Tomáš Čajka
Jana Hajšlová
Radek Kazda
Jan Poustka

Institute of Chemical Technology, Prague, Department of Food Chemistry and Analysis, Technická 3, 166 28 Prague 6, Czech Republic

Challenges of gas chromatography–high-resolution time-of-flight mass spectrometry for simultaneous analysis of polybrominated diphenyl ethers and other halogenated persistent organic pollutants in environmental samples

The potential of a gas chromatographic method employing high-resolution time-of-flight (TOF) mass spectrometry was evaluated for detection of polybrominated diphenyl ethers (PBDEs) in the environmental matrices represented by fish and river sediment. Two ionisation techniques, viz. electron ionisation (EI) and negative ion chemical ionisation (NICI), the latter with methane as a reagent gas, were used in this study. While the instrumental lowest calibration levels (LCLs) obtained in EI were in the range from 1 to 5 pg, their values ranged between 10 to 250 fg in NICI mode. This enhancement in detectability of target analytes enabled identification/quantification of even minor PBDE congeners, and consequently, improved characterisation of particular sample contamination patterns. In addition, this method allowed estimation of the PCB levels in examined samples. CB 153 was used as a contamination marker in this study.

Key Words: PBDEs; PCBs; High-resolution time-of-flight mass spectrometry; GC–MS; TOF

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1 Introduction

Brominated flame retardants (BFRs) are chemicals widely used in various products such as plastics, textiles, and furnishing foams to prevent a fire hazard. Generally, two types of such additives are in use: (i) the reactive BFRs represented mainly by tetrabromobisphenol A (TBBPA) are incorporated into the polymeric materials by covalent binding whereas (ii) the additive types, represented by polybrominated diphenyl ethers (PBDEs), polybrominated biphenyls (PBBs), and hexabromocyclododecane (HBCD), are embedded into a matrix of appropriate polymer [1].

The structural likenesses of PBDEs with polychlorinated biphenyls (PCBs) and PBBs results in a similarity of chemical properties. Due to their high lipophilicity and considerable resistance to degradation processes, PBDEs and other BFRs have become an extensive environmental concern in the last decade. Their residues have been identified in many environmental matrices such as air, sewage sludge, fish, shellfish, birds, and/or mammalia (including human adipose tissues and breast milk) [2]. However, it should be noted that levels of BFRs occurring in environmental samples, even in those classified as "fairly contaminated", are typically significantly lower than concentrations of PCBs in similarly categorised samples. Thus, low detection limits are needed for accurate measurements of BFRs.

Gas chromatography coupled to mass spectrometry (GC–MS) represents the technique most commonly used for the analysis of PBDEs [3]. Recent progress in fast detector electronics has led to "re-discovery" of the suitability of time-of-flight (TOF) mass analysers for trace analysis of organic pollutants in various matrices [4, 5]. GC–TOF MS has been demonstrated as a powerful tool not only for quantification of target analytes but also for identifying non-targeted compounds in complex matrices [6, 7]. TOF MS can be performed emphasising either high speed (unit mass resolution) or high resolution, albeit at slower acquisition speed. In the case of high-speed TOF MS, comprehensive two-dimensional gas chromatography (GC × GC) is often used for good resolution of sample components, permitting unbiased identification of sample components and achieving the low LODs needed in residue analysis [8–10].

The advantages of high-resolution TOF over common mass analysers (unit resolution quadrupoles and ion traps), can be summarised as follows [11]:

Correspondence: J. Hajšlová, Institute of Chemical Technology, Prague, Department of Food Chemistry and Analysis, Technická 3, 166 28 Prague 6, Czech Republic.
Phone/fax: +420 220 443 185. E-mail: jana.hajslova@vscht.cz.

(i) Acquisition of spectral data across a wide mass range is possible at any time during the GC run without a decrease in detection sensitivity (i. e. full spectral information searchable in spectral library is available within the elution of sample component).

(ii) Due to a high mass resolution, matrix components yielding ions with the same nominal mass as that of the target analyte can often be partially or completely resolved, and hence do not interfere.

(iii) Mass measurement accuracy permits estimation of the elemental composition of the detected ions.

In this paper, the application potential of a high-resolution time-of-flight mass spectrometer for gas chromatographic determination of PBDEs in fish tissue and river sediment is demonstrated. The possibility of determining other halogenated contaminants (in particular, PCBs) present in samples, is also discussed.

2 Experimental

2.1 Standards and chemicals

PBDE standards were supplied by Cambridge Isotope Laboratories (Andover, MA, USA). The declared purity was at least 99%. Stock solution prepared in isooctane contained following congeners: 2,4,4′-triBDE (BDE 28), 2,2′,4,4′-tetraBDE (BDE 47), 2,2′,4,5′-tetraBDE (BDE 49), 2,3′,4,4′-tetraBDE (BDE 66), 2,2′,3,4,4′-pentaBDE (BDE 85), 2,2′,4,4′,5-pentaBDE (BDE 99), 2,2′,4,4′,6-pentaBDE (BDE 100), 2,2′,4,4′,5,5′-hexaBDE (BDE 153), 2,2′,4,4′,5,6′-hexaBDE (BDE 154), and 2,2′,3,4,4′,5′,6-heptaBDE (BDE 183). Concentrations of PBDEs in calibration solutions prepared in isooctane were as follows: 0.01; 0.025; 0.05; 0.1; 0.25; 0.5; 1; 2.5; 5; 10; 25 ng/mL. The standard for α-HBCD with declared purity 98% was also supplied by Cambridge Isotope Laboratories.

Two working solutions were used for analysis of PCBs. The first one contained only seven indicator PCB congeners (28, 52, 101, 118, 138, 153, 180), while the second one contained the following 47 congeners: (i) indicator PCBs: 28, 52, 101, 118, 138, 153, 180; (ii) mono-*ortho* PCBs: 105, 114, 156, 157, 167, 189; (iii) other PCBs: 8, 18, 31, 44, 47, 49, 56, 66, 70, 74, 84, 87, 95, 97, 99, 110, 128, 129, 137, 141, 146, 149, 151, 163, 170, 183, 187, 194, 195, 199, 202, 203, 206, 209. Concentrations of PCBs in calibration solutions prepared in isooctane were as follows: 1, 2, 5, 10, 20, 50, 100, 200, 500 ng/mL. CB 112 was used as the internal (surrogate) standard. The stock solutions of these congeners were supplied by Dr. Ehrenstorfer GmbH (Augsburg, Germany). The declared purity was at least 99%.

