Determination of Brominated Flame Retardants (BFRs) in Fish Tissue using an Optimized Extraction/Cleanup Procedure and the Agilent 7000 Triple Quadrupole GC/MS System

Application Note

Food Safety

Authors

Kamila Kalachova, Jana Pulkrabova, Tomas Cajka, and Jana Hajslova
Department of Food Analysis and Nutrition
Institute of Chemical Technology, Prague
Technicka 3
166 28 Prague 6
Czech Republic

Chris Sandy
Agilent Technologies UK Ltd
610 Wharfedale Road
Winnersh Triangle
Wokingham
Berkshire
RG41 5TP
UK

Abstract

This application note shows the development and validation of a high throughput, sensitive and cost-effective analytical method for the simultaneous identification and quantification of polybrominated diphenyl ethers (PBDEs) and different types of alternative brominated flame retardants (ABFRs) in fish muscle tissue. A substantial simplification of sample processing prior to the quantitative step was achieved: after addition of water to a homogenized sample, transfer of hydrophobic analytes into ethyl acetate was supported by the addition of inorganic salts. Bulk fat, contained in the crude organic extract obtained by partition, was subsequently removed on a silica minicolumn. Finally, fish extracts were analyzed using gas chromatography coupled to tandem mass spectrometry (GC/MS/MS) in multiple reaction monitoring (MRM) mode on an Agilent 7890 GC with an Agilent 7000B Triple Quadrupole GC/MS system.

Agilent Technologies

Introduction

There is evidence that fish consumption, especially of fatty fish (such as salmon, which typically contains omega-3 polyunsaturated fatty acids, such as eicosapentaenoic (EPA) and docosahexaenoic acids (DHA)), benefits the cardiovascular system and is suitable for secondary prevention in manifest coronary heart disease. Conversely, it can increase the dietary exposure to some contaminants such as brominated flame retardants (BFRs). As a result, food products containing more than 0.1% of pentabrominated diphenyl ether (pentaBDE) and octaBDE technical mixtures were prohibited in the European Union (EU) market in August 2004, and the ban was further extended to electrical and electronic goods with decaBDE in July 2008 [1, 2]. As a consequence of these legislative acts, alternative BFRs, suitable for commercial applications as an alternative to PBDEs, have been introduced. Several of them such as 1,2-Bis(2,4,6-tribromo-phenoxy)ethane (BTBPE) have been already detected in the environment [3]. Therefore, a simple, inexpensive, rapid, and highly sensitive analytical method, that enables collection of a large set of reliable data in a short time, is needed to help control food contamination and ensure a flexible response to the Rapid Alert System for Food and Feed (RASFF) [4].

Experimental

Standards and Solutions

Calibration solutions were prepared in isooctane containing BDE 28–203, HBB, PBT, PBEB, and BTBPE at concentration levels 0.05, 0.1, 0.5, 1, 5, 10, 50, 100, and 500 ng/mL and BDE 206, 207, 209, and OBIND at 0.25, 0.5, 1, 5, 10, 50, 100, 500, and 1,000 ng/mL. Each calibration point, as well as sample extract in isooctane, contained surrogate BDE 37 and syringe standards BDE 77 and ^{13}C-BDE 209 at 10, 5, and 50 ng/mL, respectively.

Table 1. List of Target Analytes, Surrogate (SUR) and Syringe Standards (SS)

