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## Fluorinated, Chlorinated and Brominated Contaminants in Fish for Human Consumption

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## 3.2 Analysis of hexabromocyclododecane pitfalls and method comparisons<sup>5</sup>

### Abstract

Gas chromatography (GC) and liquid chromatography (LC) coupled with mass spectrometry (MS) are both used for the analysis of HBCD. An important advantage of LC-MS is the separation of individual diastereomers. This allows the use of mass labelled internal standards, which improves the accuracy of the results. The advantage of GC-MS is the simultaneous analysis with other brominated flame retardants (BFRs) such as polybrominated diphenyl ethers (PBDEs). However, until now severe discrepancies were found between GC-MS and LC-MS results. Several experiments were conducted to evaluate performance of both methods: (i) check of degradation of HBCD in the GC; (ii) measuring of HBCD response factors in the GC-MS; (iii) application of a rapid resolution column for LC separation, (iv) sensitivity test of different instruments and (v) the evaluation of GC vs. LC for the analysis of fish samples. This provided the following insights:

- In GC, degradation of HBCD diastereomers occurs, resulting in the formation of the degradation products pentabromocyclododecene and tetrabromocyclododecadiene. This leads to erroneous HBCD results. In addition, the degradation products can disturb the analysis of major PBDEs (e.g. BDE 49 and 99).
- The GC response factors of the diastereomers are different when using electron capture negative ion MS. This leads to serious quantification errors as the diastereomer profiles in standards and samples are different.
- In LC-MS, the use of a rapid LC-column (Zorbax SB-C18, 2.1 x 30 mm x 3.5 µm) reduced the run-time (5-fold) and improved sensitivity (4-fold) to 20 pg absolute.

Comparative studies in fish showed that GC results are (on average) 4.4-fold higher than the LC results. Research is not conclusive which of the methods delivers biased results (GC-MS or LC-MS or both), and this should be further elucidated. However, because of several advantages of the LC-MS method (i.e. determination of individual diastereomers, the use of mass labelled standards, good sensitivity, no thermal degradation, and simultaneous analysis with tetrabromobisphenol-A) LC-MS is currently the preferred method of analysis.

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<sup>5</sup>Based on S.P.J. van Leeuwen and J. de Boer (2008) Brominated Flame Retardants in Fish and Shellfish – Levels and Contribution of Fish Consumption to Dietary Exposure of Dutch citizens to HBCD. *Molecular Nutrition and Food Research* 52, 204-216, and on a presentation by P.E.G. Leonards and discussions at the QUASIMEME Workshop on Brominated Flame Retardants of 23-24 April 2007 (IVM, VU-University, Amsterdam, The Netherlands).

## Introduction

Hexabromocyclododecane (HBCD) is applied as an additive brominated flame retardant (BFR) used in polystyrene. In 2001, the world production totaled 16,700 ton (1). HBCD predominantly consists of 3 diastereomers ( $\alpha$ -,  $\beta$ - and  $\gamma$ -HBCD), of which  $\gamma$ -HBCD predominates in the technical product (1). Apart from these three, two (very) minor other diastereomers may also be present in the technical product ( $\delta$ - and  $\epsilon$ -HBCD) (1,2). In addition, the diastereomers consist of enantiomeric pairs (3).

HBCD can leach to the environment during production, application in products, in-service life (use of the product) and after disposal. HBCD is omnipresent in the environment and it was found in sediments, biota and humans world-wide (4). Initially, HBCD was analysed by gas chromatography, combined with mass spectrometric detection (GC-MS) (4). Later, the analysis by liquid chromatography (LC) combined with MS was introduced (LC-MS) (5,6).

In GC-MS, electron chemical negative ionization (ECNI) is generally used as ionization method for BFRs. This ionization method provides significantly better sensitivity compared with electron impact (EI) ionization, although at the cost of less selectivity (4,7). With ECNI-MS, the  $(\text{Br})^-$  isotopes are generally monitored ( $m/z$  79 and 81). With EI  $(\text{M-Br})^-$  can be monitored resulting in higher selectivity (8). The latter provides the possibility to use mass labeled standards, but as the sensitivity is too low for many environmental samples, this ionization technique is not often used.