All solvents used in experiments (dichloromethane, hexane, ethyl acetate, cyclohexane, isooctane) were of analytical grade (Scharlau, Spain; Merck, Germany). Sulphuric acid (98%) was obtained from Merck (Germany). Anhydrous sodium sulphate obtained from Penta (Czech Republic) was heated at 500°C for 5 h and then stored in a desiccator before use.

Chemicals for instrument tuning – perfluorotributylamine, chloropentafluorobenzene, 2,4,6-tris-fluoromethyl-[1,3,5]-triazine – were supplied by Sigma (USA).

2.2 Test material

Two environmental matrices were used for our experiments: different kinds of homogenised fish muscle (chub, *Leuciscus cephalus*; barbell, *Barbus barbus*; perch, *Perca fluviatilis*) and river sediments, both collected within a monitoring program at polluted sampling sites located on the Vltava (Moldau) river downstream from Prague, Czech Republic.

2.3 Sample preparation

A 20-g portion of homogenised fish muscle tissue or 25 g of wet sediment was desiccated with 100 g of anhydrous sodium sulphate. Soxhlet extraction carried out with 340 mL of a dichloromethane/hexane solvent mixture (1:1, *v/v*) for 7 hours (7–8 cycles/hour) was used for isolation of target analytes from the sample. Two millilitres of concentrated crude extracts (1.6 g/mL for fish and 0.8 g/mL for sediment) were purified by gel permeation chromatography on a Bio-Beads S-X3 (column 500 × 8 mm, styrene-divinylbenzene copolymer with particle size 200–400 mesh); cyclohexane/ethyl acetate mixture (1:1, *v/v*) was used as a mobile phase (flow-rate 0.6 mL/min). The collected fraction (15–30 mL) was evaporated to dryness and re-dissolved in 1 mL of isooctane. After addition of a few drops of sulphuric acid (removal of residual lipids), the organic layer was used for GC analysis employing different detection systems as described below. Final extracts were 3.2 and 1.6 mg/μL for fish and sediment, respectively.

2.4 GC analysis

2.4.1 GC–MS system employing high-resolution TOF analyser

Most of the experiments were performed using an Agilent 6890 GC system (Agilent, Palo Alto, CA, USA) coupled to a GCT (Micromass, Manchester, UK) high-resolution time-of-flight mass spectrometer for quantification of target analytes. The GC system was equipped with an electronic pressure control (EPC), a split/splitless injector, and a PAL Combi autosampler (CTC Analytics, Zwingen, Switzerland). The MassLynx 3.5 software was employed for data processing. Conditions of GC–TOF MS methods are summarised in **Table 1** and **Table 2**.

Table 1. Conditions of optimised GC–TOF MS method used for determination of PBDEs.

Gas chromatography	
Column:	DB-XLB (30 m × 0.25 mm × 0.1 μm)
Oven temperature program:	110°C for 1.5 min, 45 K/min to 210°C, 20 K/min to 300°C for 5 min
Helium flow rate:	1.0 mL/min (purity 99.999%)
Injection mode:	Pulsed splitless (4 mL/min for 1.5 min)
Injector temperature:	280°C
Injected volume:	1 μL
Mass spectrometry	
Ionisation gas:	Methane (purity 99.995%) at 2×10^{-4} mbar at 250°C and 1 mL/min helium flow rate
Acquisition rate:	2 spectra/s
Pusher interval:	EI mode 40 μs (25000 raw spectra/second) NICI mode 33 μs (30303 raw spectra/second)
Time-to-digital converter:	3.6 GHz
Mass range:	*m/z* 45–800 (EI mode) *m/z* 45–500 (NICI mode)
Ion source temperature:	220°C
Transfer line temperature:	280°C
Trap current:	250 μA (EI mode) 200 μA (NICI mode)
Detector voltage:	2200 V

2.4.1.1 MS with electron ionisation (EI)

The instrument was manually tuned using perfluorotributylamine ('heptacosa'). The mass resolution (>7000 FWHM, full width at half maximum) was calculated from continuum data using *m/z* 502. For exact mass calibration twelve fragments of this compound in centroid mode were used. After finishing this operation, the *m/z* 501.9711 was used as an internal reference mass (a lock mass). In initial experiments 2,4,6-tris(fluoromethyl)-[1,3,5]-triazine ('metri') was used with *m/z* 284.9949 as an internal reference mass (a lock mass). The exact mass calibration was considered successful with maximum difference between measured and theoretical masses 2 mDa.

2.4.1.2 MS with negative ion chemical ionisation (NICI)

The instrument was manually tuned using a mixture of dichloromethane, chloropentafluorobenzene, and perfluorotributylamine. The mass resolution (>7000 FWHM) was calculated from continuum data using the *m/z* 452 for perfluorotributylamine and the FWHM of this peak. For exact mass calibration, eighteen ions of these compounds in centroid mode were used. The calibration was checked using the *m/z* 201.9609 as a lock mass originating from chloropentafluorobenzene. The exact mass calibration was considered successful with maximum difference between measured and theoretical masses of 2 mDa. After finishing this operation, these compounds were completely removed from the system and only chloropentafluorobenzene was introduced. The *m/z* 201.9609 of this compound was used as an internal reference mass (a lock mass) for further experiments.

2.4.2 GC–MS system employing quadrupole analyser

For comparative analysis of PBDEs detected in NICI mode, instrumentation consisting of an HP 6890 gas chromatograph (Hewlett-Packard, CA, USA) equipped with an EPC, a split/splitless injector, an HP 7673 autosampler,

Table 2. Monitored analytes and their masses (*m/z*) in EI and NICI mode.