Abbreviation	Analyte	CAS No.
BDE 28	2,4,4′-Tribromodiphenyl ether	41318-75-6
BDE 37 (SUR)	3,4,4′-Tribromodiphenylether	147217-81-0
BDE 47	2,2′,4,4′-Tetrabromodiphenyl ether	5436-43-1
BDE 49	2,2′,4,5′-Tetrabromodiphenyl ether	243982-82-3
BDE 66	2,3′,4,4′-Tetrabromodiphenyl ether	189084-61-5
BDE 77 (SS)	3,3′,4,4′-Tetrabromodiphenyl ether	93703-48-1
BDE 85	2,2′,3,4,4′-Pentabromodiphenyl ether	182346-21-0
BDE 99	2,2′,4,4′,5-Pentabromodiphenyl ether	60348-60-9
BDE 100	2,2′,4,4′,6-Pentabromodiphenyl ether	189084-64-8
BDE 153	2,2′,4,4′,5,5′-Hexabromodiphenyl ether	68631-49-2
BDE 154	2,2′,4,4′,5,6′-Hexabromodiphenyl ether	207122-15-4
BDE 183	2,2′,3,4,4′,5′,6-Heptabromodiphenyl ether	207122-16-5
BDE 196	2,2′,3,3′,4,4′,6,6′-Octabromodiphenyl ether	N/A
BDE 197	2,2′,3,3′,4,4′,6,6′-Octabromodiphenyl ether	N/A
BDE 203	2,2′,3,4,4′,5,5′,6-Octabromodiphenyl ether	N/A
BDE 206	2,2′,3,3′,4,4′,5,5′,6-Nonabromodiphenyl ether	N/A
BDE 207	2,2′,3,3′,4,4′,5,6,6′-Nonabromodiphenyl ether	N/A
BDE 209	Decabromodiphenyl ether	1163-19-5
^{13}C-BDE 209 (SS)	^{13}C-Decabromodiphenyl ether	N/A
BTBPE	1,2-Bis(2,4,6-tribromo-phenoxy)ethane	37853-59-1
HBB	Hexabromobenzene	87-82-1
PBEB	Pentabromoethylbenzene	85-22-3
PBT	Pentabromotoluene	87-83-2
OBIND	Octabromotrimethylphenylindane	N/A

Chemicals and Materials

- Hexane (Suprasolv quality Merck, Germany or equivalent)
- Isooctane (Suprasolv quality Merck; Germany or equivalent)
- Ethyl acetate (for GC residue analysis, Sigma-Aldrich, Germany or equivalent)
- Dichloromethane (Suprasolv quality Merck, Germany or equivalent)
- Anhydrous sodium sulphate (Penta Chrudim, Czech Republic or equivalent)

Anhydrous sodium sulphate was heated at 600 °C for 7 hours and then stored in a desiccator before use. Sodium sulphate prepared and stored in this manner can be used for one month.

- Silica gel (0.063–0.200 mm) (Merck, Germany or equivalent)

Silica gel was activated by heating at 180 °C for 5 hours, then deactivated by adding 2% of deionized water, shaking for 3 hours and stored in a desiccator for 16 hours before use. Silica gel prepared and stored in this manner can be used for 14 days.

- Magnesium sulphate (Sigma Aldrich, Germany or equivalent)
- Sodium chloride (Lach-ner, Czech Republic or equivalent)
- Glass wool (Merck, Germany or equivalent)
- Polypropylene tubes, 50 mL (Merci, France or equivalent)
- Glass Pasteur pipette, D812, 230-mm length (Poulten and Graf GmbH, Germany or equivalent)

Instruments

- Tissue blender was supplied by Retsch (Haan, Germany)
- Rotary vacuum evaporator Buchi Rotavapor R-114 and R-200 with heating bath (Buchi Rotavapor, Switzerland)
- Centrifuge Rotina 35R (Hettich Zentrifugen, Germany)

Sample Preparation

A 10-g amount of fish tissue homogenate (with surrogate BDE 37 – 10 ng absolute) was mixed with 5 mL of distilled water and shaken vigorously with 10 mL of ethyl acetate in a polypropylene centrifuge tube for 1 minute. Subsequently, 4 g of magnesium sulphate and 2 g of sodium chloride were added to the mixture.

The tube was shaken for another 1 minute, centrifuged (5 minutes, 11,000 rpm), and an aliquot of 5 mL was removed from the organic layer. The solvent was carefully eliminated by evaporation under a gentle stream of dry nitrogen gas.

The evaporated extract was redissolved in 1 mL of *n*-hexane and purified using a handmade silica gel minicolumn. The fat determination and the choice of the silica minicolumn size according to the fish muscle fat content are described elsewhere [5]. Collected eluents were carefully evaporated using a vacuum rotary evaporator, and the residual solvents were removed under a gentle stream of dry nitrogen gas. Residues were redissolved in 0.5 mL of isooctane containing BDE 77 (5 ng/mL) and ^{13}C BDE 209 (50 ng/mL) used as syringe standards.

