Analysis and Significance of HBCD and TBBP-A

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Cefas Burnham Laboratory

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HBCD (Hexabromocyclododecane)

- HBCD is a high volume (20,000t worldwide), additive brominated flame retardant i.e. directly applied to the product requiring flame retardancy

- HBCD has been used for 20 years

- Major applications:
  - Extruded or expanded polysterene foam e.g rigid insulation panels, blocks for construction
  - Textile back coatings e.g upholstery
  - Electrical housing

- Exhibiting all the classic characteristics of a POP HBCD is suspected of disrupting endocrine system by impairing thyroid function and of causing liver cancer

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Complex stereochemistry

- HBCD is a technical mixture containing 16 possible stereoisomers, 6 pairs of enantiomers and 4 meso forms. (See Heeb et al Chemosphere 61 (2005) 65-73).

- For practical reasons we need only be concerned with the three dominant HBCD diasteromeric pairs (racemic mixtures) designated as α-, β- and χ-HBCD.

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Composition of technical HBCD*

Structure	Designation	Proportion in tech mixture
6a/b	α(racemic)	11.8 – 16.20 %
7a/b	β(racemic)	5.8 – 12.83 %
8a/b	χ (racemic)	70.35 - 81.6 %
3/4/9/10[b]	δ (meso)	0.5%
3/4/9/10[b]	ε (meso)	0.3%

*** After Heeb *et al***

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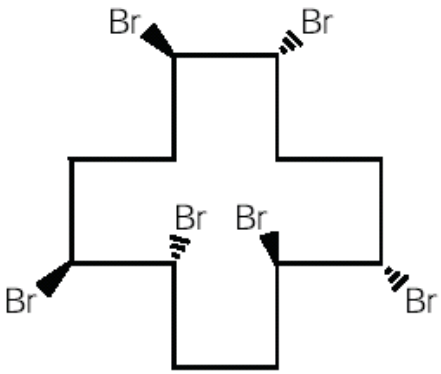
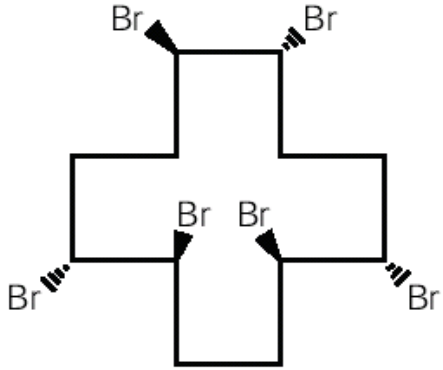
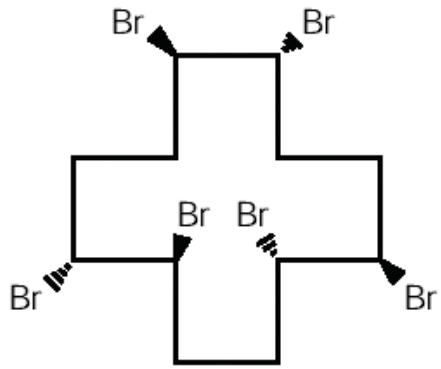
The chemical structure of α-, β- and γ-HBCD diastereoisomers







α- HBCD β-HBCD γ-HBCD

Molecular Weight = 641.6

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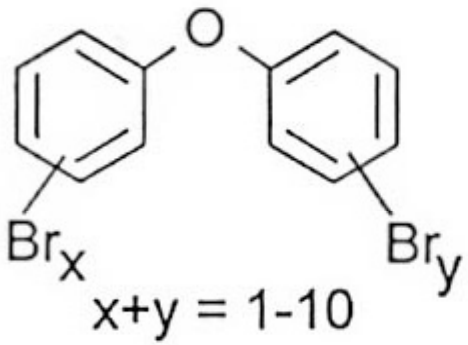
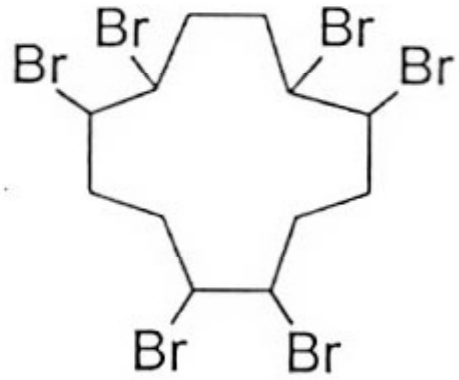
Common BFR structures

a)



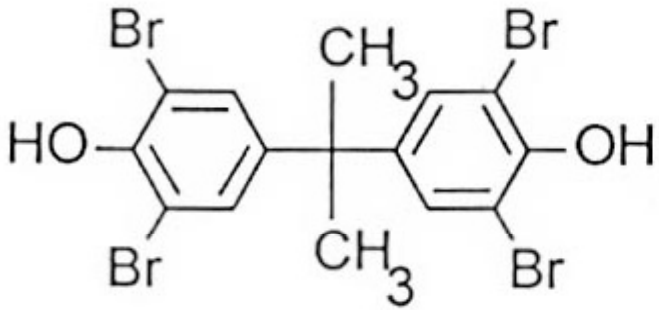
PBDE's

b)



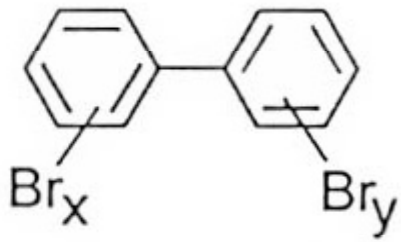
HBCD

c)



TBBP-A

d)



PBB's

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Analytical method development

- Inherent problems associated with traditional techniques (GC) used to quantify persistent organic compounds i.e. thermal rearrangements and decomposition of stereoisomers & lack of isomer resolution

- Use of LC-MS circumvents these problems

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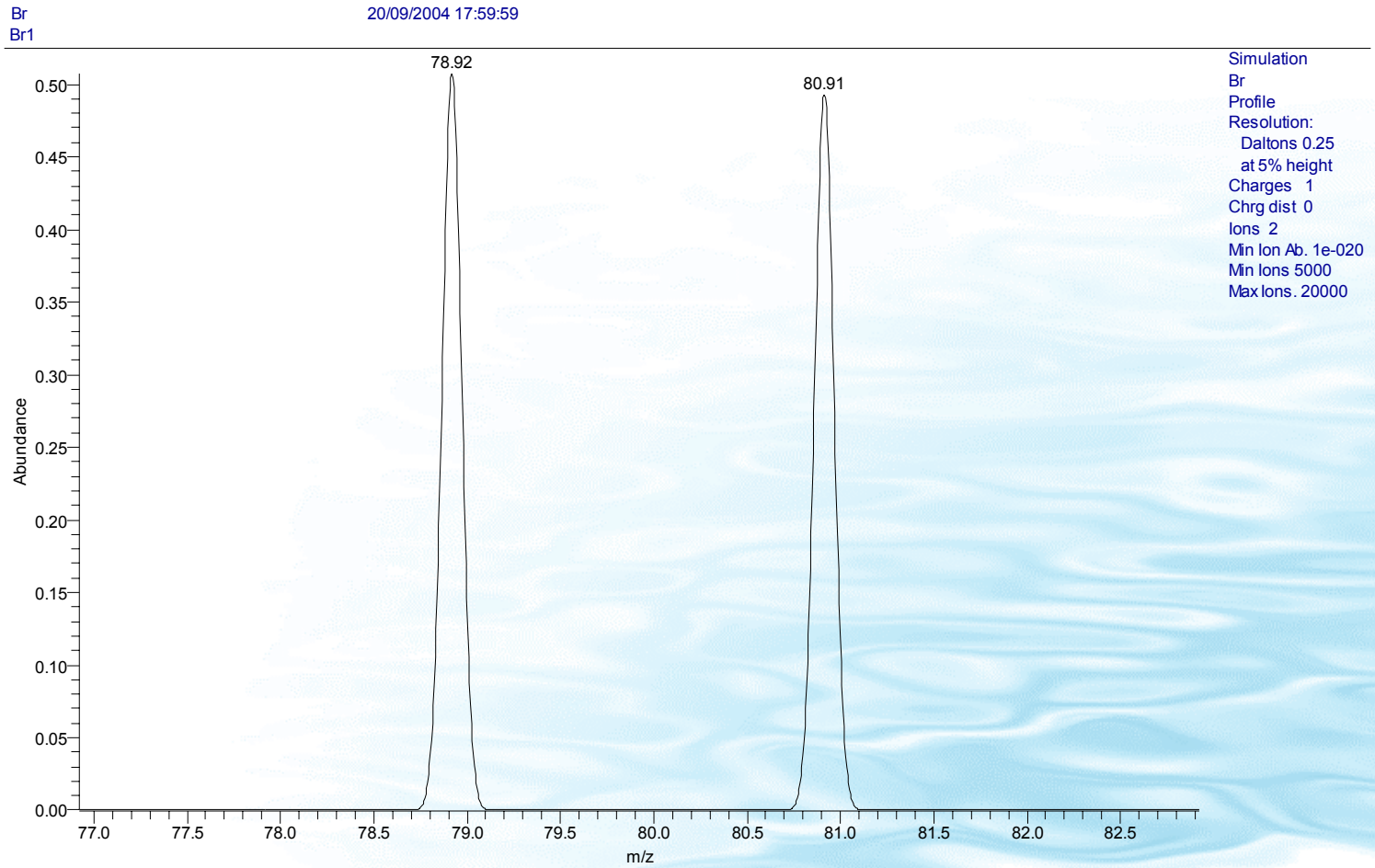
Isotopic distribution for Bromine (Br)