Mode	Homologue group	Ion	Analyte	Monitored ions [*m/z*][a)]
EI	tri-BDEs	$[M-Br_2]^+$, $[M+2]^+$, $[M+4]^+$	BDE 28	245.968, **405.803,** 407.801
	tetra-BDEs	$[M+2-Br_2]^+$, $[M+2]^+$, $[M+4]^+$	BDE 47	325.877, 483.713, **485.711**
		$[M+2-Br_2]^+$, $[M-Br_2]^+$, $[M+4]^+$	BDE 49, 66	**325.877**, 323.879, 485.711
	penta-BDEs	$[M+2-Br_2]^+$, $[M+4]^+$, $[M+6]^+$	BDE 85, 99, 100	403.787, **563.622**, 565.620
	hexa-BDEs	$[M+4-Br_2]^+$, $[M+2-Br_2]^+$, $[M+6]^+$	BDE 153, 154	**483.696**, 481.698, 643.530
	hepta-BDEs	$[M+4-Br_2]^+$, $[M+6-Br_2]^+$, $[M+6]^+$	BDE 183	**561.606**, 563.604, 721.441
	penta-CBs	$[M]^+$, $[M+2]^+$, $[M+4]^+$	CB 112 (ISTD)	323.883, **325.880**, 327.876
	hexa-CBs	$[M]^+$, $[M+2]^+$, $[M+4]^+$	CB 153	357.844, **359.842**, 361.839
	hepta-CBs	$[M]^+$, $[M+2]^+$, $[M+4]^+$	CB 180	391.805, **393.803**, 395.800
NICI	tri-BDEs to hepta-BDEs	$[^{79}Br]^-$, $[^{81}Br]^-$	BDE 28, 47, 49, 66, 85, 99, 100, 153, 154, 183	78.918, **80.916**
	penta-CBs	$[M]^-$, $[M+2]^-$, $[M+4]^-$	CB 112 (ISTD)	323.883, **325.880**, 327.876
	hexa-CBs	$[M]^-$, $[M+2]^-$, $[M+4]^-$	CB 153	357.844, **359.842**, 361.839
	hepta-CBs	$[M]^-$, $[M+2]^-$, $[M+4]^-$	CB 180	391.805, **393.803**, 395.800

[a)] Ions used for quantification purposes in bold.

an HP 5973 mass selective detector (Hewlett-Packard, CA, USA) coupled to quadrupole analyser was used. The GC conditions were as follows: DB-XLB capillary column (30 m × 0.25 mm × 0.10 μm); column temperature program: from 110°C (held for 2 min) to 300°C at 30 K/min (held 5 min); carrier gas: helium (purity 99.995%) with constant flow 1.5 mL/min; injection temperature: 280°C; injection volume: 2 μL using the pulsed splitless injection mode at 414 kPa for 2 min. The MS was operated in the selected ion monitoring (SIM) mode. The monitored ions (*m/z*) obtained by NICI were 79, 81, 159, 160 (PBDEs) and 326, 328 (CB 112, internal standard). The ion *m/z* 81 was used for quantification. Methane used as the reagent gas (purity 99.995%) was set at a pressure of 2×10^{-4} mbar. The temperatures of MSD interface, ion source, and quadrupole were 280°C, 150°C, and 105°C, respectively.

2.4.3 GC–ECD

For another comparative analysis of PCBs, instrumentation consisting of an HP 5890 Series II gas chromatograph (Hewlett-Packard, CA, USA) with an EPC, a split/splitless injector, an HP 7673 autosampler, and two parallel ^{63}Ni electron capture detectors (ECDs) was used. The GC conditions were as follows: DB-5 and DB-17 capillary columns operated in parallel (60 m × 0.25 mm × 0.25 μm); column temperature program: 60°C (2.5 min), 30 K/min to 220°C, 0.5 K/min to 240°C, 2.5 K/min to 280°C (held 10 min); carrier gas: helium (purity 99.995%) with constant flow 1.7 mL/min; injection temperature: 250°C; injection volume: 1 μL using the splitless injection mode (common injector for both columns) for 2 min; temperature of detectors: 300°C. Identification of particular PCB congeners was based on the occurrence of their signals on both columns at specific retention times (identical with those of respective standard). According to our standard operation procedure, PCB peaks obtained on the DB-5 column were used for quantification because of their lower retention times (hence better *S/N* ratio) as compared to the DB-17 column. In the case of target analytes that co-elute with other sample components, the signal measured on the DB-17 column can be used (this is the case with critical pairs such CB 153 and 163 on the DB-5 column) for quantification.

2.5 Quality assurance/quality control (QA/QC)

Calibration solutions were stored at +4°C in the refrigerator. Prior to analysis, these calibrants were transferred to amber glass vials. The analytical results were corrected for blanks and recovery by using of CB 112, added before the GPC clean-up procedure as an internal standard.

The accuracy of GC–ECD method for determination of PCBs accredited by ISO 17025 protocols was verified using certified reference material BCR 718 (wet sterilised herring muscle tissue). In addition, traceability of results was obtained by participation in the FAPAS® proficiency testing scheme (organised by the Central Science Laboratory, York, UK), *z*-scores $< |2|$ were achieved for all the PCBs involved in the test. In the case of PBDEs, the accuracy of the GC–MS method employing a quadrupole analyser was verified using candidate reference material (homogenised flounder tissue, *Platichthys flesus*). The method precision (repeatability) was determined as the relative standard deviation (RSD) for particular analytes and ranged between 4 and 13% and 2.9 and 3.2% for PBDEs and PCBs, respectively.

3 Results and discussion

As in the case of PCBs (and other persistent halogenated aromatic organic pollutants), intensive molecular and fragmentation ions (isotopic clusters) are present in the high mass region of EI spectra for any PBDE congener [12]. Thanks to a good selectivity of such ions and easy chromatographic resolution of common PBDEs, unambiguous identification and reliable quantification of these compounds is possible. On the other hand, bromine ions are typically the base peaks in NICI mass spectra of PBDEs [13]. Despite the relatively low masses of $[^{79}Br]^-$ and $[^{81}Br]^-$, their selectivity is theoretically high since only a limited number of (semi)volatile components potentially present in environmental samples are prone to yield ions capable of efficient electron capture in NICI. Thus, bromine ions are especially suitable for MS identification/quantification purposes.

As shown in **Figure 1**, a high signal-to-noise ratio can be obtained by using a narrow mass window (0.02 Da) for extraction of target PBDE ions (EI mode), when a standard mixture in pure solvent was injected. However, setting the mass window width less than 0.02 Da may result in an underestimation of peak area due to generally lower mass accuracy at low ion intensity, which is encountered at low concentration levels.

