Determination of polybrominated diphenyl ethers (PBDEs) in food

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Abstract

The study shows the steps involved in creating and optimizing an analytical method of GC-MS/MS triple quadrupole (QqQ) method for the determination of polybrominated diphenyl ethers (PBDE) in food. In the validation process the scope of the linearity detector response, limit of detection and limit of quantification for this method were defined. The verification method was performed on the basis of the analysis Interlaboratory Comparison Material for which the results were reported indications of PBDE. Next stage of testing food samples were analyzed for the content of selected congeners of PBDE.

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gas chromatography,
mass spectrometry,
salmon tissue

Introduction

Polybrominated diphenyl ethers (PDBEs) have been used since 1960s [1]. Due to the connection of the atom (s) of bromine, there are 209 PDBE congeners possible, the numbering of which complies with the recommendations of the Union of Pure and Applied Chemistry (IUPAC), Fig. 1.

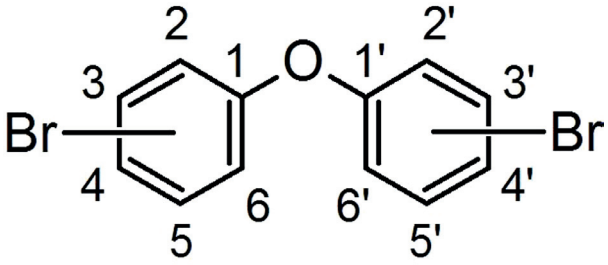

Fig. 1.
Structure of PBDE

Alaee et al. reports that polymer additives were up to 18% for pentabromodiphenyl ethers (penta-BDE), 15% for octabromodiphenyl ethers (octa-BDE) and 16% for decabromodiphenyl ether (deca-BDE) [2]. These flame retardants do not covalently bind to the matrix; belong to the group of additive flame retardants [1,3]. Under the influence of high temperatures, mechanical abrasion and improper storage and processing of materials, especially used electronic equipment, PBDE releases from the plastic to the environment [4].

In the scientists' opinion, congeners containing from 4 to 6 bromine atoms in the 2,2',4,4' position are carcinogens with properties of endocrine substances, neurotoxic and immunotoxic effects. The most common adverse effects of exposure are endometriosis, neurobehavioral developmental defects, decreased fertility, and weakening of the body's immune system [5,6]. Polybrominated diphenyl ethers bioaccumulate in fat tissue and biomagnification due to lipophilic character [1], which may lead to an increase in their amount in living organisms with food intake. The tetra-BDE, penta-BDE, hexa-BDE, hepta-BDE have been included in Annex A of the list of Persistent Organic Pollutants (POPs) of the Stockholm Convention, which means that they do not production and use [7].

The European Commission, bearing in mind the health of the citizens of the Member States on 3 March 2014, issued a Recommendation [8] on monitoring the traces of brominated flame retardants in food. The Recommendation provides for monitoring in 2014 and 2015 the following PBDE congeners: BDE-28 (2,2',4-tribromodiphenyl ether), BDE-47 (2,2',4,4'-tetrabromodiphenyl ether), BDE-49 (ether 2,2',4,5'-tetrabromodiphenyl ether), BDE-99 (2,2',4,4',5-pentabromodiphenyl ether), BDE-100 (2,2',4,4',6-pentabromodiphenyl ether), BDE-138 (2,2',3,4,4',5'-hexabromodiphenyl ether), BDE-153 (2,2',4,4',5,5'-hexabromodiphenyl ether), BDE-154 (2,2',4,4',5,6'-hexabromodiphenyl ether), BDE-183 (2,2',3,4,4',5',6-heptabromodiphenyl ether), BDE-209 (2,2',3,3',4,4',5,5',6,6'-decabromodiphenyl ether). The concentration of these ethers is to be analyzed in eggs and egg products, milk and milk products, meat and meat products, vegetable and animal fats and oils, fish and seafood, foodstuffs for particular nutritional uses and in food for infants and young children, using analytical methods with the limit of quantification 0.01 ng/g wet weight or lower [9].

