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Determination of organophosphate flame retardants and plasticizers in lipid-rich matrices using dispersive solid-phase extraction as a sample cleanup step and ultra-high performance liquid chromatography with atmospheric pressure chemical ionization mass spectrometry

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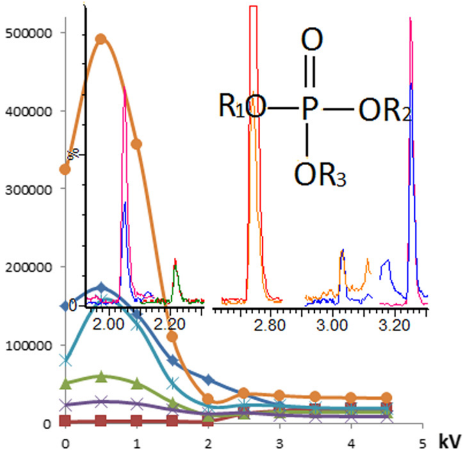
HIGHLIGHTS

- A sensitive method was developed for determination of low organophosphate ester (OPE) levels in lipid-rich samples.
- The OPE method is based on dispersive solid phase extraction and UHPLC-MS/MS-APCI(+).
- OPE quantification limits were 0.06–0.29 ng/g (egg) and 0.05 and 0.50 ng/g (liver).
- OPEs were determined at the sub-ppb levels in environmental bird egg and fish samples.

GRAPHICAL ABSTRACT



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ABSTRACT

A fast, robust and highly sensitive analysis method for determination of trace levels of organophosphate ester (OPE) flame retardants and plasticizers in lipid-rich samples was presently developed, and based on ultra-high performance liquid chromatography-tandem mass spectrometry coupled to a positive atmospheric pressure chemical ionization source (UHPLC-MS/MS-APCI(+)). The target OPEs in the sample were extracted from the biota samples, such as egg and liver, by ultrasonic extraction, and cleaned up further by dispersive solid phase extraction (d-ESP). As a result, background contamination was largely reduced. Different dispersive ESP sorbents were tested and primary secondary amine (PSA) bonded silica sorbents showed the best recoveries for these target OPEs. The recoveries obtained were in the range 54–113% (RSD < 17%), with method limits of quantification (MLOQs) ranging between 0.06 and 0.29 ng/g in egg, and 0.05 and 0.50 ng/g w.w. in liver sample. The matrix effects (MEs) associated with using APCI(+) and ESI(+) sources were investigated. APCI(+) showed much less ion suppression than ESI (+) for the determination of these OPEs. For egg and liver samples, the APCI(+) ME values ranged from 40% to 94%, while ESI(+) ME values ranged from 0% to 36%. Although APCI(+) was used for the determination of

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OPEs, the ionization mechanism might mainly be a thermospray ionization process. This UHPLC-MS/MS-APCI(+) method showed good response linearity for calibration (R2 > 0.99). The proposed method was applied to real environmental bird egg and fish samples, where several OPE were quantifiable and different OPE patterns was observed between samples.

1. Introduction

Organophosphate ester (OPE) flame retardants and plasticizers are high volume production chemicals and widely employed as additives in plastic, paints, glues, lacquers, varnishes and others to enhance their properties [1,2]. Following the phase-out of polybrominated diphenyl ethers (PBDEs), due to their persistence, bioaccumulative and toxic properties, OPEs are increasingly used as alternative flame retardants in many products [1]. In most usages, these chemicals are dispersed into the host material, not chemically bound, and they can be released from host material into the surrounding environment, especially into air and wastewater [1,3–8]. Therefore, OPEs are of great concern since being detected as pollutants in indoor environment in 1990's [3,9,10].

OPEs have been found ubiquitously in various environmental matrices. In addition to high concentration levels found in house dust samples, they have also been determined in municipal wastewater, surface and drinking water as well as human breast milk, fish and some other biota samples [3–5,9,11–17]. OPEs elicit genotoxic and neurotoxic effects [18] and some chlorinated OPEs have log *K*ow values ranging from 1 to 5 indicating the potential to persist and bioaccumulate in the environment [1,3,19].

Although GC–MS and LC-MS based methods have been widely used for determination of OPEs in dust and water samples with no sample clean-up process, analysis of very low level and trace OPEs in biotic samples remains a challenge in terms of accuracy and precision. There are relatively few studies that have investigated OPEs in wildlife and fish [13–16] and the information on OPE bioaccumulation is scarce [16,17]. The results of the first worldwide interlaboratory study on OPE flame retardants showed large coefficients of variation (CV) for spiked fish oil and sediment samples, even though relatively high concentrations of target OPEs were spiked into the sample matrices, e.g. fish oil sample spiked with 20–140 ng/g of OPEs [20]. Given that many environmentally relevant OPEs are currently in use, there are some persistent analytical problems, such as sometimes substantially high background contamination as a result of the current methods being used, which can lead to false positive results especially for samples containing very low levels of OPEs [20]. LC-MS(/MS) with electrospray ionization (ESI) source demonstrates excellent sensitivity and specificity for OPE analysis [21], but matrix interference has hampered LC-MS/MS-ESI method application in the determination of trace OPEs in lipid-rich environmental biotic samples [20–22].

