of a nitric acid solution (pH 2). Then, hemimicelles were formed on the alumina by passing a 25 mL 0.01 M nitric acid solution containing 40 mg of SDS. Recoveries of PAEs above 95% were obtained for all samples studied (raw and treated

sewage samples) and a preconcentration factor of 500 can be easily achieved by SPE of 1 L of sample and elution with 2 mL of methanol.

Last year, Jidon Li et al.[55] analyzed five phathalates by HPLC-UV after preconcentration by SPE using ionic liquid mixed hemimicelles. The authors evaluated mixed hemimicelles prepared by adsorbing 1-Hexyl-3-methyllimidazolium bromide ([C_6 min] Br) and 1-Dodecyl-3-methyllimidazolium bromide ([C_{12} min] Br) on silica surface on the phthalate concentrations. In this case, ([C_{12} min] Br)-coated silica as adsorbents was selected for the preconcentration step due to the higher capacity for the analytes and the preconcentration factor obtained was 600 folds.

Table 1, summarizes the applications and different conditions of SPE for phthalates determination preconcentration.

2.3. Solid-Phase Microextraction (SPME)

Solid-phase microextraction (SPME) in sample preparation became very popular in the late 1990s.[58] In this extraction technique, a fused silica fiber coated with a thin layer of polymer phase is immersed in the aqueous sample, while the sample is stirred. After a certain amount of time (range from minutes to hours) the coated fiber is retracted and transferred in a holder to the GC and desorbed in a hot inlet. The adsorbed compounds are desorbed and injected into the chromatographic column for the analysis. The SPME procedure (sampling, extraction concentration and sample introduction in one step) significantly reduces the risk of contamination and simplifies the overall analytical process. Several studies employing direct SPME for extraction of phthalates from water have been published. These methods used different fibers such as polyacrilate[59, 60], carbowax[61], carbowax-divinilbencene (CW-DVB)[62, 63, 64], polyaniline (PANI)[65], polydimethylsiloxane (PDMS)[66,64] or polydimethylsiloxane-divinilbencene (PDMS-DVB).[67, 68, 69, 63, 64] SPME has a great number of applications in water samples, but in recent years other applications have appeared in complex matrices, such as, vegetable oil or milk samples. For example, Holadová et al.[70] used a headspace-solid-phase microextraction for PAEs determination in vegetable oil. The authors compared the results obtained using different SPME fibers (silica fibers coated with Polydimethylsiloxane (PDMS), poliacrylate, carboxen/polidimethylsiloxane and polydimethylsiloxane-divinilbencene) and different matrix modifiers (hexane, methanol, acetonitrile, dimethylformamide, and water). The results showed that, employing PDMS 100 together with methanol as the matrix modifier, headspace SPME phthalate determination in vegetable oil samples is possible. Some applications and the experimental conditions, using SPME are summarized in the Table 2.

The main drawbacks with SPME are that extraction fibers are expensive and have a limited life[71]; sample carry-over between extractions has been reporter for some analytes[72] and for limited types of SPME fibers commercially available.

A modification of the SPME technique is in-tube SPME. This is a microextraction and preconcentration technique using an open tubular fused-silica capillary with an inner surface coating as the SPME device. The advantage of this technique is that it can be coupled on-line with HPLC, allowing complete automation, shortening analysis time and improving accuracy and precision. Mitami et al.[73] developed a method for the determination of nine phthalates

in infusion solutions in plastic containers using in-tube SPME-HPLC with limits of detection in the range of 1-10 ng/ml. In this case, the analytes were extracted from the sample directly into an open-tubular capillary (a Supel-Q Plot capillary column (60 cm x 0.32-mm i.d., 12 µm) by 20 repeated draw/eject cycles of 40 µl of sample solution. The extracted compounds were desorbed using a mobile phase flow (acetonitrile:water). The in-tube SPME method has shown a sensitivity 18-125 times higher than the direct injection method. Cháfer-Pericás et al.[74] used the same technique for DBP and DEHP determination in environmental samples. In this case, the TRB-5 coated capillary was used as an in-tube SPME device and the number of cycles to carry out the extraction was fixed at 7 using 50 µl of sample. The limits of detection obtained were 1 and 2.5 $\mu g l^{-1}$ for DBP and DEHP, respectively.

2.4. Stir Bar Sorptive Extraction

Stir bar sorptive extraction (SBSE) was first introduced by Baltussen et al. in 1999 and based on the same principles of SPME.[75] The authors used polydimethylsiloxane (PDMS) (50-300 µl), coated in a stir bar, to preconcentrate different analytes. The amount of PDMS coated in the stir bar is considerably higher than in SPME fiber; thus, the results showed high recoveries, better sensitivity and higher capacity. In the first works using this technique, once the extraction step was over, the stir bar was dried and the analytes thermally desorbed in a desorption unit, usually installed in a gas chromatograph. In this way, Prieto et al.[76] used SBSE for simultaneous preconcentration of a wide variety of organic pollutants (including DMP, DEP, DBP, BBP, DEHP and DOP) from water samples, obtaining, good sensitivity and recovery. Tan et al.[77] used the same technique for trace analysis of selected endocrine disruptors (including DEP, DBP, BBP, DEHP) in water, biosolid and sludge samples. The method has many practical advantages such as small sample volume (10 ml aqueous or <1g sludge sample) and simplicity of extraction.

An alternative to SBSE with thermal desorption (TD), is liquid desorption (LD). In this case, the analytes are desorbed using a small amount of organic solvent. LD can also be combined with GC and a large volume injection (LVI) when a thermal unit is not available. In this way, Serôdio et al.[78] developed a method for endocrine disrupter chemicals determination (including BBP and octylphthalate) in water using SBSE-LD in combination with LVI and GC coupled to mass spectrometry. In this case, the extraction procedure was performed for 60 min with a stirring speed of 750 rpm at room temperature (20ºC). After sampling the stir bars were removed with a clean tweezers dried with a lint-free tissue and placed into a 2 ml glass vial filled with 100 µl of acetonitrile ensuring the total immersion. Solvent back extraction was performed using ultrasonic treatment for 15 min at a constant temperature (25ºC). Afterward, the stir bars were removed; the acetonitrile extract was evaporated under gently purified nitrogen and after redissolved in 80 µl of ethyl acetate. The method described presents an excellent linear dynamic range for almost all endocrine disrupters chemicals from waters samples at ultra-trace level (0.025-0.400 $\mu g l^{-1}$). Later, the same authors studied a method for phthalates (DMP, DEP, DBP, BBP, DEHP, BOP) determination in drinking water, using the same technique.[79] The only difference is that in this case, methanol was used as a back extraction solvent. With the proposed method, low detections limits were obtained for all phthalates studied (3-40 $ng l^{-1}$).

Table 1. Applications of solid phase extraction (SPE) for phthalate analysis

Sample	Analyte	Sorbent Material	Sorbent conditioning	Elution	Determination	Ref
Urine	DEHP	HySphere-C18 HD (on-line)	4 mL methanol followed by 4 mL water and 4.0 mL acidified buffer	0.1% acetic acid-acetonitrile (75:25 v/v)	HPLC-MS/MS	3
Urine	MBP, MBP,MEHP	HySphere-C18 HD (on-line)	4 mL methanol followed by 4 mL water and 4 mL acidified buffer	0.1% acetic acid-acetonitrile (75:25 v/v)	HPLC-MS/MS	9
Human milk, comsumer milk and infant formula	mMP, mEP, mBP, mBzP, mEHP, mNP	Oasis HLB, Waters	Acetonitrile and basic buffer	Acetonitrile (2 mL)	LC-MS-MS	10
River Water	MMP, MBP, MEHP, DMP, DBP, DEHP	PS-2 and C18; SDB-XD	Dichlorometane, acetone, methanol and purified water; acetone	Dichlorometane	GC-MS	33
Food, water, tea, coffee	DMP, DEP, DBP, BBP, DEHP	Oasis HLB glass (6 mL; 0.5g)		2 mL methanol	GC-MS	34
Textile wastewater	DBP, Bis(2-ethylhexyl)phthalate	C18 and Isolute ENV+cartridge	7 mL of methanol followed 3 mL of water at 1ml/min	2x5 mL of methanol/hexane; 10mL of solution 5mM in triethylamine (TEA) and 5mM acetic acid/methanol (1:9) v/v	LC-APCI-MS	35
Water	DMP, DEP, DBP DEHP	C18	Deionized water followed by about 2 mL of methanol	2 mL of methanol in dichloromethane (50:50 v/v)	GC-FID	36
Extracts of sludges	DEP, DBP DEHP	C18	7 mL of methanol and 3 mL of HPLC water	Hexane/methanol 9:1	LC-MS	37
Urban wastewater samples	DMP, DEP, DBP, BBP, DEHP, DOP	LiChrolut RP-18 (C18)	5 mL diethyl ether, 5 mL methanol and 5 mL deionized water	6 mL mixture of diethyl ether-methanol (9:1; v/v)	LC-MS	38
Wastewater	DMP, DEP, DBP, BBP, DEHP, DOP	LiChrolut RP-18 (C18)	5 mL diethyl ether, 5 mL methanol and 5 mL deionized water	6 mL mixture of diethyl ether-methanol (9:1; v/v)	GC-MS	39
Drugs	DEHP, MEHP	Oasis HLB extraction column (on-line)		Acetonitrile/Water 90/10 (v/v)	LC-MS/MS	40

Table 1. (Continued)

Sample	Analyte	Sorbent Material	Sorbent conditioning	Elution	Determination	Ref
Human milk	13 phthalate metabolites	Oasis HLB column	2 mL HPLC grade methanol and 2 mL water	0.5 mL acetonitrile	HPLC-MS/MS	41
Water	BBP, DEHP	Polyestyrene based SPE column	Dichloromethane (6 mL), methanol (6 mL) and water (6-7 mL)	Methanol+dichlorometane (1+1)v/v	HPLC-UV	42
Water, landfill leachates	DMP, DEP, DBP, BBP and DEHP	Hydroxilated polyestirene-divinilbenzene polymer	5 mL of ethyl acetated followed by 5 mL of methanol and conditioned with 5 mL of acidified water	Toluene and ethyl acetate	GC-MS	45
Urine	MMP, MEP, MBP, MCHP, MEHP, MOP,MEOHP, MEHHP	Stirene-divinylbenzene methacrylate copolymer SPE cartridge	Acetonitrile (1 mL) and pH 2 phosphate buffer (1 mL, 0.14 M NaH_2PO_4 in 0.85% H_3PO_4)	1 mL of acetonitrile followed by ethyl acetate (1 mL)	HPLC-MS/MS	46
Urine	miBP, PA, mCPP, mEP, mBP, MBzP, mEHP, mEHHP and mEOHP	Stirene-divinylbenzene methacrylate copolymer SPE cartridge	Acetonitrile (1 mL) and pH 2 phosphate buffer (1 mL, 0.14 M NaH_2PO_4 in 0.85% H_3PO_4)	1 mL of acetonitrile followed by ethyl acetate (1 mL)	HPLC-ESI-MS/MS	47
Wastewater	DBP, DMP DEHP	Stirene-divinylbenzene sorbent Lichrolut EN	7 mL methanol and 3 mL water	2x5 mL acetonitrile waiting 5 min between the two aliquots	LC-APCI-MS	[48]
Water	DBP, DCHP, DOP DNP, DDP	PTFE turnings	5 mL of acetonitrile and 10 mL of Milli-Q purified water	10 mL of acetonitrile	HPLC-UV	49
River an sea water	DEP, di-n-propyl-phthalate, di-iso-butyl-phthalate and di-cyclohexyl-phthalate	Multi-walled carbon nanotubes	Washed with 5 ml of methanol and activated with 5 ml of water.	Acetonitrile (5 ml)	HPLC-UV	52
River water	BBP, DBP, DCP	*Saccharomyces cerevisiae* immobilized on silica gel	50 ml of HCl (1M) and 50 ml of water.	2.5-7.5 ml of acetone, acetonitrile, ethanol and or methanol	HPLC-UV	54
Environmental samples	DEP, DnPP, DnBP, DcHP, DEHP	([C_{12} min] Br)-coated silica	An extraction cartridge was prepared with 0.5 g of silica. Then 25 mL solution containing 25 mg of [C_{12} min] Br was pasen through the cartridge column.	3 mL methanol pH 2	HPLC-UV	55

Table 1. (Continued)

Sample	Analyte	Sorbent Material	Sorbent conditioning	Elution	Determination	Ref
Water	DEP, DBP, DEHP	AOT-?-alumina admicelles	Alumina was ultrasonically washed with 1M nitric acid for 3 min and thoroughly rinsed with water. To prepare an admicelle column, 50 mL of 0.01M nitric acid containing AOT was passed through a cartridge filled with alumina.	1 mL acet onitrile	HPLC	56
Raw and treated sewage samples	DEHP, BBP, DBP	Hemimicelles of sodium dodecyl sulphate (SDS) produced on alumina	10 mL of a nitric acid (pH=2). Then hemimicelles were formed on the alumina passing 25 mL0.01M nitric acid solution containing 40 mg SDS	2 mL of methanol	LC-MS	57
Water	DMP,DEP, BBP, DBP, DEHP, DOP	ENV+ cartridge	10 mL acetonitrile and 10 mL reagent water	10 mL acetonitrile	LC-APCI-MS	106
Water	DnBP, BBP, DEHP	LiChrolut EN	3 mL Methanol and 3 mL Milli-Q water; 5 mL acetonitrile acetonitrile and 5 mL mL Milli-Q water	On-line 100 µl ethyl acetate; 5 mL acetonitrile	GC-MS; HPLC-ESI-MS	107
Urine	MEP, MBP, MCHP, MBzP, MOP, MEHP, MDP	Oasis HLB, Waters	1 mL acetonitrile followed 2.0 mL of basic buffer	Acetonitrile (2 mL) followed Ethyl acetate (2 mL)	HPLC-APCI-MS	108
Urine	11 urinary phthalate metabolites	Nexus SPE cartridges (Varian)		Acetonitrile (2 mL) followed Ethyl acetate (2 mL)	HPLC-APCI-MS/MS	111

Table 2. SPME Methods for the phthalate analysis

Sample	Analyte	Fiber Type	Sample volumen and Treatment	Determination	Ref
Water	DnBP, DEHP	65-µm polydimethylsyloxane-divinilbencene 65-µm carbobax-divinilbencene, 85-µm polyacrylate, 75-µm carboxen-divynylbencene and 30-µm polydimethylsyloxane	3.5 mL of sample (pH 6). Extraction time 30 min at 80ºC.	GC-MS	59
Water	DMP, DEP, DnBP, BBP, DEHP, DOP	PDMS, PA	3 mL of water. Extraction during 20 min. Desorption during 5 min at 250ºC..	GC/ECD	60
Water	DBP, DEHP	Carbowax	4 mL of sample. Extraction time 15 h (stirring at 1000 rpm). Desorption with 100 µl of methanol:ethanol (80:20) during 2 min.	HPLC-ESI-MS	61
Water	DMP, DEP, DBP, BBP, DEHP, DOP, DNP	PDMS, PA, PDMS-DVB, DVB-Carboxen-PDMS, CW-DVB	5 mL of sample. Extraction time 45 min at 22ºC. The desorption time was 5 min at 270ºC	GC-MS	62
Cow milk	DMP, DEP, DBP, BBP, DEHP, DOP	PDMS, PDMS/DVB, CAR/PDMS, DVB/CAR/PDMS, PDMSDVB-StablFlex-65µm, CW/DVB-StablFlex-70µm, Polyacrylate	Five grams of cow milk weighed into a 15 mL SPME vial. A magnetic stirring bar and 2.5 g of sodium chloride were added into the vial. Extraction time 2 min at 90ºC. The extraction time 60 min at the same temperature.	GC-MS	63
Drinking water	DMP, DEP, DPP,	PDMS, PDMS/DVB, CW/DVB	5mL of sample. Extraction time 30 min at room temperature (stirring at 1200 rpm). Desorbed into the mass spectrometer during 40 s.	Fiber introduction mass spectrometry (FIMS)	64
Water	DMP, DEP, DAP, DBP, DOP	Polyaniline (PANI)	10 mL of sample. The solution was continuosly stirred during 20 min at 30ºC. The desorption temperature were 280ºC during 3 min.	GC-FID	65
Water	DEP, BBP, DOP	PDMS	30 mL of water. Extraction time: 15-60 min, Extraction temperature: 30-90ºC, Desorption time:1 -5 min	GC-MS	66
Wastewater	DMP, DEP, DBP, BBP, DEHP, DOP	CW-DVB, PDMS, PDMS-DVB, PA and CAR-PDMS	10 mL of sample. Extraction time 80 min. Desorption time was 5 min at 260ºC.	GC-MS	68
Aqueous media	DEP	PDMS/DVB, CW/TRP (carbowax/templated resin)	The extraction time was 15 min. desorption with 500 µl of the mobile phase (ecetonitrile:water 52.5:47.5) or acetonitrile	HPLC-UV	69

Table 2. (Continued)

Sample	Analyte	Fiber Type	Sample volumen and Treatment	Determination	Ref
Vegetable oil	DMP,DEP, DnBP, BBP, DEHP, DnOP	PDMS	Oil sample modified with 1 mL methanol. The sample was incubated 60 min at 40ºC. The extraction time was 20 min. Desorption temperature was 250ºC	GC/MSD and GC/ECD	70
Environmental water samples	DBP, DEHP	Capillary coated with 95% polydimethilsiloxane and 5% polydiphenylsiloxane (TRB-5)	100 µL of sample (. Flow rate for the extraction procedure 300 µl min^{-1} (30 extraction cicles). Desoprtion with 100 µl of mobile-phase	HPLC-UV	74

Another application of SBSE with LD for phthalate determination was proposed by L. Brossa et al..[80] The authors studied the extraction procedure for DEP, DNBP, DEHP BBP, DnOP determination by GC-MS. The extraction procedure was carried out with 10 ml of aqueous sample (containing the analytes), 20 g l^{1} of NaCl and 10% methanol. The stir bar was immersed in the vial containing the aqueous sample for 30 min at 50ºC and 1200 rpm. Then, the stir bar was removed and dried. The analytes were desorbed by placing the stir bar in a vial containing 0.5 ml of isooctane in the stirrer unit (1000 rpm) for 30 min at room temperature. The limits of detection obtained were between 0.02-5 $\mu g l^{-1}$.

2.5. Solid/Liquid Extraction

The analysis of PAEs in solid samples is more complex than in liquid samples due to the difficulty to extract the compounds from the solid matrix. The technique commonly used for PAEs extraction from solid samples is the Soxhlet extraction. Different authors have been reported in the literature about PAEs extraction from solid samples using this technique.[81,82,83,84] Different solvents have been used such as ethyl acetate, for the PAEs extraction from packaging films[84], n-hexane or acetone/n-hexane from soil[85,] methanol from plant matter[85], n-hexane/methyl ethyl or cetone/methanol from sludge[86], dichlorometane from dump[87] and sludges[88], acetone/hexane (1:1) from soil and biosolids[83] and hexane/dichloromethane from soil[89]. The methods reported in the literature using this technique present a good recovery, but, the main problem from Soxhlet extraction is the time needed (as higher as 10 h) for total extraction.

A modification of the Soxhlet extraction is the system used by Sablayrolles et al.[90]. These authors used a Soxtec System HT2 (Tecator, France) for PAEs extraction from sludge and vegetables. This is a semi-automated apparatus working on the Soxhlet principle, while allowing extractions which are faster, more economical (better solvent recuperation) and safer (dissociation of the extraction and heating units). The solvent used in this case for the extraction procedure was hexane.

Another alternative for the solid/liquid extraction is the ultrasonic extraction. This technique has been applied to PAEs extraction in different matrices such as suspended matter, soil and liver samples using different solvents such as acetone, acetone-petroleum ether (1:1 v/v), methanol and a mixture of acetonitrile phosphoric acid and sodium chloride.[44, 91, 92,

93, 94] The advantage of this technique compared with Soxhlet extraction is the time required. In this case, the extraction can be performed in times less than 1 h (usually 10-15 min). However, for some PAEs in soils this method is less effective than Soxhlet extraction, obtaining bad recoveries.[92]

Microwave assisted extraction (MAE) has been applied to the extraction of PAEs from solid samples such as atmospheric particle matter or sediments. MAE consists of heating the sample with the extracting solvent inside the extraction vessel with microwave energy. This technique presents advantages compared with the traditional techniques, such as being less time consuming, using lower volume of organic solvent, offering automated temperature control and capability of processing different samples in the same time. In this way, E. Cortazar et al.[95] developed a method for DEHP determination in sediment samples using this technique. In this case, the authors used 100% methanol as extracting solvent and the extraction procedure was carried out at 159 kPa for 15 min. L. Bartolomé et al.[96] developed a method for PAEs extraction from sediments using acetone as an extracting solvent using a pressure of 145 kPa during 15 min. O. Alvarez-Avilés et al.[97] determined DEP and BBP in atmospheric particulate matter using MAE-SBSE-TD-GC-MS. The optimized conditions for MAE were 20 ml of acetone at 80ºC for 10 min.

3. SEPARATION TECHNIQUES

Gas chromatography and liquid chromatography are the techniques usually used for PAEs separation in different matrices, such as environmental samples or biological samples.

3.1. Gas Chromatography

The analysis of phathalic acid esters (PAEs) is mostly performed by gas chromatography (GC). Generally, GC methods present better sensibility than HPLC methods, although this depends on the pre-treatment step, the instrumental conditions and the sample matrix [6]. Phthalates can be detected using electron capture detection (ECD)[60, 70], flame ionization detection (FID)[36 ,65, 87, 98, 99] or mass spectrometry (MS). Some official methods (US EPA methods 606 and 8060) describe the use of ECD as a detector for phthalate determination. Although ECD detectors are relatively sensitive for phthalates, the specificity is restricted. The most recommended detector for phthalate analysis is mass spectrometry detection. All types of MS analyzers, including quadrupole analysers, triple quadrupole analyzers, ion traps and magnetic sector instruments have been used for phthalates determination.[100, 101, 102, 103, 104]

The chromatographic separation was usually performed using capillary columns coated with phenyl methylpolisiloxane or dimethylpolysiloxane as stationary phase. The separations were carried out using different temperature programmes, usually varying the oven temperature from 50ºC to 300ºC. Some applications for PAEs determination using GC coupled with different detectors are shown in Table 3.

