# ENHANCED SEPARATION AND DETECTION OF TETRABROMOBISPHENOL-A AND HEXABROMOCYCLODODECANE ISOMERS USING UPLC®/MS/MS

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**Abstract**
The analysis of Tetrabromobisphenol-A (TBBPA) and Hexabromocyclododecane (HBCD) isomers using HPLC/MS/MS has been widely reported[1,2]. Whilst HPLC offers a good robust method, advantages can be gained by the use of Ultra Performance Liquid Chromatography™ (UPLC®), enhancing both the chromatographic resolution and throughput of the analytical method[3]. The shorter residence times can also minimize the potential for on-column adsorptive and degradative losses during the separation. The optimized separation resulted in a method run time of 10 minutes, with TBBPA and 5 HBCD isomers analysed (α, β, γ, δ and ε) being separated to <10% valley. Comparability of the data for real samples, to that run using a standard HPLC/MS/MS system is excellent with a variation of ~20% between the two sets of data, whilst offering a time saving of >15minutes from injection to injection, effectively doubling the throughput capability of the analysis.

**Introduction**
The use of brominated flame retardants - chemicals incorporated into materials that are potentially flammable, such as plastics, rubbers, textiles and electronic components, to slow down or inhibit the initial phase of a developing fire - has seen an exponential rise over the last two decades. Tetrabromobisphenol-A (TBBPA) and Hexbromocyclododecane (HBCD) are two of the most commonly used brominated flame retardants. However, in recent times concern has been raised about possible detrimental effects of these chemicals to the environment and to health.

HBCD is used around the world in thermal insulation foam and in textile coatings as flame-retardants preventing deaths and injuries from fire. The substance is marketed without any legislative restrictions. The main application of HBCD is as an additive flame retardant in extruded and expanded polystyrene that is used as thermal insulation in buildings. A minor application for HBCD is in upholstery textiles. The HBCD technical product is composed of a number of diasterioisomers of which the: α, β, and γ forms predominate. During manufacture, γ-HBCD is the most dominant diastereoisomer formed, contributing approximately 80% of the technical formulation [4].

A concern was identified over HBCD use in textiles where direct body contact may represent a risk over a long period of time. However, there are few suitable replacements available at the moment and the chemical has an important role in meeting fire regulations. Environmental monitoring studies have shown the presence of HBCD in aquatic systems and also in biota from surrounding areas[5]. In the wider and more remote environment HBCD has been found in predatory birds including peregrine falcons [6] and Guillemot eggs. Temporal trends studies of residue levels indicated a significant increase from 1969 to 1997 [5]

TBBPA is used to improve the fire safety of electrical and electronic equipment. It is the largest volume brominated flame retardant (BFR) in production today, for this application. TBBPA is marketed around the world without any legislative restrictions. It is not readily biodegradable and has an environmental degradation half-life of between 50 and 70 days. TBBPA has the potential to bioaccumulate (Log $K_{ow}$ of 5.9), but measured values are much lower than predicted, representing either substantial degradation or rapid loss of the parent compound.

There are currently few reports on environmental levels of TBBPA. It has been reported in sediments [7] and sewage sludge[8]. Environmental monitoring has detected varying concentrations in a range of samples [9] as far back as the early 1980s [10]. TBBPA has also been detected in aquatic biota[10] and in human tissues including blood serum [11,12] and breast milk [13] but human exposure has not been fully assessed. It is unclear whether these reported levels arise from dietary sources.

Until recently, published HBCD concentration data have been derived by gas chromatography (GC) with either negative ion chemical ionisation mass spectrometry or GC with electron capture detection. However, analysis by GC has limitations because it been unable, thus far to chromatographically resolve the different diastereoisomers using standard GC parameters. The isomers are thermally labile, with degradation or interconversion observed at temperatures greater the 160°C. Thus, HBCD values have been reported as total hexabromocyclododecane (ΣHBCD, sum of three diastereoisomers - α, β, and γ). Visible chromatographic peak broadening caused by thermal degradation and interconversion between the diastereoisomers is seen. It is desirable from a toxicological point of view and for human exposure considerations, to determine each diastereoisomer separately. In recent years some research groups have developed and used this methodology[14,15,16] using recently developed LC columns with MS detection systems particularly MS/MS in the multiple reaction monitoring mode. This paper provides more recent and improved methodology for the isomer-specific determination of HBCD and TBBPA using LC-MS/MS in conjunction with a Waters Sunfire $C_{18}$ column equipped LC system. The methodology provides for the reliable and consistent separation of 5 HBCD diastereoisomers - α, β, γ, δ and ε, as well as TBBPA in a relatively short time-scale.

**Materials and Methods**

Standards of α, β, γ, δ and ε-HBCD were supplied by Wellington Laboratories (Guelph, Canada) and diluted as single components and as a combined mixture prior to analysis, calibration curve standards and extracts were prepared at the Central Science Laboratory. All standards and extracts were analysed using a Waters Acquity UPLC® interfaced to a Waters Quattro Premier XE Tandem Quadrupole mass spectrometer, operated in the ESI- ionization mode monitoring the MRM transitions given in table 1.

