Journal of Chromatography A, 1218 (2011) 1203–1209

Contents lists available at ScienceDirect

Journal of Chromatography A

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

Simultaneous extraction and clean-up of polybrominated diphenyl ethers and polychlorinated biphenyls from sheep liver tissue by selective pressurized liquid extraction and analysis by gas chromatography–mass spectrometry

Zulin Zhang*, Ehimai Ohiozebau, Stewart M. Rhind

The Macaulay Land Use Research Institute, Craigiebuckler, Aberdeen AB15 8QH, UK

ARTICLE INFO

Article history:
Received 15 July 2010
Received in revised form
26 November 2010
Accepted 26 December 2010
Available online 1 January 2011

Keywords:
Selective pressurized liquid extraction
Polybrominated dipenyl ethers
Polychlorinated biphenyls
Gas chromatography–mass spectrometry
Sheep liver

ABSTRACT

We describe a selective pressurized liquid extraction (SPLE) method, followed by gas chromatography–mass spectrometry (GC–MS), for the simultaneous extraction and clean-up of polybrominated diphenyl ethers (PBDEs) and polychlorinated biphenyls (PCBs) in sheep liver tissue samples. The on-line clean-up of liver tissue by SPLE was tested using differing amount of acid-modified silica (sulphuric acid:silica gel, 1:2, w/w), the most effective amount being 20 g. Different extraction solvents (iso-hexane and dichloromethane), either alone or in various combinations, were used to extract these target compounds from spiked liver samples. Variables affecting the SPLE extraction efficiency, including temperature, pressure, number of extraction cycles and static extraction time were studied; the optimum parameters were 80 °C, 10.3 MPa, 2 cycles and 5 min, respectively. The SPLE based method was compared with more traditional Soxhlet, off-line PLE, ultrasonic and heating extraction methods. Overall the mean percentage recoveries for all target chemicals using SPLE were 86–103% ($n = 3$, SD < 9%), and compared favourably with the Soxhlet (63–109%, $n = 3$, SD < 8%), off-line PLE (82–104%, $n = 3$, SD < 18%), ultrasonic (86–99%, $n = 3$, SD < 11%) and heating (72–102%, $n = 3$, SD < 21%) extraction methods. The limits of detection of the proposed method were 5–96 pg g^{-1} and 2–29 pg g^{-1} for the different PBDE and PCB chemicals studied, respectively. The outputs of the proposed method were linear over the range from 0.02 to 30 ng g^{-1}, for all PCB and PBDE congeners except for PBDE 100 and 153 (0.05–30 ng g^{-1}) and PBDE 183 (0.1–30 ng g^{-1}). The method was successfully applied to sheep liver samples for the determination of the target PBDE and PCB compounds.

1. Introduction

Organohalogenated compounds (OHC) are of global concern because their physical and chemical properties render them thermostable, persistent and lipophilic. They are generally toxic to biota and can accumulate in tissues [1,2]. Polychlorinated biphenyls (PCBs) historically, had a wide range of industrial applications and despite severe restrictions on their use PCBs persist in the environment [3,4]. Another group of OHCs with similar chemical structure and environmental behavior to PCBs, are polybrominated diphenyl ethers (PBDEs); these have been used as flame retardants. Concurrent with their increasing use, environmental levels of PBDEs have risen [5–8] and, recently, PBDEs have been listed as persistent organic pollutants (POPs) under the treaty of Stockholm Convention in 2009 [9]. These compounds accumulate in both aquatic and terrestrial organisms where they exert endocrine disrupting effects [10–12]. For most of the human population, dietary intake is probably the main route of exposure to PBDEs, as for PCBs, especially through food of animal origin [13].

Sheep can accumulate limited amount of pollutants in their tissues and the animals themselves are susceptible to the pollutant effects, at least when combined with other endocrine disruptors [14–21]. However, although PBDEs and PCBs have been measured in many biological samples, including tissues from whales, seals, gulls, fish, muscle from domestic species (beef, pork and chicken) and human tissue [4,9,22–25], there is little known of the concentrations of these pollutants in sheep liver, a metabolically-active organ that accumulates and degrades contaminants, is crucial to animal health and, additionally, is a component of the human diet [21,26]. To our knowledge, there are only two publications available concerning measurement of PBDEs and PCBs in sheep liver both of which involved heating the extract as part of the sample preparation [21,26].

Attempts to develop reliable methods for the determination of these compounds in biological samples have generally involved the extraction of the analytes from matrices using Soxhlet [27,28],

* Corresponding author. Tel.: +44 1224 395124; fax: +44 1224 395010.
E-mail address: z.zhang@macaulay.ac.uk (Z.L. Zhang).

0021-9673/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2010.12.098

or sonication [29]. Alternative extraction techniques, such as microwave assisted extraction (MAE) [30,31], supercritical fluid extraction (SFE), matrix solid-phase dispersion (MSPD) [32,33], and pressurized liquid extraction (PLE) [34–36] have also been used but these extraction methods often require extensive clean-up procedures after extraction to remove matrix-interfering compounds and are usually time- and solvent-consuming [36,37].

PLE combined with the *in situ* (in cell) clean-up of the extract [38–40], a process known as on-line PLE or selective pressurized liquid extraction (SPLE), significantly reduces the need for exhaustive post-clean-up procedures, such as column and/or gel-permeation chromatography, and allows the automation of clean-up steps. In recent years, SPLE have been developed for the analysis of persistent organic pollutants (POPs) such as polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/Fs) [41,42], polychloronaphthalenes (PCNs) [43,44], PAHs [45–47], PCBs [31,39,40,48–52], PBDEs [31,37,53] and many other compounds [36,54] in environmental and food samples. Most of these previous studies have focused on the determination of single group of compounds by SPLE, i.e. PCBs [39,40,48–52] or PBDEs [37,53]. To our knowledge, only one paper has been published on the determination of both PBDE and PCB congeners simultaneously [31] and that involved SPLE coupled with gas chromatography–electron capture detector (GC–ECD) techniques to determine their concentration in fish tissue. The study focused mainly on comparing the extraction methods of MAE and PLE, and did not address method optimization and development. Accordingly, the detection limits for this protocol were relatively high (0.8–1.2 ng g^{-1} for PCBs and 1.1–2.6 ng g^{-1} for PBDEs, respectively) [31] making it unsuitable for determination of trace amounts of PBDE and PCB congeners in tissue samples at sub ng g^{-1} levels. Covaci et al. [55] reported concentrations of 0.38 ± 0.36 ng g^{-1} and 0.17 ± 0.20 ng g^{-1} for PBDE 99 and 100 in human liver and mean PCB 52 concentrations were 0.7 ± 0.5 ng g^{-1} in gull liver tissue [4].

