GC-Q-Orbitrap-based analytical method for the quantification of PBDEs in food commodities

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INTRODUCTION

Brominated flame retardants (BFRs) are chemical mixtures which are applied to products such as plastics and synthetic fibers in order to inhibit or slow down the ignition in case of fire. It has been shown that BFRs are persistent, toxic and bioaccumulative and they have been found in environment due to leaching from the products in which they were used. As a consequence, these substances have also over time reached the food chain [1-2].

The European Commission published in 2014 a Recommendation on the monitoring of traces of BFRs in foodstuff (2014/118/EU) [3]. The Recommendation established limits of quantification (LOQ) for the different groups of BFRs (polybrominated diphenyl ethers (PBDEs), hexabromocyclododecanes, tetrabromobisphenol A and derivatives, brominated phenols and derivatives, emerging and novel BFR) in order to detect them in a wide variety of food commodities.

LOQ = 0.01 ng/g wet weight or lower

- Eggs and egg products
- Milk and dairy products
- Meat and meat products
- Animal and vegetable fats and oils
- **Fish and other seafood**
- Products for specific nutritional uses
- Food for infants and small children

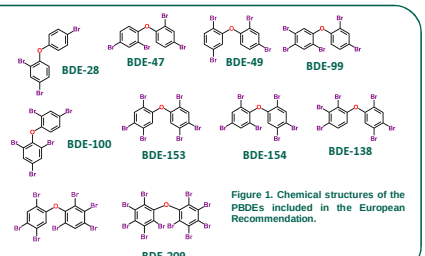


Figure 1. Chemical structures of the PBDEs included in the European Recommendation.

Polybrominated diphenyl ethers to be analyzed following Recommendation 2014/118/EU:

BDE-28 BDE-99 BDE-153 BDE-209

BDE-47 BDE-100 BDE-154

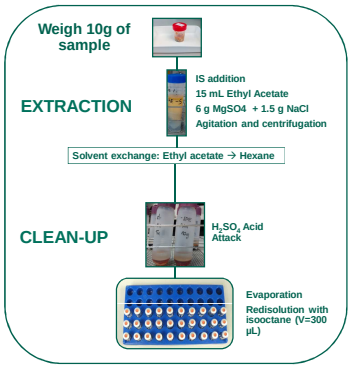
BDE-49 BDE-138 BDE-183

- **The Laboratori de l'Agència de Salut Pública (LASPB) has analyzed PBDEs in fish and seafood, as part of the official control programs since 2009**
- **Method under ISO/IEC 17025 accreditation**
- **New congeners have been included**
- **Special effort has been done in order to achieve lower LOQs (0.01 ng/g) to fulfill the Recommendation.**

EXPERIMENTAL PART

Extraction efficiency and matrix removal: evaluated by preparing replicates of spiked samples.

The protocol is shown below:



Matrixes tested:

- o Tuna
- o Mussel
- o Cuttlefish
- o Stripped Catfish
- o Salmon
- o Prawns

GC-HRMS (Q-Orbitrap): GC-Q-Exactive

5HT, 15 m, i.d. 0.25 mm, 0.10 µm

Electron impact, 50 eV

PTV, 6 µL **HR - SIM**

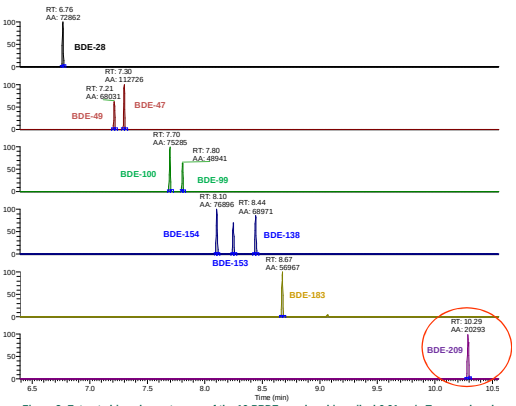
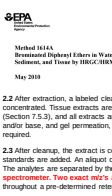


Figure 2. Extracted ion chromatogram of the 10 PBDEs analyzed in spiked 0.01 ng/g Tuna analyzed using GC-HRMS (Q-Orbitrap).



2.2 After extraction, a labeled cleanup standard is spiked into the extract and the extract is concentrated. Tissue extracts are first cleaned up using an anthropogenic isolation column (Section 7.5.3), and all extracts are cleaned up using back-extraction with sulfuric acid and/or base, and gel permeation, silica gel, and/or Florisil or alumina chromatography, as required.

2.3 After cleanup, the extract is concentrated to 20 µL and labeled injection internal standards are added. An aliquot of the extract is injected into the gas chromatograph (GC). The analytes are separated by the GC and detected by a high-resolution (≥5000) mass spectrometer. Two exact m/z's are monitored at each level of bromination (LOB) throughout a pre-determined retention time window.

With the new HRMS instruments we are capable of acquiring the full isotopic pattern without losing sensitivity and working at R: 60,000 (FWHM, *m/z* 200) or higher.

- **High-resolution (≥ 60,000 FWHM *m/z* 200) mass spectrometer**
- **Wide SIM: 50 *m/z* window to monitor the whole isotopic pattern**

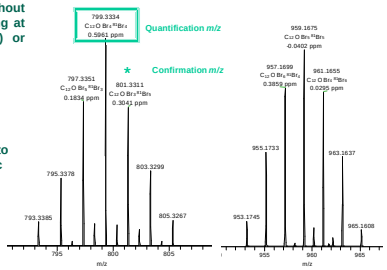


Figure 3. Isotopic pattern and molecular ion isotopic pattern for BDE-209 in GC-HRMS (Q-Orbitrap).