LC-MS detection is mostly performed on triple-quad instruments (MS/MS) using the electrospray (ESI) source. ESI-ion trap MS instruments (ITMS) have been used as well as atmospheric pressure chemical ionisation (APCI) (please refer to Covaci et al. (7) for an extensive overview of methods used). ESI is preferred over APCI by Budakowski and Tomy (6), but Suzuki and Hasegawa preferred APCI because of better S/N ratios in leachate samples (9). In the ESI source, the formation of  $(\text{M-H})^-$  takes place. The MS spectrum results in bromine clusters because of the two bromine isotopes  $m/z$  79 and  $m/z$  81 present. The most intense peak in the cluster is  $m/z$  640.7 ( $^{12}\text{C}_{12}^{1}\text{H}_{17}^{79}\text{Br}_3^{81}\text{Br}_3$ ). Triple quadrupole MS/MS instruments allow for selective detection by isolation of the mother ion  $(\text{M-H})^-$  ( $m/z$  640.6) in the first quadrupole, followed by detection of the bromine isotope  $(\text{Br})^-$  ( $m/z$  79 and/or 81) in the 3<sup>rd</sup> quadrupole. Because of the low-mass cut-off of ITMS instruments, detection of the bromine isotope is not feasible. Adding ammoniumchlorine to the mobile phase can solve this selectivity drawback. This promotes (and stabilises) the chlorine adduct formation ( $(\text{M+Cl})^-$  ion of  $m/z$  676.6), which allowed for MS/MS analyses by monitoring the  $676.6 \rightarrow 640.7$  transition ( $(\text{M+Cl})^- \rightarrow (\text{M-H})^-$ ), in that way creating a selective method.

A major advantage of LC-ESI-MS(/MS) over GC-ECNI-MS is the option of using  $^{13}\text{C}$  labelled internal standards. These standards allow correction for losses during extraction and clean up. Furthermore, several studies showed that

these labelled standards effectively correct for matrix suppression or enhancement occurring in the ESI source (10-12).

The results obtained by both techniques for biota samples showed large discrepancies. GC results were on average 4.4-fold higher than LC results (13). To explore the causes of these differences, the following experiments were conducted: (i) determination of GC-MS response factors of individual diastereomers; (ii) determination of degradation in the GC-MS. In addition, a short LC column was tested for improving speed and sensitivity of the LC-MS system. The results were discussed at the QUASIMEME workshop on the analysis of BFRs (23-24 April 2007, VU University, IVM, Amsterdam, The Netherlands).

Table 3.12 Advantages and disadvantages of HBCD analysis by LC-MS and GC-ECNI-MS (6,7,12,14,15).

	Advantage	Drawback
LC-ESI-MS(/MS)	<ul style="list-style-type: none"> <li>- Determination of individual diastereomers</li> <li>- Determination of enantiomers</li> <li>- Use of <math>^{13}\text{C}_{12}</math> and/or <math>^2\text{H}_{18}</math> mass labeled internal standards</li> <li>- Simultaneous analysis with TBBP-A</li> <li>- Selective detection (MS/MS)</li> </ul>	<ul style="list-style-type: none"> <li>- Matrix effects may occur in the ESI source, leading to erroneous results<sup>1</sup></li> <li>- Ion suppression reduces sensitivity</li> <li>- Lower sensitivity compared to GC</li> </ul>
GC-ECNI-MS	<ul style="list-style-type: none"> <li>- Simplicity (single value)</li> <li>- Sensitivity</li> <li>- Simultaneous analysis of other BFRs (e.g. PBDEs)</li> </ul>	<ul style="list-style-type: none"> <li>- Semi-selective detection ((Br)<sup>-</sup>, m/z 79 and 81)</li> <li>- No separation of diastereomers</li> <li>- Interconversion of diastereomers and degradation in injector and oven &gt;160°C</li> </ul>

<sup>1</sup> Matrix effects can be controlled by using mass labeled internal standards

### Degradation of HBCD in the GC-MS

The GC analysis of HBCD, the HBCD degradation products and the polybrominated diphenylethers (PBDEs) were performed on a GC-ECNI-MS (Agilent 6890, Wilmington, USA) (Instrument 1, Table 3.13). The following samples were run: (A) standard solution of a technical HBCD mixture (500 ng/mL); (B) a BDE standard solution containing BDE 47, 49, 58 (internal standard), 66, 71, 75, 77, 85, 99, 100, 119, 138, 153, 154, 183 and the methyl derivative of TBBP-A (500 ng/mL) and (C) a Western Scheldt sediment extract. Extraction and clean-up was performed according to (16).

### Different GC-ECNI-MS response factors for each HBCD diastereomer

This experiment was also performed on the aforementioned GC-ECNI-MS system. The HBCD diastereomer response factors were determined by injection of 0.53 ng ( $\alpha$ -HBCD), 0.54 ng ( $\beta$ -HBCD) and 0.55 ng ( $\gamma$ -HBCD). The responses were corrected for the slight differences in injected amounts.

Table 3.13 Instruments used.

