Determination of phthalates in food simulants and liquid samples using ultrasound-assisted dispersive liquid–liquid microextraction followed by solidification of floating organic drop

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A B S T R A C T

A simple, inexpensive, reliable and environmentally friendly method based on ultrasound-assisted dispersive liquid–liquid microextraction followed by solidification of floating organic drop and gas chromatography–flame ionization detector was developed for the simultaneous determination of five phthalates in food simulants and different food and water samples. Parameters affecting the extraction process were studied and optimized by univariate analysis and experimental design. Under optimum conditions, method showed good linearity in the selected range (R^2 from 0.993 to 0.995). Limits of detection (LOD) ranged from 0.64 to 2.82 μg L^{-1} and enrichment factors from 854 to 1893. Precision of the method, expressed as relative standard deviation, was checked at two levels obtaining good results (2.7–9.3%). Accuracy of the method was checked in food simulants also obtaining good results. The method allowed determination of phthalates in food simulants at lower concentrations than the migration limits established by the European Union. The developed method was also applied to real water, wine, vinegar and soft drink samples obtaining acceptable results.

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1. Introduction

Phthalate esters (PAEs) are used in a wide range of industrial and domestic applications. Particularly, they are widely used as plasticizers in polymeric materials to increase their flexibility through weak secondary molecular interactions with polymer chains. Since PAEs are only physically bound to the polymer chains, they can be easily released from products and migrate into the food, beverages or water in direct contact (Hongyuan, Baomi, Jingjing, & Kyung, 2010; Hongyuan, Xiaoling, & Baomi, 2011; Hongyuan, Xiaoling, & Kuo, 2012). In addition, in the food packaging industry, they are not used only as plasticizers, but also as adhesives, offset printing inks and lacquers. Migration of PAEs has received considerable attention in recent years because of their effect in human health, being considered endocrine disruptors and possible carcinogens among others (Batlle & Nerín, 2004; United States Environmental Protection Agency, 2005). Indeed, due to their ubiquity and their potential risk for human health and environment, several of them have been included in the priority list of pollutants of the United States Environmental Protection Agency (2013). In Europe, restrictions on the quantities of substances able to migrate into the food are imposed on materials used for food packaging (Commision regulation (EU) N° 10/2011, 2011). These restrictions are known as specific migration limits (SML) and they are defined as "the maximum permitted amount of a given substance released from a material or article into food or food simulants" and expressed in mg substance per kg food. Compliance with these limits has to be checked in food simulants as models for different food categories. In addition, determination of the PAEs in real water, beverages and food samples arriving at consumers is also important.

Owing to the low concentration of PAEs and to the complexity of sample matrices, a preconcentration and separation step is often required prior to analysis. Recent trends in sample preparation include miniaturization of classical extraction techniques, getting generally simpler, faster and greener techniques. In this way, liquid phase microextraction (LPME) emerged as a solvent-minimized version of the classic liquid–liquid extraction in which only several microliters of extractant are used. From LPME introduction, different approaches classifiable into three main categories have been developed: single drop microextraction (SDME), dispersive liquid–liquid microextraction (DLLME) and

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hollow-fiber liquid-phase microextraction (HF-LPME) (Asensio-Ramos, Ravelo-Pérez, González-Curbelo, & Hernández-Borges, 2011).

In DLLME (Rezaee et al., 2006), dispersion of the extractant is achieved by the addition of a third solvent (dispersant), miscible with both phases. Due to the formed cloudy solution, superficial area in contact between these two phases is larger, and thus, extraction faster. After extraction, sample must be centrifuged in order to separate both phases. Ultrasonic radiation is used for the acceleration of mass transfer process, in ultrasound-assisted dispersive liquid—liquid microextraction (UA-DLLME) (Lv et al., 2014). The cloudy solution can also be only caused by ultrasound radiation in the called ultrasound-assisted emulsification micro-extraction (USAEME) (Regueiro, Llompart, Garcia-Jares, Garcia-Monteagudo, & Cela, 2008).

LPME avoids the problem of the large solvent volumes in classical liquid—liquid extraction, but extraction solvents used in this technique are still generally toxic. In this respect, a new approach based on the solidification of floating organic drop (SFOD) was proposed, in which an extractant with lower density than water, low toxicity and proper melting point (10—30 °C) was used (Khalili Zanjani, Yamini, Shariati & Jönsson, 2007). In that way, after extraction, the organic droplet is solidified in an ice bath and then, easily collected with a spatula, melted and conducted to analytical determination. These type of solvents have been used in different LPME techniques, giving rise to different combined techniques (Viñas, Campillo, & Andruch, 2015), such as DLLME-SFOD (Leong & Huang, 2008), UA-DLLME-SFOD (Wang, Zhu, Cui, Miao, & Chen, 2014) and USAEME-SFOD (Bordagaray, Garcia-Arrona, & Millán, 2014). Those techniques combine the advantages of both former techniques, being all of them environmentally friendly due to the use of low volumes of practically non toxic solvents.

