Optimization of Analytical Conditions for Improved Sensitivity in the Analysis of Deca-BDE on a DFS High Resolution GC/HRMS system

Dirk Krumwiede
1.) Analysis of PBDE
   – special aspects and requirements

2.) Parameter optimization for the analysis of the Deca–BDE using SSL and PTV injectors

3.) A dual column setup combining highest sensitivity for Deca–BDE and good separation efficiency for all other congeners
Part 1: Analysis of PBDE
What is special with PBDE analysis?

- $M^+$ peaks are not the most intense ions for all bromination degrees

- A high mass range has to be covered: (m/z 248 to 799)
  - large electric jumps for window defining runs
  - finding suitable reference masses for the high mass range

- adaptation of temperatures for the high boiling PBDE:
  
  Injector, oven, transfer line and ion source temperatures

- Deca–BDE is thermolabile and high boiling
Full scan results comparing $M^+$ and $[M-2Br]^+$ isotope peak intensities
Mass/Intensity List for PBDE
relative intensities of $M^+$ versus $[M-2Br]^+$

<table>
<thead>
<tr>
<th># Br</th>
<th>m</th>
<th>m+2</th>
<th>m+4</th>
<th>m+6</th>
<th>m+8</th>
<th>m+10</th>
<th>m+12</th>
<th>Int.</th>
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<th>$M^+$</th>
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<th>$M-2Br^+$</th>
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<td>801,331</td>
<td>78</td>
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Mass Chromatogram of a 6 window run
Separation by bromination degree on a 15 m column

Tri-BDE
405.803

Tetra-BDE
485.711

Penta-BDE
563.622

Hexa-BDE
483.696

Hepta-BDE
561.606

Deca-BDE
799.333
CS3-E: BDE 2-10 brominated on 15 m column / SSL inj.

Instrumental Parameters:
**DFS:**
EI ionization, 40 eV, source 290°C
R = 10,000 (10 % valley)

**Trace GC Ultra:**
DB5MS (15/0.25/0.1)
constant flow 1 ml/min
splitless injection 1.5 min, 270°C
100°C/ min (2) - 205°C/15 – 330°C/6
transfer line temperature 280°C

2-BDE / 3-BDE
4-BDE / 5-BDE
6-BDE
7-BDE
8-BDE
9-BDE
10-BDE
CS3-E: BDE tetra separation on 15 m column / SSL inj.

1 pg

RT: 10.99 - 12.89

RT: 11.59
AA: 26947

RT: 11.27
AA: 17923

RT: 11.95
AA: 12721

RT: 12.52
AA: 24820

RT: 11.59
AA: 990

RT: 11.26
AA: 23821

RT: 11.95
AA: 19998

RT: 12.51
AA: 35255

NL:
1.39E4
m/z= 483.6642-483.7610 MS ICIS dk_PBDEdemo o14

NL:
2.05E4
m/z= 485.6620-485.7592 MS ICIS dk_PBDEdemo o15

NL:
1.23E6
m/z= 495.7033-495.8025 MS ICIS dk_PBDEdemo o15

NL:
1.83E6
m/z= 497.7010-497.8006 MS ICIS dk_PBDEdemo o15

NL:
1.39E4
m/z= 483.6642-483.7610 MS ICIS dk_PBDEdemo o14

NL:
2.05E4
m/z= 485.6620-485.7592 MS ICIS dk_PBDEdemo o15

NL:
1.23E6
m/z= 495.7033-495.8025 MS ICIS dk_PBDEdemo o15

NL:
1.83E6
m/z= 497.7010-497.8006 MS ICIS dk_PBDEdemo o15

100 fg (1/10 diluted)

