Analysis of HBCD and TBBPA by GC-MS versus LC-MS-MS – indications of systematic differences in obtained results

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Introduction.

Tetrabromobisphenol A (TBBPA) is the most used BFR worldwide (145,113 t yr^{-1}, 2003), while hexabromocyclododecane (HBCD) is the third most used BFR (21,951 t yr^{-1}, 2003) (BSEF 2006). Currently, the use of TBBPA and HBCD has not been regulated anywhere in the world though they are included as 'priority' compounds in monitoring and assessment programmes in several countries (BSEF 2006). Previous studies have shown that HBCD is present in a wide variety of environmental matrices e.g. sediment, fish, and marine mammals (Muir et al. 2006; Sellstrom et al. 1998; Verreault et al. 2005). So far the reportings of TBBPA in environmental biota samples are scarce (Law et al. 2006; Morris et al. 2004). The possible metabolite, dimethyl-TBBPA (Me-TBBPA), has only been subject to a limited number of studies resulting in detection of Me-TBBPA in both biotic and abiotic matrices (Sellstrom and Jansson 1995; Vorkamp et al. 2005; Watanabe et al. 1983).

Previously, the dominating analytical method for HBCD and TBBPA was GC-MS (Sellstrom and Jansson 1995). When analyzing TBBPA by GC-MS, derivatisation is needed in order to obtain adequate response and avoid peak tailing. In the last few years a shift towards LC-MS(-MS) analysis has taken place (Budakowski and Tomy 2003; Hayama et al. 2004). LC-MS allows separation of the HBCD-isomers and analysis of TBBPA without derivatisation. The aim of the current study was to combine the analysis of HBCD, TBBPA, and Me-TBBPA in biota samples. In the method development both GC-MS and LC-MS-MS was applied in order to find the optimal analytical solution. The complete method development is described in Frederiksen et al. (subm.). This abstract compares the two different analytical approaches, GC-MS and LC-MS-MS, and discuss the outcoming results.

Materials and Methods.

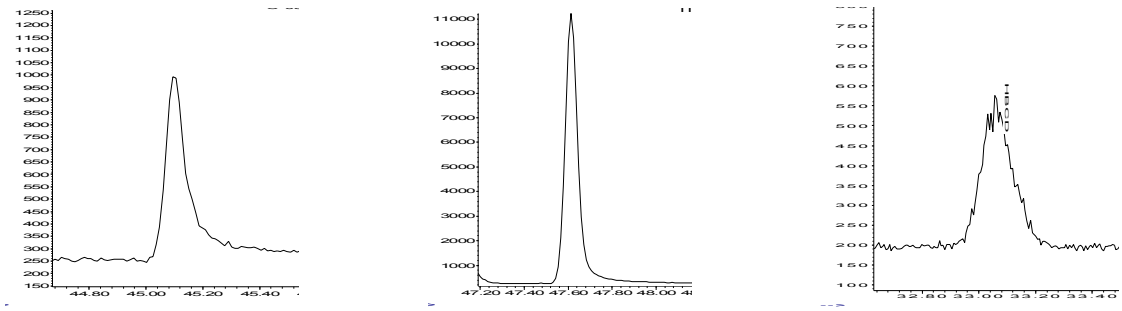
An entire analytical process including sample extraction, clean-up, and analysis was developed and a preliminary screening of Arctic biota has been described elsewhere (Frederiksen et al. subm.). Briefly, biota samples were Soxhlet extracted (hexane:acetone 3:1) and purified by gel permeation chromatography (GPC) on a 600x25 mm polystyrene/divinyl benzene column (ethyl acetate: cyclohexane 1:1), addition of sulphuric acid and adsorption chromatography on silica gel columns (isooctane +isooctan:diethyl ether 85:15). The analysis of HBCD, TBBPA, and Me-TBBPA was optimized on both analytical instruments, GC-MS and LC-MS-MS, with respect to sensitivity (ions, injection volume, temperature etc.) and separation of analytes. The limit of detection (LOD) of the instrument was determined as three times the signal-to-noise ratio (S/N-ratio). The reproducibility of the two analytical methods was tested by analysis of several replicates of the same sample.

Results and Discussion

The calibration curve of ΣHBCD on GC-MS was linear in the range from 1.3 ng/ml to 100 ng/ml. When analysing the HBCD-isomers by LC-MS-MS the calibration curves were observed to be non-

linear, this phenomenon is often observed with negative electrospray ionisation and is mainly to saturation of the signal at high concentrations. However, a good power regression could be obtained for each isomer in the range of 1 to 100 ng/ml.

Without derivatisation TBBPA resulted in a broad and tailing peak with a poor response in the GC-MS analysis. However, when TBBPA was derivatised with acetic acid anhydride (to form Ac-TBBPA) the response was increased by up to a factor of 10 (Fig. 1a and 1b). When analysing HBCD by GC-MS it is not possible to separate the three isomers which are present in a technical mixture. The response of HBCD is relatively low (Fig. 1c). In addition HBCD is thermally labile and thus degraded in the inlet and on the column of the GC-MS (Barontoni et al. 2001). The degradation that occurs in the inlet can be significantly reduced by lowering the temperature of the inlet e.g. to 220ºC, thus reducing the number and intensity of peaks from degradation products. Some degradation still occurs on the column, which results in a broad peak originating from the degradation in the inlet and not from the presence of three isomers. Good response and chromatography was observed for Me-TBBPA. The GC-MS optimization resulted in a temperature program for the complete separation of ΣHBCD, TBBPA, Me-TBBPA, and Ac-TBBPA (derivatized TBBPA). All GC-MS analyses were performed on a J&W column of 60 m with the following temperature program: 90º (2 min), 15ºC/min to 220ºC, 3ºC/min to 300ºC, 300ºC for 13 min. The temperature of the inlet was 220ºC.

