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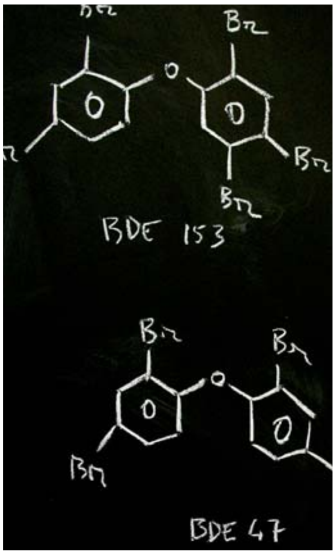

Determination of PBDEs in Sediment Samples using Pressurized Solvent Extraction and Automated Soxhlet Extraction

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Determination of PBDEs in Sediment Samples using Pressurized Solvent Extraction and Automated Soxhlet Extraction

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The efficiency of pressurized solvent extraction (PSE) was investigated by comparing PSE with automated Soxhlet extraction. Sediment samples containing polybrominated diphenylethers (PBDEs) were processed in the Speed Extractor E-914 for PSE and in the Extraction System B-811 for automated Soxhlet extraction. Quantification of PBDEs was performed by GC-MS. This study shows that both techniques are comparable in terms of accuracy, recovery and reproducibility and confirms that PSE is a much faster technique consuming much less solvent than automated Soxhlet extraction.

Introduction

PBDEs are organobromine compounds used as flame retardants to improve fire safety in domestic and commercial applications. They have attracted considerable attention due to their resemblance to the very toxic dibenzodioxins and dibenzofuranes. PBDEs are categorized into three groups based on the average number of bromine atoms in the molecule (Figure 1): penta-BDEs are typically used in polyurethane foam such as in mattresses, seat cushions and rigid insulation; octa-BDEs are typically used in high-impact plastics such as computers, automobile trim and telephones; deca-BDEs are typically used in televisions, computers, cable insulation, adhesives and textile coating [1].

The production of PBDEs has increased rapidly the last 30 years due to the growing popularity of personal computers and other electronic equipment. By 2004, penta- and octa-BDEs were phased out of production by manufactures in the United States and Europe, but deca-BDE continues to be used in high amounts worldwide [2-3].

PBDEs are non-covalent additives and leach from items through use, volatilization or abrasion and enter the environment. Because of their persistence and lipophilicity, PBDEs enter the food chain and accumulate in biologic tissues. They have been found in the air, soil, oceans, lakes, and in animal tissues throughout the world with increasing levels over time. In humans, contamination was observed in blood, breast milk and fat tissues. Animal studies show that exposure to PBDEs causes endocrine disrupting effects, affects reproductive processes, reduces immune system performance, and there is also evidence of neurotoxicity [4-6].

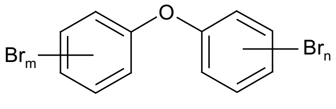

Fig. 1: Chemical structure of PBDEs

Automated Soxhlet extraction is a proven technique for extraction of polyhalogenated organic pollutants such as PCBs and PBDEs in semi-solid and solid matrices [7]. Buchi's SpeedExtractor is an alternative to Soxhlet. The SpeedExtractor is an automated parallel pressurized solvent extraction system which allows to process up to four or six samples simultaneously. The extraction is performed at elevated temperatures and pressures in order to improve solvation kinetics and penetration of the sample matrix. As a result, the analytes diffuse from the matrix to the extraction solvent more quickly and smaller amounts of solvent are required. The U.S. EPA method 3545A is applicable to the extraction of semivolatile organic compounds, organophosphorous pesticides, organochlorine pesticides, chlorinated herbicides, PCBs, PCDDs/PCDFs, and polyhalogenated organic compounds from soils, clays, sediments, sludges, and waste solids [8].