RT: 10.99 - 12.89

RT: 11.31
AA: 1715

RT: 11.64
AA: 2454

RT: 12.56
AA: 2121

RT: 11.99
AA: 1232

RT: 11.69
AA: 1783

RT: 11.36
AA: 3833

RT: 11.59
AA: 39681

RT: 12.51
AA: 35255

RT: 11.63
AA: 23821

RT: 12.56
AA: 3200

RT: 11.90
AA: 18937

RT: 11.99
AA: 12721

RT: 12.52
AA: 24820

RT: 11.62
AA: 23821

RT: 12.56
AA: 35255

RT: 11.99
AA: 1232

RT: 11.63
AA: 3833

RT: 11.36
AA: 23821

RT: 12.56
AA: 35255

NL:
1.28E3
m/z= 483.6642-483.7610 MS ICIS dk_PBDEdemo o14

NL:
1.96E3
m/z= 485.6620-485.7592 MS ICIS dk_PBDEdemo o15

NL:
1.09E5
m/z= 495.7033-495.8025 MS ICIS dk_PBDEdemo o15

NL:
1.59E5
m/z= 497.7010-497.8006 MS ICIS dk_PBDEdemo o14
DFS – PBDE analysis in low fg range (like PCB, dioxin/furans)
Application Notes on PBDE

DFS - Analysis of Brominated Flame Retardants with High Resolution GC/MS

Introduction
Polybrominated diphenyl ethers (PBDEs) are among the most important and widely used flame retardants in a variety of different industrial products. They are found worldwide in matrices, moving them into the focus of recent legislation banning certain PBDE congeners (Directive 2003/11/EC) for the member states of the European community.

As a result of analysis of PBDEs, a new area of interest has emerged due to their known toxicity. Similar to dioxins/furans and PCBs, PBDEs are polychlorinated biphenyls, polybrominated diphenyl ethers that exist in a wide range of congeners (209) (Figure 1).

Figure 1: PBDE chemical structure, here Deca-BDE

The most efficient technique by far for three application areas is high resolution GC/MS using isotope dilution technique for quantitation with highest precision and significance.
Part 2: Optimized analysis for Deca–BDE using SSL and PTV injectors

Parameters influencing sensitivity for Deca–BDE:

- liner type and diameter
- injector type SSL ↔ PTV
- pressure pulse / surge
- splitless time

- oven program
- column length
- column flow

- source temperature
- ionization energy (eV)
SSL injector: splitless time and pressure pulse

SSL injector with 5 mm I.D. liner

Too low splitless time causes significant loss of intensity for Deca-BDE.

DB5 15 m; 0.25mm; 0.1 um film, 1 ml/min He

Hepta-BDE

Deca-BDE

Splitless time: 0.7 min

Splitless time: 1.0 min

Splitless time: 1.6 min

Pressure pulse: 20 psi
SSL or PTV for Deca-BDE analysis? Decrease of sensitivity over time

SSL injector: first analysis

Hepta-BDE

Deca-BDE

SSL injector: later analysis

SSL injector: next day

PTV injector: same sensitivity after 30 runs

DB5 15 m; 0.25mm; 0.1 um film, 1 ml/min He

SSL injector with 5 mm I.D. liner

PTV injector with 2 mm I.D. metal liner

⇒ PTV offers better sensitivity and stability for Deca - BDE analysis.
Column length and Deca–BDE response

DB5 15 m / 30 m; 0.25mm; 0.1 um film;
Flow: 0.8 ml/min He

Deca - BrDPE

Hepta - BrDPE

120 °C (2 min) –
15 °C/min -> 205 °C
-6 °C/min -> 330 °C

Hepta - BrDPE

Deca - BrDPE

120 °C (2 min) –
20 °C/min -> 230 °C
-6 °C/min -> 330 °C

NL:
8.65E3
m/z=
561-563 F:
+ m/z=
798-800 MS
ICIS
pbrdpe_614

NL:
9.26E3
m/z=
561-563 F:
+ m/z=
798-800 MS
ICIS
pbrdpe_611
Oven program and Deca–BDE response
same 15 m column / two different oven programs

