

# DEVELOPMENT AND COMPARISON OF METHODS FOR THE SIMULTANEOUS DETERMINATION OF VOLATILE BROMINATED FLAME RETARDANTS AND DERIVATES IN WATER

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## Introduction

Flame retardants (FRs) are structurally diverse chemicals that suppress the combustion process. Thirty-nine percent of FRs are based on bromine (1). Some are phenolic compounds, such as 4-bromophenol, 2,4-dibromophenol and 2,6-dibromophenol, which may occur as by-products from the photochemical degradation of 3,5,3',5'-tetrabromobisphenol A in water (2,3). Bromophenols (BPhs) can degrade into corresponding bromoanisoles via *O*-methylation by bacterial micro-organisms (4). BPhs and brominated anisoles are of considerable interest because of their extremely low taste thresholds (sub-ng/L in water) (5). 4-Bromoaniline, 2,4-dibromoaniline, 2,6-dibromoaniline and 2,4,6-tribromoaniline are reported as intermediates in the formulation of FRs. Bromotoluenes and aliphatic bromides may be also formed during the thermal decomposition of flame-retarded polystyrene (6) or in water treatment processes. Hexabromocyclododecane (HBCD) has been reported to biodegrade into 1,5,9-cyclododecatriene as the final product (7).

Few studies have been conducted to determine bromoanisoles, BPhs, bromoanilines, bromotoluenes and aliphatic bromides in water. Gas chromatography-mass spectrometry (GC-MS) has been recognized as the method of choice for these brominated flame retardants (BFRs) and derivatives. A pre-concentration technique is recommended. The simultaneous determination of BPhs, bromotoluenes, bromoanilines, bromoanisoles and the isomers of 1,5,9-cyclododecatriene in water samples has not been reported yet. The aim of the present study was to develop and compare different methods for the simultaneous analysis of these BFRs and derivatives. Four different extraction techniques, liquid-liquid extraction (LLE), solid phase extraction (SPE), headspace (HS) and solid phase micro-extraction (SPME), combined with GC-MS in EI and ECNI were tested, using a multi-factor experimental design. The feasibility of the most reliable and robust methods was evaluated by analyzing water samples from the river Western Scheldt (The Netherlands).

## Materials and methods

*n*-Decyl bromide (DeBr, 98%) and *n*-cetyl bromide (CeBr) were supplied by Eurobrom B.V (Amsterdam, The Netherlands). 2-Bromotoluene (2BT, 99%), 3-bromotoluene (3BT), benzal bromide (BBr), 4-bromotoluene (4BT, 98%), 2-bromoanisole (2BA, 97%), 4-bromoanisole (4BA, 99%), 3,5-dibromotoluene (3,5DBT, 97%), 2,5-dibromotoluene (2,5DBT, 98%), 2,4-dibromophenol (2,4DBPh, 95%), 2,6-dibromoaniline (2,6DBA), 2,6-dibromophenol (2,6DBPh, 99%), 2,4-dibromoaniline (2,4DBA, 98%), 2,4-dibromoanisole (2,4DBAl), *trans*-, *trans*-, *trans*-1,5,9-cyclododecatriene (EEE), *trans*-, *trans*-, *cis*-1,5,9-cyclododecatriene (EEZ, 98%), *trans*-, *cis*-, *cis*-1,5,9-cyclododecatriene (EZZ), 2,4,6-tribromotoluene (TBT, 98%), 2,4,6-tribromoanisole (TBAI, 99%), 2,4,6-tribromoaniline (TBA, 98%) and 2,3,4,5,6-pentabromotoluene (PBT, 99%) were supplied by Sigma-Aldrich Chemie B.V. (Zwijndrecht, The Netherlands). All solvents used were suitable for organic residue analysis. Standard mixtures of approx. 2 µg/mL in acetone were weekly prepared from individual stock solutions in dichloromethane.

Millipore 47 mm glass vacuum filtration apparatus (Bedford, MA, USA) was used for SPME with glass microfiber filters GC/C from Whatmann (Maidstone, UK) on Empore<sup>TM</sup> SDB-XC (3M, St. Paul, MN, USA) high performance extraction disks (Varian Benelux, Middelburg, The Netherlands) (8). SPME manual holders and fibers were from

Supelco (Bellefonte, PA, USA). Two fibers, 100  $\mu\text{m}$  polydimethyl siloxane (PDMS) and 85  $\mu\text{m}$  carboxen-polydimethylsiloxane (CAR-PDMS) (3, 9-11) were tested.

*Liquid-liquid extraction procedure.* For the method validation experiments, 1 L of HPLC water was spiked with 1 mL of a mixture solution containing all the target compounds. The three dichloromethane phases were pooled, and concentrated to 1 mL.

*Solid-phase extraction procedure.* SDB-XC is a 100% spherical, porous polystyrene divinyl benzene copolymer used as a reversed phase sorbent for solid phase extraction. It provides a unique selectivity, especially in the retention of moderately polar, water-soluble analytes.