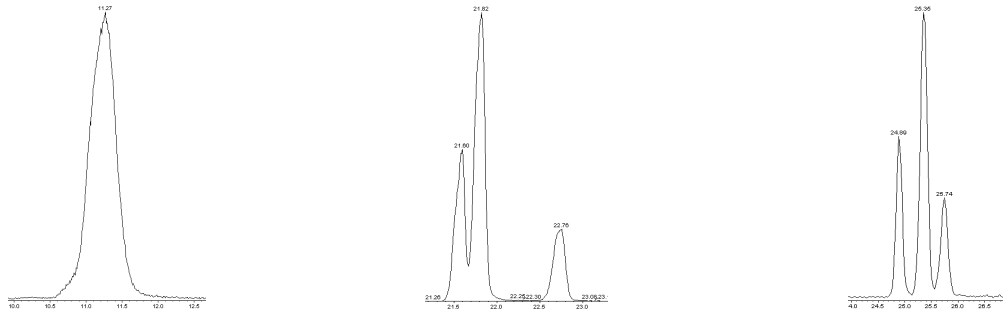


a b c

Figure 1. a) TBBPA (112 ng/ml) peak tailing and poor response. b) Ac-TBBPA (112 ng/ml). c) HBCD (5 ng/ml) broad peak as a result of degradation.

Figure 2a-c illustrates the importance of both column and eluent optimisation in LC-MS analysis. Separation of the three HBCD-isomers with the final gradient program is shown in figure 2c, the final gradient program with H_2O and methanol was: 1 min at 80/20, ramped to 5/95 in 15 min, 5/95 held for 10 min, ramped to 80/2 in 4 min, held for 10 min, at the same conditions the chromatography of TBBPA was good. It is possible that an even better separation could be obtained by the application of a mixture of water, methanol, and acetonitrile. It was not possible to ionize Me-TBBPA to a significant degree neither by electrospray nor atmospheric pressure ionisation, thus Me-TBBPA cannot be analysed by LC-MS-MS (with the two ionisation options as mentioned).

LC-MS-MS is the more sensitive method, with lower levels of detection (LOD) than GC-MS. LOD was calculated as three times the S/N-ratio, where the noise was determined as the maximum noise observed in a real sample immediately prior to the analyte peaks. The resulting values of LOD are shown in table 2.



a b c

Figure 2. LC-MS-MS separation of the HBCD isomers (1 µg/ml, equal amounts of α, β, and γ-HBCD). a) 5 cm C_{18} water/methanol. b) 15 cm C_{18} w. water/acetonitrile. c) 15 cm C_{18} w. water/methanol (final method).

Table 2. Limit of detection of the analytes in GC-MS and LC-MS-MS analyses in ng/ml.

Instrument\Compound	**Me-TBBPA**	**TBBPA**	**A-HBCD**	**β-HBCD**	**γ-HBCD**
GC-MS	1.28	2.6*	ΣHBCD: 5.92		
LC-MS-MS	-	1.53	1.02	0.46	1.59

*Estimated for Ac-TBBPA from the response factor and LOD of Me-TBBPA.

Reproducibility was determined for three real samples of sand eel oil which had undergone extraction and clean-up prior to instrumental analysis. This means that the results include variations in the processing of the samples withim one batch. The relative standard deviations of the GC-MS analysis were 5.7% and 6.6% for Me-TBBPA and ΣHBCD, respectively. The relativestandard deviation of the LC-MS-MS analysis of three replicate samples within the same batch was 12.4%, 4.7%, 4.2%, and 13% for TBBPA, α-, β-, and γ-HBCD, respectively.

As part of the method development, samples of salmon liver *(Salmo salar)* and harbour seal *(Phoca vitulina)* were analysed. The results showed consistently higher concentrations for ΣHBCD when determined by GC-MS compared with LC-MS-MS. For example the concentration of ΣHBCD in salmon liver was 10 ng/g lw when analysed by LC-MS-MS while the results of GC-MS analysis indicated that the samples contained 30 ng/g lw. In the preliminary experiments, compound losses during sample evaporation were observed, but could be minimized by the use of silanized vials. Still, the results obtained by GC-MS were on average 40% higher than that of the same samples analyzed by LC-MS-MS (Frederiksen et al. subm.) although recovery of the two methods were similar. These differences could arise from differences in susceptibility of matrix effects in the two methods e.g. alteration in degradation on column of samples compared to standards on GC-MS. Furthermore, the thermal rearrangement of the HBCD-isomers which occurs above 160ºC (Peled et al. 1995), might be incomplete in the standards, resulting in greater degradation when large amount of γ-HBCD is present. Thereby a standard of an equal mixture of α, β, and γ-HBCD is not representative of the biota samples which mainly consist of α-HBCD, which would lead to an overestimate of samples containing mainly α-HBCD. The broad peak of ΣHBCD in GC-MS could result in greater uncertainty in determination; however, it would not result in a consistent overestimation as is observed. In any case, this issue has not received any attention in the literature, and results obtained by various analytical methods are directly compared.

The current and previous studies (Morris et al. 2004) selected to analyze TBBPA by LC-MS-MS because of the advanges of not needing to derivatize, but this study also showed that better detection

limits could be obtained by applying LC-MS-MS. In the case of HBCD LC-MS-MS offered not only the advantages of isomeric separation but also better detection limits. In the case of Me-TBBPA LC-MS-MS did not offer an alternative to the the well working GC-MS analysis. The question of camparability of the results obtained by the different methods, should be adressed and discussed in the future.

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