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Improving the sensitivity of liquid chromatography–tandem mass spectrometry analysis of hexabromocyclododecanes by chlorine adduct generation☆

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ABSTRACT

It is well documented and experimentally confirmed that hexabromocyclododecanes (HBCDs) tend to associate with several anions forming different adducts that can affect the sensitivity and the accuracy of the determinations. In the present work, two different approaches for HBCD determination have been optimised and characterised based on their repeatability and intermediate precision, linear calibration ranges, sensitivity, limits of detection and quantification and application to commercial food samples. Both methods involve the use of a triple quadrupole mass spectrometer coupled to a liquid chromatograph and the addition of different ammonium salts to the mobile phase, i.e. ammonium chloride or ammonium acetate, in order to encourage (Cl method) or try to inhibit (Ac method), respectively, the formation of the chlorine adducts of the molecular ion. Precision of the two methods investigated was similar and both approaches presented a comparable behaviour for the analysis of food samples. However, the Cl method showed higher sensitivity and the limits of detection (0.23–0.41 pg on column) and quantification (0.77–1.35 pg on column) were up to 14 times lower than those obtained applying the Ac method. All these facts make the Cl method the best choice for the quantification of HBCDs in food samples with low concentration levels.

1. Introduction

Brominated flame retardants (BFRs) are a group of organobrominated compounds that added to different commercial products can decrease the likelihood and intensity of fire in said materials. Due to their undeniable usefulness, nowadays they are widely used on a daily basis in every day life. However, their high usage causes important amounts of these compounds to be released into the environment causing pollution problems and risks for human health. For this reason, during the last decade, the use of some BFRs, such as penta- and octa-technical mixtures of polybrominated diphenyl ethers (PBDEs), has been banned in the European Union, leading to an increase in the demand for replacement BFRs, such as hexabromocyclododecanes (HBCDs). HBCD is an additive BFR generally used in expanded and extruded polystyrene foams and in textiles for household and furniture appliances. Due to the fact that it is not chemically bound to the materials to which it is added, it is susceptible to transfer from said materials to the environment [1]. From a chemical point of view, HBCD is a non-aromatic, brominated cyclic alkane with a complex stereochemistry [2]. The marketed mixture is mainly composed of γ-HBCD diastereoisomer (75–89%) while the rest of the diastereoisomers (primarily α- and β-HBCD) are presented in a lower percentage (10–13% and 0.5–12%, respectively). Moreover, each of these diastereoisomers, presents two enantiomeric forms that should be determined for a better understanding of the enantioselective behaviour of HBCD. It is important to note that HBCDs suffer a significant decomposition at temperatures above 200–220 °C [3,4] and their diastereoisomers also undergo a thermal rearrangement at temperatures above 160 °C [4,5] which leads to a lack of resolution between peaks corresponding to each diastereoisomer when gas chromatography (GC) is used as separation technique. This fact makes the use of liquid chromatography (LC) mandatory to obtain HBCD diastereoisomer/enantiomer specific data.

Regarding detection systems, mass spectrometry (MS) is the preferred analytical technique, because of its high selectivity and sensitivity. Among different types of MS analysers, the triple quadrupole is the most used [3,6–18], monitoring the specific transitions m/z 640.6 → m/z 78.9 and 80.9. Some authors have also used single quadrupole mass analysers monitoring the $[M-H]^-$ ion [12] and new hybrid triple quadrupole/linear ion trap mass spectrometers monitoring the same transitions previously mentioned

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[19–21]. However, several authors [5,22,23] have described that variable amounts of an ion at *m/z* 676.6, corresponding to the chlorine adducts of HBCDs, are usually detected in the mass spectra of this compound, affecting the accuracy of the quantification. Some of these authors proposed the use of ammonium acetate as an eluent modifier to control and even slightly block the chlorine adduct formation without losing sensitivity in comparison with no eluent modifier addition. This approach is generally followed for HBCD determinations [5,8,9,11,12,16,18,24], nevertheless, it does not completely prevent the chlorine adduct formation but it triggers the formation of an additional acetate adduct.

Taking all of these issues into account, other alternatives for eluent modifiers and mass analysers should be investigated. However, very few methods have been published in this sense. For example, Gómara et al. [23] and Kuiper et al. [25] described the enantiomer and diastereoisomer specific determination of HBCDs, respectively, using high-performance liquid chromatography coupled, by an electrospray ionisation source, to a three-dimensional ion trap mass spectrometry system working in its tandem operation mode (LC–ESI-MS/MS). Contrary to the idea previously mentioned, these methods are based on the specific formation of the chlorine adduct (*m/z* 676.6) of the (±)α-, (±)β-, and (±)γ-HBCD enantiomers/diastereoisomers and their further fragmentation into their stable $[M-H]^-$ ion (*m/z* 640.6). In these methods, good results were obtained, mainly solving those problems related to the variable amounts of other adduct peaks in the mass spectra. However, in food samples, the HBCD concentrations are quite low and when a three-dimensional ion trap mass spectrometer is used, could be difficult to reach the necessary limits of detection and reproducibility. In these cases, the use of a triple quadrupole mass spectrometer could overcome at a reasonable price these difficulties.