Materials and reagents

Standard BDE-CS5 (Wellington Laboratories) containing BDE-3, BDE-7, BDE-15, BDE-17, BDE-28, BDE-47, BDE-49, BDE-66, BDE-71, BDE-77, BDE-85, BDE-99, BDE-100, BDE-119, BDE-126, BDE-138, BDE-153, BDE-154, BDE-183 at 400 ng/mL, BDE-209 at 4000 ng/mL and congeners with isotope C-13 [13C]12-BDE-3, [13C]12-BDE-15, [13C]12--BDE-28, [13C]12-BDE-47, [13C]12-BDE-99, [13C]12-BDE-139, [13C]12-BDE-153, [13C]12--BDE-154, [13C]12-BDE-183, [13C]12-BDE-209 at 100 ng/mL. Internal standard BDE-ES2 (Wellington Laboratories) containing congeners with isotope C-13 [13C]12-BDE-28, [13C]12-BDE-47, [13C]12--BDE-99, [13C]12-BDE-153, [13C]12-BDE-154, [13C]12-BDE-183 at 250 ng/mL, [13C]12-BDE-209 at 1250 ng/mL. Syringe standard DF-ISS2 (Wellington Laboratories) containing [13C]12-1,2,3,4--tetrachlorodibenzo-p-dioxin ([13C]12-TCDD) and [13C]12-1,2,3,7,8,9-hexachlorodibenzo-p-dioxin at 40 ng/mL. Other reagent acetonitrile (POCh), argon 5.0 (Linde), helium 6.0 (Linde), sodium chloride reagent-grade (POCh), dichloromethane HPLC grade (POCh), n-hexane HPLC grade (POCh), 98% sulfuric acid reagent-grade (J.T. Baker), primary/secondary amine to QuEChERS (Agilent), anhydrous sodium sulfate reagent-grade (J.T. Baker), anhydrous magnesium sulfate reagent-grade (POCh), octadecyl modified silicato QuEChERS (Agilent), silica gel (0.063-0.200 mm) for chromatography columns (Merck), sodium hydroxide reagent-grade (J.T. Baker).

Method validation

The development of methodology for the analysis of PBDE congeners using a gas chromatograph coupled with a GC-MS/MS tandem mass spectrometer

(Shimadzu GCMS-TQ8040, auto injector AOC-20i) was started by analysing the BDE-CS5 standard solution in the full range of ion scans (full scan) from 100 to 1000 Thomson (m/z). Chromatography column: DB-5ms, stationary phase 5%-phenyl-95%-methylpolysiloxane (30m × 0.25mm × 0.25µm). Temperature programme: initial temperature 80°C, this temperature is maintained for 4 minutes, an increase of 35°C to 180°C, subsequent increment of 10°C to 320°C, this temperature is maintained for 20 minutes. Total analysis time: 40.86 minutes: carrier gas: helium, injector temperature: 260°C, splitless. Ionization mode: ionization with a beam of electrons (EI), energy -70eV, ion source temperature: 250°C, transfer line temperature: 280°C. The next step was the analysis of the ion products of the fragmentation (ion product scan), i.e. daughter ions. This mode of analysis employing tandem mass spectrometry (MS/MS) is aimed at quantifying the unambiguous fragmentation reaction, described by the equation:

parental ion → daughter ion (1)

The parental ions selected from the spectrum of full scan were isolated on the first quadrupole and subjected to collision with argon (collisional gas) in the second quadrupole (collision chamber). The fragmentation (daughter) ions were analysed on the third quadrupole, which was a mass analyser (Q3 scan). An example of mass spectrum for the BDE-47 congener, 326 m/z was selected as the parental ion, Fig. 2. Method validation was carried out only for congeners recommended by the European Commission.

The selection of fragmentation reactions in accordance with the equation (1) allowed for the collection of data (ion scans) in the mode of monitoring selected fragmentation reactions – SRM (single reaction monitoring). This tandem mass spectrometry mode is characterized by better sensitivity, because out of the whole range of ions only those with an appropriate ratio of m/z are scanned, which moreover yield daughter ions with a given (predefined) ratio of m/z. The fragmentation reaction does not depend on the position of bromine atoms, but only on their number in the molecule of polybrominated diphenyl ether.