The aim of the present study was to develop and optimize an efficient and simple analytical method for simultaneous determination of trace concentrations of environmentally-important PBDE and PCB compounds, in sheep liver, using SPLE and gas chromatography mass spectrometry (GC–MS). We initially determined whether or not the SPLE parameters (the amount of retainer, solvents, temperature, pressure, cycles and static time etc.) affected the extraction efficiency with this tissue and these parameters were further optimized. The final aim was to validate the established method for the determination of target chemicals in sheep tissue samples.

2. Materials and methods

2.1. Reagents and materials

All glassware was machine washed and then baked at 450 °C for 12 h to eliminate organic contamination before use.

All solvents used were of HPLC grade (Rathburns, Walkerburn, UK). Seven PBDE compounds were combined to produce a stock solution (approximately 20 μg mL^{-1} for each component) including 2,4,4′-tribromodipenyl ether (PBDE 28), 2,2′,4,4′,-tetrabromodiphenyl ether (PBDE 47), 2,2′,4,4′,5-pentabromodiphenyl ether (PBDE 99), 2,2′,4,4′,6-pentabromodiphenyl ether (PBDE 100), 2,2′,4,4′,5,5′-hexabromodiphenyl ether (PBDE 153), 2,2′,4,4′,5,6′-hexabromodiphenyl ether (PBDE 154), 2,2′,3,4,4′,5′,6-heptabromodiphenyl ether (PBDE 183) and the internal standard (^{13}C-2,2′,4,4′,5-pentabromodiphenyl ether, ^{13}C-PBDE 99: 50 μg mL^{-1}) (AccuStandard, New Haven, CT, USA). Six PCB compounds (stock solution, approximately 10 μg mL^{-1} for each component) including 2,4,4'-trichlorobiphenyl (PCB 28), 2,2′,5,5′-tetrachlorobiphenyl (PCB 52), 2,2′,4,5,5′-pentachlorobiphenyl (PCB 101), 2,2′,3,4,4′,5′-hexachlorobiphenyl (PCB 138), 2,2′,4,4′,5,5′-hexachlorobiphenyl (PCB 153), 2,2′,3,4,4′,5,5′-heptachlorobiphenyl (PCB 180) plus 2,3′,4,4′,5-pentachlorobiphenyl (PCB 118, neat, being prepared to stock solution approximately 3 μg mL^{-1}) and an internal standard (^{13}C-2,2′,3,4,4′,5′-hexachlorobiphenyl, ^{13}C-PCB 138: 50 μg mL^{-1}) were also supplied by AccuStandard (New Haven, CT, USA). These standard stock solutions were stored at 4 °C and diluted with dichloromethane (DCM) before use.

Silica gel (70–230 mesh; VWR, Leicestershire, UK) and anhydrous sodium sulfate (12–60 mesh, Mallinchrodt Baker, Deventer, Netherlands) used for SPLE were washed in DCM, by Soxhlet, for 6 h then baked in a muffle furnace at 550 °C overnight before use. Acid-modified silica was made at a ratio of 1:2 (98%) sulphuric acid (Fisher Scientific, Loughborough, UK):silica gel, and then mixed on a roller for 1 h and used immediately.

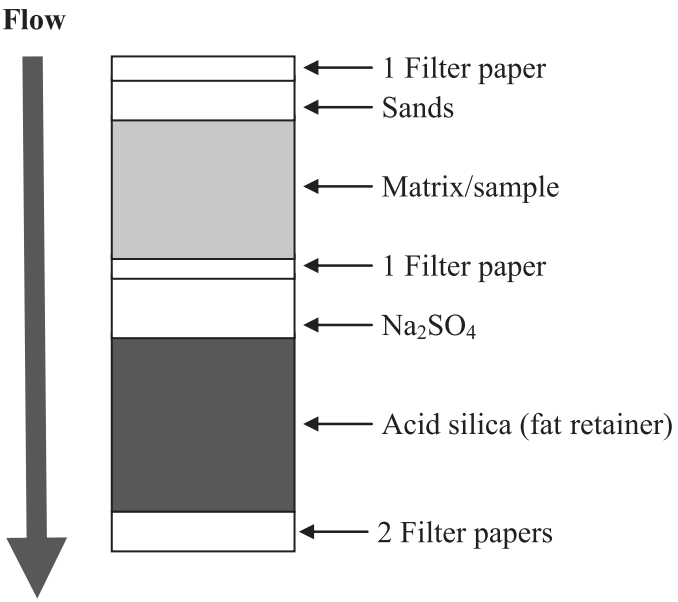

Fig. 1. Packing of the extraction cell.

2.2. Selective pressurized liquid extraction

SPLE was conducted using a fully automated Dionex ASE 200 system. The SPLE conditions were optimized for the extraction of PBDE and PCB congeners from liver tissue sample as discussed below in Section 3. All PLE extraction cells were washed twice with methanol, with ultrasonication (10 min), prior to use. Cell loading was conducted in the following sequence (Fig. 1): a 33 mL extraction cell was loaded by inserting two filter papers (Dionex, Leeds, UK) into the cell outlet followed by a weighed amount of acid-modified silica (either 5, 10, 15 or 20 g), topped by 1 g of sodium sulfate for in-cell clean-up, followed by another filter paper. 0.8 g (dry weight) of sheep liver tissue sample spiked with organic standards was then added (freeze-dried and ground sheep liver tissue, spiked with target chemicals and left for 1 h to allow the solvent to evaporate and mix, thoroughly, before extraction), followed by 0.5 g sand which had been heated in a muffle furnace at 550 °C overnight to remove traces of organic matter. Cells were prepared in triplicate (for each test) and inserted into the cell tray for extraction.

PLE was automated and allowed unattended extraction of up to 24 samples. The machine was programmed to run a series of extractions and individual parameters were changed as required. These included extraction temperature (40, 60, 80, 100 and 120 °C), pressure (3.45, 6.89, 10.3 and 13.8 MPa), static time (5, 10 and 15 min) and number of extraction cycles (1, 2 and 3). The extraction solvent

was also altered. After extraction, the solvents were evaporated down to 0.1 mL under a gentle stream of nitrogen gas at between 30 and 35 °C. These extracts were transferred to GC vials prior to analysis by GC–MS (see below).