When analysing real-world samples, the situation changed dramatically. Besides BFRs (and other groups of common contaminants of aquatic ecosystem such as PCBs, organochlorine pesticides, musk compounds, etc.) co-extracted matrix components not completely removed by the purification step are unavoidably contained in examined samples. All these compounds can be responsible for problems when the GCT TOF MS detector is operated in a high-resolution mode. To understand the essence of this issue it should be emphasised that to achieve the required mass measurement accuracy of target analytes a single point correction of the base mass calibration has to be carried out using a selected peak (lock mass) from the mass spectrum of a reference compound. In this way a slight drift of the calibration scale caused by temperature

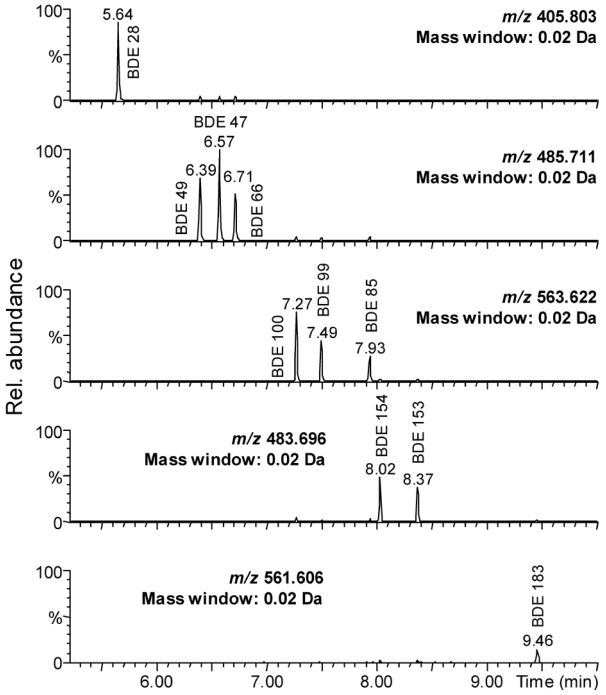

Figure 1. GC–TOF MS chromatograms of PBDE standard solution in EI mode (10 pg of each analyte injected). The target ions were extracted using a 0.02 Da mass window.

fluctuations in the flight tube and instabilities of the power supplies are compensated. Typically, the *m/z* 284.9949 from EI fragmentation of the 'metri' compound (see Section 2.4.1) is used as the lock mass. However, as more analytes are targeted and volatile and semi-volatile co-extracted compounds occur, it becomes more likely that their ions and the lock mass ion can overlap. An example of worsened mass measurement accuracy that was encountered under unfavourable experimental conditions is illustrated in **Figure 2.A**. Employing lock mass *m/z* 284.9949 from the 'metri' reference compound and setting a narrow 0.02 Da mass window for extraction of the target ion *m/z* 485.711 resulted in the failure to detect BDE 47 although it was the dominant congener contained in the fish extract. Under these experimental conditions detection of this congener was possible only by application of a wider mass window (1 Da), see **Figure 2.B**. A significant improvement in mass measurement accuracy was obtained by replacement of the 'metri' reference compound by 'heptacosa'. As shown in **Figure 2.C**, when the ion *m/z* 501.9711 from 'heptacosa' was used as a lock mass, the application of a narrow mass window (0.02 Da) for the extraction of particular ion (*m/z* 485.711) provided unbiased detection. However, one must be aware that 'heptacosa' yields more fragmentation ions as compared to 'metri'. Hence the risk of interferences with ions of target analytes is increased, especially at lower ion masses.

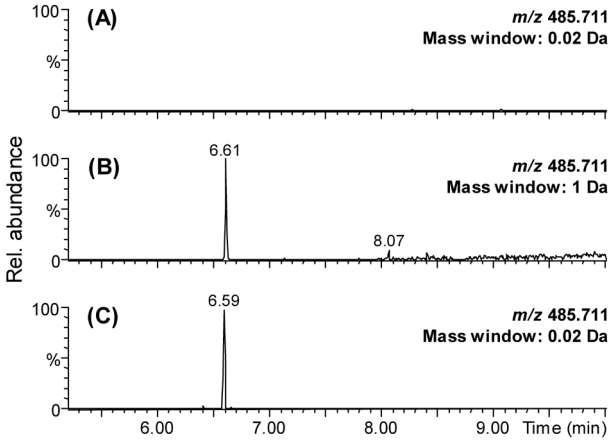

Figure 2. GC–TOF MS chromatograms of fish extract in EI mode (equivalent to 3.2 mg of original matrix injected). Detection of BDE 47: (A) mass window 0.02 Da used to search for quantification ion, 'metri' used as a lock mass (*m/z* 284.9949); (B) mass window 1 Da used to search for quantification ion, 'metri' used as a lock mass (*m/z* 284.9949); (C) mass window 0.02 Da used to search for quantification ion, 'heptacosa' used as a lock mass (*m/z* 501.9711).

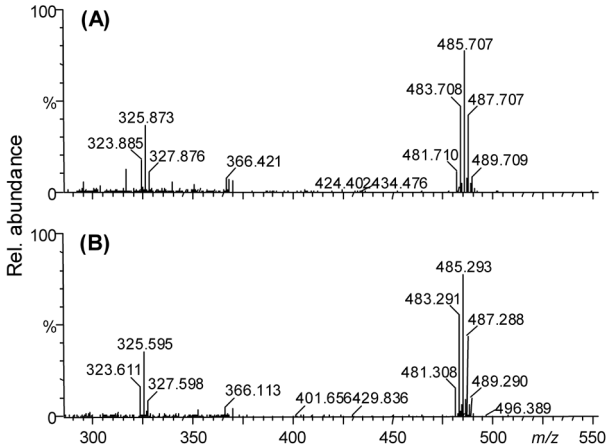

Figure 3. Mass spectra of BDE 47 in EI mode measured in fish extract (3.2 mg sample equivalent injected). (A) 'Heptacosa' (*m/z* 501.9711) used as a lock mass; (B) 'Metri' (*m/z* 284.9949) used as a lock mass.