The last stage during the creation of the analytical methodology enabling the quantification of selected PBDE congeners was the quantification of collision energy – CE. The collision energy can be changed using the voltage on the cylindrical bars in the second quadrupole, which constitutes the collision chamber. The amount of energy should be selected in such a way that the surface area expressed in conventional units of surface of the chromatographic peak has the largest possible value (optimization). The voltage for natural congeners and ones marked with isotopes of carbon C-13 is the same because the collision energy depends on the number of bromine atoms in individual PBDE congeners, while the ratio of m/z ions subjected to fragmentation in the same ether group has no significant effect during fragmentation.

The quantification of BDE-209 proved impossible due to the degradation of the congener in the chromatographic column Fig. 3. This is confirmed in the literature: the authors postulate application of a short chromatographic column [10], and even separate procedures in the preparation of samples in which BDE-209 is to be determined [11].

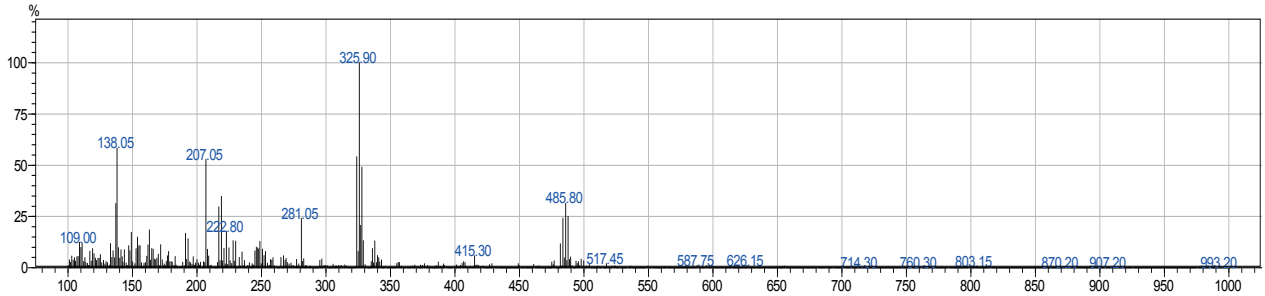

Fig. 2
The mass spectrum of the BDE-47 congener obtained in the full scan mode

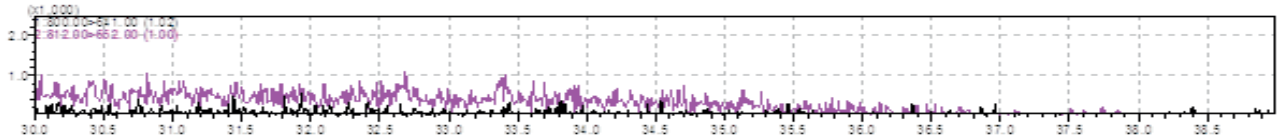

Fig. 3.
Extracted chromatogram for BDE-209 (black) and $[^{13}C]_{12}$-BDE-209 (purple)

Table 1 contains a summary of retention time (t_R), for the ratio *m/z* of parent and daughter ions and optimal collision energy of individual PBDE congeners, low resolution MS, and also of the recovery pattern $[^{13}C]_{12}$-TCDD.

The obtained equations of the calibration curves allowed to determine the relative analyte response ratios with respect to internal standards (*Rrf*), i.e. marked PBDE congeners. Table 2, summarises correlation coefficients, (R^2), which determine the linear response of the detector and the response coefficients of the analytes.

Determined detection limits (LOD) and quantification limits (LOQ) are summarized in Table 3. The numerical values of the presented limits refer to the analytical equipment, not the analytical procedure, because the tested standards have not been subjected to the full procedure of preparing samples for analysis.

