UTILIZING THE SPEED AND RESOLUTION OF UPLC TO ENHANCE THE MS/MS DETECTION OF HBCD AND TBBP-A DIASTEREOMERS

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AIM OF WORK

To provide a UPLC/MS/MS solution for

- **Shorter run times and**
- **Increased resolution**

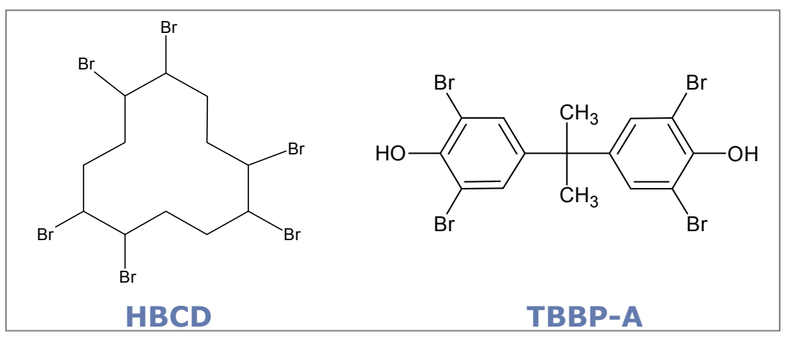
for the analysis of hexabromocyclododecane (HBCD) and tetrabromobisphenol-A (TBBP-A) diastereomers using UPLC/MS/MS.

INTRODUCTION

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

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

Both HBCD and TBBP-A are currently marketed around the world without any legislative restrictions. However, as emerging contaminants or Persistent Organic Pollutants (POPs), the importance of continuous monitoring to quantify the impact of these chemicals on human health and the environment is paramount.



GC analysis of these compounds is currently limited as it is unable to chromatographically resolve the different diastereoisomers, whereas with LC separation is possible. LC enables more specific toxicological studies to be performed.

There are robust methodologies reported in the literature[1,2] for the analysis of TBBP-A and HBCD diastereomers using HPLC/MS/MS. However, advantages can be gained by the use of Ultra Performance Liquid Chromatography™ (UPLC®) through enhanced chromatographic resolution and throughput of the analytical method[3].

The optimised separation used in this method resulted in a cycle time of 10 minutes, with the 5 HBCD diastereomers and TBBP-A analysed being separated to <10% valley.

METHOD

LC conditions

LC System:	Waters® ACQUITY UPLC®
System Column:	ACQUITY UPLC BEH C18 Column
	2.1 x 50 mm, 1.7 µm
	2.1 x 150 mm, 1.7 µm
Flow Rate:	500 µL/min
Column temp:	60C
Mobile Phase A:	Water
Mobile Phase B:	Methanol

MS conditions

MS System:	Waters Quattro Premier™ XE
Ionization Mode:	ESI negative polarity

Compound	Transition	Cone Voltage (V)	Collision Energy (eV)
TBBP-A	542.6 > 419.7	55	40
TBBP-A	542.6 > 447.6	55	35
13C-TBBP-A	554.6 > 80.9	55	55
13C-TBBP-A	554.6 > 430.6	55	40
HBCD	640.4 > 78.9	15	15
HBCD	640.4 > 80.9	15	15
13C-HBCD	652.4 > 78.9	15	15
13C-HBCD	652.4 > 80.9	15	15

EXPERIMENTAL

Extracts of marine origin and calibration solutions containing BFRs and 13C-Labelled BFRs (internal standards) were provided by the Central Science Laboratory, (CSL) York, UK. The α, β, γ, δ, ε HBCD and TBBP-A single compound standards were supplied by Wellington Laboratories. The methodology used for sample preparation and HPLC/MS/MS analysis are described elsewhere[4].

RESULTS

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.

During development, two UPLC methods were assessed with comparisons being made to the original HPLC based method, shown in Figure 1. Direct transfer from HPLC to UPLC gave better chromatographic resolution, reducing the cycle time from 25 minutes to 5 minutes using the 50mm length column. This UPLC method would allow a lab to increase its throughout from 2.4 to 12 samples /hr/ instrument — an increase of 5 times.