	Instrument 1	Instrument 2
Method	GC-ECNI-MS	LC-ESI-MS/MS
Instrument	Agilent 6890 GC Agilent 5973 MSD	Agilent 1200 HPLC pump Agilent 6410 QQQMS
Column	CP-Sil-8CB (50 m x 0.25 mm id x 0.25 $\mu$ m film)	Zorbax Eclipse (150 mm*2.1 mm ID, 3.5 $\mu$ m)
Ionisation	ECNI	ESI-
Ions	(Br) <sup>-</sup> m/z 79/81	(M-H) <sup>-</sup> >(Br) <sup>-</sup> m/z 640.7>79
	Instrument 3	Instrument 4
Method	LC-ESI-MS/MS	LC-ESI-ion trapMS/MS
Instrument	Shimadzu LC-10 AD Micromass Quattro 2000 QQQMS	ThermoFinnigan LCQ Advantage
Column	Zorbax Eclipse (150 mm*2.1 mm ID, 3.5 $\mu$ m)	Zorbax XDB-C18 (150 mm x 2.1 mm ID, 3.5 $\mu$ m)
Ionisation	ESI-	ESI-
Ions	(M+Cl) <sup>-</sup> >(M-H) <sup>-</sup> m/z 676.7>640.7	(M+Cl) <sup>-</sup> >(M-H) <sup>-</sup> m/z 676.7>640.7

### Evaluating LC separation on a rapid resolution column and sensitivity of different instruments

The sensitivity of different instruments (Table 3.13) was evaluated by injection of  $\alpha$ -HBCD standard solutions. The detection limit was set at a signal-to-noise ratio of 3:1. In addition to the column mentioned in Table 3.13 (instrument 2), the separation and sensitivity of a rapid resolution column (Zorbax SB-C18 column (Agilent, Wilmington, USA, 2.1 mm x 30 mm x 3.5  $\mu$ m particles)) was evaluated. In this case, a mixture of 3 diastereomers was injected.

### Comparison of GC versus LC results

The comparison of GC and LC was carried out during a survey on BFRs in Dutch fish samples (chapter 4.2). Briefly, samples were extracted and cleaned-up by gel permeation chromatography. After additional clean up over a silica column, the extracts were concentrated and analysed by GC-ECNI-MS monitoring (Br)<sup>-</sup> m/z 79 and 81 (Table 3.13, instrument 1). To enable LC analysis, the GC extract was evaporated to dryness, followed by a solvent change to methanol. At that stage <sup>13</sup>C-HBCD internal standards (for all 3 diastereomers) were added and the extract was subsequently analysed by LC-ion trap MS/MS (Table 3.13, instrument 4) monitoring m/z 676.7 (M+Cl)<sup>-</sup> (chlorine adduct)  $\rightarrow$  m/z 640.7 (M-H)<sup>-</sup>. More details on the analytical approach can be found in chapter 4.2.

## Results and discussion

### Degradation of HBCD in the GC-MS

Although there are some benefits of the GC analysis (see Table 3.12), several serious disadvantages are connected to GC-MS. An important issue is the instability of HBCD at elevated temperatures applied in the GC injector and oven. At these temperatures (>160°C), the thermally labile HBCD diastereomers can rearrange and this may considerably affect the results (4). Even more important, at elevated injector and oven temperatures HBCD may degrade to pentabromocyclododecene (PBCDe) and tetrabromocyclododecadiene (TBCDe). This is demonstrated for a standard solution of  $\alpha$ -,  $\beta$ - and  $\gamma$ -HBCD diastereomers (Figure 3.10, top) and was also recently reported by Abdallah et al. (15). Figure 3.10 shows an example of the degradation of HBCD on a CP-Sil-8 column (50 m x 0.25 mm x 0.25  $\mu$ m) that was extensively used (hundreds of environmental samples had been analysed before on this column). The degradation is most likely caused by the active sites in the column. Furthermore, active sites in a dirty GC liner may cause degradation as well (17). The HBCD degradation results in serious errors in the HBCD determination, because of the reduced peak area of HBCD in the standard solution. It is not known if HBCD degrades to the same extent in sample extracts where other co-extracted interferences are present. Poole recently reviewed 'matrix induced signal enhancement' in GC analysis (18). He showed that thermolabile compounds could be degraded in hot vaporizing injectors. However, in a sample extract, matrix components can also 'shield' the compound, minimizing degradation. Possibly, this plays a role in the HBCD analysis as well, which requires further investigations. Obviously, degradation on the GC column should be avoided, and liners should be replaced regularly.

The degradation products (PCBDe and TBCDe) can disturb the determination of BDEs when using ECNI-MS for detection (monitoring the  $m/z$  79 and 81 ions). On a CP-Sil 8 column, TBCDe interferes with BDEs 49, 71 and 75 and PCBDe interferes with the important congener BDE 99 (Figure 3.10, bottom). This hinders an accurate determination of these BDEs. Obviously, the co-elutions may be omitted when selecting a GC column with different polarity. However, given the broad PCBDe and TBCDe peaks, it may be very difficult to reduce their influence. In addition, more polar columns will lead to an increase of HBCD degradation. Abdallah et al. distinguished four TBCD isomers<sup>6</sup> in dust samples (by LC-MS) (15). This may explain the broad peak, which is observed in the GC chromatogram in Figure 3.10. In addition, they found two PCBDe isomers. Possibly, the actual number of isomers is even higher, than the six they determined.