Table 1.
Parameters of the developed SRM method

Compound	t_R [min]	Parent ion [*m/z*]	Daughter ion [*m/z*]	collision energy [V]
$[^{13}C]_{12}$-BDE-28	14.969	258.00	150.00	27
BDE-28	14.974	246.00	139.00	27
BDE-49	16.709	326.00	217.00	29
$[^{13}C]_{12}$-BDE-47	17.023	338.00	228.00	29
BDE-47	17.028	326.00	127.00	29
BDE-100	18.539	404.00	297.00	31
BDE-99	19.025	404.00	297.00	31
$[^{13}C]_{12}$-BDE-99	19.029	416.00	308.00	31
BDE-154	20.210	484.00	377.00	37
$[^{13}C]_{12}$-BDE-154	20.213	496.00	386.00	37
BDE-153	20.835	484.00	377.00	37
$[^{13}C]_{12}$-BDE-153	20.837	496.00	386.00	37
BDE-138	21.730	484.00	377.00	37
BDE-183	22.865	564.00	455.00	39
$[^{13}C]_{12}$-BDE-183	22.869	576.00	466.00	39
$[^{13}C]_{12}$-TCDD	15.932	331.00	258.00	22

Table 2.
Correlation coefficients and relative response factors

Analyte/ internal standards	R^2	Response factor (*Rrf*)
BDE-28/ [13C]12-BDE-28	0.9997	1.0688
BDE-47/ [13C]12-BDE-47	0.9972	1.1348
BDE-49/ [13C]12-BDE-47	0.9987	1.3186
BDE-99/ [13C]12-BDE-99	0.9997	1.4348
BDE-100/ [13C]12-BDE-99	0.9995	1.0195
BDE-138/ [13C]12-BDE-153	0.9988	1.2539
BDE-153/ [13C]12-BDE-153	0.9999	1.7060
BDE-154/ [13C]12-BDE-154	1	1.7231
BDE-183/ [13C]12-BDE-183	0.9996	1.0914

Table 3.
Limits of detection and quantification for individual PBDE congeners

Congener	LOD [ng/mL]	LOQ [ng/mL]
BDE-28	0.024	0.072
BDE-47	0.095	0.28
BDE-49	0.093	0.28
BDE-99	0.036	0.11
BDE-100	0.18	0.53
BDE-138	0.074	0.22
BDE-153	0.078	0.24
BDE-154	0.074	0.22
BDE-183	0.071	0.21

Results and discussion

The correctness of the analytical method was verified using material from interlaboratory comparisons, for which the results of PBDE quantification were reported. The material was derived from the worldwide InterCIND QA/QC inter-laboratory comparison program from 2013 (the first edition), it was a homogenate of fish tissue, stored in a dark and cool place in the original dark glass packaging. The homogenate sample was weighed, the BDE-ES2 internal standard was added, and anhydrous sodium sulphate was added, then it was extracted in a Soxhlet apparatus with dichloromethane, in the next step the solvent was replaced with n-hexane. The extract was purified on two chromatography columns filled with silica gel modified with sodium hydroxide, neutral silica gel, and silica gel modified with sulfuric acid. The contents of the individual natural PBDE congeners obtained basing on the analysis of the prepared sample together with reported laboratory results are presented in Table 4.

Our results obtained at the dynode voltage of 1.2 kV are similar to those reported after laboratory comparisons. BDE-49, BDE-100, BDE-138 and BDE-183 congeners are outside the minimum and maximum values reported by laboratories. The explanation of this fact can be seen in the persistent supersaturation of the detector despite decreasing the voltage on the dynode through conversion.

Table 4.
Summary comparison of the obtained results with reports provided by laboratories

Congener	Concentration [ng/g]	*R* [%]	Statistics of the reported results				
			Average [ng/g]	Mediana [ng/g]	Min [ng/g]	Max [ng/g]	SD
BDE-28	0.672	53.3	0.7113	0.7090	0.4830	0.9350	0.0993
BDE-47	41.7	40.4	48.50	49.05	40.00	56.12	3.56
BDE-49	0.623	40.4	0.4864	0.4971	0.3240	0.5572	0.0658
BDE-99	4.52	57.1	4.9342	4.9880	3.8400	6.0666	0.4419
BDE-100	8.51	57.1	14.213	14.050	10.641	17.392	1.510
BDE-138	0.103	47.0	0.0307	0.0283	0.0156	0.0572	0.0099
BDE-153	2.66	47.0	2.1293	2.1829	1.5200	2.7000	0.2322
BDE-154	2.09	67.5	3.3448	3.3591	1.9942	4.1980	0.4993
BDE-183	0.293	42.3	0.1714	0.1700	0.1020	0.2489	0.0346

R **–** recovery of the internal standard
Min – minimum value
Max – maximum value
SD – standard deviation

Results of the analysis of real samples

A **rapeseed** sample was treated with an internal standard of BDE-ES2 and extracted with dichloromethane in a Soxhlet apparatus, the extract was concentrated and the solvent was replaced with n-hexane. The extract was purified with the use of membranes (SPM), the receiving liquid was hexane. The concentrated dialysate was transferred into a column filled with silica gel modified with sulfuric acid and silica gel modified with sodium hydroxide, in the next stage the eluate was transferred into a column filled with aluminium oxide.