Therefore, the aim of this work is to study the reliability of a new approach of an LC–ESI-MS/MS method for the determination of HBCDs and to compare it with the conventional method reported in the literature. Briefly, the new approach presented is based on the formation of a chlorine adduct as precursor ion, $[M+Cl]^-$, and its further fragmentation into the $[M-H]^-$ ion using a triple quadrupole mass spectrometer. The different experimental parameters affecting the formation and ionisation of the precursor ions and the MS/MS detection of the product ions formed were studied. Performance of both approaches was also investigated and evaluated in terms of limits of detection, linear working concentration ranges, precision and applicability for the analysis of commercial food samples.

To the best of our knowledge, it is the first time in the literature that an approach based on the formation of the chlorine adduct and its further fragmentation into the $[M-H]^-$ ion using a triple quadrupole mass spectrometer is described in depth and compared with the conventional method used for HBCD determinations.

2. Experimental

2.1. Reagents and standards

Methanol and acetonitrile of LC–MS Chromasolv grade, supplied by Riedel-de Haën (Seelze, Germany), were used. Milli-Q water was obtained using a Millipore system (Bedford, MA, USA). The three native HBCD diastereoisomers (α-, β- and γ-HBCD) standards were purchased from Cambridge Isotope Labs. (Andover, MA, USA) while the three $^{13}C_{12}$-labelled HBCDs from Wellington Labs. (Guelph, Canada). Ammonium chloride (99.8% purity) and ammonium acetate (99.0% purity) were obtained from Sigma–Aldrich (St. Louis, MO, USA).

All reagents used for sample treatment were of trace analysis grade. *n*-Hexane, sulphuric acid (95–97%) and silica gel were supplied by Merck (Darmstadt, Germany) and granular anhydrous sodium sulphate by J.T. Baker (Deventer, The Netherlands). Acetone, dichloromethane and toluene were supplied by SDS (Peypin, France).

2.2. Instrumental determination

All MS experiments were carried out using an Accela pump (Thermo Fisher Scientific, San José, CA, USA) with a quaternary gradient system coupled to a TSQ Quantum Access triple quadrupole mass spectrometer (Thermo Fisher Scientific) using an ESI interface. Spray voltage was set at 3.0 kV, nitrogen (99.5% purity) was used as a sheath and auxiliary gas, and argon (99.9990% purity) was employed as a collision gas. Mass spectra were acquired in the negative ion mode.

Among the three HBCD diastereoisomers previously mentioned, the most abundant in the technical mixture (i.e., γ-HBCD [10]) was selected for the optimisation of both the ion transmission into the analyser and the MS/MS parameters. To carry out these experiments, a methanolic solution of γ-HBCD (2 ng μL^{-1}) was infused into the mass spectrometer at a flow rate of 5 μL min^{-1} using the syringe pump included in the TSQ instrument and mixing it with 100 μL min^{-1} of water:methanol (10:90, v/v) by means of a zero-dead volume T-piece. Sheath and auxiliary gases were set at 0.6 and 6 L min^{-1}, respectively.

The rest of the MS parameters were optimised using a flow injection analysis-mass spectrometry (FIA-MS). For that, a 5 μL sample of γ-HBCD (0.2 ng μL^{-1}) dissolved in methanol was injected by an Accela autosampler (Thermo Fisher Scientific) into the LC system and carried through in the eluent at a flow rate of 500 μL min^{-1}. In this case, the sheath and auxiliary gases were set at 0.9 and 6 L min^{-1}, respectively.

The separation of the HBCD enantiomers, published elsewhere [23], was performed at 25 °C on a chiral LC column Nucleodex β-PM (permethylated-β-cyclodextrin, 200 mm × 4 mm, 5 μm) purchased from Macherey-Nagel (Düren, Germany). A mobile phase consisting of water:methanol:acetonitrile:ammonium salt (38:30:30:2, v/v/v/v) at a flow rate of 500 μL min^{-1} was maintained for 0.5 min, after which a linear gradient to methanol:acetonitrile:ammonium salt (28:70:2, v/v/v) over 18 min was applied and the final conditions were maintained for 6.5 min. An injection volume of 20 μL was used. As previously mentioned, two different ammonium salts were tested, one of them being ammonium chloride, which encourages the formation of the chlorine adduct (Cl method hereafter), the other one being ammonium acetate, which tries to inhibit the formation of this adduct (Ac method hereafter). The salt concentration was an important parameter and for this reason was also optimised.

2.3. Sample treatment

The food samples used for the study were selected as follows: two of them (salmon and eel) belonged to the "Interlaboratory Comparison on Dioxins in Food 2006 and 2007" and three of them (beef meat, cow milk and chicken eggs) were real-life samples commercially available at local supermarkets in Spain. Once at the laboratory, the non-edible part of the food samples was removed and the edible part was stored in stable conditions (freeze-dried) until analysis. Samples were extracted by matrix solid-phase dispersion (MSPD) as previously described in detail elsewhere [26]. Briefly, freeze-dried samples (approximately 6 g of fat) were homogenised with silica gel:anhydrous sodium sulphate powder (1:4, w/w). The mixture was ground to a fine powder, loaded into a column and extracted with acetone:*n*-hexane mixture (50:50, v/v). Further clean-up and lipid removal were achieved by using acid and basic impregnated silica gel multilayer columns (25 g of 44% (w/w) and 13 g of 22% (w/w) of silica modified with sulphuric