The mass spectra of BDE 47 as measured in fish extract utilising two alternative reference compounds for mass correction are shown in **Figure 3**. The difference between the theoretical (*m/z* 485.711) and experimental value of the quantification ion was only 4 mDa when using 'heptacosa' for mass correction while application of 'metri' for the same purpose provided *m/z* underestimation as large as 418 mDa.

It should be noted that only GPC followed by sulphuric acid treatment was used for sample preparation prior to the GC step. Under these conditions, the bulk lipids together with other high molecular weight compounds that

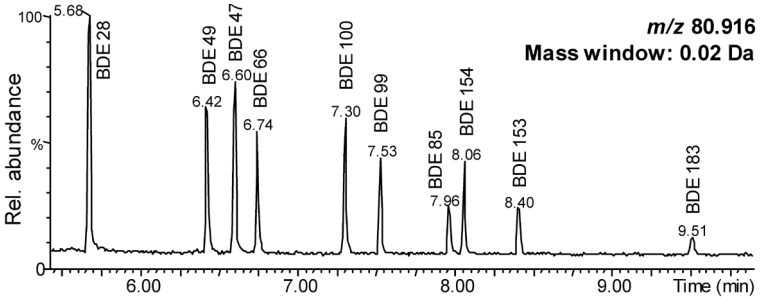

Figure 4. GC–TOF MS chromatogram of PBDEs standard solution in NICI mode (0.25 pg of each analyte injected). The target ion (*m/z* 80.916) was extracted using a 0.02 Da mass window.

can interfere in GC–MS analysis of PBDEs were eliminated; however, this purification procedure does not separate BFRs from some other lower molecular weight sample components including other organohalogenated compounds (e.g. organochlorine pesticides and PCBs). In most studies concerned with BFR analysis, an additional clean-up step employing neutral sorbents such as silica gel, alumina, and/or Florisil® is often used for fractionation of contaminants present in GPC eluate into different classes [12]. To demonstrate the potential of the high-resolution TOF mass analyser to identify/quantify in a single GC run various target analytes occurring in complex mixtures of environmental pollutants typically present in real-world samples, utilisation of a second clean-up step was omitted. Unavoidably a simple clean-up procedure such as that used in our study might result in relatively poor mass accuracy. This was indeed the case of data acquired in experiments conducted in EI mode. However, employing the more selective ionisation technique (NICI) allowed improving performance characteristics of the method (see below).

Unfortunately, on employing EI for examination of real-world samples only three BDE congeners (BDE 28, BDE 47, and BDE 100) exceeded LOD values and could be identified. Moreover, in the case of the most abundant BDE 47 (and similarly CB 153 and CB 180 in non-diluted extract) underestimated results were obtained compared to those data obtained in NICI mode because of high "background" saturation of the time-to-digital converter (this phenomenon is explained below).

Table 3 lists the lowest calibration levels (LCLs) of standards in pure solvent in EI mode. As documented in this table, significant improvement of BDE congener detectability was achieved when the NICI technique was employed; therefore, the performance characteristics of analytical procedures aimed at trace analysis of PBDEs and other persistent organic pollutants were evaluated for the MS detector operated in this mode.

Figure 4 shows a typical NICI chromatogram of PBDEs standard mixture. A similar signal-to-noise ratio was obtained regardless of the width of the mass window (1 Da or 0.02 Da) used for $[Br]^-$ ion extraction of particular

Table 3. Lowest calibration levels (LCLs) of 10 major PBDEs and 2 indicator PCBs (standards in pure solvent) attainable by an optimised GC–MS procedure employing GCT TOF MS.

	EI mode	NICI mode
Analyte	LCL [pg/μL]	LCL [pg/μL]
BDE 28	1	0.01
BDE 47	1	0.01
BDE 49	1	0.01
BDE 66	1	0.01
BDE 85	5	0.05
BDE 99	2.5	0.05
BDE 100	2.5	0.025
BDE 153	2.5	0.05
BDE 154	2.5	0.05
BDE 183	5	0.25
CB 153	2	10
CB 180	2	1

congeners. In any case, NICI provided more selective detection (absence of detectable chemical noise in system) as compared to EI.

As shown in **Figure 5**, not only the target analytes (BDE 28, BDE 49, BDE 66, BDE 99, BDE 100, BDE 154, BDE 153, BDE 183) but also other brominated compounds were present in the fish extract. However, unequivocal identification of these chemicals was not feasible because in NICI spectra, only peaks corresponding to abundant but non-specific ions $[Br]^-$ (*m/z* 79 and 81) and $[HBr_2]^-$ (*m/z* 159, 161 and 163) or $[Br_2]^-$ (*m/z* 158, 160, 162) were present. In addition, an assessment of the elemental composition of these "unknown" compounds based on the exactly measured *m/z* values of ions with higher masses (e.g. molecular ions) was impossible due to their low intensity. These spectral profiles are rather different as compared to other studies in which occurrence of more intensive ions in the higher mass region of NICI mass spectra was reported [5, 9]. However, in our experiments the instrument was manually tuned with the

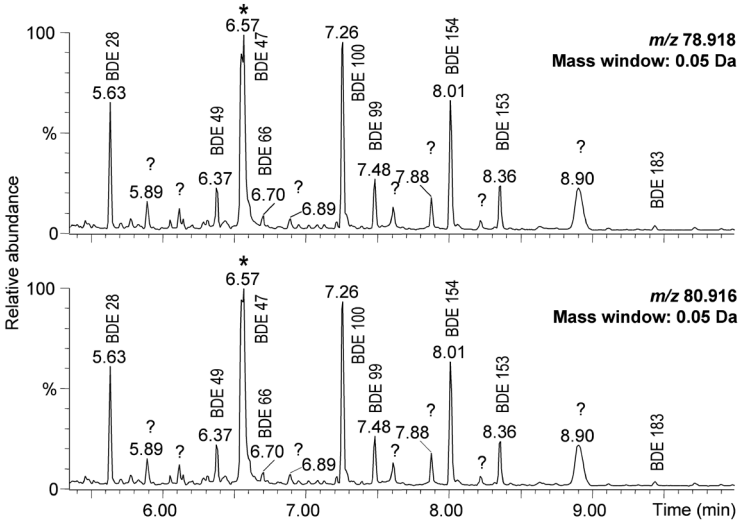

Figure 5. GC–TOF MS chromatograms of fish extract in NICI mode (sample equivalent of 3.2 mg injected). The saturated peak is marked by an asterisk and "unknown" compounds are marked by question marks. The target ions (*m/z* 78.918 and 80.916) were extracted using a 0.05 Da mass window.

