LIQUID CHROMATOGRAPHY/NEGATIVE ION ELECTROSPRAY TANDEM MASS SPECTROMETRY METHOD FOR THE QUANTIFICATION OF HBCD ENANTIOMERS

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Abstract
During the last decade, the use of some brominated flame retardants (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 hexabromociclododecanes (HBCD). Due to the fact that HBCD is not chemically bound to the materials that it is added to, it is susceptible to easy transfer from said materials to the environment. HBCD is a nonaromatic, brominated cyclic alkane with a complex stereochemistry. To obtain HBCD isomer specific data it is mandatory to use liquid chromatography (LC) since gas chromatography (GC) does not allow the separation of the different isomers as individual GC peaks. Regarding detection systems, mass spectrometry (MS) is the preferred analytical technique, because of their high selectivity and sensitivity.
In this study, a new method for the enantiomer specific determination of HBCDs by using high performance liquid chromatography coupled, by an electrospray ionization source, to an ion trap mass spectrometer working in its tandem operation mode (LC-ESI-MS/MS) has been developed.

Introduction
During the last decade, the use of some brominated flame retardants (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 hexabromociclododecanes (HBCD). 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 that it is added to, it is susceptible to easy transfer from said materials to the environment. On the other hand, the consumption in European countries in 2001 of different BFRs was estimated to be 9,500 metric tons of HBCDs and 8,360 metric tons of PBDE technical products, which made HBCDs a matter of special concern in European countries [1]. HBCD is a nonaromatic, brominated cyclic alkane with a complex stereochemistry [2]. The marketed mixture is mainly composed of γ-HBCD diasteroisomer (75-89%) while the rest of the diasteroisomers are presented in a lower percentage. To obtain HBCD isomer specific data it is mandatory to use liquid chromatography (LC) since gas chromatography (GC) does not allow the separation of the different isomers as individual GC peaks due to the fact that HBCD suffers thermal rearrangement at temperatures above 160 ºC [3] which leads to a lack of resolution between GC peaks corresponding to each isomer. In addition, a significant decomposition of HBCD was observed at temperatures above 200 ºC [4]. Regarding detection systems, mass spectrometry (MS) is the preferred analytical technique, because of their high selectivity and sensitivity.
We present here a method for the enantiomer specific determination of HBCDs by using high performance liquid chromatography coupled, by an electrospray ionization source, to an ion trap mass spectrometer working in its tandem operation mode (LC-ESI-MS/MS). The different parameters affecting the separation, ionization and the MS/MS detection conditions were studied.

Materials and Methods

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, USA). The three HBCD diasteroisomers (α-, β- and γ-HBCD) standards were purchased from Cambridge Isotope Laboratories (Andover, USA).

The LC experiments were carried out on a Finnigan Surveyor pump (Thermo Electron, San José, CA, USA). The separation was performed on a chiral LC column NUCLEODEX β-PM (permethylated-β-cyclodextrin, 200 mm x 4 mm, 5 µm) purchased from Macherey-Nagel GmbH&Co. (Düren, Germany). The column was pre-washed using a methanol:water (50:50) mixture for 24 hours before its first use. For every analysis a flow rate of 500 µL/min was used and 20 µL were injecting by a Finnigan Surveyor autosampler (Thermo Electron, San José, CA, USA).

The mass spectrometry and tandem mass spectrometry experiments were carried out on a Finnigan LCQ Deca (Thermo Electron, San José, CA, USA) ion trap mass spectrometer using an electrospray ionization (ESI) interface. Spray voltage was set at 4.5 KV, nitrogen (99.5 % purity) was used as the sheath and auxiliary gas, and helium (99.9990 % purity) was the collision gas. Mass spectra were acquired in the negative mode.