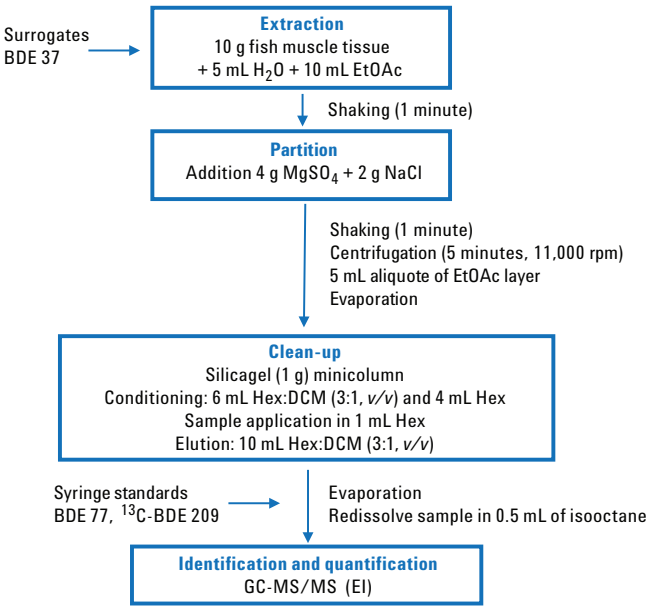

Figure 1. General scheme of the extraction and clean-up of the fresh fish muscle tissue. (Hex – n-hexane, DCM – dichloromethane, EtOAc – ethyl acetate).

Instrumental Analysis

All GC/MS experiments were performed using a gas chromatograph Agilent 7890A GC (Agilent Technologies, Santa Clara, CA, USA) coupled to a triple quadrupole mass spectrometer Agilent 7000B MS (Agilent Technologies) operated in electron ionization (EI) mode. The GC system was equipped with an Agilent 7693A auto-sampler (Agilent Technologies) and a carbon dioxide cooled multimode inlet (MMI). For the separation, a DB-XLB capillary column (15 m × 0.18 mm, 0.07 µm film thickness; Agilent Technologies) was used. Optimized conditions of GC analysis are summarized in Table 2.

Table 2. Optimized Conditions of GC Analysis

Agilent 7890 GC conditions	
Column	15 m × 0.18 mm, 0.07 µm DB-XLB (custom column, p/n N/A)
Autosampler	Agilent 7693A Automated Liquid Sampler
Injection	2 µL cold splitless using CO_2 cooled Multimode Inlet (MMI)
Injection port liner	2 mm id dimpled deactivated liner (p/n 5190-2296)
Injector temperature program	80 °C (0.20 minute), 600 °C/min to 285 °C
Injection mode	Cold pulsed splitless
Injection pulse pressure	50 psi
Splitless period	1.5 minutes
Purge flow to split vent	50 mL/min at 1.0 minute
Carrier gas	Helium
Carrier gas flow	1.5 mL/min (11 minutes), 15 mL/min to 3 mL/min
Oven temperature program	110 °C (1.5 minutes), 30 °C/min to 320 °C, (3.5 minutes)
Run time	12 minutes

The 7000B Triple Quadrupole GC/MS was operated in MS/MS electron ionization (EI) mode, and analytes detected/confirmed using Multiple Reaction Monitoring (MRM) detecting two transitions per analyte as listed in Table 3. The temperatures of the transfer line, the ion source, first quadrupole, and second quadrupole were 300 °C, 280 °C, 150 °C, and 150 °C, respectively. The collision cell gases were nitrogen (1.5 mL/min) and helium (2.25 mL/min). The electron multiplier (EM) gain values are shown in Table 3. Both MS resolutions were 1.2 amu full width at half maximum. The dwell times were adjusted to 20–80 ms depending on the number of transitions per time window to achieve five cycles/s (Hz).

MassHunter quantative analysis software (version B.04.04) (Agilent Technologies) was used for data processing.

Table 3. Optimized Conditions of the MS/MS Method

Compound name	Precursor ion	MS1 resolution	Product ion	MS2 resolution	CE	RT window (min)	EM gain
BDE 28	405.8	Wide	246	Wide	20	0.3	10
	407.8	Wide	248.1	Wide	22	0.3	10
PBT	406.7	Wide	325.8	Wide	16	0.3	10
	406.7	Wide	246.8	Wide	24	0.3	10
PBEB	499.7	Wide	484.6	Wide	19	0.3	10
	499.7	Wide	420.5	Wide	11	0.3	10
BDE 37	405.8	Wide	246.0	Wide	20	0.3	10
	407.8	Wide	248.1	Wide	22	0.3	10
BDE 49	485.7	Wide	326.1	Wide	28	0.3	10
	483.7	Wide	324.1	Wide	32	0.3	10
HBB	551.7	Wide	472.5	Wide	26	0.3	10
	551.7	Wide	391.5	Wide	34	0.3	10
BDE 47	485.7	Wide	326.0	Wide	28	0.3	10
	483.7	Wide	324.1	Wide	32	0.3	10
BDE 66	485.7	Wide	326.0	Wide	28	0.3	10
	483.7	Wide	324.1	Wide	32	0.3	10
BDE 77	485.7	Wide	326.0	Wide	28	0.3	10
	403.8	Wide	269.9	Wide	35	0.3	10

