Determination of PBDEs using liquid chromatography - negative ionization-atmospheric pressure photoionisation-tandem mass spectrometry (LC-NI-APPI-MS/MS): validation and application to house dust.

Mohamed Abdallah & Stuart Harrad.

Division of Environmental Health and Risk Management.
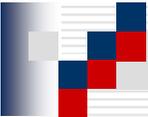
University of Birmingham

Adrian Covaci

Toxicological centre

University of Antwerp

UNIVERSITY OF BIRMINGHAM

Why?

➢ It's only possible to analyze higher PBDEs (7-10 Br atoms) accurately by GC-ECNI-MS while lower PBDEs (1-6 Br atoms) can be analyzed using GC-EI-MS.

➢ Analysis of BDE-209 requires columns with higher isothermal temperature limits, shorter lengths, and higher phase ratios to avoid on-column degradation.

➢ Additionally, optimized injection techniques (i.e. on-column injection, pressure pulses, split vent timing) are necessary to minimize the time spent in the heated zones of the injector to avoid thermal decomposition.

➢ It's very difficult to assess BDE-209 debromination - a key issue with respect to its environmental risk assessment.

UNIVERSITY OF BIRMINGHAM

Why?

➢Even using all the above, nona BDEs (especially 207 and 208) can't be quantified accurately. Problem worsens as the GC liner becomes dirty.


(A) Clean liner

(C) Very dirty liner

(B) Slightly dirty liner

UNIVERSITY OF BIRMINGHAM
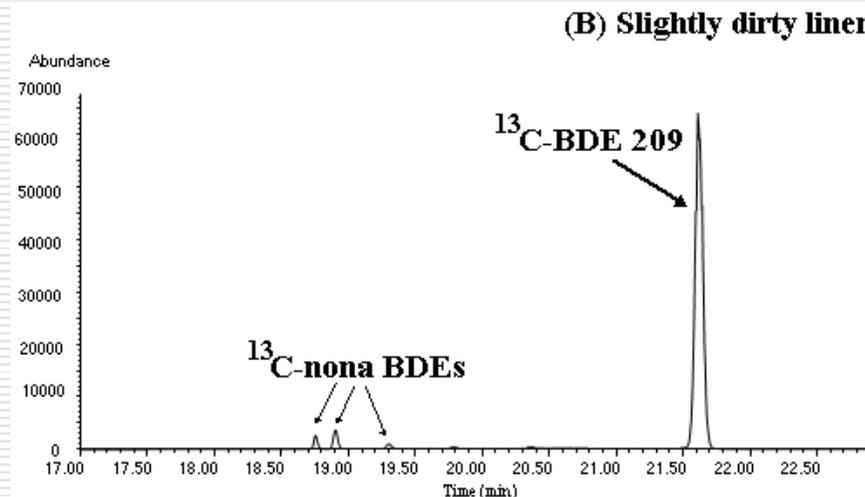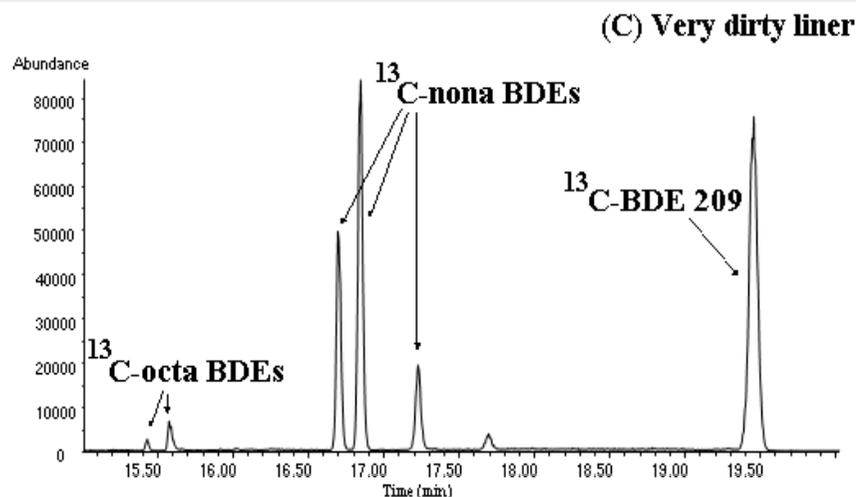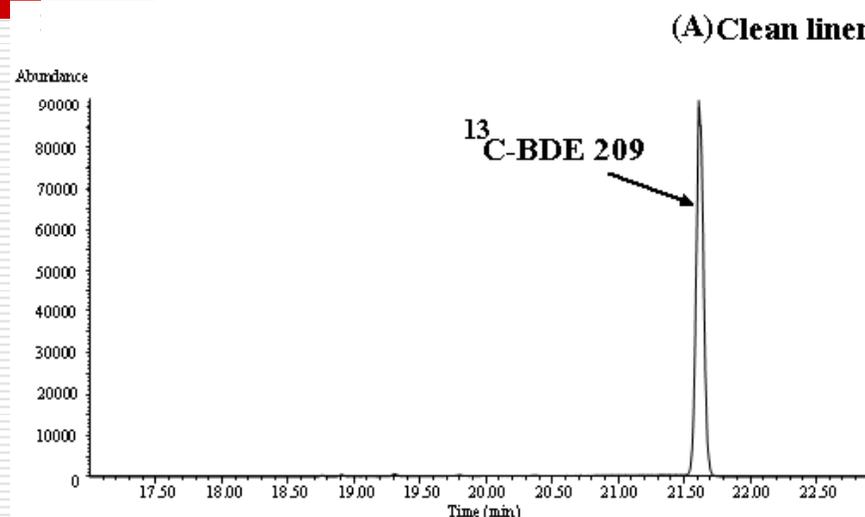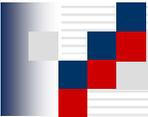
Background

➤ GC thermal decomposition issues (e.g. for HBCDs and PBDEs) are avoided in analysis using liquid chromatography.

➤ Debrauwer et al[1]. reported APPI ion source used in NI mode to give "promising" results in the analysis of tetra-deca BDEs using LC-MS/MS (quadrupole ion trap). No such results were obtained using ESI or APCI sources. However, no quantitative applications were made.

