# COMPARING GC-MS AND LC-MS FOR THE DETERMINATION OF HBCD IN FOOD – RESULTS FROM AN INTERLABORATORY COMPARISON STUDY

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**Introduction**

1,2,5,6,9,10-Hexabromocyclododecane (HBCD) is the third most widely used brominated flame retardant in the world. HBCD has gained attention in the field of environmental surveillance[1] and has been shown to bioaccumulate in food chains[2-3]. Toxicological studies suggest that HBCD can disrupt the thyroid function and may have developmental neurotoxic effects[4-5]. Nevertheless, the technical use of HBCD might increase as a replacement for the Penta- and Octa-PBDE flame retardant formulations that have been banned in Europe and are being phased out in North America.

The term HBCD refers to the commercial product consisting of a mixture of mainly three diastereomers (α-, β- and γ-HBCD)[6]. While technical mixtures contain 70-90% of γ-HBCD, α-HBCD is often the dominating isomer in wildlife samples, especially at high trophic levels[3,7-8].

GC-MS or LC-MS are commonly used for the quantitative determination of HBCD. However, both of these well-established techniques have limitations. GC-MS operated in the electron-capture negative-ion mode is a very sensitive technique. However, HBCD stereoisomers undergo thermal rearrangement and decomposition at elevated temperatures used for GC separation and the isomers can not be chromatographically resolved. In contrast, reversed-phase chromatography easily separates the HBCD-stereoisomers and HPLC coupled to electrospray ionisation MS is a versatile tool for the isomer-specific determination of HBCD. However, the sensitivity of LC-MS is lower than that of GC-MS, and HBCD can only be detected in samples with relatively high contamination levels.

In order to assess the quality of the determination of HBCD, the Department of Analytical Chemistry at the Norwegian Institute of Public Health, Oslo, Norway organised an interlaboratory comparison study, which took place from fall 2005 to spring 2006.

The purposes of this study were to a) assess the comparability of results from the different analytical techniques, b) to provide a quality assurance instrument for the participating laboratories, and c) to assess the readiness of expert laboratories to determine HBCD in biological environmental samples.

**Materials and Methods**

The chosen test materials were Herring from the Baltic Sea (15g), Cod liver oil (~5g) and of a standard solution of 0.500 ng/µl of α-HBCD in toluene.

The laboratories were requested to determine either the total HBCD concentration (GC-MS), or the concentrations of α-HBCD, β-HBCD and γ-HBCD (LC-MS or LC-MS/MS), or both.

Laboratories should perform the determinations using their own methods for sample preparation and instrumental analysis, their own standards and quantification procedures, and their own method for lipid determination.

**Results and Discussion**

*Methods*

Of the 19 laboratories invited world-wide, 12 laboratories registered and 10 reported results. Three laboratories reported results determined by both GC-MS and LC-MS. Four laboratories made their determinations only by LC-MS and three only by GC-MS.

A wide variety of extraction and cleanup procedures were used among the laboratories. The lipid extraction procedures used are well known methods like ASE, Soxhlet extraction, liquid-liquid extraction and cold-column extraction. Lipid removal was performed using partitioning with sulphuric acid, column extraction on silica based columns and/or Florisil columns or by gel permeation chromatography.

*Reported results*

In the tables below the results for detected isomers or total HBCD are presented for each laboratory. In the statistical calculations the non-detects as well as outliers according to Dixons Q-test have been excluded.

Table 1. Individual results for α-HBCD in standard solution in pg/µl.

| | | GC, pg/µl | LC, pg/µl | both methods, pg/µl |
|---|---|---|---|---|
| Lab code | 2 | 502 | | 502 |
| | 3 | | 482 | 482 |
| | 4 | 553 | | 553 |
| | 5 | | 463 | 463 |
| | 7 | 490 | | 490 |
| | 7 | | 510 | 510 |
| | 8 | 482 | | 482 |
| | 9 | 506 | | 506 |
| | 11 | | 521 | 521 |
| | 12 | 599 | | 599 |
| | target value | 500 | 500 | 500 |
| | min | 482 | 463 | 463 |
| | max | 599 | 521 | 599 |
| | median | 504 | 496 | 504 |
| | mean | 522 | 494 | 511 |
| | std dev | 45 | 26 | 40 |
| | RSD % | 9 | 5 | 8 |

*Evaluation of total HBCD*

The reported results for GC, LC and both GC/LC were found to be normally distributed. When comparing the results determined by GC-MS and LC-MS using a t-test (p=0.05), no statistically significant differences were found, although the results determined by GC-MS were slightly higher than the LC-MS results both for herring, cod liver oil and the standard solution. Thus, the possibility of degradation of HBCD during GC does not seem to influence the quantification. Due to the wide variety of extraction and cleanup methods used, the impact of the sample preparation on the results could not be evaluated.

The calculated mean of the total HBCD concentration on fresh weight basis including data from both methods, were 0.40 ng/g (range 0.20 ng/g - 0.64 ng/g) in herring and 7.0 ng/g (range 5.0 ng/g – 9.7 ng/g) in cod liver oil. The RSDs were 35% and 21% for herring and cod liver oil, respectively. For comparison, the RSD for the standard solution was 8%.

