Introduction
Brominated Flame Retardants (BFRs) have been widely used in various commercial products to prevent and reduce the extent of fires. 1,2,5,6,9,10-hexabromocyclododecane (HBCD) is the third most produced BFR worldwide (16700 tons in 2001) and the second most used BFR in Europe. Technical grade HBCD mixtures are obtained via bromination of cyclododeca-1,5,9-triene and consist mainly of three diastereomeric pairs of enantiomers called α, β and γ-HBCD, with γ-HBCD being the major constituent (more than 70 %). The different diastereomers have somewhat different properties. For example, the water solubilities of α, β and γ-HBCD are 48.8, 14.7 and 2.1 µg/L respectively.

Like other additive BFRs, HBCD may be released into the environment through emissions during production, by leakage from consumer products during use or following disposal. Due to its low degradability and high octanol-water partition coefficient (Kow), this substance has a relatively high bioaccumulation potential in the adipose tissue of living organisms.

HBCD long range transportability has been testified in many studies, thus environmental pollution caused by the production and usage of HBCD could possibly be spread over great distances. As substantial and irreversible harm induced by HBCD may occur due to its massive use, environmental persistence and biological toxicity, HBCD has been testified as potential contamination.

Most of the environmental data published so far have been obtained by gas chromatography (GC). The HBCD diastereomers do not separate on the commonly used GC columns, thus, the result obtained is the sum concentration of all HBCD isomers. A new method dedicated to separate level measurement of α, β and γ-HBCD diastereomers using reverse-phase liquid chromatography (LC) has been developed in this study.

Experimental Conditions
Three different columns were investigated for the chromatographic separation and the Thermo Scientific Hypersil GOLD 1.9 µm, 100 mm × 2.1 mm column was selected for giving the best separation of α, β and γ-HBCD isomers and the best sensitivity.

Elution solvents were Acetonitrile and Ammonium Acetate 20 mM. Several mobile phase programs and flow rates from 0.3 mL/min to 0.5 mL/min were tested. The conditions considered suitable as regards the results were:

<table>
<thead>
<tr>
<th>Column</th>
<th>Part Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypersil™ GOLD 1.9 µm, 100 mm × 2.1 mm</td>
<td>25002-102130</td>
</tr>
</tbody>
</table>

LC Conditions
Mobile Phase: A: Ammonium acetate 20 mM
B: Acetonitrile
Gradient:

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>%B</th>
</tr>
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<tbody>
<tr>
<td>0</td>
<td>20</td>
</tr>
<tr>
<td>2</td>
<td>20</td>
</tr>
<tr>
<td>3.5</td>
<td>100</td>
</tr>
<tr>
<td>6.5</td>
<td>100</td>
</tr>
<tr>
<td>7.5</td>
<td>20</td>
</tr>
<tr>
<td>10</td>
<td>20</td>
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</tbody>
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Flow Rate: 0.4 mL/min
Injection Volume: 10 µL

MS Conditions
Negative electrospray ionization (ESI-).
Target compounds were determined by multiple reactions monitoring (MRM). The following diagnostic signals were monitored for α, β and γ-HBCD:

* m/z 640.7 → m/z 78.9 for C₁₂-HBCD isomers
* m/z 652.7 → m/z 78.9 for C₁₂-labelled HBCD isomers
* m/z 435.3 → m/z 255.2 for fluorometholone (external standard)
* m/z 640.7 → m/z 80.9 for C₁₂-HBCD isomers as qualifier ion signals to confirm the presence of α, β or γ-HBCD
**Results**

The retention times of the three chromatographic peaks in extract chromatograms for samples (Figure 1b) were consistent with those of α, β and γ-HBCD in standard solutions (Figure 1a). α-HBCD is eluted before β and γ-HBCD, and all isomers come out within three minutes.

**Conclusions**

The analysis of HBCD level in environment has been a new hot research topic. GC has shown limitations in the determination of α, β and γ-HBCD, as these diastereomers are not separated and have to be quantified as the total amount. The LC/MS method described here can chromatographically resolve the three diastereomers and therefore quantify them individually. Optimization of the chromatographic conditions provided a sensitive method with baseline resolution of the three diastereomers in 3 minutes run time.

Figure 1: Chromatogram for HBCD standard solution (a) and for a liver sample (b)