Results and Discussion

Optimisation of the MS conditions

The γ-HBCD diasteroisomer was selected for the optimisation of the MS/MS conditions due to its abundance in the technical mixture, higher than 70% [5]. To determine the ions to be monitored, a methanolic solution of γ-HBCD (2 ng/µL) was infused into de mass spectrometer at a flow rate of 5 µL/min using the syringe pump included in the LCQ instrument and was mixed with 100 µL/min of mobile phase by means a zero-dead volume T-piece. The mass spectra obtained (Figure 1.a.) presented an important contribution of a chlorine adduct cluster, fact already reported by other authors [3,6], and some of them tend to reduce its formation by the addition of ammonium acetate [7], but its complete inhibition has not been reported. In the present paper, the objective was the opposite and, for this reason, different percentages of ammonium chloride were add to the mobile phase for increasing and stabilising the amount of the chlorine adduct formed. Ammonium chloride concentrations between 0 and 400 µM (in the whole mobile phase) were tested and the best results were achieved using 80 µM (Figure 1.b.).

Due to the thermal instability of HBCD, the heated capillary temperature was also investigated in order to obtain the best signal to noise ratios (S/N). As shown in Figure 2, the highest S/N values were accomplishing using 180 ºC, therefore, this temperature was set for the following experiments.

Once these general conditions were fixed, the parameters connected with the isolation and fragmentation of the precursor ion and the storage of the product ion were investigated. First of all, an isolation window of eight units over the cluster central value was selected for the trapping of the three most abundant ions of the adduct cluster, i.e. *m/z* 674.6, 676.6 and 678.6. Wider isolation windows increased the background noise without a significant increment in the total response. It is well known that the stability of the trajectories of the ions trapped into the analyser depends on the parameter q_z. In this paper, the stability range of both precursor and product ions was investigated in order to choose the optimum value for both. Figure 3 shows the variation of the signal corresponding to precursor (*m/z* 676.6) and product (*m/z* 640.6) ions as a function of the q_z values. Product ion presented a narrower stability range than precursor ions, the optimum value being q_z = 0.225. This value remained fixed for the following experiments.

The fragmentation of the precursor ions isolated in the trap took place by collision induced dissociation (CID) and the effect of the CID voltage necessary for parent ion dissociation was also studied. Figure 4 shows the intensities of precursor and product ions for different normalised collision energy values. As expected, a decrease in the response of the precursor ion was observed when increasing the CID voltage, which resulted in a progressive increase in the response of the corresponding product ions formed. The normalised collision energy providing the maximum intensity for the product ions formed was selected at the top of the narrow peak obtained for the variation of product ion response (Figure 4).

Optimisation of the enantiomer separation

Different mobile phases were tested in order to improve the separation of the three diasteroisomers and the two enantiomers of each diasteroisomer from each other. Firstly, mobile phases containing different percentages of water:methanol and water:methanol:isopropanol were studied but all of them failed to separate the six

enantiomers under study. Taking into account some studies previously published [4], acetonitrile was added to the mobile phase as an additional solvent and different gradients were tested. In the end, the following gradient programme was selected. A mobile phase consisted of water:methanol:acetonitrile:ammonium chloride (2mM) (38:28:30:4) at a flow rate of 500 µL/min was maintained for 0.5 min, after which a linear gradient to methanol:acetonitrile:ammonium chloride 2mM (26:70:4) over 18 min was applied and the final conditions were maintained for 7 min.

Column temperature effect was also investigated. Temperatures lower than room temperature (25 ºC) led to a longer time of analysis without improving the separation. On the other hand, temperatures higher than 25 ºC produced a decrease in the response obtained and a worsening of the resolution between enantiomers. Therefore, a column temperature equal to 25 ºC remained as the optimum temperature.

Table 1 summarises the experimental conditions selected for the whole LC-ESI-MS/MS method as well as the range investigated for each studied parameter. Under these optimum conditions, the developed method allowed the baseline separation and the detection of the six HBCD enantiomers under study by means of the formation of the chlorine adduct and its subsequent fragmentation into the quasi-molecular ion $[M-H]^-$ which was finally monitored for qualitative and quantitative purposes.

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Figure 1. Mass spectra of γ-HBCD (a) without any additive and (b) with 80 µM of ammonium chloride.

