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

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INTRODUCTION

The following method describes the analysis of the Brominated Flame Retardant (BFR) diastereomers Hexabromocyclododecane (HBCD) and Tetrabromobisphenol-A (TBBP-A) using UPLC/MS/MS. This solution provides increased chromatographic resolution and shorter run times over and above current methods. All five HBCD diastereomers were identified in samples of marine origin.

BFRs are chemicals commonly used in many domestic and industrial appliances, equipment and textiles to increase their resistance to fire.

The use of BFRs has seen an exponential rise over the last two decades with HBCD and TBBP-A being two of the most common chemicals used in the highest levels. There is current concern as to the persistent nature of these chemicals and consequently, the detrimental effects they may have on human health and the environment.

The ratios of the three commonly detected HBCD diastereomers in manufactured flame retardant products is different to the ratio detected in sample extracts, indicating that the compounds are metabolised by biological entities.

Current HPLC based methods are becoming more isomer specific[1,2] to enable more specific toxicological studies to be performed. Until recently, published HBCD concentration data have been derived by GC techniques.

However, GC analysis is currently limited as it is unable to resolve the HBCD diastereomers using standard parameters. They are thermally labile, with degradation or interconversion observed at temperatures greater than 160 °C. Thus, historically values have been reported as total HBCD.

METHODS

The methodology used for sample preparation and HPLC/MS/MS analysis are described elsewhere[2].

UPLC CONDITIONS

LC system: Waters® ACQUITY UPLC® System

Column: ACQUITY UPLC BEH C18 2.1 x 150 mm, 1.7 µm
Column temp: 60 °C
Flow rate: 500 µL/min
Mobile phase A: Water
Mobile phase B: Methanol
Gradient:
0.00 min 20% A
5.00 min 20% A
6.00 min 0% A
8.00 min 0% A
8.10 min 20% A
Total run time: 10 min
Injection volume: 10 µL, full loop

MS CONDITIONS

MS system: Waters Quattro Premier™ XE

Ionization mode: ESI negative polarity
Capillary voltage: 2.5 kV
Desolvation gas: Nitrogen, 1000 L/Hr, 400 °C
Cone gas: Nitrogen, 20 L/Hr
Source temp: 120 °C
Acquisition: Multiple Reaction Monitoring (MRM)
Transitions: See Table 1
Collision Gas: Argon at 3.5 x 10^{-3} mBar

Compound	Transition	Cone Voltage	Collision Energy
TBBP-A	542.6 > 419.7 542.6 > 447.6	55	45 35
^{13}C-TBBP-A	554.6 > 80.9 554.6 > 430.6	55	55 40
HBCD	640.4 > 78.9 640.4 > 80.9	15	15 15
^{13}C-HBCD	652.4 > 78.9 652.4 > 80.9	15	15 15

Table 1. MRM parameters

ACQUISITION/PROCESSING METHODS

The data were acquired using Waters MassLynx™ Software v. 4.1. The data was processed using TargetLynx™ Application Manager.

RESULTS & DISCUSSION

During development, two UPLC methods were assessed with comparisons being made to the original HPLC based method.

The initial evaluation was a direct transfer of the separation achieved by HPLC. The 25 minute chromatogram for TBBP-A, α–, β– and γ-HBCD using HPLC separation is shown in Figure 1.

It was possible to achieve improved chromatographic resolution, while reducing the total run time to 5 minutes by using an ACQUITY UPLC BEH C18, 2.1 x 50 mm, 1.7 µm column (Figure 1). UPLC separation would allow the laboratory to increase throughput from 2.4 samples per hour to 12 samples per hour.

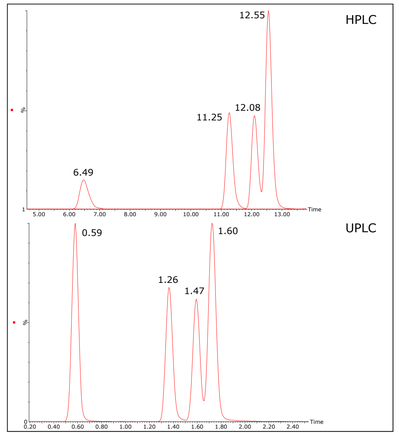
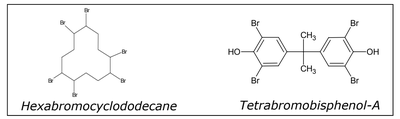


Figure 1. HPLC versus UPLC separation of TBBP-A, α–HBCD, β–HBCD and γ-HBCD

After optimization of the rapid separation, a mixed standard containing the five HBCD diastereomers and TBBP-A was analyzed.

To achieve the required separation, an ACQUITY UPLC BEH C18, 2.1 x 150 mm, 1.7 µm column was required, which resulted in a total run time of 10 minutes or 6 samples per hour (Figure 2).