2.3. Pressurized liquid extraction (off-line)

Sheep liver samples (0.8 g, dry weight) were weighed and placed in stainless-steel extraction cells (11 mL capacity) prepared for PLE as follows: filter paper was placed at the bottom of the cell to prevent clogging of the metal frit and then the tissue sample spiked with organic standard was added, followed by 0.5 g of muffled sand. The cells were then sealed and inserted into the cell tray for extraction. After the extraction, the solvents (DCM) were evaporated down to 0.2 mL under a gentle stream of nitrogen gas at between 30 and 35 °C. These extracts were then subjected to further clean up (see below) and analysed by GC–MS (see below).

2.4. Soxhlet extraction

Soxhlet extraction for comparison of the SPLE results was conducted by placing 0.8 g of liver tissue (dry weight) into 28 mm × 100 mm cellulose thimbles (Whatman, Kent, UK) and extracted with 150 mL DCM for 8 h, with a reflux cycle time of approximately 10 min. Following extraction, the sample extracts were concentrated by rotary evaporation and subjected to the clean-up procedure, and then analysed by GC–MS.

2.5. Ultrasonic extraction method

The liver tissue samples (0.8 g, dry weight) were placed in 30 mL glass vials to which 20 mL DCM was added. The samples were sonicated for 30 min then allowed to settle for 20 min, after which the liquid phase (DCM) was removed to another tube. The residual liver tissue was then subjected to a second 30 min sonication with a further 20 mL DCM which was removed and added to the first extract. The combined extract was concentrated by rotary evaporation and then subjected to the clean-up procedure and GC–MS analysis.

2.6. Heating extraction method

0.8 g of liver tissue sample (dry weight) was weighed in 30 mL glass tubes, 20 mL DCM was added and the tubes capped. The samples were heated to 55 °C and kept at this temperature for 2 h. The extract solution was then filtered through cellulose filter papers (150 mm, Whatman, Kent, UK) pre-washed with DCM and the extract was concentrated by rotary evaporation and then subjected to the clean-up procedure and GC–MS analysis.

2.7. Clean-up

The silica gel used for clean-up was activated by heating overnight at 550 °C. The sodium sulfate, used in the clean-up columns, was heated in a muffled furnace at 550 °C overnight to remove water and organic contamination. Cotton wool used to pack the columns was washed by Soxhlet extraction in DCM. Glass columns (i.d. 30 mm, length 220 mm) with Teflon stopcocks, plugged with cotton wool were packed with 10 g of acid-modified silica and 1 g sodium sulfate. After extraction with off-line PLE, Soxhlet, ultrasonication or heating, the extract was concentrated to 0.2 mL by rotary evaporation and/or nitrogen flow and transferred to the top of an acid-modified silica column. The column was eluted with iso-hexane (C_6H_{14}: primarily 2- and 3-methylpentanes; 100 mL) and concentrated to 1 mL by rotary evaporation, and then to dryness under a nitrogen stream. The residue was dissolved in 100 μL iso-hexane for GC–MS analysis.

2.8. Fat determination

To determine the fat content of the liver samples, 5 g of homogenized sample were Soxhlet-extracted with 200 mL iso-hexane for 6 h. The solvent was removed by rotary evaporation and the lipid content determined gravimetrically with an analytical balance Mettler AT250 (Brash, Glasgow, UK).

2.9. GC–MS analysis

An Agilent 5975C MSD (mass selective detector) linked to 7890A GC with an autosampler (7683B), was used for PBDE and PCB analysis with selected ion mode. The capillary column was ZB-5MS (30 m × 0.25 mm i.d. × 0.25 μm film thickness, Phenomenex, Macclesfield, UK). For PBDEs analysis, the initial oven temperature was 70 °C which was held for 1 min. It was then ramped to 170 °C at 30 °C min^{-1}, then ramped to 300 °C at 8 °C min^{-1} and then held at the final temperature for 5 min. For PCBs analysis, the initial oven temperature was 120 °C which was held for 1 min; the temperature was then ramped at 4 °C min^{-1} to 280 °C and held for 1 min and then ramped to 320 °C at 30 °C min^{-1} and held for 5 min. The carrier gas was helium and the flow rate was held constant at 1 mL min^{-1}. The samples were injected in splitless mode (injection volume: 3 μL; splitless time: 1 min). The injector and mass spectrometer were held at 250 °C and 200 °C, respectively. The electron impact energy was set 70 eV for mass spectrometer.

Before sample analysis, relevant standards were analysed to check instrument performance, peak height and resolution. With each set of samples analysed, reference standard mixtures, quality control samples, and procedural blanks were run in sequence to check for contamination, instrument performance, peak identification and quantification. Compounds were identified mainly by selected ion and their retention times. All results of liver sample analysis were reported on a dry-weight basis.

3. Results and discussion

3.1. Effects of fat/fat retainer ratio on extraction efficiency

Most animal tissues, like sheep liver, contain fat. The acid-modified silica in the extraction process served as a fat retainer providing an on-line clean-up during PBDE/PCB extraction such that the extracts were clean enough to be analysed using GC/MS. Since it is crucial that sufficient fat retainer is applied to the extraction cell [49], five different fat retainers were investigated using the Dionex system ASE 200 with 33 mL cells. Sulphuric acid-impregnated silica gave very clean extracts when extracting PCB with n-hexane at 100 °C from fishmeal, while the other fat retainers investigated (three types of alumina and Florisil) resulted in coloured extracts. To give fat-free extracts, a fat to fat retainer ratio (FFR) of at least a 1:40 (0.025) was required; this is consistent with the FFR value of 0.024 presented in the Dionex Application Note 322 [36,49,56]. Thus, in this experiment sulfuric acid-impregnated silica was used as fat retainer and tested with FFR ratios of 0.05, 0.025, 0.017 and 0.0125 (using 10% DCM-iso-hexane as solvent and 100 °C). Using 0.8 g of sheep liver for all the extractions, and with the fat content of sheep liver found to be 31%, FFR ratios of 0.05, 0.025, 0.017 and 0.0125 required 5, 10, 15 and 20 g acid-modified silica, respectively (greater weights could not be tried because of the finite volume of the extraction cell (33 mL)). The packing of the extraction cell is summarized in Fig. 1. When opening the extraction cells after a completed extraction, a band of oxidized fat was present which declined in optical density further down the tube and was

absent at the bottom of the cell (e.g. for 20 g acid-modified silica cell), indicating that there was no co-elution of fat in the extraction [36,37,49].