Table 2. Individual results for herring in pg/g fresh weight[a].

| | | total HBCD | | | α-HBCD | β-HBCD | γ-HBCD |
|---|---|---|---|---|---|---|---|
| | | GC, pg/g | LC, pg/g | both methods, pg/g | pg/g | pg/g | pg/g |
| Lab code | 2 | 391 | | 391 | | | |
| | 3 | | 299 | 299 | 299 | | |
| | 4 | 640 | | 640 | | | |
| | 4 | | 1430[b] | 1430[b] | 1280[b] | | 150 |
| | 5 | | 199 | 199 | 199 | | |
| | 7 | 370 | | 370 | | | |
| | 7 | | 400 | 400 | 350 | 20 | 30 |
| | 8 | 584 | | 584 | | | |
| | 9 | 472 | | 472 | 407[c] | 14[c] | 51[c] |
| | 11 | | 382 | 382 | 352 | | 30 |
| | 12 | 250 | | 250 | | | |
| | min | 250 | 199 | 199 | 199 | 14 | 30 |
| | max | 640 | 400 | 640 | 407 | 20 | 150 |
| | median | 432 | 341 | 387 | 350 | 17 | 40 |
| | mean | 451 | 320 | 399 | 321 | 17 | 65 |
| | std dev | 145 | 92 | 138 | 78 | 4 | 57 |
| | RSD % | 32 | 29 | 35 | 24 | 23 | 88 |

[a] Lab 6 did not detect any isomers above their detection limit and are left out from the table.
[b] Outlier, excluded according to Dioxins Q- test.
[c] The relative contribution of the isomers is determined by LC-MS, their amount is based on the total quantity determined by GC-MS.

Table 3. Individual results for cod liver oil in pg/g fresh weight[a].

| | | total HBCD | | | α-HBCD | β-HBCD | γ-HBCD |
|---|---|---|---|---|---|---|---|
| | | GC, pg/g | LC, pg/g | both methods, pg/g | pg/g | pg/g | pg/g |
| Lab code | 2 | 5865 | | 5865 | | | |
| | 3 | | 5910 | 5910 | 5910 | | |
| | 4 | 7095 | | 7095 | | | |
| | 4 | | 6400 | 6400 | 4700 | | 1700 |
| | 5 | | 4992 | 4992 | 4992 | | |
| | 7 | 8860 | | 8860 | | | |
| | 7 | | 8250 | 8250 | 7450 | 400 | 400 |
| | 8 | 7155 | | 7155 | | | |
| | 9 | 9697 | | 9697 | 8691[b] | 399[b] | 607[b] |
| | 11 | | 7710 | 7710 | 7060 | 332 | 315 |
| | 12 | 5500 | | 5500 | | | |
| | min | 5500 | 4992 | 4992 | 4700 | 332 | 315 |
| | max | 9697 | 8250 | 9697 | 8691 | 400 | 1700 |
| | median | 7125 | 6400 | 7095 | 6485 | 399 | 504 |
| | mean | 7362 | 6652 | 7039 | 6467 | 377 | 756 |
| | std dev | 1644 | 1327 | 1481 | 1541 | 39 | 642 |
| | RSD % | 22 | 20 | 21 | 24 | 10 | 85 |

[a] Lab 6 did not detect any isomers above their detection limit and are left out from the table.
[b] The relative contribution of the isomers is determined by LC-MS, their amount is based on the total quantity determined by GC-MS.

*Evaluation of the isomer specific determination*
The relative contribution in percent of α-, β- and γ-HBCD to the total HBCD was found to be 80:4:16 and 85:5:10 for herring and cod liver oil, respectively. As can be seen from tables 2 and 3, the variation of the α-HBCD and β-HBCD results is comparable to the variations for the total HBCD concentration. However, the RSDs for γ-HBCD in herring and cod liver oil are considerably higher. The reason for this is that one of the reported values is much higher than all the others, but due to the limited number of reported values for γ-HBCD this value was not identified as an outlier.

*Sensitivity*
All laboratories using GC-MS were able to detect HBCD in both samples. All, except one laboratory using LC-MS, detected α-HBCD, while just two and three of six laboratories were able to detect β-HBCD in herring and cod liver oil, respectively. Four of six laboratories detected γ-HBCD in both herring and cod liver oil. The reported detection limits for β- and γ-HBCD were 10 – 2800 pg/g for herring and 25-13300 pg/g for cod liver oil.

*Summary*
No statistically significant differences were found between results obtained by LC-MS and GC-MS for total HBCD. The quality of the determinations were good, with few detected outliers and RSDs in the same range as for tetra to hepta PBDEs obtained in another study on the same cod and herring sample (*Interlaboratory Comparison on Dioxins in Food 2005*)[9]. The present study has shown that laboratories are able to determine HBCD in biological environmental samples with satisfactory quality.

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