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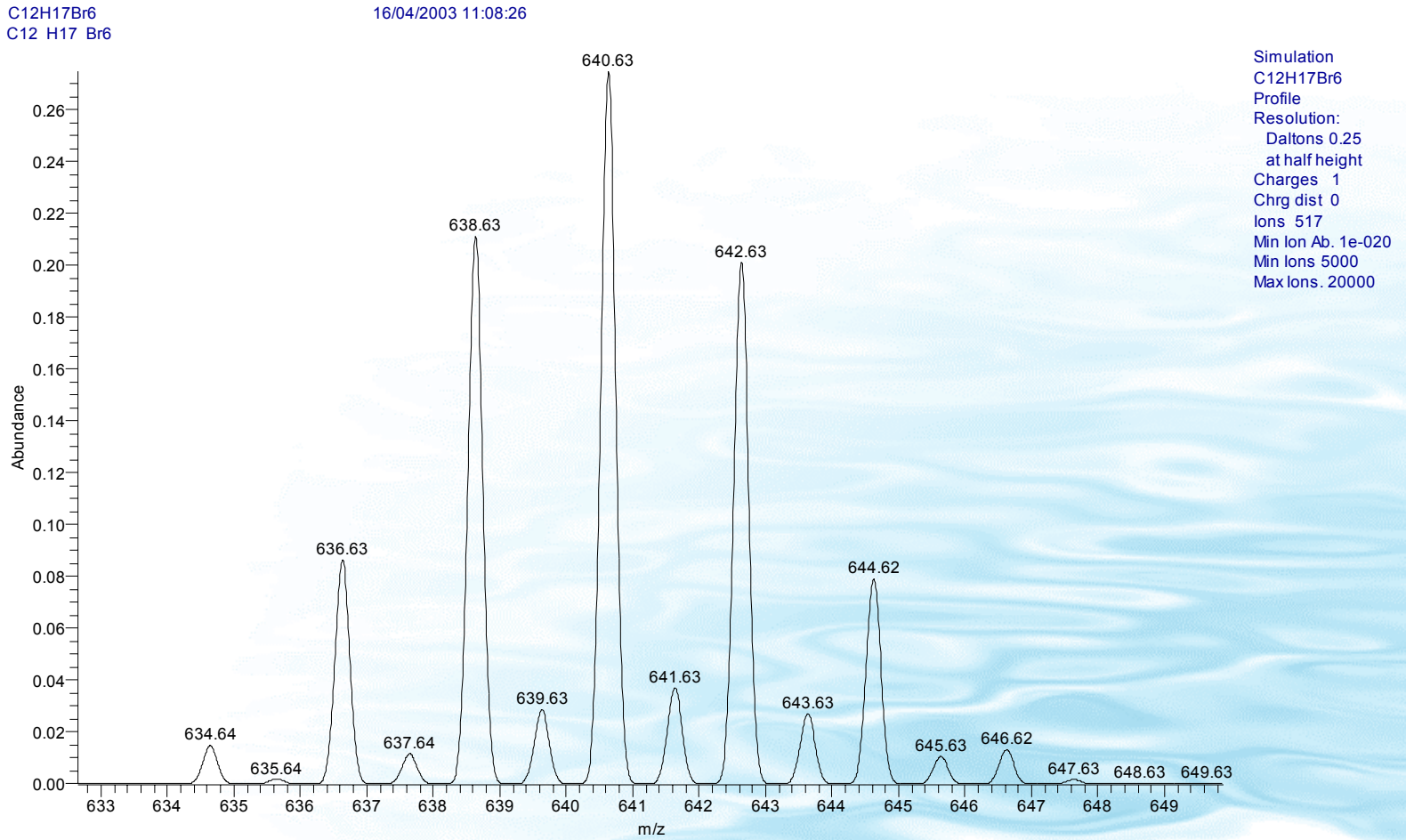
Isotopic distribution for HBCD anion [M-H]



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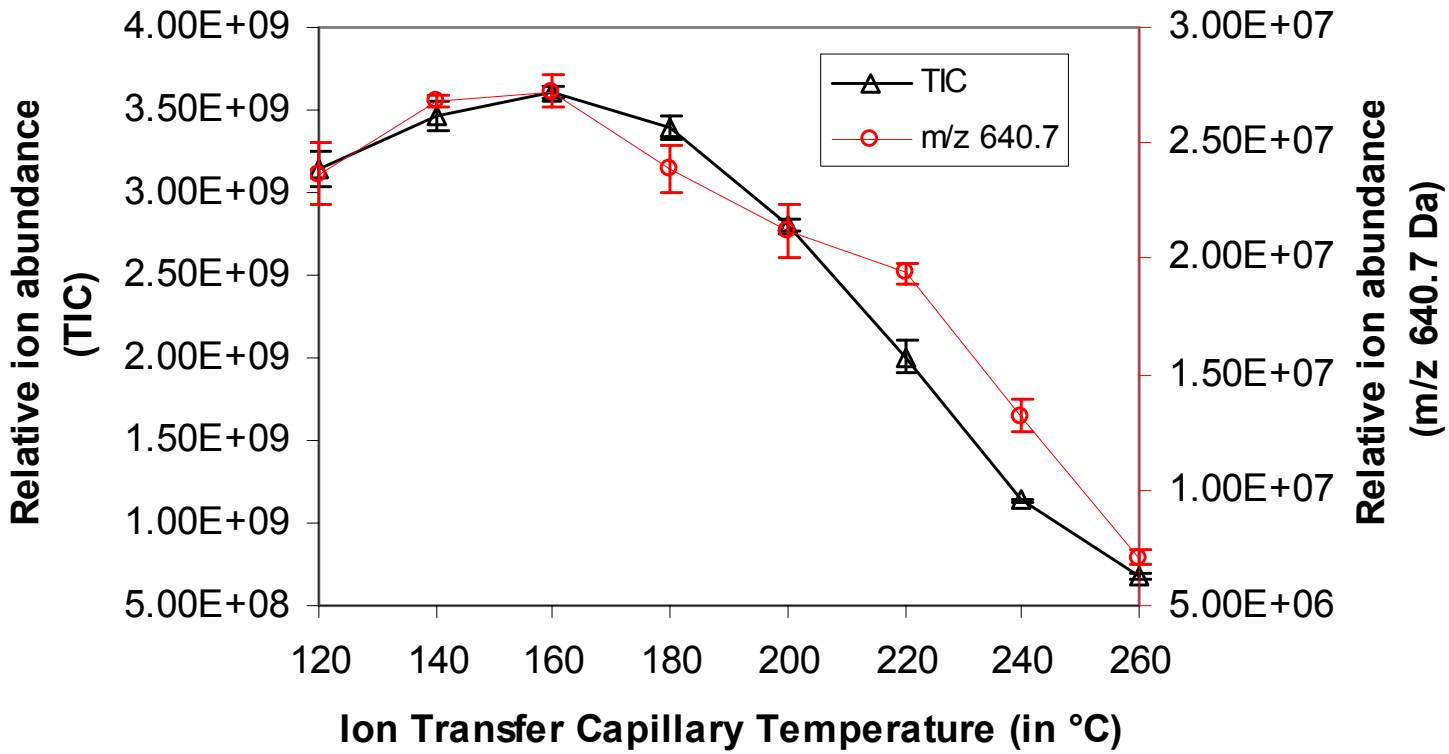
Ion Trap MS Detector parameters optimisation