➤ Recently Lagalante and Oswald[2] reported on the use of LC-APPI-MS/MS for determination of 8 PBDEs in dust using an external standard method (i.e. no internal standard used).

[1] Rapid Commun. Mass Spectrom. 2006; 20: 2133–2142.

[2] Anal. Bioanal. Chem. 2008; 391:2249–2256.

UNIVERSITY OF BIRMINGHAM
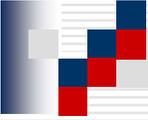
Aims

➢ Develop and validate a new analytical method using LC-NI-APPI-MS/MS for determination of 14 PBDEs in one run using ^{13}C-labelled isotopes as internal standards.

➢ Apply the developed method for determination of PBDEs in dust.

➢ Compare the results of the developed method to those obtained using a well established GC-ECNI-MS method.

UNIVERSITY OF BIRMINGHAM

Principles of Photospray Ionisation.

Ionisation energy supplied by a Krypton UV-lamp (10 eV).

➤ Photoionisation of Dopant (toluene-IE < 10 eV)

➤ Ionised dopant produces charged solvent clusters.

➤ Ionised dopant and/or clusters ionise the analyte molecules.
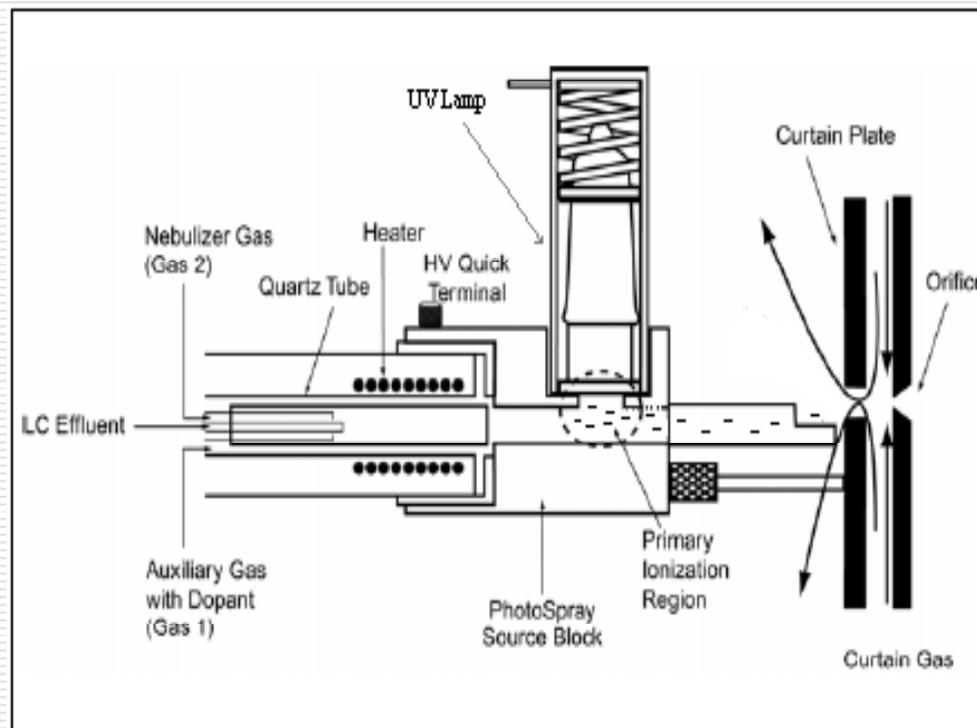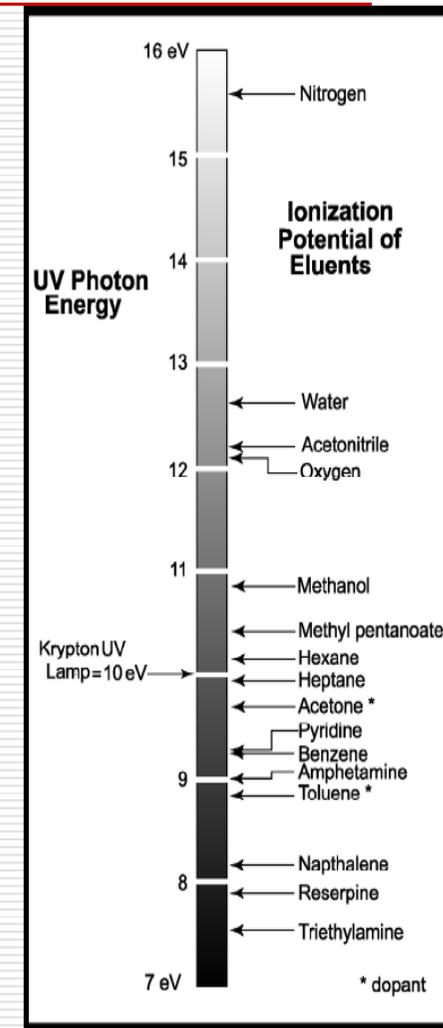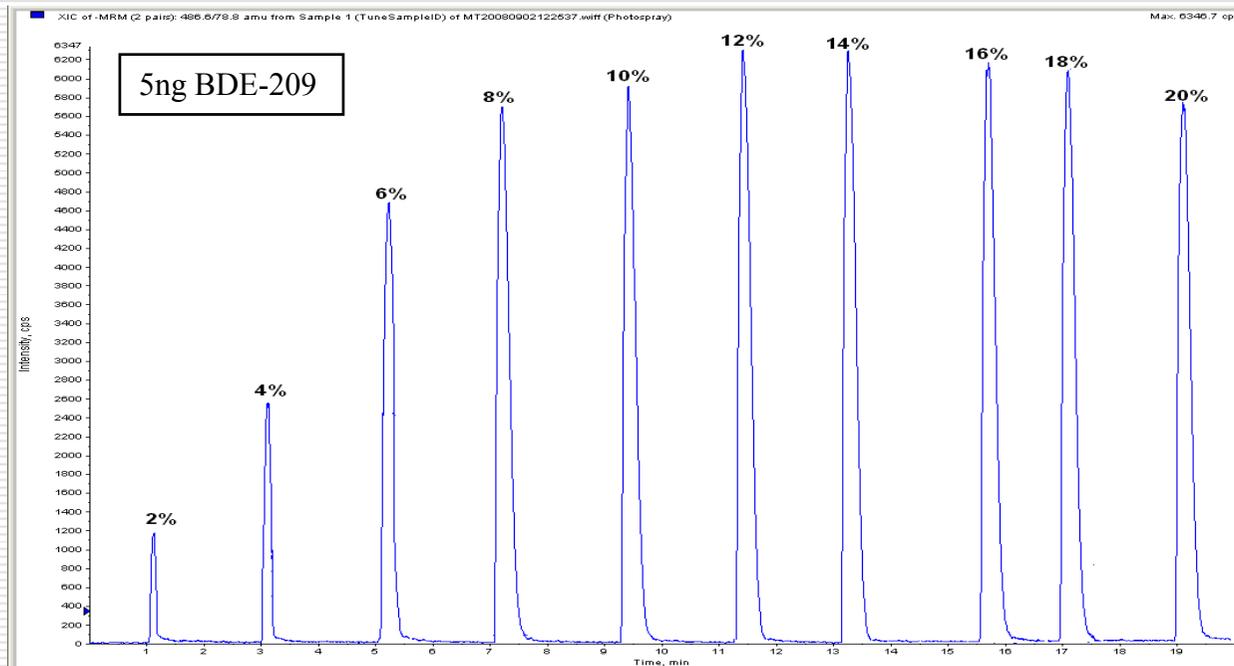