Recoveries of PBDEs (5–133%) and PCBs (1–35%) were poorest for 5 g acid-modified silica compared to the other three FFR ratios, particularly for PCB 101, PCB 138, PCB 153, PCB 180 and PBDE 28 for which recoveries were less than 10%. Although the recoveries for PBDE (except for PBDE 47) were not much increased with additional acid-modified silica above 10 g, the recoveries for PCBs in 20 g acid-modified silica (69–81%) were much better than those of 10 g (23–68%) and 15 g (38–66%). Resolutions of chromatogram peaks were improved, with lower contamination rates and less background noise for the target chemicals with increasing amounts of fat retainer. The FFR of 0.0125 (20 g acid-modified silica) was therefore the preferred ratio of fat/fat retainer ratio for the remaining testing in this study.

3.2. Solvent optimization for SPLE

In general, physico-chemical properties such as polarity and specific density, both of which influence penetration into the matrix, are relevant when determining the extraction solvent of choice. The extraction solvent must be able to solubilize the analytes of interest, minimizing the co-extraction of other matrix components [57]. The major challenge in the present study was the selection of a solvent system that allows the simultaneous extraction and clean-up of PBDEs and PCBs [38]. Previous studies suggest that hexane, DCM or mixture of DCM and hexane [37,39,40,49,50,53] are generally the preferred solvents for PBDE or PCB extraction and elution from classic sorbents (such as silica gel/acid-modified silica). Although several previous studies have addressed solvent selection for either PCBs [39,40,49,50] or PBDEs [37,53], none has focused on optimization of these solvents for simultaneous extraction and clean-up of both PBDE and PCB compounds by SPLE. In this study DCM, iso-hexane and various mixtures of them were assessed for extraction efficiency using 20 g acid-modified silica and 100 °C.

The DCM extracts were very dark, indicating co-elution of contaminants, and were not analysed further. The recovery results showed that the iso-hexane: DCM (9:1, v/v) mix gave better extraction efficiency (73–108%) for PBDEs and PCBs than the other three individual solvents or mixtures of iso-hexane (52–94%), iso-hexane:DCM (8:2, v/v; 47–156%) and iso-hexane:DCM (6:4, v/v; 47–89%). In addition, the optical density of the extract increased with the proportion of DCM in iso-hexane (especially with >20% DCM in iso-hexane). Previous studies [36,37] suggested that increasing the proportion of DCM in hexane increased the recovery rates of PBDEs while hexane (heptane or pentane) was the preferred solvent for PCBs measured by SPLE [39,40,49]. However, a higher DCM content resulted in greater co-extraction of material [36,37,40]. This co-extracted material, may have been fat from tissue samples or acid-modified silica degradable material [36,37]. Thus, to achieve a balance between complete PBDE/PCBs extraction and minimal co-extracted material, an iso-hexane:DCM ratio of 9:1 (v/v) was chosen as the extractant for use in further optimization tests.

3.3. Optimization of number of extraction cycles, pressure and static time for SPLE

The number of extraction cycles is another important factor affecting extraction efficiency. Single cycle extraction of PCB and chlorinated pesticide, previously, resulted in a low extraction efficiency [58] but the application of a second extraction cycle, for some matrices, resulted in an improvement of 10% in PCB recovery [59]. In our study, the recovery (data not presented) of PBDE 100 and PCB 28 were improved with 2 cycles and all resulted in a high extraction efficiency but there were no significant further improvements when using an additional third cycle without increased co-extraction of other compounds. Thus, considering the time taken and solvent consumption, use of 2 cycles was considered optimal, as concluded by Suchan et al. [57] who investigated extraction efficiency for PCBs and chlorinated pesticides in fish. No significant influence of pressure on the recovery rates was observed (range applied: 3.45–13.8 MPa; data not shown); this is in agreement with previous reports [48,50,53] which also showed that pressure is a parameter without significant effect on the extraction process. The main purpose of applying an increased pressure is to keep the solvent liquid from boiling. Therefore the default pressure of 10.3 MPa was selected for further optimization experiments.

There was no effect of increased static time (from 5 to 15 min) on the PBDE/PCB extraction efficiency by SPLE (data not presented). This is perhaps not unexpected since Ramos et al. [45] indicated an increased efficiency with an increase in static time from 3 to 5 min but little improvement from 5 min to 10 min and no further effect at 20 min and Zhang et al. [60] and Losada et al. [37] concluded that 5 min of static time was adequate for efficient PLE extraction and minimum solvent use.

3.4. Optimization of temperature for SPLE

In general, increased temperature disrupts the strong solute–matrix interactions resulting from Van der Waals forces, hydrogen bonding or dipole attractions between solute molecules and active sites in the matrix and is therefore a factor affecting recovery rates. Higher temperature also reduces the viscosity of solvents, thus facilitating better penetration into matrix particles, enhancing extraction efficiency [54,57]. Higher extraction efficiency has been achieved with increased temperature for the PLE extraction [54,61,62]. On the other hand, higher temperature will also result in co-extraction of interference materials (e.g. co-extracted fat or acid-modified silica degradable material) from the matrix which may adversely affect GC/GC–MS analysis [39,51]. Consequently, to achieve optimum parameters a compromise between high PBDE/PCB extraction efficiency at high temperatures and low interference from co-extracted material at low temperatures is required. The extraction efficiency was tested at different temperature (40, 60, 80, 100 and 120 °C) using 20 g acid-modified silica as a fat retainer and 10% DCM-iso-hexane for solvent in this experiment.