*Headspace experimental design.* HS was conducted using a Combi Pal (CTC Analytics, Zwingen, Switzerland). CTC Pal is a multifunctional autosampler for HS and liquid GC injection. The extraction conditions were optimized by means of a multifactorial design.

*Solid-phase micro-extraction design.* The type of fiber (PDMS or CAR-PDMS), extraction mode (headspace or direct immersion), extraction temperature, salt content, extraction time and agitation speed were optimized in two steps using a multi-factorial design. Desorption time was set at 2 min in the injection port at 250  $^{\circ}\text{C}$  for PDMS and at 300  $^{\circ}\text{C}$  for CAR-PDMS. Additional desorption was 15 minutes in the heater unit of the CTC Pal, thus reducing the carryover.

*Gas chromatography-mass spectrometry.* HS and SPME analyses were performed on an Agilent GC 6890N, (Palo Alto, CA, USA) equipped with a CTC Pal sample injector and an Agilent 5973N MS. Extracts from SPE and LLE were also analyzed by a 6890N GC/5975XL ECNI-MS. The analyses using the 5973 MSD were performed on a 25 m length x 0.22 mm I.D, 0.25  $\mu\text{m}$  SGE BPX5 column (SGE, Bester, Amstelveen, The Netherlands). The injector (200  $^{\circ}\text{C}$ ) was operated in the splitless mode (2 min) The injector temperature for liquid injection, HS and SPME injection was 250  $^{\circ}\text{C}$ . The ECNI-MS analyses were performed on a 60 m x 0.25 mm, 0.25  $\mu\text{m}$  thickness, CP-Sil8 CB column (Varian). The injector (200  $^{\circ}\text{C}$ ) was operated in the pulsed splitless (1.5 min).

*Validation procedure.* The validation of the different methods was performed with HPLC water according to EURACHEM and ISO 5725 guidelines. The linearity was established in the 0.05-1000 ng/mL range. Statistical analysis (ANOVA) was performed to check the goodness-of-fit and linearity (12). Precision, (intra-day repeatability and between-day precision over three days), was calculated at two concentration levels, three replicates at each level. Trueness was evaluated by spiking HPLC water with two different concentrations of analytes, depending of the extraction methods. All the measurements were done in triplicate.

*Sample collection.* Four water samples were collected from two locations in the Western Scheldt (The Netherlands), near the mouth of the Ghent-Terneuzen Canal. The sampling was performed twice with one month in between. Water samples were filtered and poured into 1L-amber glass bottles with polypropylene screw caps and stored at -18 $^{\circ}\text{C}$ . Each sample was analyzed in triplicate.

## Results and discussion

As an example of the substantial volume of results, those of the SPME-GC-EIMS method are given in Table 1. The major ions formed in the EI mode were  $[\text{M}^{\dagger}]$  and  $[\text{M-Br}]^{\dagger}$ . EI provides a much better sensitivity for mono-bromine substitutes. The better sensitivity of ECNI strongly depends on the bromine content. Hence, the three isomers of 1,5,9-cyclododecatriene gave no ECNI signal. The differences in sensitivity among the two DBTs and benzyl bromide are due to the position of bromine in the molecule. The parameters affecting the micro-extraction process, the type of fiber coating (100  $\mu\text{m}$  PDMS and 75  $\mu\text{m}$  CAR-PDMS), the extraction temperature (40, 60, 80  $^{\circ}\text{C}$ ) and the extraction mode (direct immersion DISPME and headspace HSSPME) were evaluated by a multifactor categorical 2\*3\*2 design involving 12 runs and three replicates was selected. The most significant factor for the majority of the compounds was either the fiber (A) or the interaction between fiber and temperature (AC). The extraction temperature (C) was found to be relevant for 17 analytes, among them the monobromotoluenes and the aliphatic bromide, while the mode of

**Table 1. Method performance figures of SPME-GC-(EI)-MS method.**

Compound	LODs (ng/mL)	Linear range (ng/mL)	<sup>a</sup> F <sub>ext</sub> /p	R <sup>2</sup>	<sup>b</sup> Repeatability (RSD% n=3)	
2BT	0.029	0.029-87.9	7381/<0.001	0.9993	19%	22%
4BT	0.034	0.034-113	3032/<0.001	0.9984	22%	22%
3BT	0.023	0.023-109	3618/<0.001	0.9986	18%	22%
4BA	0.040	0.040-101	1548/<0.001	0.9968	20%	21%
2BA	0.028	0.028-101	1252/<0.001	0.9960	21%	17%
EEE	0.008	0.008-117	5219/<0.001	0.9990	9%	16%
EEZ	0.014	0.014-98.5	6431/<0.001	0.9992	11%	16%
3,5DBT	0.009	0.009-115	9332/<0.001	0.9995	75%	20%
2,5DBT	0.011	0.011-110	4756/<0.001	0.9989	19%	20%
EZZ	0.011	0.011-101	10121/<0.001	0.9995	11%	16%
BBr	0.168	0.168-104	2610/<0.001	0.9985	71%	16%
DeBr	5.54	0.554-96.7	1901/<0.001	0.9989	n.d	12%
2,4DBPh	4.16	4.16-52.8	42.4/0.096	0.977	n.d	18%
2,6DBPh	0.041	0.041-71.6	2907/<0.001	0.9986	21%	22%
2,4DBAl	0.0006	0.0006-113	9280/<0.001	0.9992	27%	21%
2,4DBA	0.185	0.185-121	720/<0.001	0.9931	7%	19%
TBT	0.002	0.002-121	51682/<0.001	0.9999	16%	16%
TBAI	0.00086	0.00086-103	2244/<0.001	0.9978	19%	18%
TBA	0.007	0.007-93	617/<0.001	0.9936	24%	16%
CeBr	0.006	0.006-8.69	4414/<0.001	0.9991	16%	5%
PBT	0.00077	0.00077-9.34	330/<0.001	0.9910	19%	26%