Analytical methods for determination of the PAEs are mainly based on chromatographic techniques, such as gas chromatography (GC) or high pressure liquid chromatography (HPLC). Mass spectrometry based detectors have been widely applied for the PAEs determination by these two techniques, but less sensitive and more affordable techniques such as HPLC-DAD and GC-FID have also been used (Lv, Hao, & Jia, 2013; Yang et al., 2015). A wide range of combinations of these detection techniques with different pre-treatment methods have been used for the phthalate determination in food or water samples (Farajzadeh, Sorouraddin, & Afshar Mogaddam, 2015).

The aim of this work was to develop a simple, low cost and reliable analytical method for simultaneous determination of five PAEs in food simulants and liquid food and water samples using UA-DLLME-SFOD as a preconcentration technique followed by GC-FID. Up to our knowledge, amongst phthalates, only di-(2-ethylhexyl) phthalate has been determined combining the solvents used in SFOD techniques and GC-FID (Yamini, Ghambarian, Khalili-Zanjani, Faraji, & Shariati, 2009) and often determination of the PAEs using a dispersive technique is carried out using highly toxic chlorinated solvents (Cinelli, Avino, Notardonato, Centola, & Russo, 2013; Hongyuan et al., 2012; Xue, Zhang, Wang, Wang, & Du, 2014). The UA-DLLME-SFOD technique combines advantages of both DLLME and SFOD techniques; it is rapid due to the high superficial area between phases and it is environmentally friendly due to the solvents used. In this work, influence of different parameters in extraction was investigated with the aid of experimental design. After optimization, procedure was validated and it was finally applied to the determination of the PAEs in food simulants, and different water and liquid food samples.

2. Experimental

2.1. Reagents and standards

Dibutyl phthalate (DBP, 99%), benzyl butyl phthalate (BBP, 98%), dicyclohexyl phthalate (DCHP, 99%), bis(2-ethylhexyl) phthalate (DEHP, 99.5%), di-n-octyl phthalate (DNOP, 99.5%), 1-undecanol (99%), 2-dodecanol (99%), n-hexadecane (99%), Br-hexadecane (97%), 1-chlorooctadecane (96%) and 1,10-dichlorodecane (99%) were purchased from Sigma—Aldrich (Barcelona, Spain). 1-Dodecanol (98%), methanol (99.8%), acetonitrile (99.7%), ethanol absolute (99.5%), acetone (99.5%) and sodium chloride (99.5%) were supplied by Panreac (Madrid, Spain). Doubly distilled water was used throughout this work.

Individual stock solutions of PAEs and a mixed stock solution (1 g L^{-1} of each analyte) were prepared in methanol and stored in amber-colored vials in the refrigerator. Working solutions were prepared weekly by dilution of the stock one with methanol, and they were preserved in the refrigerator.

All the glassware used in this research was previously soaked and washed with acetone and dried at 240 °C for at least 4 h.

2.2. Samples

Food simulants were prepared in the laboratory as described in regulation (Commission regulation (EU) No 10/2011, 2011): Simulant B (3% (w/v) acetic acid/water solution) and simulant C (20% (v/v) ethanol water solution).

Different commercial samples (three mineral water, three vinegars, four wines (2 packed in glass bottles and 2 in Tetrapak box), three soft drinks and one sangria) were purchased from one local shopping center. Recovery tests in commercial samples were carried out using appropriate dilutions. Samples were spiked adding 50 μL of a working solution containing all the analytes to the final solution.

2.3. Instrumentation and chromatographic conditions

Chromatographic analyses were performed on a HP 6890N (Agilent Technologies, Wilmington, DW, USA) gas chromatographer equipped with a split/splitless injector used in splitless mode and a flame ionization detector (FID). Injector temperature was 300 °C and splitless time was 3 min. The column used was a HP-5 (30 m × 0.250 mm × 0.25 μm film thickness) capillary column (Agilent Technologies). The carrier gas was helium with a 1.3 mL min^{-1} flow. The oven temperature program was: 160 °C for 1 min, increased to 200 °C at a rate of 10 °C min^{-1}, and then a ramp of 2 °C min^{-1} to 255 °C. Detector temperature was 300 °C.

Extractions were carried out in a Bandelin Sonorex Digitec DT100H ultrasound bath (ALLPAX, Papenburg, Germany) with 35 kHz ultrasound frequency. Centrifugation was performed on a Selecta centrifuge (Barcelona, Spain). The cooling bath was a Julabo F26 (Augsburg, Germany). The heating bath was a Lauda ecoline re104. Experimental design was performed and evaluated with Statistica software (StatSoft, Tulsa, USA).

2.4. UA-DLLME-SFOD procedure

10 mL sample solution containing 25 g L^{-1} NaCl was placed in a 40 mL glass vial. 0.75 mL of acetonitrile and a mixed solution of PAEs standards were spiked, and the resulting solution was placed in a thermostatic bath for 5 min at 35 °C. Then, 15 μL of n-hexadecane (extraction solvent) was added to the solution, it was gently shaken by hand and placed into an ultrasonic bath for sonication at 35 kHz and 35 °C ± 1 °C during 5 min. As a result,