Table 3. GC methods for PAEs determination

Sample	Analyte	Analytical column	Oven temperature programme	Detector	Limit of detection	Ref
Blood, milk and urine	DBP.	Fused silica column phenyl methyl siloxane (30 m x 0.25 mm, 0.25 µm)	No linear gradient from 80ºC to 280ºC.	MS: m/z: 149		16
Water	DMP, DEP, DAP, DnBP, BBP, DCHP, DEHP	DB-5MS-fused silica column (30 m x 0.25 mm, 0.25 µm)	No linear gradient from 60ºC to 285ºC.	MS: m/z: 163/194, 149/177, 149/189, 149/223 149/206, 149/167, 149/279	0.02-0.05 µg/l	25
Food simulantes	DMP, DEP; DiPP, DBP, DiBP, DEHP, DOP,	DB-5 fused silica column (30 m x 0.32 mm, 0.25 µm)	No linear gradient from 60ºC to 285ºC.	FID	0.02-0.40 µg/l	27
Water	DMP, DEP, DBP, BBP, DEHP, DOP	HP-5MS capillary column (30 m x 0.25 mm, 0.25 µm)	No linear gradient from 60ºC to 300ºC.	MS. m/z: 163/194, 149/177, 149/223, 149/206/91, 167/149/279, 149/279	0.003-0.01 µg/l	28
Wine	DMP, DEP, DBP, BBP, iBP, DEHP	Restek RTX-5MS capillary column (5% diphenyl 95% dimethypolysiloxane) (30 m x 0.25 mm, 0.25 µm)	No linear gradient from 70ºC to 280ºC.	MS. m/z: 163 for DMP, 149 for the other PAEs	0.015-0.018 µg/ml	32
River water	MMP, MEP, MiPRP, MPRP, MIBP, MBP, MPEP, MCHP, MEHP, MBZP, MOP	HP-5MS capillary column (30 m x 0.25 mm, 0.25 µm)	No linear gradient from 50ºC to 300ºC.	MS. m/z: 163/167 for quantification of monoesters. 149 for quantification of phthalic acid diesters excep DMP, in which m7z 163 was used.	0.010-0.030µg/l	33
Egypcians traditional foods and driks	DMP, DEP, DBP, BBP, DEHP	HP-5MS capillary column (5% diphenyl 95% dimethypolysiloxane) (80 m x 0.25 mm, 0.25 µm)	No linear gradient from 60ºC to 230ºC	MS. m/z: 163.770, 149.177, 149.150, 149.910, 149.167, 129.112	40-100 ng/l	34
Wastewater	DBP, DEHP	DB-17-HT (30 m x 0.25 mm, 0.1 µm)	40ºC increasing to 350ºC at 8ºC/min.	MS		35
Water	DMP, DEP, DBP, DEHP	PTE™-5 (30 m x 0.25 mm, 0.25 µm)	No linear gradient from 180ºC to 280ºC.	FID	28.5, 27.2, 42.7, 60.6 ng/l	36
Water	DMP, DEP, DnBP, BBP, DEHP, BnOP,	HP-5MS (cross-linked 5% methyl silicone) (30 m x 0.25 mm, 0.25 µm)	60ºC increased to 280ºC at 20ºC/min; hold for 5 min.	MS. m/z: 149 for all phthalate esters except for DMP (163)	2-27 ng/l	59

Table 3. (Continued)

Sample	Analyte	Analytical column	Oven temperature programme	Detector	Limit of detection	Ref
Water	DMP, DEP, DnBP, BBP, DEHP, DnOP	DB-35 (35% phenyl 65% polymethylsiloxane) (30 m x 0.25 mm, 0.15 µm)	No linear gradient from 45ºC to 270ºC. Total run time 50 min.	ECD	LOQ: 0.003-0.05 µg/l	60
Water	DMP, DEP, DBP, BBP, DEHP, DOP, DNP	HP-5MS (5% phenylmethylsiloxane) (30 m x 0.20 mm, 0.25 µm)	60ºC (5 min), increased to 280ºC at 15ºC/min; hold for 5 min.	MS. m/z: 163/194, 149/177, 149/223, 149/206/91, 167/149/279, 149/279, 149/167.	0.005-0.04 µg/l	62
Cow milk	DMP, DEP, DBP, BBP, DEHP,DOP	DB-5 (30 m x 0.25 mm, 0.1 µm)	No linear gradient from 55ºC to 280ºC.	MS. m/z: 163/77/194, 149/177/104, 149/223/104, 149/91/206, 149/167/279, 149/279/104	0.01-4.7 ng/g	63
Water	DEP, BBP, BBP, DEHP, DOP	HP-5 capillary column (30 m x 0.32 mm, 0.25 µm)	No linear gradient from 70ºC to 280ºC.	MS. m/z:149.177, 149.104, 149.91, 149.167, 149.279	0.07-3.15 µg/l	66
Vegetable oil	DMP, DEP, DnBP, BBP, DEHP, DnOP	DB-35 (35% phenyl 65% polymethylsiloxane) (30 m x 0.25 mm, 0.15 µm)	No linear gradient from 45ºC to 270ºC. Total run time 50 min.	ECD	LOQ: 0.2-0.5 mg/kg	70
Water	DMP, DEP, DBP, BBP, DEHP, DOP	HP-5MS (30 m x 0.25 mm, 0.25 µm) (5% diphenyl, 95% dimethylpolysiloxane)	No linear gradient from 50ºC to 290ºC during 39.33 min	MS. m/z: 77/163, 149/177, 104/149, 91/ 149, 149/167, 149/ 279	0.1-10 ng/l	76
Water, biosolid and sludge	DEP, DBP, BBP, DEHP	DB-5MS-fused silica column (30 m x 0.25 mm, 0.25 µm)	No linear gradient from 50ºC to 300ºC during 40 min	MS. m/z: 149/177, 149/223, 149/206, 149/279.	2.0 ng/l and 0.02 ng/g	77
Water	BBP, MOP	HP-5MS (30 m x 0.25 mm, 0.25 µm) (5% diphenyl, 95% dimethylpolysiloxane)	No linear gradient from 50ºC to 280ºC during 40 min	MS. m/z: 149 and 91, 149 and 279	<25 ng/l	78
Drinking water	DMP, DEP, DBP, BBP, DEHP, BOP	TRB-5MS (30 m x 0.25 mm, 0.25 µm) (5% diphenyl, 95% dimethylpolysiloxane)	No linear gradient from 70ºC to 280ºC during 24.50 min	MS. m/z: 163/77, 149/177, 149/150, 149/91, 129/112, 149/279	0.15-0.6 µg/l	79
Water	BBP, DOP	HP-5MS capillary column (30 m x 0.25 mm, 0.25 µm)	No linear gradient from 60ºC to 290ºC. Total run time 30.64 min.	MS.	0.02 µg/l	80

Table 3. (Continued)

Sample	Analyte	Analytical column	Oven temperature programme	Detector	Limit of detection	Ref
Soils and biosolids	DMP, DEP, DnBP, DuBeP, DEHP, DnOP	DB-5 MS-fused silica column (30 m x 0.25 mm, 0.25 µm)	No linear gradient from 50ºC to 280ºC during 13.73 min	MS. m/z: 163 for DMPand DnOP, and 149 for other PAEs.	0.1-5.0 µg/l	83
Soil	DMP, DEP, DBP, DEHP,	Col-elite5 (39 m x 0.25 mm, 0.25 µm)	No linear gradient from 180ºC to 280ºC during 13.73 min	FID		87
Sewage sludge	DEHP	TRB-Meta X5 Tracer, Technokroma, (30 m x 0.25 mm, 0.25 µm)	220ºC 12 min hold, 20ºC/min to 300ºC, 7 min hold.	MS: m/z: 149/167		88
Sludge end vegetables	DMP, DEP, DBP, BBP, DEHP, DOP	Restek RTX-5MS capillary column (5% diphenyl, 95% dimethylpolysiloxane) (30 m x 0.25 mm, 0.25 µm)	Started at 50ºC for 1 min, followed by an increase of 20ºC/min to 310ºC which is manteined for 6 min.	MS.	0.003 µg/ml	90
Soil	DMP, DEP, DiBP, DBP, BMEP, BMPP, DAP, DHP, BBP, HEHP, BBEP	DB-5 fused silica column (30 m x 0.25 mm, 0.25 µm)	No linear gradient from 50ºC to 280ºC.	MS. m/z: 163/149 for DMP and 149 for other PAEs.	0.001-0.022µg/g	92
Sediments	DMP, DEP, DBP, BBP, DEHP, DOP	HP-5 capillary column (30 m x 0.25 mm, 0.25 µm)	No linear gradient from 800ºC to 280ºC.	MS. m/z:163/77, 149/177, 149/223, 149/91, 149/167, 149/279	<25 ng/kg	95
Sediments	DMP, DEP, DBP, BBP, DEHP, DOP	HP-5 fused silica column (30 m x 0.25 mm, 0.25 µm)	No linear gradient from 60ºC to 290ºC.	MS. m/z: 77/163, 149/177, 104/149, 91/149, 149/167, 149/279	0.5-22 ng	96
Atmospheric particulate matter	DEP, BBP	DB-5 fused silica column (30 m x 0.32 mm, 0.25 µm)	No linear gradient from 40ºC to 280ºC.	MS	90, 20 ng/l	97
Food pakaging films	DMP, DEHP, DMTP, DPP, DNP, DEP, DnBP, BBP	Supelco column MDN-5 (95% polydimethylsiloxane, 5% polydiphenylsiloxane) (30 m x 0.25 mm, 0.25 µm)	No linear gradient from 50ºC to 310ºC.	FID	13.88-87.63mg/l	98
Water	DMP, DEP, DPP, DIBP, DBP, DEHP	HP-5 capillary column (30 m x 0.32 mm, 0.25 µm)	No linear gradient from 100ºC to 270ºC.	FID	0.2-4 µg/l	99

Table 3. (Continued)

Sample	Analyte	Analytical column	Oven temperature programme	Detector	Limit of detection	Ref
Olive oil	DMP, DEP, DDBP, BBP, DEHP, DOP	Varian Factor Four 5-ms (95% polydimethylsiloxane, 5% polydiphenylsiloxane) capillary column (30 m x 0.25 mm, 0.25 µm)	No linear gradient from 100ºC to 250ºC.	MS-MS. m/z: 149/135, 149, 121/81, 121/65, 121/65, 121/65	4.6-168 µg/kg	101
Migration from toys and childcare articles	DINP, DIDP, DEHP	DB-17HT (50% dimethyl-50% diphenyl polysiloxane) (30 m x 0.25 mm, 0.15 µm)	60ºC for 3 min, ramped at 10ºC/min to 290ºC, and finally held for 10 min.	MS. m/z: 149	0.1 µg/ml	102
Medical polyvinyl chloride tubing	DEHP	DB-1 capillary column (30 m x 0.25 mm, 0.1 µm)	From 50ºC to 300ºC at 20ºC/min, with an initial isotherm of 3 min and final isotherm of 10 min. Total run time 22.5 min.	MS.		103
Watewater	DMP, DEP, DBP, BBP, BEHP, DOP	ZB-5 MS Zebron (30 m x 0.32 mm, 0.25 µm)	No linear gradient from 120ºC to 260ºC.	MS. m/z: 194/163/133, 177/149, 223/205/149, 205/149, 279/149, 279/149	6-90 ng/l	104

Table 4. HPLC-UV methods for PAEs determination

Sample	Analyte	Analytical column	Mobile phase	Detector	Limit of detection	Ref
Nail cosmetics	DMP, DEP, DPP, DiBP, DEHP	Zorbax Eclipse XDB C18 (150 mmx 4.6 mm i.d. 3.5 µm particle diameter)	Linear gradient elution with ethanol-water starting from 50 to 95% ethanol in 30 min.	UV. Wavelength: 254 nm.	0.4-0.6 µg ml^{-1}	8
Parenteral nutrition and plasma	DEHP	Waters Spherisorb C18 column (150 mmx 4.6 mm i.d. 5 µm particle diameter)	Mixture acetonitrile –aqueous buffer (0.08% triethanolamine adjusted to pH 2.8 with 1 M phosphoric acid) mixture (88:12, v/v) at flow-rate of 1.0 mL/min.	UV. Wavelength: 202 nm.	LOQ= 20 ng/ml	13
Intravenous parenteral emulsions containing fat	DEHP	Waters Spherisorb C18 column (150 mmx 4.6 mm i.d. 5 µm particle diameter)	Mixture acetonitrile –aqueous buffer (0.08% triethanolamine adjusted to pH 2.8 with 1 M phosp horic acid) mixture (88:12, v/v) at flow-rate of 1.0 mL/min.	UV. Wavelength: 202 nm.		14
Blood of haemodialyzed patients	DEHP	Waters Spherisorb C18 column (150 mmx 4.6 mm i.d. 5 µm particle diameter)	Mixture acetonitrile –aqueous buffer (0.08% triethanolamine adjusted to pH 2.8 with 1 M phosphoric acid) mixture (88:12, v/v) at flow-rate of 1.0 mL/min.	UV. Wavelength: 202 nm.		15
Landfill leachates	DMP. DEP, DnBP	Venusil XBC18 column (250mmx4.6mm, i.d.:5µm).	Mixture methanol water (80:20 v/v). Flow rate 1.0 ml min^{-1}. Temperature 25ºC	UV. Wavelength: 280 nm	0.0012, 0.0014, 0.0022 mgl^{-1}	24
Water	DMP, DEP, DnBP	Zorbax SB C8 (150 mmx 4.6 mm i.d. 5 µm particle diameter)	Mixture methanol-water (75:25 v/v)at flow rate of 0.8 mL min^{-1}. Temperature: 25ºC.	UV. Wavelength: 280 nm	2.0, 1.0 5.0 $ngmL^{-1}$	29
Waters	DBP, DCHP, DOP DNP, DDP	Zorbax Eclipse XDB C8 (150 mmx 4.6 mm i.d. 5 µm particle diameter)	Mixture acetonitrile/water (97:3)	UV. Wavelength: 226nm.		49
River an sea water	DEP, DnPP, DiPP DcHP	Agilent Zorbax Eclipse XDB-C8 column (150 mmx 4.6 mm i.d. 5 µm particle diameter)	Mixture acetonitrile/water (67:33) at flow rate of 1 ml/min.	UV. Wavelength: 226nm		52
Wastewater	DBP, DEHP	Inertsil ODS 2 column (4.6 mm i.d. x 250 mm, 5 µm particle size) and Speriorex ODS (1.0 mm i.d. x 150 mm, 5 µm particle size)	Mixture methanol/water. Flow rate: 0.5 and 50µl min^{-1} for colums of 4.6 and 1.0 mm i.d. respectively	UV. Wavelength: 254nm.	Limit of quantification: = 0.1 and 0.5 ng/mL	53

Table 4. (Continued)

Sample	Analyte	Analytical column	Mobile phase	Detector	Limit of detection	Ref
River water	BBP, DBP, DCP	MIGHTYSIL RP-18 GP 150 (150 mmx 4.6 mm i.d.)	Mixture ethanol/water (67:33) at flow rate of 0.7 ml/min.	UV. Wavelength: 254nm		54
Water samples	DEP, DnPP, DnBP, DcHP, DEHP	Diamonsil-C18 (250 mmx 4.6 mm i.d. 4 µm particle diameter)	Mixture acetonitrile/water (75:25) at flow rate of 1 ml/min.	UV. Wavelength: 226nm	0.12-0.17 µg/l	55
Aqueous samples	DEP	ChromCart colum (250 x 3.0 mm i.d.) packedwith Nucleosil C18-50 dp 5 µm. Thermostatted at 30ºC.	Mixture acetonitrile/water (52.5:47.5) at flow rate of 0.5 ml/min.	UV. Wavelength: 254nm.	Limit of quantification: 5 ng/ml	69
Aqueous samples	DEP, DPP, BBP, DBP, DAP, DCHP, DHP, DEHP, DOP	Hypersil ODS (150 mmx 4 .6 mm i.d. 5 µm particle diameter)	Linear gradient elution with acetonitrilel-water starting from 65 to 75% at 1.5 mL/min for 5 min run, from 75% to 95% at 1.5-2.0 mL/min for a 5 min run, and held 95% at 2.0 mL/min for 2 min.	UV. Wavelength: 225 nm.		73
Environmental water samples	DBP, DEHP	A Genesis C18 (5 cmx4.6 mm i.d. 4 µm particle diameter)	Mixture acetonitrile-water in gradient elution mode at flow rate of 1 mL min -1. The elution program was initiated with 40% of water and it was maintained constant for 1 min. At 5 min the content of acetonitrile was 100%, it was maintained constant until the end of the chromatogram.	UV. Wavelength: 230 nm	1 and 2.5 µg L^{-1}	74
Liver samples	MEHP, DEHP	Altima C18 column (150 mmx 4.6 mm i.d. 5 µm particle diameter)	A gradient elution range of 60% to 100% acetonitrile with a gradient time of 5 min at a flow rate of 1 mL min^{-1}, then increased to 2mL min for 3 min while keeping the final solvent composition at 100 % acetonitrile. pH 3 with 25 mM $NaH_2PO_4.H_2O$ buffer.	UV. Wavelength: 235 nm.	0.57, 1.37 µg mL^{-1}.	93

Table 4. (Continued)

Sample	Analyte	Analytical column	Mobile phase	Detector	Limit of detection	Ref
Standard solutions	DPP, DIBP, DMP, DEP, DBP, BBP, DCHP, DEHP, DNOP, DAP, DHP	Acquity UPLC BEH phenyl column (Waters) (50mmx2.1mm 1.7µm particle size thermostatized at 45ºC. Agilent SB-phenyl column (250mmx4.6mm, 5µm particle size thermostatized at 25ºC.	The mobile phase was a no linear gradient prepared from methanol (component A) and water (component B). From 50% to 100% of A.	UV. Wavelength: 225 nm		105
Physiological saline, distilled water for injection and glucose solution	DEHP	Shodex C18-5A (150 mmx 4.6 mm i.d.)	Mixture acetonitrile/methanol /water (60:100:25)	UV. Wavelength: 225nm.		109

Table 5. HPLC-MS or MS-MS methods for PAEs determination

Sample	Analyte	Analytical column	Mobile phase	Detector	Limit of detection	Ref
Urine	DEHP, MEHP, DOP, DBP, BBzP, DEP	Luna Phenyl-Hexil column (3µm, 150 mm x 4.6 mm i.d.)	Non linear solvent gradient. Mobil phase A: 10 % acetonitrile containing 1.0 % (v/v) acetic acid. Mobil phase B: 90% acetonitrile containing 1.0 % (v/v) acetic acid. Flow rate 1 ml/min. Mobil phase C: 100 % acetonitrile	ESI-MS-MS	0.25-1.0 µg/l	1
Urine	DEHP metabolites	Inertsil ODS-3 (2.1 x 50 mm i.d., particle size 5 µm)	Non linear solvent gradient. Mobil phase A: 0.1 % (v/v) acetic acid aqueous solution. Mobil phase B: acetonitrile.	ESI-MS-MS.	0.7-1.1 ng/ml	3
Urine	MBP, MBzP, MEHP	Inertsil ODS-3 (2.1 x 50 mm i.d., particle size 5 µm)	Non linear solvent gradient. Mobil phase A: 0.1 % (v/v) acetic acid aqueous solution. Mobil phase B: acetonitrile.	ESI-MS-MS. Precursor ion ? product ion: m/z, 221? 71 for MBP, 255? 183 for MBzP and 277? 134 for MEHP.	1.3, 1.7, 0.7 ng/ml	9
Milk	MMP, MEP, MBP, MBzP MEHP, NNP	Betasil phenyl column (3µm, 100 mm x 2 mm)	Non linear solvent gradient from 100 % mobile phase A (0.1% acetic acid in water) to 100 % mobile phase B (0.1% acetic acid in acetonitrile)	ESI-MS-MS. ESI in negative ion mode.	0.01-0.5 µg/l	10
Milk and milk products	DBP, BBP, DEHP, DiNP, DiDP	Luna C5 100A column (5 µm, 50 mm x 2.0 mm i.d.)	Isocratic mode. Mobile phase: 2.0 % v/v water in methanol/ acetonitrile (1+1)	ESI-MS-MS.	9, 4, 6, 5, 5 µg/kg	11

Table 5. (Continued)

Sample	Analyte	Analytical column	Mobile phase	Detector	Limit of detection	Ref
Sewage sludge	DEP, DBP, DEHP	Lichrospher 100 RP-18 (250 x 4 mm and 5 µm particle size)	Non linear solvent gradient. Mobil phase A: 50% methanol/50% ecetonitrile. Mobil phase B: water. Both with 0.5 % of acetic acid.	APCI-MS. ESI in positive ion mode. m/z values: 149.	15, 25, 50 ng/g	37
Urban wastewater	DMP, DEP, DBP, DEHP, DOP	Gemini C18 (150 mm x 4.6 mm i.d., 5 µm particle size)	Non linear solvent gradient. Mobil phase A: 1.0 % (v/v) acetic acid aqueous solution. Mobil phase B: acetonitrile.	APCI (or) ESI-MS. m/z values: 149 for all phthalates except DMP, 199, 163 for DMP	LOQ (ng/L): 57, 12, 25, 19, 41, 26.	38
Drugs	DEHP, MEHP	Mightysil® RP-18 GP column (5 mm x 2.1 mm, 25 µm particle size)	Acetonitrile/water (90/10 v/v) at a flow rate of 0.2 ml/min	ESI-MS-MS. Precursor ion ? product ion: m/z, 391? 149 for DEHP, 277? 134 for MEHP		40
Human milk	13 Phthalate metabolites	Betasil Phenyl column (3µm, 100 mm x 2.1 mm)	Non linear solvent gradient. Mobil phase A: water Mobil phase B: acetonitrile. Both with 0.1 % of acetic acid.	ESI-MS-MS.	0.2-1.9 ng/ml	41
Urine	16/22 phthalate metabolites	Betasil phenyl column (3µm, 150 mm x 2.1 mm)	Non linear solvent gradient. Mobil phase A: water containing 0.1 % (v/v) acetic acid. Mobil phase B: acetonitrile containing 0.1 % (v/v) acetic acid	ESI-MS-MS	0.16-4-30 ng/ml	46 31
Urine	MMP, MEP, MBP, MCHP, MEHP, MOP,MiNP, MiDP, MEOHP	Betasil phenyl column (3µm, 100 mm x 2 mm)	Non linear solvent gradient from 100 % mobile phase A (0.1% acetic acid in water) to 100 % mobile phase B (0.1% acetic acid in acetonitrile)	ESI-MS-MS. ESI in negative ion mode. Multiple reaction monitoring mode	0.23-1.59 ng/ml	47
Industriale fluents	DBP, DMP, DEHP	Hypersil Green ENV (5µm, 150 mm x 5mm)	Non linear solvent gradient. Mobil phase A: water containing 0.5 % (v/v) acetic acid. Mobil phase B: acetonitrile containing 0.5 % (v/v) acetic acid. Flow rate 1 ml/min	APCI-MS. m/z values: 149.	0.06-0.08 µg/l	48

Table 5. (Continued)

Sample	Analyte	Analytical column	Mobile phase	Detector	Limit of detection	Ref
Raw and treated sewage samples	DEP, DEHP, BBP, DBP	Hypersil C18 (150 mmx 4.6 mm i.d. 5 µm particle diameter)	Mixture methanol/water in gradient mode: (1) 40-90% of methanol in 25 min. (2)10 min with 100% methanol	APCI-MS. m/z values: 313 for BBP, 279 for DBP and 391 for DEHP.	0.07 µg L-1 for BBP, 0.01 µg L-1 for DBP and DEHP.	57
Sludges and sediment samples	DBP, DEHP	Supelcosil LC-8 column of 25 cm x 1.0 mmi.d., 5 µm particle size.	Methanol (0.1 % acetic acid) at 0.1 ml/min	ESI-MS. Target ions: 279 (+), 391 (+) for DBP and DEHP respectively.		61
Water and sediment samples	DMP, DEP, BBP, DBP, DEHP, DOP	Kromasil 100 C18 (5µm, 25 mm x 0.46 mm)	Non linear solvent gradient. Mobil phase A: water Mobil phase B: acetonitrile.	APCI(positive mode)-MS. m/z values: 163 for DMP, 177 for DEP, 313 for BBP, 279 and 205 for DBP and 391 and 371 for DEHP and DOP	0.05-1 µg/l	106
Urine	MEP, MBP, MCHP, MBzP, MEHP, MOP, MNP, MDP	Betasil phenyl column (5µm, 50 mm x 3 mm)	Linear gradient from 100% buffer A (6 mM aqueous ammonium acetate pH 6.5) to 100% buffer B (90% acetonitrile in 6 mM aqueous ammonium acetate, pH 6.5). Flow rate 1.2 ml/min	APCI-MS-MS	0.5-2 ng/ml	108
Urine	mMP, mEP, mBP, mCHP, mBzP, mEHP, mOP, mNP, mDP, mEOHP, mEHHP	Betasil phenyl column (5µm, 50 mm x 2mm)	Non linear solvent gradient. Mobil phase A: water containing 0.1 % (v/v) acetic acid. Mobil phase B: acetonitrile containing 0.1 % (v/v) acetic acid. Flow rate 0.6 ml/min	APCI-MS-MS	0.7-1.6 ng/ml	111
Human serum	MEHP and DEHP	Wakosil3C18, 2.0 x 100 mm, 3 µm)	Acetonitrile/water (15/85 v/v) at 0.2 ml/min.	ESI-MS-MS. Precursor ion ? product ion: m/z, 277? 134 for MEHP, 391? 149 for DEHP.	5 and 14 ng/ml.	112
Urine	Five metabolites of DEHP	Betasil phenyl-hexyl column (3µm, 150 mm x 4.6mm)	Non linear solvent gradient. Mobil phase water: Water with 1% of acetic acid and acetonitrile.	ESI-MS-MS		113

Table 5. (Continued)

Sample	Analyte	Analytical column	Mobile phase	Detector	Limit of detection	Ref
Urine	MBP, MCHP, MBeP, MEHP, MIDP	Primesphere $5C_{18}$HC (250 x 3.2 mm)	Non linear solvent gradient. Mobil phase A: 0.05 M ammonium acetate containing 0.1 % (v/v) acetic acid. Mobil phase B: methanol containing 0.1 % (v/v) acetic acid	APCI(negative mode)-MS.	10-40 ng/ml	118

3.2. Liquid Chromatography

High-performance liquid chromatography (HPLC) can be used as an alternative technique and is especially useful for analysis of isomeric mixtures and metabolites of phthalates without derivatisation.[47] Ultraviolet detection has been used for phthalate determination in environmental and biological samples [8, 42, 49, 52, 55, 56, 61, 69, 93, 105,73], but the use of mass spectrometry has increased in recent years, operating with single spectrometer[35, 37, 38, 48, 57, 106, 107, 108, 109, 110] or using mass spectrometer in tandem[3, 9, 110, 40, 41, 46, 47, 111, 112] with applications in different matrix samples (sludge, urban wastewater, urine, milk and drugs). Although GC-MS offered higher sensitivity for phthalate determination than LC-MS, LC-MS approach offered some advantages compared with GC-MS, such as, higher selectivity, more reliable quantification of PAEs isomeric mixtures, simpler cleanup procedures and shorter analysis time.[100]

Separation of phthalates using liquid chromatography is usually performed in reverse phase using C18 or C8 columns, but some applications appear in the literature using other columns such as phenyl columns.[10, 105, 113] Tables 4 and 5 summarized different methods for PAEs determination in different matrices using HPLC as a separation technique coupled with different detectors. The separation conditions, including type of column, mobile phase, flow rate etc., as well as the detectors used and the limits of detection obtained, are also listed in the tables.