Ionization by UV light-Dopant needed.

UNIVERSITY OF BIRMINGHAM

Optimisation of NI-APPI parameters

1. **Dopant choice:**

   Toluene was preferred to Acetone due to its favourable ionization energy and lower proton affinity that facilitate proton transfer reaction with the analytes.
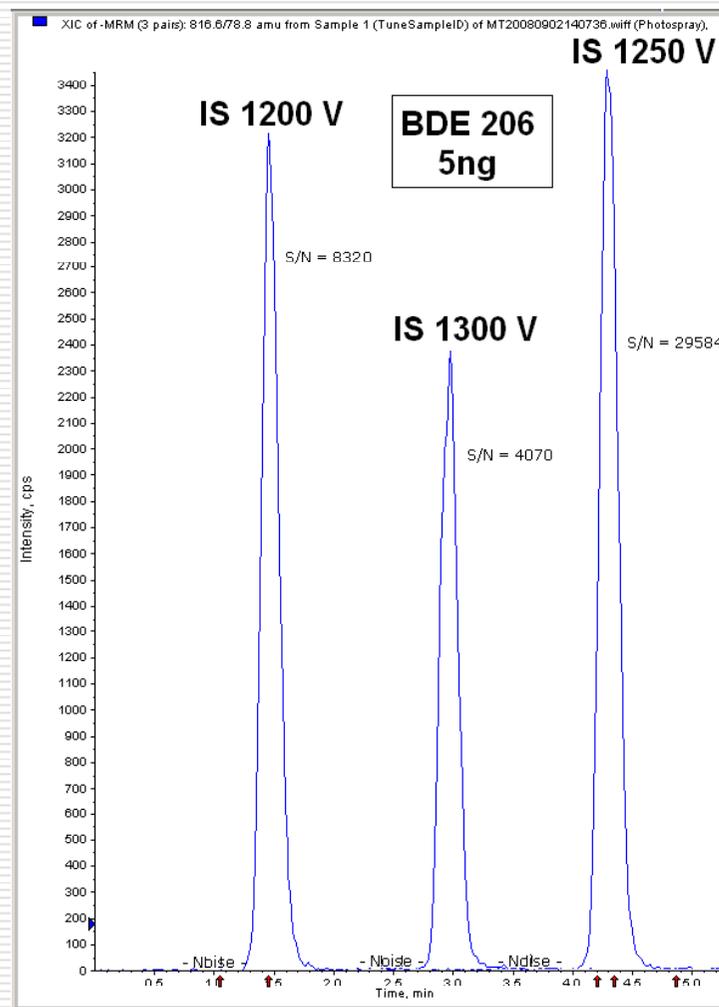
2. **Dopant percent:** 12%

Optimization of NI-APPI parameters (continued)

3. Source parameters:

Parameter	Value
Curtain gas	25 psi
Collision (CAD) gas	High
Ion transfer voltage (IS)	1250 V
APPI probe temperature	400 ºC
Probe nebuliser gas	60 psi
Auxillary gas	30 psi

XIC of -MRM (3 pairs): 816.6/78.8 amu from Sample 1 (TuneSampleID) of MT20080902140736.wiff (Photospray),

IS 1250 V

IS 1200 V

BDE 206
5ng

S/N = 8320

IS 1300 V

S/N = 29584

S/N = 4070

Optimization of NI-APPI parameters (continued)