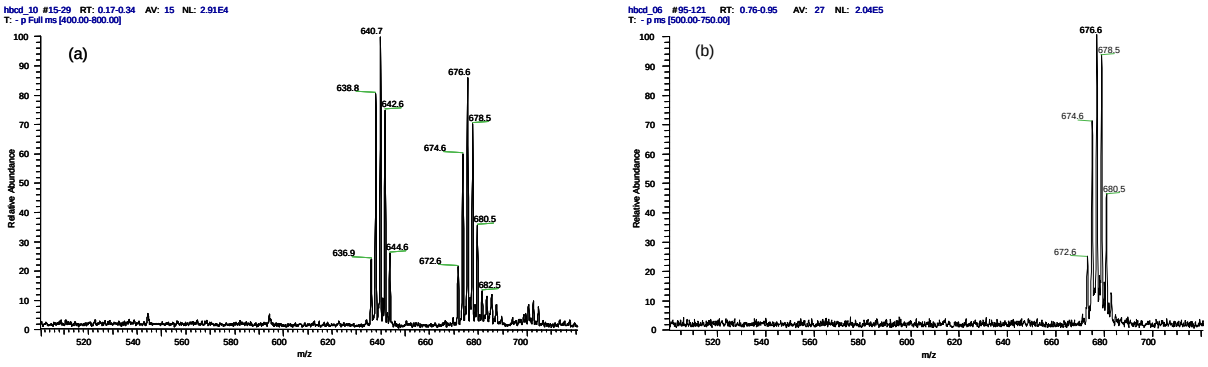

Figure 2. Stability of the precursor ion $[M+Cl]^-$ with the heated capillary temperature.

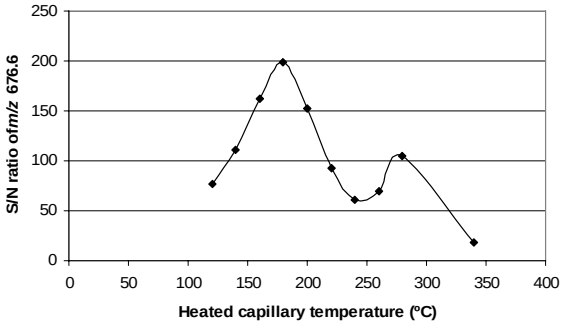

Figure 3. Trapping efficiency of the precursor and product ions for different q_z values in MS/MS experiments.

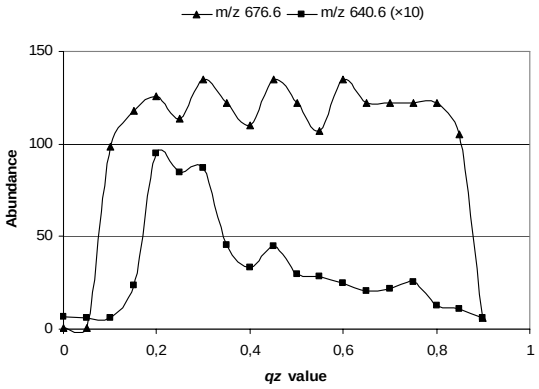

Figure 4. Optimisation of the normalized collision energy for MS/MS experiments

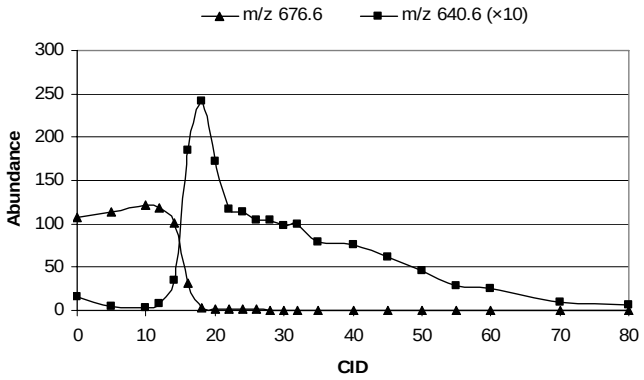

Table 1. Ranges and optimum values of the different parameters under study for the whole LC-ESI-MS/MS method.

Parameter	Range	Optimum value
	MS/MS	
Concentration of ammonium chloride (μM)	0 – 400	80
Heated capillary temperature (ºC)	120 – 340	180
q_z value	0 – 0.9	0.225
Normalised collision energy (%)	0 – 80	18
	HPLC	
Mobile phase composition	Different combinations of water, methanol, isopropanol and acetonitrile	water:methanol:acetonitrile
Column temperature (ºC)	15 – 45	25