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Simultaneous determination of 16 brominated flame retardants in food and feed of animal origin by fast gas chromatography coupled to tandem mass spectrometry using atmospheric pressure chemical ionisation☆

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ABSTRACT

A gas chromatography tandem mass spectrometry method using atmospheric pressure chemical ionisation was developed for the monitoring of 16 brominated flame retardants (7 usually monitored polybromodiphenylethers (PBDEs) and BDE #209 and 8 additional emerging and novel BFRs) in food and feed of animal origin. The developed analytical method has decreased the run time by three compared to conventional strategies, using a 2.5 m column length (5% phenyl stationary phase, 0.1 mm i.d., 0.1 μm f.t.), a pulsed split injection (1:5) with carrier gas helium flow rate at 0.48 mL min^{-1} in one run of 20 min. For most BFRs, analytical data were compared with the current analytical strategy relying on GC/EI/HRMS (double sector, R = 10000 at 10% valley). Performances in terms of sensitivity were found to meet the Commission recommendation (118/2014/EC) for nBFRs. GC/APCI/MS/MS represents a promising alternative for multi-BFRs analysis in complex matrices, in that it allows the monitoring of a wider list of contaminants in a single injection and a shorter run time.

1. Introduction

Brominated Flame Retardants (BFRs) such as polybromodiphenylethers (PBDEs) were used in the industry for their fire resistance properties. As they are suspected to present a risk for Human health [1,2], the use of penta- and octa-BDEs was first restricted in the European Union (Directive 2003/11/EU and Commission Decision 2005/618/EU [3,4]). As they bioaccumulate in the food web, the European Union has recommended their monitoring in food items (2014/118/EC [5]) in order to assess human exposure and record time and space trends. The scientific community has progressively studied novel and emerging BFRs [6,7], such as pentabromobenzene (PBBz), hexabromobenzene (HBBz), 2,3,5,6-tetrabromo-*p*-xylene (pTBX), tetrabromo-o-chlorotoluene (TBCT), pentabromoethylbenzene (PBEB), pentabromotoluene (PBT), hexabromocyclopentenyldibromocyclooctane (HCBDCO) and octabromotrimethylphenyllindane (OBIND). The scientific opinion published by the European Food Safety Authority (EFSA) panel on contaminants in the food chain [8] warrants the attention of scientists to reveal the potential occurrence of these compounds in food items.

As other halogenated contaminants monitored at sub ng g^{-1} in complex matrices, BFRs require appropriate analytical methods to guarantee the reliability of the results. Gas Chromatography coupled to High Resolution Mass Spectrometry (GC/EI/HRMS) was proposed in standardized analytical procedures (EPA Method 1614 [9]) to characterize PBDEs in water, soil, sediment and tissue samples. This strategy remains widely used to determine the occurrence of PBDEs in biological samples of human origin such as human plasma [10], serum [11] or breast milk [11–15] but also in adipose tissue [11], biological tissue [16], fish [17] or bivalves [18]. GC/EI/HRMS allows to be selective enough to detect organohalogen compounds in complex extracts because of their mass defect. Signal to noise ratios are thus enhanced and PBDEs can be quantified down to several fg level on column. One drawback of GC/EI/HRMS

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(double sector) remains its inability to deal with large m/z range analyses and then to cover a single run of BFRs with low and high molecular masses. As the electromagnetic field capability does not exist outside m/z range M to 2 M, the monitoring of tri-BDE (# 47, $[M]^{+\cdot}$ m/z 405.8027) with deca-BDE (#209, $[M\text{-}Br_2]^{+\cdot}$ m/z 801.3315) is tricky in a single run. Another drawback of GC/EI/HRMS concerns the GC part. Indeed, the stability of the highly brominated PBDEs is impacted by the GC temperature. A fast temperature ramp combined with high helium flow rates (which still have to be compatible with high vacuum sources) on a shorter column is in favour of BDE #209 sensitivity [19]. However, it has a negative impact on resolution, which is the key parameter for efficient separation of several congeners' pairs like Hexa-BDE # 154 and 153. As a consequence, BDE #209 is often analyzed in a specific run separated from the seven other PBDEs (BDE #28, #47, #99, #100, #153, #154 and #183) according to the EPA method 1614 [9].

A new analytical tool is then required to prevent degradation in the GC oven and to analyze a wide range of m/z simultaneously. The GC/APCI/QqQ system was selected for this work because it allows the use of adapted chromatographic columns with huge flow rates and a mass window extended from m/z 50–1200 thanks to its quadrupole capabilities. This instrument is already described to be an efficient alternative for highly brominated congeners analysis (Portoles, 2015 [20]) in introducing simultaneously PBDEs and two highly brominated and emerging BFRs, i.e. decabromodiphenylethane and 1,2-bis(2,4,6-tribromophenoxy)ethane. In this method, limits of detection were on the dozen fg level, which could be compatible with their potential occurrence levels in food samples.