Table 3. Optimized Conditions of the MS/MS Method (Continued)

Compound name	Precursor ion	MS1 resolution	Product ion	MS2 resolution	CE	RT window (min)	EM gain
BDE 100	565.7	Wide	405.8	Wide	28	0.3	10
	403.8	Wide	269.9	Wide	35	0.3	10
BDE 99	565.7	Wide	405.8	Wide	28	0.3	10
	403.8	Wide	269.9	Wide	35	0.3	10
BDE 85	565.7	Wide	405.8	Wide	28	0.3	10
	403.8	Wide	269.9	Wide	35	0.3	10
BDE 154	643.6	Wide	483.8	Wide	20	0.3	10
	483.7	Wide	374.9	Wide	40	0.3	10
BDE 153	643.6	Wide	483.8	Wide	20	0.3	10
	483.7	Wide	374.9	Wide	40	0.3	10
BDE 183	561.7	Wide	454.9	Wide	45	0.3	100
	721.6	Wide	561.8	Wide	17	0.3	100
BTBPE	356.8	Wide	277.8	Wide	13	0.3	100
	356.8	Wide	328.6	Wide	11	0.3	100
BDE 197	801.7	Wide	641.5	Wide	14	0.3	100
	641.7	Wide	534.5	Wide	47	0.3	100
BDE 203	801.7	Wide	641.5	Wide	14	0.3	100
	641.7	Wide	534.5	Wide	47	0.3	100
BDE 196	801.7	Wide	641.5	Wide	14	0.3	100
	641.7	Wide	534.5	Wide	47	0.3	100
BDE 207	719.6	Wide	559.6	Wide	49	0.3	100
	879.8	Wide	719.6	Wide	9	0.3	100
BDE 206	719.6	Wide	559.6	Wide	49	0.3	100
	879.8	Wide	719.6	Wide	9	0.3	100
OBIND	406.7	Wide	327.7	Wide	25	0.3	100
	852.7	Wide	771.7	Wide	14	0.3	100
13C- BDE 209	651.5	Wide	543.6	Wide	34	0.3	100
	811.8	Wide	651.4	Wide	44	0.3	100
BDE 209	799.4	Wide	639.5	Wide	44	0.3	100
	639.6	Wide	530.7	Wide	36	0.3	100

Results and Discussion

Using the newly developed high throughput sample preparation method together with a 7890 GC and a 7000B Triple Quadrupole GC/MS system for the instrumental analysis, it is possible to analyze 21 representative BFRs. In addition to the eight PBDE congeners of primary interest (BDE 28, 47, 99, 100, 153, 154, 183, and 209) included by the EFSA CONTAM panel [6, 7] in the core group of BFRs that should be monitored, an additional eight PBDEs congeners (BDE 49, 66, 85, 196, 197, 203, 206, and 207) and five alterative BFRs (PBEB, PBT, HBB, BTBPE and OBIND) were also measured.

Chromatographic separation

When analyzing PBDEs, not only the separation of target analytes, but also potential coelutions with other nontarget compounds have to be taken into consideration since many isomers may occur in real-world samples. For these reasons, 30-m long capillary columns are typically employed. However, when highly brominated thermo-degradable compounds such as BDE 209 have to be analyzed, shorter (10–15-m) columns are often required. Therefore, the risk of coelutions may arise. Moreover, when NCI is employed, in which case the bromine isotope pattern (*m/z* 79 and 81) is detected, other brominated compounds might easily interfere. Conversely, EI generates more specific $[M^+]$ and $[M–Br_2]^+$ ions, and ^{13}C-labelled standards can be used to facilitate accurate quantification, but higher quantification limits are commonly achieved when compared to NCI.