4. **Multiple reaction monitoring (MRM):**

a) **[M-Br+O]⁻** as precursor ion for all the studied BDEs except BDE 209, ^{13}C-BDE-209 and BDE 208 where a higher intensity ion cluster was observed for **[C₆Br₅O]⁻** ions.
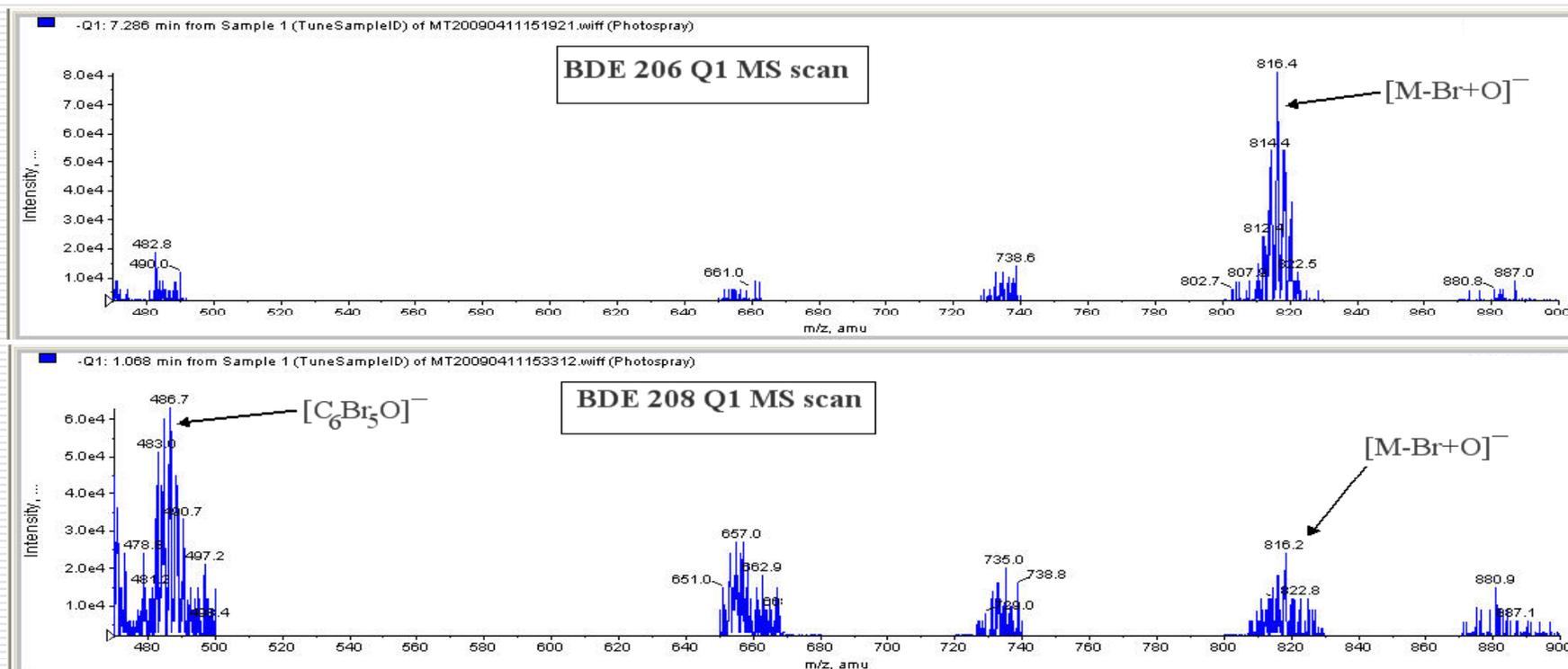
Optimization of NI-APPI parameters (continued)
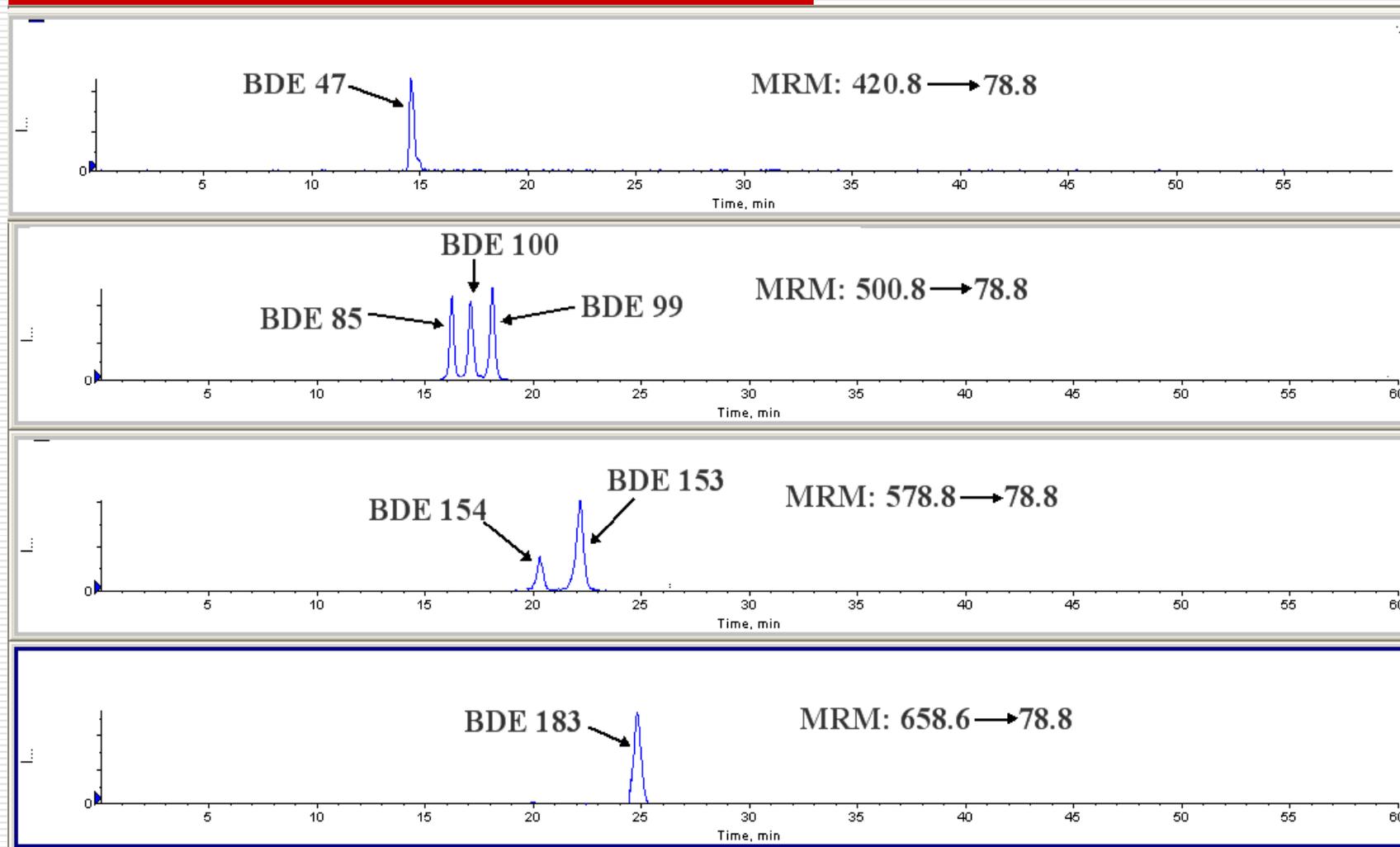
4. **Multiple reaction monitoring (MRM):**

b) $[M-Br+O]^-$ can fragment to produce $[M'-HBr]^-$ or $[M'-Br_2]^-$ ions. However $[Br]^-$ as fragment ions affords maximum sensitivity.