3.3. Capillary Electrophoresis

Capillary electrophoresis (CE) is a separation technique that provides several advantages such as speed, high efficiency and high sensitivity. Although, this technique is not usually used for PAEs determination, in recent years some authors have developed methods for PAEs determination in sediments and urine using this technique. Bao-Yuan Guo et al.[89] developed a method for DMP, DEP, DBP, DEHP and DOP from sediments using micellar electrokinetic chromatography (MEKC). The limits of detection obtained were within in a range of 0.050-0.063 mg/kg. The phthalates contents determined by MEKC were comparables to those obtained by CG.FID. In 2008, Yong-Lai Feng et al.[114] developed a method for MMP, MEP, MBP, MEHP and MEHHP in urine samples, using capillary zone

electrophoresis (CZE) coupled with mass spectrometry. The limits of detection obtained were within a range of 0.53-1.3 ngl^{-1}.

4. CONTAMINATION PROBLEMS

The major problem in phthalate analysis is contamination, resulting in false positive results or over-estimated concentrations. The risk of contamination is present in the whole analytical scheme, including sampling, sample preparation and chromatography analysis. Due to the fact that phthalates are widely used, they are present in the air, water, organic solvents, plastic and adsorbed onto glass or other materials.[115]

A recent study carried out by Ried et al.[116] shows significant quantities of phthalates from various components commonly found in the environment of the analytical laboratory. Consequently, plastic syringes, pipette tips, plastic filters and all type of plastic material must be avoided, and glass material must be used instead. Once plastic materials containing phthalates are avoided, the main sources of contamination are phthalates present as vapors or part of the particle matter in air, contaminating all surfaces, particularly glassware, plastic objects and our skin.[117]

Due to the fact that the sources of contamination can vary from one laboratory to another and depend on factors such as season, weather and ventilation of the laboratory[117], general recommendation for avoiding contamination are not possible. Franhauser-Noti et al.[117] studied the blank problems in trace analysis of DEHP and DBP by gas chromatography-mass spectrometry. These authors applied a test to identify the sources of system contamination in a systematic manner and described a list of measures to reduce phthalates contamination. The major improvement was obtaining by adding aluminium oxide into the solvents the reservoirs. Another critical factor is the quality of caps for the autosampler vials. These caps can also contain phthalates. As a general precaution, only one injection should be made from each vial.

Different cleaning methods have been proposed to avoid the contamination problems due to the phthalates from the material used in the laboratory. In all of them glass material is rinsed with organic solvents after a rigorous washing.[106, 109, 118, 119, 120,.121]

C. Pérez Feás et al.[110] cleaned the glassware material prior to analysis according to recommendations specified in EPA method 506 in order to reduce the background contamination. All the material was washed with hot water and soap and rinsed with technical-grade acetone. Then, the glassware was sealed with aluminum foil and stored in a clean environment to avoid adsorption of phthalates from air.

6. CONCLUSION

The interest for PAEs determination in different matrix samples (water, sediments, sludge, and biological samples) has increased in recent years due to the toxicity of these compounds.

The main problem in PAEs determination is the contamination of the sample, due to the presence of high levels of these compounds in the laboratory environment. A solution to this

problem would be the use of methods in which the sample pretreatment is performed out in a closed system or on-line with the detection technique. Both approaches minimize sample preparation.

Different methods have been developed, using a variety of preconcentration and extraction techniques, such as SPE, LLE, SPME, SLE, etc. For aqueous samples SPE and SPME were the most commonly used techniques obtaining good recoveries. For solid samples, Soxhlet extraction was the most usual technique but new methods using ultrasound or microwave energy are nowadays more popular because they shorted the extraction time.

CG and HPLC as separation techniques coupled with different detectors were the main techniques for PAEs determination employed in the literature. Results showed that methods using GC-MS or HPLC-MS-MS presented lower detection limits.

6. REFERENCES

[1] Koch, H. M., González-Reche, L. M. & Angerer, J. (2003). *J. Chromatogr.*, B, *784*, 169.

[2] Merodio, P. & Nogueira, J. M. F. (2006). *Water. Res.*, *40*, 2572.

[3] Kato, K., Yamauchi, T., Higashimaya, K. & Nakazawa, H. (2003). *J. Liq. Chromatogr. Relat. Technol.*, *26*, 2167.

[4] Jones-Lepp, T., Gerlach, C. L. & Cooter, E. J. (2000). *Trenes Anal. Chem.*, *19*, 286.

[5] Jobling, S., Raynolds, T., White, R., Parker, M. & Sumpter, J. P. A. (1995). Variety of environmentally persistent chemicals, including some phthalate plastizers, are weakly estrogenic. *Environ. Health Perspect.*, *103*, 582.

[6] Communication from the Comisión to the Council and the European Parliament on the implementation of the community strategy for endocrine disrupters-a range of substances suspected of interferinf with the hormona systems of humans and wildlife, COM (1999) 706; COM (2001) 262 final, Brussels, 2001.

[7] US Environmental Protection Agency (EPA), Protection Agency, *Introduction to Water Policy Standards*, Office of Water, Washinton, DC, 1999.

[8] De Orsi, D., Gagliardi, L., Porrá, R., Berri, S., Climenti, P., Granese, A., Carpani, I. & Tonelli, D. (2006). *Anal. Chim. Acta*, *555*, 238.

[9] Kato, K., Shoda, S., Takahashi, M., Doi, N., Yoshimura, Y. & Nakazawa, H. (2003). *J. Chromatogr.*, B, 788, 407.

[10] Mortensen, G. K., Main, K. M., Anderson, A. M., Leffers, H. & Skakkebaek, N. E. (2005). *Anal. Bioanal. Chem.*, *382*, 1084.

[11] Soresen, L. K. (2006). Rapad Commu. *Mass spectrom.*, *20*, 1135.

[12] Faouzi, M. A., Dine, T., Gressier, B., Kambia, K., Luyckx, M., Pagniez, D., Brunet, C., Cazin, M., Babeles, A. & Cazin. J. C. (1999). Inter. *J. Pharma.*, *180*, 113.

[13] Kambia, K., Dine, T., Gressier, B., Germe, A. F., Luyckx, M., Brunet, C., Michaud, L., Gottrand, F. (2001). *J. Chromatogr.*, B, *755*, 297.

[14] Kambia, K., Dine, T., Gressier, B., Bah, S., Germe, A. F., Luyckx, M., Brunet, C., Michaud, L. & Gottrand, F. (2003). *Inter. J. Pharma.*, *262*, 83.

[15] Kambia, K., Dine, T., Azar, R., Gressier, B., Luyckx, M. & Brunet, C. (2001). *Inter. J. Pharma.*, *229*, 139.
[16] Ji-an Chen, (2008). Huijie Liu, Zhiqun Qiu, Weiqun Shu, *Environ. Poll.*, *156*, 849.
[17] Orssi, D. D., Gagliardi, L., Porrà, R., Berri, S., Climenti, P., Granesse, A., Carpani, I. & Tonelli, D. (2006). *Analytica Chim. Acta*, *555*, 238.
[18] Hou, L. & Lee, H. K. (2004). *J. Chromatogr*., A, *1152*, 37.
[19] Liu, S. & Dasgupta, P. K. (1995). anal. *Chem*., 67, 2042.
[20] Cardoso, A. A. & Dasgupta, P. K. (1995). *Anal. Chem*., *67*, 2562.
[21] Jeannot, M. A. & Cantwell, F. F. (1996). *Anal. Chem*., *68*, 2236.
[22] Jeannot, M. A. & Cantwell, F. F. (1997). *Anal. Chem*., *69*, 235.
[23] Jeannot, M. A., Cantwell, F. F. (1997). *Anal. Chem*., *69*, 2935.
[24] Jirong Yao, (2008). Hui Xu, Lili Lv, Dandan Song, Yangfang Cui, Taozhi Zhan and Yu-Qi FENA, *Anal. Chim. Acta*, *616*, 42.
[25] Farahni, H. , Reza Ganjali, M., Dinarvand, R. & Norouzi, P. (2008). *Talanta*, *76*, 718.
[26] Khalili Zanjan, M. R., Yamini, Y., Shariati, S. & Jonson, J. A. (2007). *Anal. Chim. Acta*, *585*, 286.
[27] Batlle, R. & Nerín, C. (2004). *J. of Chromatogr*., A, *1045*, 29.
[28] Psillakis, E. & Kalogerakis, N. (2003). *J. Chromatogr*., A, *999*, 145.
[29] Pie Liang, Qian Li, Ping Xu, Dan Du, (2008). *Chromatographia*, *68*, 393.
[30] Holadová, H. & Hajslová, J. (1995). Int. J. Environ. *Anal. Chem*., *59*, 43.
[31] Silva, M. J., Preau, J. L., Jr., Needham, L. L., Calafat, A. M. (2008). *J. Chromatogr.,* B, *873*, 180.
[32] del Carlo, M., Pepe, A., Sacchetti, G., Compagnone, D. & Mastrocola, D. (2008). Cichelli, *A. Food Chemistry*, *111*, 771.
[33] Suzuki, T., Yaguchi, K. & Suzuki, S. (2001). *Environ. Sci. Technol*., *35*, 3757.
[34] Mohamed, M. A. & Ammar, A. S. (2008). *American J. of Food and Techn*., 1.
[35] Castillo, M. & Barceló, D. (2001). *Anal. Chim. Acta, 426*, 253-264.
[36] Fatoki, O. S., Noma, A. & Afr, S. (2001). *J. Chem*., *54*, 69.
[37] Petrovic, M. & Barceló, D. (2000). *Anal. Chem*., *72*, 4560.
[38] Zafra-Gómez, A., Ballesteros, O., Navalón, A. & Vilches, J. L. (2008). *J. Microchem*., *88*, 87.
[39] Ballesteros, O., Zafra, A., Navalón, A., Vílches, J. L. (2006). *J. Chromatogr. A*, *1121*, 154.
[40] Ito, R., Seshimo, F., Miura, N., Kawaguchi, M., Saito, K., Nakazawa, H. (2005). *J. Pharmaceutical and Biomedical Analysis, 39*, 1036.
[41] Calafat, A. M., Slakman, A. R., Silva, M. J., Herbert, A. R., Needham, L. L. (2004). *J. Chromatogr*., B, *805*, 49.
[42] Jara, S., Lysebo, C., Greibrokk, T. & Lundanes, E. (2000). *Anal. Chim. Acta*, *407*, 165.
[43] Davi, M. L., Liboni, M. & Malfatti, G. (1999). Int. *J.Environ. Anal. Chem*., *74*, 155.
[44] Suzuki, T., Yaguchi, K. & Suzuki, S. (2001). *Sci. Technol*., *35*, 3757.
[45] Jonsson, S. & Borén, H. (2002). *J. Chromatogr*., A, *963*, 393.
[46] Kato, K., Silva, M. J., Needham, L. L. & Calafat, A. M. (2005). *J. Chromatogr*., B, *814*, 355.
[47] Silva, M. J., Slakman, A. R., Reidy, J. A., Preau, J. L. Jr., Herbert, A. R., Samandar, E., Needham, L. L. & Calafat, A. M. (2004). *J. Chromatogr. B*, *805*, 161.

[48] Castillo, M., Alpendurada, M. F. & Barceló, D. (1997). *J. Mass Spectrometry*, *32*, 1110.
[49] Ya-Qi Cai, (2003). Guibin Jiang and Jinfu Liu *Anal. Scien*., *19*, 1491.
[50] Luna, S. C., Yanagisawa, Y., Ford, T. E. & Spengler, J. D. (2000). *Chmosphere*, *41*, 1857.
[51] Doong, R., Chang, S. & SPNG, Y. (2000). *J. Chromatogr. Sci*., *38*, 528.
[52] Ya-Qi Cai, Gui-Bin Jiang, Jing-Fu Liu, Qing-Xiang Zhou, (2003). *Anal*. *Chim. Acta*, *494*, 149.
[53] Yoshihiro Saito, Yuji Nakao, (2002). Motohiro Imaizumi, Yoriko Morishima, Yoshiaki Kiso, Kiyokatsu Jinno, Anal. *Bioanal. Chem*., *373*, 81.
[54] katsumata, H., Begum, A., Kaneco, S., Suzuki, T. & Ohta, K. (2004). *Anal. Chim. Acta*, *502*, 167.
[55] Jindon Li, Yaqui Cai, Yali Shi, Shifen Mou, (2008). *Guibin Jian Talanta*, *74*, 498.
[56] Sayito, T., Matsushima, S. & Hiraide, M. (2004). *J. Chromtogr. A*, *1040*, 185.
[57] López-Jimenez, F. J., Rubio, S. & Pérez-Bendito, D. (2005). *Anal. Chim. Acta*, *551* 142.
[58] Pawliszyn, J. (1999). Applications of solid phase microextraction, *RSC Chromatography monographs.* Royal Society of Chemistry, Letchworth, UK (ISBN 0-85404-525-2).
[59] Peñalver, A., Pocurull, E., Borrull, F. & Marcé, R. M. (2000). *J. Chromatogr. A*, *872*, 191.
[60] Prokupková, G., Holandová, K., Poustka, J. & Hajslová, J. (2002). *Anal. Chim. Acta 457*, 211.
[61] Möder, M., Popp, P. & Pawliszyn, J. (1998). *J. Microcolumn Separations*, *10(2)*, 225.
[62] Luks-Betlej, K., Popp, P., Janozska, B. & Paschke, H. (2001). *J. Chromatogr.*, A *938*, 93.
[63] Feng, Y. L., Zhu, J. & Sensenstein, R. (2005). *Anal. Chim. Acta*, *538*, 41.
[64] Silva, R. C., Meurer, E. C., Eberling, M. N. & Augusto, F. (2005). The Analist, *130*, 188.
[65] Xiang, Li, Ming Zhong, Shifen Xu, Cheng Sun, (2006). *J. Chromatogr. A*, *1135*, 101.
[66] Cortazar, E., Zuloaga, O., Sanz, J., Raposo, J. C., Etxebarria, N. & Fernández, L. A. (2002). *J. Chromatogr. A*, *978*, 165.
[67] Peñalver, A., Pocurull, E., Borrull, F. & Marcé, R. M. (2001). *J. Chromatogr.*, A922, 377.
[68] Polo, M., Llompart, M., García-Jares, C., Cela, R. (2005). *J. Chromatogr.*, *1072*, 63.
[69] Kelly, M. T. & Larroque, M. (1999). *J. Chromatogr.*, A, *841*, 177.
[70] Holadová, K., Prokupková, G., Hajslová, J. (2007). *J. Poustka, Anal. Chim. Acta*., *528*, 24.
[71] Haberhauer, C., Crnoja, M., Rosenberg, E. & Grasserbauer, M. (2000). *Fresenius J. Anal. Chem*., *366*, 329.
[72] de Fatima, M. (2000). *J. Chromatogr.*, A 889, 3.
[73] Mitani, K., Izushi, F. & Kataoka, H. (2004). *J. Anal. Toxicology*, *28*, 575.
[74] Cháfer-Pericás, C., Campís-Falcó, P., Prieto-Blanco, M. C. (2008). *Anal. Chim. Acta*, *610*, 268.
[75] Baltussen, E., Sandra, P., David, F. & Cramers, C. (1999). *J. Microcol*., Sep, *11*, 737.

[76] Prieto, A., Telleira, O., Etxebarria, N., Fernández, L. A., Usobiaga, A. & Zuloaga, O. (2008). *J. Chromatogr.* A 1214, 1.
[77] Tan, B. L. L., Hawker, D. W., Müller, J. F., Tremblay, L. A., Chapman., H. F. (2008). *Water Research*, *42*, 404.
[78] Serodio, P., Nogueira, J. M. F. (2004). *Anal. Chim.*, Acta, *517*, 21.
[79] Seròdio, P. & Nogueira, J. M. (2006). *Water Research*, *40*, 2572.
[80] Brossa, L., Marcé, R. M., Borull, F. & Pocurull, E. (2005). *Chromatographia*, *61.*
[81] Aranda, J. M., O´Connor, G. A., Eicement, G. A. (1989). *J. Environ. Qual*., *18*, 45.
[82] Strachan, S. D., Nelson, D. W., Sommers, L. E. (1983). *J. Environ.*, Qual, *12*, 69.
[83] Gibson, R., Min-Jian Wang, E. & Padgett, A. (2005). *J. Beck, Chemosphere*, *61*, 1336.
[84] Bonini, M., Errani, E., Zerbitani, G., Ferri, E. & Girotti, S. (2008). *Michrochem J.*, *90*, 31.
[85] Aranda, J. M., O´Connor, G. A. & Eiceman, G. A. (1989). *J. Environ. Qual*., *18*, 45.
[86] Strachan, S. D., Nelson, D. W., Sommers, L. E. (1983). *J. Environ. Qual, 12*, 69.
[87] Adeniyi, A., Dayomi, M., Siebe, P. & Okedeyi, O. (2008). *Chem. Central J.*, *2*, 1.
[88] Bagó, B., Martín, Y., Mejía, G., Broto-Puig, F., Díaz Ferrero, J. & Agut, L. (2005). *Comellas, Chemosphere*, *59*, 1191.
[89] Bao-Yuan Guo, Bei Wen, Xiao-Quan Shan, Shu-Zheng Zhang, Jin-Ming Lin, (2005). *J. Chromatogr. A*, *1095*, 189.
[90] Sablayrolles, C., Montréjaud-Vignoles, M., Benanou, D., Patria, L. & Treihou, M. (2005). *J. Chromatogr.*, *1072*, 233.
[91] Kirchmann, H., Astrom, H. & Jonsall, G. (1991). *Swedish J. Agric. Res.*, *21*, 107.
[92] Ling Ling, Der. Muir, Xue Tong Wang, Xiao Bai Xu, (2005). Int. *J. Environ. Anal. Chem.*, *85(2)*, 89.
[93] Debatir Sicar, Sargon J. Albazi, Yousef Atallah and William Pizza, (2008). *J. Chromatogr. Sci.*, *46*, 627.
[94] Errekatxo, A., prieto, A., Zuloaga, O., Usobiaga, A., Etxebarria, N. & Fernández, L. A. (2008). *Anal. Bioanal. Chem.*, *392*, 1471.
[95] Cortazar, E., Bartolomé, L., Delgado, A., Etxebarria, N., Fernández, L. A., Usobiaga, A. & Zuloaga, O. (2005). *Anal. Chim. Acta*, 534, 247.
[96] Bartolomé, L., Cortazar, E., Raposo, J. C., Usobiaga, A., Zuloaga, O., Etxebarría, N., Fernández, L. A. (2005). *J. Chromatogr.* A, *1068*, 229.
[97] Alvarez-Avilés, O., Cuadra-Rodríguez, L., González-Illán, F., Quiñones-González, J., Rosario, O. (2007). *Anal. Chim. Acta*, *597*, 273-281.
[98] Bonini, M., Errari, E., Zerbinati, G., Ferri, E. & Girotti, S. (2008). *Microchem. J.*, *90*, 31.
[99] Kotowska, U., Garbowska, K., Isidorov, V. A. (2006). *Anal. Chim. Acta*, *560*, 110.
[100] David, F., Sandra, P., Tienpont, B., Vanwalleghem, F. (2003). In: C. A., Staples, (Eds.), The Handbook of Environmental Chemistry. Part Q. Phthalate Esters, vol. 3, *Springer, Berlin*, 15.
[101] Cavaliere, B., Macchione, B., Sindona, G. & Tagarelli, A. (2008). *J. Chromatogr.*, A, *1205*, 137.
[102] Earls, A. O., Axford, I. P. & Braybrook, J. H. (2003). *J. Chromatogr.*, A 983, 237.
[103] Inoue, K., Okumura, H., Tae Higuchi, H., Oka, Y. & Yoshimura, H. (2002). Nakazawa, *Clin. Chim. Acta*, *325*, 157.

[104] Ballesteros, O., Zafra, A., Navalón, A. & Vílchez, J. L. (2006). *J. Chromatogr.*, *1121*, 154.
[105] Tin Wu, Chao Wang, Xing Wang, Haiqing Xiao, Qiang Ma, Qing Zhang, 2008, *Chromatographia*, *68*, 803.
[106] Gimeno, R. A., Marcé, R. M. & Borrull, F. (2003). *Chromatographia*, *58*, 37.
[107] Brossa, L., Marcé, R. M., Borrull, F. & Pocurull, E. (2005). *Environ. Toxicol. Chem*. *24*, 261.
[108] Blount, B. C., Milgram, K. E., Silva, M. J., Malek, N. A., Reidy, J. A., Needham, L. L. & Broca, J. W. (2000). *Anal. Chem.*, *72*, 4127.
[109] Hanawa, T., Muramatsu, E., Asakama, K., Tanaka, M., Kawano, K., Seki, T., Juni, K. & Nakajima, S. (2000). *Int. J. Pharm*., *210*, 109.
[110] Pérez Feás, C., Barciela Alonso, M. C., Peña-Vázquez, E., Herbello Hermelo, P., Bermejo Barrera, P. (2008). *Talanta*, *75*, 1184.
[111] Silva, M. J., Malek, N. A., Hodge, C. C., Reidy, J. A., Kato, K., Barr, D. B., Needham, L. L., Broca, J. W. (2003). *J. Chromatogr*., B, *789*, 393.
[112] Takatori, S., Kitagawa, Y., Kitagawa, M., Nakazawa, H., Hori, S. (2004). *J. Chromatogr*., B, *804*, 397.
[113] Preus, R., Koch, H. M. & Angerer, J. (2005). *J. Chromatogr*. , B, *816*, 269.
[114] Yon-Lai Feng, Jiping Zhu, (2008). *Electrophoresis*, *29*, 1965.
[115] David, F., Sandra, P., Tienpont, B. & Vanwalleghem, F. (2003). In: C. A., Staples, (Eds.), *The Handbook of Environmental Chemistry. Part Q Phthalate Esters*, *vol 3*, *Springer, Berlin*, 15.
[116] Reid, A. M., Brougham, C. A., Fogarty, A. M. & Roche, J. J. (2007). Int. *J. Environ. Anal. Chem*., *87*, 125.
[117] Fankhauser-Noti, A. & Grob, K. (2007). *Anal. Chim. Acta*, *582*, 353.
[118] Anderson, W. A. C., Barnes, K. A., Castle, L., Damant, A. P. & Scotter, M. J. (2002). *Analyst*, *127*, 1193.
[119] Jonson, S., Ejlertsson, J., Ledin, A., Mersiowsky, I. & Svensson, B. H. (2003). *Water Res*., *37*, 609.
[120] Faozi, M. A., Khalfi, F., Dine, T., Luyckx, M., Brunet, C., Gressier, B., Groudaliez, F., Cazin, M., Belabed, A. & Cazin, J. C. (1999). *J. Pharm. Biomed. Anal*., *21*, 923.
[121] Cai, Y. Q., Jiang, G. B., Liu, J. F. (2003). q.X. *Zhou, Anal. Chim*., Acta, *494*, 149.

Available online at www.sciencedirect.com
ScienceDirect
Talanta 75 (2008) 1184–1189
Talanta
www.elsevier.com/locate/talanta

Phthalates determination in physiological saline solutions by HPLC–ES-MS

C. Pérez Feás, M.C. Barciela Alonso, E. Peña-Vázquez, P. Herbello Hermelo, P. Bermejo-Barrera *

Department of Analytical Chemistry, Nutrition and Bromatology, University of Santiago de Compostela, Santiago de Compostela 15782, Spain

Received 14 September 2007; received in revised form 2 January 2008; accepted 9 January 2008
Available online 19 January 2008

Abstract

Phthalates are a group of chemical compounds with increasing interest from the analytical point of view. The risks for human health associated with some of these compounds have unleashed the necessity to develop analytical methods with great sensitivity that allow us to detect their presence at trace levels in order to assure protection for the population.

