Journal of Chromatography B, 965 (2014) 164–172

Contents lists available at ScienceDirect

Journal of Chromatography B

journal homepage: www.elsevier.com/locate/chromb

Simultaneous solid phase extraction coupled with liquid chromatography tandem mass spectrometry and gas chromatography tandem mass spectrometry for the highly sensitive determination of 15 endocrine disrupting chemicals in seafood

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ARTICLE INFO

Article history:
Received 4 April 2014
Received in revised form 20 June 2014
Accepted 21 June 2014
Available online 28 June 2014

Keywords:
Simultaneous pretreatment
Phthalate esters
Monoalky phthalate esters
Alkylphenols
Bisphenol A
Seafood

ABSTRACT

This study aimed to develop a sensitive and reliable multi-residue method for the determination of trace amounts of endocrine disrupting chemicals including five phthalate esters (PAEs), five monoalky phthalate esters (MPEs), four alkylphenols (APs) and bisphenol A (BPA) in seafood. Ultrasonic liquid extraction was selected for extraction based on acetonitrile, instead of frequently-used n-hexane, due to its lower background of PAEs. Application of solid phase extraction (SPE) with primary secondary amine (PSA, 1 g/6 mL) cartridge achieved the relatively low matrix effects for MPEs and BPA in seafood. To our knowledge, it is the first study reporting about simultaneous extraction and purification of PAEs, MPEs, APs and BPA in biota samples. To obtain the maximum sensitivity, both liquid chromatography tandem mass spectrometry (LC–MS/MS) and gas chromatography tandem mass spectrometry (GC–MS/MS) were applied for analysis. This method was validated and tested on fish, mollusk and prawn. Sufficient linearity was verified by Mandel's fitting test for the matrix-matched calibrations used in this study for MPEs, APs and BPA, between 0.5 ng/g and 200 ng/g or 400 ng/g. And correlation coefficients of all calibrations suppressed 0.99 for all analytes. Good recoveries were obtained, ranging from 60% to 127% for most compounds. The sensitivity was good with method detection limits (MDLs) of 0.015–2.2 ng/g wet weight (ww) for all compounds. Most MDLs are much lower than those in previous reports. The sensitive method was then applied on real fish, mollusk and prawn samples from the Yangtze River Delta sea area (China), and all the target compounds were detected with the maximum concentrations of PAEs, MPEs, APs and BPA up to 219.3 ng/g ww, 51.4 ng/g ww, 62.0 ng/g ww and 8.6 ng/g ww, respectively.

1. Introduction

Phthalate esters (PAEs), alkylphenols (APs) and bisphenol A (BPA), which consist of one or two benzene rings, are typical compounds of endocrine disrupting chemicals (EDCs). They are manufactured mostly as additives or precursors in products such as plastics, surfactants and resin [1,2]. Because of their huge output, wide dispersive use and low removal efficiency, these compounds are found in many matrices and cannot be avoided in our environment. Occurrence of PAEs, APs and BPA in environmental matrices, especially in water and food, has aroused considerable concern [3]. Previous reports showed that many EDCs are likely to cause reproductive, teratogenic and developmental toxicities even at low concentrations [4]. In recent years, study of the main primary metabolites of PAEs, monoalky phthalate esters (MPEs), has also been carried out because PAEs can be easily degraded in vivo [5] and MPEs could reflect the internal exposure of biota to PAEs [6]. Many countries have strengthened regulations on use of PAEs, APs and BPA. In China, the quality limits for PAEs in food and food additives are set at 1.5 mg/kg for di (2-ethylhexyl) phthalate (DEHP) and 0.3 mg/kg for di-n-butyl phthalate (DBP) according to the announcement of Chinese Ministry of Health in June, 2011. In 2010, Canada first listed BPA as toxic compound [7]. The European Council Directive (2003/53/EC) on the marketing and use of nonylphenol (NP) intends to decrease its consumption [8].