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<sup>6</sup> Although the definition of "isomers" was used to differentiate between the structures, it may be more correct (just as with the mother compound) to use the definition of 'diastereomer'.

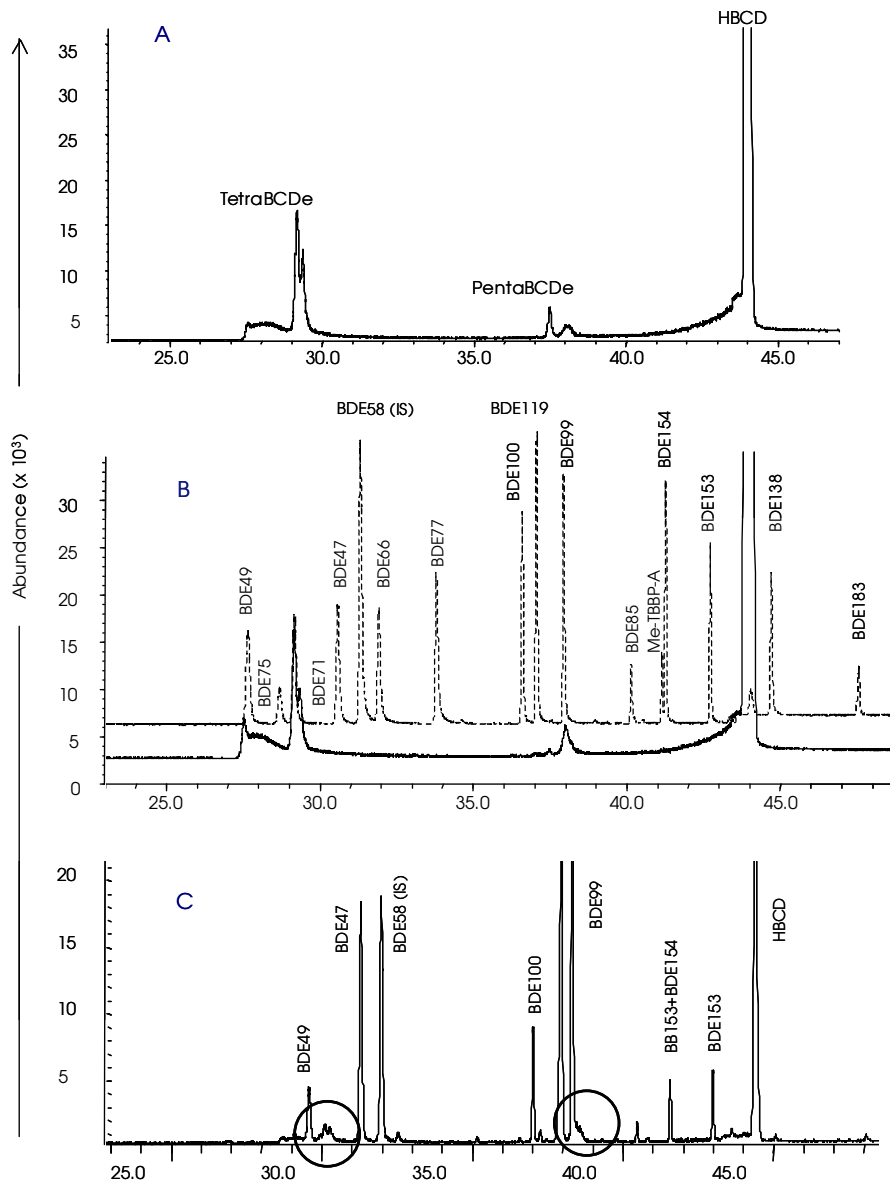


Figure 3.10 Degradation of HBCD to pentabromocyclododecene (PBCDe) and tetrabromocyclododecadiene (TBCDe) on a GC- column (CP-Sil-8CB, 50 m x 0.25 mm x 0.25  $\mu$ m). A: standard solution of HBCD with TBCDe and PBCDe degradation products. B: TBCDe and PBCDe degradation products superimposed on a PBDE standard solution (dotted line), showing the co-elutions. C: co-elutions with BDE 49 and 99 in a Western Scheldt sediment extract. See text for instrumental conditions.

Apart from degradation in the GC, PBCDe and TBCDe may also be present as impurities in technical mixtures (19). Furthermore, PBCDe was reported as a metabolite/degradation product by Hiebl and Vetter (20). This shows that PBCDe and TBCDe may be target compounds themselves, which is an additional reason to avoid degradation of HBCD.