Hepta - BrDPE

Deca - BrDPE

DB5 15 m; 0.25mm;
0.1 um film; 1 ml/min He

120 °C (2 min) –
15 °C/min -> 205 °C
-6 °C/min -> 268 °C
-40 °C/min -> 330 °C

120 °C (2 min) –
15 °C/min -> 205 °C

-6 °C/min -> 330 °C
Carrier gas flow and Deca–BDE response

DB5 15 m; 0.25mm; 0.1 um film

Oven program:
120 °C (2 min) –
15 °C/min -> 205 °C
- 6 °C/min -> 330 °C

⇒ Nearly a factor 2 between low and high flow setting.
Deca – BDE optimized sensitivity on a 15 m DB5
1 pg Deca (1 ul 1:10 diluted CS1 standard)

DB5 15 m; 0.25mm; 0.1 um film, 1 ml/min He

PTV injector:
Glas liner 1 mm I.D.; 120 °C (0.2) – 8 °C/min – 320 °C; 1 min splitless

Oven:
120 °C (2 min) – 15 °C/min -> 205 °C
- 6 °C/min -> 330 °C

Source / transfer line:
280 °C

Lock / Cali mass (PFK):
754.95; 766.95
Parameters influencing sensitivity for Deca–BDE:

- liner type and diameter
- injector type SSL ↔ PTV
- pressure pulse / surge
- splitless time

- oven program
- column length
- column flow

- source temperature
- ionization energy (eV)

- Rule of thumb: Elution temperature for Deca-BDE should be as low as possible
  ➔ 270 – 280 °C
  ➔ same as for dioxins (35 / 40 eV)

- No significant difference from liner I.D.
- PTV is preferable for Deca-BDE.
- Pressure pulse useful for SSL / PTV
- Assure sufficient splitless time
Part 3: A dual column setup for PBDE analysis combining sensitivity and separation efficiency

Following some minor adaptations of source and transferline parts two columns were installed into the same GC (two injectors) and source using a Y-shaped dual column adaptor.

This setup allows to inject from the same vial in subsequent runs on two different columns.

For PBDE:

SSL injector $\rightarrow$ 30 m (0.25; 0.1) DB5 column

PTV injector $\rightarrow$ 6 m (0.20; 0.1) DB5 column

Trace GC (dual injector)  Transfer line with 2 column adaptor
Dual column setup for PBDE analysis
Typical chromatogram
Separation on DB5 (here 30 m) by bromination degree

Instrumental Parameters:
DFS:
EI ionization, 40 eV, source 270°C
R = 10,000 (10 % valley)

Trace GC Ultra:
DB5MS (30/0.25/0.1)
constant flow 0.8 ml/min
splitless injection, 280°C
120°C/ min - 230°C/20 – 330°C/6
transfer line temperature 280°C

Di-BDE
327.892

Tri-BDE
405.803

Tetra-BDE
485.711

Penta-BDE
563.622

Hexa-BDE
483.696

Hepta-BDE
561.606
Deca–BDE response on 15m / 6m column (15m – single column / 6 m – dual column setup)

DB5 15 m; 0.25mm; 0.1 um
DB5 6 m; 0.2mm; 0.1 um

120 °C (2 min) – 15 °C/min -> 205 °C
-6 °C/min
-> 330 °C (15 m)
-> 305 °C (6 m)

For display:
4.48 min offset on Ret. Times of 6 m Chromatogram
Deca–BDE response on 15m / 6m column
(15m – single column / 6 m – dual column setup)

DB5 15 m; 0.25mm; 0.1 um
DB5 6 m; 0.2mm; 0.1 um

120 °C (2 min) –
15 °C/min -> 205 °C
-6 °C/min
-> 330 °C (15 m)
-> 305 °C (6 m)

For display:
4 min offset on Ret. Times
of 6 m Chromatogram
Deca-BDE linearity on 6m column (dual column setup) / 0.25, 0.5, 1, 10 pg native Deca

Area (QM) vs. Specified Amount

CF = 0.999830
Thermal degradation products of Deca-BDE
100 pg native only BDE-209 was injected

Optimized splitless injection
for minimized thermal decomposition.

