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

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1. Introduction

Brominated flame retardants (BFRs) are chemical mixtures that are applied to manufactured products 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 reached the food chain [1-2].

The European Commission Recommendation 2014/118/EU [3] on the monitoring of BFRs in foodstuff establishes that five classes of BFRs should be analyzed: polybrominated diphenyl ethers, hexabromocyclododecanes, tetrabromobisphenol A and derivatives, brominated phenols and derivatives, emerging and novel BFR

According to the Recommendation, up to ten polybrominated diphenyl ethers (PBDEs) congeners (BDE-28, 47, 49, 99, 100, 138, 153, 154, 183 and 209) should be analyzed in a wide variety of food commodities: 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 and food for infants and small children. The limit of quantification of the analytical methods should be 0.01 ng/g or lower.

The Laboratori de l'Agència de Salut Pública de Barcelona (LASPB) has analyzed PBDEs in fish and seafood since 2009. The method is currently included in the scope of the accreditation of the laboratory following ISO/IEC 17025 requirements. However, new congeners have been included and a special effort has been done in order to get lower limits of quantification to fulfill the Recommendation requirements. For this purpose, an analytical method based on an extraction with ethyl acetate and gas chromatography (GC) coupled to a hybrid Q-Orbitrap has been optimized. The new method has been in-house validated.

2. Materials and Methods

2.1. Standards and reagents

Certified standards of individual PBDE congeners were obtained from Sigma-Aldrich (Steinheim, Germany). In addition, ^{13}C-PBDE-47 and ^{13}C-PBDE-209 used as surrogate internal standards were supplied by Wellington Laboratories (Wellington, Canada). A standard mixture in methanol containing the selected PBDE congeners was used for the preparation of extracted matrix-matched calibration solutions ranging from 0.01 to 0.3 ng/g wet weight.

2.2. Samples and Extraction procedure

In this study, a sample treatment method was optimized for the analysis of PBDEs in fish and seafood samples (mussel, catfish stripped, cuttlefish, tuna fish and salmon). Briefly, 10 g of sample was mixed with 5 mL of distilled water and shaken vigorously with 15 mL of ethyl acetate (EA) in a Falcon tube. Subsequently, partition induced was carried out with EA and the addition of salts (6g $MgSO_4$ and 1.5g of NaCl). An aliquot of 10 mL was removed from the upper organic layer and the solvent was evaporated to the last drop under a stream of nitrogen.
The extract residue was re-dissolved in 6 mL of n-hexane and 3mL of sulfuric acid was added. The extracts were frozen at -20ºC during 1 hour. The upper layer fraction was separated and evaporated under a stream of nitrogen, re-dissolved in 0.3 mL of isooctane and filtered with a 0.45 µm filter into an amber vial before analysis.

2.3. GC-EI-Q-Orbitrap analysis

All GC-EI-Q-Orbitrap experiments were performed in a GC Trace 1310 coupled to Q Exactive GC mass spectrometer (GC-Q-Orbitrap) (Thermo Fisher Scientific, Germany). For GC separation of the target compounds, a TG-5HT fused-capillary column (15 m x 0.25 mm I.D., 0.1 µm of film thickness) was used. GC-EI-Q-Orbitrap was operated in SIM mode (width window *m/z* 50.0). The resolution was set at 30,000 (*m/z* 200, FWHM) at a scan rate 4 Hz, the automatic gain control (AGC, maximum number of ions to fill the C-Trap) was set at 1e6, with an automatic injection time (IT, ion trap opening time).The temperatures of the transfer line and ion source were 250ºC and 280ºC, respectively and helium was used as carrier gas at a flow rate of 1.5 mL/min. The GC oven temperature program was as follows: 40.0 ºC (2 min); 40ºC/min to 350ºC (6 min). Programmed temperature vaporization (PTV) was used as injection mode, with a temperature program as follows: injection at 40ºC (0.1min) to 320 ºC at 5ºC/sec (14 min) and 6 µL were injected.

3. Results and Discussion

3.1. Instrumental analysis

The analysis of PBDEs, especially of the highly brominated congeners is a real challenge for many laboratories mostly due to the high sensitivity required. Of special concern is the analysis of BDE-209.

In order to achieve these extremely low limits, different analytical strategies have been tested in the LASPB. Promising results have been obtained with GC-Q-Orbitrap, but some critical issues, during the method development had to be addressed: GC column type, injection method and injection parameters, acquisition modes, ion source tune and parameters (e.g. emission current, electron voltage). The two most intense ions of the halogenated pattern were selected in a wide SIM window, to include the complete isotopic pattern.

We were capable to detect and quantify all the congeners at 0.01 ng/g in the different matrixes analyzed. The selectivity and sensitivity of the high resolution technology make this new mass spectrometer a good choice for routine analysis of these compounds. An example is shown in **Figure 1**.