The purpose of this study was to investigate the efficiency of the SpeedExtractor E-914 for the extraction of PBDEs in environmental matrices in comparison to automated Soxhlet extraction. For this, various PBDEs were extracted of two sediment samples with the SpeedExtractor E-914 and the automated Soxhlet Extraction System B-811: a certified sediment sample (CRM BROC-02, RIVO, The Netherlands) and a real sample from Lake Maggiore, Italy. This real sample was used to test the reproducibility at low concentrations in the SpeedExtractor E-914. Then the results obtained with the SpeedExtractor E-914 were compared to those obtained with the Extraction System B-811.

Experimental

Samples

Certified material:
Certified Reference Material (CRM BROC-02) was obtained from the Netherlands Institute for Fishery Research (RIVO) [9]. The sample was collected in the vinicity of a brominated flame retardants (BFR) chemical factory situated in Western Scheldt, The Netherlands. It contained the ten congeners BDE-28/33, 47, 49, 66, 85, 99, 100, 153, 154, and 183 with concentrations in the range of 0.29–14.2 ng/g (dry weight) and the congener BDE-209 with a concentration of 1163.7 ng/g (dry weight) [10]. CRM BROC-02 was delivered lyophilized and 90 µm sieved, ready for extraction.

Sediment sample from Pallanza bay:
Lake Maggiore is the second largest (212 km^2) and deepest (372 m) lake in Italy. Situated 194 meters above sea level, Lake Maggiore is surrounded by the Italian regions of Lombardy (east) and Piedmont (west) and by the Swiss canton Ticino (north). In recent investigations in Italy and Switzerland, PBDEs were found in various environmental compartments of Lake Maggiore, such as sediments and mussels [11]. Bay and estuarine sediments are reservoirs of

toxic compounds and are therefore very appropriate sites to collect samples and to evaluate the contamination level of PBDEs in the environment. A sediment core was collected in Pallanza bay near Bevano in March 2007 by the Institute of Ecosystem Study (ISE) of the CNR Water Research Institute, Brugherio, Italy, in the frame of a study on DDT and other dangerous substances in the Lake Maggiore ecosystem [12]. The sediment core was collected with a ponar gravity corer (transparent PVC tube, 1000 mm, 630 mm i.d.). Subsamples were obtained by slicing the core in 1 cm intervals. Prior to analysis, sediments were lyophilized (liophylizator BVL2/FM, Brizio Basi & C., Milan) and sieved to 90 µm with a vibratory sieve shaker (Fritsch, Germany).

Fig. 2: Extraction System B-811

Chemicals and materials

All solvents used for the analyses (*n*-hexane, acetone, iso-octane, and dichloromethane) were pesticide grade and were purchased from Sigma-Aldrich (Steinheim, Germany). Silica gel for column chromatography (70-230 mesh) was supplied by Sigma-Aldrich (Steinheim, Germany). Florisil adsorbent for chromatography (100-200 mesh) was obtained from Fluka (Steinheim, Germany). Quartz sand (particle size 0.3-0.9 mm) was supplied by BÜCHI Labortechnik AG (Flawil, Switzerland). Concentrations of BDE-28/33, 47, 49, 66, 85, 99, 100, 153, 154 and 183 were determined by external calibration using the standards mixtures EO-5099 (Cambridge Isotope Laboratories, Andover, MS, USA) containing congeners BDE-1, 2, 3, 7, 8, 10, 11, 12, 13, 15, 17, 25, 28, 30, 32, 33, 35, 37, 47, 49, 66, 71, 75, 77, 85, 99, 100, 116, 118, 119, 126, 138, 153, 154, 155, 166, 181, 183 and 190 and BDE-CM AccuStandards (New Haven, CT, USA) containing congeners BDE-28/33, 47, 99, 100, 153, 154, 183 and 209. Decabromodiphenyl (BB-209) was used as internal standard for decabromodiphenyl ether (BDE-209) and was purchased from AccuStandards (New Haven, CT, USA).

