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FAST GAS CHROMATOGRAPHY COUPLED TO TANDEM MASS SPECTROMETRY USING ATMOSPHERIC PRESSURE CHEMICAL IONISATION FOR BROMINATED FLAME RETARDANTS MONITORING

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1. Introduction

Brominated Flame Retardants (BFRs) such as polybromodiphenylethers (PBDEs) were used in the industry for their fire protection properties. As they are suspected to represent a risk for Human health, their use was first restricted in the European Union (1). Industries have then replaced PBDEs by novel and emerging BFRs. As they bioaccumulate in the food web, the European Union has recommended their monitoring in food items (2). In parallel, the sanitary and biomonitoring agencies have paid attention to circulating BFR levels in human biological fluids to assess the population internal exposure.

The monitoring of BFRs at sub $ng.g^{-1}$ in complex matrices requires appropriate methods to guarantee the quality of the results. 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 to 1200 thanks to the quadrupole capabilities. This instrument is already described as an efficient alternative for highly brominated congeners analysis (3), as itallows to simultaneously measure PBDEs and two highly brominated and emerging BFRs, i.e. decabromodiphenlyethane (DBDPE) and 1,2-bis(2,4,6-tribromophenoxy)ethane (BTBPE). 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 markers of exposure monitoredsimultaneously . This work was dedicated to the chromatographic introduction and separation with, as main objective, to ensure fast separation without any compromise on target analyte separation and sensitivity. The originality of this work lies in the short column used and its specific associated parameters.

2. Materials and Methods

2.1. Chemicals and standards

PBDE congeners #28, #47, #100, #99, #154, #153, #183 and #209, both native and 13C12-labelled standards, pentabromobenzene (PBBz), hexabromobenzene (HBBz), including native and 13C6-labelled standards, 2,3,5,6-tetrabromo-p-xylene (pTBX), tetrabromo-o-chlorotoluene (TBCT), pentabromoethylbenzene (PBEB), pentabromotoluene (PBT), hexabromocyclopentenyldibromocyclooctane (HCBDCO) and octabromotrimethylphenyllindane (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. All solutions were stored at +4 °C.

2.2. GC/APCI/MS/MS (QqQ) analysis (Figure 1)

A gas chromatograph A7890 (Agilent Technologies, Palo Alto, CA, USA) coupled to a triple quadrupole mass spectrometer Xevo TQS (Waters, Milford, US) using an atmospheric pressure interface was used. Injector temperature was 275 °C. Transfer line temperature was set at 350°C with a sheath nitrogen gas at 400 mL min^{-1}. Source temperature was 150 °C. Auxiliary and cone gas flow rates were 50 and 225 L h-1. APCI was operated in the positive mode under dry conditions. A 2 µA intensity was set for the corona needle. Cone voltage was set at 30 V. An ultra-inert simple gooseneck 2 mm i.d. liner (Sky, Restek) and a short GC column of 2.5 m x 0.1 mm, 0.1 µm (Optima 5, Macherey-Nagel, Hoerdt, France) were installed. Sample was injected in pulsed split mode (1 µL, 40 psi during 0.85 min, split 1:5). The oven temperature was programmed at 80°C (1 min), 15 °C min^{-1} until 310 °C (0 min) and 40 °C min^{-1} until 350 °C (1 min).

3. Results and Discussion

The multi-BFRs method was developed taking into account the chromatographic efficiency and thermostability of all target compounds in a single run. The efficiency of separation was first assessed on two BDE congeners #154 and #153 (Figure 2). The best resolution was obtained at the 50 cm s^{-1} velocity which only required a carrier gas flow rate of 0.48 mL min^{-1}. The sensitivity was then interpreted first on BDE #154 and temperature gradients of 10 and 20 °C min^{-1} gave similar results. A temperature ramp of 15 °C min^{-1} was selected for future analyses on this column.

The stability of heavier BFRs such as BDE #209 was then assessed. The maximum BDE #209 sensitivity was observed above a carrier gas velocity of 60 cm s-1 whatever the temperature gradient. The level of sensitivity was maintained at higher velocities with a higher temperature ramp (more than 20 °C min^{-1}). However, these conditions could affect the separation of BDE #154 and #153 and were therefore not acceptable for this new method. We decided then to keep the carrier gas velocity at 50 cm s-1 in the column and to increase the carrier gas velocity in the liner. Actually, as the time spent by BDE #209 in the injector would directly impact its degradation (placed at 275 °C in our experiment), we decided to minimise this time. A 2 mm i.d. simple gooseneck liner was then used instead of a 4 mm i.d. one. The pulsed splitless mode (40 psi, 0.85 min) was selected to take into account the expansion volume of 1 µL toluene injected and the capacity of the liner. After installation of the new liner, the sensitivity (measured in absolute abundance in this case) was multiplied by ten for the decabrominated compounds and remained the same for the lower brominated flame retardants. This technical solution was kept for the analysis of food and feed samples on the 2.5 x 0.1 mm column.