A simple and rapid method for determining a group of phthalate esters in aqueous samples was developed. The method was based on high-performance liquid chromatography–(electrospray)-mass spectrometry (HPLC–ES-MS), working in positive ionisation (PI) mode. A gradient elution was performed with acetonitrile–ultrapure water starting from 5 to 75% acetonitrile in 5 min followed by isocratic elution during 5 min. Standard calibration curves were linear for all the analytes over the concentration range 10–500 ng mL^{-1} .The LOD values found for DMP, DEP, BBP and DBP were 0.8, 3.4, 0.6 and 1.2 ng mL^{-1} respectively. The relative standard deviation ranged from 0.8 to 1.7%, which indicated good method precision.

The proposed analytical method has been applied to the analysis of commercial physiological saline solutions in order to check the presence of phthalates and to determine their concentration.

Keywords: Phthalates; HPLC–ES-MS; Physiological saline solutions

1. Introduction

Diesters of phthalic acid, commonly referred to as phthalates, are a group of chemical compounds widely used in industry and commerce due to their large variety of uses. Because of their properties to improve softness and flexibility to the plastics they are used mainly as plasticizers to give products to consumer and industry versatiles, durables and accessibles such as medical devices, children's toys and all kind of packaging. Furthermore, phthalates are also used as industrial solvents and lubricants, as additives in textile industry and pesticides and also in personal care products such as deodorants, lotions and perfumes, to retain the colour and fragrance [1–4].

Approximately 93% of all plasticizers are phthalates, the remaining 7% corresponding to esters and polyesters based on adipate, phosphoric acid, sebacic acid, etc. [1]. The world production of these compounds is estimated at several million tonnes per year. Phthalates are not chemically bound in the plastics; therefore, they can be lost from plastic and released to the environment [5].

Consistent toxicological evidence indicates association between several of these phthalate esters and risks for human health and the environment. In particular, dibutyl phthalate (DBP), butyl benzyl phthalate (BBP), and di-(2-ethylhexyl) phthalate (DEHP) are in the list of the proposed substances suspected to produce endocrine alterations published by European Union (EU) [6].

Section 307 of the US Clean Water Act establishes that dimethyl phthalate (DMP), diethyl phthalate (DEP), butyl benzyl phthalate, dibutyl phthalate, di-(2-ethylhexyl) phthalate and dioctyl phthalate (DOP) must be considered priority toxic pollutants [7]. These concerns have been further aggravated by recent analysis of human blood and urine samples, where traces of various phthalates (or their metabolites) have been found [8,9]. For

* Corresponding author.
E-mail address: pbermejo@usc.es (P. Bermejo-Barrera).

0039-9140/$ – see front matter © 2008 Published by Elsevier B.V.
doi:10.1016/j.talanta.2008.01.019

these reasons, the interest in the study of this type of chemical substances has increased during the last few years, and therefore it is essential to develop a reliable and sensible analytical method that allows us to determine and quantify this group of compounds at trace levels.

Several methods have been developed for their determination in different matrices, including water (drinking water, surface water, wastewater), soil, sediment, sludge, dust, air and biota (vegetation, milk, fish, etc.) [2,10–12].

The analysis of phthalic acid esters is mostly performed by gas chromatography (GC) [13–17]. Generally, GC methods present better sensibility than HPLC methods, although depend on the pre-treatment step, the instrumental conditions and the sample matrix [6]. High-performance liquid chromatography (HPLC) can be used as an alternative technique and is especially useful for analysis of isomeric mixtures and metabolites of phthalates without derivatisation [18].

Phthalates can be detected using UV detection [8,19–21], flame ionisation detection (FID) [22,23], electron capture detection (ECD) [24] or mass spectrometry (MS) [10,11,25,26]. Some official methods (US EPA methods 606 and 8060) describe the use of ECD for the phthalate determination. Although ECD detectors are relative sensitive for phthalates, the specificity is restricted. The most important detector for phthalate analysis is mass spectrometric detection. All types of MS analysers, including quadrupole analysers, triple quadrupole analysers, ion traps and magnetic sector instruments have been used for phthalates determination [27].

The major problem in phthalate analysis is the contamination, resulting in false positive results or over-estimated concentrations. The risk of contamination is present in the whole analytical scheme, including sampling, sample preparation and chromatographic analysis. Due to the fact that phthalates are widely used, they are present in air, water, and organic solvents and plastic and adsorbed on glass or other materials [27].

A recent study carried out by Reid et al. [28] shows significant quantities of phthalates from various components commonly found in the environmental of analytical laboratory. Consequently, plastic syringes, pipette tips, plastic filters and all type of plastic material must be avoided, and glass material must be used instead. Once plastic materials containing phthalates are avoided, the main source of contamination are phthalates present as vapours or part of the particulate matter in air, contaminating all surfaces, particularly glassware, plastic objects and our skin [29].

As a result of the contribution of all these sources of contamination, the experiments to reduce its produce confusing results because, the sources of contamination vary from one laboratory to another and depend on factors such as season, weather and ventilation of the laboratory [29].

Different cleaning methods have been proposed to avoid the contamination problems due to the phthalates from the material used in the laboratory. In all of them glass material is rinsed with organic solvents after a rigorous washing [11,30–35].

The aim of this work is to develop a method for phthalates determination presents in trace levels in physiological saline solutions, using HPLC–ES-MS.

2. Experimental

2.1. Reagents and standards

All reagents used were of analytical reagent-grade. Dimethyl phthalate and butyl benzyl phthalate were obtained from Supelco (Bellefonte, PA, USA). Diethyl phthalate and dibutyl phthtalate were obtained from Riedel-de Haën (Seelze, Germany). The purity of these reagents was over 98%.

Lichrosolv gradient grade acetonitrile and methanol were purchased from Merck (Darmstadt, Germany). Technical-grade acetone and acetic acid glacial (HPLC) for instrumental analysis were purchased from Panreac (Barcelona, Spain). Ultrapure (resi-analyzed) water for environmental inorganic and organic trace analysis was supplied by J.T. Baker (Phillipsburg, NJ, USA).

Individual standard solutions of each phthalate ester at a concentration of 1000 mg L^{-1} were prepared in methanol, preserved of light and stored at 4 °C in a Teflon-capped glass vial. From these solutions, a working mixture in methanol was prepared weekly containing all standards of concentration 100 mg L^{-1} each. All the working solutions were prepared daily by diluting this solution.

Special care was taken to avoid the contact of reagents and solvents with plastic materials. In order to reduce background contamination, all glassware was cleaned prior to the analysis according to the recommendations specified in EPA method 506. All material was washed with hot water and soap, rinsed with tap and ultrapure water and finally thorough rinsed with technical-grade acetone. Then, glassware was sealed with aluminium foil and stored in a clean environment to avoid adsorption of phthalates from the air.

2.2. Instrumentation

Phthalates separation and quantification was carried out using liquid chromatography/electrospray ionisation-mass spectrometry system.

The HPLC system used was an 1100 Series equipped with an automatic injector (Agilent Technologies, Waldbronn, Germany) that is coupled to an API 150 EX single quadrupole mass spectrometer equipped with a Turboionspray interface (PE Biosystems, Concord, Canada).

The analytical column was a ZORBAX Eclipse XDB-C_8 of 50 mm length and 2.1 mm internal diameter (particle size 3.5 μm) supplied by Agilent Technologies.

2.3. Chromatographic and mass spectrometry conditions

The binary mobile phase consisted of ultrapure water and acetonitrile, both solvents containing 0.1% (v/v) acetic acid. The elution gradient started with 95% of ultrapure water, which was reduced linearly to 25% in 5 min. Then, this composition was maintained for 5 min before returning to the initial conditions. The column was equilibrated for 10 min.

The flow rate and the injection volume were 200 μL min^{-1} and 10 μL, respectively and the chromato-

Table 1
Optimal values of the compound parameters for the four phthalates studied

Compound	Acronym	*m/z*	Potentials		
			DP	FP	EP
Dimethyl phthalate	DMP	163.25	40.38	73.87	8
Butyl benzyl phthalate	BBP	91.15	25	225	6
Diethyl phthalate, dibutyl phthalate	DEP, DBP	149.05	25	290	8.5

graphic separation was carried out at room temperature. Under these conditions the separation time was less than 10 min.

Electrospray ionisation was performed in positive ion mode. The operational parameters were the same for all of analytes with an ionspray voltage of 5500 V; nitrogen was used as nebulizer and curtain gas at a pressure of 14 psi in both cases; air current at 450 °C and 7000 cc min^{-1} was used as turbo heater gas.

The compound parameters such as declustering potential (DP), focusing potential (FP) and entrance potential (EP) were optimized for each analyte. The optimal conditions are shown in the Table 1.

2.4. Sample preparation

Samples were injected directly in the chromatograph, it wasn't necessary any sample preparation process.

3. Results and discussion

3.1. ES-MS optimization

Four phthalate esters (DMP, DEP, BBP and DBP) were selected for this study.

To evaluate the mass spectral fragmentation pattern of each compound and to optimize the set of parameters used, a standard solution (100 mg L^{-1}) of each compound was analyzed by direct injection in the spectrometer. For these experiments, a KD Scientific, model 100, syringe pump (New Hope, MN, USA) at 15 μL min^{-1}, was used.

Full-scan data acquisition was performed from 80 to 400 *m/z*, with the target mass fixed to the following *m/z* values: 91.15 for BBP, 149.05 for DEP and DBP and 163.25 for DMP. The spectral data provided ions in accordance with previous studies reported in literature [2,15,16,36,37]. The selected ions were chosen to attain the best response in the SIM mode acquisition. Characteristics as molecular weight, identification ions and retention time corresponding to these compounds are given in Table 2.

3.2. Optimization of HPLC separation

After optimizing the detection conditions, the following experiments were conducted to optimize the chromatographic separation of the analytes.

Experiments were carried out using different mobile phases reported in the literature (methanol:water [38], acetonitrile:water [20], acetonitrile (1%methanol):water [21]), working in isocratic mode. The best resolution was obtained using acetonitrile:water as a mobile phase. These results agree with the experiments developed by López-Jiménez et al. [10]. In order to improve the resolution and to decrease the time of analysis, different experiments were carried out working in gradient mode. The best results were obtained started with 95% of ultrapure water and decreasing this percentage to 25% in 5 min. Then, this composition was maintained for 5 min before returning to the initial conditions. Finally, the column was equilibrated during 10 min before each injection. Other parameters optimized were the percentage of acetic acid and the flow rate of the mobile phase. The optimal conditions were 0.1% (v/v) acetic acid and a flow rate of 200 μL min^{-1}.

The chromatogram obtained for a mixed of these compounds under the optimized conditions is shown in the Fig. 1.

3.3. Analytical performance

To evaluate the linearity of the method, a direct calibration curve was realized. Ten microliters of standard solutions in ultrapure water with concentrations ranging from 10 to 500 ng mL^{-1} were injected by triplicate. Detector signals, measured in arbitrary units (peak areas), were plotted versus the amount of analyte injected, expressed in ng mL^{-1} and background levels were subtracted from de results. The equations obtained for each compound were as follows:

$$\text{DMP}: \quad Q_A = 46399\,C + 377217 \quad r = 0.9964$$

$$\text{DEP}: \quad Q_A = 1784\,C + 16643 \quad r = 0.9987$$

Table 2
Molecular weight, selected ions and retention time to the analysis of the target phthalates

Phthalate	Molecular weight	SIM ion	Identification ions	RT (min)
Dimethyl phthalate	194	163	149, 163, 181	6.90
Diethyl phthalate	222.24	149	149, 177, 195	7.59
Butyl benzyl phthalate	312.40	91	91, 149, 205, 223, 247	9.18
Dibutyl phthalate	278.35	149	149, 205, 223	9.44

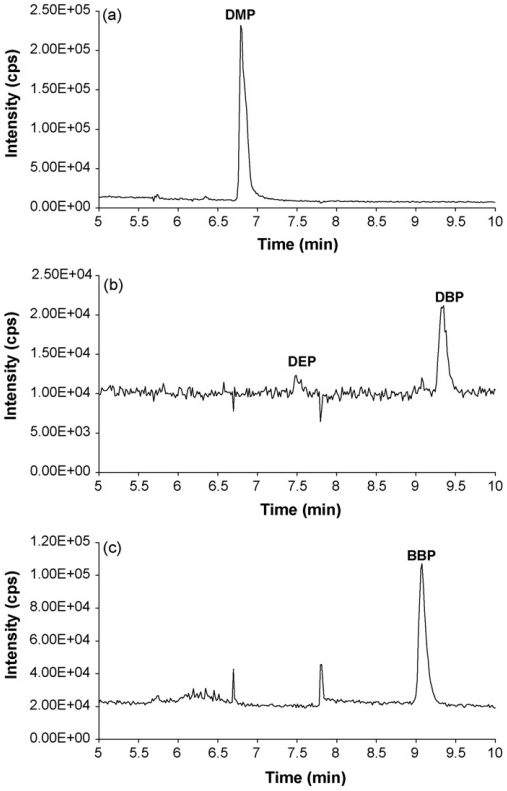


Fig. 1. LC/MS extracted ion chromatogram obtained from a standards solution (100 ng mL^{-1}) in a physiological saline sample purchased in a glass bottle to the following *m*/*z* values: (a) 163.25 for DMP, (b) 149.05 for DEP and DBP, and (c) 91.15 for BBP.

$$\text{BBP}: \quad Q_A = 18218\,C + 153056 \quad r = 0.9978$$

$$\text{DBP}: \quad Q_A = 5166\,C + 66474 \quad r = 0.9963$$

where Q_A is the peak area and C is the concentration in ng mL^{-1}.

Standard addition method was applied over the same range of concentrations using a commercial physiological saline solution purchased in a glass bottle. The equations obtained for each compound were as follows:

$$\text{DMP}: \quad Q_A = 12334\,C + 12308 \quad r = 0.9998$$

$$\text{DEP}: \quad Q_A = 114\,C + 1686 \quad r = 0.9979$$

$$\text{BBP}: \quad Q_A = 5319\,C - 20441 \quad r = 0.9988$$

$$\text{DBP}: \quad Q_A = 639\,C - 8128 \quad r = 0.9985$$

Table 3
Linear range, correlation coefficients, LODs and LOQs values obtained from the standard addition method in physiological saline solutions

Phthalate	Linear range (ng mL^{-1})	Correlation coefficient (r)	LODs (ng mL^{-1})	LOQs (ng mL^{-1})
DMP	10–500	0.9998	0.99	3.29
DEP	10–500	0.9979	22.13	73.78
BBP	10–500	0.9988	5.32	17.73
DBP	10–500	0.9985	24.07	80.23

To compare slopes of the calibration and addition graphs for the four compounds, the *t*-test (95% significance levels) [39] was applied and differences were observed for all compounds. This means that the sample matrix had influence in the sensitivity of the method, so, standard addition graphs had been used to analyze these samples.

The limit of detection (LOD) and limit of quantification (LOQ) for the method were calculated according with the equations:

$$\text{LOD} = \frac{3\text{S.D.}}{m}; \quad \text{LOQ} = \frac{10\text{S.D.}}{m}$$

where S.D. is the standard deviation of 11 measurements of a blank and m is the slope of the addition graph. The commercial physiological saline solution purchased in a glass bottle was used as a blank. The results obtained for LODs and LOQs are shown in the Table 3. As can be seen in the Table 3, LODs are between 0.99 and 24.07 ng mL^{-1} for all compounds, and the highest levels obtained were for DEP and DBP.

To check the precision an interday assay was developed. A physiological saline solution sample purchased in a glass bottle and spiked with three concentration levels (50, 100 and 300 ng mL^{-1}) were analyzed during different days (six determinations per concentration each day) for all compounds studied. The results obtained are shown in the Table 4. The R.S.D. values were between 1.9 and 10.9% so, the method is precise for all studied compounds.

The recovery of the method was evaluated by injection of the physiological saline solution purchased in a glass bottle spiked with three different concentrations of these compounds. The solutions were injected by triplicate and the recovery calculated using the standard addition graph. The results obtained are shown in Table 5. The average recoveries were 101.5%, 94.7%, 108.3% and 101.4% for DMP, DEP, BBP and DBP, respectively.

Table 4
Relative standard deviation (%) obtained for three concentration levels (based on six determinations) in interday assay

Phthalate	R.S.D. (%)		
	50 ng mL^{-1}	100 ng mL^{-1}	300 ng mL^{-1}
DMP	10.9	3.5	3.6
DEP	8.0	4.5	4.1
BBP	5.6	6.2	2.2
DBP	5.1	7.5	1.9

Table 5
Recovery percentage for physiological saline solutions ± standard deviation

Phthalate	% Recovery		
	50 ng mL^{-1}	100 ng mL^{-1}	300 ng mL^{-1}
DMP	103.7 ± 1.4	105.4 ± 1.0	95.0 ± 1.2
DEP	89.7 ± 3.3	99.5 ± 3.5	95.1 ± 3.1
BBP	111.3 ± 9.5	104.2 ± 2.0	109.5 ± 1.5
DBP	105.8 ± 2.7	89.8 ± 2.1	108.7 ± 3.5

Table 6
Concentrations (ng mL^{-1}) ± standard deviation (based on three replicates) found in different physiological saline solutions

Physiological saline solutions	DMP	DEP	BBP	DBP
Brand A	5 ± 1	335 ± 5	<LOD	50 ± 2
Brand B	<LOD	<LOD	<LOD	<LOD
Brand C	<LOD	<LOD	5 ± 1	<LOD
Brand D	153 ± 2	<LOD	<LOD	<LOD

<LOD: lower than the detection limit.

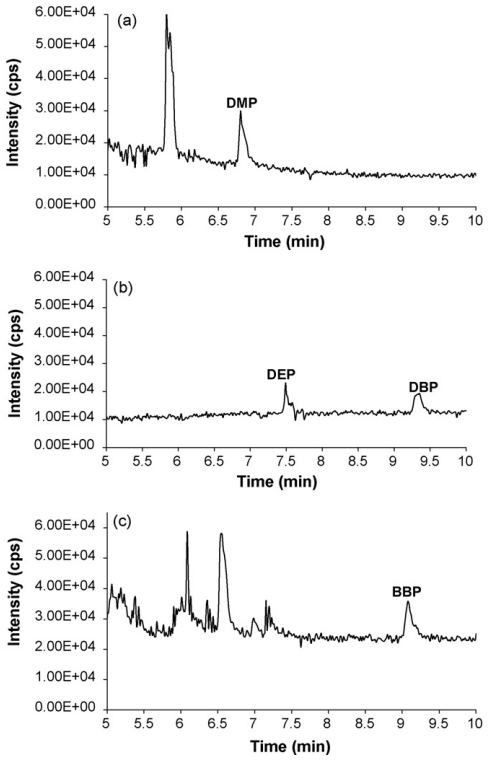


Fig. 2. LC/MS extracted ion chromatogram obtained from brand A physiological saline solution to the following *m*/*z* values: (a) 163.25 for DMP, (b) 149.05 for DEP and DBP, and (c) 91.15 for BBP.

3.4. Application to physiological saline solutions

The proposed analytical method has been applied to the analysis of four commercial physiological saline solutions in order to check the presence of these phthalates and to determine their concentration. Samples were injected directly in the chromatograph, it wasn't necessary any sample preparation process.

The original recipients containing three of these physiological saline solutions were made from plastic material. The other one was in a glass bottle. The phthalate esters are used in the manufacture of the plastic recipients, so the influence of the material on the concentration of the phthalates has been evaluated.

Physiological saline solutions were analyzed in order to verify the presence of different peaks at the same retention time as the compounds studied. Some peaks appeared at the retention times corresponding to DMP, DEP, BBP and DBP. The spectra of these peaks confirmed that they correspond to these four phthalates.

The results obtained for these phthalates in the four samples are given in the Table 6. The levels of these compounds in the brand B, was less than the LODs of the method. The absence of these compounds in this sample can be attributed to that this sample is distributed in a glass bottle. Fig. 2 shows the LC/MS ion chromatograms obtained from brand A of physiological saline solution.

4. Conclusions

A method for the determination of different phthalates by HPLC–ES-MS was developed. The method is rapid (the separation and determination was realized in less than 10 min), precise and accurate.

Four commercial physiological saline solutions from different brands were analyzed using the proposed method. The results shown, that these compounds are present only in the samples distributed in plastic bottles. In physiological saline solution distributed in glass bottle, these compounds were not detected (<LODs).

Acknowledgement

C. Pérez Feás wishes to thank Ministerio de Educacion y Ciencia for her FPU grant.

References

[1] H.M. Koch, L.M. Gonzalez-Reche, J. Angerer, J. Chromatogr. B 784 (2003) 169.
[2] P. Serodio, J.M.F. Nogueira, Water Res. 40 (2006) 2572.
[3] K. Kato, T. Yamauchi, K. Higashimaya, H. Nakazawa, J. Liq, Chromatogr. Relat. Technol. 26 (2003) 2167.
[4] T. Jones-Lepp, C.L. Gerlach, E.J. Cooter, Trends Anal. Chem. 19 (2000) 286.
[5] A. Gómez-Hens, M.P. Aguilar-Caballos, Trends Anal. Chem. 22 (2003) 847.
[6] Communication from the Commission to the Council and the European Parliament on the implementation of the community strategy for endocrine disrupters—a range of substances suspected of interfering with the hormone

systems of humans and wildlife, COM (1999) 706; COM (2001) 262 final, Brussels, 2001.
[7] US Environmental Protection Agency (EPA), Protection Agency, Introduction to Water Policy Standards, Office of Water, Washington, DC, 1999.
[8] D. De Orsi, L. Gagliardi, R. Porrá, S. Berri, P. Chimenti, A. Granese, I. Carpani, D. Tonelli, Anal. Chim. Acta 555 (2006) 238.
[9] K. Kato, S. Shoda, M. Takahashi, N. Doi, Y. Yoshimura, H. Nakazawa, J. Chromatogr. B 788 (2003) 407.
[10] F.J. López-Jiménez, S. Rubio, D. Pérez-Bendito, Anal. Chim. Acta 551 (2005) 142.
[11] R.A. Gimeno, R.M. Marcé, F. Borrull, Chromatographia 58 (2003) 37.
[12] G.K. Mortensen, K.M. Main, A.M. Andersson, H. Leffers, N.E. Skakkebaek, Anal. Bioanal. Chem. 382 (2005) 1084.
[13] A. Peñalver, E. Pocurull, F. Borrull, R.M. Marcé, J. Chromatogr. A 872 (2000) 191.
[14] L. Brossa, R.M. Marcé, F. Borrull, E. Pocurull, Chromatographia 61 (2005) 61.
[15] K. Luks-Betlej, P. Popp, B. Janozska, H. Paschke, J. Chromatogr. A 938 (2001) 93.
[16] M. Polo, M. Llompart, C. García-Jares, R. Cela, J. Chromatogr. A 1072 (2005) 63.
[17] L. Brossa, R.M. Marcé, F. Borrull, E. Pocurull, Environ. Toxicol. Chem. 24 (2005) 261.
[18] M.J. Silva, A.R. Slakman, J.A. Reidy, J.L. Preau Jr., A.R. Herbert, E. Samandar, L.L. Needham, A.M. Calafat, J. Chromatogr. B 805 (2004) 161.
[19] K. Mitani, F. Izushi, H. Kataoka, J. Anal. Toxicol. 28 (7) (2004) 575.
[20] N. Hata, E. Yuwatini, K. Ando, M. Yamada, I. Kasahara, S. Taguchi, Anal. Sci. 20 (2004) 149.
[21] S. Jara, C. Lysebo, T. Greibrokk, E. Lundanes, Anal. Chim. Acta 407 (2000) 165.
[22] O.S. Fatoki, A. Noma, J. Chem. 54 (2001) 69.
[23] U. Kotowska, K. Garbowska, V.A. Isidorov, Anal. Chim. Acta 560 (2006) 110.
[24] G. Prokupková, K. Holadová, J. Poustka, J. Hajslová, Anal. Chim. Acta 457 (2002) 211.
[25] M. Castillo, A. Oubiña, D. Barceló, Environ. Sci. Technol. 32 (1998) 2180.
[26] K. Kato, M.J. Silva, L.L. Needham, A.M. Calafat, Anal. Chem. 77 (2005) 2985.
[27] F. David, P. Sandra, B. Tienpont, F. Vanwalleghem, in: C.A. Staples (Ed.), The Handbook of Environmental Chemistry. Part Q. Phthalate Esters, vol. 3, Springer, Berlin, 2003, p. 15.
[28] A.M. Reid, C.A. Brougham, A.M. Fogarty, J.J. Roche, Int. J. Environ. Anal. Chem. 87 (2007) 125.
[29] A. Fankhauser-Noti, K. Grob, Anal. Chim. Acta 582 (2007) 353.
[30] W.A.C. Anderson, K.A. Barnes, L. Castle, A.P. Damant, M.J. Scotter, Analyst 127 (2002) 1193.
[31] S. Jonsson, J. Ejlertsson, A. Ledin, I. Mersiowsky, B.H. Svensson, Water Res. 37 (2003) 609.
[32] M.A. Faouzi, F. Khalfi, T. Dine, M. Luyckx, C. Brunet, B. Gressier, F. Goudaliez, M. Cazin, A. Belabed, J.C. Cazin, J. Pharm. Biomed. Anal. 21 (1999) 923.
[33] S. Takatori, Y. Kitagawa, M. Kitagawa, H. Nakazawa, S. Hori, J. Chromatogr. B 804 (2004) 397.
[34] T. Hanawa, E. Muramatsu, K. Asakama, M. Suzuki, M. Tanaka, K. Kawano, T. Seki, K. Juni, S. Nakajima, Int. J. Pharm. 210 (2000) 109.
[35] Y.Q. Cai, G.B. Jiang, J.F. Liu, Q.X. Zhou, Anal. Chim. Acta 494 (2003) 149.
[36] R. Kumar, A Mass Spectral Guide for Quick Identification of Phthalate Esters, American Laboratory, 1999, p. 32.
[37] E. Psillakis, N. Kalogerakis, J. Chromatogr. A 999 (2003) 145.
[38] Y. Saito, Y. Nakao, M. Imaizumi, Y. Morishima, Y. Kiso, K. Jinno, Anal. Bioanal. Chem. 373 (2002) 81.
[39] R. Cela (Ed.), Avances en Quimiometría práctica, Universidad de Santiago de Compostela,Santiago de Compostela, Spain, 1994, p. 171.