A sample of a **rapeseed meal** was treated with a BDE-ES2 internal standard solution and extracted with a dichloromethane/acetone blend in a Soxhlet apparatus. The extract was transferred into a column filled with a silica gel modified with sulfuric acid and silica gel modified with sodium hydroxide, in the next stage it was transferred into a column filled with aluminium oxide.

The three samples of **fodder material** of plant origin (vegetable fatty acids) were treated with the BDE-ES2 internal standard solution. The analytes were isolated by dialysis (SPM) for 24 hours, the receiving fluid was n-hexane. The concentrated dialysate was transferred into a column filled with silica gel modified with sulfuric acid and silica gel modified with sodium hydroxide, in the next stage the eluate was transferred into a column filled with aluminium oxide.

A sample of **fish oil** was treated with the BDE-ES2 internal standard solution. The purification method was the one described for the sample of fodder material.

The analyses were carried out with the voltage on the conversion dynode being 2.0 kV, which is why it was possible to apply the *Rrf* coefficients from the obtained calibration curves. The results of analyses of the samples described above are summarized in Table 5.

Two salmon tissue samples were derived from a commercial product purchased in Kraków. 20 µL of the BDE-ES2 internal standard solution was added to homogeneous samples (sample I 10.0 g, sample II 10.1 g). In the first stage, the QuEChERS method [12] was used for the isolation of analytes:

Table 5.
Obtained results of the analysis of real samples

Material	Rapeseed	Rapeseed meal	Fodder material	Fodder material	Fodder material	Fish oil
Mass of sample [g]	82.6	61.8	25.1	34.3	32.5	2.1
Added internal standard BDE-ES2 [µL]	20	20	20	20	20	20
Congener	**Concentration [ng/g]**					
BDE-28	0.000094	0.00026	0.0037	0.0019	0.0039	0.54
BDE-47	0.0016	0.00075	0.044	0.079	0.067	9.0
BDE-49	0.0014	0.00074	0.052	0.068	0.058	3.5
BDE-99	0.0011	0.00016	0.064	0.051	0.039	1.1
BDE-100	0.00028	0.000078	0.010	0.014	0.012	3.8
BDE-138	-	0.000037	0.0090	0.0016	0.0018	0.015
BDE-153	0.00026	0.000034	0.0029	0.016	0.010	0.14
BDE-154	0.000088	0.000020	0.0021	0.0042	0.0027	1.0
BDE-183	0.00029	0.000061	0.0064	0.017	0.0080	0.045
suma PBDE	0.0051	0.0021	0.19	0.25	0.20	19

extraction with acetonitrile with sodium chloride, anhydrous magnesium sulphate, in the second stage the extract was purified using anhydrous magnesium sulphate, primary or secondary amine and modified silica gel octadecyl groups (dispersion extraction to the solid phase). The final stage was the purification of the extract on a chromatography column filled with an inactive silica gel and modified sodium hydroxide and sulfuric acid. The content of the quantified congeners was found to be very low, therefore higher dynode voltage of 2.0 kV is a better solution during the analysis. Both samples consisted of the same material, so the content of congeners should be similar. This result was obtained during the analysis at 2.0 kV voltage. Fig. 4 shows the chromatograms of the total ion current and the chromatogram extracted for sample I at the voltage on the conversion dynode being 2.0 kV.