Instrumentation

Automated Soxhlet extraction:
Automated Soxhlet extraction was performed with the Extraction System B-811 (Figure 2). The system offers operation in 4 different modes (Soxhlet Standard, Soxhlet Warm, Hot Extraction, and Continuous Extraction) without rebuilding. Each mode involves a fully automated extraction process consisting of extraction, rinsing, and drying, designed for unattended operation and minimum system handling.

The design of the Extraction System B-811 is very appropriate for environmental applications. The four extraction positions are independent, which prevents cross-contamination. All parts coming in contact with the solvent and sample are inert. Electronic monitoring of extraction, rinsing and drying ensures reproducible conditions and reliable results. The optimized extraction method can be saved and reused for a new batch of samples.

The sediment sample (approx. 0.6 g) was mixed with sand (2-3 g). Then the sediment/sand mixture was placed into a thimble (borosilicate glass fiber, Advanced MFS Inc., USA) and the internal standards were added on top. The samples were extracted with the Extraction System B-811 using the Soxhlet Warm extraction mode (Figure 3), with 100 ml of a mixture of *n*-hexane/acetone (75%/25%). The extraction parameters are shown in Table 1.

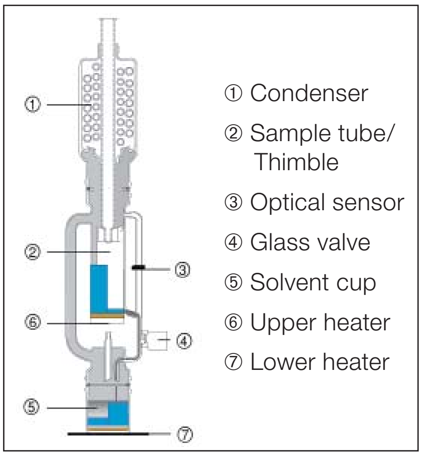

Fig. 3: Soxhlet Warm mode

The solvent is heated up by the lower heating element (7) and evaporated. The vapor rises up to the condenser (1). The condensed solvent flows down along the condensation tube into the extraction chamber (2) with the sample inside. The condensed solvent is collected in the extraction chamber while the glass valve (4) is closed. The sample is extracted. The solvent level rises up to the optical sensor (3). Each time the solvent level

Tab. 1: Extraction parameters of the Extraction System B-811, Soxhlet Warm

Extraction System B-811 (4 positions)	
Extraction solvent Volume	*n*-hexane/acetone 75% / 25% ~100 ml
Step 1: extraction	Upper heating level 5 Lower heating level 10 25 cycles
Step 2: rinse	Lower heating level 10 Time 5 min
Total run time	~150 min

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reaches the optical sensor (3), the solvent containing the extracted compounds is released into the solvent cup (5) by opening the magnetic valve (4). The glass valve (4) remains open, until the extraction chamber is empty. One cycle is finished.

After the first cycle is finished (1st solvent level detection at the optical sensor (3), additionally the upper heating element (6) is activated and warms up the solvent in the extraction chamber.
Since the solvent is heated up in the extraction chamber, the solubility of the analytes is increased resulting in better mass transfer and finally a remarkable reduction of the extraction time.

PSE:
PSE was performed with the SpeedExtractor E-914 (Figure 4). This extraction system has been primarily designed for environmental extraction. Samples are no longer extracted one after another, but up to four samples can be run in parallel. This allows for incoming batches to be processed immediately and under identical temperature and pressure conditions, which ensures very high precision. Each position is equipped with valves at the inlet and outlet of the extraction cell and individual pressure gauges for each position (Figure 5).

Fig. 4: SpeedExtractor E-914

This concept prevents cross-contamination, and blocked or leaky positions are immediately identified and can be deactivated. The extraction cells are sealed automatically. In addition to significant time savings, this setup also provides a high degree of safety as it eliminates handling errors and ensures reproducibility.

Sample preparation and extraction

The sediment sample (approx. 0.6 g) was mixed with sand (2-3 g) and this mixture was placed into an extraction cell of 40 ml already half-filled with sand. Then the cell was filled up with sand and the internal standards were added on top (Figure 6). The sediment samples were extracted in the SpeedExtractor E-914 using the optimized extraction parameters shown in Table 2. Extraction was carried out at T = 100 °C and p = 100 bar with a mixture of *n*-hexane/acetone (75%/25%). The static extraction time (hold time) was 13 min.