Journal of Chromatography B, 879 (2011) 231–235

Contents lists available at ScienceDirect

Journal of Chromatography B

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

Direct LC–ES-MS/MS determination of phthalates in physiological saline solutions

C. Pérez Feás, M.C. Barciela-Alonso, P. Bermejo-Barrera *

Department of Analytical Chemistry, Nutrition and Bromatology, University of Santiago de Compostela, Avd de las Ciencias, Santiago de Compostela 15782, Spain

ARTICLE INFO

Article history:
Received 30 September 2010
Accepted 1 December 2010
Available online 10 December 2010

Keywords:
Phthalates
LC–ES-MS/MS
Physiological saline solutions

ABSTRACT

A method for determining a group of phthalic esters (PAEs) in physiological saline solutions has been developed. The PAEs studied were dimethyl phthalate, diethyl phthalate, butyl benzyl phthalate and dibutyl phthalate. These groups of phthalates were determined by liquid chromatography–electrospray ionization-tandem mass spectrometry, working in positive ion mode. The compounds were separated by liquid chromatography working in gradient mode with acetonitrile–ultrapure water as a mobile phase. The separation was performed starting with 5% of acetonitrile and increasing to 75% in 5 min, followed by isocratic elution for 8 min. The method was precise (with relative standard deviation (RSD) from 1.0 to 6.8%) and sensitive, with LODs of 0.05, 0.38, 0.05 and 0.82 $\mu g\,L^{-1}$ for DMP, DEP, BBP and DBP, respectively. The proposed analytical method has been applied to determine these compounds in different physiological saline solutions commercialized in plastic bottles.

1. Introduction

Phthalates (PAEs) are a group of chemical compounds widely used in industry and commerce. Due to the ability to improve the softness and flexibility of plastics, they are widely used as polymer additives in plastics. These compounds are present in a wide variety of consumer products including children toys, cosmetics, personal care products, packaging, etc. [1–3]. Phthalates are not chemically bound to plastic; thus, they can be easily released from the plastic packaging to the contents and the environment [4].

The interest in the study of these types of chemical substances has increased in recent years because some of these compounds, such as dibutyl phthalate (DBP), butyl benzyl phthalate (BBP) and diethyl hexyl phthalate (DEHP), are suspected to be endocrine disruptors and carcinogenic to humans [5,6]. Therefore, it is essential to develop reliable and sensitive methods for determining this group of compounds at trace levels.

Several methods have been developed for PAEs determination in different matrices such as, biological samples, pharmaceutical drugs and environmental samples. The analysis of PAEs is mostly performed by gas chromatography (GC). Generally, GC methods present better sensitivity than HPLC methods, although these depend on the pre-treatment step, the instrumental conditions and the sample matrix [7]. Phthalates can be detected using electron capture detection (ECD) [8,9], flame ionization detection (FID) [10–12] and mass spectrometry (MS) [13–15]. HPLC can be used as an alternative technique and is especially useful for analysis of isomeric mixtures and phthalates metabolites without derivatization [16]. HPLC can be used in combination with different detectors such as UV [17–19], mass spectrometry [20–24] and using tandem mass spectrometry [16,25–28].

In some cases, due to the low levels of these compounds in the samples, a clean up/preconcentration step is necessary before the instrumental analysis. These sample pre-treatments include liquid–liquid extractions (LLE) [24,29,30], liquid-phase microextraction (LPME) [31], single drop microextraction (SDME) [32], solid phase extraction (SPE) [25,33], solid phase microextraction (SPME) [34,35], stir bar sorptive extraction (SBSE) [36,37] and solid–liquid extraction (SLE) [38]. The major problem in phthalate determination is the sample contamination during the sample pre-treatment. Due to the fact that these compounds are widely used, they are present in the environment and can be adsorbed onto the glass and other material. This problem can be diminished using different methods proposed in the literature to prevent phthalate contamination problems [20,21,27] and by reducing the number of steps necessary to prepare the sample.

The aim of this work was to develop a high sensitive method for phthalates determination in physiological saline solution samples by LC–ES-MS/MS without any sample pre-treatment.

2. Experimental

2.1. Reagents and standards

All reagents used were of analytical reagent-grade. Dimethyl phthalate (DMP) and butyl benzyl phthalate (BBP) were obtained from Supelco (Bellefonte, PA, USA). Diethyl phthalate (DEP)

* Corresponding author. Tel.: +34 881 814 266, +34 600 942 346; fax: +34 981 595 012.
E-mail address: pilar.bermejo@usc.es (P. Bermejo-Barrera).

1570-0232/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.jchromb.2010.12.002

and dibutyl phthalate (DBP) were obtained from Riedel-de Haën (Seelze, Germany). The purity of these reagents was over 98%.

Stock standard solutions of each phthalate ester at a concentration of 1000 mg L^{-1} were prepared in methanol, kept in darkness and stored at 4 °C in a Teflon-capped glass vial. From these solutions, a working standard solution in methanol was prepared weekly containing all standards at concentrations of 100 mg L^{-1} each. Diluted working standard solutions were prepared daily by diluting the working solution.

Lichrosolv gradient grade acetonitrile and methanol were purchased from Merck (Darmstadt, Germany). Technical-grade acetone and glacial acetic acid (HPLC) for instrumental analysis were purchased from Panreac (Barcelona, Spain). Ultrapure (resi-analysed) water for environmental inorganic and organic trace analysis was supplied by J.T. Baker (Phillipsburg, NJ, USA).

Special care was taken to avoid the contact of reagents and solvents with plastic materials. Because of the ubiquity of plasticizers and the tendency of residues to persist, all glassware was cleaned prior to the analysis according to the recommendations specified in U.S. EPA Method 506 [39]. All material was washed with hot water and soap, rinsed with tap and ultrapure water and finally thoroughly rinsed with technical-grade acetone. Glassware was then sealed with aluminium foil and stored in a clean environment to avoid adsorption of phthalates from the air.

2.2. Instrumentation

Phthalates separation and quantification was performed using liquid chromatography/electrospray ionization-tandem mass spectrometry system.

A Series 1100 liquid chromatograph from Agilent Technologies (Waldbronn, Germany) was coupled to an API 4000™ Triple Quadrupole Mass spectrometer (Applied Biosystems, Concord, Canada) equipped with a Turbo Ionspray™ ionization source. Mass Spectrometry data were processed with Analyst 1.4.2 software.

A ZORBAX Eclipse XDB-C_8 column (2.1 mm × 50 mm, 3.5 μm particle size) supplied by Agilent Technologies was used for the separation of these compounds.

2.3. Chromatographic conditions

Ultrapure water and acetonitrile (both solvents containing 0.1%, v/v acetic acid) were used as a binary mobile phase. Phthalates were separated by LC working in gradient mode with acetonitrile–ultrapure water as a mobile phase. The separation was performed starting with 5% of acetonitrile and increasing to 75% in 5 min, followed by isocratic elution for 8 min and increasing to 75% in 5 min, remaining at this composition for 8 min.

The flow rate and the injection volume were 200 μL min^{-1} and 10 μL, respectively, and the chromatographic separation was performed at 40 °C. Under these conditions the separation time was less than 13 min. These optimal conditions are shown in Table 1.

Table 1
Operational conditions for LC–MS/MS.

HPLC (Agilent 1100)	
Column	Zorbax Eclipse XDB-C8 (3.5 μm 2.1 mm × 50 mm)
Mobile phase	Ultrapure water:acetonitrile (0.1%, v/v acetic acid)
Mode	Gradient
Flow rate	200 μl/min
Oven temperature	40 °C
Injection volume	10 μl
MS/MS (API 4000)	
Ion spray voltage	5500 V
Ionization mode	ESI-positive
Curtain gas	25 psi (nitrogen)
GS1 (nebulizer gas)	50 psi
GS2 (auxiliary gas)	60 psi
Ion source temperature	450 °C
CAD (collisionally activated dissociation)	

2.4. Sample preparation

The samples were injected directly into the chromatograph, without any previous sample preparation process.

3. Results and discussion

3.1. ES-MS/MS conditions

The ES-MS/MS conditions for DMP, DEP, BBP and DBP determination by ES-MS/MS were studied. The ion source dependent (turbo ion spray) conditions were the same for all the analytes with an electrospray needle voltage of 5500 V in the positive ion mode. Nitrogen as a nebulizer and turbo heater gas (at 450 °C) was set as a pressure of 50 and 60 psi, respectively. The pressure of the curtain gas was also optimized selecting 25 psi as the optima pressure. Ion source collision-activated dissociation (CAD) was studied during the development of the method, selecting 4 V as the optimum condition.

To establish the MS/MS operating conditions used to determine these phthalates by ES-MS/MS, a standard solution (100 mg L^{-1}) of each phthalate were used. These solutions were infused directly into the MS/MS system using the syringe pump system of the API 4000. The phthalates studied were monitored at *m/z* 195, 223, 313 and 279, working in the scan mode, which were assigned to $[M+H]^+$. Moreover, in the product ion MS/MS measurement, the selective reaction monitoring ions (SRM) of DMP, DEP, BBP and DBP were set depending on their precursor ions. The combinations of precursor ion and product, as well the optimum potentials, are shown in Table 2.

3.2. Optimization of LC separation

After optimizing the detection conditions, the following experiments were conducted to optimize the chromatographic separation of the analytes.

Table 2
Optimal values of the compound parameters for the four phthalates studied, *m/z* transition selected and retention time (DP: declustering potential; EP: enhance potential; CE: collision energy; CXP: collision cell exit potential).

Compound	Acronym	*m/z* transition	Potentials optimization				t_R (min)
			DP	EP	CE	CXP	
Dimethyl phthalate	DMP	195 → 163	31	10	13	14	8.4
Diethyl phthalate	DEP	223 → 149	36	10	23	12	9.2
Butyl benzyl phthalate	BBP	313 → 91	41	10	23	6	11
Dibutyl phthalate	DBP	279 → 205	50	9	11	10	11.2

Table 3
Linear range, correlation coefficients, LODs and LOQs values obtained from the standard addition method in physiological saline solutions.

Phthalate	Linear range ($\mu g\,L^{-1}$)	Correlation coefficient (r)	LOD ($\mu g\,L^{-1}$)	LOQ ($\mu g\,L^{-1}$)
DMP	0.5–50	0.9996	0.05	0.16
DEP	1–50	0.9978	0.38	1.27
BBP	1–50	0.9986	0.05	0.16
DBP	1–150	0.9956	0.82	2.74

Experiments were performed using acetonitrile:water, both solvents containing 0.1% (v/v) acetic acid as a mobile phase. This mobile phase was selected based on a previous work developed in our research group for phthalates determination in physiological saline solutions by LC–ES-MS [20]. Experiments were developed using a physiological saline solution spiked with 25 $\mu g\,L^{-1}$ of DMP, DEP and BBP, and 100 $\mu g\,L^{-1}$ of DBP. The best results were obtained starting the elution with 5% of acetonitrile, which was then increased linearly to 75% in 5 min. This composition was maintained for 8 min before returning to initial conditions. The column was equilibrated for 10 min.

Other parameters optimized were the temperature of the chromatographic column and the flow rate of the mobile phase. The optimum conditions selected were at temperature of 40 °C and a flow rate of 200 $\mu L\,min^{-1}$.

The chromatogram obtained for the physiological saline solution, spiked with 25 $\mu g\,L^{-1}$ of DMP, DEP and BBP, and 100 $\mu g\,L^{-1}$ of DBP, under the optimized conditions is shown in Fig. 1.

3.3. Analytical performances

After selection of the optimum conditions for LC–ES-MS/MS, the method was evaluated using DMP, DEP, BBP and DBP standard solutions.

The linearity of the response of this method was evaluated using a standard addition method. This addition was performed at seven different concentrations of the standard solution of these phthalates, using a commercial physiological saline solution supplied in a glass bottle. Linear regression was performed by plotting the peak area versus concentration, and was linear over the range of 0–50 $\mu g\,L^{-1}$ for DMP, DEP and BBP, and of 0–150 $\mu g\,L^{-1}$ for DBP. The equations obtained for each compound were as follows:

$$\text{DMP}: Q_A = 273725\,C + 265276 \quad r = 0.9996$$

$$\text{DEP}: Q_A = 325956\,C + 208430 \quad r = 0.9978$$

$$\text{BBP}: Q_A = 255127\,C + 185647 \quad r = 0.9986$$

$$\text{DBP}: Q_A = 129571\,C + 263706 \quad r = 0.9956$$

where Q_A is the peak area and C is the concentration in $\mu g\,L^{-1}$.

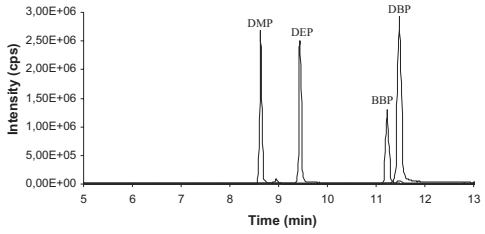


Fig. 1. LC–MS/MS ion chromatogram obtained from a physiological saline solution spiked with 25 $\mu g\,L^{-1}$ of DMP, DEP and BBP, and 100 $\mu g\,L^{-1}$ of DBP.

The limit of detection (LOD) and limit of quantification (LOQ) were calculated based on the IUPAC definition:

$$\text{LOQ} = \frac{10SD}{m} \qquad \text{LOD} = \frac{3SD}{m}$$

where SD is the standard deviation of ten blank solutions and m is the slope of the addition graph. A commercial physiological saline solution supplied in a glass bottle was used as a blank. The results obtained for LODs and LOQs are shown in Table 3. The LODs obtained are between 0.05 and 0.82 $\mu g\,L^{-1}$. The highest LOD obtained was for DBP. These LODs are lower than those obtained in a previous study to determine these compounds in the same type of samples by LC–MS [20]. Moreover, the method presents better or comparable sensitivity than other methods proposed in the literature for the determination of these phthalates using GC-MS in water samples. Serodio and Nogueira [2] developed a method for phthalates determination using stir bar sorptive extraction with liquid desorption followed by large volume injection and GC-MS obtaining LODs from 0.15 to 0.60 $\mu g\,L^{-1}$. Peñalver et al. [40] obtained LODs from 15 to 50 $\mu g\,L^{-1}$ for these phthalates using GC-MS, and obtained LODs from 0.007 to 0.17 $\mu g\,L^{-1}$ using SPME previous to the determination by GC-MS. Koch et al. [1] obtained LODs from 0.25 to 1.0 $\mu g\,L^{-1}$ for the determination of these phthalates in urine samples by LC–ES-MS/MS. The advantage of the proposed method is that present a good sensitivity when analyzing the sample directly, without any requiring preparation steps (e.g. preconcentration step).

Assays were developed to check intra- and interday precision. For the intraday study, aliquots of a physiological saline solution purchased in a glass bottle were spiked with two concentration levels of all phthalates studied and analysed six times in the same run. The interday assay was performed in the same way analyzing 12 aliquots of spiked samples in two different days. The results obtained for the intra- and interday assays are shown in Table 4. The RSD values were between 1.2 and 5.0% in the intraday assay and between 1.0 and 6.8% in the interday assay; thus, the method is precise for all the compounds studied.

The analytical recovery of the method was calculated using a blank sample (physiological saline solution commercialized in a glass bottle) spiked with three different concentrations of these compounds (5, 25 and 50 $\mu g\,L^{-1}$ for DMP, DEP and BBP and 40, 100 and 150 $\mu g\,L^{-1}$ for DBP). The spiked samples were prepared twice and analysed three times, and the recovery calculated using the standard addition graph. The recovery percentages obtained are shown in Table 5. The average analytical recoveries were 106.7, 92.6, 102.9 and 96.4% for DMP, DEP, BBP and DBP, respectively.

3.4. Application to physiological saline solution samples

The proposed analytical method has been applied to the analysis of different physiological saline solution samples, commercialised in plastic bottles, in order to check the presence of these phthalates and determine their concentration. Samples were directly injected into the chromatographic system; and no sample preparation process was necessary.

The results obtained for DMP, DEP, BBP and DBP are given in Table 6. The concentration levels obtained for BBP are lower than the LOD for all samples studied, and DBP was only detected in

Table 4
Results of intra- and interday assays to validate proposed LC–MS/MS method.

Phthalate	Intraday (n = 6)			Interday (n = 12)		
	Detected average (ng mL^{-1})	SD	RSD (%)	Detected average (ng mL^{-1})	SD	RSD (%)
DMP	29.86	0.36	1.20	29.88	0.29	0.97
	49.10	2.02	4.12	50.23	1.94	3.86
DEP	24.88	0.31	1.25	26.52	1.81	6.84
	46.24	1.04	2.25	46.95	1.33	2.84
BBP	24.28	0.46	1.89	24.44	0.61	2.51
	48.04	2.41	5.01	49.51	2.28	4.61
DBP	92.06	2.01	2.19	97.88	6.55	6.70
	148.91	6.91	4.64	146.74	5.40	3.68

Table 5
Recovery percentage for physiological saline solutions ± standard deviation to validate proposed LC–MS/MS method (n = 3).

Phthalate	% Recovery		
	5 μg L^{-1}	25 μg L^{-1}	50 μg L^{-1}
DMP	100.3 ± 2.5	118.3 ± 0.8	101.4 ± 3.2
DEP	81.4 ± 1.6	101.4 ± 0.5	94.9 ± 0.6
BBP	111.5 ± 2.8	98.7 ± 0.8	98.6 ± 2.7
Phthalate	% Recovery		
	40 μg L^{-1}	100 μg L^{-1}	150 μg L^{-1}
DBP	92.2 ± 2.2	93.6 ± 1.2	103.4 ± 1.6

Table 6
Concentration (μg L^{-1}) ± standard deviation (based on three replicates) found in different physiological saline solutions. <LOD: lower than the detection limit.

Physiological saline solution	DMP	DEP	BBP	DBP
Brand 01	17.4 ± 0.6	14.5 ± 0.4	<LOD	7.7 ± 0.6
Brand 02	0.4 ± 0.1	<LOD	<LOD	<LOD
Brand 03	19.2 ± 1.5	3.9 ± 0.2	<LOD	<LOD
Brand 04	346.8 ± 0.8	2.7 ± 0.1	<LOD	<LOD

brand 1. The concentration levels varied from 0.4 to 346 μg L^{-1} for DMP and from 0.4 to 14.5 μg L^{-1} for DEP. The brand 2 sample presented the lowest concentration of phthalates, being DMP the only phthalate detected. Phthalate esters are used in the manufacture of plastic containers; thus, the presence of phthalates in the samples can be attributed to the release of these compounds from the plastic containers. As an example, the chromatogram obtained when analyzing the brand 1 sample is shown in Fig. 2.

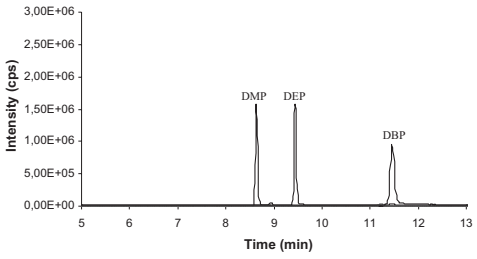


Fig. 2. LC–ES-MS/MS ion chromatogram obtained from brand 1 physiological saline solution.

4. Conclusion

A rapid (less than 13 min), sensitive and accurate method for the determination of DMP, DEP, BBP and DBP by LC–ES-MS/MS was developed. The main advantage of this method, compared with the methods proposed in the literature, is that the compounds can be detected at very low concentration without any sample pre-treatment. Moreover, the limits of detection obtained are comparable with the LODs found in the literature for determining of these phthalates by researches who performed a preconcentration step before the determination by GC-MS. Another advantage is that the reduction of the number of sample pre-treatment steps decreases the risk of the sample contamination during the analysis, which is a very common problem in the analysis of phthalates.

The method was applied for the determination of these compounds in four physiological saline solutions commercialized in plastic bottles. The presence of these compounds in the samples can be attributed to the different compositions of the plastic containers. Thus, control of material used in the manufacture of the plastic containers is essential to avoid human exposure to these toxic contaminants.

Acknowledgement

The authors wish to thank the Xunta de Galicia (Grupo de Referencia Competitivos 2007 (000047-0) for financial support.

References

[1] H.M. Koch, L.M. Gonzalez-Reche, J. Angerer, J. Chromatogr. B 784 (2003) 169.
[2] P. Serodio, J.M.F. Nogueira, Water Res. 40 (2006) 2572.
[3] T. Jones-Lepp, C.L. Gerlach, E.J. Cooter, Trends Anal. Chem. 19 (2000) 286.
[4] A. Gómez-Hens, M.P. Aguilar-Caballos, Trends Anal. Chem. 22 (2003) 847.
[5] I. Colón, D. Caro, J. Carlos, C.J. Bourdony, O. Rosario, Environ. Health Prespect. 108 (2000) 895.
[6] R.M. Sharpe, Int. J. Androl. 26 (2003) 2.
[7] Communication from the Commission to the Council and the European Parliament on the implementation of the community strategy for endocrine disrupters-a range of sustances suspected of interfering with the hormona systems of humans and wildlife, COM (1999) 706; COM (2001) 262 final, Brussels, 2001.
[8] G. Prokupková, K. Holandová, J. Poustka, J. Hajslová, Anal. Chim. Acta 457 (2002) 211.
[9] K. Holadová, G. Prokupková, J. Hajslová, J. Poustka, Anal. Chim. Acta 528 (2007) 24.
[10] O.S. Fatoki, A. Noma, S. Afr, J. Chem. 54 (2001) 69.
[11] X. Li, M. Zhong, S. Xu, C. Sun, J. Chromatogr. A 1135 (2006) 101.
[12] A. Adeniyi, M. Dayomi, P. Siebe, O. Okedeyi, Chem. Cent. J. 2 (2008) 1.
[13] B. Cavaliere, B. Macchione, G. Sindona, A. Tagarelli, J. Chromatogr. A 1205 (2008) 137.
[14] A.O. Earls, I.P. Oxford, J.H. Braybrook, J. Chromatogr. A 983 (2003) 237.
[15] O. Ballesteros, A. Zafra, A. Navalón, J.L. Vílches, J. Chromatogr. A 1121 (2006) 154.
[16] M.J. Silva, A.R. Slakman, J.A. Reidy, J.L. Preau Jr., A.R. Herbert, E. Samandar, L.L. Needham, A.M. Calafat, J. Chromatogr. B 805 (2004) 161.
[17] S. Jara, C. Lysebo, T. Greibrok, E. Lundanes, Anal. Chim. Acta 407 (2000) 165.
[18] Y.-Q. Cai, G. Jiang, J. Liu, Anal. Sci. 19 (2003) 1491.
[19] D. Sicar, S.J. Albazi, Y. Atallah, W. Pizza, J. Chromatogr. Sci. 46 (2008) 1471.