Extraction efficiency increased with the temperature from 40 (69–91%) to 80 °C (92–103%), except for PBDE 28, suggesting that, in general, higher extraction efficiency was achieved with the higher temperatures. However, the extraction recoveries were reduced with further temperature increases to 100 (73–96%) and 120 °C (18–73%) for all compounds, except for PBDE 99 (for which recoveries increased up to 100 °C and then declined at 120 °C). At 120 °C, the PBDE/PCB recoveries were lowest, the chromatograms showed poor peak resolution and identification was more difficult. They also showed greater contamination and more background noise, especially when the temperature was increased from 80 to 120 °C for PCBs (Fig. 2). This was attributable to the presence of more co-extracted material in the extracts at higher temperatures (100 °C and 120 °C). These findings differ from previous studies; when Bjorklund et al. [49] extracted PCBs from fishmeal with acid-modified silica, all seven of the indicator PCBs were quantitatively recovered at 100 °C. However, this can be explained through their use of the apolar solvent, n-hexane, which is suitable for PCBs extraction but not for PBDEs, as stated above [36,37,60]. Apolar solvents (e.g. n-hexane, n-heptane and n-pentane etc.) can withstand the harsh treatment associated with integrated clean-up approaches using acid-modified silica, even at temperatures up to

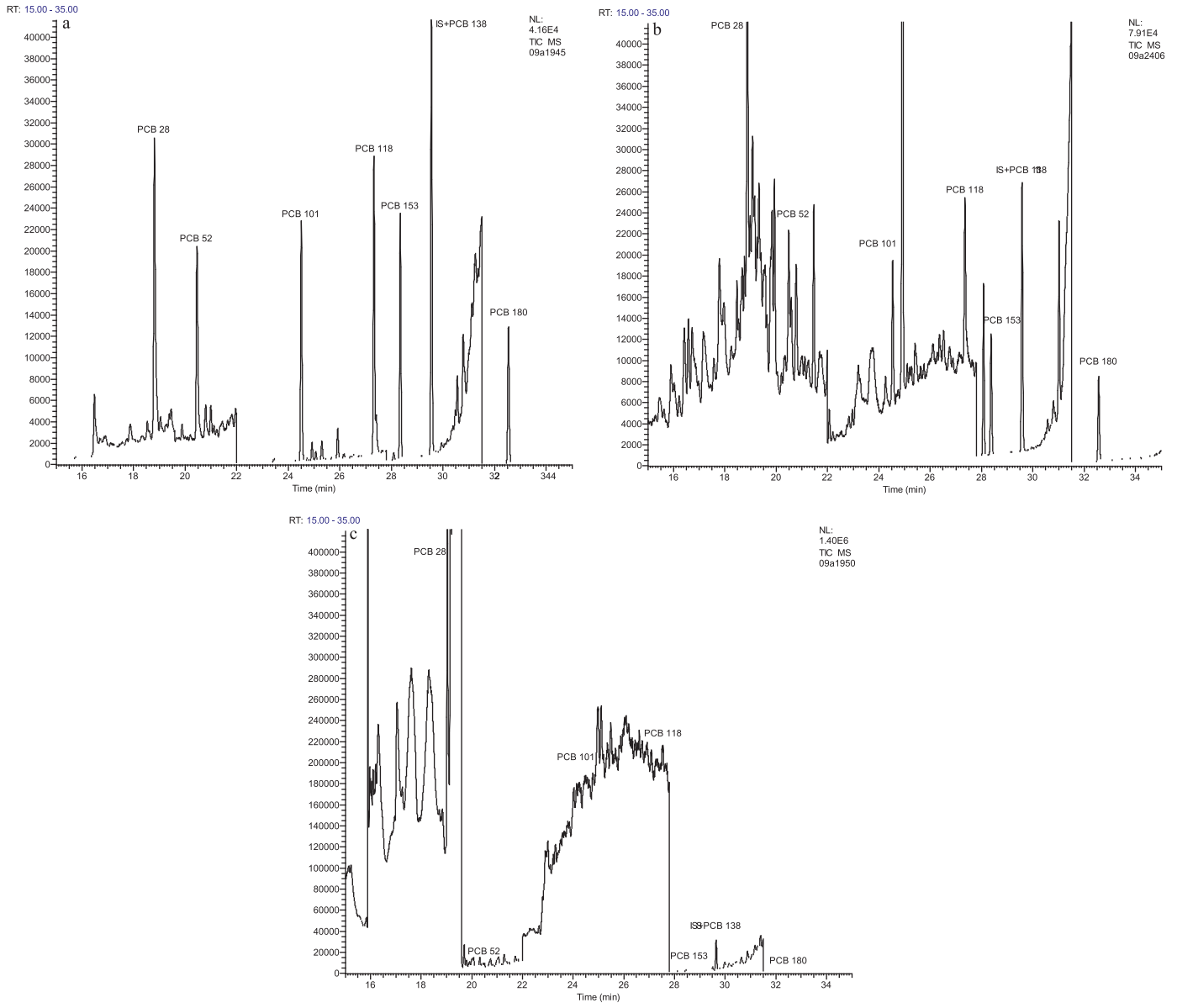

Fig. 2. PCB chromatograms obtained from using different temperature: (a) 80 °C; (b) 100 °C; (c) 120 °C.

150 °C [36,39]. On the basis of these results, 80 °C was selected for the further optimization and validation of the extraction method.

3.5. Comparison of Soxhlet extraction, ultrasonication, heating extraction, and off-line PLE with SPLE

The average recoveries using the SPLE method (86–102% with SD: 2–9%) were comparable to those of Soxhlet extraction (63–109% with SD: 4–8%), ultrasonication (86–98% with SD: 4–8%), heat extraction (72–93% with SD: 6–21%) and off-line PLE (83–103% with SD: 5–18%) for PBDEs. Results for PCBs were similar with the average recoveries being 93–103% (SD: 3–5%) for SPLE and 82–104% (SD: 3–12%) for the other four methods. The proposed SPLE extraction method compares favourably with other methods previously used. It showed good recoveries, does not require exhaustive post-clean-up procedure and allows 24 samples a day to be handled (higher throughput than Soxhlet, ultrasonication, heat extraction or off-line PLE).

