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Simultaneous pressurized liquid extraction and clean-up for the analysis of polybrominated biphenyls by gas chromatography–tandem mass spectrometry

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ABSTRACT

This paper describes a fast and simple pressurized liquid extraction (PLE) method combined with gas chromatography coupled to ion trap tandem mass spectrometry (GC–ITMS-MS) for the determination of polybrominated biphenyls (PBBs) in fish samples. The method is based on a simultaneous extraction/clean-up step to reduce analysis time and solvent consumption. The effect of several PLE operating conditions, such as solvent type, extraction temperature and time, number of cycles, and lipid retainer, was optimized to obtain maximum recovery of the analytes with the minimum presence of matrix-interfering compounds. The best conditions were obtained at 100 °C with n-hexane using 15 g of silica modified with sulphuric acid (44%, w/w) as sorbent for lipid removal. Quality parameters of the GC–ITMS-MS method were established, achieving good linearity ($r > 0.998$), between 1 and 500 ng ml^{-1}, and low instrumental limits of detection (0.14–0.76 pg injected). For the whole method, limits of detection ranging from 0.03 to 0.16 ng g^{-1} wet weight and good precision (RSD < 16%) were obtained.

1. Introduction

Polybrominated biphenyls (PBBs) are a large group of brominated flame retardants (BFRs) that have been used as additives in textiles, electric and electronic components, plastics, clothing, building materials and other commercial products to decrease the likelihood and intensity of fire [1]. PBBs are easily released into the environment owing to their lack of covalent link to the polymeric material or textile with which they are mixed to improve fireproof properties [2,3]. Despite their merits in fire protection, the threat of PBBs was recognized in 1973 after the Michigan disaster, when they were accidentally mixed with cattle feed [4–6]. Shortly after this event, in 1974, PBB producers in the USA voluntarily ceased to manufacture these compounds and this action was followed worldwide few years later. In spite of a continuous reduction of the annual production of PBBs, the continued presence of these compounds in biological and environmental samples has been reported [7–11]. The toxicological concern about exposure to PBBs is related to their potential disruptor endocrine and, in some cases, to carcinogenic properties [12,13]. In addition, the combustion of PBBs generates compounds of higher toxicity such as polybrominated dibenzo-*p*-dioxins (PBDDs) and dibenzofurans (PBDFs) [14,15].

The analysis of PBBs is currently performed using methods similar to those applied for polychlorinated biphenyls (PCBs) and other persistent organic pollutants. These methods generally involve the extraction of the analytes from solid matrices using classical techniques such as Soxhlet [16–18], solvent extraction [19,20] or sonication [21,22], followed by extensive clean-up procedures to remove matrix-interfering compounds. The main drawback of these techniques is that they often require large amounts of solvents and are usually time-consuming. Pressurized liquid extraction (PLE) has become one of the most promising techniques to address these drawbacks and it has been successfully applied to the analysis of organic pollutants [23,24,28]. However, in most of the PLE applications reported, an exhaustive clean-up of the extracts before analysis by gas chromatography is required. In an attempt to decrease sample handling time and to increase sample throughput, several authors propose selective pressurized liquid extraction (SPLE) using sorbents such as modified silica, Florisil or alumina [26–29]. In recent years, SPLE has been developed for the analysis of persistent organic pollutants such as PCBs [26–28,30–34], polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD/Fs) [25,35,36], polybrominated diphenylethers (PBDEs) [32,37–42], polychloronaphtalenes (PCNs) [43,44], polycyclic aromatic hydrocarbons (PAHs) [45–48] and many other compounds [25,49] in environmental and food samples. To our knowledge, PBB extraction by PLE using on-line clean-up has not been described. Therefore one of the objectives of the present study was to develop a simultaneous extraction/clean-up method based on selective PLE for the analysis of PBBs.