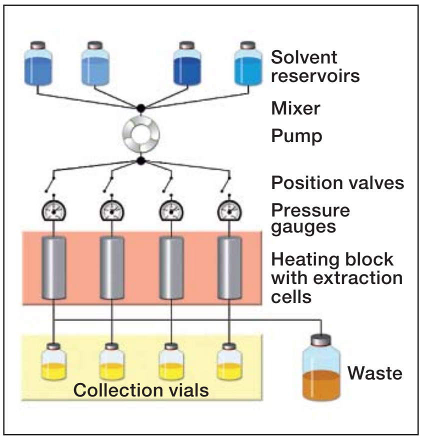

Fig. 5: Illustration of the parallel extraction system E-914

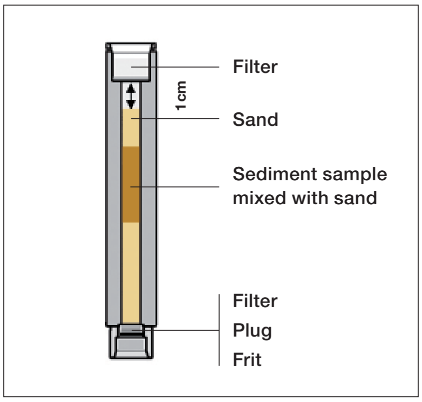

Fig. 6: Cell preparation

Optimization of the method

The efficiency of PSE depends on several factors: solvent, pressure, temperature, matrix, number of cycles, and extraction time.

For comparative reasons, the extraction solvent mixture *n*-hexane/acetone (75%/25%) chosen for automated Soxhlet was also used for PSE. Pressure and temperature were set according to the EPA recommendations for pressurized solvent extraction, which stipulate that best recoveries are achieved at 100 bar and 100°C. Temperature above 100°C may result in co-extraction of undesired organic compounds embedded in the matrix and degradation or decomposition of the target analytes [8].

A good understanding of the extraction process is necessary to obtain the optimal extraction time. During the extraction process, samples go through several automated steps. In a first heat-up step, the pressure and temperature inside the extraction cell are slowly increased to the set parameters of the extraction program.

During the hold step, these parameters remain constant. After this step the extracts are discharged and collected in collection vials via pressure compensation. All three steps can be repeated several times based on the extraction program (Figure 7).

The extraction of the target analytes occurs during the heat-up and hold steps. The time used for the heat-up step is a device-dependent constant. The heat-up step is a fixed instrument parameter and depends on the size of the cell and the instrument configuration. The hold-time, however, can be modified by the operator and directly affects the extraction recovery and time. Another important factor of recovery is the number of cycles. At the end of each cycle, the extract is discharged into the collection vial and fresh solvent is pumped into the extraction cell to begin a new cycle.

Tab. 2: Extraction method of the SpeedExtractor E-914

SpeedExtractor E-914 (4 positions)	
Temperature	100 °C
Pressure	100 bar
Solvent	*n*-hexane 75%, acetone 25%
Cells	40 ml
Vials	240 ml
Cycles	1
Heat-up	3 min
Hold	13 min
Discharge	2 min
Flush with solvent	1 min
Flush with gas	2 min