As shown in Figures 2 and 3, using a 7890 GC with a 7000B Triple Quadrupole system equipped with an Agilent DB-XLB (15 m × 0.18 mm, 0.07 µm) capillary column operated in EI mode, all 21 target BFRs were resolved in less than 12 minutes compared to a 17.5 minutes GC run using the single quadrupole in NCI mode (Figure 4). The reduced time needed for the instrumental analysis results from the high separation efficiency of a DB-XLB column and triple quadrupole mass analyzer operated in EI-MRM, in which case separation of PBDEs from other brominated compound using highly selective precursor and product ions was possible. As seen in Figure 2, the coelution of PBEB and BDE 37 could be easily resolved using different MS/MS (EI) ion transitions. However, it was not possible to resolve the coelution of BDE 28 and PBT in Figure 4, when GC/MS (NCI) was employed.

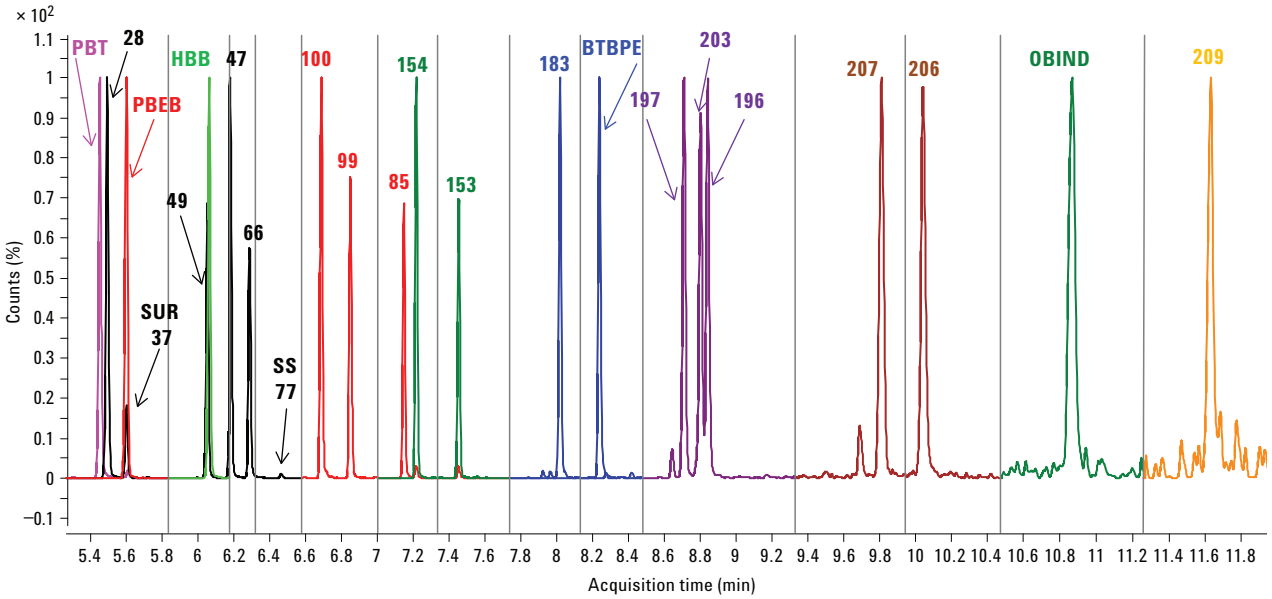

Figure 2. An example of chromatogram (GC–EI–MS/MS) of fish muscle tissue spiked at 5 µg/kg.