UNIVERSITY OF BIRMINGHAM
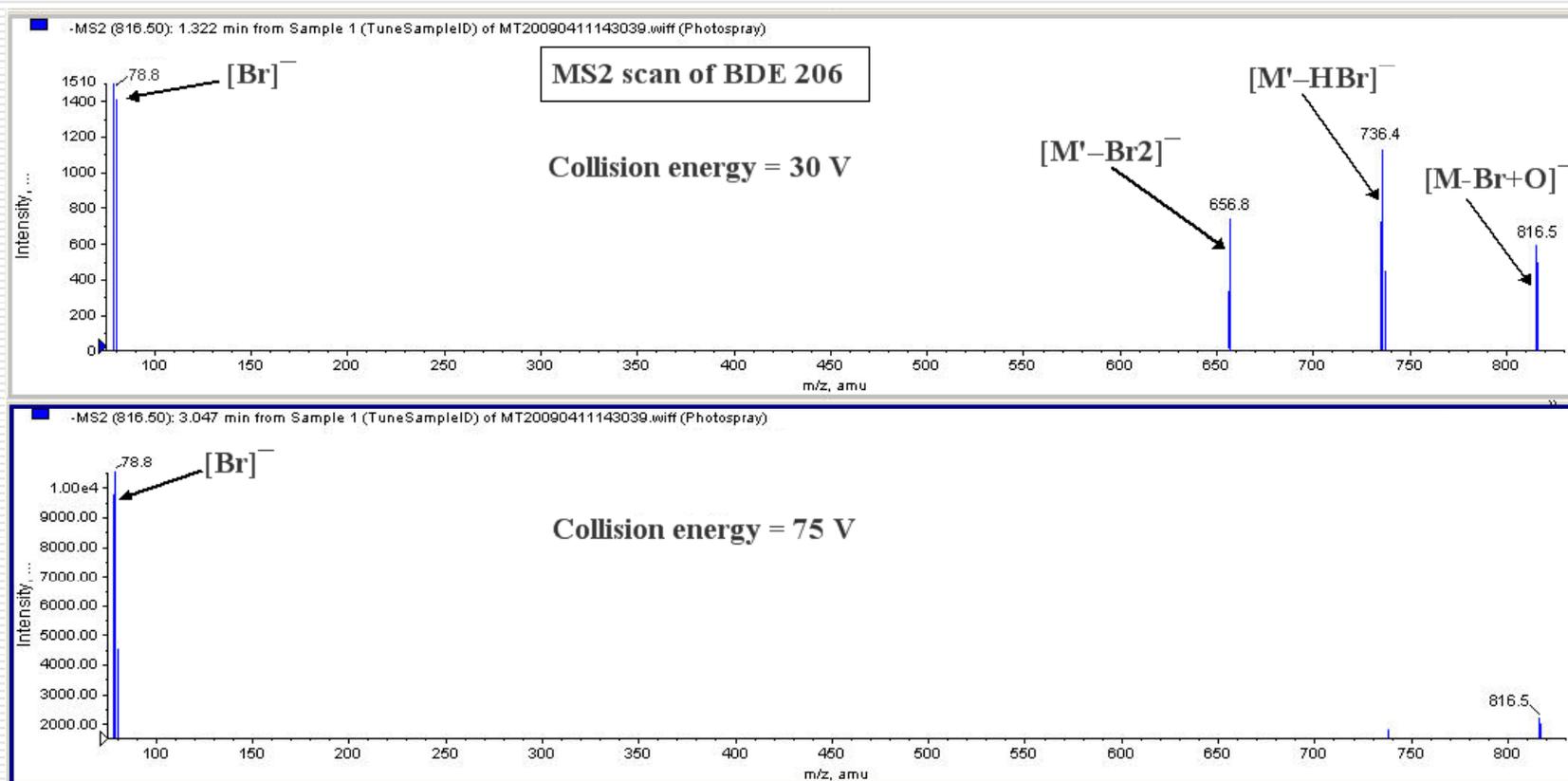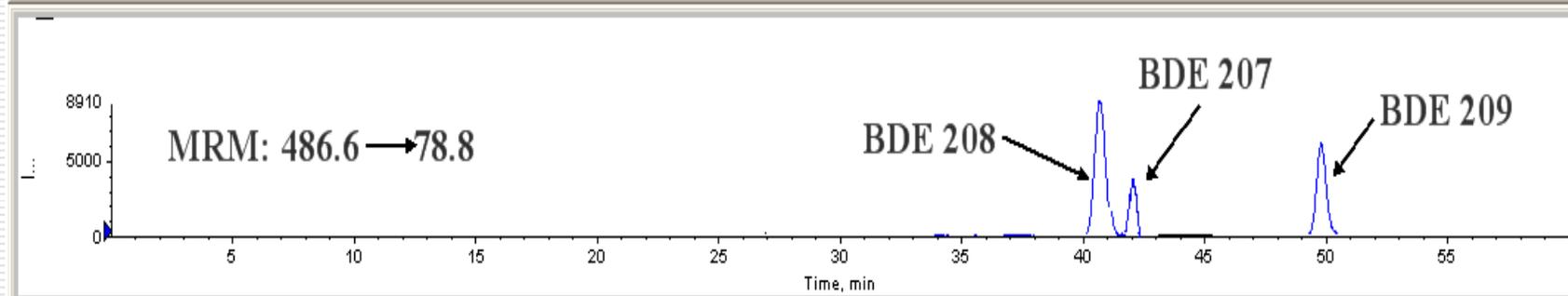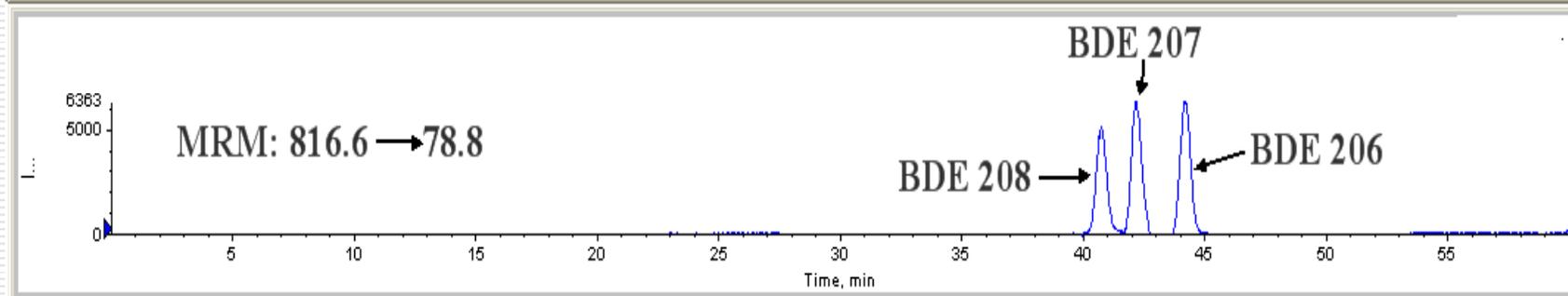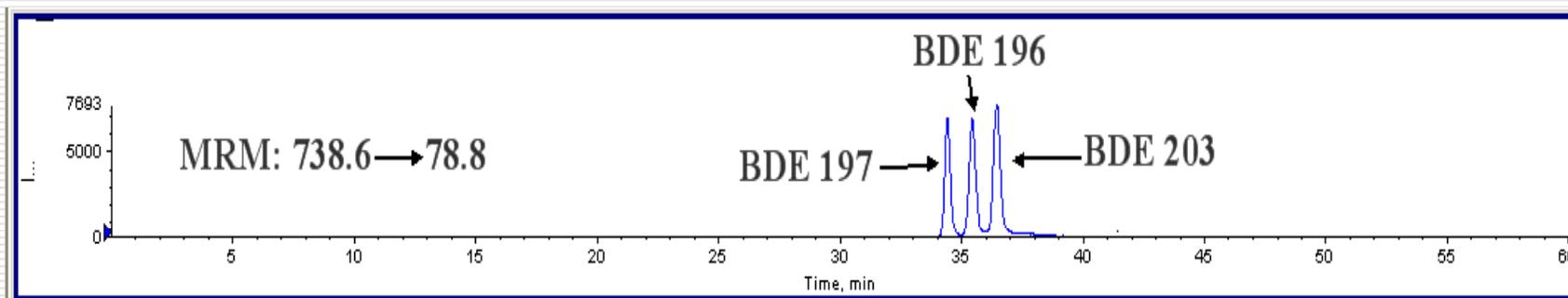
Compound Specific parameters