Separation was performed using an Acquity BEH $C_{18}$ 2.1 x 150mm, 1.7µm column at a flow rate of 0.5ml $min^{-1}$ and a temperature of 60ºC using the following solvent gradient:-

Solvent A = Water

Solvent B = Methanol

| Time | %A | %B |
|---|---|---|
| 0min | 20 | 80 |
| 5min | 20 | 80 |
| 6min | 0 | 100 |
| 8min | 0 | 100 |
| 8.1min | 20 | 80 |
| 10min | 20 | 80 |

| Compound | Transition | Cone Voltage (V) | Col.Energy (eV) |
|---|---|---|---|
| TBBPA | 542.60 > 419.70 | 55 | 40 |
| TBBPA | 542.60 > 447.60 | 55 | 35 |
| 13C-TBBPA | 554.60 > 80.90 | 55 | 55 |
| 13C-TBBPA | 554.60 > 430.60 | 55 | 40 |
| HBCD | 640.40 > 78.90 | 15 | 15 |
| HBCD | 640.40 > 80.90 | 15 | 15 |
| 13C-HBCD | 652.40 > 78.90 | 15 | 15 |
| 13C-HBCD | 652.40 > 80.90 | 15 | 15 |

Table 1. MRM transitions acquired for native and $^{13}C$ labeled TBBPA and HBCD

After optimization of ionization and MS/MS conditions a mixed standard containing the five HBCD isomers (α, β, γ, δ and ε) plus TBBPA was acquired for assessment of chromatographic separation. The optimal separation and sensitivity was achieved using the 150mm BEH $C_{18}$ column.

The single component HBCD isomer solutions were then acquired to identify the elution order. Following this analysis, a bracketed calibration curve containing both native and $^{13}$C-labelled α, β, γ-HBCDs and TBBPA with native concentrations from 5ng ml$^{-1}$ to 600 ng ml$^{-1}$ was acquired with a number of food extracts, all injections were of a 10µL volume. Details of the methodology for the extraction and purification of HBCD/TBBPA from food samples as well as operating conditions for the HPLC/MS/MS measurements have been described elsewhere[16].

**Results and Discussion**

Acquisition of the five single component HBCD standards resulted in the elution order of α, δ, β, ε, γ being deduced, with peak widths of 0.15 minutes. The TIC for the eluting peaks, including TBBPA is presented in Figure 1, where the valley of <10% between β and ε HBCD can be observed.

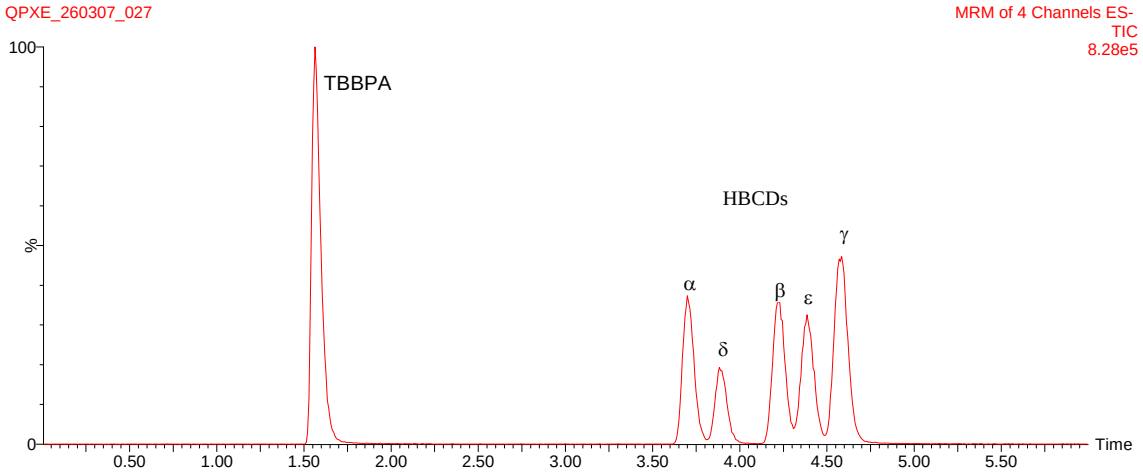


Figure 1, separation and elution order for TBBPA (1.56mins) to γ-HBCD (4.58mins), with the valley between β-HBCD (4.22mins) and ε-HBCD (4.39mins) being <10%.