#### Different GC-ECNI-MS response factors for each HBCD diastereomer

GC-ECNI-MS response factors are different for the different diastereomers. After injection of equal amounts of the individual diastereomers on the GC-ECNI-MS column, the responses for  $\alpha$ -,  $\beta$ - and  $\gamma$ -HBCD were 100, 71 and 73%, respectively (see Figure 3.11). When determining total HBCD in biota (mainly  $\alpha$ -HBCD) while using a standard consisting of equal concentrations of  $\alpha$ -,  $\beta$ - and  $\gamma$ -HBCD, errors of 10-20% in the final result can be made. Errors become even more pronounced (up to 40%) when using a technical HBCD mixture (containing mainly  $\gamma$ -HBCD) as a standard. It is therefore recommended, when doing GC analysis, to match the diastereomer profile in the sample with the profile in the standard.

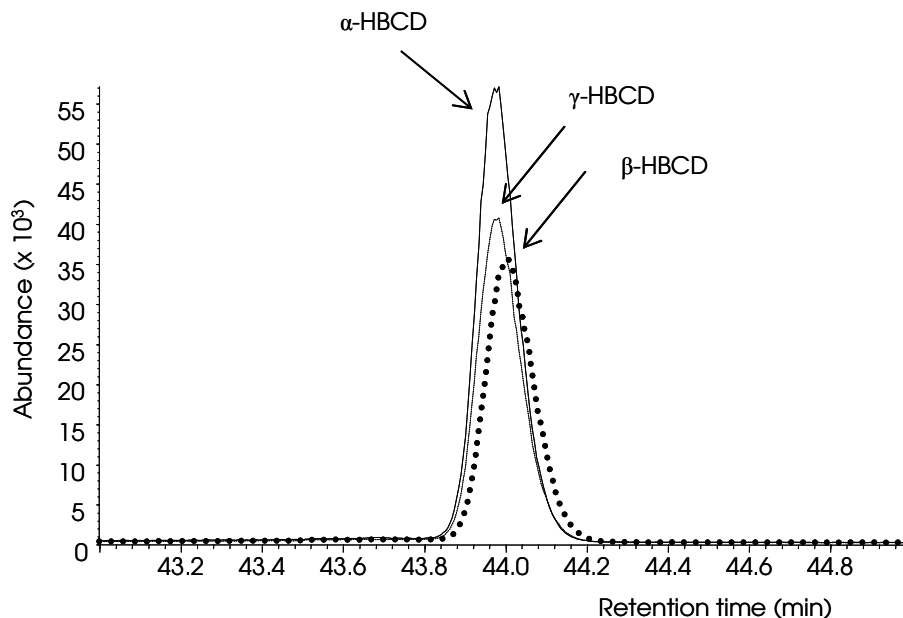


Figure 3.11 GC-ECNI-MS response of  $\alpha$ -,  $\beta$ -,  $\gamma$ -HBCD diastereomers. Injected amounts were 0.53 ng ( $\alpha$ -HBCD), 0.54 ng ( $\beta$ -HBCD) and 0.55 ng ( $\gamma$ -HBCD). Relative responses are 100, 71 and 73% for  $\alpha$ -,  $\beta$ -,  $\gamma$ -HBCD.



### Evaluation of a rapid resolution column

The separation of HBCD diastereomers is typically achieved by a gradient of water and a modifier, typically methanol or acetonitrile (or a combination of both), using a reversed phase column (C18) (21). Some authors have used ammonium acetate or chloride in the water phase to aid the ionization (5,12,22,23). A typical run takes 20 to 30 minutes in order to complete the gradient elution and to return to the initial conditions. Leonards (24) explored the possibilities for improving sensitivity by focusing the peaks on a short column. He successfully separated the diastereomers on a 30 mm column (Zorbax SB-C18, 2.1 x 30 mm x 3.5  $\mu\text{m}$ ) using isocratic elution. The optimal solvent composition was found to be methanol-acetonitrile-ammonium acetate (0.01 mM) mixture (38:38:24%). This resulted in a reduced analytical runtime, from 11 to 6.5 minutes for elution of the three diastereomers (Figure 3.12).