BDE #	Precursor (m/z)	Fragment (m/z)	DP (V)	CE (V)	Linearity (0.02-5 ng/µl)	LOD on column (pg)
47	420.8	78.8	-12	-55	$r^2 = 0.997$	29.8
85	500.8	78.8	-8	-60	$r^2 = 0.996$	26.1
99	500.8	78.8	-11	-60	$r^2 = 0.996$	12.3
100	500.8	78.8	-14	-63	$r^2 = 0.998$	15.2
153	578.8	78.8	-8	-65	$r^2 = 0.996$	18.3
154	578.8	78.8	-8	-65	$r^2 = 0.997$	20.1
183	658.6	78.8	-12	-61	$r^2 = 0.998$	16.2
196	738.6	78.8	-14	-71	$r^2 = 0.996$	15.7
197	738.6	78.8	-12	-71	$r^2 = 0.997$	14.9
203	738.6	78.8	-15	-71	$r^2 = 0.997$	14.2
206	816.6	78.8	-11	-75	$r^2 = 0.996$	13.6
207	816.6	78.8	-12	-69	$r^2 = 0.996$	13.3
208	486.6	78.8	-10	-71	$r^2 = 0.997$	13.8
209	486.6	78.8	-8	-75	$r^2 = 0.996$	12.7

Chromatographic separation (RP-C$_{18}$- 25 x 4.6 mm; 3μm)

UNIVERSITY OF
BIRMINGHAM

Chromatographic separation (RP-C$_{18}$- 25 x 4.6 mm; 3μm)

UNIVERSITY OF
BIRMINGHAM

Application to dust sample

~0.2 g dust (25-500 μm)

+ ^{13}C-IS

PLE extraction

Concentration

Acid Silica clean-up

+ RDS

LC-APPI-MS/MS analysis

- ^{13}C-BDE 47 (25 ng; 432.6 → 78.8)
- ^{13}C-BDE 99 (25ng; 512.4 → 78.8)
- ^{13}C-BDE 153 (25ng; 590.6 → 78.8)
- BDE 128 (25ng; 578.6 → 78.8)
- ^{13}C-BDE 209 (75ng; 494.7 → 78.8)

- 13C-BDE 100 (25 ng; 512.6 → 78.8)