[20] C. Pérez Feás, M.C. Barciela Alonso, E. Peña Vázquez, P. Herbello Hermelo, P. Bermejo Barrera, Talanta 75 (2008) 1184.
[21] R.A. Gimeno, R.M. Marcé, F. Borrull, Chromatographia 58 (2003) 37.
[22] L. Brossa, R.M. Marcé, F. Borrul, E. Pocurull, Environ. Toxicol. Chem. 24 (2005) 261.
[23] B.C. Blount, K.E. Milgram, M.J. Silva, N.A. Malek, J.A. Reidy, L.L. Needham, J.W. Broca, Anal. Chem. 72 (2000) 4127.
[24] C. Pérez-Feás, M.C. Barciela Alonso, A. Sedes-Díaz, P. Bermejo-Barrera, Anal. Bioanal. Chem. 397 (2010) 529.
[25] K. Kato, M.J. Silva, L.L. Needham, A.M. Calafat, J. Chromatogr. B 814 (2005) 355.
[26] M.J. Silva, N.A. Malek, C.C. Hodge, J.A. Reidy, K. Kato, D.B. Barr, L.L. Needham, J.W. Broca, J. Chromatogr. B 789 (2003) 393.
[27] S. takatori, Y. Kitagawa, M. Kitagawa, H. Nakazawa, S. Hori, J. Chromatogr. B 804 (2004) 397.
[28] R. Ito, F. Seísmo, N. Miura, M. Kawaguchi, K. Saito, H. Nakazawa, J. Pharmaceut. Biomed. Anal. 39 (2005) 1036.
[29] K. Kambia, T. Dine, B. Gressier, A.F. Germe, M. Luyckx, C. Brunet, L. Michaud, F. Gottrand, J. Chromatogr. B 755 (2001) 297.
[30] C. Ji-an, L. Huijie, Q. Zhiqun, S. Weiqun, Environ. Pollut. 156 (2008) 849.
[31] H. Farahani, M. Reza, R. Dinarvand, P. Norouzi, Talanta 76 (2008) 718.
[32] R. Battle, C. Nerón, J. Chromatogr. A 1045 (2004) 29.
[33] M. Del Carlo, A. Pepe, G. Sacchetti, D. Compagnone, D. Mastrocola, A. Cichelli, Food Chem. 111 (2008) 771.
[34] A. Peñalver, E. Pocurull, F. Borull, R.M. Marcé, J. Chromatogr. A 922 (2001) 377.
[35] K. Holadová, G. Prokupková, J. Hajslová, J. Poustka, Anal. Chim. Acta 582 (2007) 24.
[36] P. Serodio, J.M.F. Nogueira, Anal. Chim. Acta 517 (2004) 21.
[37] A. Prieto, O. Telleira, N. Etxebarria, L.A. Fernández, A. Usobiaga, O. Zuloaga, J. Chromatogr. A 1214 (2008) 1.
[38] M. Bonini, E. Eran, G. Zerbinati, E.S. Ferri, S. Girotti, Michrochem. J. 90 (2008) 31.
[39] USEPA Method 506 revision 1.1 (1995). "Determination of Phthalates and Adipate Esters in Drinking Water by Liquid–Liquid Extraction or Liquid–Solid Extraction and Gas Chromatography with Photoionization Detection. National Exposure Research Laboratory Office of Research and Development U.S. Environmental Protection Agency. Cincinnati, OH 45268.
[40] A. Peñalver, E. Pocurull, F. Borrull, R.M. Marcé, J. Chromatogr. A 872 (2000) 191.

Anal Bioanal Chem (2010) 397:529–535
DOI 10.1007/s00216-009-3337-0

ORIGINAL PAPER

Phthalates determination in pharmaceutical formulae used in parenteral nutrition by LC-ES-MS: importance in public health

Cristina Pérez-Feás · María Carmen Barciela-Alonso · Antía Sedes-Díaz · Pilar Bermejo-Barrera

Received: 29 September 2009 / Revised: 13 November 2009 / Accepted: 21 November 2009 / Published online: 19 December 2009

Abstract A method for determining a group of phthalate esters in pharmaceutical formulae used in parenteral nutrition samples (with and without vitamins) has been developed. The phthalic acid esters (PAEs) studied were dimethyl phthalate, diethyl phthalate, butyl benzyl phthalate, dibutyl phthalate, di-(2-ethylhexyl) phthalate, and dioctyl phthalate. This group of phthalates was determined by high performance liquid chromatography (HPLC)–electrospray ionization–mass spectrometry, working in positive ion mode. The phthalates analyzed were extracted from the sample using hexane and sodium hydroxide. The hexane was then evaporated, and the compounds were redissolved in acetonitrile. The compounds were separated by HPLC working in gradient mode with acetonitrile-ultrapure water starting from 5% to 75% acetonitrile in 5 min, followed by isocratic elution for 27 min. Standard calibration curves were linear for all the analytes over the concentration range 10–250 μg L^{-1}. The method was precise (with RSD from 3.3% to 12.9%) and sensitive. The proposed analytical method has been applied to the analysis of these compounds in different pharmaceutical formulae (with different compositions) for parenteral nutrition samples in order to check the presence of phthalates and determine their concentration.

Keywords Phthalates · LC-ES-MS · Parenteral nutrition

C. Pérez-Feás · M. C. Barciela-Alonso · A. Sedes-Díaz · P. Bermejo-Barrera (✉)
Department of Analytical Chemistry, Nutrition and Bromatology, University of Santiago de Compostela, Santiago de Compostela 15782, Spain
e-mail: pilar.bermejo@usc.es

Introduction

Phthalates or phthalic acid esters (PAEs) are a group of chemical compounds widely use in industry and commerce due to their large variety of uses. Due to the ability to increase the softness and flexibility of plastics, they are used mainly as plasticizers in a wide variety of products including medical devices, children's toys, and all types of packaging. The main drawback of the use of PAEs is that they can migrate from the material to the environment and pollute water, soil, and food products. Furthermore, certain phthalate esters and or their metabolites are suspected to be human carcinogenic agents and endocrine disruptors, which make their trace determination particularly important [1, 2]. The interest of the determination of these compounds has increased in recent years due to results obtained in the studies concerning in human blood and urine samples where trace levels of various phthalates (or their metabolites) have been found [3, 4].

Several techniques have been used for PAEs determination in different matrices. In order to detect PAEs at sub ppm levels in different samples, a cleanup/preconcentration step is necessary before instrumental analysis. Different methods have been developed with this purpose such as liquid–liquid extraction (LLE) [5–10], liquid-phase microextraction [11], single drop microextraction [12], solid phase extraction [13–17], solid phase microextraction [18–20], stir bar sorptive extraction [21, 22], and solid/liquid extraction [23, 24].

Gas chromatography (GC) and high-performance liquid chromatography (HPLC) are the techniques usually used for PAEs separation in different matrices, such as environmental or biological samples. Generally, GC presents higher sensitivity than HPLC methods, although depending on the pre-treatment step, the instrumental conditions, and

the sample matrix [1]. HPLC can be used as an alternative technique and is especially useful for analysis of isomeric mixtures and metabolites of phthalates without derivatization [25]. Ultraviolet detection has been used for phthalate determination in environmental and biological samples [8, 9, 17, 26, 27]. However the use of mass spectrometry has increased in recent years, either operating with a single spectrometer [28, 29] or using a mass spectrometer in tandem [4, 16, 30, 31] with applications in different matrix samples (sludge, urban wastewater, urine, milk, and drugs). Although CG-MS offered higher sensitivity for phthalate determination than LC-MS, LC-MS approach offered some advantages, compared with GC-MS, such as superior selectivity with molecular weight information for the isomeric mixtures, more reliable quantification of PAEs isomeric mixtures, simpler cleanup procedures, and shorter analysis time. Moreover, phthalic acid monoesters can be analyzed without derivatization by HPLC [32].

The major problem in phthalate analysis is contamination, resulting in false-positive results or over-estimated concentrations. The risk of contamination is present in the whole analytical scheme, including sampling, sample preparation, and chromatographic analysis. Due to the fact that phthalates are widely used, they are present in air, water, organic solvents and plastic, and adsorbed onto glass or other materials [32].

Different cleaning methods have been proposed to prevent phthalate contamination problems from material used in the laboratory. In most of these methods, glass material is rinsed with organic solvents after a rigorous washing [17, 31, 33].

The aim of this work was the development of a method for phthalates determination in parenteral nutrition samples by HPLC-ES-MS.

Experimental

Reagents and standards

All reagents used were of analytical reagent grade. Dimethyl phthalate (DMP), butyl benzyl phthalate (BBP), dipentyl phthalate (DPeP), and dioctyl phthalate (DOP) were obtained from Supelco (Bellefonte, PA, USA). Diethyl phthalate (DEP) and dibutyl phthtalate (DBP) were obtained from Riedel-de Haën (Seelze, Germany). Diethylhexyl phthalate (DEHP) was obtained from Merck (Darmstad, Germany). The purity of these reagents was over 98%.

Individual standard solutions of each phthalate ester at a concentration of 1,000 mg L^{-1} were prepared in methanol, protected from light, and stored at 4 °C in a Teflon-capped glass vial. From these solutions, a working mixture in methanol was prepared weekly, containing all standards of concentration 100 mg L^{-1} each. All the working solutions were prepared daily by diluting this solution.

Hexane (PA-ACS-ISO; Panreac, Barcelona, Spain) and sodium hydroxide (Merck, Darmstadt, Germany) were used in the liquid–liquid extraction.

Lichrosolv gradient grade acetonitrile and methanol were purchased from Merck (Darmstadt, Germany). Technical-grade acetone and acetic acid glacial (HPLC) for instrumental analysis were purchased from Panreac (Barcelona, Spain). Ultrapure (resi-analized) water for environmental inorganic and organic trace analysis was supplied by J.T. Baker (Phillipsburg, NJ, USA).

Special care was taken to avoid contact of reagents and solvents with plastic materials. In order to reduce background contamination, all glassware was cleaned prior to analysis according to the recommendations specified in EPA method 506. All material was washed with hot water and soap, rinsed with tap and ultrapure water, and thoroughly rinsed with technical-grade acetone. Glassware was then sealed with aluminum foil and stored in a clean environment to prevent adsorption of phthalates from air.

Instrumentation

Phthalates separation and quantification were carried out using a liquid chromatography/electrospray ionization mass spectrometry system.

The HPLC system used was a 1100 Series equipped with an automatic injector (Agilent Technologies, Waldbronn, Germany) that is coupled to an API 150 EX single quadrupole mass spectrometer equipped with a Turboionspray interface (PE Biosystems, Concord, Canada).

The analytical column was a ZORBAX Eclipse XDB-C_8 of 50 mm length and 2.1 mm internal diameter (particle size 3.5 μm) supplied by Agilent Technologies.

A centrifuge Selecta (Barcelona, Spain) working at 3,500 rpm was used in the liquid–liquid extraction procedure.

Chromatographic and mass spectrometry conditions

The binary mobile phase consisted of ultrapure water and acetonitrile, both solvents containing 0.1% (*v*/*v*) acetic acid. The elution gradient started with 5% of acetonitrile, which was increased linearly to 75% in 5 min. This composition was maintained for 27 min before returning to the initial conditions. The column was then equilibrated for 10 min. The flow rate and the injection volume were 200 μL min^{-1} and 10 μL, respectively, and the chromatographic separation was carried out at room temperature. Under these conditions, the separation time was less than 30 min.

Electrospray ionization was performed in positive ion mode using the operational parameters shown in Table 1. The compound parameters such as declustering potential

Table 1 ES-MS parameters

Compound	*m/z*	DP	FP	EP
DMP	163.25	40.38	73.87	8
BBP	91.15	25	225	6
DEP, DBP, DPeP, DEHP, DOP	149.05	25	290	8.5

Nebulizer and curtain gas (N_2), 14 psi; heater gas, 7,000 cm^3/min; ES temperature, 450 °C; ionspray voltage, 5,500 V; mode: positive

DP declustering potential, *FP* focusing potential, *EP* enhance potential

(DP), focusing potential (FP), and enhance potential (EP) were optimized for each analyte. The optimal conditions are displayed in Table 1.

Sample preparation

The six phthalate esters studied in this work were extracted from the sample using a liquid–liquid extraction procedure. Thus, a volume of 1 mL of parenteral nutrition and 1 mL of NaOH 0.1 M were introduced into a conical glass tube. The mixture was vortexed for 2 min, and then 2 mL of hexane was added. The solution was shaken for 3 min in samples without vitamins and 5 min in samples with vitamins. In samples with vitamins, a centrifugation step at 3,500 rpm for 10 min was used to improve phase separation. The organic layer (fraction 1) was separated and transferred into another clean conical glass tube. The aqueous phase was extracted again with 2 mL of hexane, and the mixture was treated as above. The separated organic phase (fraction 2) was combined with fraction 1, and the total organic phase was evaporated to dryness using a hot water bath under argon stream. The residue was reconstituted with 500 μL of acetonitrile containing 250 μg L^{-1} of DPeP (internal standard (IS)) and shaken for 1 min; finally, 10 μL of solution was injected in the HPLC-ES-MS system.

Results and discussion

ES-MS optimization

Six phthalate esters (DMP, DEP, BBP, DBP, DEHP, and DOP) were selected for this study. DPeP was used as an internal standard.

To evaluate the mass spectral fragmentation pattern of each compound and to optimize the set of parameters used, a standard solution (100 μg L^{-1}) of each compound was analyzed by direct injection in the spectrometer. For these experiments, a KD Scientific, model 100, syringe pump (New Hope, MN, USA) at 15 μL min^{-1}, was used.

Full-scan data acquisition was performed from 80 to 400 *m/z*, with the target mass fixed to the following *m/z* values: 91.15 for BBP, 149.05 for DEP, DBP, DPeP, DEHP, and DOP and 163.25 for DMP. The spectral data provided ions in accordance with previous studies reported in the literature [18, 34–37]. The selected ions were chosen to attain the best response in the SIM mode acquisition.

Optimization of HPLC separation

After optimizing the detection conditions, the following experiments were conducted to optimize the chromatographic separation of the analytes.

Experiments were carried out using different mobile phases reported in the literature (methanol/water [38], acetonitrile/water [39], and acetonitrile (1% methanol)/water[40]), working in isocratic mode. The best resolution was obtained using acetonitrile/water as a mobile phase. These results agree with the experiments developed by López-Jimenez et al. [41]. In order to improve the resolution and to decrease the time of analysis, different experiments were carried out working in gradient mode. The best results were obtained starting with 5% of acetonitrile and increasing this percentage to 75% in 5 min. This composition was maintained for 27 min before returning to the initial conditions. Finally, the column was equilibrated during 10 min before each injection. Other parameters optimized were the percentage of acetic acid and the flow rate of the mobile phase. The optimal conditions were 0.1% (*v/v*) acetic acid and a flow rate of 200 μL min^{-1}.

The chromatogram obtained for a mixture of these compounds under the optimized conditions is shown in Fig. 1.

Phthalate separation by liquid–liquid extraction

The phthalate esters studied were separated using a LLE procedure. The sample was treated with sodium hydroxide, to digest the fat contained in the sample and to favor the migration of phthalates toward the extracting agent, and hexane was used as an organic phase.

The initial conditions used to develop this study were selected based on of the results obtained by Kambia et al. [8]. Thus, 1 mL of parenteral nutrition and 1 mL of 1 M sodium hydroxide were introduced into a conical glass tube. The mixture was vortexed for 2 min, and then 2 mL of hexane was added. The solution was shaken for 5 min and centrifuged for 10 min at 3,500 rpm. The organic layer (fraction 1) was separated and transferred into another clean conical glass tube. The aqueous phase was extracted again with 2 mL of hexane, and the mixture was treated as above. The separated organic phase (fraction 2) was combined with fraction 1, and the total organic phase was evaporated

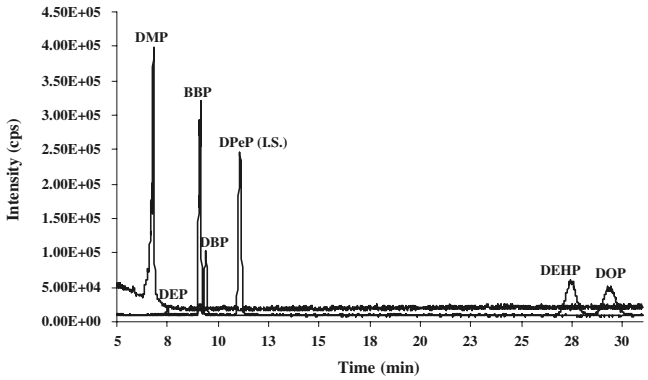


Fig. 1 LC/MS extracted ion chromatogram obtained from a standards solution (100 μg L^{-1}) in acetonitrile. Retention times (*RT*; min): DMP 6.72, DEP 7.43, BBP 9.02, DBP 9.29, DPeP 10.94, DEHP 26.71, DOP 28.62

to dryness using a hot water bath under argon stream. The residue was reconstituted with 500 μL of acetonitrile containing 250 μg L^{-1} of IS and shaken for 1 min; finally, 10 μL of solution was injected in the HPLC-ES-MS system. The parameters studied in this work were sodium hydroxide concentration, volume of hexane, and agitation time. The experiments were developed with two types of parenteral nutrition containing aminoacids, glucose, and electrolytes. The only difference was the presence or absence of vitamins, which confer the lipophilic character to the sample.

The first parameter studied was the volume of hexane. Experiments were carried out using a blank sample of parenteral nutrition (with and without vitamins) spiked with 100 μg L^{-1} of all phthalates studied and varying the volume of hexane between 1 and 3 mL in each extraction (extraction by duplicate). In these experiments, the other parameters were fixed at 1 mL of 1 M NaOH and agitation time of 5 min. The results obtained show that the signals remain practically constant with the volume of hexane, for compounds such as DEP, but in general there is an improvement using 2 mL of hexane in each extraction. The results were similar in the two types of samples studied; thus, 2 mL of hexane was selected to develop this study.

Another parameter studied was the sodium hydroxide concentration. For this purpose, experiments were carried out varying the NaOH concentration from 0 to 1.5 M. The results obtained show that, in general, the extraction procedure is improved by increasing the NaOH concentration until 0.1 M in both types of samples; concentrations higher than 0.1 M decreased the percentage of extraction for all compounds studied. Therefore, 0.1 M sodium hydroxide was selected for this study. The result obtained disagrees with the result obtained by Kambia et al., which used 1 M sodium hydroxide in the extraction procedure. This different result may be attributed to the different composition of the samples analyzed.

The following parameter studied was the agitation time during the extraction procedure. The experiment was carried out varying the agitation time between 1 and 7 min. The best results were obtained using agitation times of 3 min for all compounds in parenteral nutrition samples without vitamins and 5 min for all compounds in parenteral nutrition samples with vitamins.

Analytical performance

To evaluate the linearity of the method, a direct calibration was performed. Ten microliters of standard solutions in acetonitrile with concentrations ranging from 10 to 250 μg L^{-1} was injected by triplicate. Relative areas (analyte peak area/IS peak area) were plotted versus the amount of analyte injected, expressed in μg L^{-1}, and the background levels were subtracted from the results. The results obtained show good correlation coefficients (r^2> 0.9964) for all the studied compounds.

The standard addition method was applied over the same range of concentrations using a parenteral nutrition sample (with and without vitamins), obtaining good correlation coefficients (r^2>0.9910) for all the studied compounds.

To compare slopes of direct calibration and addition graphs for the six compounds, the *t* test (95% significance level) [42] was applied. Results for samples without vitamins have shown statistically differences for DMP, whereas statistically differences were observed for all compounds for samples with vitamins. This means that the sample matrix had influence in the sensitivity of the method; thus, standard addition graphs have been used to analyze the samples in all cases.

The limit of detection (LOD) and limit of quantification (LOQ) for the method were calculated using 11 measurements of an acetonitrile extract of a blank sample. This blank sample was prepared with the same compounds that as the parenteral nutrition and stored in a glass bottle to

Table 2 LODs and LOQs obtained for the six phthalates in parenteral nutrition samples (without and with vitamins)

	DMP	DEP	BBP	DBP	DEHP	DOP
Nutrition without vitamins						
LOD (μg L^{-1})	1.1	7.0	1.3	7.4	5.0	1.1
LOQ (μg L^{-1})	3.6	23.5	4.5	24.8	16.8	3.7
Nutrition with vitamins						
LOD (μg L^{-1})	0.1	13.2	0.9	10.8	2.5	1.0
LOQ (μg L^{-1})	0.5	44.1	2.9	35.9	8.3	3.3

avoid phthalates contamination and was then treated with the liquid–liquid extraction procedure described in the "Sample preparation" section. Limits of detection of target compounds in the parenteral nutrition samples were calculated from the instrumental detection limit, taking into account the amount of sample extracted, the volume of the organic phase used, and the recovery of the method. The results obtained for LODs and LOQs for the different samples (with and without vitamins) are shown in Table 2. The limits of detection are between 0.1 and 10.8 μg L^{-1}, and the highest levels obtained were for DEP and DBP. The LOQ obtained for DEHP is lower than those obtained by Kambia et al. [8] (20 ng mL^{-1}) for the determination of this compound in this type of sample using HPLC-UV.

To check, the intra- and interday precision assays were developed. For the intraday study, six aliquots of a parenteral nutrition sample with vitamins and another six without vitamins, spiked with 100 μg L^{-1} of all compounds studied, were subjected to the extraction procedure described above. The extracts were analyzed in the same day for all compounds studied, and the relative standard deviation was calculated. The interday assay was carried out in the same way by subjecting 12 aliquots of spiked samples (with and without vitamins) to the extraction procedure in two different days. The RSD values were between 2.4% and 9.4% in the intraday assay and between 3.3% and 12.9% in the interday assay; thus, the method is precise for all studied compounds.

The recovery of the method was calculated using a blank sample (with and without vitamins) spiked with three different concentrations of these compounds (50, 100, and 200 μg L^{-1}). The extractions were carried out by duplicate and analyzed by triplicate and the recovery calculated using the standard addition graphs. The average analytical recoveries were 56.0%, 93.0%, 96.6%, 94.1%, 74.4%, and 74.3% for DMP, DEP, BBP, DBP, DEHP, and DOP, respectively, in nutrition samples without vitamins, and 78.6%, 56.3%, 97.6%, 99.2%, 67.8%, and 105.2% for DMP, DEP, BBP, DBP, DEHP, and DOP, respectively, in nutrition samples with vitamins.

Application to parenteral nutrition samples

The proposed analytical method has been applied to the analysis of different parenteral nutrition samples, with and without vitamins, used in the public health system, in order to check the presence of these phthalates and to determine their concentrations.

All samples studied were prepared by the Hospital Clínico Universitario de Santiago de Compostela Pharmacy Department. These samples were prepared for neonates admitted to the intensive care unit and stored in ethyl vinyl acetate bags at −4 °C until analysis. The analytes were extracted from the sample using the extraction procedure described in the "Sample preparation" section.

The results obtained for these phthalates in the samples (with and without vitamins) analyzed are shown in Table 3. Figure 2 shows the chromatograms obtained for two parenteral nutrition samples studied.

As can be seen in Table 3, the phthalates were not detected in samples without vitamins or were detected at very low concentrations.