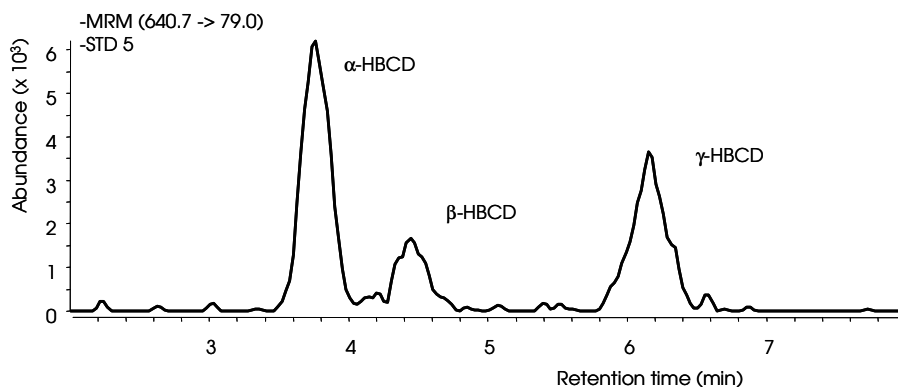


Figure 3.12 Analysis of HBCD diastereomers using LC-ESI-MS/MS (Agilent 6410) by isocratic elution on a rapid resolution column (Zorbax SB-C18, 2.1 x 30 mm x 3.5  $\mu\text{m}$ , 100 pg of each diastereomer injected). Experimental details mentioned in the text.

Because no conditioning of the column is required, the next sample can be injected directly after elution of the last diastereomer, which results in an additional gain in time. It should be noted that with real samples, slowly eluting matrix components could accumulate on the column, leading to a decreased chromatographic performance. However, these matrix components can be removed by running a gradient to e.g. 100% acetonitrile after multiple sample injections.

In addition to the reduced runtime, the limit of detection (LOD) improved 5-fold compared to that of the 150 mm column. This resulted in a LOD of 20 pg absolute (Table 3.14), which is one order of magnitude less sensitive than GC-ECNI-MS (Table 3.13, instrument 1). The sensitivity of other instruments (Table 3.13, instrument 2-4) was evaluated by analysis of HBCD standard solutions.

The LODs were based on a minimum signal-to-noise ratio of 3. The LOD of the Agilent 6410 instrument (instrument 2) is only slightly better (using a 150 mm column) compared to the other LC-MS instruments (instrument 3 and 4). The main reason is that  $m/z$  640.7>79 (instrument 2) is a low yield transition, whereas the yield of the 676.7>640.7 transition (instrument 3 and 4) is higher, resulting in a higher sensitivity. The most efficient transition is instrument dependent. Although small amounts of the chlorine adduct could be detected on the Agilent 6410 instrument (instrument 1), it was too low for producing a sensitive 676.7>640.7 transition. Therefore, the  $m/z$  640.7>79 was used for detection and confirmation on this instrument.

Table 3.14 Sensitivity differences for the  $\alpha$ -HBCD diastereomer (pg absolute)

Method	Agilent GC-ECNI-MS (Br) <sup>-</sup> m/z 79/81	LCQ Advantage LC-ITMS/MS (M+Cl) <sup>-</sup> > (M-H) <sup>-</sup> m/z 676.7>640.7	Quattro 2000 LC- QQQ (M+Cl) <sup>-</sup> > (M-H) <sup>-</sup> m/z 676.7>640.7	Agilent 6410 LC-QQQ (M-H) <sup>-</sup> > (Br) <sup>-</sup> m/z 640.7>79
HBCD per isomer	2 <sup>1</sup>	100	100	75/20 <sup>2</sup>
TBBP-A	1	100	200	n.a.

<sup>1</sup> Sum of 3 diastereomers

<sup>2</sup> LOD 75 pg for the 150 mm column and 20 pg for the rapid resolution (30 mm) column

### Evaluation of HBCD concentrations measured by GC and LC

A small number of studies have compared results obtained by GC and LC based methods. In chapter 4.2,  $\alpha$ -,  $\beta$ - and  $\gamma$ -HBCD were determined by LC-ESI-MS/MS, whereas total-HBCD was measured by GC-ECNI-MS. The LC results show that  $\alpha$ -HBCD is the predominant isomer in the samples analysed, followed by  $\gamma$ - and  $\beta$ -HBCD (Figure 3.13). The samples plotted in this figure only include those for which the LC-ESI-MS/MS result was above the limit of quantification.

Figure 3.13 shows concentrations of total-HBCD determined by GC-ECNI-MS (printed on top of the bars) and the sum of the three diastereomers determined by LC-ESI-MS/MS (indicated by the bars). In most fish samples, the GC results were higher than the LC results. The correlation line of the results of both methods shows a slope of 0.23 meaning that the LC-result = 0.23 x GC-result. Or, the GC-based results are 4.4-fold higher compared to the LC based results. There can be several causes for this phenomenon. On the GC-side, the thermally labile HBCD diastereomers can rearrange above oven temperatures of 160°C (22), resulting in different response. Furthermore, HBCD can degrade (Figure 3.10). It's important to note that the magnitude of these phenomena may be different in a real sample as compared to a standard solution. On the LC-side, the issue of different response factors is not relevant because the diastereomers are separated chromatographically.