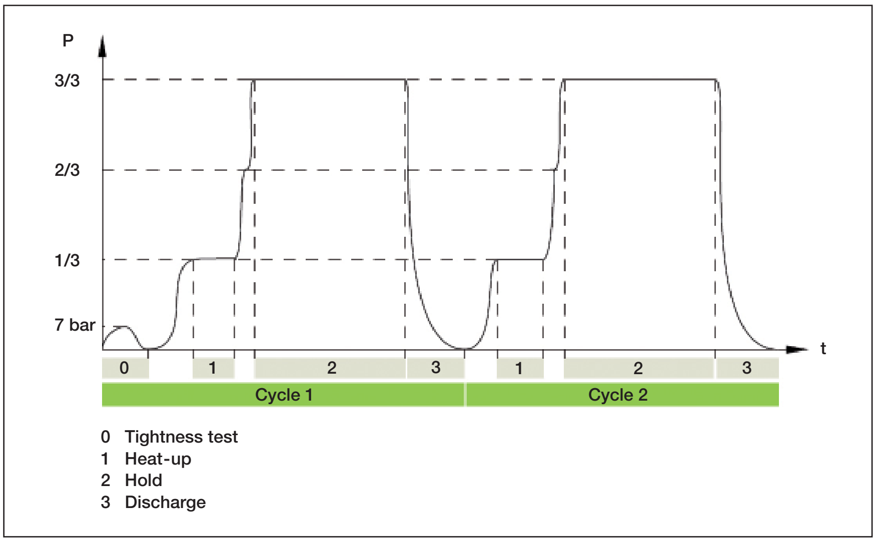

Fig. 7: Extraction method stages

Hence, for highly concentrated samples, the use of more than one cycle is necessary. However, when saturation of the analyte is not an issue, excellent recovery can be obtained by increasing the hold time only.

In order to optimize the extraction method in terms of recovery and time, a balance between the number of cycles and the hold time has to be found. To do so, the number of cycles was first kept constant at 2 and the hold time was changed to 7, 9, 13, and 18 min. The fractions of the first and second cycle were collected separately thanks to the vial change function of the SpeedExtractor. This function allows for exchanging the collection vials between cycles separately to collect extracts of different cycles.

The concentration of the congener BDE-209 in the extracts of fractions 1 and 2 was determined to illustrate the efficiency of each cycle.

Clean-up

The extracts were concentrated and transferred onto a multilayer silica gel column filled (from the top) with 0.5 g acidified silica (30% concentrated sulfuric acid, w/w), 0.5 g potassium silicate, 1 g acidified silica (30% concentrated sulfuric acid, w/w), and 0.5 g activated Florisil. The elution was carried out with 10 ml of a mixture of *n*-hexane/dichloromethane (75%/25%). The eluate was concentrated to near dryness by a gentle stream of nitrogen and resolubilized in 1 ml of iso-octane. The final extract was concentrated to 0.10 ml by a gentle nitrogen stream.

Quantification by GC-MS

The determination of *tri-* to *hepta*-BDEs was performed by GC/MS. A TRACE GC 2000 gas chromatograph coupled with a PolarisQ Ion-Trap mass spectrometer equipped with a PTV injector and an AS autosampler (Thermo Electron, Austin, Texas) was used. 2 µl aliquots were injected into the GC/MS under the following conditions: column 18 m, 0.18 mm i.d., 0.18 µm film thickness (Rtx-5MS, Restek, Bellefonte, PA, USA); injection port: 60°C for 6 s, 5°C/s to 320°C, hold 2 min; oven at 125°C for 0.5 min; 20°C/min to 320°C, hold 0.5 min; carrier gas helium at 0.6 ml/min; injection flow 0.6 ml/min; transfer flow 1.2 ml/min. Samples were analyzed using MS/MS acquisition mode: transfer line: 300°C, damping gas at 1 ml/min, ion source at 260°C. The determination of BDE-209 was performed by a TRACE gas chromatograph Ultra equipped with a cold on-column injector and an ECD-40 detector (Thermo Electron, Austin, Texas). 1 µl aliquots were injected into the GC-ECD under the following conditions: column 7 m, 0.32 mm i.d. (Rtx-5MS, Restek, Bellefonte, PA, USA) coupled to a retention gap 0.5 m, 0.53 mm i.d., 0.10 µm film thickness (DB-1MS, J&W Scientific, Palo Alto, CA, USA); oven at 80°C for 1 min; 40°C/min to 285°C, hold 12 min; carrier gas helium at 2.5 ml/min.