The results obtained for parenteral nutrition samples with vitamins were very different. All compounds studied,

Table 3 Concentration (μg L^{-1}) ± standard deviation (base on three replicates) found in different parenteral nutrition samples (without and with vitamins)

	DMP	DEP	BBP	DBP	DEHP	DOP
Samples without vitamins						
Sample 1	nd	nd	1.3±0.1	nd	nd	nd
Sample 2	nd	nd	nd	13.3±3.7	7.0±0.5	nd
Sample 3	2.40±0.9	nd	nd	nd	9.3±3.2	nd
Sample 4	nd	nd	11.6±0.2	12.9±5.4	22.2±1.8	nd
Sample 5	nd	nd	nd	nd	19.5±7.0	nd
Sample 6	nd	nd	nd	nd	17.2±0.5	nd
Sample 7	nd	nd	nd	21.6±4.4	36.5±0.8	nd
Samples with vitamins						
Sample 1	6.8±0.1	nd	6.1±0.3	35.3±1.5	215.1±3.9	nd
Sample 2	7.1±0.1	nd	5.9±0.1	42.4±4.7	993.7±4.2	nd

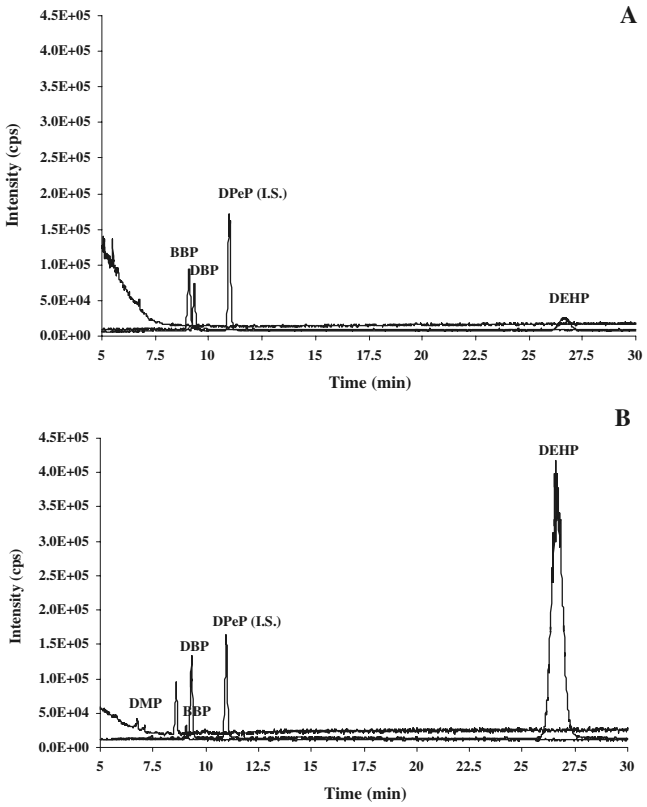


Fig. 2 LC/MS extracted ion chromatogram obtained from parenteral nutrition sample without vitamins (**a**) and with vitamins (**b**)

except DEP and DOP, were detected in the four samples studied. The highest concentrations were obtained for DEHP in all samples studied. This demonstrates that the lipid content of the parenteral nutrition increases the release of phthalates from the packaging to the sample.

Table 4 shows the results corresponding to the same sample with vitamins before (sample A) and after (sample B) passing through the administration tube. In this case, an increment in the concentration of DEP and DEHP was observed. This means that these types of components are employed in the manufacture of infusion lines. As reported, these compounds are usually present in plastics to improve their flexibility, and this can entail a risk to the health of the patients.

Conclusion

A sensitive and precise method to separate and determine six phthalates in parenteral nutrition samples by HPLC-ES-MS was optimized. An LLE method to separate and preconcentrate these compounds in the samples was studied using sodium hydroxide and hexane as an organic phase. The proposed method was applied to the determination of these compounds in parenteral nutrition samples with different compositions. The only difference of these groups of samples is the lipid content (samples with and without vitamins). The results obtained show that the presence of vitamins in the sample increases the release of these

Table 4 Concentration (μg L^{-1}) ± standard deviation (base on three replicates) found in a sample with vitamins before (sample A) and after (sample B) to pass through the administration tube

Samples	DMP	DEP	BBP	DBP	DEHP	DOP
Sample A	6.7±0.1	35.0±7.9	5.3±0.1	29.6±1.5	1,605.6±9.1	nd
Sample B	7.8±0.1	120.2±1.9	5.3±0.1	17.8±2.2	1,910.8±12.0	nd

compounds from the infusion bags to the sample. This is due to the fact that the lipid content (some vitamins) favors the release of these compounds from the bag because phthalates are lipid soluble and are not chemically bound to plastics. Moreover, an increase of the DEHP and DEP was observed in the sample passed through the administration tube used to supply the nutrition to the patient. The results confirm previous findings [43–45] and show that infusion lines leach plasticizers in substantial amounts. This large amount of phthalates (especially DEHP) is a cause of worry because it may affect the most vulnerable patients.

Control of material used in the manufacture of medical devices is important to avoid exposure to toxic contaminants, like phthalates, that may produce several complications in patients.

Acknowledgments The authors wish to thank the Xunta de Galicia (Grupo de Referencia Competitivos 2007 (000047-0)) for financial support. We are also grateful to the Neonatology Service and the Pharmacy Department from Complejo Hospitalario Universitario de Santiago, CHUS, for supplying the parenteral nutrition. Cristina Pérez Feás wishes to thank the Ministerio de Educación y Ciencia for her FPU grant.

References

1. Communication from the Commission to the Council and The European Parliament on the implementation of the community strategy for endocrine disrupters-a range of substances suspected of interfering with the hormone system of humans and wildlife, COM (1999) 706; COM (2001) 262 final, Brussels, 2001
2. US Environmental Protection Agency (EPA) (1999) Protection agency. Introduction to water policy standards. Office of Water, Washington, DC
3. De OD, Gagliardi L, Porrá R, Berri S, Chimenti P, Granese A, Carpani I, Tonelli D (2006) Anal Chim Acta 555:238–241
4. Kato K, Shoda S, Takahashi M, Doi N, Yoshimura Y, Nakazawa H (2003) J Chromatogr B 788:407–411
5. Mortensen GK, MainK M, Anderson AM, Leffer H, Skakkeback NE (2005) Anal Bioanal Chem 382:1084–1092
6. Soresen LK (2006) Rapad Commu Mass Spectrom 20:1135–1143
7. Faouzi MA, Dine T, Gressier B, Kambia K, Luyckx M, Pagniez D, Brunet C, Cazin M, Babeles A, Cazin JC (1999) Inter J Pharma 180:113–121
8. Kambia K, Dine T, Gressier B, Germe AF, Luyckx M, Brunet C, Michaud L, Gottrand F (2001) J Chromatogr 755:297–303
9. Kambia K, Dine T, Gressier B, Germe AF, Luyckx M, Brunet C, Michaud L, Gottrand F (2003) Inter J Pharma 262:83–91
10. Ji-an C, Huijie L, Zhiqun Q, Weiqun S (2008) Environ Poll 156:849–853
11. Farahani H, Reza M, Dinarvand R, Norouzi P (2008) Talanta 76:718–723
12. Batlle R, Nerin C (2004) J Chromatogr A 1045:29–35
13. Silva MJ, Preau JL Jr, Needham LL, Calafat AM (2008) J Chromatogr B 873:180–186
14. Del Carlo M, Pepe A, Sacchetti G, Compagnone D, Mastrocola D, Cichelli A (2008) Food Chem 111:771–777
15. Zafra-Gómez A, Ballesteros O, Navalón A, Vilches JL (2008) Michrochem J 88:87–94
16. Kato K, Silva MJ, Needham LL, Calafat AM (2005) J Chromatogr B 814:355–360
17. Cai Ya-Qi, Jiang G-B, Liu Ping-Fu, Zhou P-X (2003) Anal Chim Acta 494:149–156
18. Luks-Betlej K, Popp P, Janozska B, Paschke H (2001) J Chromatogr A 938:93–101
19. Peñalver A, Pocurull E, Borrull F, Marcé RM (2001) J Chromatogr A 922:377–384
20. Holadová K, Prokupková G, Hajslová J, Poustka J (2007) Anal Chim Acta 582:24–33
21. Serodio P, Nogueira JMF (2004) Anal Chim Acta 517:21–32
22. Prieto A, Telleira O, Etxebarria N, Fernández LA, Usobiaga A, Zuloaga O (2008) J Chromatogr A 1214:1–10
23. Gibson R, Wang M-J, Padgett E, Beck AJ (2005) Chemosphere 61:1336–1344
24. Bonini M, Errani E, Zerbinati G, Ferri E, Girotti S (2008) Michrochem J 90:31–36
25. Silva MJ, Slakman AR, Reidy JA, Preau JL, Herbert AR, Samandar E, Needham LL, Calafat AM (2004) J Chromatogr B 805:161–167
26. Li J, Cai Y, Shi Y, Mou S, Jian G (2008) Talanta 74:498–504
27. Sahitoh T, Matsushima S, Hiraide M (2004) J Chromatogr A 1040:185–191
28. Pérez Feás C, Barciela Alonso MC, Peña Vázquez E, Herbello Hermelo P, Bermejo Barrera P (2008) Talanta 75:1184–1189
29. Hanawa T, Muramatsu E, Asakama K, Tanaka M, Kawano K, Seki T, Juni K, Nakajima S (2000) Int J Pharm 210:109–115
30. Silva MJ, Malek NA, Hodge CC, Reidy JA, Kato K, Barr DB, Needman LL, Broca JW (2003) J Chromtogr B 789:393–404
31. Takatori S, Kitagawa Y, Kitagawa M, Nakazawa H, Hori S (2004) J Chromatogr B 804:397–401
32. David F., Sandra P., Tienpont B., Vanwalleghem, in C.A., Staples (ED.), The Handbook of Environmental Chemistry. Part Q. Phthalate Esters, vol. 3, Springer, Berlin, 2003, p.15
33. Gimeno RA, Marcé RM, Borrull F (2003) Chromatographia 58:37–41
34. Serodio P, Nogueira JMF (2006) Water Res 40:2572–2582
35. Polo M, Llompart M, García-Jares C, Cela R (2005) J Chromatogr A 1072:63–72
36. Kumar R (1999) A mass spectral guide for quick identification of phthalates esters. American Laboratory, Shelton, p 32
37. Psillakis E, Kalogerakis N (2003) J Chromatogr A 999:145–153
38. Saito Y, Nakao Y, Imaizumi M, Morishima Y, Kiso Y, Jinno K (2002) Anal Bioanal Chem 373:81–86
39. Hata N, Yuwatini E, Ando K, Yamada M, Kasahara I, Taguchi S (2004) Anal Sci 20:149–152
40. Jara S, Lysebo C, Greibokk T, Lundanes E (2000) Anal Chim Acta 407:165
41. López-Jimenez FJ, Rubio S, Pérez-Bendito D (2005) Anal Chim Acta 551:142–149
42. Cela R (ed) (1994) Avances en Quimiometría práctica. Universidad de Santiago de Compostela, Santiago de Compostela, Spain, p 171
43. Loff S, Kabs F, UIT K, Sartoris J, Mandi B, Niessen KH, Waag KL (2000) J Pediatric Surgery 12:1775–1781
44. Loff S, Subotic U, Reinicke F, Wischmann BJ (2004) J Pediatr Gastroenterol Nutr 39:341–345
45. Subotic U, Animan T, Kiss M, Brade J, Breitkopf K, Loff S (2007) J Pediatr Gastroenterol Nutr 44:71–76

Microchemical Journal 99 (2011) 108–113

Contents lists available at ScienceDirect

Microchemical Journal

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

Presence of phthalates in contact lens and cleaning solutions

C. Pérez-Feás, M.C. Barciela-Alonso, P. Bermejo-Barrera *

Department of Analytical Chemistry, Nutrition and Bromatology, University of Santiago de Compostela, Santiago de Compostela 15782, Spain

ARTICLE INFO

Article history:
Received 29 March 2011
Accepted 7 April 2011
Available online 15 April 2011

Keywords:
Phthalates
Contact lenses
Cleaning solutions
LC–MS/MS

ABSTRACT

A fast and simple method using liquid chromatography–tandem mass spectrometry (LC–MS/MS) has been applied to identify and quantify four phthalic acid esters (PAEs) in different contact lens cleaning solutions. A migration study of these compounds from contact lenses has also been performed. The PAEs studied were dimethyl phthalate, diethyl phthalate, butyl benzyl phthalate and dibutyl phthalate. The migration of PAEs from contact lenses was performed by suspending each contact lens in an artificial tear solution at 37 °C and shaking it at 130 rpm for 24 h. The purpose of this study was to determine a possible migration of these compounds to the eyes as a result of the use of contact lenses and their cleaning solutions. The method was precise (with relative standard deviation (RSD) from 2.2 to 11.9%). It was also sensitive, with LODs of 0.03, 0.19, 0.31 and 2.62 μg L^{-1} for DMP, DEP, BBP and DBP respectively. The results obtained confirm the presence of these substances in some types of contact lens cleaning solutions. Furthermore, DBP and BBP were liberated from the contact lenses during the migration study.

1. Introduction

Worldwide production of phthalates and their frequent application in different products for daily use has resulted in their widespread presence in all parts of the environment. They are used to make plastics more flexible and resilient, and are often referred to as plasticizers.

Plastics are made of monomers and other starting substances which are chemically reacted to a macromolecular structure, the polymer, forming the main structural component of the plastics. Different additives are added to the polymer to achieve defined technological effects. Potential health risks may occur from non- or incompletely reacted monomers and other starting substances and from low molecular weight additives which are transferred via migration from the plastic [1].

PAEs increase the flexibility of plastics only through weak secondary molecular interactions with polymer chains. These compounds are not covalently bound to the vinyl polymer matrix, and can thus be released fairly easily from these products. These plasticizers are found in products such as construction materials, medical devices, toys, and food packaging. Some of these compounds are also used in cosmetics, fragrances and personal care products [2].

A large number of these compounds have been identified as priority hazardous substances by the European Union (EU), the US Environmental Protection Agency (EPA) and by several international organizations [3–5]. Plasticizers can affect several aspects of human health especially the reproductive, endocrine and respiratory systems and can also produce dermatological problems [6–11]. Some phthalates such as benzyl butyl phthalate (BBP), dibutyl phthalate (DBP), di-n-pentyl phthalate (DnPeP) and di-(2-ethylhexyl) phthalate (DEHP) are classified as toxic to reproduction (category 2) by the European Union [12]. The results of toxicological studies have led to the prohibition in 1999 of the addition of phthalates to prepare plastics intended for toys [13,14]. Recently, the European Commission has published a new regulation on plastic materials and articles which will come into contact with food (Commission Regulation (EU) Nª10/2011 of 14 January 2011). This Regulation substitute Commission Directive 2002/72/EC and establishes the specific rules for plastic materials and articles to be applied for their safe use. This Regulation includes the Union list which contains substances authorized to be used in the manufacture of plastics which will come into contact with foods [1].

The determination of phthalates is not an easy task, in fact their widespread presence in laboratory environment, including air, glassware and reagents can produce false positive outputs [15–17]. Therefore, the risk of contamination is present in the whole analytical scheme, including sampling, sample preparation and analysis.

Several methods for PAEs determination at very low concentrations in different matrices are found in the literature (water [16,18–20], food [21–23], sediments [24], soils [25,26], biological samples [27–29], toys [30–32], cosmetics [33], etc.). Different sample treatments, extraction and preconcentration steps such as liquid–liquid extraction (LLE) [34–37], solid phase extraction (SPE) [38–41], solid phase microextraction (SPME) [42–44], stir bar sorptive extraction (SBSE) [45,46] and solid/liquid extraction (SLE) [47–49] have been used before the instrumental analysis to determine these compounds in these types of samples.

* Corresponding author. Tel.: +34 600942346.
E-mail address: pilar.bermejo@usc.es (P. Bermejo-Barrera).

0026-265X/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.microc.2011.04.006

GC and HPLC as separation techniques coupled with different detectors are the main techniques used in the literature for PAE determination. The coupling of these separation techniques with mass spectrometry and tandem mass spectrometry increases the sensitivity of the method. The results found in the literature indicate that GC–MS or HPLC–MS/MS present the lowest limits of detection [50].

In recent years, some authors have also focused their research to study the migration of phthalates from different matrices. Earls et al. [30] study the migration of some phthalates from toys and childcare articles to saliva simulants. The authors determined the phthalates in saliva by GC–MS. Bonini et al. also studied the migration of this type of compounds from food packaging films by GC–FID [49]. The objective of the present work is to evaluate the level of exposition to phthalates and the risks to human health by studying the presence of these compounds in contact lens cleaning solutions at sub ppm level, and by performing a migration study of these compounds from contact lenses to artificial tear solutions.

2. Experimental

2.1. Reagents and standards

All reagents used were of analytical reagent-grade. Dimethyl phthalate (DMP) and Butyl benzyl phthalate (BBP) were obtained from Supelco (Bellefonte, PA, USA). Diethyl phthalate (DEP) and Dibutyl phthalate (DBP) were obtained from Riedel-de Haën (Seelze, Germany). The purity of these reagents was over 98%.

Lichrosolv gradient grade acetonitrile and methanol were purchased from Merck (Darmstadt, Germany). Technical-grade acetone and glacial acetic acid (HPLC) for instrumental analysis were purchased from Panreac (Barcelona, Spain). Ultrapure (resi-analyzed) water for environmental inorganic and organic trace analysis was supplied by J.T. Baker (Phillipsburg, NJ, USA).

2.2. Instrumentation

A Series 1100 liquid chromatograph from Agilent Technologies (Waldbronn, Germany) was coupled to an API 4000™ Triple Quadrupole Mass spectrometer (Applied Biosystems, Concord, Canada) equipped with a Turbo Ionspray™ ionization source. A Zorbax Eclipse XDB-C_8 column (3.5 μm, 2.1 mm × 50 mm) from Agilent Technologies was used for the separation. Data acquisition and processing were performed using Analyst Software 1.4.2 (Applied Biosystems).

A Boxcult incubator situated on a Rotabit orbital-rocking platform shaker (J.P. Selecta, Barcelona, Spain) was used to perform the migration test from the contact lenses.

2.3. Glassware cleaning

Special care was taken to avoid the contact of reagents and solvents with plastic materials. Because of the ubiquity of plasticizers and the tendency of residues to persist, all glassware was cleaned prior to the analysis according to the recommendations specified in U.S. EPA Method 506 [51].

All materials were washed with hot water and soap, rinsed with tap and ultrapure water and finally thoroughly rinsed with technical-grade acetone. Glassware was then sealed with aluminum foil and stored in a clean environment to avoid adsorption of phthalates from the air.

2.4. Standard preparation

Stock standard solutions of each phthalate ester at a concentration of 1000 mg L^{-1} were prepared in methanol, kept in darkness, and stored at 4 °C in a Teflon-capped amber glass bottles until use. From these solutions, a working standard solution in methanol was prepared weekly containing all standards at concentrations of 100 mg L^{-1} each. Diluted working standard solutions were prepared daily.

2.5. Sample preparation

Two types of samples were studied: contact lens cleaning solutions and artificial tear solutions. All samples were purchased in pharmacies.

The contact lens cleaning solutions were injected directly into the liquid chromatography–tandem mass spectrometry system without any pre-treatment step. The artificial tear solution was used in the migration study of these compounds from contact lenses, using the procedure described in Section 2.6. The artificial tear solution used in this study was also injected directly into the LC–MS/MS system.

2.6. Migration test

The objective of this work was to check the presence of these phthalates in contact lenses and to study their migration from contact lenses to artificial tear solutions. The method is based on orbital–horizontal shaking of the contact lenses with artificial tear solution, under strictly controlled conditions of temperature, mode of mechanical agitation, contact time and volume of artificial tear solution. The method is aimed at representing the human eye environment as far as it is possible in the laboratory.

The contact lenses were put into the Teflon-capped amber glass vials with 1 mL of artificial tear solution. The vials were incubated at 37 °C with orbital–horizontal shaking at 130 rpm for 24 h. The solutions were then transferred to another glass vial and directly injected into LC–MS/MS system. No sample preparation process was necessary.

2.7. LC–MS/MS conditions

The LC–MS/MS conditions for DMP, DEP, BBP and DBP determinations in aqueous samples were studied in a previous work developed by our research group [52].

Ultrapure water and acetonitrile, both solvents containing 0.1% (v/v) acetic acid, were used as a binary mobile phase. Phthalates were separated by LC working in gradient mode. The separation was performed starting with 5% of acetonitrile and increasing to 75% in 5 min, which was increased linearly to 75% in 5 min. This composition was maintained for 8 min before returning to initial conditions. The column was equilibrated for 10 min. Ten microliters of each sample was injected using the HPLC autosampler configured with syringe washes between injections to eliminate carryover. The flow rate was 200 μL min^{-1} and the column oven was maintained at 40 °C. Under these conditions the separation time was less than 13 min. These optimal conditions are shown in Table 1.

ESI in the positive ion mode was used to form the positively charged analyte ions at the interface under fixed instrument settings (Table 1). The combinations of precursor ion and product ions were as follows: DMP (precursor ion → product ion, m/z 195 → 163), DEP (m/z 223 → 177), BBP (m/z 313 → 91) and DBP (m/z 279 → 149).

3. Results and discussion

Phthalates (DMP, DEP, BBP and DBP) were determined in contact lens cleaning solutions and artificial tear solutions by LC–ESI–MS/MS. The instrumental conditions used in this work were optimized in a previously work [52]. The working conditions are described in Section 2.7 and summarized conditions are in Tables 1 and 2.

One of the main problems involved in the determination of phthalates is laboratory contamination [15]. It was not possible to obtain zero method blanks for the phthalates analyzed. However, the contamination level was reduced to a low and rather constant level by

Table 1
Operational conditions for LC–MS/MS.

HPLC (Agilent 1100)	
Column	Zorbax Eclipse XDB-C8 (3.5 μm 2.1 mm × 50 mm)
Mobile phase	Ultrapure water : acetonitrile (0.1% (v/v) acetic acid)
Mode	Gradient
Flow rate	200 μL/min
Oven temperature	40 °C
Injection volume	10 μL
MS/MS (API 4000)	
Ion spray voltage	5500 V
Ionization mode	ESI-positive
Curtain gas	25 psi (nitrogen)
GS1 (nebulizer gas)	50 psi
GS2 (auxiliary gas)	60 psi
Ion source temperature	450 °C
CAD (collisionally activated dissociation)	4

using high quality solvents combined with thorough rinsing of all glassware with ultrapure water and technical-grade acetone. The blank results were always subtracted to correct experimental values.

3.1. Analytical performances

Before the determination of these phthalates in contact lens cleaning solutions and artificial tear solutions, the analytical characteristics were studied.

The calibration was performed by the standard addition method, using an artificial tear solution and working in a concentration range from 0.5 to 100 μg L^{-1} for DMP and DEP, from 5 to 100 μg L^{-1} for BBP and from 40 to 250 μg L^{-1} for DBP.

All the standard solutions were analyzed in triplicate. Linear regression was performed by plotting the peak area versus concentration. The coefficients of correlation (r) obtained were higher than 0.9985, indicating adequate linearity. The equations obtained for each compound are shown in Table 3.

The sensitivity of the method was determined by calculating the limit of detection (LOD) and the limit of quantitation (LOQ). LOD and LOQ were assessed based on the IUPAC definition:

$$LOD = \frac{3SD}{m} \quad LOQ = \frac{10SD}{m}$$

Where *SD* is the standard deviation of ten blank solutions and *m* is the slope of the addition graph. A commercial artificial tear solution was used as a blank. The results obtained for LODs and LOQs are shown in Table 3. The LODs obtained are between 0.03 and 2.62 μg L^{-1}. The LODs and LOQs obtained in the present work were compared with values found in the literature. To the best of our knowledge, there are no published papers for the determination of these phthalates in contact lens cleaning solutions and artificial tear solutions; thus, the values obtained can be compared with the results obtained in saline or water samples. Koch et al. [53] determined these phthalates in urine samples by LC–MS/MS obtaining LODs from 0.25 to 1.0 μg L^{-1}. Gimeno et al. [54] obtained LODs from 0.01 to 1 μg L^{-1} for these phthalates in water samples using solid phase extraction previous to the determination by LC–MS. In general, the LODs obtained in this work are comparable or in some cases better than the values found in the literature. The advantage of the proposed method is its simplicity and speed because no preconcentration step is necessary. As sample manipulation is minimized, the contamination problems are greatly reduced, allowing phthalate determination at ppb levels.

Table 2
Phthalates and their precursor and product ion transitions, potential optimization (DP: declustering potential; EP: enhance potential; CE: collision energy; and CXP: collision cell exit potential) and retention times (RT).

Analyte	Acronym	Precursor/product ions (m/z)	Potential Optimization				
			DP	EP	CE	CXP	RT (min)
Dimethyl phthalate	DMP	195/163	31	10	13	14	8.6
Diethyl phthalate	DEP	223/177	36	10	23	12	9.6
Butyl benzyl phthalate	BBP	313/91	41	10	23	6	11.6
Dibutyl phthalate	DBP	279/149	50	9	11	10	11.9

Table 3
Linear range, correlation coefficients, LOD and LOQ values obtained from the standard addition method in artificial tear solution.

Analyte	Linear range (μg L^{-1})	Correlation coefficient (r)	LOD (μg L^{-1})	LOQ (μg L^{-1})
DMP	0.5–100	0.9993	0.03	0.09
DEP	0.5–100	0.9992	0.19	0.63
BBP	5–100	0.9988	0.31	1.03
DBP	40–250	0.9985	2.62	8.74

The within-run precision was studied using an artificial tear solution spiked with four concentrations of each phthalate (10, 25, 50 and 75 μg L^{-1} for DMP, DEP and BBP and 60, 100, 150 and 200 μg L^{-1} for DBP). Each solution was analyzed six times in the same run. The results obtained are shown in Table 4. The relative standard deviations (RSD) were between 2.2 and 11.9%; thus, the method is precise for all the compounds studied.