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http://dx.doi.org/10.1016/j.jchromb.2014.06.024
1570-0232/

The relatively low concentrations of PAEs, MPEs, APs and BPA in marine biota and the complexity of matrices make the enrichment and cleanup steps unavoidable prior to instrumental analysis. To the present, some sample pretreatment methods, like ultrasonic extraction (USE) [9], coupled with gel permeation chromatography (GPC) [10] or solid phase extraction (SPE) [11] have been frequently used for extraction and purification of PAEs, MPEs, APs and BPA in seafood. Furthermore, many studies carried out simultaneous sample pretreatment of these compounds in water, urine, food and biota. Mortazavi et al. [12] used Soxhlet extractor to extract 4-nonylphenol (4-NP), octylphenol (OP) and BPA in fish for 10 h with dichloromethane followed with purification process based on silica gel column. Niu et al. [13] applied liquid extraction based on acetonitrile followed with on-line SPE system to pretreat NP, OP and BPA in cereals. Sun et al. [14] developed temperature controlled ionic liquid dispersive liquid–liquid microextraction to pretreat DEHP and mono-2-ethylhexyl ester (MEHP) in human urine. Wang et al. [15] and Chen et al. [16] used SPE to prepare urine samples for the determination of MPEs and BPA. Bono-Blay et al. [17] used SPE cartridge to extract PAEs, APs and BPA in water for more than 1 h. However, to the best of our knowledge, none of these researches has reported about the simultaneous sample preparation of PAEs, MPEs, APs and BPA in seafood before. The sample pretreatment is always time-consuming and may take about 61% of the whole chemical analysis time [18]. It required a long time to pretreat PAEs, MPEs, APs and BPA separately in seafood. In order to shorten the pretreatment period, simultaneous sample pretreatment method for these compounds in seafood is needed.

In addition, many high sensitive analytical techniques, like liquid chromatography tandem mass spectrometry (LC–MS/MS) and gas chromatography tandem mass spectrometry (GC–MS/MS) [19,20], have been used for analysis of these analyses in recent years. GC–MS/MS has been frequently used for the analysis of PAEs due to the high volatility. As for MPEs, APs and BPA, the low volatility limits the direct application of GC–MS/MS. Although derivation steps can help to enhance the volatility, they are time-consuming and also, however, result in the transformation of MPEs [5]. Thus LC–MS/MS is always applied in analysis of MPEs, APs and BPA [16,19]. Simultaneous detection would be more convenient, but we would still use different techniques to obtain low method detection limits (MDLs).

This study aimed to develop a simple, sensitive and robust method to determine PAEs, MPEs, APs and BPA in seafood. Simultaneous sample pretreatment of 15 target compounds including five PAEs, five MPEs, four APs and BPA, based on USE followed by SPE purification, were developed, and various operational conditions such as selection of extraction solvent and SPE cartridge, elution of SPE and the gradient of liquid phase were optimized. USE was applied to the extraction procedure in this study because USE with glass centrifuge tube got the least blank contamination of PAEs compared to other extraction techniques with plastic materials. Acetonitrile replaced frequently-used n-hexane [21] and dichloromethane [12] in USE because of the high background levels of PAEs in n-hexane in our study and the toxicity of dichloromethane. As for SPE procedure, separate elution on PSA cartridge reduced matrix effects for MPEs and BPA, significantly. Reversed-phase high performance liquid chromatography/electrospray ionization in negative mode coupled triple quadrupole mass spectrometry (HPLC/ESI-MS/MS) was selected for the analysis of MPEs, APs and BPA, and gas chromatography/electron impact ionization in positive mode coupled triple quadrupole mass spectrometry (GC/EI-MS/MS) was selected for PAEs to obtain the maximum sensitivity. In addition, the developed method was validated by assessing linearity, precision and accuracy as well as the matrix effect, and further applied to seafood samples, including fish, prawn and mollusk, from coastal areas of Zhejiang Province in China.