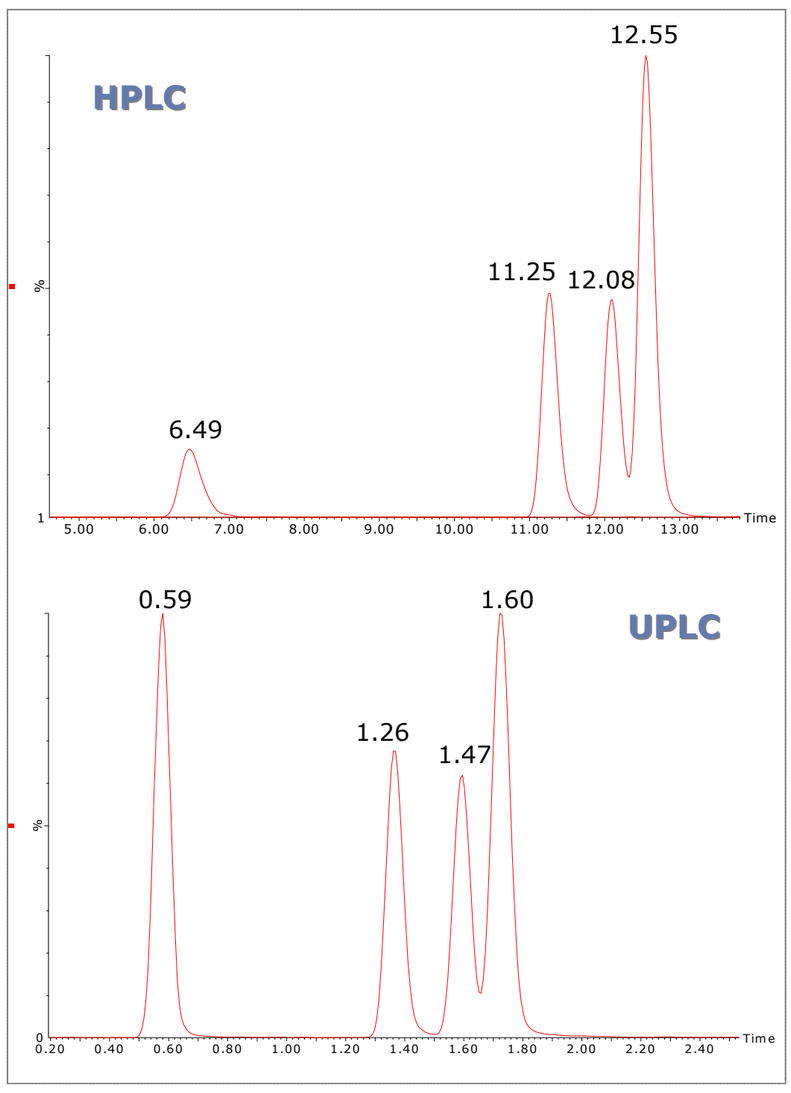


Figure 1. UPLC reduces run-time for TBBP-A (0.59mins), α-HBCD (1.26 mins), β-HBCD (1.47 mins) and γ-HBCD (1.60 mins) - direct method transfer from HPLC to UPLC

The second method developed focused on the resolution optimisation of the five HBCD diastereomers and TBBP-A - this was achieved using the 150mm length column giving a cycle time of 10 minutes (6 samples/ hr).

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 chromatogram for the eluting peaks, including TBBP-A is presented in Figure 2, where the valley of <10% between β and ε HBCD can be observed.

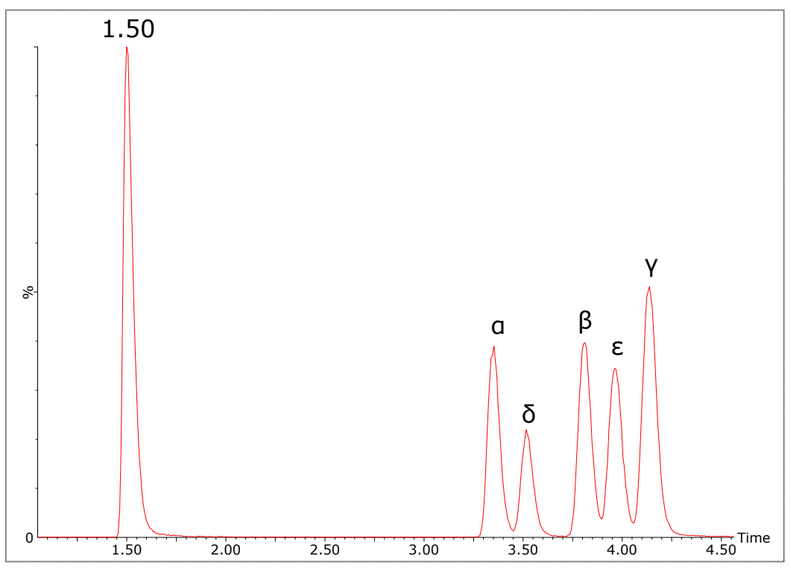
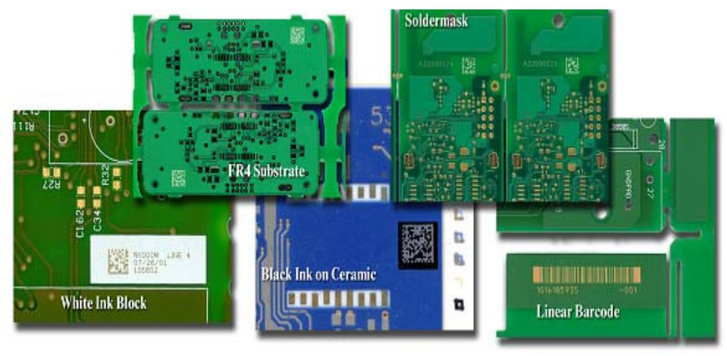


Figure 2. Optimised UPLC separation for TBBP-A (1.50 mins) and resolution of five HBCD diastereomers, (final peak elutes <4.25 mins)



CONCLUSION

The use of Waters ACQUITY UPLC with Quattro Premier XE, enabled a significant improvement in chromatographic resolution and run-time over and above current methods.

All 5 HBCD diastereomers and TBBP-A could be determined rapidly, with added confidence given through TargetLynx data processing.

This vastly increases a cost conscious laboratories 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 work flow and lab efficiency leading to maximised asset utilisation and a faster return on investment.

REFERENCES

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Thank-you to Central Science Laboratory, (CSL) York, UK who provided the marine origin and calibration solutions containing BFRs and 13C-Labelled BFRs (internal standards). Also thank-you to Wellington Laboratories for supplying the α, β, γ, δ, ε HBCD and TBBP-A single compound standards.

Only a limited amount of the work has been shown on this poster, but the full application note can be downloaded at www.waters.com

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