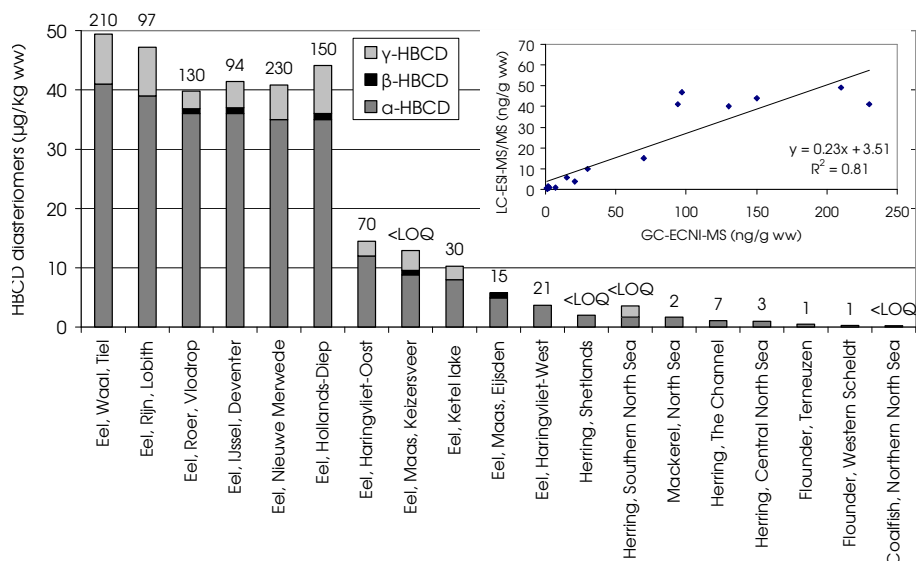


Figure 3.13 HBCD diastereomers ( $\alpha$ ,  $\beta$ , and  $\gamma$ ) profile as determined by LC-ESI-MS/MS. For comparison, the value determined by GC-ECNI-MS is plotted at the top of each bar. Only the fish samples with at least 1 diastereomer concentration above LOQ are plotted. The regression plot (inlay) shows the correlation between the GC and LC data.

However, signal suppression may occur in the electrospray ionisation due to co-eluting matrix constituents. These effects were found to be insignificant by Dodder *et al.* (21) although Tomy *et al.* did report matrix effects occurring in the LC-ESI-MS/MS (which were overcome by using  $^{13}\text{C}$ -labeled internal standards) (12). In the present study compensation for matrix effects was achieved by using a  $^{13}\text{C}$ -labeled HBCD internal standard for each diastereomer. Also, the samples have been submitted to a very thorough clean up, which strongly reduces the chance of ion suppression in the MS. For these reasons, the LC results are regarded the most accurate ones. Obviously, the additional diastereomer profile information is beneficial and crucial when assessing the fate and behaviour of HBCD. Roosens *et al.* recently presented a comparison of five different methods (LC and GC based) for analyzing HBCD in eel from the river Scheldt basin (8). The concentrations in those samples were high (400–1400 ng/g wet weight). The high concentrations enabled the detection of a more specific ion (i.e. (M-Br) $^-$ ) for GC-MS quantification, and this enabled the use of  $^{13}\text{C}$ -labeled  $\alpha$ -HBCD for correcting the GC results. They concluded that the results obtained were very well comparable between methods. In fact, their study shows that these methods were capable of producing data that lie within a 2-fold difference from each other, and this is better than the results from our study (Figure 3.13). However, a few remarks should be made here:

- Their methods 3 (GC-EI-MS), 4 (GC-ECNI-MS), 5 (LC-ITMS) and 6 (LC-ESI-MS/MS) all used  $^{13}\text{C}$ -HBCD as internal standard. Nevertheless, differences between these methods were up to 53% (minimum vs. maximum) for the six eel samples analysed. It is therefore surprising that the results were not closer under these conditions.
- The differences between methods were not consistent. For example, compared to the other methods, LC-ESI-MS/MS (method 6) produced the lowest HBCD concentration in eel sample L5p1, whereas it produced the nearly highest result in eel sample L5.
- Because the levels were high in these eel samples, they were able to report data within a 2-fold difference. However, in the majority of the biota samples, concentrations are 1-3 orders of magnitude lower. In those cases, mass labelled standards can no longer be used for GC-MS quantification and most likely the same level of agreement between the GC and LC methods can not be maintained.