The aim of our study was to propose an efficient analytical tool which could both save time and increase the number of contaminants monitored. The first part of this work focused on the ionisation conditions of the APCI source as well as on the determination of the most relevant transition of each of the BFRs included in the method. The second part of this work was dedicated to the chromatographic introduction and separation with, as main objective, to ensure fast separation without compromising target analyte separation and sensitivity. Several food and feed items were investigated both using GC/EI/HRMS (electromagnetic field or BE) and GC/APCI/MS/MS (triple quadrupole or QqQ); data were compared both in terms of robustness and sensitivity. Quality control analysis, analytical contamination considerations in blank samples and quantification at sub-ppb levels obtained with the GC/APCI/MS/MS method were the performances which were reported and discussed in this paper in comparison with the GC/EI/HRMS ones.

2. Material and methods

2.1. Chemicals and standards

Chemicals and solvents were of high quality grade for trace analysis. Acetone, diethyl ether, ethanol, n-hexane, n-pentane and toluene were purchased from Promochem (Molsheim, France), anhydrous sodium sulfate and dipotassium oxalate monohydrate from Merck (Darmstadt, Germany). Ultrapure water was obtained using a Nanopure system from Barnstead (>18 MΩ cm, Waltham, MA, USA).

The PBDE congeners #28, #47, #100, #99, #154, #153, #183 and #209, both native and $^{13}C_{12}$-labelled standards, PBBz, HBBz, including native and $^{13}C_6$-labelled standards, pTBX, TBCT, PBEB, PBT, HCBDCO and OBIND were provided by Wellington Laboratories (Guelph, Canada). Their purities were higher than 98%. A solution containing all the native BFRs at 10 pg μL^{-1} was prepared in toluene for the GC method development. For the analysis of feed/food samples, five calibration standard solutions were prepared in toluene. Concentrations of OBIND and BDE #209 were 1, 10, 50, 200 and 1000 pg μL^{-1}; other native BFRs were at 2, 10, 20, 100 and 200 pg μL^{-1}. Labeled BFRs were added in each calibration solution at 20 pg μL^{-1} except $^{13}C_{12}$-BDE-209 which was diluted at 50 pg μL^{-1} in all calibration solutions. The labeled compound stock solution was a mixture of all labeled compounds at 8 pg μL^{-1} in toluene and $^{13}C_{12}$-BDE-209 at 20 pg μL^{-1}. The labeled injection standard solution was prepared in toluene at 1 pg μL^{-1} for $^{13}C_{12}$-BDE-77 and 138. All solutions were stored at +4 °C.

2.2. Samples

Tested food and feed came from the 2015 French monitoring plan. It included feed (fish meal and fish oil, n = 7), crustacean and mollusks (n = 6), milk (n = 3), eggs (n = 3), fish (n = 9), ovine liver (n = 2) and muscle from different species (n = 9) samples. Blank samples were made of extraction solvent and internal standard solution without any matrix addition. The QA/QC sample consisted in fish oil naturally contaminated with PBDE and spiked with 1000 pg of OBIND and 400 pg of other emerging BFRs. For routine analysis, one blank sample was extracted in each batch of fourteen samples whereas one QA/QC sample was extracted in every second batch.

2.3. Sample treatment

Laboratory glassware was always rinsed with dichloromethane prior to use and the analyses were carried out in a protected area (over-pressurized room) to limit environmental contamination.

Before extraction, solid samples were freeze-dried and ground in order to obtain a homogeneous thin powder. Fat matrices such as oils were directly dissolved in an appropriate solvent (i.e. hexane) for subsequent clean-up without any extraction step. Further details on all the sample preparation steps have already been described in a previous publication [21]. Briefly, fifty microliters of labeled compound stock solution were added to the samples. Lipids were extracted from freeze-dried samples by Pressurized Liquid Extraction (SpeedExtractor E-914/E-916, Büchi, Rungis, France) using a toluene/acetone mixture (70:30, v/v). Purification steps were achieved on successive columns manually packed with neutral and acidic silica gel; analytes were eluted in hexane and successively purified on Florisil® and carbon. After evaporation of the solvent, extracts were reconstituted in 20 μL of labeled injection standard solution for recovery determination.

2.4. GC/EI/HRMS (BE) analysis

The system used was a gas chromatograph A7890 (Agilent Technologies, Palo Alto, CA, USA) coupled to a high resolution mass spectrometer MS-800D (Jeol, Tokyo, Japan). The instrument was calibrated at a resolution of 10000 (10% of the valley). Samples were injected twice, one acquisition being dedicated to the lowest brominated analytes (LBFR method) and the other method to the highly brominated ones (BDE #209 and OBIND, HBFR method).

LBFR method: PBDE analysis was conducted on a DB5MS column (30 m, 0.25 mm, 0.25 μm) (Agilent Technologies, Palo Alto, CA, USA). Helium was used as carrier gas at 1 mL min^{-1}. Injector and transfer line temperature were set at 280 °C. Samples were injected in the splitless mode (2 μL). The oven temperature was programmed at 120 °C (2 min), 10 °C min^{-1} until 215 °C (0 min), 3 °C min^{-1} until 270 °C and 10 °C min^{-1} until 310 °C (4 min). Electron ionisation was set at 38–40 eV with an emission current of 500 ± 100 μA. The source temperature was set at 280 °C. Two diagnostic ions were recorded per analyte and internal standard in HRSIM mode (Table 1).

HBFR method: BDE #209 and OBIND were separated on an RTX-1614 column (15 m × 0.25 mm, 0.10 μm) (Restek, Lisses, France).