Nevertheless, one issue remained unsolved at this stage: a peak broadening phenomenon, amplified with the velocity, was observed for the most volatile BFRs. We hypothesized the toluene volume as a potential cause. Indeed, we observed that 1 µL of toluene was not loaded on the same column length whether the internal diameter was 0.25 mm or 0.1 mm. Taking into account the thermal expansion coefficient of toluene, we then determined a theoretical length of 139 mm in the 2.5 m x 0.1 mm i.d. column on which the volume of toluene was loaded, which probably affected negatively the peak shape of the most volatile analytes. A solution was found by injecting the solvent in the split mode (1:5). Peak broadening disappeared with these new parameters, even for the most volatile analytes and a gain in sensitivity was clearly demonstrated with the chromatographic peak symmetry improvement. Typical resulting diagnostic chromatograms are presented in Figure 2.

4. Conclusion

The multiple origins and sources of BFR exposure highlighted the need for a multi-residue method to monitor BFRs in food, feed and biological matrices. The aim of our study was to develop a unique, alternative and reliable analytical method for this purpose. Our work was focused on a simplification of the analytical process, taking into account the stability of each compound separately. The final GC method was based on a pulsed split injection combined with a short column (2.5 m length) with an internal diameter of 0.1 mm and a type 5 stationary phase (0.1 µm film thickness). The MS parameters used two transitions per analyte and internal standard. This new analytical tool allows the monitoring of 16 BFRs simultaneously in less than 15 min, using less helium volume than a conventional 15 m x 0.25 mm i.d. column in a single run. Moreover, the column cost is highly decreased with this novel approach as a 10 m length column is cut three times to obtain 4 analytical columns. The developed methodology is now on-going to be applied for a first inventory of food contamination levels and human internal exposure on the French scale.

5. Aknowledgements

The authors would like to thank the French Ministry of Agriculture for its financial support.

6. References:

1. Directive 2003/11/EC relating to restrictions on the marketing and use of penta-BDE and octa-BDE.
2. Commission recommendation 2014/118/EU on the monitoring of traces of BFRs in food.
3. Portoles T et al.. Anal Chem. 2015 Oct 6; 87(19):9892-9.

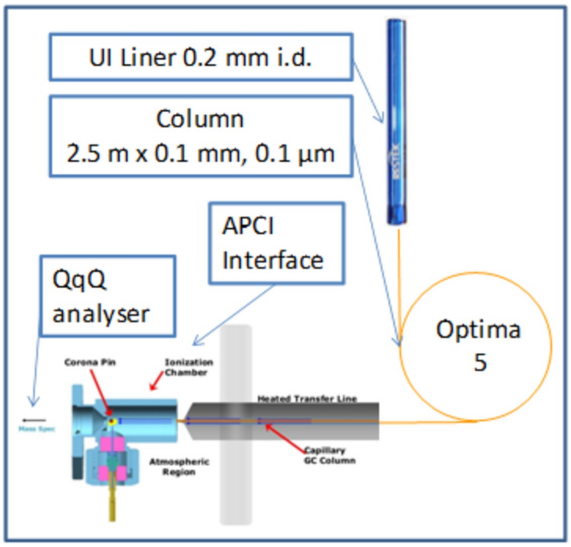

Figure 1: Diagram of the GC/APCI/MS/MS configuration

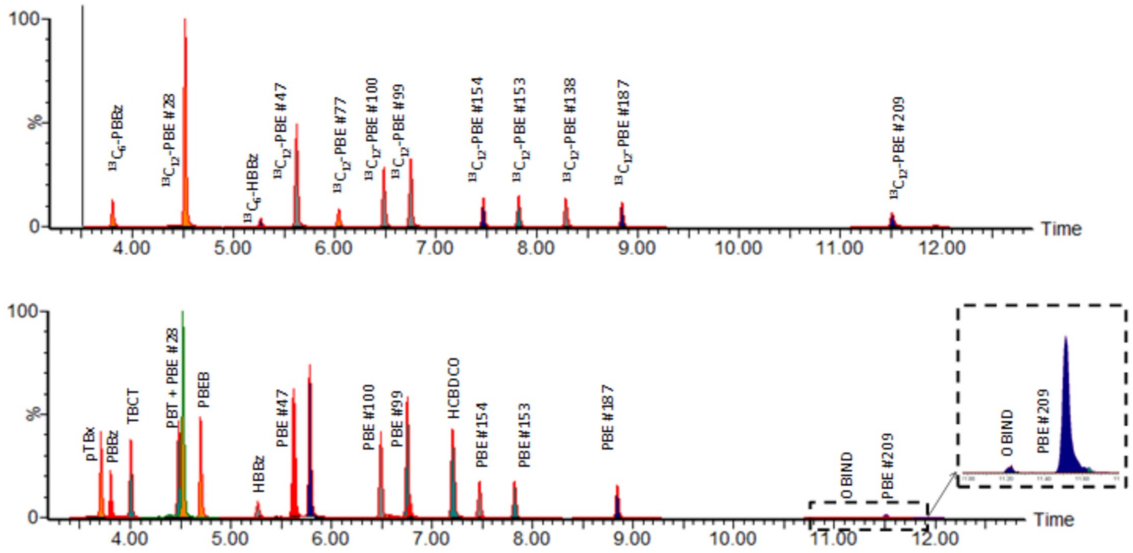

Figure 2: Diagnostic GC/APCI/MS/MS ion chromatograms obtained for labeled (top) 25 pg injected except 50 pg for 13C12-BDE #209 (split 1:5; 5 pg and 10 pg on-column respectively) and native BFRs (down) 100 pg injected except 50 pg for BDE #209 and OBIND (split 1:5; 20 pg and 10 pg on-column respectively)