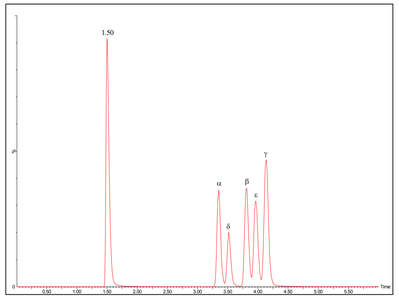


Figure 2. Optimized UPLC separation of TBBP-A and the five HBCD diastereomers

The reduction in peak width achieved using UPLC resulted in significant sensitivity gains when compared with HPLC (Figure 3):

- TBBP-A peak width reduced from 0.67 min to 0.15 min
- HBCDs peak widths reduced from 0.53 min to 0.16 min

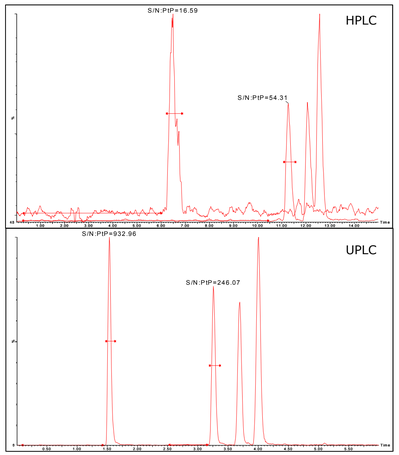


Figure 3. HPLC versus UPLC sensitivity of TBBP-A, α–HBCD, β–HBCD and γ-HBCD

TBBP-A was detected in all of the marine origin samples analyzed, with α–, β– and γ-HBCDs being detected in most extracts, δ- and ε-HBCD were not detected in any.

The α-enantiomer dominated in all the samples analyzed. This profile is characteristic of marine biota and probably arises as a result of selective metabolism and/or biotransformation of the enantiomers.

A typical marine origin extract with concentrations of 0.56, 0.07 and 0.06 ng/g for α–, β– and γ-HBCD, respectively is shown in Figures 4 and 6.

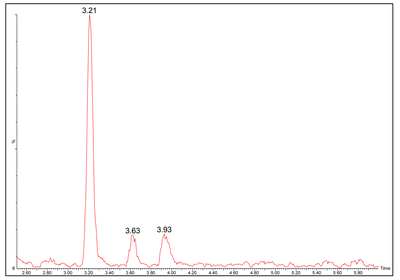


Figure 4. Typical extract of marine origin showing low levels of TBBP-A and HBCDs

All results were compared between the two methods. The mean deviation between HPLC and UPLC was <20%. α-HBCD results across the batch are shown in Figure 5, with all data shown with 15% error bars, conforming to a typical measurement uncertainty.

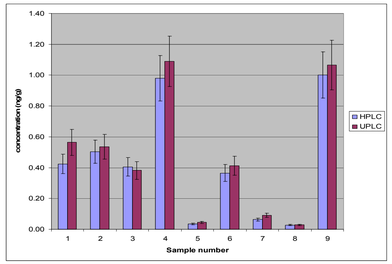


Figure 5. Comparison of TargetLynx determined concentrations in samples of marine origin for α-HBCD using HPLC and UPLC

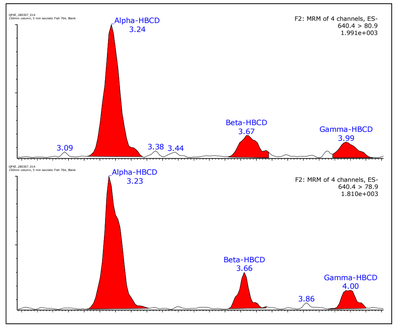


Figure 6. TargetLynx view of a typical extract of marine origin showing low levels of TBBP-A and HBCDs

CONCLUSION

- **The use of the Waters ACQUITY UPLC with Quattro Premier XE enabled a significant improvement in chromatographic resolution and run time over and above current methods.**
- **All five HBCD diastereomers and TBBP-A could be determined rapidly in a run time of 10 minutes; a throughput improvement of up to five times, with all the diastereomers being separated to <10% valley.**
- **This vastly increases a cost conscious laboratory's productivity by reducing both run time and acquisition-to-report time. Also, cost and environmental impact will be reduced through lower solvent usage required with UPLC.**
- **Final results compare favourably with an established fully validated (ISO 17025) method ensuring confidence in results.**
- **This methodology, carried out on the Waters ACQUITY UPLC with Quattro Premier XE, will result in increased sample capacity, flexibility in workflow, and increased lab efficiency leading to maximized asset utilization and a faster return on investment.**

References

1. Fernandes A., Driffield M. et al., Molecular Nutrition and Food Research, 2007 (in press).
2. Fernandes A., Driffield M. et al., Submitted to Food Addit. Contam. 2007.

Acknowledgements

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