The linearity of measurement over the range 5ng ml$^{-1}$ to 600 ng ml$^{-1}$ was good for the 4 compounds determined quantitatively (TBBPA, α, β, γ-HBCDs) with all coefficients of determination ($r^2$) being >0.999 for the un-weighted curves. The raw chromatograms for the 5ng ml$^{-1}$ (50pg on column) standard are given in figure 2

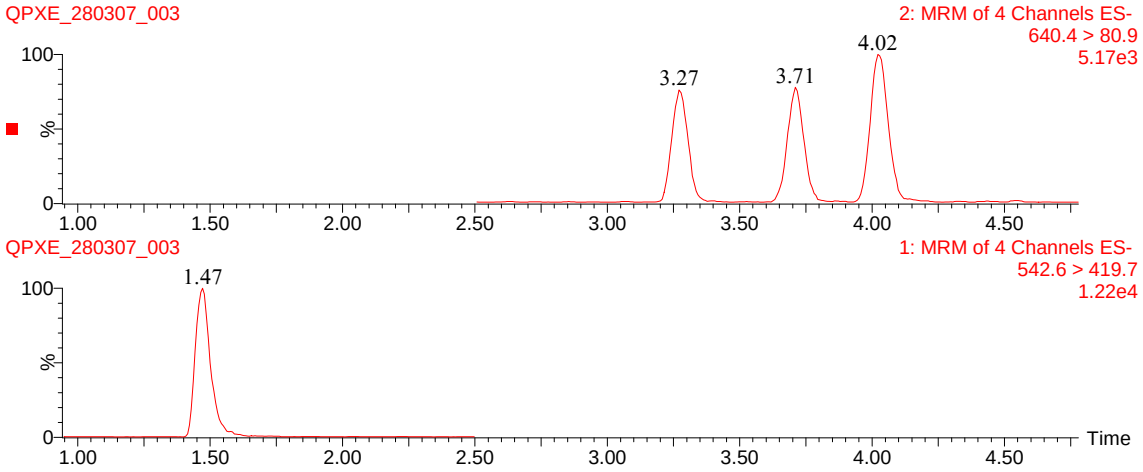


Figure 2, 50pg on column standard injection of TBBPA, α, β, γ-HBCDs

TBBPA was detected in all of the food samples, all of which were of marine origin, with α, β, γ-HBCDs being detected in most samples, δ and ε-HBCD were not detected in any of the food extracts. The α-enantiomer dominated in all the samples analysed, and as observed in other reports[5, 14, 15]. This profile is characteristic of marine biota and probably arises as a result of selective metabolism of the different enantiomers and/or biotransformation processes. A typical fish extract with concentrations of 0.38, 0.056 and 0.032 ng $g^{-1}$ for α, β, γ-HBCD respectively is given in figure 3.

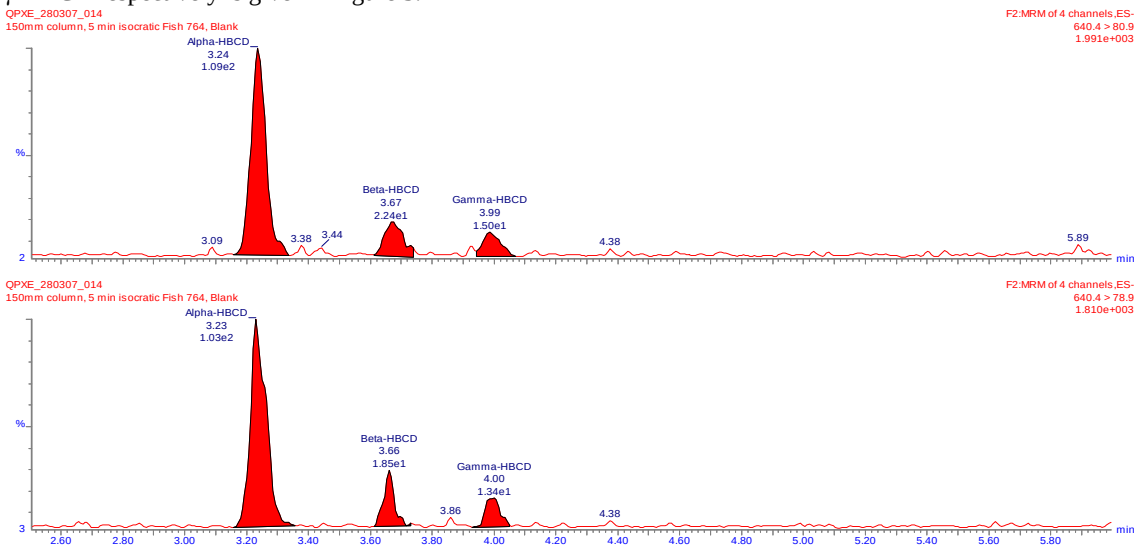


Figure3, UPLC-MS/MS separation of α, β and γ-HBCD in fish; δ, ε enantiomers and TBBPA were not detected.

A comparison of the data obtained using this method and the conventional methodology used shows good agreement with a mean variation of ~20 % between the two data sets. However the advantage in terms of analysis time is >15 minutes.

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