3.6. Validation and application of the developed method

The limits of detection (LOD) of the proposed method were considered to be values corresponding to 3 times the standard deviations of the background noise obtained for blank samples, whereas the limits of quantification (LOQ) were deemed to be the analyte concentration corresponding to a signal/noise ratio of 10. LOD varied from 5 pg g^{-1} to 96 pg g^{-1} for PBDEs and from 2 to 29 pg g^{-1} for PCBs, (Table 1). As no previous LOD or LOQ data were available for sheep liver analysis it was compared with the outcomes of analytical methods applied to other animal tissue (e.g. fish). The LOD values of the current method were lower than many of those reported by other authors. For example, for the PCBs used in this study, LODs of 0.03–0.2 ng g^{-1} were reported in pork and chicken meat [40] while in fish samples, LODs of either 0.3 ng g^{-1} [49] or 0.8–1.2 ng g^{-1} have been reported for PCBs and LODs of 1.1–2.6 ng g^{-1} for PBDEs [31]. Losada et al. [37], using fish samples, reported LOD values similar in range to the current study of sheep liver of 10–34 pg g^{-1} for PBDEs and 0.002–0.07 ng g^{-1} for PCBs, although these were based on wet weight [50].

Table 1
Linearity, limit of detection (LOD) and limit of quantification (LOQ) for PBDE and PCB of the proposed method.

Chemicals	LOD (pg g^{-1})	LOD (pg g^{-1})	Linear range (ng g^{-1})	Correlation coeffients (r)
PBDE 28	5	16	0.02–30	0.999
PBDE 47	8	26	0.02–30	0.999
PBDE 99	15	49	0.02–30	0.999
PBDE 100	47	156	0.05–30	0.999
PBDE 153	34	114	0.05–30	0.994
PBDE 154	24	79	0.02–30	0.999
PBDE 183	96	319	0.1–30	0.998
PCB 28	10	32	0.02–30	0.999
PCB 52	7	24	0.02–30	0.999
PCB 101	6	19	0.02–30	0.999
PCB 118	2	8	0.02–30	0.996
PCB 138	12	39	0.02–30	0.999
PCB 153	12	41	0.02–30	0.999
PCB 180	29	98	0.02–30	0.999

Table 2
Recoveries (%) on different spiked level of PBDEs and PCBs in sheep liver samples.

Spiked level (ng g^{-1})	PBDE28	PBDE47	PBDE99	PBDE100	PBDE153	PBDE154	PBDE183	PCB28	PCB52	PCB101	PCB118	PCB138	PCB153	PCB180
0.02	79	112	90	n/a	n/a	79	n/a	76	79	75	50	51	83	55
0.05	89	83	97	58	47	91	n/a	79	90	50	74	85	56	83
0.10	86	63	96	57	70	83	66	79	84	50	68	67	67	67
0.20	69	67	86	68	57	84	53	78	57	75	69	78	78	85
0.50	81	66	90	70	108	88	58	125	80	101	75	96	83	65
1.00	86	57	55	70	111	69	76	93	60	78	67	93	76	63
5.00	77	73	88	75	103	82	82	98	89	85	69	94	84	77
10.0	86	98	95	101	99	102	92	93	93	100	99	103	102	99
20.0	77	76	101	80	92	90	102	100	96	93	90	102	98	87
30.0	75	80	100	81	102	96	109	101	97	99	93	102	93	84

The linearity of the method was examined by plotting the concentrations of PBDEs and PCBs found in the spiked samples against the concentration added. The concentrations of PBDE and PCB in the sheep tissue were calculated, and this value was subtracted from the concentrations found in the spiked samples. Both the results from the initial validation and the second round, when investigating the intermediate precision, were incorporated when evaluating the linearity. As can be seen from the regression results presented in Table 1, the correlation coefficients were >0.99 for all compounds.

To further validate the method, a series of recovery tests (using 20 g acid-modified silica as fat retainer, 10% DCM in iso-hexane, 10.3 MPa, 80 °C, 2 cycles and 5 min for static time) were conducted by spiking sheep liver tissue samples with different concentrations of the standard mixture. For spiked tissue samples, the recovery rates were 50–125% for all PCB compounds at spiking rates of 0.02–30 ng g^{-1}, 55–112% for PBDE 28, 47, 99 and 154 at spiking rates of 0.02–30 ng g^{-1}, 47–111% for PBDE 100 and 153 at spiking rates of 0.05–30 ng g^{-1}, and 53–109% for PBDE 183 at spiking rates of 0.1–30 ng g^{-1} (Table 2).

The results demonstrated that PBDEs and PCBs can be extracted and determined from sheep liver tissue samples by the proposed method, with good accuracy and precision. The combined method of SPLE and GC–MS method developed can be applied to sheep liver samples containing the target PCBs and PBDEs at concentrations as low as pg g^{-1} level.

After validation, the method was applied to the analysis of target PBDE/PCBs in sheep liver samples from Hartwood, Lanarkshire, UK. Most target compounds were detected although some values were below the limits of detection (LOD) for PBDE 99, 100, 153, 154, 183 and PCB 101 (Table 3). The concentrations of PBDEs and PCBs were 0.80–2.17 ng g^{-1} and 1.91–5.55 ng g^{-1}, respectively. PBDE 47 (0.35–1.37 ng g^{-1} mean: 0.65 ng g^{-1}) and PCB 118 (0.39–3.79 ng g^{-1}, mean: 1.68 ng g^{-1}) were detected in all samples and were the predominant congeners.

Since the only two reports of PBDE/PCBs concentrations in sheep liver (similar concentration to this study) are from our previous work [21,26], we could only compare our data with concentrations in human or other animal tissues to assess the level of liver contamination in sheep. The PBDEs and PCBs concentrations were much lower than those reported in human liver samples (PBDEs: 47.69–541.1 ng g^{-1} and PCBs: 257.9–455.1 ng g^{-1}) although PBDE 47 was identified as the predominant congener, in terms of concentration [25] as found in human milk, blood and tissue samples [55,63,64]. This may be because PBDE 47 is one of the main isomers in commercial penta-BDE products. The contamination level of PBDEs (3.6 ± 2.1 ng g^{-1}) in human liver samples from

Table 3
PBDE and PCB content of sheep liver samples collected from Hartwood, Lanarkshire, UK.