Figure 5. Effect of Ion Transfer Capillary Temperature on HBCD ion abundance



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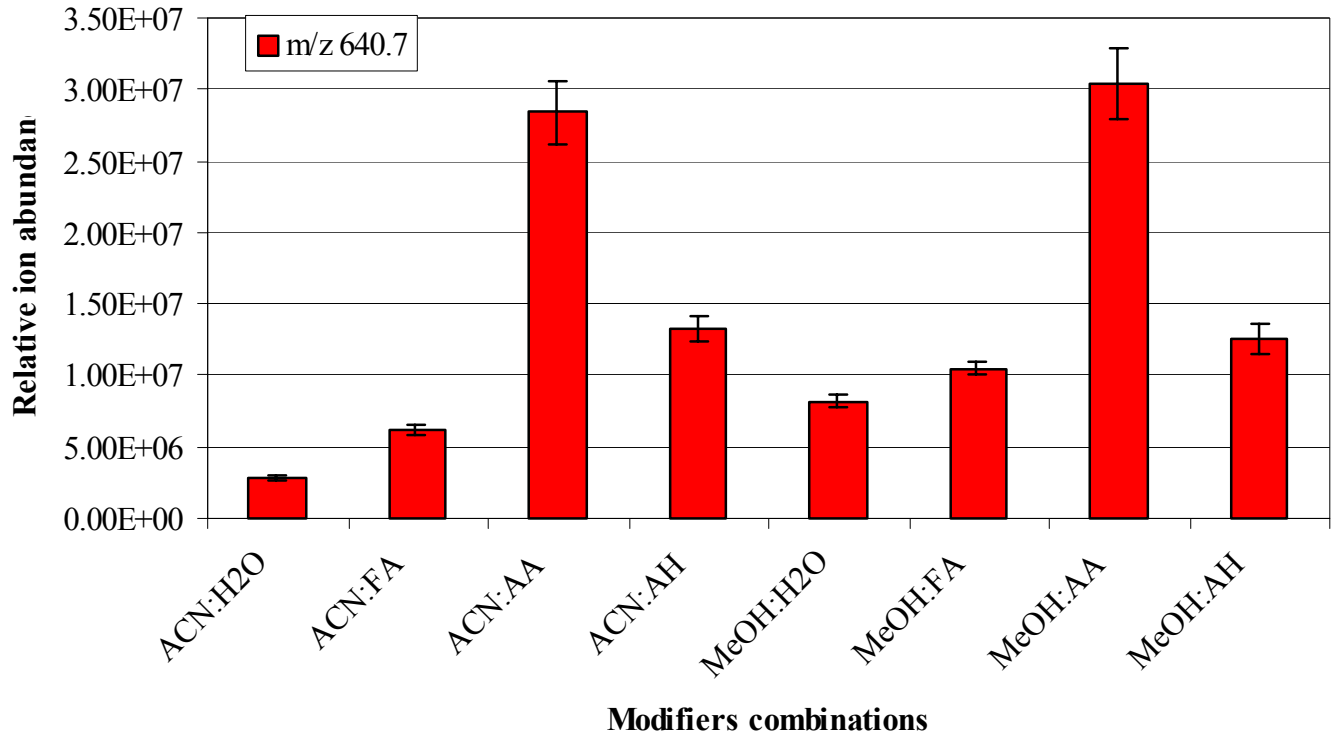
Ion trap MS Detector parameters optimisation

Figure 2. Effects of mobile phase modifiers on HBCD parent ion $[M-H]^-$ (*m/z* 640.7) intensity



***Key*: ACN: acetonitrile; FA: formic acid; AA: Ammonium acetate; AH: Ammonium hydroxide; MeOH: Methanol**

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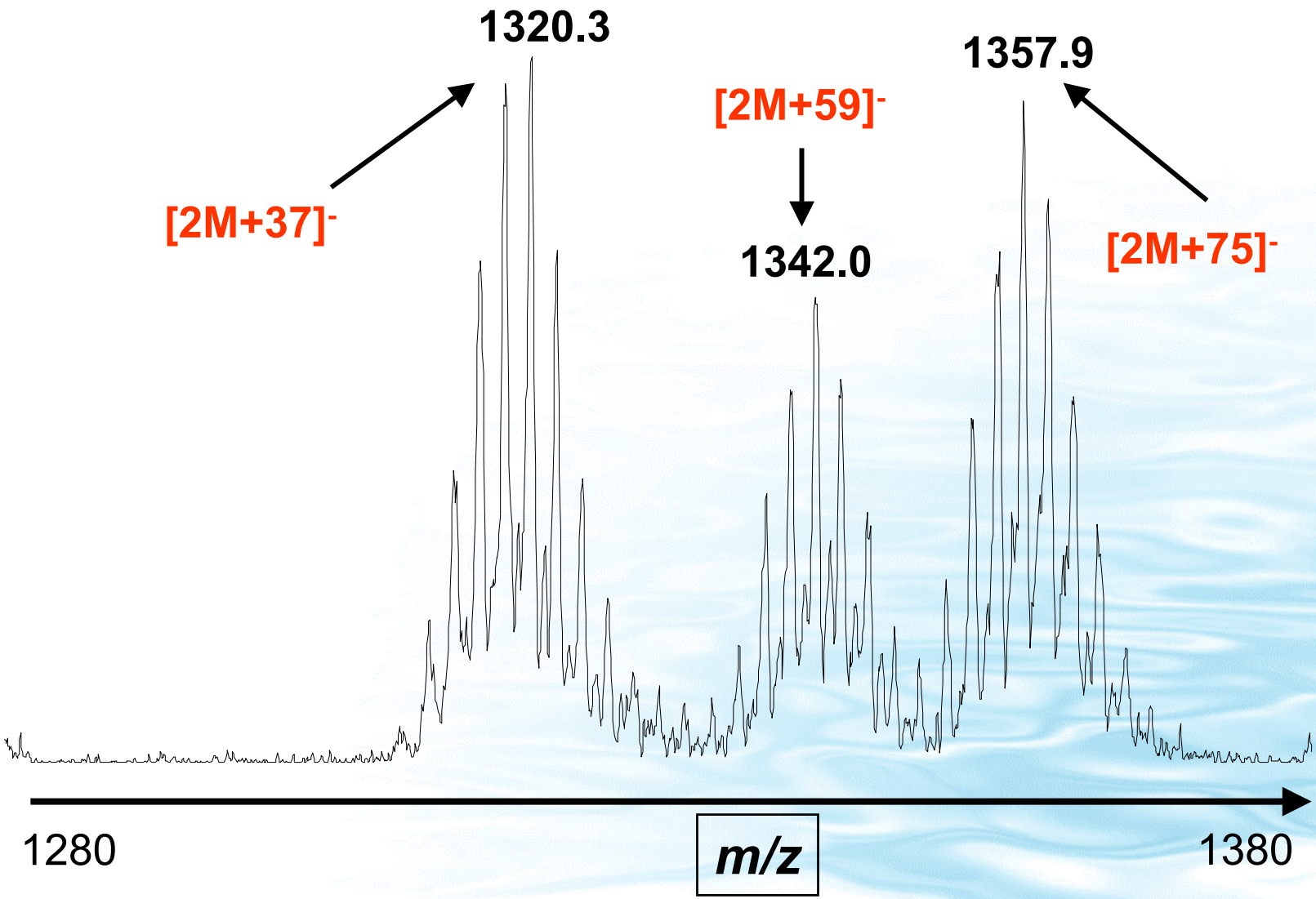
γ-HBCD DIMERIC ADDUCT ION SPECTRA



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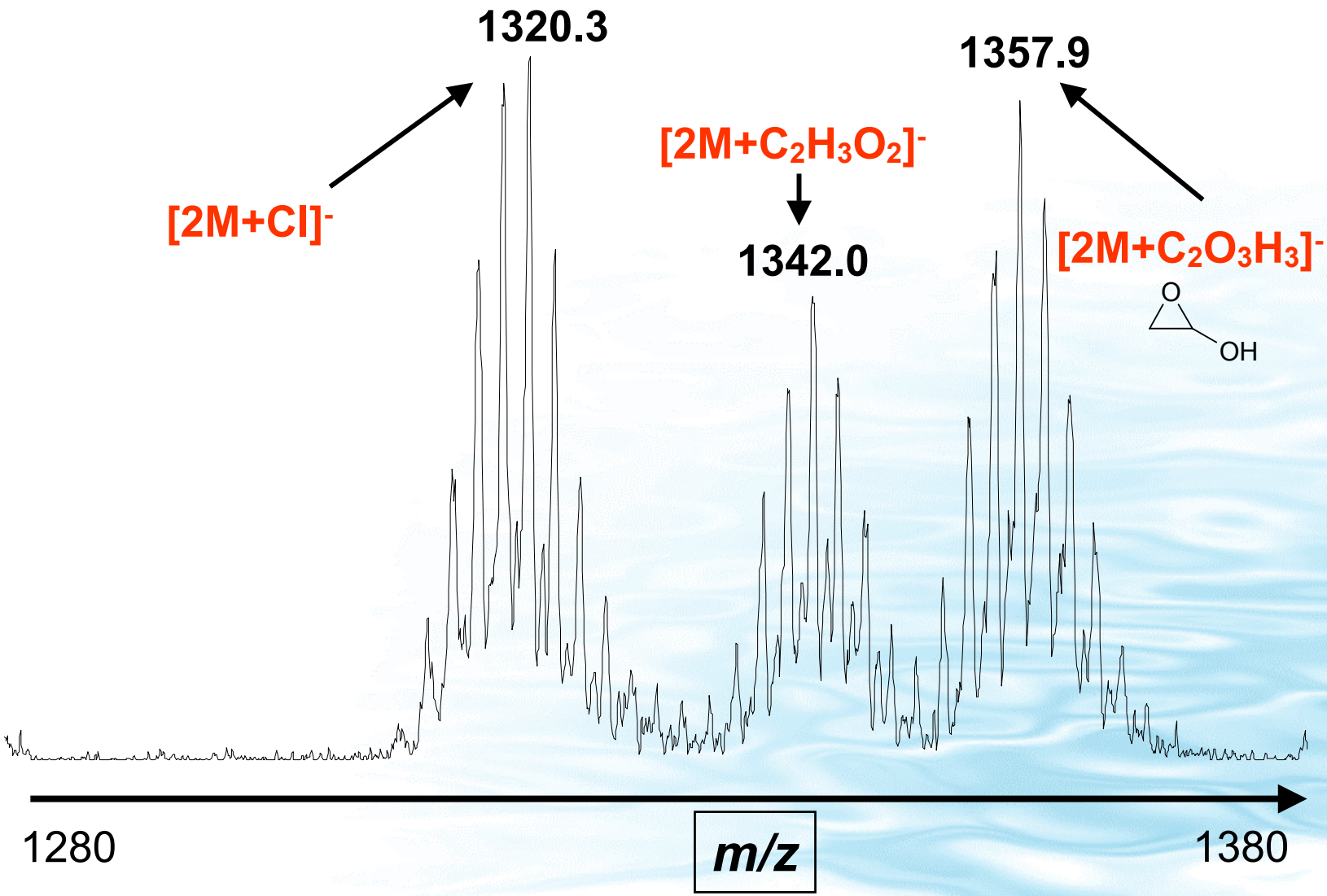
γ-HBCD DIMERIC ADDUCT ION SPECTRA