Haug et al. (25) recently reported on a comparison of GC-MS and LC-MS results from two interlaboratory studies (2005 and 2007). The test materials provided to the laboratories were (in the order of decreasing concentrations) a cod liver oil, Baltic Sea herring fillet, salmon fillet, butter and chicken meat. They observed that in the cod liver oil and herring fillet the mean and median GC-MS results were 10-40% higher as compared to LC-MS results. Although the differences were statistically insignificant, this is also an indication that GC based results (in fish) can be higher compared to LC based results. In salmon, this difference was less pronounced. Judging from the higher GC values, the authors concluded that thermal degradation in the GC did not influence the results. However, this statement is debatable as a multitude of experimental and instrumental factors can have influenced the final result, both in GC and LC (as discussed above). Unfortunately, the LC datasets and GC datasets originated from different laboratories, and none of the labs used both techniques, which hampered an in-depth analysis of the different techniques. For the butter and chicken sample insufficient LC results were obtained to allow a comparison with GC. The method sensitivities of most LC laboratories were insufficient for the low HBCD levels in the butter and chicken sample. These studies show that in fish samples (mostly) higher signals are observed with GC-MS as compared to LC-MS. Possibly, on the GC side, a matrix effect occurs leading to elevated results. Poole recently reviewed 'matrix induced signal enhancement' in GC analysis (18). He showed that thermolabile compounds can be degraded in hot vaporizing injectors. However, in a sample extract, matrix components can 'shield' the compound, minimizing degradation.

In a study on house-dust, Abdallah et al. (15) compared LC-ESI-MS/MS of diastereomers with GC-ECNI-MS. He evaluated the use of different internal standards and daughter ions by the following methods:

1. Quantifying on the m/z 561 (daughter) and using  $^{13}\text{C}_{12}$ -HBCD diastereomers as internal standards (m/z 573)
2. Quantifying on the m/z 561 (daughter) and using BDE 128 as internal standard
3. Quantifying on m/z 79 (daughter) and using BDE 128 as the internal standard.

In house dust, both  $\alpha$ - and  $\gamma$ -HBCD may be present in substantial amounts. They detected 14-67%  $\alpha$ -HBCD of total-HBCD in dust samples (26). Therefore, they also explored the above-mentioned three methods in combination with quantification using  $\alpha$ -HBCD or  $\gamma$ -HBCD response factors. The closest match between GC-ECNI-MS and LC-ESI-MS/MS was obtained with method 1 using the  $\alpha$ -HBCD response factor and method 3 using the  $\gamma$ -HBCD response factor. The GC vs. LC slope values were 1.05 and 0.97, respectively. This shows that the match between GC-MS and LC-MS may depend on analytical factors on the GC side (i.e. choice of native standard, choice of internal standard) and may also be dependent of the composition of individual samples. The authors recommended LC-ESI-MS/MS as the method of choice and proposed the use of GC-ECNI-MS for screening of samples with very low HBCD concentrations (e.g. human samples). This avoids extensive GC method optimisations and comparison studies.

### Issues for further research

Recently, new GC stationary phases were introduced based on ionic liquids with completely different retention characteristics (27). Possibly, these phases enable separation of the HBCD diastereomers allowing further study on diastereomer quantification by GC-ECNI-MS. Nevertheless, GC should be used with caution due to possible degradation and because no mass labeled internal standards can be used.

The LC-ESI-MS methods would benefit from further lowering of LODs. However, the m/z 640.7>79 transition has low yields, resulting in reduced sensitivity as compared to the chlorine adduct transition (676.6>640.7). More research is needed to find out if the yield of m/z 640.7>79 can be improved. Alternatively, the chlorine adduct formation may be used. However, as the formation varies from instrument to instrument, more efforts are needed to find out what instrument (and eluent) conditions determine the adduct formation and how they could be influenced for improving instrument sensitivity. The study by Roosens et al. (8) showed that even when using  $^{13}\text{C}$ -labeled internal standards for the analysis of eel samples, considerable different results were obtained between different LC-ESI-MS instruments. For a full acceptance of LC-ESI-MS, more efforts are needed to explain the experimental differences between the LC- and GC-MS. A further acceptance of LC-ESI-MS is supported by future method comparison (interlaboratory) studies that unambiguously

show that different methods and LC-MS detection techniques provide comparable answers.

## Conclusions

This study showed that different diastereomer response factors and degradation of HBCD to PBCDe and TBCDe can significantly reduce the accuracy of the GC based results for HBCD. In addition, the degradation products can also add to the  $m/z$  79 signals of major BDE congeners like 49 and 99. Because no mass labelled standards can be used, especially in low contaminated samples, corrections for inaccuracies are difficult. An LC-MS method overcomes these problems as mass labelled internal standards can be used that allow accurate quantification. Furthermore, the use of a short LC column provides additional sensitivity and speed. This results in a LOD of 20 pg for our LC-MS method which is only 10-fold higher than for GC-ECNI-MS. Therefore, LC-MS is currently the preferred method for determination of HBCD.

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