Concentration (ng g^{-1})	PBDE28	PBDE47	PBDE99	PBDE100	PBDE153	PBDE154	PBDE183	PCB28	PCB52	PCB101	PCB118	PCB138	PCB153	PCB180
A	0.24	0.35	0.11	<LOD	0.11	0.07	0.77	0.75	0.98	0.01	2.08	0.24	1.26	0.38
B	0.14	0.49	0.04	<LOD	0.04	0.03	0.45	0.38	0.01	0.02	1.24	0.25	0.58	0.16
C	0.17	0.57	0.18	<LOD	0.06	<LOD	0.24	0.66	0.16	0.01	3.79	0.14	0.60	0.19
D	0.01	0.66	0.02	<LOD	0.11	<LOD	<LOD	0.63	0.21	0.05	2.07	0.51	0.70	0.26
E	0.10	0.74	<LOD	<LOD	<LOD	<LOD	<LOD	0.57	0.09	0.02	0.39	0.10	0.54	0.20
F	0.15	1.37	0.05	<LOD	0.04	0.05	0.51	0.52	0.18	<LOD	0.44	0.19	0.58	0.14
G	0.21	0.36	0.33	<LOD	0.05	0.05	0.55	0.83	0.03	0.05	1.77	0.34	0.60	0.15

Belgium was similar to that of observed in our study but that of PCBs (259 ± 205 ng g^{-1}) was higher than in our study although it should be noted that they were expressed in terms of lipid weight [55]. Kannan et al. [65] reported similar concentrations of PBDEs (0.71–1.3 ng g^{-1}) and higher concentrations of PCBs (28–35 ng g^{-1}) in the liver tissue of dolphins from the Irrawaddy River in India [65]; they also found that PBDE 47 was present in higher concentrations than any other PBDE congener [65]. The species differences in tissue accumulation are likely to be reflection of species differences in environmental exposure and rates of uptake, metabolism and excretion by the animal.

4. Conclusion

A method based on SPLE and GC–MS for the determination of sheep liver concentrations of various brominated and chlorinated congeners was optimized. For optimal sample extraction, 20 g of acid-modified silica and a DCM: iso-hexane ratio of 1:9 (v/v) were found to be appropriate for extraction and on-line clean-up by SPLE. An extraction temperature of 80 °C resulted in high recoveries and low fat co-extraction with good chromatograms. The other optical PLE parameters were 2 cycles, 10.3 MPa and 5 min for static time. The optimized method has been successfully applied to the analysis of sheep liver samples.

Acknowledgements

This work was funded by the Scottish Governments' Rural and Environment Research and Analysis Directorate. We thank Mrs. Carol Kyle for her help with the preparation of sheep liver tissue samples.