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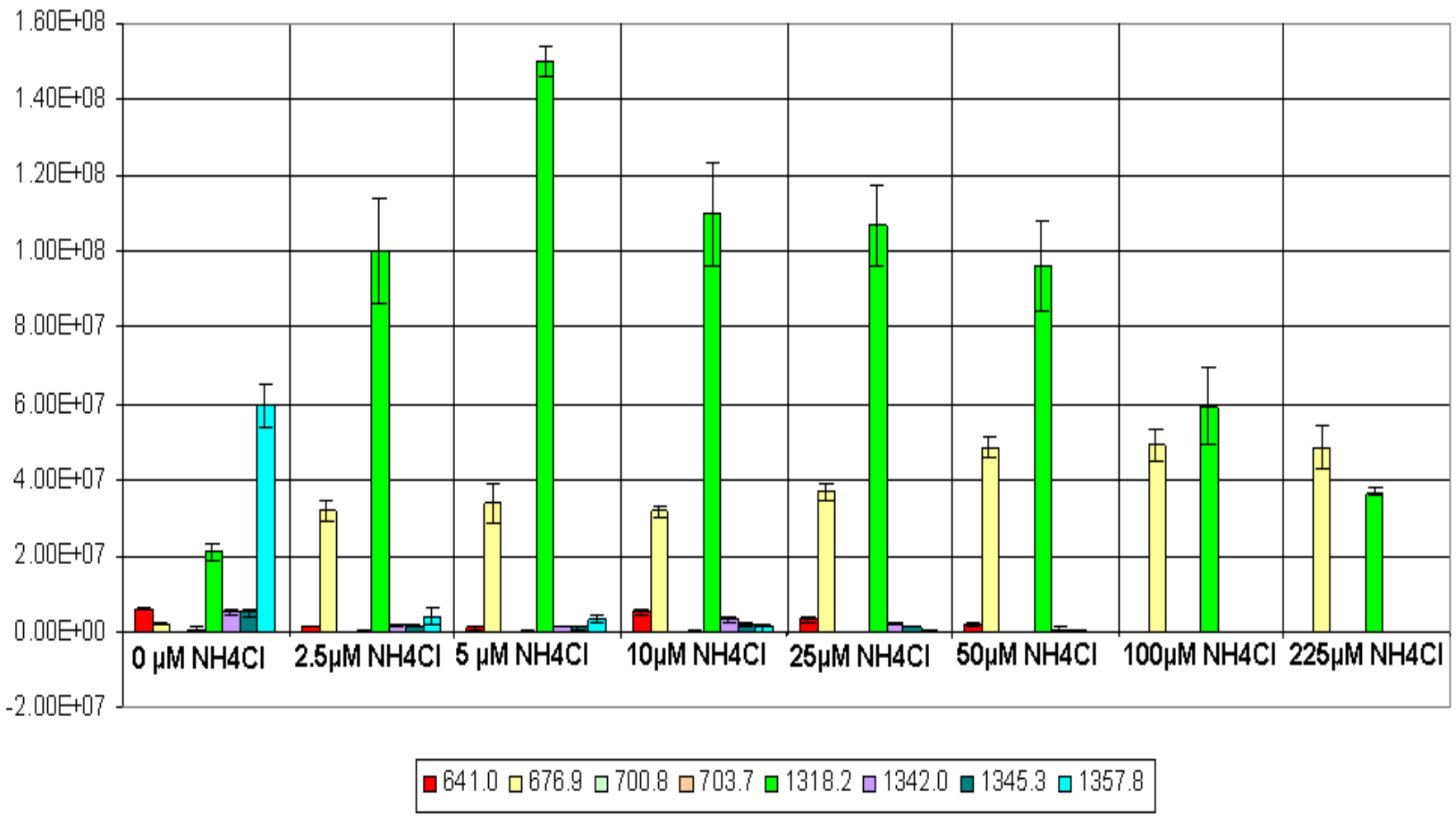
Control of dimer formation by promoting HBCD-Cl clusters (m/z 677 and m/z 1318) using NH_4Cl as a mobile phase additive



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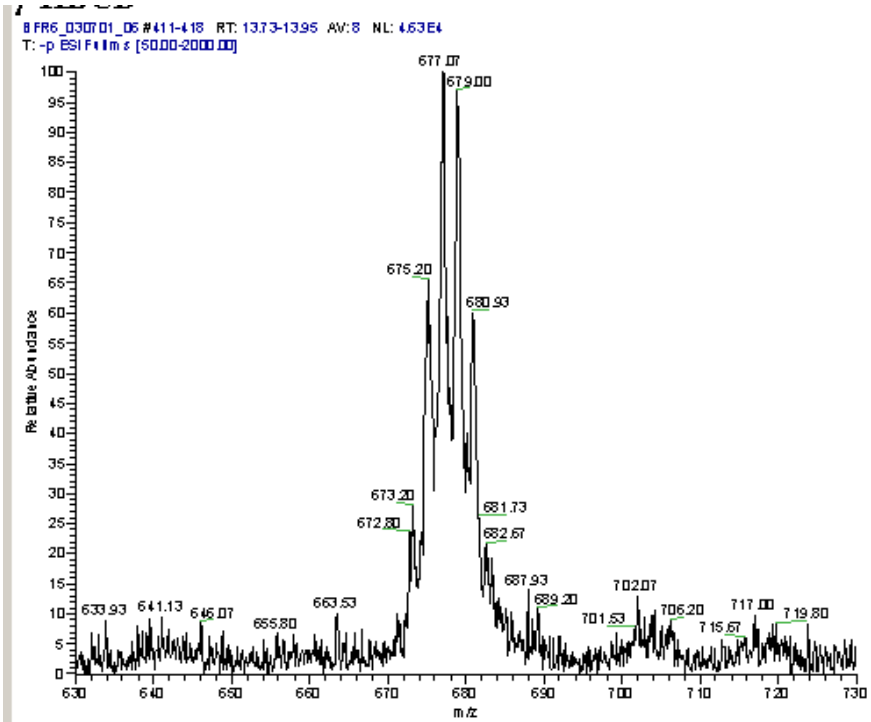
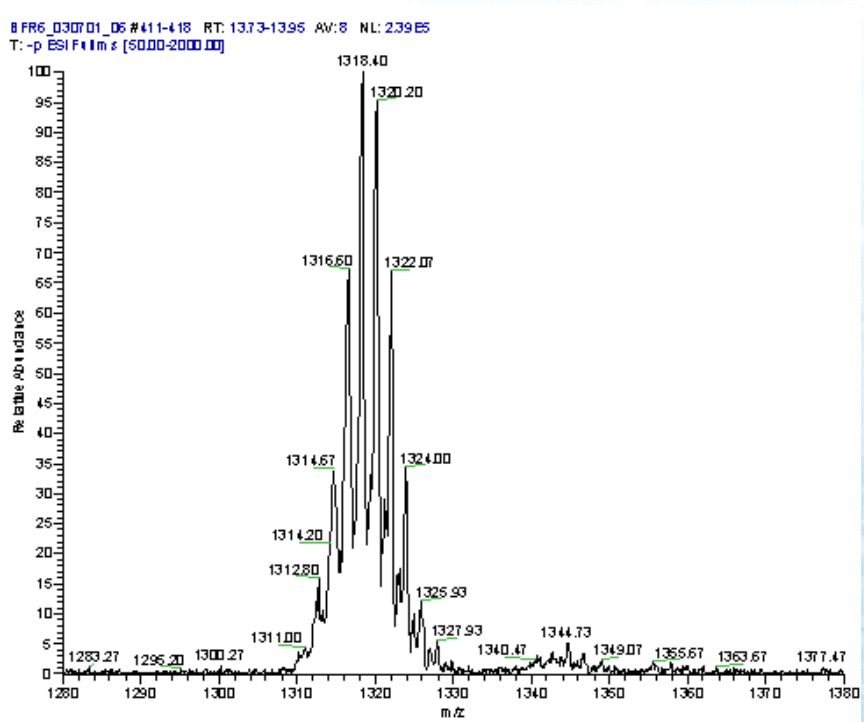
Mass spectra for γ-HBCD separated by HPLC in the presence of 10 μM NH_4Cl

$[M+Cl]^-$



$[2M+Cl]^-$



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ITMS CHARACTERISATION OF ANALYTES

LCQ Advantage in negative ESI and SIM mode

TBBP-A (MW = 543.9) → **m/z 541 & m/z 543**

α-, β- & γ- HBCD (MW = 641.7) → **m/z 677 & m/z 1318**
$[M+Cl]^-$ and $[2M+Cl]^-$

$^{13}C_{12}$ TBBP-A (MW = 555.8) → **m/z 555 & m/z 553**

d18 α-, β- & γ- HBCD (MW = 659.7) → **m/z 695 & m/z 1354**
$[M+Cl]^-$ and $[2M+Cl]^-$

