

Application Note

010/2009



SpeedExtractor E-914

**Determination of PBDEs in Sediment Samples
using the SpeedExtractor E-914**

Determination of PBDEs in Sediment Samples using the SpeedExtractor E-914

Polybrominated diphenyl ethers (PBDEs) are flame retardants used in a myriad of applications and are monitored worldwide because of health concerns. In this application, PBDE congeners were extracted from two sediment samples (certified sample and sediment core from Lake Maggiore) according to EPA 3545A [1] using the SpeedExtractor E-914 and determined by GC-MS/GC-ECD. The results were comparable to the certified values and showed high reproducibility and recovery.

Introduction

PBDEs are brominated flame retardants used in a myriad of applications (Figure 1). They are monitored worldwide because of health concerns. Bay and estuary sediments store toxic compounds and are therefore very appropriate sites to evaluate the contamination level of PBDEs in the environment.

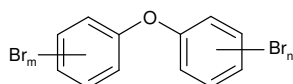


Figure 1: Chemical structure of PBDEs

This application note describes the extraction of PBDEs from two sediment samples using the SpeedExtractor E-914: one certified sediment sample BROCC-02 (RIVO, The Netherlands), one real sample from lake Maggiore, Italy. The PBDE congeners were determined by GC-MS/GC-ECD.

Experimental

Instrumentation: SpeedExtractor E-914 with 40 ml cells, Trace 2000 GC / PolarisQ ion-trap mass spectrometer (Thermo), Trace GC Ultra / ECD-40 detector (Thermo).

Sample: Certified reference material (CRM) BROCC-02 provided by the Research Institute for Fisheries (RIVO), The Netherlands, sediment sample collected from Pallanza Bay, Lake Maggiore, Italy.

The extraction of both samples was performed four-fold using the parameters given in Table 1. This extraction method requires an overall time of 26 min and consumes approx. 30 ml of solvent *per* position.

Table 1: Extraction method of the SpeedExtractor E-914

Temperature	100 °C
Pressure	100 bar
Solvent	<i>n</i> -hexane 75%, acetone 25%
Cells	40 ml
Vials	240 ml
Cycle	1
Heat-up	3 min
Hold	13 min
Discharge	2 min
Flush with solvent	1 min
Flush with gas	2 min

The extract was concentrated and cleaned on a multi-layer silica gel/florisil column. The determination of tri- to hepta-BDEs was performed by GC-MS. The congener BDE-209 was determined by GC-ECD.

Results

The mean values (n=4) obtained for BROCC-02 are displayed in Table 2. They are comparable to the certified values. The calculated RSDs are generally below 20%. The recoveries of the ¹³C-labeled internal standards fall in the range of 69-97% (data shown in Application Note 010/2009).

Table 2: Mean values (n=4) (ng/g) for eleven PBDE congeners

PBDE	Certified Sample BROCC-02		Certified Values [ng/g]
	Mean (n=4) [ng/g]	RSD [%]	
BDE-28/33	0.52	23	0.63
BDE-47	10.7	8	10.1
BDE-49	2.63	8	2.75
BDE-66	0.18	11	0.29
BDE-85	0.68	10	0.66
BDE-99	12.9	12	14.2
BDE-100	3.03	13	3.04
BDE-153	1.24	7	1.93
BDE-154	1.46	3	1.71
BDE-183	0.39	15	0.45
BDE-209	1'207	8	1'164

The mean concentrations obtained for the sediment sample from Pallanza Bay are displayed in Table 3. They range from 0.43 to 3.6 ng/g with RSDs below 15%.

Table 3: Mean values (n=4) (ng/g) for 4 PBDE congeners

PBDE	Sediment Sample from Pallanza Bay	
	Mean (n=4) [ng/g]	RSD [%]
BDE-47	0.43	14
BDE-99	0.43	10
BDE-100	0.16	13
BDE-209	3.60	14

Conclusion

The SpeedExtractor E-914 delivered results with high accuracy, reproducibility and recovery. The data show that this procedure is perfectly suitable for the extraction of PBDEs in sediment.

Acknowledgement

We sincerely thank the CNR Water Research Institute in Italy.

References

[1] U.S. Environmental Protection Agency. Method 3545A, Pressurized Fluid Extraction (PFE)

SpeedExtractor E-914 operation manual

For more detailed information refer to Application Note 010/2009

1 Introduction

PBDEs are organobromine compounds used as flame retardants to improve fire safety in domestic and commercial applications. Three types are defined based on the average number of bromine atoms in the molecule: 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 [1,2]. The production of PBDEs increased rapidly the last 30 years due to the growing popularity of personal computers and other electronic equipment. The global market demand was about 67,000 tons in 2001 and it is still increasing at 4% per year despite of restrictions following risks assessment reports [3]. 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 [4].