UNIVERSITY OF
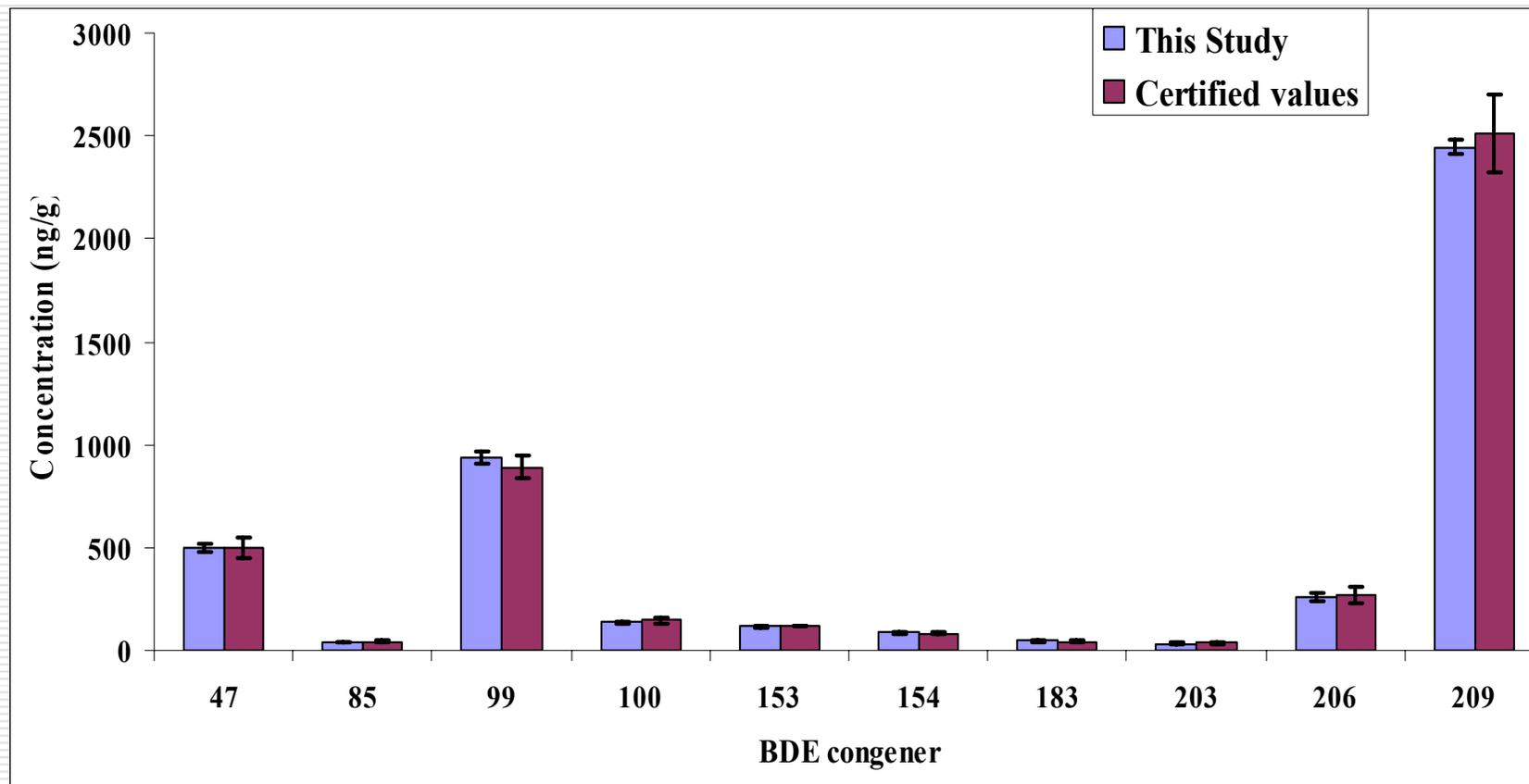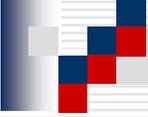BIRMINGHAM
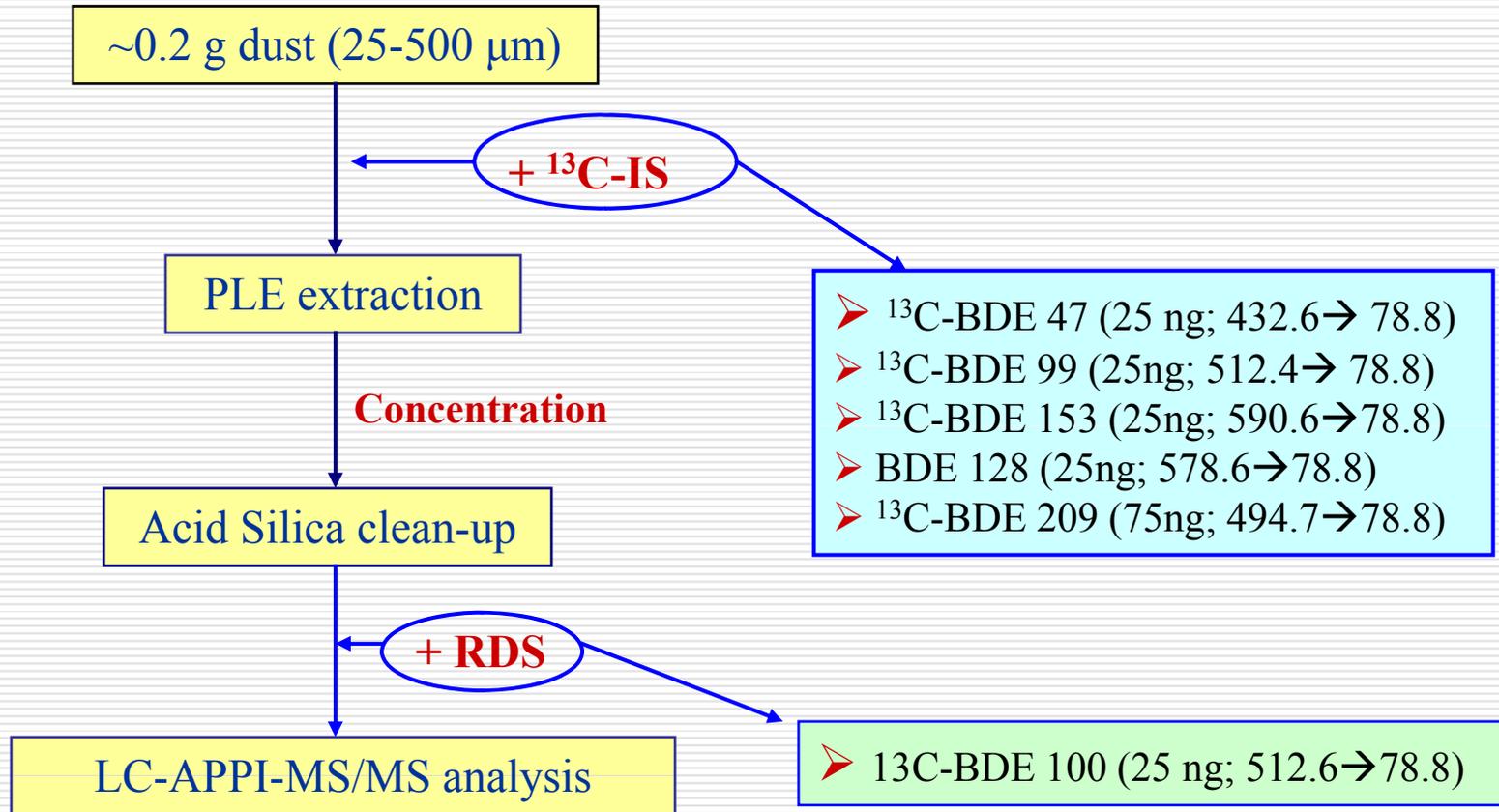
Results of NIST SRM2585

Comparison of certified PBDE concentrations (ng/g) for SRM2585 with the average values (n=5) obtained in this study.