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LIQUID CHROMATOGRAPHIC SEPARATION OF TBBP-A & HBCDs

LC	***ThermoFinnigan Surveyor*** **Quarternary Pump**
Column	***Phenomenex*** **'Luna' C18 (2 x 150 mm; 3μm) + (2 x 4 mm)** ***Security Guard*** **cartridge**
Column Temp	**40 °C**
Flow Rate	**200 μL min^{-1}**
Injection vol.	**20 μL**

Mobile Phase

Time (minutes)	20 mM ammonium acetate (%)	methanol (%)	0.05 mM ammonium chloride (%)	acetonitrile (%)
0.0	70	20	10	0
3.5	19	66	10	5
18.0	19	66	10	5
18.1	0	0	0	100
30.0	0	0	0	100
30.1	70	20	10	0
40.0	70	20	10	0

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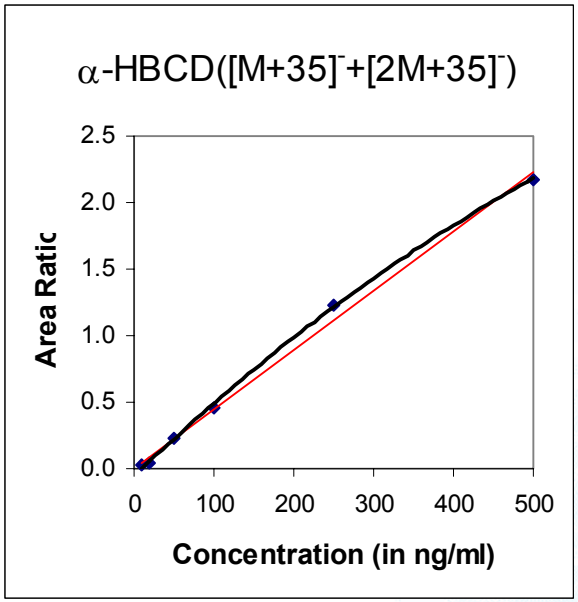
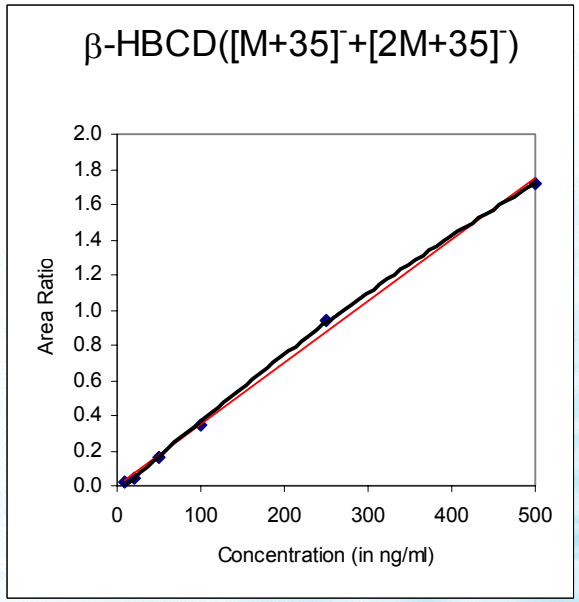
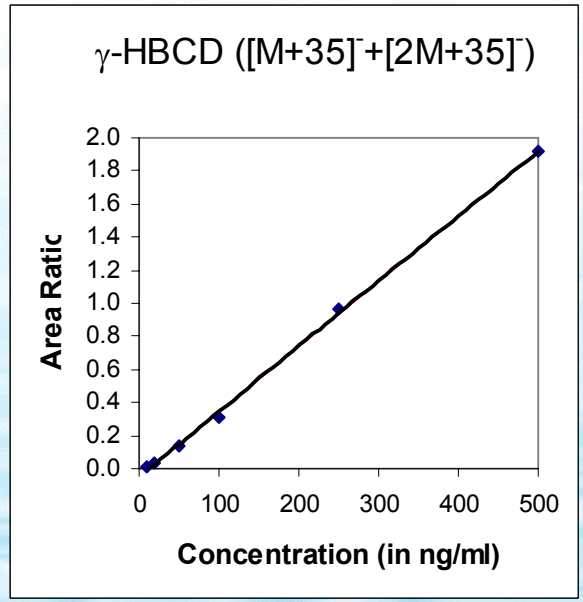
Calibration curves obtained for α-, β-, γ- HBCD using d18-HBCDs internal standards and 5 µM NH_4Cl in the mobile phase

HBCDs produce 2nd order calibration curves with a linear range from 10 to *ca* 500 ng/ml







R^2 = 0.995

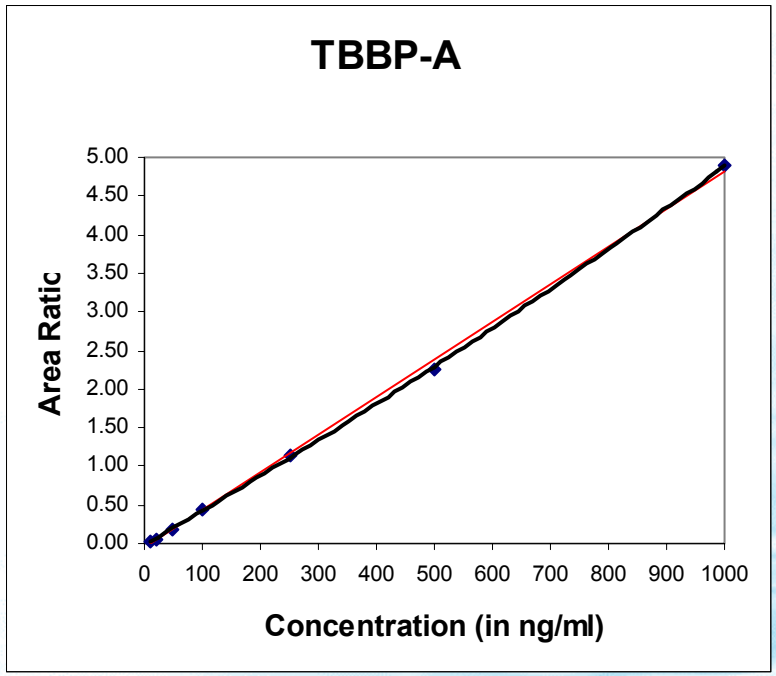
R^2 = 0.997

R^2 = 0.999

Cefas

Calibration curves obtained for TBBP-A using $^{13}C_{12}$ TBBP-A as an internal standards and 5 µM NH_4Cl in the mobile phase

TBBP-A produces linear calibration curves over the 10 - 1000 ng/ml range



R^2 = 0.999

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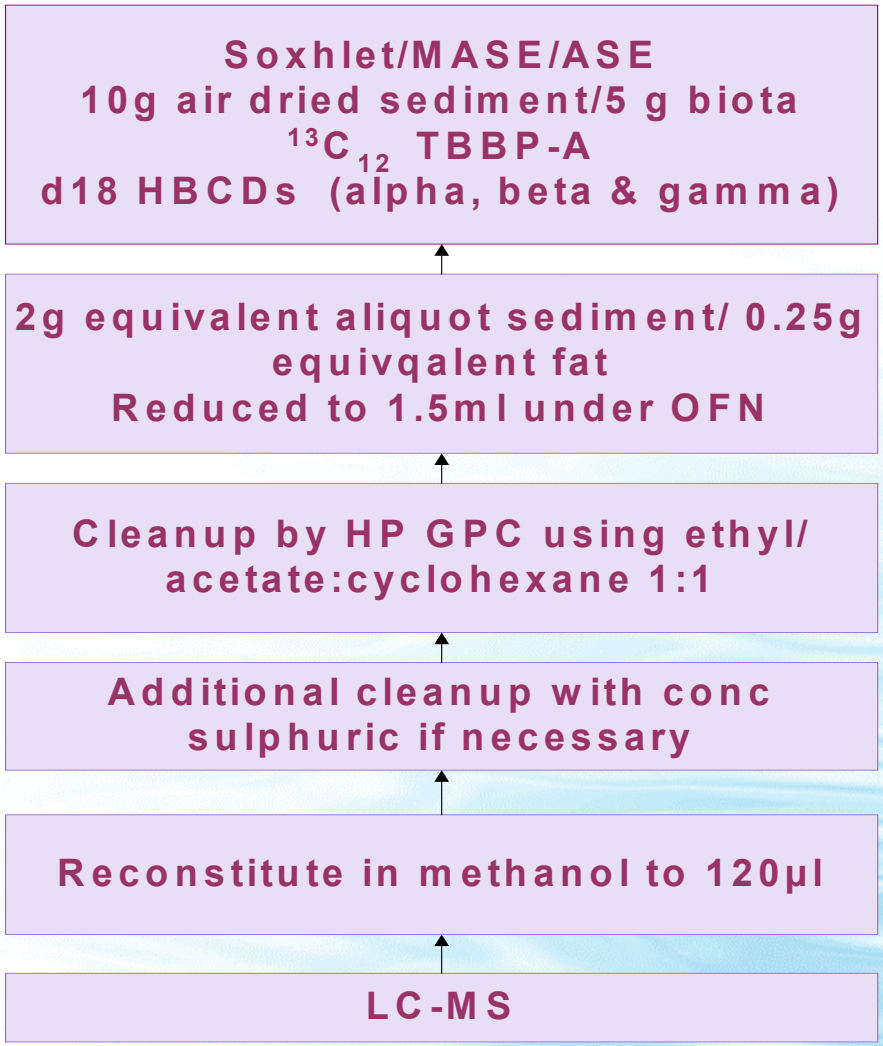
Analytical scheme for HBCD & TBBP-A