Table 6.
Results of the quantification of PBDE congeners

Concentration [ng/g]						
Congener	Analysis at a voltage of 1.2 kV			Analysis at a voltage of 2.0 kV		
	Rrf	**Sample I**	**Sample II**	***Rrf***	**Sample I**	**Sample II**
BDE-28	1.1043	0.029	0.034	1.0688	0.027	0.034
BDE-47	1.2270	0.39	0.76	1.1348	0.37	0.40
BDE-49	0.99180	0.62	0.68	1.3186	1.1	1.2
BDE-99	1.1925	0.031	0.047	1.4348	0.020	0.022
BDE-100	1.5316	0.13	0.14	1.0195	0.13	0.15
BDE-138	0.77699	0.0033	0.036	1.2539	0.00034	0.0013
BDE-153	1.3253	0.0064	0.0256	1.7060	0.0043	0.0064
BDE-154	1.2258	0.0695	0.0622	1.7231	0.034	0.038
BDE-183	0.94380	0.0031	0.017	1.0914	0.016	0.017
Sum of PBDE		1.3	1.8	Sum of PBDE	1.7	1.9

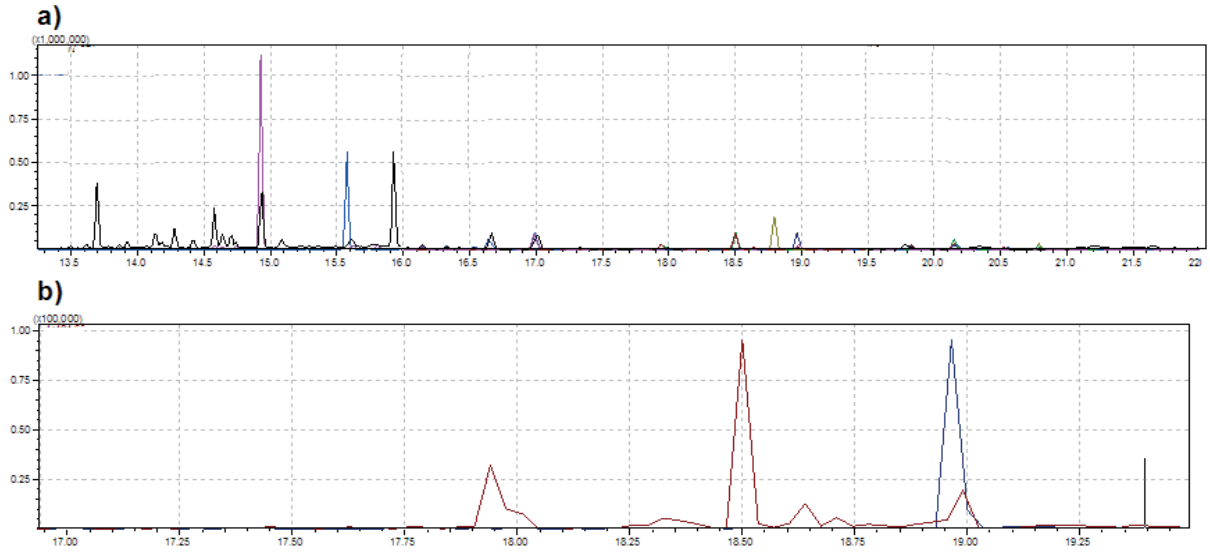

Fig. 4.
Chromatograms (a) of total ion current and (b) extracted chromatogram for penta-BDE

Conclusions

The CG-MS/MS method using triple quadrupole during the investigation of selected fragmentation reactions allowed for obtaining the results of the material analysis from interlaboratory comparisons similar to those reported by laboratories. It is characterized by a linear response of the detector in a wide range of analyte concentrations. The estimated limits of quantification exhibit different values for each PBDE congener and range from 0.072 ng/mL to 0.53 ng/mL. Increasing the voltage on the conversion dynode allows for quantifying PBDE at a lower concentration level by amplifying the signal. The use of internal standards marked with isotopes $[^{13}C]_{12}$-PBDE makes it possible to define the content of natural PBDE congeners without the need to accurately measure the volume of the sample – the isotope dilution method. The method cannot be used for quantifying the BDE-209 congener, because it undergoes degradation in the chromatographic column. In all food samples, the PBDE congeners recommended by the European Commission were also quantified in a plant-based products. The compounds found in the highest concentrations in the tested samples are tetrabromodiphenyl ethers: BDE-47 and BDE-49.

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