UNIVERSITYOF
BIRMINGHAM

Quality assurance/ quality control

➢ Internal standard recoveries (71-87%).

➢ Repeatability (n=5): RSD <5%.

➢ Precision (n=5): RSD <10%.

➢ Inter- and Intra-day variability of results : RSD < 5%.

➢ Method blanks (i.e. no dust): None of the studied PBDEs above LOD.

➢ Field blanks (i.e. 0.2g Na_2SO_4 vaccumed instead of dust): None of the studied PBDEs above LOD.

UNIVERSITY OF BIRMINGHAM

Comparison with GC-ECNI-MS results

Statistical comparison between PBDEs concentrations obtained by GC-ECNI-MS and LC-APPI-MS/MS.

Statistical parameter* (n=25)	BDE 183	BDE 197	BDE 203	BDE 196	BDE 209
t-test** paired for means	1.85	1.54	1.96	1.95	0.44
F-test*** for variance	0.41	0.43	0.38	0.39	0.26
Pearson correlation (r)	0.998	0.999	0.997	0.998	0.999
Slope GC vs LC	1.03	1.03	1.06	1.05	0.99

* Confidence level set at 95%
** t-critical = 2.06
*** F-critical = 0.52

UNIVERSITY OF BIRMINGHAM

Investigation of BDE 209 debromination in car dust.

- 208/209 = 1.46%.

- Σ nona BDEs/209 = 4.62%

- detection of octa BDE 202.

- 197/201 = 1.72 %.

UNIVERSITY OF
BIRMINGHAM
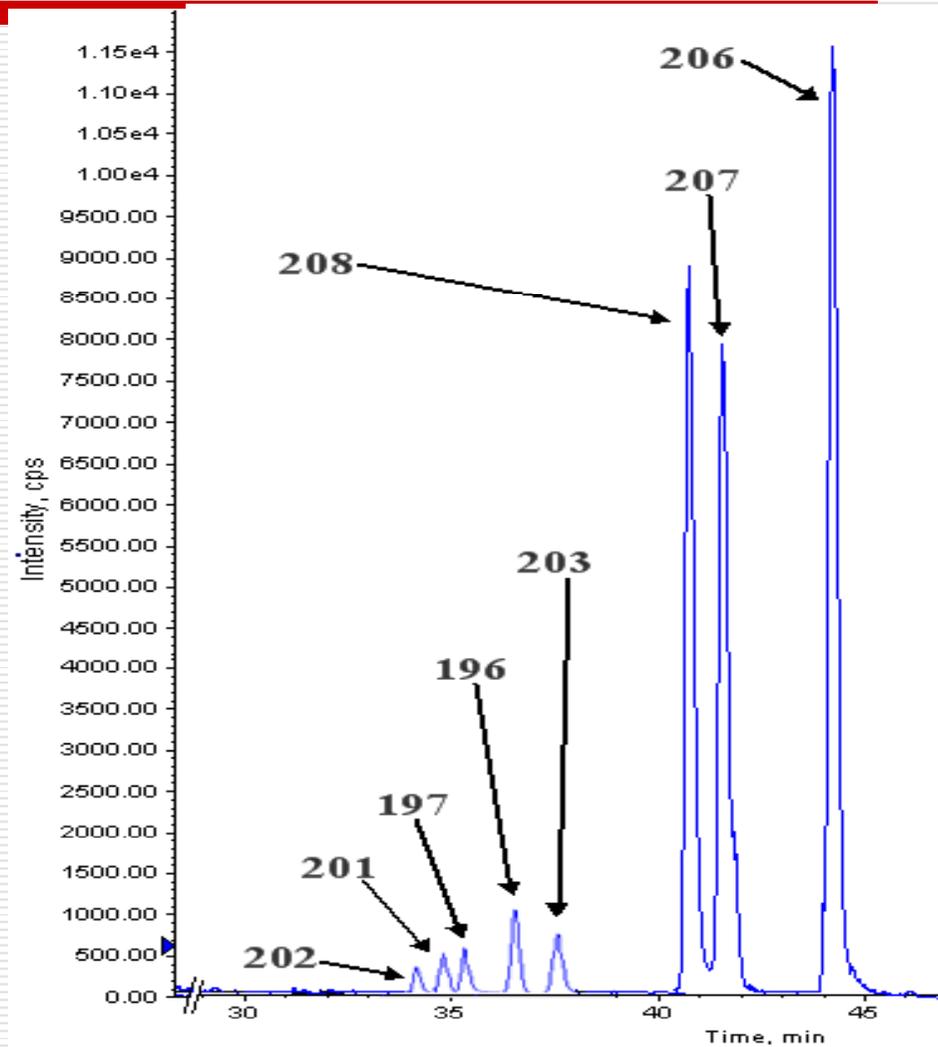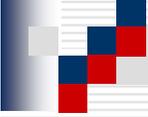
Conclusions

➢ A new isotope-dilution method was developed for quantitative determination of 14 tetra-deca BDEs (in one run) using LC-NI-APPI-MS/MS and was applied successfully to house dust.

➢ The accuracy of the method was validated using NIST SRM2585 and favourable results were obtained. Excellent precision was obtained due to the use of isotopically labelled ^{13}C-IS that can compensate for any instrumental variability in response during the course of analysis.

➢ No thermal degradation problems of the higher brominated compounds were encountered.

➢ The method proved useful for investigation of BDE 209 debromination in house dust.

UNIVERSITY OF BIRMINGHAM
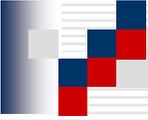
ACKNOWLEDGEMENT

➢ *Mohamed Abdallah* *gratefully acknowledges the Egyptian ministry of higher education for funding his PhD studentship*

➢ *Adrian Covaci* *acknowledges the Research Scientific Foundation of Flanders (FWO) for postdoctoral fellowship.*

THANK YOU

UNIVERSITY OF BIRMINGHAM

PBDEs ^{13}C-Internal Standard

UNIVERSITY OF
BIRMINGHAM