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HBCD QC using a Cod LRM (*n*=10)

	α-HBCD	β-HBCD	γ-HBCD	Σ-HBCD
LRM concentration (µg/kg)	***4.0***	***3.2***	***17.6***	***24.8***
Concentration (µg/kg)	**3.5**	**3.0**	**13.4**	**19.9**
SD	**0.7**	**0.3**	**2.7**	**2.2**
% RSD	**21**	**9**	**20**	**11**
% Recovery	**87**	**93**	**76**	**80**

LOQ = 1 ng/g

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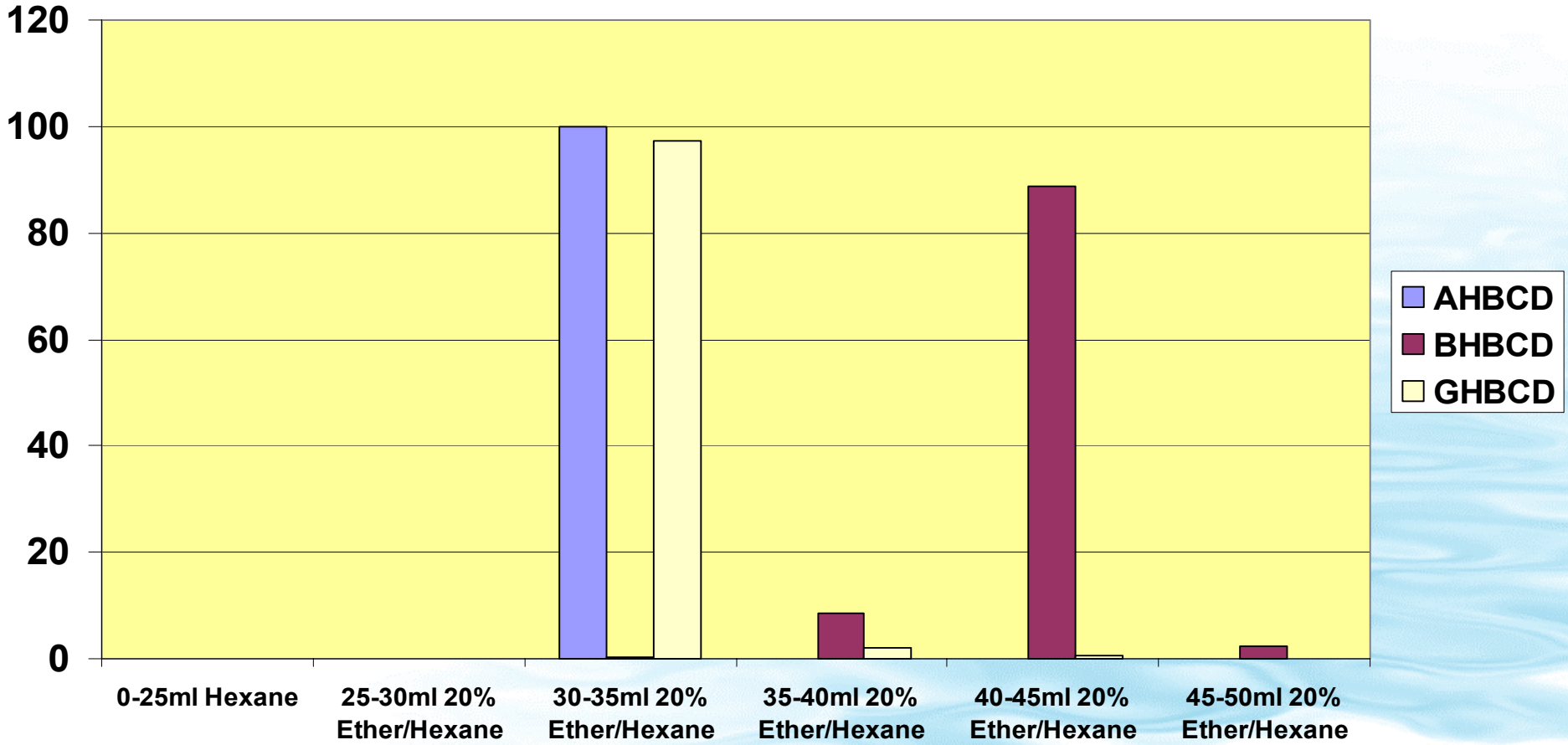
HBCD Recovery from Silica
(3g 3% Deactivated)



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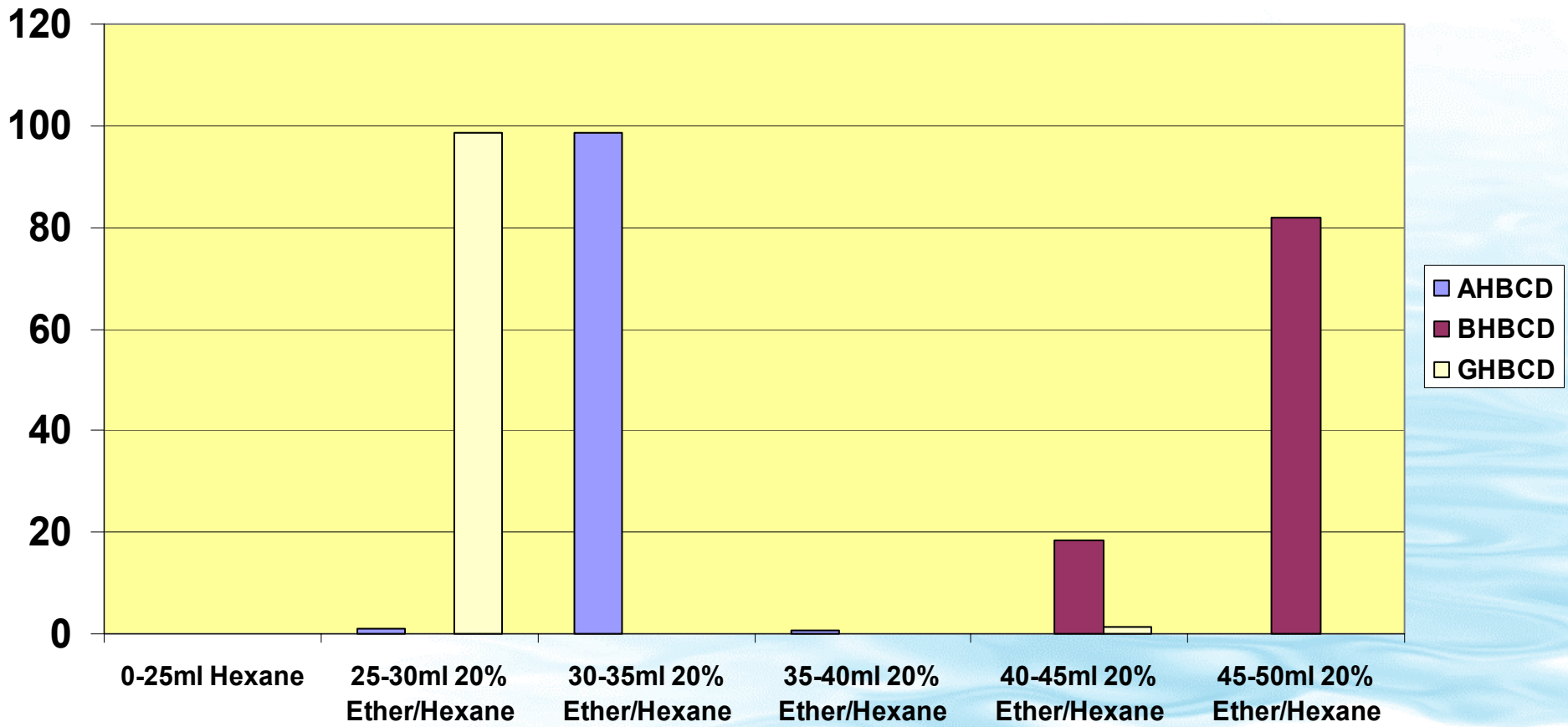
HBCD Recovery from Alumina (5% deactivated)