Verification of the method

Verification of BDE analyses was assured using certified sediment material (CRM BROC-02). ^{13}C-labeled congeners supplied by Wellington Laboratories Inc., Ontario, Canada (mix MBDE-MXA and MBDE-MXB) containing [$^{13}C_{12}$]BDE-28, 47, 99, 153, 154, and 183 were used as internal standards. The labeled congeners were added to the certified material before extraction.

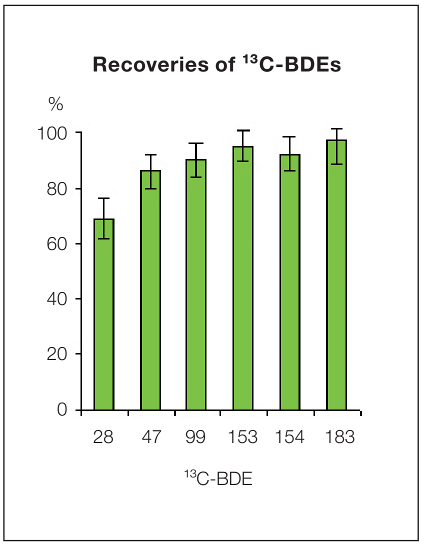

Fig. 8: Recoveries (%) of ^{13}C-labelled internal standards of PBDEs in sediment sample BROC-02 (n = 4).

Results and discussion

Recoveries of the internal standards were found to be in the range of 69 to 97% (Figure 8), and therefore meet the acceptance criteria established by the U.S. EPA method 1614 which describes the analysis of PBDEs in water, soil, sediment and tissue [13]. The relative standard deviations (RSDs) were smaller than 9%. The limits of quantification (LOQ) for BDEs were determined using signal-to-noise ratio method (>10:1). The limits of quantification were calculated to be 0.10 ng/g for *tri-* to *hepta*-BDEs and 0.5 ng/g for BDE-209.

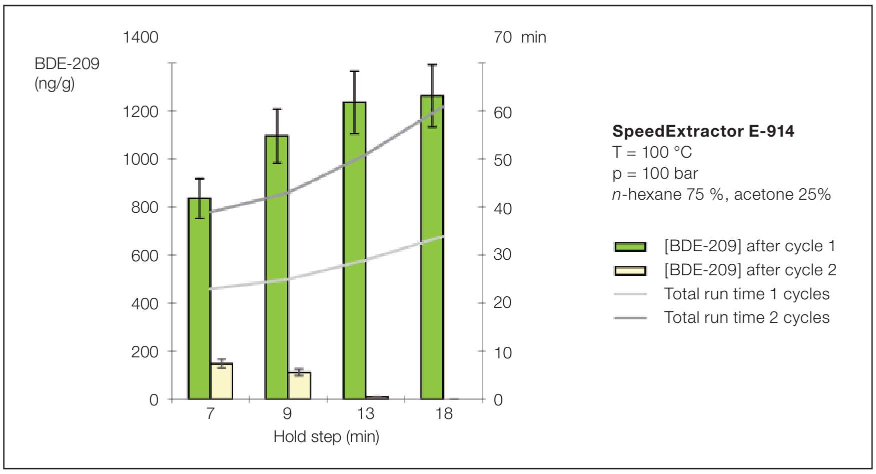

Fig. 9: Optimization of PSE: recovery of BDE-209 against number of cycles and hold time

Tab. 3: Contents of PBDEs (ng/g dry weight) in CRM BROC-02

	CRM BROC-02				
	SpeedExtractor E-914		Extraction System B-811		Certified values
PBDE	Mean [ng/g] n = 4	RSD [%]	Mean [ng/g] n = 7	RSD [%]	Mean [ng/g]
BDE-28/33	0.52	23	0.55	14	0.63
BDE-47	10.7	8	10.3	6	10.14
BDE-49	2.63	8	3.15	9	2.75
BDE-66	0.18	11	0.22	4	0.29
BDE-85	0.68	10	0.74	18	0.66
BDE-99	12.9	12	12.7	10	14.2
BDE-100	3.03	21	3.28	7	3.04
BDE-153	1.24	7	1.39	11	1.93
BDE-154	1.46	3	1.27	13	1.71
BDE-183	0.39	15	0.28	21	0.45
BDE-209	1207	8	1197	11	1164