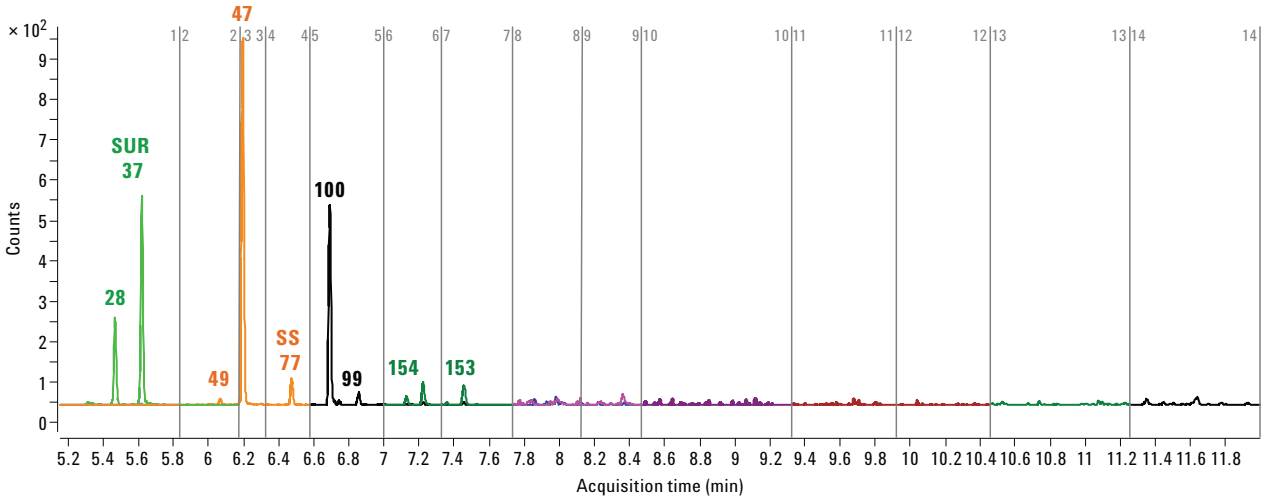

Figure 3. An example of chromatogram (GC–EI–MS/MS) of naturally contaminated fish muscle tissue.

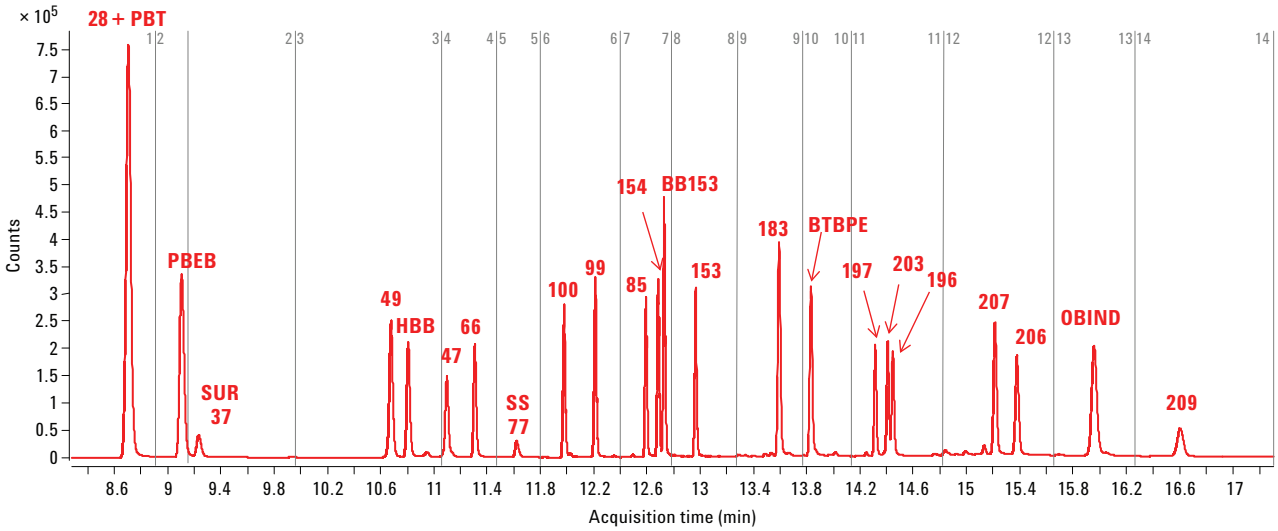

Figure 4. An example of chromatogram (GC–NCI–MS) of fish muscle tissue spiked at 5 µg/kg.

Method performance characteristics

Using the final GC/MS/MS setup, the repeatability of instrument analysis was tested on the standard mixture of all target compounds in isooctane at a concentration of 100 ng/mL (corresponding to 10 µg/kg fish muscle tissue). The repeatability of GC/MS/MS response for all target compounds, expressed as a relative standard deviation (RSD, %), was in the range of 1–7%.

The sample preparation method and optimized GC/MS/MS analysis conditions detailed in the Experimental section of this application note were evaluated in a validation study and the overview of validation data (recovery, repeatability, Limit of Quantification (LOQ), and linearity of the system) is summarized in Table 4. In order to validate the entire analytical method, samples of fish muscle tissue were spiked with all target analytes at two concentration levels (1 and 5 µg/kg) and then prepared and analyzed. With each batch of samples, the procedural blank was prepared (that is, the sample was processed in the same way, but without the use of test matrix). The recovery (%) was calculated as an absolute recovery (not corrected to the recovery of surrogate standard) and repeatability (%) was expressed as a relative standard deviation (RSD). The recoveries (%) and RSD (%) were in the range: 78–115% (RSD 2–14%).