specific goal of achieving maximum intensity of low masses (i.e. ions below *m/z* 100). The intensity of molecular or other high mass ions was largely suppressed for this reason. As can be seen in Figure 5, a rather broad peak as compared to the shapes of PBDEs was present in the chromatogram at retention time t_R = 8.90 min. Considering similar GC records of BFR mixture shown by Covaci et al. [12] and their discussion of the different chromatographic behaviour of HBCD compared to PBDEs, the identity of this bromine-containing compound was then confirmed by injection of a certified standard of α-HBCD. It is worthy of note that in technical HBCD, which consists of α, β, and γ diastereomers, the last-named one is the predominant component (up to 89%). The isomerisation process, which occurs in this mixture at temperatures above ca. 160°C, results in a significant increase of α-HCBD content (up to 78% in equilibrium). Since thermal conversion of HCBD residues contained in real-world samples unavoidably takes place under GC conditions the use of α-isomer standard for the identification/quantification purpose is justified [12]. Although several other (minor) brominated compounds were recorded in the sample, their identification in the NICI mode was impossible without analytical standard; in the EI mode this was not possible either, mainly due to poor intensity of their signals.

Another problem we encountered was the impossibility of quantifying the most abundant BDE 47 in NICI when fish extract equivalent to 3.2 mg/μL of original matrix was injected into GC since the concentration of this congener exceeded the GCT TOF MS linear range. The reason for this phenomenon was saturation of the time-to-digital converter (TDC), which is used for determination of the arrival time of particular ions. This ion counting system only allows events separated by a sufficient time interval (i.e. dead time) to be distinguished. When two ions arrive at the detector in a sequence within this dead time interval, the TDC does not count the latter ion (dead time loss). A dead time correction algorithm, which is inherently applied, allows the dynamic range and exact mass capability to be extended only by up to about one order of magnitude. However, when two or more ions arrive at the detector simultaneously in one flight cycle, the TDC counts them as one ion, which results in mass spectral signals with lower intensities than expected and, consequently, inaccurate mass assignment. At around $2-4 \times 10^5$ ion events per second acquired within the selected mass range the transfer buffer of the TDC becomes saturated (partial saturation caused by single ion of an analyte may arrive at around 2×10^4 ion events per second). The practicable way to improve this situation is to increase the "inhibit push" value in the TDC settings. The higher "inhibit push" value promotes registration of higher *m/z* ions by the TDC. However, the fragmentation ions still hit the microchannel plate detector (MCP) although they are not registered. Changing the TDC function cannot avoid MCP saturation occurring at around 10^6 ion events per second. **Figure 6** shows the chromatogram obtained by analysis of standard solutions containing PBDE congeners at different concentration levels. The effect of an increasing number of "not detected" ions (due to the TDC saturation) on mass measurement accuracy is well illustrated here. As far as concentrations of the bromine ions $[^{79}Br]^-$ and $[^{81}Br]^-$ were complied within the dynamic range, the measurement errors were only −0.7 and 0.3 mDa, respectively. Serious saturation of the TDC resulted in measurement errors as much as −6.3 and −9.3 mDa for bromine ions.

The implication of the TDC saturation was the need to reanalyse the fish extract after its dilution (10 times) to obtain accurate determination of the most abundant congener BDE 47. All of the detected analytes could be quantified in diluted sample except for congeners BDE 66 and

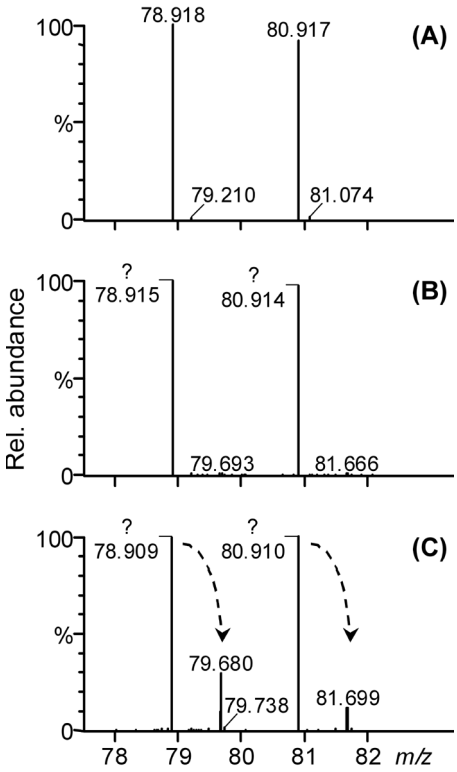

Figure 6. The effect of the TDC saturation on mass measurement accuracy of bromine ions (theoretical *m/z* 78.9183 and 80.9163) in NICI mode: (A) peaks at concentration level within the dynamic range; (B) saturated peaks (marked by question-marks); moderate saturation resulted in the shift to lower mass; (C) saturated peaks (marked by question-marks); serious saturation resulted in the shift to lower mass together with presence of secondary peaks due to saturation.

BDE 183, which were below LOQs. A summary of the quantitative data is given in **Table 4**. Relatively good agreement between these two NICI measurements (i.e. diluted and non-diluted extract) was obtained. The concentration value obtained for congener BDE 47 by extrapolation led to an underestimation of this congener (concentration instead of 2.23 ng/g only 0.96 ng/g muscle). This limitation was common for all the analysed fish samples since the concentrations of minor and major BDE congeners were on fairly different levels.

As already emphasised, one of the main advantages of TOF over common mass analysers is the continuous acquisition of spectral data across a wide mass range without sacrificing any detection sensitivity as encountered, e.g., when switching the quadrupole analyser operated in SIM mode into full scan one. We attempted to identify selected PCBs in fish extract, which commonly occur in this type of environmental samples. Retrieval of stored data enabled us to identify/quantify reliably two of seven indicator PCB congeners (CB 153 and CB 180) that

Table 4. Average ($n = 2$) levels of PBDEs (ng/g muscle) determined in fish extract (chub, *Leuciscus cephalus*) by GC–TOF MS.