References

[1] K.C. Jones, P. DeVoogt, Environ. Pollut. 100 (1999) 209.
[2] N. Basu, A.M. Scheuhammer, M. O'Brien, Environ. Pollut. 149 (2007) 25.
[3] K.J. Fernie, R.J. Letcher, Environ. Sci. Technol. 44 (2010) 3520.
[4] K. Sagerup, L.S. Helgason, A. Polder, H. Strom, T.D. Josefsen, J.U. Skare, G.W. Gabrielsen, Sci. Total Environ. 407 (2009) 6009.
[5] E. Eljarrat, A.D.L. Cal, D. Raldua, C. Duran, D. Barcelo, Environ. Sci. Technol. 38 (2004) 2603.
[6] A. Koizumi, T. Yoshinaga, K. Harada, K. Inoue, A. Morikawa, J. Muroi, S. Inoue, B. Eslami, S. Fujii, Y. Fujimine, N. Hachiya, S. Koda, Y. Kusaka, K. Murata, H. Nakatsuka, K. Omae, N. Saito, S. Shimbo, K. Takenaka, T. Takeshita, H. Todoriki, Y. Wada, T. Watanabe, M. Ikeda, Environ. Res. 99 (2005) 31.
[7] H.M. Stapleton, Anal. Bioanal. Chem. 386 (2006) 807.
[8] S.D. Shaw, M.L. Berger, D. Brenner, D.O. Carpenter, L. Tao, C.S. Hong, K. Kannan, Chemosphere 71 (2008) 1422.
[9] H.B. Moon, K. Kannan, M. Choi, J. Yu, H.G. Choi, Y.R. An, S.G. Choi, J.Y. Park, Z.G. Kim, J. Hazard. Mater. 179 (2010) 735.
[10] H.A. Anderson, P. Imm, L. Knobeloch, M. Turyk, J. Mathew, C. Buelow, V. Persky, Chemosphere 73 (2008) 187.
[11] R. Bergonzi, C. Specchia, M. Dinolfo, C. Tomasi, G. De Palma, T. Frusca, P. Apostoli, Chemosphere 76 (2009) 747.
[12] S.M. Rhind, Phil. Trans. R. Soc. B 364 (2009) 3391.
[13] J.G. Li, H.F. Yu, Y.F. Zhao, G. Zhang, Y.N. Wu, Chemosphere 73 (2008) 182.
[14] M. Bellingham, P. Fowler, M. Amezaga, S. Rhind, C. Cotinot, B. Madon-Pepin, R. Sharpe, N. Evans, Environ. Health Perspect. 117 (2009) 1556.
[15] M. Bellingham, P.A. Fowler, M.R. Amezaga, C.M. Whitelaw, S.M. Rhind, C. Cotinot, B. Mandon-Pepin, R.M. Sharpe, N.P. Evans, J. Neuroendocrin 22 (2010) 527.
[16] P.A. Fowler, N.J. Dora, H. McFerran, M.R. Amezaga, D.W. Miller, R.G. Lea, P. Cash, A.S. McNeilly, N.P. Evans, C. Cotinot, R.M. Sharpe, S.M. Rhind, Mol. Hum. Reprod. 14 (2008) 269.
[17] P.M. Lind, M. Gustafsson, S.A.B. Hermsen, S. Larsson, C.E. Kyle, J. Örberg, S.M. Rhind, Sci. Total Environ. 407 (2009) 2200.
[18] P.M. Lind, D. Öberg, S. Larsson, C.E. Kyle, J. Örberg, S.M. Rhind, Sci. Total Environ. 408 (2010) 2340.
[19] C. Paul, S.M. Rhind, C.E. Kyle, H. Scott, C. McKinnell, R.M. Sharpe, Environ. Health Perspect. 113 (2005) 1580.
[20] R.M. Rhind, C.E. Kyle, G. Telfer, E.I. Duff, A. Smith, Environ. Health Perspect. 113 (2005) 447.
[21] S.M. Rhind, C.E. Kyle, C. Mackie, L. McDonald, J. Environ. Monit. 11 (2009) 1469.
[22] S. Ohta, D. Ishizuka, H. Nishimura, T. Nakao, O. Aozasa, Y. Shimudzu, F. Ochiai, T. Kida, M. Nishi, H. Miyata, Chemosphere 46 (2002) 689.
[23] J.K. Huwe, G.L. Larsen, Environ. Sci. Technol. 39 (2005) 5606.
[24] R.J. Letcher, W.A. Gebbink, C. Sonne, E.W. Born, M.A. McKinney, R. Dietz, Environ. Int. 35 (2009) 1118.
[25] G.F. Zhao, Z.J. Wang, H.D. Zhou, Q. Zhao, Sci Total Environ. 407 (2009) 4831.
[26] S.M. Rhind, C.E. Kyle, C. Mackie, L. McDonald, Z. Zhang, E. Duff, M. Bellingham, M. Amezaga, B. Mandon-Pepin, B. Loup, C. Cotinot, N. Evans, R. Sharpe, P. Fowler, J. Environ. Monit. 12 (2010) 1582.
[27] B. Johnson-Restrepo, K. Kannan, D.P. Rapaport, B.D. Rodan, Environ. Sci. Technol. 39 (2005) 5177.
[28] D.C.G. Muir, S. Backus, A.E. Derocher, R. Dietz, T.J. Evans, G.W. Gabrielsen, J. Nagy, R.J. Norstrom, C. Sonne, I. Stirling, M.K. Taylor, R.J. Letcher, Environ. Sci. Technol. 40 (2006) 449.
[29] M.N. Jacobs, A. Covaci, A. Gheorghe, P. Schepens, J. Agric. Food Chem. 52 (2004) 1780.
[30] S. Bayen, H.K. Lee, J.Ph. Obbard, J. Chromatogr. A 1035 (2004) 291.
[31] N. Tapie, H. Budzinski, K. Le Menach, Anal. Bioanal. Chem. 391 (2008) 2169.
[32] B. Gomara, L. Herrero, M.J. Gonzalez, Environ. Sci. Technol. 40 (2006) 7541.
[33] A. Martinez, M. Ramil, R. Montes, D. Hernanz, E. Rubi, I. Rodriguez, R. Cela Torrijos, J. Chromatogr. A 1072 (2005) 83.
[34] E. Bjorklund, T. Nilsson, S. Bowadt, Trends Anal. Chem. 19 (2000) 434.
[35] S. Harrad, S. Hazrati, C. Ibarra, Environ. Sci. Technol. 40 (2006) 4633.
[36] E. Bjorklund, S. Sporring, K. Wiberg, P. Haglund, C. van Holst, Trends Anal. Chem. 25 (2006) 318.
[37] S. Losada, F.J. Santos, M.T. Galceran, Talanta 80 (2009) 839.
[38] R. Carabias-Martinez, E. Rodriguez-Gonzalo, P. Revila-Ruiz, J. Hernandez-Mendez, J. Chromatogr. A 1089 (2005) 1.
[39] S. Sporring, E. Bjorklund, J. Chromatogr. A 1040 (2004) 155.
[40] J.J. Ramos, C. Dietz, M.J. Gonzalez, L. Ramos, J. Chromatogr. A 1152 (2007) 254.
[41] M. Nording, S. Sporring, K. Wiberg, E. Bjorklund, P. Haglund, Anal. Bioanal. Chem. 381 (2005) 1472.
[42] K. Wiberg, S. Sporring, P. Haglund, E. Bjorklund, J. Chromatogr. A 1138 (2007) 55.
[43] I. Wiater-Protas, B. Van Bavel, A. Parczewski, Chem. Anal. 47 (2002) 659.
[44] K. Saito, A. Sjodin, C.D. Sandau, M.D. Davis, H. Nakazawa, Y. Matsuki, D.G. Paterson, Chemosphere 57 (2004) 373.
[45] L. Ramos, J.J. Vreuls, U.A.T. Brinkman, J. Chromatogr. A 891 (2000) 275.
[46] S. Lundstedt, P. Haglund, L. Oberg, Anal. Chem. 78 (2006) 2993.
[47] J.K. Houessou, C. Delteil, V. Camel, J. Agri. Food Chem. 54 (2006) 7413.
[48] A. Muller, E. Bjorklund, C. van Holst, J. Chromatogr. A 925 (2001) 197.
[49] E. Bjorklund, A. Muller, C. Von Holst, Anal. Chem. 73 (2001) 4050.
[50] J.L. Gomez-Ariza, M. Bujalance, I. Giraldez, A. Velasco, E. Morales, J. Chromatogr. A 946 (2002) 209.
[51] S. Sporring, C. van Holst, E. Bjorklund, Chromatographia 64 (2006) 553.
[52] I. Kania-Korwel, H.X. Zhao, K. Norstrom, X.S. Li, K.C. Hornbuckle, H.J. Lehmler, J. Chromatogr. A 1214 (2008) 37.
[53] A. de la Cal, E. Eljarrat, D. Barcelo, J. Chromatogr. A 1021 (2003) 165.
[54] M.M. Schantz, Anal. Bioanal. Chem. 386 (2006) 1043.
[55] A. Covaci, S. Voorspoels, L. Roosens, W. Jacobs, R. Blust, Chemosphere 73 (2008) 170.
[56] Dionex, Application Note ASE 322, Dionex Corporation, Sunnyvale, CA, 1996.
[57] P. Suchan, J. Pulkrabova, J. Hajslova, V. Kocourek, Anal. Chim. Acta 520 (2004) 193.
[58] M.M. Schantz, J.J. Nichols, S.A. Wise, Anal. Chem. 69 (1997) 4210.
[59] E. Bjorklund, S. Bowadt, T. Nilsson, L. Mathiasson, J. Chromatogr. A 836 (1999) 285.
[60] Z.L. Zhang, M. Shanmugam, S.M. Rhind, Chromatographia 72 (2010) 535.
[61] L. Turrio-Baldassarri, C.L. Battistelli, A.L. Iamiceli, Anal. Bioanal. Chem. 375 (2003) 589.
[62] C. von Holst, A. Muller, F. Serano, S. Sporring, E. Bjorklund, Chromatographia 61 (2005) 391.
[63] D. Meironyte Guvenius, A. Bergman, K. Noren, Arch. Environ. Contam. Toxicol. 40 (2001) 564.
[64] R.A. Hites, Environ. Sci. Technol. 38 (2004) 945.
[65] K. Kannan, K. Ramu, N. Kajiwara, R.K. Sinha, S. Tanabe, Arch. Environ. Contam. Toxicol. 49 (2005) 415.