Gas chromatography coupled to mass spectrometry, working in either electron ionization (EI-MS) or negative ion chemical ionization (NICI-MS) modes, is the technique most commonly used

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for the analysis of PBBs. Of these, NICI-MS is by far the most popular approach because it provides higher sensitivity than EI-MS. Nevertheless, NICI-MS offers lower selectivity because the dominating bromine isotope ions (m/z 79 and 81), which are often the base peak in full scan mass spectra, are selected for quantification [50]. In contrast, EI provides higher quality structural information, giving the molecular ions and the sequential losses of bromine atoms [51–53]. Recently, to overcome the lack of selectivity of the GC–NICI-MS method for the analysis of PBBs, a triple-quadrupole mass analyzer working in tandem mode has been used [54–56]. Nevertheless, PBDE mass interferes on the labelled PBBs, making difficult the quantification. Gas chromatography coupled to high resolution mass spectrometry (GC–HRMS) in EI mode provides the most selective method and reasonable sensitivity for the determination of these compounds [57–59]. However, GC–HRMS is relatively expensive and requires skilled personnel and heavy maintenance on a regular basis to ensure proper functioning. Gas chromatography coupled to ion-trap tandem mass spectrometry (GC–ITMS-MS) has also been proposed for the analysis of PBBs, but a few numbers of congeners has been studied [60–63].

Here we developed a simple and rapid PLE method in combination with gas chromatography coupled to GC–ITMS-MS for the analysis of PBBs at low concentrations in fish samples. A one-step extraction and clean-up method was optimized in order to reduce the analysis time and solvent consumption. For this purpose, several sorbents for lipid removal were studied and PLE parameters were optimized. In addition, MS–MS working conditions that provided maximum sensitivity and selectivity for the analysis of PBBs were established. Finally, quality parameters were determined and the applicability of the method was evaluated.

2. Experimental

2.1. Chemicals and standards

Individual analytical-reagent grade PBB congeners were supplied with a purity higher than 99% by AccuStandard (New Haven, CT, USA): 4,4′-dibromobiphenyl (BB-15), 2,4′,5-tribromobiphenyl (BB-31), 2,2′,4,5′-tetrabromobiphenyl (BB-49), 2,2′,5,5′-tetrabromobiphenyl (BB-52), 3,3′,4,4′-tetrabromobiphenyl (BB-77), 2,2′,4,5′,6-pentabromobiphenyl (BB-103), 2,2′,4,4′,5,5′-hexabromobiphenyl (BB-153), and 2,2′,4,4′,6,6′-hexabromobiphenyl (BB-155). Individual stock standard solutions of each PBB congener at 10 $\mu g\,ml^{-1}$ were prepared in isooctane. In addition, individual standard solutions of 3,3′,5,5′-tetrabromobiphenyl (BB-80), 2,2′,4,5,5′-pentabromobiphenyl (BB-101) and 3,3′,4,4′,5,5′-hexabromobiphenyl (BB-169) at a concentration of 10 $\mu g\,ml^{-1}$ in isooctane were obtained from Dr. Ehrenstorfer (Augsburg, Germany). A standard solution containing the eleven PBB congeners was prepared in isooctane from the respective individual stock standard solutions at a concentration of 1 $\mu g\,ml^{-1}$ of each compound. A standard solution of isotopically $^{13}C_{12}$-labelled CBs 28, 52, 101, 138, 153, 180 and 209 (MBP-MXE), supplied by Wellington Laboratories (Guelph, Canada), at 5 $\mu g\,ml^{-1}$ of each congener was used as internal standard for quantification by isotopic dilution. In addition, a standard solution of isotopically $^{13}C_{12}$-labelled BDE 139 at a concentration of 2.5 $\mu g\,ml^{-1}$, supplied by Wellington Labs, was used as syringe standard for recovery determination. Seven calibration standard solutions containing a mixture of the eleven PBB congeners at concentrations ranging from 1 to 500 $ng\,ml^{-1}$ and the isotopically labelled internal standards for recovery and quantification at 100 $ng\,ml^{-1}$ were prepared by dilution of the corresponding standard stock solutions in isooctane.