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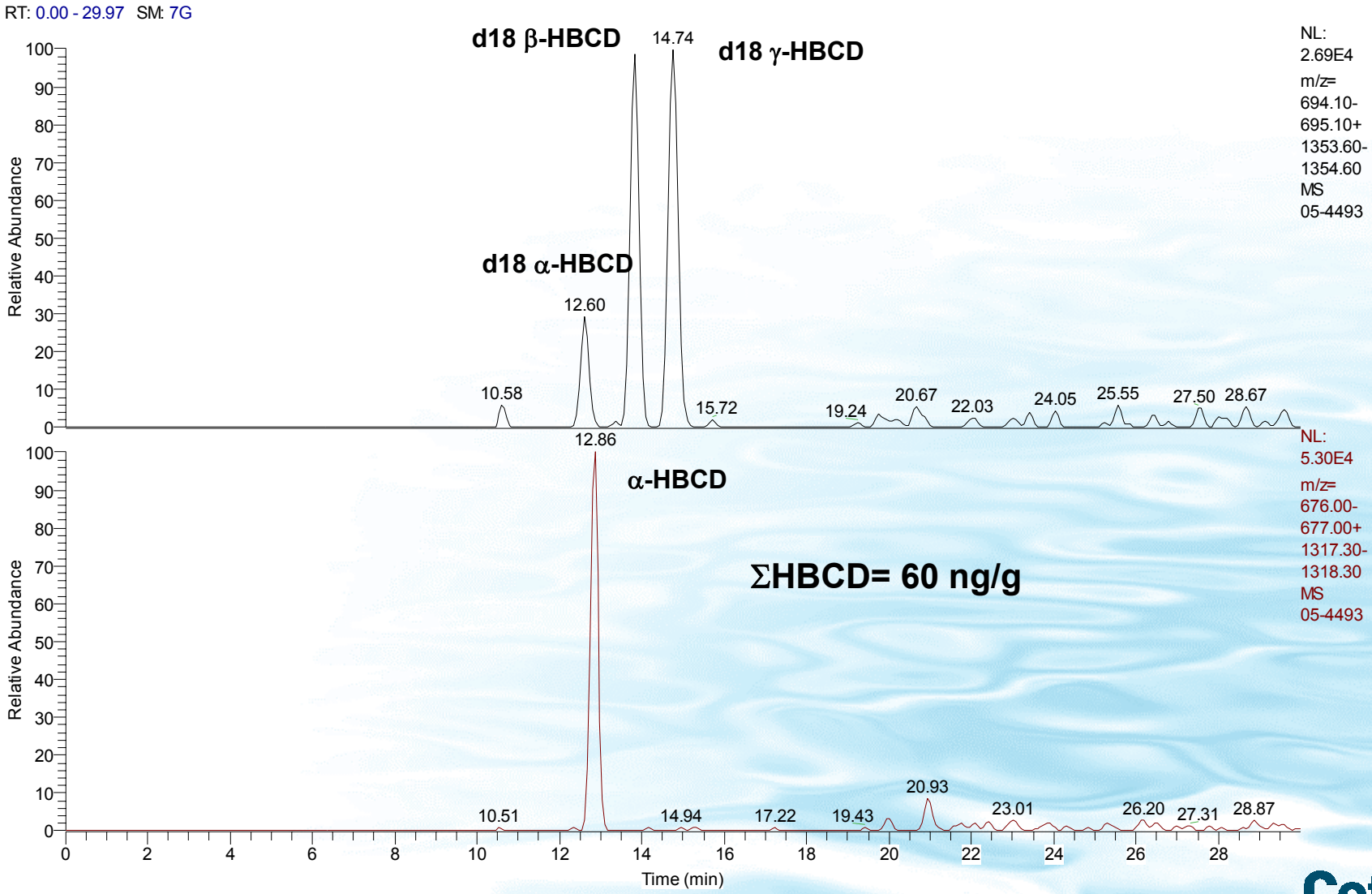
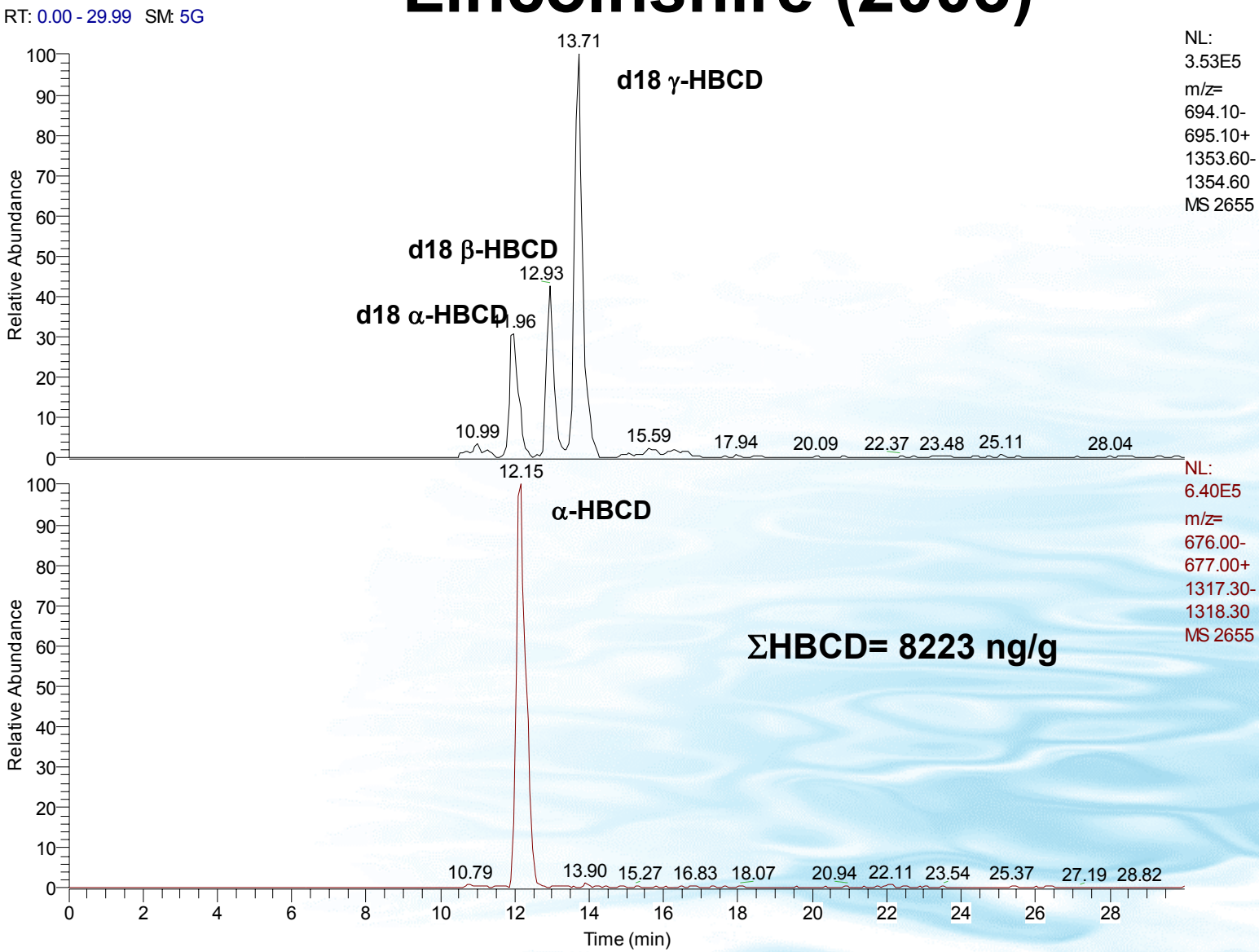
Eel sample from the river Thames (2005)



Stranded porpoise - Gibraltar point, Lincolnshire (2003)



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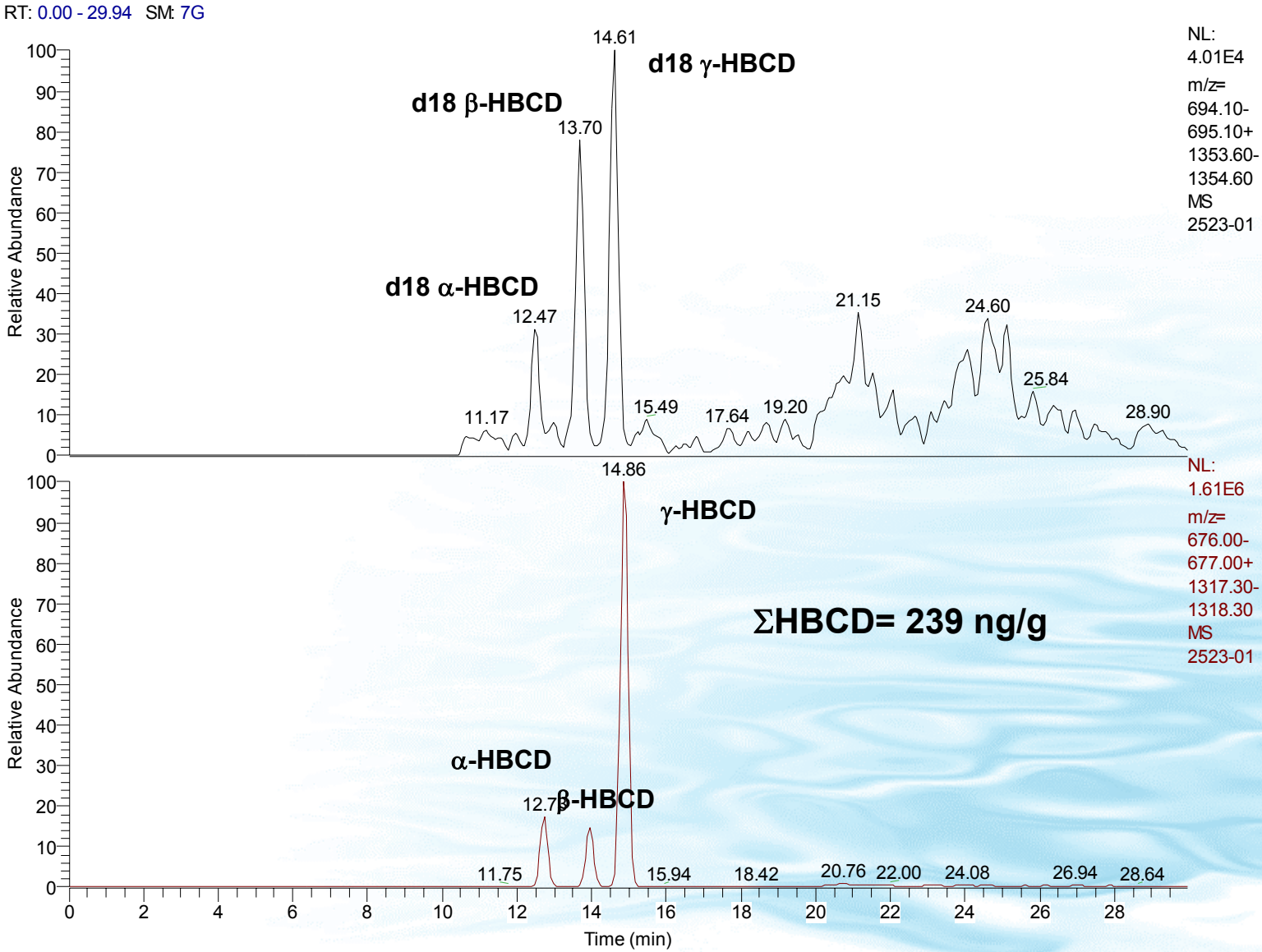
Cork Sewage Sludge sample