Injector temp.: 250 °C
Pressure pulse: 150 kPa

1.6E4 deca peak height

Enforced / increased thermal decomposition
Injector temp.: 310 °C

5.04E3 deca peak height
Reducing thermal degradation of Deca-BDE in the injector comparison Deca-BDE in splitless / PTV on column injection

**DB5 15 m; 0.25mm; 0.1 um film**
Flow: 1 ml/min He
Same oven program:
120 °C (2 min) – 15 °C/min -> 205 °C
- 6 °C/min -> 330 °C

### Splitless injection
290 °C
no precolumn

### PTV on column like injection
80°C(0.2)-5°C/min-
320 °C (20)
precolumn (1.8*0.53)
Conclusions

- estimation for Deca-BDE sensitivity with SSL/PTV:
  
  - 30m: \(> 10 \text{ pg}\)
  - 15m: ca. 1 – 5 pg
  - 6m: ca. 0.1 – 0.5 pg

- dual column setup: combining
  a.) good separation efficiency
  b.) sensitivity for Deca-BDE

- Deca thermal decomposition due to oven temperature probably reduced to the limit on 6 m column

- on column injection reduces decomposition in the injector strongly
ThermoFisher Scientific
DFS
High Resolution Sector Field MS

PTV LVI for POPs analysis
80 ul dioxins/furans with PTV LVI
Recovery and peakshape

PTV LVI: 80 ul CS3 (1/80)

PTV splitless: 1 ul CS3 (undil.)

Overlay
80 ul dioxins/furans PTV LVI on samples
peregrine falcon egg extract / eal extract - tcdd

Ratio mass
Quan mass

PTV LVI: 80 ul sample extract (1/80)

splitless (SSL): 1 ul sample extract (undil.)

Ratio mass
Quan mass

PTV LVI: 80 ul sample extract (1/40)

splitless (SSL): 2 ul sample extract (undil.)
80 ul and 1 ul calibration curve: tcdf
average response EPA 1613 standards: CSL, CS 0.5, CS1 – CS4

80 ul calibration curve

1 ul calibration curve
ThermoFisher Scientific
DFS
High Resolution Sector Field MS

DFS dual data acquisition
DFS – Dual data option
up to two fold increased sample throughput

- 2 GCs attached to 1 DFS
- 1 extra wide Autosampler covering both GC’s
The standard way to inject samples single GC system: sequence of 3 injections

One GC

Wasted time

injection

Wasted time

injection

Wasted time

injection
Dual GC system the standard way to inject samples same time for 3 injections as on 1 GC system
Dual Data acquisition – dual GC system
how to achieve higher throughput

⇒ Staggered injection !!:
Inject next sample on GC2 already during elution of peaks on GC 1

Red dotted line:
Injection time on GC2
Dual Data acquisition – dual GC system
5 samples injected in the same time as before 3
Dual Data acquisition – dual GC system

5 samples injected in the same time as before.
Dual Data acquisition – dual GC system
5 samples injected in the same time as before 3
ThermoFisher Scientific
DFS
High Resolution Sector Field MS

Targetquan: new Quantification software
TargetQuan – New Quantification Software package
**TargetQuan – The New Peak Browser**

*By Compound One Analysis Sequentially Selected Entry*

*By Analysis One Compound Sequentially Selected Files*

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**Xcalibur Analysis Sequence:**

1. Loads Sequence Data Files
2. For Calibrations and Unknowns
TargetQuan – The New Screen Layout

Customized Windows:
1. Any Combination Possible
2. Store as Screen Layout
3. Fast Switching Chro ⇔ Spreadsheet

Spreadsheet Individual Layout
TargetQuan – The ISTD Dependancy Tree

The Isotope Dilution Tool:
1. Drag & Drop to Build
2. Hierachy of Standards
Thank you for your attention!

To learn more:

www.thermo.com/dfs