2. Experiment

2.1. Reagents and materials

GC grade acetonitrile was supplied by Scharlau (Barcelona, Spain). HPLC grade methanol (MeOH), acetone, acetic acid (99% purity) and formic acid (96% purity) were purchased from Tedia (OH, USA). Analytical reagent ammonia solution (25–28% purity) and sodium chloride (NaCl) were supplied by Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China). Ultrapure water was produced by a Millipore Milli-Q system (MA, USA).

The divinylbenzene/N-vinylpyrrolidone copolymer (Oasis HLB, 3 cc/60 mg) glass cartridges were purchased from Waters (MA, USA); the primary secondary amine (ProElut PSA, 1 g/6 mL) glass cartridges were supplied by Dikma (Beijing, China); AccuBond Florisil cartridges were purchased from Agilent Technologies (CA, USA).

The analytical standards dimethyl phthalate (DMP) (99.5% purity), diethyl phthalate (DEP) (99.5% purity), DBP (99.0% purity), DEHP (98.5% purity), di-n-octyl phthalate (DNOP) (99.5% purity), mono-methyl ester (MMP) (98.0% purity), mono-n-butyl ester (MBP) (98.0% purity), MEHP (91.0% purity) were obtained from Dr. Ehrenstorfer (Augsburg, Germany). Mono-ethyl ester (MEP) and mono-n-octyl ester (MNOP) were supplied by Accustandard (CA, USA) and Toronto Research Chemicals Inc. (Ontario, Canada), respectively. BPA, 4-NP, 4-n-nonylphenol (4-n-NP), 4-octylphenol (4-OP) and 4-tert-octylphenol (4-t-OP) were purchased from Sigma–Aldrich (Poole, UK), and the purities are all above 98%.

Individual standard stock solutions of the 15 target compounds with concentration of 1000 mg/L were all prepared in acetonitrile and stored in a refrigerator in the dark at −20 °C. Standard native solutions were prepared from the stock solutions by acetonitrile dilution and eight points were set at 0.5, 5, 10, 25, 50, 100, 200 and 400 μg/L for working solutions. Matrix-matched standard solutions were also obtained to compensate for matrix effects at 0.5, 5, 10, 25, 50, 100, 200 and 400 ng/g by spiking extracts of blank matrix, including fish, prawn and mollusk. These solutions were all stored in the dark at −4 °C prior to use.

All glasswares used in experiment were washed by acetone twice and NaCl was baked at 500 °C for 5 h to elude the contained target compounds prior to use.

2.2. Instrumentation

2.2.1. LC–MS/MS analysis

Separation and quantification of MPEs, APs and BPA were performed by using a liquid chromatography (1200 series, Agilent Technologies, CA, USA), equipped with an automatic injector (CTC analytics, Swiss), coupled to a triple-stage quadrupole mass spectrometer (API5000 QTrap, ABSciex, USA). The injection volume was set at 10 μL. As for APs, the chromatographic separation was carried out with a Waters Xbridge C_{18} column (5 μm, 100 mm × 2.1 mm (i.d.)) (Waters, MA, USA) at a flow rate of 0.25 mL/min. The column temperature was kept at 40 °C. The mobile phases were (A) 0.3% ammonium hydroxide in MilliQ water and (B) MeOH with the following gradient: from 20% to 65% (B) in 2 min; from 65% to 100% (B) in 4 min; keeping 100% (B) for 6 min; from 100% to 20% (B) in 0.2 min; keeping 20% (B) for 6.8 min. The column for BPA and MPEs was an Agilent Eclipse XDB C_8 column (5 μm, 100 mm × 4.6 mm (i.d.)) (Agilent technologies, CA, USA). And column was kept at 30 °C. The mobile phase compositions were: (A) 0.01% acetic acid in MilliQ water; (B) acetonitrile. The solvents were mixed as follows: 0 min,