METHOD VALIDATION

The method was validated using spiked blank tuna fish samples based on 657/2002/EU. The following parameters were tested: linearity, precision, trueness, selectivity, limit of quantification and uncertainty.

Samples were spiked at 3 differents levels: 0.01 ng/g, 0.025 ng/g and 0.1 ng/g.

Table 1. Quality parameters obtained during validation.

	Linearity	Precision: RSD (%)	LOQs (ng/g)	U (%)
BDE-28	0.9991	10.3	0.01	25.3
BDE-49	0.9987	12.7	0.01	25.8
BDE-47	0.9989	10.7	0.01	21.3
BDE-100	0.9930	15.6	0.01	32.1
BDE-99	0.9945	14.2	0.01	30.2
BDE-154	0.9990	12.8	0.01	25.3
BDE-153	0.9984	13.0	0.01	25.7
BDE-138	0.9910	13.5	0.01	26.7
BDE-183	0.9924	15.8	0.01	35.3
BDE-209	0.9993	13.9	0.01	29.6

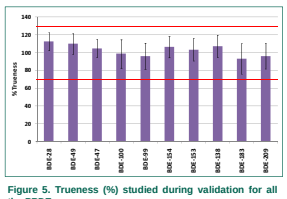


Figure 5. Trueness (%) studied during validation for all the PBDEs.

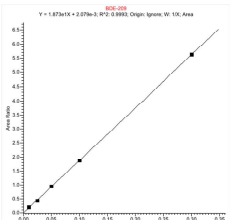


Figure 4. Matrix-matched calibration curve prepared on TUNA fish for BDE-209

- **Linearity was studied between the LOQ: 0,01 ng/g and 0,3 ng/g.**
- **Precision expressed as RSD (%) was always better than 22 %.**
- **Uncertainty (U %) was always better than 44 %.**
- **Trueness (%) was always between 70-130 %.**

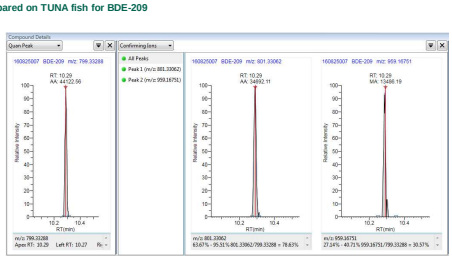


Figure 6. BDE-209: Extracted ion chromatogram for the quantification ion (*m/z* 799.33288) and the confirmation ion (*m/z* 801.33062). The molecular ion (*m/z* 959.16751) is also displayed, showing the specificity of the technique.

MONITORING ON THE PRESENCE OF PBDE IN FOOD

2016: 50 % of the samples (fish and seafood commodities) were found positive for PBDEs by GC-HRMS (Q-Orbitrap).

Table 2 shows results for the analysis of 52 samples: for each congener maximum and minimum concentrations, average and mean values are shown.

Table 2. Average, maximum, minimum and mean concentration (ng/g wet weight) of each PBDE congener found in the fifty-two samples analyzed during 2016-campaign.

	BDE-28	BDE-49	BDE-47	BDE-100	BDE-99	BDE-154	BDE-153	BDE-138	BDE-183	BDE-209
Average	0.010	0.020	0.037	0.031	0.016	0.027	< 0.010	< 0.010	< 0.010	0.031
Max. conc.	0.010	0.035	0.100	0.068	0.019	0.038	< 0.010	< 0.010	< 0.010	0.093
Min. conc.	0.010	0.010	0.011	0.012	0.014	0.012	< 0.010	< 0.010	< 0.010	0.010
Mean	0.010	0.020	0.022	0.013	0.015	0.028	< 0.010	< 0.010	< 0.010	0.020

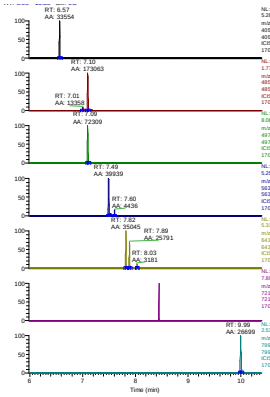


Figure 7. Extracted ion chromatogram of the PBDEs analyzed in a Salmon fish sample during 2016-campaign using GC-HRMS (Q-Orbitrap).

For 2017 new food commodities included in the Recommendation will be analyzed reflecting consumption habits in order to give an accurate estimation of exposure.

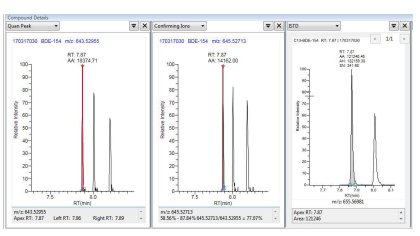


Figure 8. Extracted ion chromatogram of the hexa-PBDEs spiked at 0.01 ng/g in cheese sample during 2017-campaign using GC-HRMS (Q-Orbitrap).

REFERENCES

1. Schechter, et al., Environmental Health Perspectives, 2006, 114 (10), 1515-1520.
2. Schechter, et al., Toxicology and Applied Pharmacology, 2010, 243 (2), 217-224.
3. Recommendation of 3 March 2014 (2014/118/EU) on the monitoring of traces of brominated flame retardants in food.

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CONCLUSIONS

- **The in-house developed extraction method based on QuEChERS methodology was successfully applied to the analysis of fish and seafood.**
- **The LOQs required in the Recommendation (2014/118/EU) were achieved for all the PBDEs using GC-HRMS (Q-Orbitrap).**
- **In 2016, 50 % of samples contained PBDEs with levels above the recommended LOQ (0.01 ng/g), which reveals the exposure for humans to PBDEs through the food chain.**
- **New food commodities are being analyzed during 2017-campaign using the same methodology.**

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