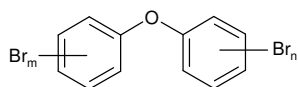


Figure 1: Chemical structure of PBDEs

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 PBDEs exposure causes endocrine disrupting effects, affects reproductive processes, reduces immune system performance and there is evidence of neurotoxicity [5-10].

This application note describes the extraction of PBDEs from two sediment samples using the SpeedExtractor E-914: one certified sample BROCC-02 (RIVO, The Netherlands), one real sample from Lake Maggiore, Italy. The PBDE congeners were analyzed by GC-MS/GC-ECD. The following extraction procedure meets requirements of the U.S. EPA method 3545A [11].

2 Equipment

- SpeedExtractor E-914, with 40 ml cells
- Ponar gravity corer (PVC tube, length 1000 mm, i.d. 630 mm)
- Lyophilizator BVL2/FM (Brizio Basi & C., Milan)
- Vibratory sieve shaker (Fritsch, Germany)
- Thermo Electron TRACE GC 2000 coupled with a polarisQ ion-Trap mass spectrometer equipped with a PTV injector and a AS 3000 autosampler
- TRACE GC Ultra equipped with a cold on-column injector and an ECD-40 detector (Thermo Electron, Austin, Texas)

3 Chemicals and Materials

- *n*-Hexane, analytical grade, Sigma-Aldrich (52765)
- Acetone, analytical grade, Sigma-Aldrich (650501)
- Isooctane, analytical grade, Sigma-Aldrich (650439)
- Dichloromethane, analytical grade, Sigma-Aldrich (323993)



- ^{13}C labeled internal PBDE standards provided by Wellington Laboratories Inc. (Ontario, Canada): concentrations of BDE-28/33, 47, 49, 66, 85, 99, 100, 153, 154, 183, 209 were determined by external calibration using the standards mixtures EO-5099, Cambridge Isotope Labs (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 Inc. (New Haven, CT, USA) containing congeners BDE-28, 47, 99, 100, 153, 154, 183 and 209. BB-209 was used as internal standard for BDE-209 and was obtained from AccuStandards Inc. (New Haven, CT, USA)
- Silica gel (70-230 mesh), Sigma Aldrich (288624)
- Florisil (100-200 mesh), Sigma Aldrich (220736)
- Quartz Sand, particle size 0.3-0.9 mm, Buchi (037689)
- Capillary column Restek Rtx-5MS, 18 m x 0.18 mm i.d, x 0.18 μm film thickness (Bellefonte, PA, USA)
- Capillary column Restek Rtx-5MS, 7 m x 0.32 mm i.d, x 0.25 μm film thickness (Bellefonte, PA, USA) coupled to a retention gap 0.5 m x 0.53 mm i.d., 0.10 μm film thickness (DB-1MS, J&W scientific, Palo Alto, CA, USA).

4 Samples

- Certified Reference Material (CRM BROCC-02) purchased from the Research Institute for Fisheries (RIVO), The Netherlands. The sample was collected near a brominated flame retardants (BFR) factory in Western Scheldt, The Netherlands.

The CRM BROCC-02 was delivered lyophilized and 90 μm sieved, ready for extraction.

- Sediment sample collected in Pallanza Bay by the Institute of Economy Study (ISE) of CNR in March 2007. Sampling station "13" located near Baveno. Contribution to the project: "Research on DDT and other dangerous substances in the Lake Maggiore ecosystem", International Committee for the Protection of Italian-Swiss Waters [12].

The sample was collected with a ponar gravity corer (transparent PVC tube of internal diameter 630 mm and length of 1000 mm developed by the ISE). Subsamples were obtained by slicing the core in 1 cm intervals.

5 Procedure

The extraction and analysis of PBDEs in sediment includes the following steps:

- Preparation of sample
- Preparation of cells
- Extraction with the Speed Extractor E-914
- Determination of the congeners by GC-MS and GC-ECD

5.1 Preparation of the cells

- Fill half of the cell with sand
- Weigh in approx. 0.6 g of sample and mix it with approx. 2-3 g of sand
- Add the mixture sample/sand into the cell
- Fill up the cell with sand
- Add the internal standards on top of the sand

5.2 Extraction with the SpeedExtractor E-914

The parameters used for the extraction of PBDEs are displayed on Table 1. Both samples, CRM BROCC-02 and the sediment sample from Pallanza bay, were four-fold extracted. The total time of the following extraction method is 26 min. It consumes 30 ml of solvent *per* position.