Based on preliminary GC/MS/MS measurements using matrix samples contaminated at low concentrations, the LOQs in fish muscle tissue were in the following range: 0.005 µg/kg corresponding to 0.05 ng/mL (higher values were achieved for highly brominated BFRs).

With regards to a wide concentration range of target analytes occurring in fresh fish tissue, it is necessary to use an extensive scale of working standard solutions for calibration 0.05–500 ng/mL (0.25–1,000 ng/mL in case of BDE 206, 207, 209, and OBIND). Weighted linear regression (1/x) was used and the regression coefficient (R^2) was calculated for the calibration curve from the LOQ up to the highest calibration point (500 ng/mL or 1,000 ng/mL). Within these experiments, all target analytes fulfil the linearity in calibration range mentioned above with regression coefficient (R^2) higher than 0.99.

Table 4. Overview of Validation Data Obtained Within the Validation Study of Sample Preparation Method and Optimized GC/MS/MS Analysis

Analyte	1 µg/kg		5 µg/kg		LOQ (µg/kg)	Linearity (R^2)*
	REC (%)	RSD (%)	REC (%)	RSD (%)		
BDE 28	89	2	92	6	0.005	0.9990
BDE 47	78	7	83	5	0.005	0.9983
BDE 49	100	6	97	4	0.005	0.9993
BDE 66	100	6	96	5	0.005	0.9989
BDE 85	107	7	106	6	0.005	0.9984
BDE 99	106	8	100	5	0.005	0.9982
BDE 100	102	8	98	5	0.005	0.9981
BDE 153	107	10	101	7	0.05	0.9985
BDE 154	99	10	93	6	0.005	0.9984
BDE 183	100	8	104	10	0.05	0.9936
BDE 196	83	14	81	8	0.1	0.9964
BDE 197	86	12	93	12	0.1	0.9990
BDE 203	79	12	81	12	0.1	0.9929
BDE 206	79	10	86	13	1	0.9987
BDE 207	85	12	89	14	0.5	0.9963
BDE 209	81	8	79	11	1	0.9985
PBT	115	10	114	5	0.05	0.9994
PBEB	105	7	105	4	0.01	0.9959
HBB	102	12	103	3	0.05	0.9969
BTBPE	113	13	113	12	0.01	0.9973
OBIND	104	11	107	10	1	0.9929

* The regression coefficient (R^2) was calculated for the calibration curve from the LOQ up to the highest calibration point (500 ng/mL = 50 µg/kg and 1,000 ng/mL = 100 µg/kg in case of BDE 206, 207, 209, and OBIND).

Conclusions

A newly developed procedure based on an ethyl acetate–aqueous sample suspension partition step followed by the SPE minicolumn silica cleanup, laboratory throughput can be improved; up to six samples can be prepared in less than 1 hour compared to several hours needed for Soxhlet extraction followed by other common cleanup techniques. In addition, the volume of extraction solvents is also significantly reduced when applying the new sample processing strategy, therefore not only reducing cost but also providing a more environmentally friendly analysis.

Method performance characteristics of the sample preparation and optimized GC/MS/MS analysis conditions detailed in the Experimental section of this application note agreed with the SANCO document No. 12495/2011 [8], originally designed for pesticide residue analysis but commonly applied to other organic food contaminants (recoveries in the range 70–120% and repeatability less than 20%). The recoveries of all target analytes were in the range 78–115% and repeatabilities (expressed as relative standard deviation, RSD) did not exceed 14% even at the lower spiking level. Under the optimized GC/MS/MS (EI) conditions the LOQs were 0.005 µg/kg (higher values were achieved for highly brominated BFRs).

Triple quadrupole MS operated in EI represents a good alternative to routine single quadrupole MS, because precursor and product ions in the high *m/z* region can be selected, providing less interference and improved selectivity. Using this approach, analysis of even trace levels of BFRs, which are necessary for reliable data assessment conducted within exposition studies, is feasible. Further details relating to this application are also available in a recently published journal article [9].

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