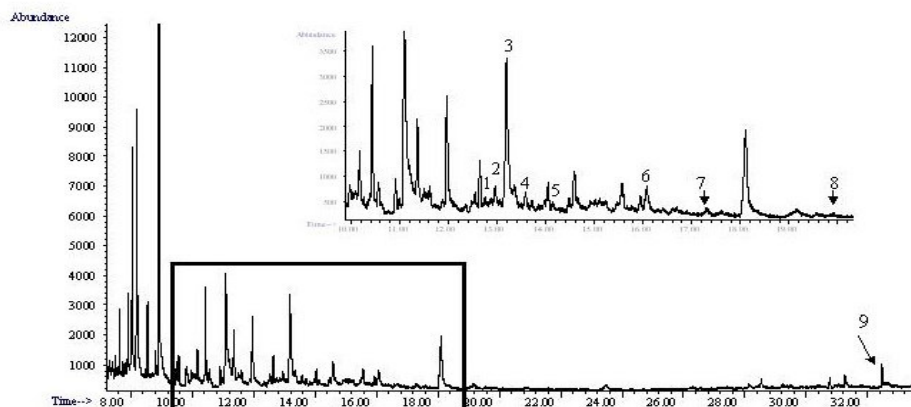
<sup>a</sup>Linearity validation: one-way ANOVA; F<sub>exp</sub>: experimental F-value; p: p-value from F(1, n-2) one-tailed test

<sup>b</sup>Spiked at 2 ng/mL and 20 ng/mL; n.d.: not detected.

extraction (B) was to be considered when extracting bromoanisoles and bromoaniline. Data were also analyzed statistically using partial least squares regression (PLS)(13). Analyzing all information, a compromise should be reached to achieve the optimal conditions for all the target compounds due to their diverse in behaviour and physico-chemical properties. Despite the better response for the most volatile compounds with CAR-PDMS, PDMS was the selected fiber. In addition to its high efficiency for heavier compounds, it was the only one capable of sampling 2,4-DBA and it did not show so much carryover as the CAR-PDMS. When PDMS is used, low temperatures and immersion seemed to be the most effective conditions, except for PBT and CeBr, whose extraction yield increased with temperature. Taking into account the overall tendency, immersion mode and 60 °C were the values selected for the other two significant factors. In a second optimization step by Box Behnken design (13), the optimal SPME extraction conditions were found to be 30 % salt content, 600 rpm agitation speed and 15 min extraction.

The methods were validated and compared in terms of detection limits, linearity, precision and trueness under the optimized conditions (SPME and HS procedures). SPE and LLE samples were analysed with EI and ECNI-MS. Table 1 gives the results for the SPME-GC-(EI)-MS method.

The SPE-GC-(ECNI)MS method allowed the identification and quantification of 2,5DBT, 3DBT, BBr, 2,4DBPh, 2,6DBPh, 2,6DBA, 2,4DBAl, TBT, TBAI, TBA and PBT in the samples from the Western Scheldt estuary ([Figure 1](#)).



**Figure 1.** Chromatogram of a water sample (Peak identification: 1. 3,5DBT+2,5DBT, 2. BBr, 3. 2,4DBPh, 4. 2,6DBPh, 5. 2,6DBA, 6. 2,4,4.DBA, 7. TBAI, 8. TBA, 9. PBT)

The presence of TBT, TBAI and TBA was confirmed with SPME-GC-(EI)MS. The other compounds were < LOD. The 2,4DBPh levels are below its odour threshold. One sample exceeded the taste threshold of 2,6DBPh (0.5 ng/L). Most samples exceeded the TBAI threshold of 0.03 ng/L (7,15), which was confirmed by the characteristic earthy-musty odour.

SPE-GC-(ECNI)MS, SPE-GC-(EI)MS and SPME-GC-(EI)MS are the preferred methods for the simultaneous determination of bromoanisoles, bromoanilines, bromotoluenes, bromophenols, aliphatic bromides and the isomers of 1,5,9-cyclododecatriene in water, because of their low detection limits, and good linearity, precision, trueness and selectivity. ECNI was more sensitive for the analysis of compounds with more than two bromine atoms, whereas EI is more sensitive for compounds with one or no bromine atoms. To our knowledge, this is the first time that such a wide suite of compounds is analyzed in a single run.

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