Congener	Ionisation technique (sample)			
	EI	EI	NICI	NICI
	(purified extract)	(extract 10 × diluted)	(purified extract)	(extract 10 × diluted)
BDE 28	< LOQ	< LOQ	0.09 ± 0.01	0.10 ± 0.01
BDE 47	0.47 ± 0.04 [a)]	1.65 ± 0.15 [a)]	0.96 ± 0.08 [b)]	2.23 ± 0.20
BDE 49	< LOQ	< LOQ	0.04 ± 0.00	0.05 ± 0.00
BDE 66	< LOQ	< LOQ	0.01 ± 0.00	< LOQ
BDE 85	< LOQ	< LOQ	< LOQ	< LOQ
BDE 99	< LOQ	< LOQ	0.12 ± 0.01	0.09 ± 0.01
BDE 100	< LOQ	< LOQ	0.37 ± 0.03	0.40 ± 0.03
BDE 153	< LOQ	< LOQ	0.20 ± 0.02	0.12 ± 0.01
BDE 154	< LOQ	< LOQ	0.32 ± 0.03	0.18 ± 0.02
BDE 183	< LOQ	< LOQ	0.02 ± 0.00	< LOQ
CB 153	2.3 ± 0.1 [a)]	21.4 ± 1.1	20.5 ± 1.0	19.9 ± 1.0
CB 180	1.2 ± 0.1 [a)]	12.0 ± 1.0	12.3 ± 1.0 [b)]	9.92 ± 0.81

[a)] The TDC was saturated due to a high background chemical noise, underestimation of concentration of particular analyte occurred. Values shown in this field were calculated using calibration curves (standards in pure solvent) not influenced by the TDC saturation.

[b)] At this concentration level saturation of the TDC by analyte occurred, which resulted in either underestimation or overestimation of its concentration. Values shown in this field were calculated using calibration curves (standards in pure solvent) not influenced by the TDC saturation.

eluted in a similar retention time region as the target PBDEs, see **Figure 7** (two types of PCB calibration solutions were used for quantification purposes to exclude possible co-elution with isomeric congeners).

Since congener CB 153 is considered as a good marker of the extent of PCB contamination (correlation between this compound and total PCBs was shown in several studies [14–17]), the possibility of obtaining information on this persistent organic pollutant level without any modification of instrument setting, simply by retrieving of stored data, brings an additional advantage resulting from utilisation of the GC–TOF MS system.

River sediment was another matrix examined for the occurrence of PBDEs by the GC–TOF MS method. Congeners BDE 49, BDE 47, BDE 66, BDE 100, BDE 99, BDE 85, BDE 153, and BDE 183 were identified by this approach. As shown in **Figure 8**, the chromatogram of river sediment extract contained more “unknown” signals compared to that of fish extract.

The GCT TOF MS instrument performance characteristics, such as linearity, lowest calibration level, repeatability

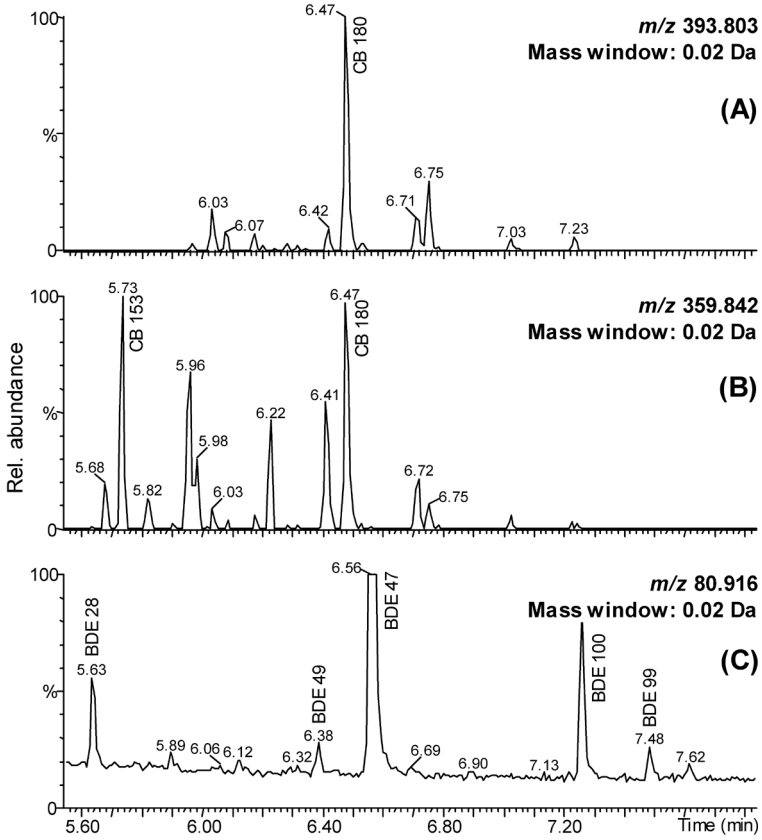

Figure 7. GC–TOF MS chromatograms of selected PCBs and PBDEs in diluted fish extract (0.32 mg of original matrix equivalent injected) in NICI mode. Ions extracted by a mass window of 0.02 Da. (A) Chromatogram of ion *m/z* 393.803 corresponding to heptachlorobiphenyls. (B) Chromatogram of ion *m/z* 359.842 corresponding to hexachlorobiphenyls. (C) Chromatogram of ion *m/z* 80.916 corresponding to PBDEs.