Dichloromethane, acetone, isooctane and n-hexane of residue analysis grade and sulphuric acid of analytical reagent grade (95–97%) were purchased from Merck (Darmstadt, Germany). Sodium sulphate (p.a., purity >99%), silica gel (Gel 60) and Florisil were also obtained from Merck. Before use, silica gel and Florisil were baked at 400 °C for 10 h and 675 °C for 12 h, respectively. Silica gel modified with sulphuric acid (44%, w/w) was prepared by slowly adding an appropriate amount of sulphuric acid to the silica at room temperature. Glass microfibre filters GF/A for covering PLE cell caps were provided by Whatman (Maidstone, UK). All glass materials were cleaned with AP-13 Extran alkaline soap (Merck, Darmstadt, Germany) for 24 h, rinsed consecutively with Milli-Q water and acetone, and dried overnight before use.

2.2. Samples and sample treatment

A set of five fish samples (trout, salmon, horse mackerel, sardine and gilthead seabream) purchased from a local supermarket were selected for the analysis of PBBs using the PLE and GC–ITMS-MS method. These fish samples were selected to cover a wide range of lipid content and are among those most frequently found in the Spanish diet [64]. Trout and salmon were of aquaculture origin, while horse mackerel, sardine and gilthead were caught in the Mediterranean Sea. Initially, each fish was washed and the non-edible parts were removed to obtain clean tissues. The edible part of the fish was triturated, homogenized, frozen and lyophilized. The dried sample was then ground in a glass mortar to obtain a fine powder, which was preserved in glass vials and stored at 4 °C in a dry place in darkness before analysis.

A standard reference material, SRM 1945 (whale blubber), obtained from the National Institute of Standards and Technology (NIST, Boulder, CO, USA), was used to validate the whole method. This reference material is certified for selected PCBs, organochlorine pesticides and PBDEs, although information about the presence of the BB-153 has been reported by Zhu and Hites [65].

2.3. On-line extraction and clean-up of fish samples using PLE

The simultaneous extraction and clean-up of the samples was performed on an ASE-100 Accelerated Solvent Extraction System (Dionex, Sunnyvale, CA, USA). Before PLE extraction, 1 g of the freeze-dried fish sample was spiked with isotopically $^{13}C_{12}$-labelled PCBs and was kept overnight at room temperature to equilibrate. The sample was then mixed with sodium sulphate at a Na_2SO_4/fish ratio of 3:1 (w/w) in a mortar until a homogenous mixture was obtained. Florisil and silica modified with sulphuric acid (44%, w/w) were tested as sorbents for lipid removal. The extraction cell was loaded by inserting two cellulose filters into the cell outlet, followed by the sorbent material and the sample, using anhydrous sodium sulphate to fill up the dead volume of the extraction cell. After sealing the cell with the top cell cap, the extraction cell was placed in the ASE system. Samples were extracted at 100 °C with n-hexane as extraction solvent and silica modified with sulphuric acid (44%, w/w) as lipid retainer, applying 3 static cycles of 5 min each and a flush volume of 60%. Further details about the optimization of the extraction parameters are described in the results and discussion section. The final extracts were then solvent-reduced to approximately 2 ml, adding isooctane as a keeper. Afterwards, the final volume of the extracts was carefully concentrated under a gentle nitrogen stream to ca. 50 μl. The extracts were then analyzed by GC–ITMS-MS after the addition of isotopically $^{13}C_{12}$-labelled BDE 139 used as syringe standard.

The lipid content of fish samples was determined by PLE (without sorbent for lipid removal) of an additional sub-sample and further gravimetric measurements. A solvent mixture of n-hexane:dichloromethane (1:1, v/v) was used. The percentage of lipids determined for the fish samples (wet weight) was as follows: 0.7% for trout, 2.1% for horse mackerel, 10.1% for gilthead, 9.8% for sardine and 14.6% for salmon.