The analytical recovery of the method was determined using an artificial tear solution spiked with three different concentrations of these compounds (10, 25 and 50 μg L^{-1} for DMP, DEP and BBP and 60, 100 and 150 μg L^{-1} for DBP). The spiked samples were analyzed three times, and the recovery calculated using the standard addition graph. The recovery percentages obtained are shown in Table 5. The average analytical recoveries were 101.0, 100.1, 102.5 and 103.9% for DMP, DEP, BBP and DBP, respectively.

The advantage of the proposed method is that it presents good sensitivity and precision, allowing us to detect trace levels of these phthalates with reduced analysis time. Moreover, the simplicity of the method avoids contamination problems, very frequent in phthalates determinations.

3.2. Application of the proposed method to contact lenses cleaning solution

The proposed analytical method has been applied to the analysis of different cleaning solutions commercialized in plastic bottles to check the presence of these phthalates and determine their concentrations. Samples analyzed in the study are available in two different plastic packages, single-dose (samples 4 and 5) and multidose (samples 1, 2 and 3). All samples studied are all-in-one solutions that clean, rinse, disinfect, store, remove proteins and lubricate soft contact lenses. The samples were directly injected into the chromatographic system; no sample preparation process was necessary.

Table 4
Within-run precision assays.

Analyte	% RSD			
	10 μg L^{-1}	25 μg L^{-1}	50 μg L^{-1}	75 μg L^{-1}
DMP	8.6	5.1	3.2	6.4
DEP	11.9	7.4	4.1	10.7
BBP	2.5	4.1	2.4	3.7
	60 μg L^{-1}	100 μg L^{-1}	150 μg L^{-1}	200 μg L^{-1}
DBP	8.5	4.7	2.2	5.4

Table 5
Recovery percentage ± standard deviation (n = 3).

Analyte	% Recovery		
	10 μg L^{-1}	25 μg L^{-1}	50 μg L^{-1}
DMP	98.9 ± 4.9	100.4 ± 5.2	103.8 ± 3.0
DEP	106.4 ± 0.5	93.7 ± 7.7	100.4 ± 4.4
BBP	108.7 ± 1.7	97.2 ± 2.8	101.5 ± 3.0
	60 μg L^{-1}	100 μg L^{-1}	150 μg L^{-1}
DBP	98.5 ± 0.9	99.5 ± 0.6	113.8 ± 3.4

Results obtained are given in Table 6. Analysis of the results shows that the phthalates studied were not detected in the cleaning solutions purchased in multidose containers (60–120 mL). These results can be attributed to the fact that the multi-dose containers are rigid and they do not require the use of plasticizers in their manufacture.

However, in samples purchased in single-dose containers (10 mL) DEP, BBP and DBP were detected and quantified. DMP was not detected in any of the cleaning solutions studied. DEP levels of 9.1 and 0.9 μg L^{-1} were detected in sample 4 and sample 5 respectively. Sample 4 also presented DBP at concentrations of 19.7 μg L^{-1}. BBP was only detected in sample 5 in a concentration of 2.6 μg L^{-1}.

The only differences between sample 1 and sample 4 are the packaging (multidose or single-dose) and the wetting agent used (sodium hyaluronate and polyvinylpyrrolidone, respectively). Both samples are the same brand, but sample 1 was purchased in plastic bottle of 60 mL, and sample 4 was purchased in a single-dose unit of 10 mL. The difference in the results obtained can be attributed to the release of DEP and DBP, used in the manufacturing process to provide elasticity to the packaging of sample 4. Figs. 1 and 2 show the chromatograms obtained when analyzing both samples; significant differences were found.

3.3. Migration study of phthalates from contact lenses to artificial tear solution

In the second part of the study soft contact lenses were subjected to a migration test. The aim was to evaluate the possible release of phthalates from the contact lenses to artificial tear solution and determine their concentrations. The method is based on orbital–horizontal shaking of the contact lenses with artificial tear solution, under strictly controlled conditions of temperature, mode of mechanical agitation, contact time and volume of artificial tear solution. The method is aimed at representing the human eye environment as far as it is possible in the laboratory.

Soft contact lenses classified by the FDA as non-ionic contact lenses containing between 51 and 80% of water were studied. The main component of this type of lens is t hydrogel, which consists of a solid phase (polymer) dispersed in an aqueous phase.

Table 6
Concentration (μg L^{-1}) ± standard deviation (based on three replicates) found in different contact lens cleaning solutions.

Cleaning solutions	DMP	DEP	BBP	DBP
Sample 1	n.d.	n.d.	n.d.	n.d.
Sample 2	n.d.	n.d.	n.d.	n.d.
Sample 3	n.d.	<LOD	n.d.	n.d.
Sample 4	n.d.	9.10 ± 0.47	n.d.	19.66 ± 2.60
Sample 5	n.d.	0.92 ± 0.13	2.62 ± 0.01	n.d.

<LOD: lower than the detection limit. n.d.: not detected.

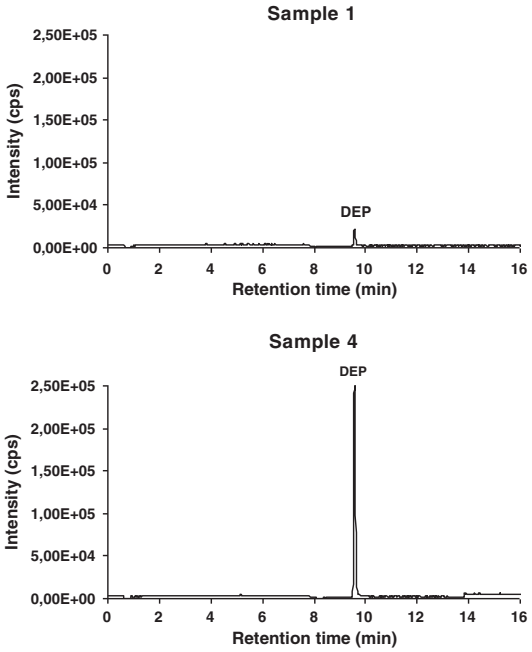


Fig. 1. LC–ES–MS/MS ion chromatogram obtained for DEP in sample 1 (cleaning solution multidose) and sample 4 (cleaning solution single-dose).

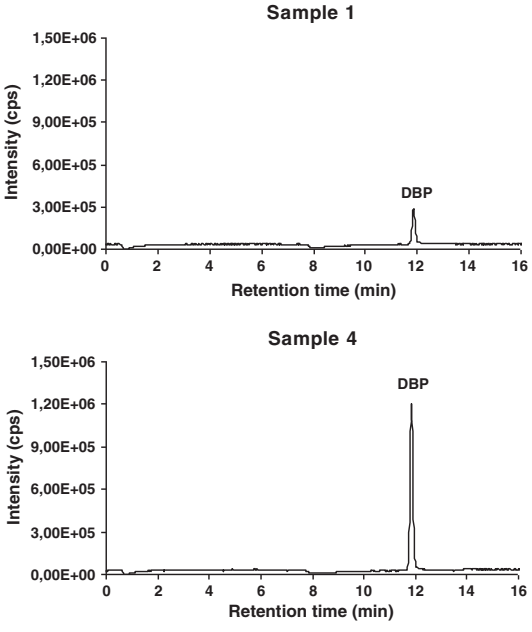


Fig. 2. LC–ES–MS/MS ion chromatogram obtained for DBP in sample 1 (cleaning solution multidose) and sample 4 (cleaning solution single-dose).

Table 7
Concentration ($\mu g\ L^{-1}$) ± standard deviation (based on three replicates) found in artificial tear solution after applying the migration test to the contact lenses.

Cleaning solutions	DMP	DEP	BBP	DBP
Artificial tear solution	n.d.	n.d.	n.d.	n.d.
Sample A	n.d.	<LOD	3.13 ± 0.05	169.85 ± 2.61
Sample B	n.d.	0.27 ± 0.04	3.95 ± 0.04	184.19 ± 1.94

<LOD: lower than the detection limit. n.d.: not detected.

These contact lenses are disposable; each manufacturer sets the period of use, which ranges between 1 and 30 days. Sample A was Omafilcon A contact lens which contains 59% of water and its main monomer is oxietilfosforilcolina methacrylate. Sample B was Hioxifilcon A contact lens (55% of water) that is made of a copolymer of GMA (glycerol monomethacrylate) with HEMA (hydroxyethyl methacrylate).

Each contact lens was introduced into the Teflon-capped amber glass vial with 1 mL of artificial tear solution. Another vial with artificial tear solution but without a contact lens was used as a blank. The vials were incubated at 37 °C with orbital–horizontal shaking at 130 rpm for 24 h. The solutions were then transferred to another glass vial and directly injected into LC–ESI–MS/MS system. No sample preparation process was necessary before the LC–ESI–MS/MS determination. The results obtained are given in Table 7. Results show a significant release of some of the phthalates studied. DBP levels above 165 $\mu g\ L^{-1}$ were detected in both cases. BBP was also detected. The concentrations found were 3.1 and 3.9 $\mu g\ L^{-1}$ for samples A and B respectively. DEP was only detected in sample B at concentrations near the LOD. DMP was not detected in any samples studied. The results obtained in this migration study indicate that the daily use of these contact lenses can be an important source of exposure to these compounds. Research should be developed to minimize the release of these compounds from the contact lenses, or to manufacture contact lenses with materials free of phthalates.

4. Conclusion

To the best of our knowledge, this is the first time that the presence of phthalates was studied in contact lenses and their cleaning solutions. These products are used every day by many consumers who are not aware of the impact of phthalates on human health.

A fast and simple method using LC–ESI–MS/MS has been applied. Results demonstrate the presence of different phthalates in contact lens cleaning solutions purchased in single-dose units. No phthalates were detected in cleaning solutions packaged in semi-rigid or rigid containers. These results are attributed to the plasticizers used in the manufacture of single-dose packaging to make them softer.

Moreover, the migration test confirms the release of some phthalates from soft contact lenses. The phthalate released in the highest concentration was DBP with concentrations above 165 $\mu g\ L^{-1}$ in contact lenses A and B. BBP was also detected in the two contact lenses and the levels found were below 5 $\mu g\ L^{-1}$. DEP was only detected in sample B at levels near the LOD.

The results obtained in this study indicate that the use of certain soft contact lenses might be of concern. Toxicological evidence indicates an association between several of these phthalate esters and risks to human health; therefore, more research studies are needed to know in great detail the release mode and the maximum amount of phthalates that can be released from these products.

Acknowledgment

The authors wish to thank the Xunta de Galicia (Grupo de Referencia Competitivos 2007 (000047-0)) for financial support.

References

[1] 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. L. 12 (2011) 1–89.
[2] A. Gómez-Hens, M.P. Aguilar-Caballos, Social and economic interest in the control of phthalic acid esters, TrAC Trends Anal. Chem. 22 (2003) 847–857.
[3] W. Brack, H.J.C. Klamer, M. Lopez de Alda, D. Barcelo, Effect-directed analysis of key toxicants in European river basins. A review, Environ. Sci. Pollut. Res. Int. 14 (2007) 30–38.
[4] M. Coquery, A. Morin, A. Becue, B. Lepot, Priority substances of the European Water Framework Directive: analytical challenges in monitoring water quality, TrAC Trends Anal. Chem. 24 (2005) 117–127.
[5] P. Roose, U.A.Th. Brinkman, Monitoring organic microcontaminants in the marine environment: principles, programmes and progress, TrAC Trends Anal. Chem. 24 (2005) 897–926.
[6] B.G. Lake, R.J. Price, M.E. Cunnigham, D.G. Walters, Comparison of the effects of di (2-ethylhexyl)adipate on hepatic peroxisome proliferation and cell replication in the rat and mouse, Toxicology 123 (1997) 217–226.
[7] EC-CSTEE, Opinion on the Toxicological Characteristics and Risks of Certain Citrates and Adipates used as Substitute for Phthalates as Plasticizers in Certain Soft PVC Products, Scientific Committee on Toxicity, Ecotoxicity and the Environment (CSTEE), Brussels, 1999.
[8] K.W. Seo, K.B. Kim, Y.J. Kim, J.Y. Choi, K.T. Lee, K.S. Choi, Comparison of oxidative stress and changes of xenobiotic metabolizing enzymes induced by phthalates in rats, Food Chem. Toxicol. 42 (2004) 107–114.
[9] M. Dalgaard, U. Hass, A.M. Vinggaard, K. Jarkfelt, H.R. Lam, I.K. Sorensen, H.M. Sommer, O. Ladefoged, Di(2-ethylhexyl)adipate (DEHA) induced developmental toxicity but non antiandrogenic effects in pre- and postnatally exposed Wistar rats, Reprod. Toxicol. 17 (2003) 163–170.
[10] M. Wormuth, M. Scheringer, M. Vollenweider, K. Ungerbühler, What are the sources of exposure to eight frequently used phthalic acid esters in Europeans? Risk Anal. 26 (2006) 803–824.
[11] K. Miyata, K. Shiraishi, S. Houshuyama, N. Imatanaka, T. Umano, Y. Minobe, K. Yamasaki, Subacute oral toxicity study of di(2-ethylhexyl)adipate based on the draft protocol for the "Enhanced OECD test guideline no. 407", Arch. Toxicol. 80 (2006) 181–186.
[12] 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, Off. J. Eur. Union. L. 396 (2006) 1–849 (Appendix 6).
[13] Commission Decision 1999/815/EC of 7 December 1999 adopting measures prohibiting the placing on the market of toys and childcare articles intended to be placed in the mouth by children under three years of age made of soft PVC containing one or more of the substances di-iso-nonyl phthalate (DINP), di(2-ethylhexyl) phthalate (DEHP), dibutyl phthalate (DBP), di-iso-decyl phthalate (DIDP), di-n-octyl phthalate (DNOP), and butylbenzyl phthalate (BBP), Off. J. Eur. Union. L. 315 (1999) 46–49.
[14] Directive 2005/84/EC of the European Parliament and of the Council of 14 December 2005 amending for the 22nd time Council Directive 76/769/EEC on the approximation of the laws, regulations and administrative provisions of the Member States relating to restrictions on the marketing and use of certain dangerous substances and preparations (phthalates in toys and childcare articles), Off. J. Eur. Union. L. 344 (2005) 40–43.
[15] A. Fankhauser-Noti, K. Grob, Blank problems in trace analysis of diethylhexyl and dibutyl phthalate: investigation of the sources, tips and tricks, Anal. Chim. Acta 582 (2007) 353–360.
[16] G. Prokupková, K. Holadová, J. Poustka, J. Hajslová, Development of a solid-phase microextraction method for the determination of phthalic acid esters in water, Anal. Chim. Acta 457 (2002) 211–223.
[17] A.M. Reid, C.A. Brougham, A.M. Fogarty, J.J. Roche, An investigation into possible sources of phthalate contamination in the environmental analytical laboratory, Int. J. Environ. Anal. Chem. 87 (2007) 125–133.
[18] O.S. Fatoki, A. Noma, Determination of phthalate esters in the aquatic environment, S. Afr. J. Chem. 54 (2001) 1–15.
[19] L. Brossa, R..M. Marcé, F. Borrull, E. Pocurull, Occurrence of twenty-six endocrine-disrupting compounds in environmental water samples from Catalonia, Spain, Environ. Toxicol. Chem. 24 (2005) 261–267.
[20] K. Luks-Betlej, P. Popp, B. Janozska, H. Paschke, Solid-phase microextraction of phthalates from water, J. Chromatogr. A 938 (2001) 93–101.
[21] Y.-L. Feng, J. Zhu, R. Sensenstein, Development of a headspace solid-phase microextraction method combined with gas chromatography mass spectrometry for the determination of phthalate esters in cow milk, Anal. Chim. Acta 538 (2005) 41–48.
[22] K. Kambia, T. Dine, B. Gressier, A.F. Germe, M. Luyckx, C. Brunet, L. Michaud, F. Gottrand, High-performance liquid chromatographic method for the determination of di(2-ethylhexyl) phthalate in total parenteral nutrition and in plasma, J. Chromatogr. B 755 (2001) 297–303.
[23] B. Cavaliere, B. Macchione, G. Sindona, A. Tagarelli, Tandem mass spectrometry in food safety assessment: the determination of phthalates in olive oil, J. Chromatogr. A 1205 (2008) 137–143.
[24] E. Cortazar, L. Bartolomé, A. Delgado, N. Etxebarria, L.A. Fernández, A. Usobiaga, O. Zuloaga, Optimization of microwave-assisted extraction for the determination of nonylphenols and phthalate esters in sediment samples and comparison with pressurized solvent extraction, Anal. Chim. Acta 534 (2005) 247–254.

[25] Guo Bao-Yuan, B. Wen, Shan Xiao-Quan, Zhang Shu-Zheng, Lin Jin-Ming, Separation and determination of phtalates by micelar electrokinetic chromatography, J. Chromatogr. A 1095 (2005) 189–192.
[26] A. Adeniyi, M. Dayomi, P. Siebe, O. Okedeyi, An assessment of the levels of phthalate esters and metals in the Muledane open dump, Thohoyandou, Limpopo province, South Africa, Chem. Central J. 2 (2008) 9–17.
[27] K. Kato, S. Shoda, M. Takahashi, N. Doi, Y. Yoshimura, H. Nakazawa, Determination of three phthalate metabolites in human urine using on-line solid-phase extraction–liquid chromatography–tandem mass spectrometry, J. Chromatogr. B 788 (2003) 407–411.
[28] K. Kato, M.J. Silva, L.L. Needham, A.M. Calafat, Determination of total phthalates in urine by isotope-dilution liquid chromatography–tandem mass spectrometry, J. Chromatogr. B 814 (2005) 355–360.
[29] S. Takatori, Y. Kitagawa, M. Kitagawa, H. Nakazawa, S. Hori, Determination of di (2-ethylhexyl)phthalate and mono(2-ethylhexyl)phthalate in human serum using liquid chromatography–tandem mass spectrometry, J. Chromatogr. B 804 (2004) 397–401.
[30] A.O. Earls, I.P. Axford, J.H. Braybrook, Gas chromatography–mass spectrometry determination of the migration of phthalate plasticisers from polyvinyl chloride toys and childcare articles, J. Chromatogr. A 983 (2003) 237–246.
[31] T. Yuzawa, Ch. Watanabe, R.R. Freeman, S. Tsuge, Rapid and simple determination of phthalates in plastic toys by a thermal desorption–GC/MS method, Anal. Sci. 25 (9) (2009) 1057–1058.
[32] T. Rothenbacher, W. Schwack, Rapid and nondestructive analysis of phthalic acid esters in toys made of poly(vinyl chloride) by direct analysis in real time single-quadrupole mass spectrometry, Rapid Commun. Mass Spectrom. 23 (17) (2009) 2829–2835.
[33] D. De Orsi, L. Gagliardi, R. Porra, S. Berri, P. Chimenti, A. Granese, I. Carpani, D. Tonelli, A environmentally friendly reversed-phase liquid chromatography method for phthalates determination in nail cosmetics, Anal. Chim. Acta 555 (2006) 238–241.
[34] C. Pérez-Feás, M.C. Barciela-Alonso, A. Sedes-Díaz, P. Bermejo-Barrera Phthalates, Determination in pharmaceutical formulae used in parenteral nutrition by LC–ES–MS: importance in public health, Anal. Bioanal. Chem. 397 (2010) 529–535.
[35] G.K. Mortensen, K.M. Main, A.M. Andersson, H. Leffers, N.E. Skakkebaek, Determination of phthalate monoesters in human milk, consumer milk, and infant formula by tandem mass spectrometry (LC–MS/MS), Anal. Bioanal. Chem. 382 (2005) 1084–1092.
[36] L.K. Soerensen, Determination of phthalates in milk and milk products by liquid chromatography/tandem mass spectrometry, Rapid Commun. Mass Spectrom. 20 (2006) 1135–1143.
[37] M.A. Faouzi, T. Dine, B. Gressier, K. Kambia, M. Luyckx, D. Pagniez, C. Brunet, M. Cazin, A. Belabed, J.C. Cazin, Exposure of hemodialysis patients to di-2-ethylhexyl phthalate, Int. J. Pharm. 180 (1999) 113–121.
[38] M.J. Silva, J.L. Preau Jr., L.L. Needham, A.M. Calafat, Cross validation and ruggedness testing of analytical methods used for the quantification of urinary phthalate metabolites, J. Chromatogr. B. 873 (2008) 180–186.
[39] M. Del Carlo, A. Pepe, G. Sacchetti, D. Compagnone, D. Mastrocola, A. Cichelli, Determination of phthalate esters in wine using solid-phase extraction and gas chromatography–mass spectrometry, Food Chem. 111 (2008) 771–777.
[40] M.A. Mohamed, A.S. Ammar, Quantitative analysis of phthalates plasticizers in traditional Egyptian foods (Koushary and Foul Medams), Black tea, instant coffee and bottled waters by solid phase extraction–capillary gas chromatography–mass spectroscopy, Am. J. Food Technol. 3 (2008) 341–346.
[41] A. Zafra-Gómez, O. Ballesteros, A. Navalón, J.L. Vilches, Determination of some endocrine disrupter chemicals in urban wastewater samples using liquid chromatography–mass spectrometry, Microchem. J. 88 (2008) 87–94.
[42] K. Holadová, G. Prokupková, J. Hajslová, J. Poustka, Headspace solid-phase microextraction of phthalic acid esters from vegetable oil employing solvent based matrix modification, Anal. Chim. Acta 582 (2007) 24–33.
[43] M.T. Kelly, M. Larroque, Trace determination of diethyl phthalate in aqueous media by solid-phase microextraction–liquid chromatography, J. Chromatogr. A 841 (1999) 177–185.
[44] C. Cháfer-Pericás, P. Campíns-Falcó, M.C. Prieto-Blanco, Automatic in-tube SPME and fast liquid chromatography: a cost-effective method for the estimation of dibutyl and di-2-ethylhexyl phthalates in environmental water simples, Anal. Chim. Acta 610 (2008) 268–273.
[45] A. Prieto, O. Telleria, N. Etxebarria, L.A. Fernández, A. Usobiaga, O. Zuloaga, Simultaneous preconcentration of a wide variety of organic pollutants in water samples: comparison of stir bar sorptive extraction and membrane-assisted solvent extraction, J. Chromatogr. A 1214 (2008) 1–10.
[46] B.L.L. Tan, D.W. Hawker, J.F. Mueller, L.A. Tremblay, H.F. Chapman, Stir bar sorptive extraction and trace analysis of selected endocrine disruptors in water, biosolids and sludge samples by thermal desorption with gas chromatography–mass spectrometry, Water Res. 42 (2008) 404–412.
[47] R. Gibson, M.J. Wang, E. Padgett, A.J. Beck, Analysis of 4-nonylphenols, phthalates, and polychlorinated biphenyls in soils and biosolids, Chemosphere 61 (2005) 1336–1344.
[48] J.M. Aranda, G.A. O'Connor, G.A. Eiceman, Effects of sewage sludge on bis(2-ethylhexyl) phthalate uptake by plants, J. Environ. Qual. 18 (1989) 45–50.
[49] M. Bonini, E. Errani, G. Zerbinati, E. Ferri, S. Girotti, Extraction and gas chromatographic evaluation of plasticizers content in food packaging films, Microchem. J. 90 (2008) 31–36.
[50] P. Bermejo Barrera, M.C. Barciela Alonso, C. Pérez Feas, E. Peña Vázquez, P. Herbello Hermelo, Bisphenol A and Phthalates: Uses, Health Effects and Environmental Risks. Chapter 2978-1-60741-701-9, 2011.
[51] USEPA Method 506 revision 1.1, Determination of Phthalates and Adipate Esters in Drinking Water by Liquid–Liquid Extraction or Liquid–Solid Extraction and Gas Chromatography with Photoionization Detection, National Exposure Research Laboratory Office of Research and Development U.S. Environmental Protection Agency, Cincinnati, Ohio 45268, 1995.
[52] C. Pérez-Feás, M.C. Barciela-Alonso, P. Bermejo-Barrera, Direct LC–ES–MS/MS determination of phthalates in physiological saline solutions, J. Chromatogr. B 879 (2011) 231–235.
[53] H.M. Koch, L.M. Gonzalez-Reche, J. Angerer, On-line clean-up by multidimensional liquid chromatography–electrospray ionization tandem mass spectrometry for high throughput quantification of primary and secondary phthalate metabolites in human urine, J. Chromatogr. B 784 (2003) 169–182.
[54] R.A. Gimeno, R.M. Marcé, F. Borrull, Determination of plasticizers by high-performance liquid chromatography and atmospheric pressure chemical ionization mass spectrometry in water and sediment samples, Chromatographia 58 (2003) 37–41.