Optimization of the method (PSE)

After cycle 1, the extraction was not completed within hold times of 7 and 9 minutes since the BDE-209 content in the extracts of cycle 2 was still considerable. With an extraction hold time of 13 minutes, the extraction was almost completed after cycle 1, with an insignificant concentration of BDE-209 extracted in cycle 2. Recoveries were not significantly improved with a hold step of 18 minutes (Figure 9). Using minimum time and solvent, while providing excellent recovery, PSE with one cycle and an extraction hold time of 13 minutes, T = 100°C and p = 100 bar, was therefore chosen as the optimal method for the extraction of PBDE congeners in sediment samples.

Reproducibility

Reproducibility of PSE was very good; RSDs were generally below 20% (Table 3). As expected higher RSDs were found for the low concentrated BDE (BDE 28/33 and BDE 183).

Comparison of PSE with automated Soxhlet

The concentrations of PBDEs obtained by PSE were very similar to those obtained by automated Soxhlet extraction and all congeners showed good agreement (Table 3). This indicates that the recoveries of PSE within the selected conditions are comparable to those of automated Soxhlet extraction. The efficiency however, is better for PSE as the total extraction time is 26 minutes compared to 150 minutes for automated Soxhlet extraction. PSE results showed also good comparability to the certified values with two exceptions (Table 3). PSE gave lower values for congeners BDE-66 and BDE-153 (62 and 64% from the certified value). Since the automated Soxhlet extraction also exhibited lower values (76 and 72%), this can likely be attributed to a stronger adsorption of these two congeners to the matrix.

Extraction time and solvent

The selected extraction conditions were suitable for PBDE extraction since the PSE results were very similar to the automated Soxhlet values and the certified values. The extraction time of 26 min was sufficient to deliver very good recoveries of PBDEs. Compared to the 150 min of the automated Soxhlet extraction system, PSE was 5 times faster. PSE only used 30 ml of solvent per position, compared to 100 ml for automated Soxhlet extraction.

Extraction of PBDEs in a real sample from Pallanza bay

The sample collected from Pallanza bay was processed in the SpeedExtractor E-914 to test the reproducibility of the instrument at low concentration levels since previous studies showed that sediment samples from this locality contained low levels of PBDEs [11]. Four congeners were analyzed (BDE-47, BDE-99, BDE-100, and BDE-209). The mean values and the RSDs are reported in Table 4. The reproducibility was excellent: all RSDs were below 15%.

Conclusion

The PBDE contents obtained with the SpeedExtractor E-914 and the Extraction System B-811 were comparable. Both instruments delivered extractions with very high accuracy, recovery and reproducibility.

The extraction time with the SpeedExtractor E-914 was much shorter with 26 min compared to 150 min for automated Soxhlet extraction and the amount of solvent was reduced to 30 ml/sample.

Consuming a minimum of solvent, delivering extractions with high reproducibility, accuracy and speed PSE is an alternative to automated Soxhlet extraction for the extraction of PBDEs from sediment samples.

Tab. 4: Contents of PBDEs (ng/g dry weight) in a sediment sample from Pallanza bay

	Sediment – Pallanza bay	
	SpeedExtractor E-914	
PBDE	Mean [ng/g] n = 4	RSD [%]
BDE-47	0.43	14
BDE-99	0.46	10
BDE-100	0.16	13
BDE-209	3.60	14

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Application Note 010/2009. Determination of PBDEs in Sediment Samples using the SpeedExtractor E-914.

Application Note 014/2009. Determination of PBDEs in Sediment Samples using the Extraction System B-811.

Operation manual Extraction System B-811

Operation manual SpeedExtractor E-914

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