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Simultaneous determination of 16 organophosphorus flame retardants and plasticizers in fish by liquid chromatography-tandem mass spectrometry

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ABSTRACT

In this work a method to analyze simultaneously sixteen organophosphorus flame retardants (OPFRs) by liquid chromatography-quadrupole-linear ion trap mass spectrometry (LC-QqLIT-MS) in fish samples was successfully developed. Sample preparation strategies, including different extraction techniques and clean-ups were tested. The chosen methodology is based on the extraction of 0.25 g of dried fish by ultrasound and clean-up by solid phase extraction (SPE) with a tandem of C18 and basic alumina cartridges. Recoveries were between 45 to 115%, with RSDs lower than 25%. mLODs and mLOQs were between 0.34–11.6 ng/g lw and 1.12–38.8 ng/g lw, respectively, with the exception of Tris(tribromoneopentyl)phosphate (TBNPP) (37.4 and 125 ng/g lw, respectively) and Tris(isopropylphenyl)phosphate (IPPP) (51.6 and 172 ng/g lw, respectively) which had higher limits. The developed method was applied to twelve river fish samples. Thirteen out of sixteen analyzed compounds were detected. At least, one of the sixteen studied OPFRs was detected in all the analyzed samples, with ΣOPFR levels up to 2423 ng/g lw. This is the first study reporting IPPP and Isodecyldiphenyl phosphate (IDPP) levels in biota samples. Moreover, levels found for IPPP are quite high (up to 601 ng/g lw) and thus it is important to consider in the future development of analytical methodologies for OPFR analysis.

1. Introduction

The plastic industry is one of the most important nowadays. Unfortunately, even though plastics have made our lives easier, the contamination produced by them is one of the most known, and not only for the material itself, but for the chemicals used for its manufacturing. In order to give stability to these polymers, some chemicals called plasticizers are added into the mixture as well as flame retardants (FRs) to satisfy the safety standards. These chemicals are not really bonded to the polymer, which increases their release into the environment. These FRs are used to increase the fire resistance of a wide variety of materials, not only plastics. If we put together the fact that plastics are used everywhere and they are contaminated with plasticizers and FRs, and at the same time these FRs are used in all kind of materials, we have a big and wide source of contamination for the ecosystems and humans. One of these chemicals used as plasticizer and FR are the organophosphorus FRs (OPFRs). These compounds have been in the industry for at least four decades [1,2] and their consumption has grown over the years. Years ago, the most produced brominated FRs (BFRs) were the polybrominated diphenyl ethers (PBDEs) which were banned by the Stockholm convention in 2009 [3]. In 2011, 20% of the worldwide production of FRs was due to BFRs and 15% was due to OPFRs. However, and having into account the ban of PBDEs, OPFRs production could rise in the coming years.

The knowledge about the occurrence and behavior of these OPFRs in the environment is still scarce and the amount of published works is limited. A recent published review showed the concentration levels usually found in different environmental compartments such as air, water, sediment and soil [4]. However, limited information is available about the OPFR occurrence in biota samples. There are few works where the presence of OPFRs is confirmed in fish from different world locations specifically in the Netherlands [5], Canada [6], China [7], Norway [8], Philippines [9] and Sweden [10]. All these works (except for the Norwegian study) showed high concentrations of OPFRs in comparison to the average

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levels found for PBDEs, which indicates that OPFRs could be somehow bioaccumulated, even though their K_{ow} are not as big as those of PBDEs or other persistent organic pollutants. Besides this supposed bioaccumulation, some of these compounds are neurotoxic [11], endocrine disruptors [12] and carcinogenic [13].

Advances on the analytical determination of OPFRs in biota are only described in few publications. Methodologies for the OPFR analysis include a variety of extraction techniques and instrumental analysis. Pressurized liquid extraction (PLE) [10,14], microwave assisted extraction [7], shaking [8] and high speed solvent extraction [15] are some examples of extraction techniques applied to OPFR analyses. Since the lipid co-extraction is a well-known problem in biological samples, and OPFRs cannot be treated with acid, other cleanups were applied such as solid phase extraction (SPE) and filtration [14] or gel permeation chromatography (GPC) [7]. The instrumental analysis includes gas chromatography (GC) coupled to low resolution mass spectrometry (LRMS) [7] or high resolution MS (HRMS) [10], and liquid chromatography (LC) coupled to tandem MS (MS–MS) [14]. Some OPFRs such as Diphenyl cresyl phosphate (DCP), Tris(isopropyl-phenyl)phosphate (IPPP) and Tricresyl phosphate (TMCP) have different isomers. While GC allows an isomeric specific analysis, i.e. for *ortho-* *meta-* and *para-* isomers of TMCP, LC allows the analysis of some OPFRs that are degraded inside the GC column such as DCP and IPPP, which are degraded to Triphenyl phosphate (TPHP). It is important to note that these methods comprised a maximum of fourteen compounds, although OPFR market greatly exceeds that number.

The aim of this work was to develop an analytical methodology for the simultaneous analysis of a wide range of OPFRs, specifically sixteen compounds (Table 1) in samples of fish samples by LC–MS–MS. Analytical parameters such as recoveries, reproducibility, limits of detection (LODs) and limits of quantification (LOQs) of the method were evaluated and compared with previous published methods. Finally, and in order to check the applicability of the developed method, twelve biota samples from a Spanish river basin were analyzed.