3.2. Extraction procedure optimization

Based on our experience and according to published literature [5-6], we considered the following factors to optimize the extraction procedure: sample amount, sample composition (water and fat content), type of extraction solvent, volume of extraction solvent, inorganic salts for partition induced, clean-up. The extraction efficiency and the matrix removal were evaluated by preparing replicates of spiked samples.

For the extraction step, different solvents were tested and ethyl acetate (EA) was the most efficient extraction solvent. Further optimization focused on the extraction step with EA was done, because different salts ($MgSO_4$, NaCl, NaAcetate) and amounts of them were tested. It was observed that extraction was more efficient if $MgSO_4$ and NaCl were used, with no significant differences when the quantity was modified.

The classical clean-up approach for the analysis of PBDEs combines an acid addition with a Silica or Florisil SPE step [5]. We have also optimized the clean-up step trying different strategies: d-SPE, SPE, acid attack and combination of them. The most effective protocol consisted of sulfuric acid addition. Several tests were carried out with different SPE sorbents to evaluate if it was worth adding this additional step. Signal intensity was not enhanced (neither signal-to noise ratio) and the removal of further matrix interferences was not observed. Moreover, when using this extra step, method reproducibility was slightly worse and the time of analysis was enlarged. For this reason, it was decided that the final clean-up protocol would only consist on a sulfuric acid treatment.

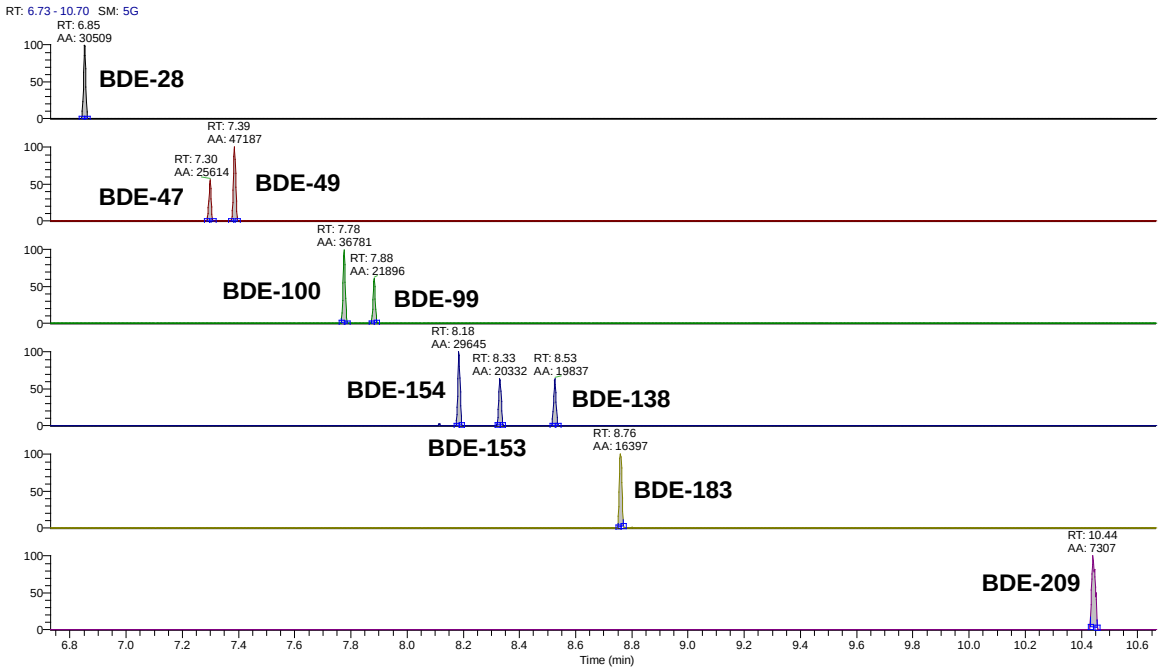

Figure 1. Extracted ion chromatogram of the 10 PBDEs analyzed in tuna fish spiked at 0.01 ng/g analyzed using GC-Q-Orbitrap.

3.3. 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.

3.4. Analysis of real samples

Official control campaigns are every year organized by the Food Health Quality Research Program of ASPB, in order to evaluate whether marketed foods comply with the absence and/or established tolerance levels of specific parameters. What is more, the European Food Safety Authority (EFSA) requested new data on PBDEs as demanded on the Recommendation 2014/118/EU. For these reasons, during 2016, 52 fish and seafood samples were analyzed. Almost 50% of samples of 2016-campaing were found to contain PBDEs. In 2017, a new sampling campaign is starting and new food commodities are being analyzed using the analytical method presented above.

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