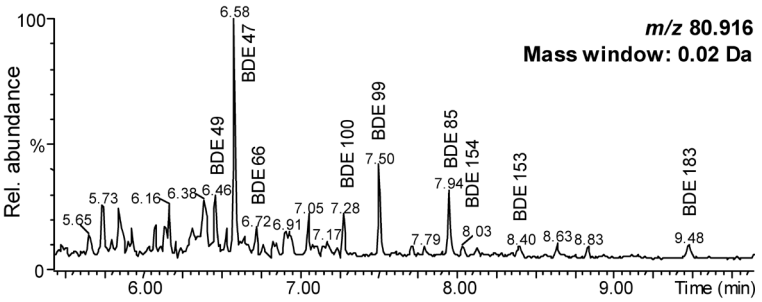

Figure 8. GC–TOF MS chromatogram of sediment extract in NICI mode. Sample equivalent corresponding to 1.6 mg of dry matrix injected.

of response (area), and retention time, were assessed for each particular BDE and CB congener determined in NICI mode. The linear range for all the PBDEs and PCBs was relatively narrow, not exceeding 2–3 orders of magnitude. As already discussed above for fish, real-world samples with widely differing levels of target analytes have to be re-analysed after dilution to enable accurate quantification of the most abundant analyte responses that fall out of the linear range in the first run.

The repeatability of responses and retention times was determined as a relative standard deviation (RSD,%) from 6 repetitive analyses of standard solutions of PBDEs (1 ng/mL) and PCBs (20 ng/mL) using a 2 Hz spectral acquisition rate; 3–9 points per peak, depending on position of the particular BDE congener in the chromatogram and its concentration, were obtained with this set-up. RSDs of peak areas and retention times of target analytes were in the range 4.9–8.2% (no internal syringe standard was used for data correction) and less than 0.4%, respectively.

The lowest calibration levels (LCLs) of standards in pure solvent of PBDEs ranged between 0.01–0.25 pg, i.e. 0.002–0.04 ng/g muscle. These values are 20–100 times lower compared to those obtained by employing EI (the same GC conditions applied), see Table 3. This improvement of detectability is in agreement with results reported for a quadrupole analyser operated in SIM by Eljarrat et al. [18], who obtained detection limits between 30 fg and 1.72 pg when using NICI and only between 0.53 and 32.09 pg on employing EI. As regards the LCLs achieved

Table 5. Comparison of levels (mean ± SD) of PBDEs and PCBs (ng/g muscle) in fish extract (chub, *Leuciscus cephalus*) employing alternative detection techniques ($n = 2$).

	Detection system		
Congener	TOF[a, b, c)]	Quadrupole[b, d)]	ECD[e)]
BDE 28	0.10 ± 0.01	0.10 ± 0.01	n.a.
BDE 47	2.23 ± 0.20	2.30 ± 0.22	n.a.
BDE 49	0.05 ± 0.00	0.05 ± 0.00	n.a.
BDE 66	0.01 ± 0.00	< LOQ	n.a.
BDE 85	< LOQ	< LOQ	n.a.
BDE 99	0.09 ± 0.01	0.12 ± 0.01	n.a.
BDE 100	0.40 ± 0.03	0.30 ± 0.03	n.a.
BDE 153	0.12 ± 0.01	0.16 ± 0.01	n.a.
BDE 154	0.18 ± 0.02	0.24 ± 0.02	n.a.
BDE 183	0.02 ± 0.00	< LOQ	n.a.
CB 153	19.9 ± 1.0	n.a.	20.3 ± 0.6
CB 180	9.92 ± 0.81	n.a.	9.93 ± 0.30

a) Except for BDEs 66 and 183 values present results from diluted fish extract.
b) Instrument operated under NICI conditions.
c) 0.32 mg sample equivalent injected.
d) 6.4 mg sample equivalent injected.
e) 3.2 mg sample equivalent injected.
n.a. = not analysed.

for CB 153 and CB 180, these ranged between 1–10 pg, i. e. ca 0.17–1.7 ng/g.

For the assessment of the quality of measured data, criteria listed in the EU Directive [19] concerning the performance of analytical methods and the interpretation of results were applied. According to this document, the maximum permitted tolerance for ions with abundance >50% of the base peak is ±20% when generated in NICI mode; ions with >50% and >20% to 50% of base peak obtained by EI are allowed to vary in the range ±10% and ±15%, respectively. All these criteria were met in our study. The maximal deviation of NICI ions was 5%, and in the case of EI 3% and 6%, respectively.

The PBDEs and PCBs results as measured under optimised experimental settings of high-resolution TOF MS instrument in NICI mode were also compared to data obtained using alternative detection systems – a quadrupole MS analyser operated in NICI mode for PBDEs and an electron capture detector for PCBs. Good agreement was obtained between the results generated by high-resolution TOF and measurements realised by conventional detection approaches mentioned above (see **Table 5**).

4 Conclusions

The potential of high-resolution time-of-flight mass spectrometry in a gas chromatographic determination of polybrominated diphenyl ethers was evaluated in this study. Simultaneous analysis of polychlorinated biphenyls occurring in fish extract and/or river sediment could be carried out thanks to the availability of full spectral information even at very low concentration levels. This unique performance feature of TOF MS, which is inconceivable when working with quadrupole and/or ion trap analysers without compromising signal intensity or acquisition speed settings (which may result in a rather poor spectra/peak quality), enables confirmation of respective analyte identity at its trace level.

GC–TOF MS employing EI allowed identification and quantification of major PBDEs occurring in real-world samples on the basis of higher *m/z* fragmentation ions; however, LOQs of target analytes were not low enough to analyse some of the minor congeners. The application of NICI resulted in a 20–100-fold decrease of LCLs of target compounds; nevertheless, it did not enable unambiguous identification of particular PBDE peaks since low molecular bromine ions were used for identification/quantification in this case. However, this ionisation technique was more selective since only a limited number of compounds was ionised (i.e. provided an analytical signal). To obtain such remarkable detection potential, tuning of detector electronics was targeted on the low mass region and, consequently, besides bromine ions that were used for identification/quantification practically no higher diagnostic ions were available in recorded NICI spectra.

Due to a limited linear range of the GCT TOF MS instrument and taking into account a typically large concentration range of persistent organohalogen pollutants in environmental matrices, it was often possible to obtain accurate quantification of major congeners only by re-analysis of diluted sample. In so far as the impossibility of quantifying BDEs 66 and 183 occurring typically at ultratrace levels can be tolerated, most of the TDC saturation problems can be prevented/minimised by injection of a very small sample equivalent ("diluted sample"). The quality of the generated data was still comparable to that obtained by a quadrupole analyser when the amount of sample taken for analysis was higher by one order of magnitude (instruments operated in NICI mode compared).

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