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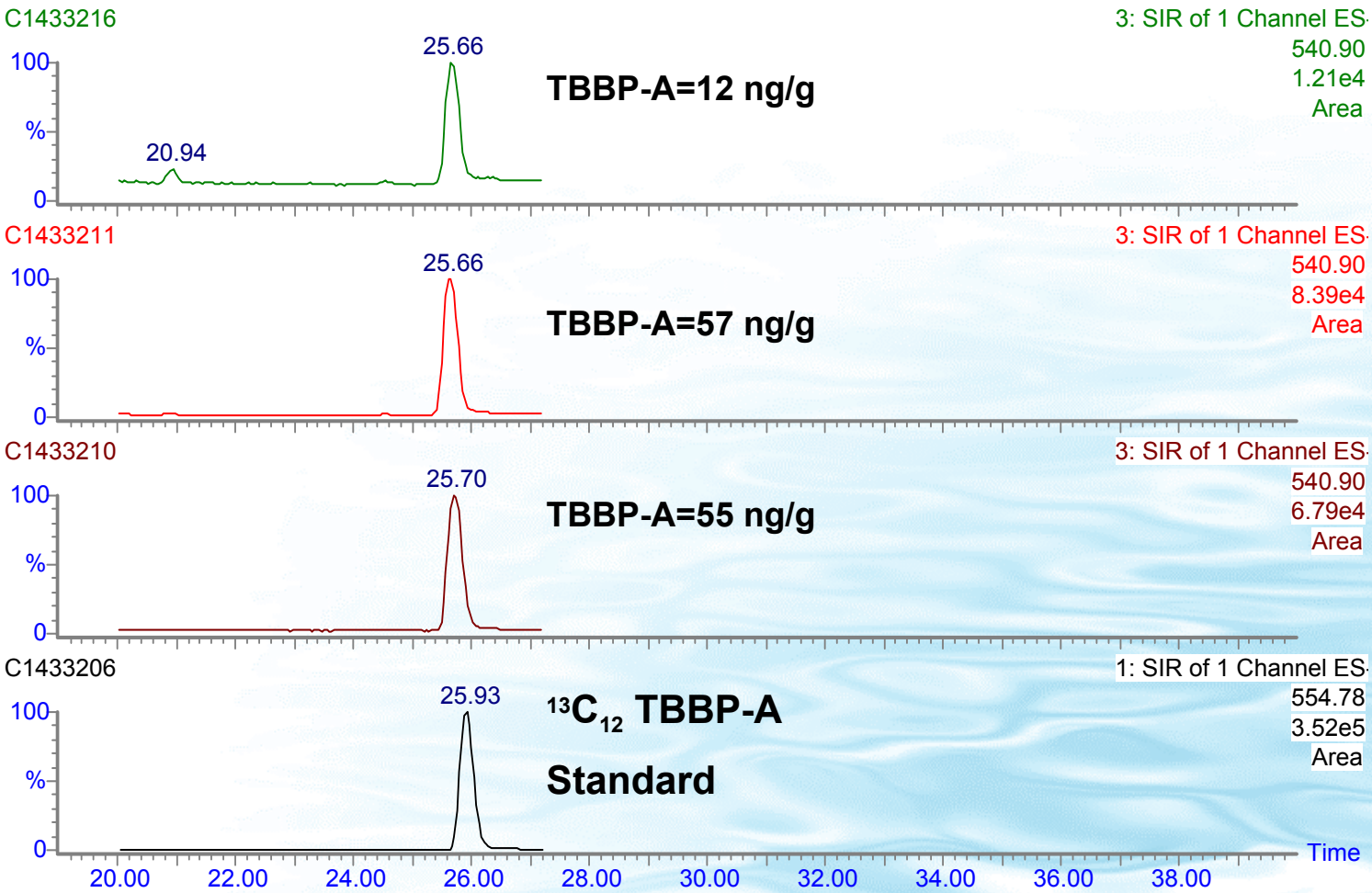
SELECTED CHROMATOGRAMS OF TEES SEDIMENT EXTRACTS - TBBP-A



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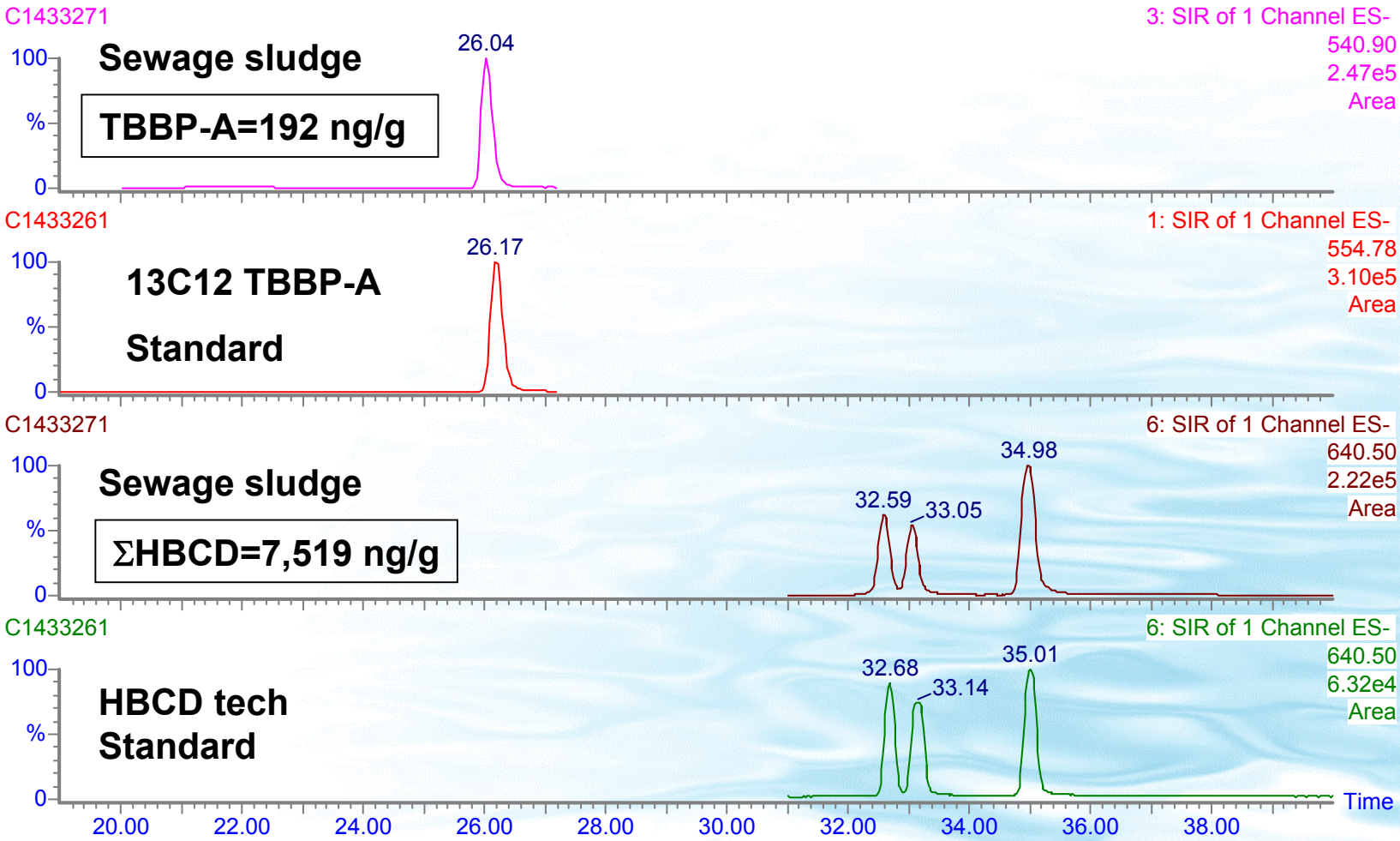
SEWAGE SLUDGE EXTRACTS



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Summary

- LC-MS in single quad , triple quad or ion trap configuration allows robust determination of TBBP-A & isomeric based HBCD measurement and is to be preferred over GC-MS techniques without isomeric resolution.
- The techniques presented has been fully validated and applied to a range of environmental matrices from biota (fish, shellfish, marine mammals) to abiotic materials (sediments and sewage sludge)

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