Table 1: Extraction method of the SpeedExtractor E-914

Parameter	Value
Temperature	100 °C
Pressure	100 bar
Solvent	<i>n</i> -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



5.3 Clean-up

- Concentrate the extracts to 2-3 ml by a gentle nitrogen stream
- Filtrate the residue through 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), 0.5 g activated Florisil
- Elute the column collecting 10 ml of a mixture hexane 50% / dichloromethane 50%
- Concentrate the eluates to near dryness by a gentle nitrogen stream
- Resolubilize the eluates in 1 ml of isooctane
- Concentrate the final extracts to 0.10 ml by a gentle nitrogen stream

5.4 Quantification by gas chromatography

The determination of tri- to hepta-BDEs was performed by GC-MS with a Thermo Electron TRACE GC 2000 gas chromatograph coupled with a PolarisQ Ion-Trap mass spectrometer and equipped with a PTV injector and a AS 3000 autosampler.

2 µl aliquots were injected to the GC-MS under the following conditions: Column 18 m, 0.18 mm ID, 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: 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: 260 °C.

The determination of BDE-209 was performed by GC-ECD equipped with a cold on-column injector. 1 µl aliquots were injected into the GC-ECD under the following conditions: Column 7 m, 0.32 mm ID (Rtx-5MS, Restek, Bellefonte, PA, USA) coupled to a retention gap 0.5 m, 0.53 mm ID, 0.10 µm film thickness

(DB-1MS, J&W scientific, Palo Alto, CA, USA); oven: 80 °C for 1 min; 40 °C/min to 285 °C, hold 12 min; carrier gas helium at 2.5 ml/min.

6 Results

The results obtained for the certified sample CRM BROC-02 are displayed in Table 2. The values obtained with the SpeedExtractor and the certified values are comparable. The repeatability was good with RSDs generally below 20%. As expected, higher RSDs were found for low concentrated BDE (BDE 28/33 and BDE 183). The recoveries of the ¹³C-labeled internal standards were good for the analyzed congeners BDE-28, BDE-47, BDE-99, BDE-153, BDE-154 and BDE-183 (Table 3): recovery rates were situated in the range of 69-97%, which meet acceptance criteria established by the U.S. EPA method 1614 describing analysis of polyhalogenated compounds in water, soil, sediment and tissue [13].



Table 2: Mean values (n = 4) (ng/g) and RSDs for the determined PBDE congeners in BROC-02

PBDE	Sample BROC-02		
	SpeedExtractor E-914		Certified values [14]
	Mean (n = 4) [ng/g]	RSD [%]	Mean [ng/g]
BDE-28/33	0.52	23	0.63
BDE-47	10.7	8	10.1
BDE-49	2.63	8	2.75
BDE-66	0.18	11	0.29
BDE-85	0.68	10	0.66
BDE-99	12.9	12	14.2
BDE-100	3.03	13	3.04
BDE-153	1.24	7	1.93
BDE-154	1.46	3	1.71
BDE-183	0.39	15	0.45
BDE-209	1'207	8	1'164

Table 3: Recoveries (%) of ¹³C-labelled internal standards of PBDEs in sediment sample BROC-02

	Positions in E-914				Mean	RSD [%]
	1	2	3	4		
[¹³ C ₁₂]BDE-28	78	71	67	61	69	10
[¹³ C ₁₂]BDE-47	92	84	89	78	86	7
[¹³ C ₁₂]BDE-99	97	88	83	91	90	6
[¹³ C ₁₂]BDE-153	101	98	92	89	95	5
[¹³ C ₁₂]BDE-154	99	89	94	85	92	6
[¹³ C ₁₂]BDE-183	106	103	91	89	97	8

The mean concentrations obtained for the sediment sample from Pallanza Bay are displayed in Table 4. They range from 0.43 to 3.6 ng/g. These results show that for low concentrations, the repeatability is still very good with RSDs below 15%.

Table 4: Mean values (n=4) (ng/g) for 4 PBDE congeners

PBDE	Sediment-Pallanza Bay	
	Mean (n=4) [ng/g]	RSD [%]
BDE-47	0.43	14
BDE-99	0.43	10
BDE-100	0.16	13
BDE-209	3.60	14



7 Conclusion

PBDEs were extracted from a certified sediment sample and a real sample from Pallanza bay using the SpeedExtractor E-914. The congeners were subsequently analyzed by GC-MS/GC-ECD. The SpeedExtractor E-914 gave accurate and reproducible results. The extraction needed an overall time of 26 min and it consumed 30 ml of solvent *per* position. The method shows that this procedure is perfectly suitable for the extraction of PBDEs in sediment.

8 Acknowledgements

We sincerely thank Claudio Roscioli and Licia Guzzella of the CNR Water Research Institute, Brugherio, Italy, for their help in the development of this application note.

9 References

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SpeedExtractor E-914 operation manual

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