2. Materials and methods

2.1. Standards and reagents

Tris(2-butoxyethyl)phosphate (TBOEP), Tris(chloroethyl)-phosphate (TCEP), Tris(chloroisopropyl)-phosphate (TClPP), Trihexyl phosphate (THP) and Tris(2-ethylhexyl)phosphate (TEHP) were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Tetrekis(2-chlorethyl)dichloroisopentyl-diphosphate (V6), 2-ethylhexyldiphenyl phosphate (EHDP), Isodecyldiphenyl phosphate (IDPP), Tris(tribromoneopentyl)phosphate (TBNPP) were purchased from AccuStandard (New Haven, CT, USA). DCP, Tributyl phosphate (TBP), TPHP, Triphenylphosphine oxide (TPPO) and Tris(1,3-dichloro-2-propyl)phosphate (TDCPP) were purchased from Sigma–Aldrich (St. Louis, MO, USA). TMCP was purchased from Dr. Ehrenstorfer (Augsburg, Germany). IPPP was purchased from Chiron (Trondheim, Norway). TDCPP-d_{15}, TBP-d_{27}, TCEP-d_{12}, $^{13}C_2$-TBOEP were purchased from Wellington Laboratories Inc. (Guelph, ON, Canada). TPHP-d_{15} was obtained from Cambridge Isotope Laboratories Inc. (Andover, MA, USA). C18, neutral alumina, Florisil® and silica cartridges were obtained from Biotage (Uppsala, Sweden). Basic alumina was purchased from Interchim (Montluçon, France). Acetone, acetonitrile, dichloromethane and hexane solvents for organic trace analysis were purchased from J.T. Baker (Center Valley, PA, USA). Methanol and water solvent for trace analysis as well as ammonium acetate and formic acid were obtained from Merck (Darmstadt, Germany).

2.2. Sample collection and sample preparation

Samples analyzed in this study were fish, specifically 6 barbels (*Barbus graellsii*), 5 carps (*Cyprinus carpio*) and 1 trout (*Salmo trutta*), collected by DC electric pulse along the Llobregat River (northwest of Spain). All the samples were transferred to the laboratory wrapped in aluminum foil at a temperature of 4 °C. They were crushed and placed in clean amber glass containers and frozen at −20 °C before being freeze dried. All samples were kept at −20 °C until analysis.

When working with OPFRs, there is an important issue to take into account: the blank contamination. Indoor is contaminated with OPFRs and therefore the contamination during the sample preparation process is an important factor. To solve this problem, the non-volumetric material was heated at 340 °C and wrapped with aluminum foil and lastly rinsed with an appropriate solvent just before use. The volumetric material was always rinsed before use with an appropriate solvent. Even taking these precautions, the blank signal was inevitable and uncontrollable, and was different from day to day. The blank signal can come from different places that cannot be controlled, like the ambient air or the nitrogen from the evaporator. A realistic goal is to minimize as much as possible the blank signal. In the first worldwide inter-laboratory study of OPFRs [16], there is a more in depth explanation about the blank signal and how to minimize it.

Different experiments were carried out in order to optimize the sample preparation procedure. First of all, sample amount was checked in order to reach a compromise between sensitivity and reduction in the complexity of the purification. Different amounts of sample were tested: 0.1, 0.25, 0.5, 1 and 3 g dried weight (dw) of lyophilized sample. Then, three different extraction procedures were tested: shaking, ultrasounds and PLE. As suggested by the literature [7,15] a mixture of hexane:acetone (1:1) was chosen as solvent extraction. Finally, different tests were performed in order to optimize the cleanup procedure by SPE. Three different cartridges were evaluated: Florisil®, neutral alumina and silica gel. Extracts were passed through the cartridges previously conditioned with 20 mL of hexane and then eluted with a mixture of hexane:dichloromethane (1:2). Additionally, scavenging tandems of cartridges of basic alumina, neutral alumina and Florisil® in tandem with C18 cartridges were tested. Extracts were completely dried and reconstituted with 60 mL of acetonitrile and then passed through the tandem of cartridges previously conditioned with 20 mL of acetonitrile. The collected extracts were reduced under a gentle nitrogen stream at 30 °C, and re-dissolved with 200 μL of methanol. Prior to analysis by LC–MS–MS, 10 ng of TCEP-d_{12}, TDCPP-d_{15}, TBP-d_{27}, TPHP-d_{15} and $^{13}C_2$-TBOEP were added as internal standards (IS).

2.3. Instrumental analysis

Instrumental analysis was performed by LC, using a Symbiosis™ Pico (SP104.002, Spark Holland), connected in series with a 4000 QTRAP Hybrid Triple Quadrupole—Linear Ion Trap-MS (Applied Biosystems-Sciex, Foster City, CA, USA) with a TurboIonSpray source. Two different chromatographic columns were tested: a Purosphere Star RP-18 (125 mm × 2.0 mm, particle size 5 μm) and a RP-8 (150 mm × 4.6 mm, 5 μm). Moreover, tests adding a volatile salt in the mobile phase were carried out to obtain a proper chromatographic separation.

For target quantitative analyses, data acquisition was performed in selected reaction monitoring (SRM) in order to increase sensitivity. Two SRM transitions between the precursor ion and the two most abundant product ions were selected for each OPFR, one for quantification and the second one for confirmation. The MS–MS conditions were optimized to provide the highest relative