Effectively extracting and separating OPEs from complex matrices is a challenge as they have a wide range of physical properties, from very polar to very hydrophobic. Common clean-up methods for biotic samples, including gel permeation chromatography and solid phase extraction (SPE), use large volumes of solvent and several processing steps, which can result in serious blank/background contamination. Dispersive SPE (d-SPE) is more "green" analytical chemistry approach and is a straightforward sample preparation technique suitable for a wide variety of environmental, food and agricultural product samples [23,24]. One specific method that has become popular is the QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) technique. d-SPE sorbents such as PSA (primary secondary amine) bonded silica, active carbon or C18 (octadecyl) bonded silica, are added directly to the sample extract as a dispersive-phase agent instead of being packed in SPE cartridges, to remove interfering matrix components but leaving the target analytes in solution. This technique uses much less solvent and overall simplifies the clean-up process, which can largely reduce the possibility of blank contamination. However, and to our knowledge, there are currently no published analytical methods applying d-SPE techniques for the determination of OPEs in lipid-rich samples.

The aim of this project was to develop an UHPLC-MS/MS and d-SPE based quantitative analytical method for the reliable, rapid and sensitive determination of low level and trace OPE (triesters) in lipid-rich biota samples as represented by bird egg and animal tissues. Different extraction and clean-up methods were tested and compared. ESI and APCI sources were evaluated for their sensitivities and matrix effects in the analysis of these lipid-rich samples.

2. Materials and methods

2.1. Chemicals and reagents

A summarization of the names, abbreviations and CAS numbers of the thirteen target organophosphate esters (OPEs) are as follows: tris(2-chloroethyl) phosphate (TCEP; CAS 115-96-8), tripropyl phosphate (TPrP; CAS 513-08-6), tris(2-chloroisopropyl) phosphate (TCIPP; CAS 13674-84-5), tris(1,3-dichloro-2-propyl) phosphate (TDCIPP; CAS 13674-87-8), triphenyl phosphate (TPHP, CAS 115-86-6), tris(2,3-dibromopropyl) phosphate (TDBPP; CAS 126-72-7), tributyl phosphate (TNBP; CAS 126-73-8), tris(methylphenyl) phosphate (TMPP; CAS 1330-78-5), 2-ethylhexyl-diphenyl phosphate (EHDPP; CAS 1241-94-7), tris(2-butoxyethyl) phosphate (TBOEP; CAS 78-51-3), tris(2-ethylhexyl) phosphate (TEHP; CAS 78-42-2), triethyl phosphate (TEP; CAS 78-40-0) and 2,2-bis (chloromethyl_propane-1,3-diyl-tetrakis(2-chloroethyl) bis(phosphate) (V6; CAS 38051-10-4). TEP, TPrP, TNBP, TCEP, TBOEP, TPHP, TEHP, TDBPP, and EHDPP were all purchased from Sigma–Aldrich (St. Louis, MO, USA). TDCIPP and TMPP were purchased from TCI America (Portland, OR); TCIPP was purchased from AK Scientific (Union City, CA); V6 was generously donated by Dr. Heather Stapleton (Duke University, NC). Five deuterium labeled OPEs were used in this study as internal standards; d_{27}-TNBP and d_{15}-TEP were purchased from Cambridge Isotope Laboratories (Tewksbury, MA, USA), and d_{15}-TPHP was purchased from Wellington Laboratories (Guelph, ON, Canada). d_{12}-TCEP and d_{15}-TDCIPP were synthesized by Dr. Vladimir Belov (Max Planck Institute for Biophysical Chemistry, Germany).

All four d-SPE dispersive-phase agents, which includes PSA bonded silica, Discovery DSC-18, Z-Sep+ and Supelclean ENVI-Carb, were purchased from Sigma–Aldrich.

CHROMASOLV® Plus hexane (HEX) and dichloromethane (DCM) and methanol LC-MS Ultra CHROMASOLV® (Fluka) were obtained from Sigma–Aldrich. Ultra-pure water produced with a Milli-Q system (Millipore; Bedford, MA, USA). All other chemicals and reagents were American Chemical Society (ACS) reagent grade or better.

A mixed working solution containing 50 ng/mL each of the 13 target OPEs was prepared by dilution of stock solutions with MeOH. A mixed OPE internal standard working solution containing