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DFS

High Resolution Sector Field MS

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

Dirk Krumwiede

Presentation Overview

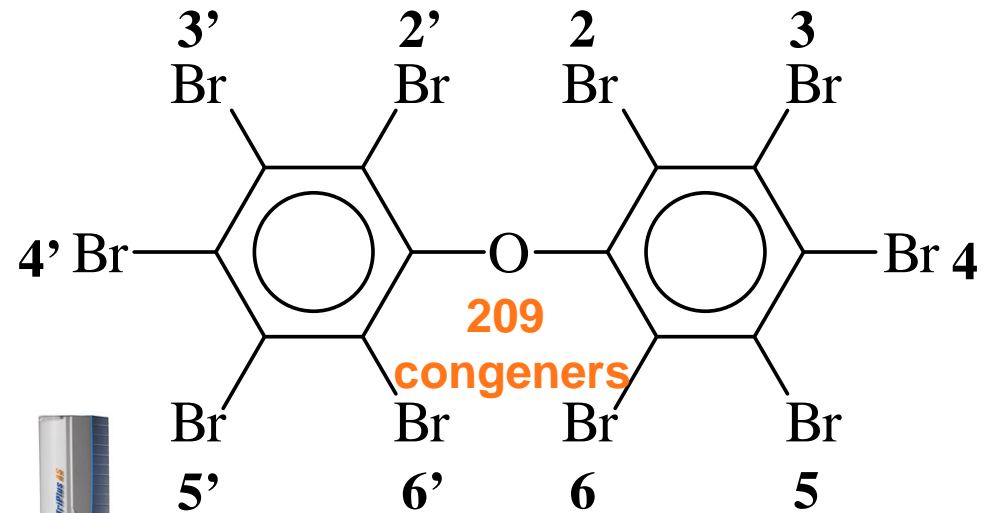
Analysis of polybrominated Diphenylethers (PBDE)

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



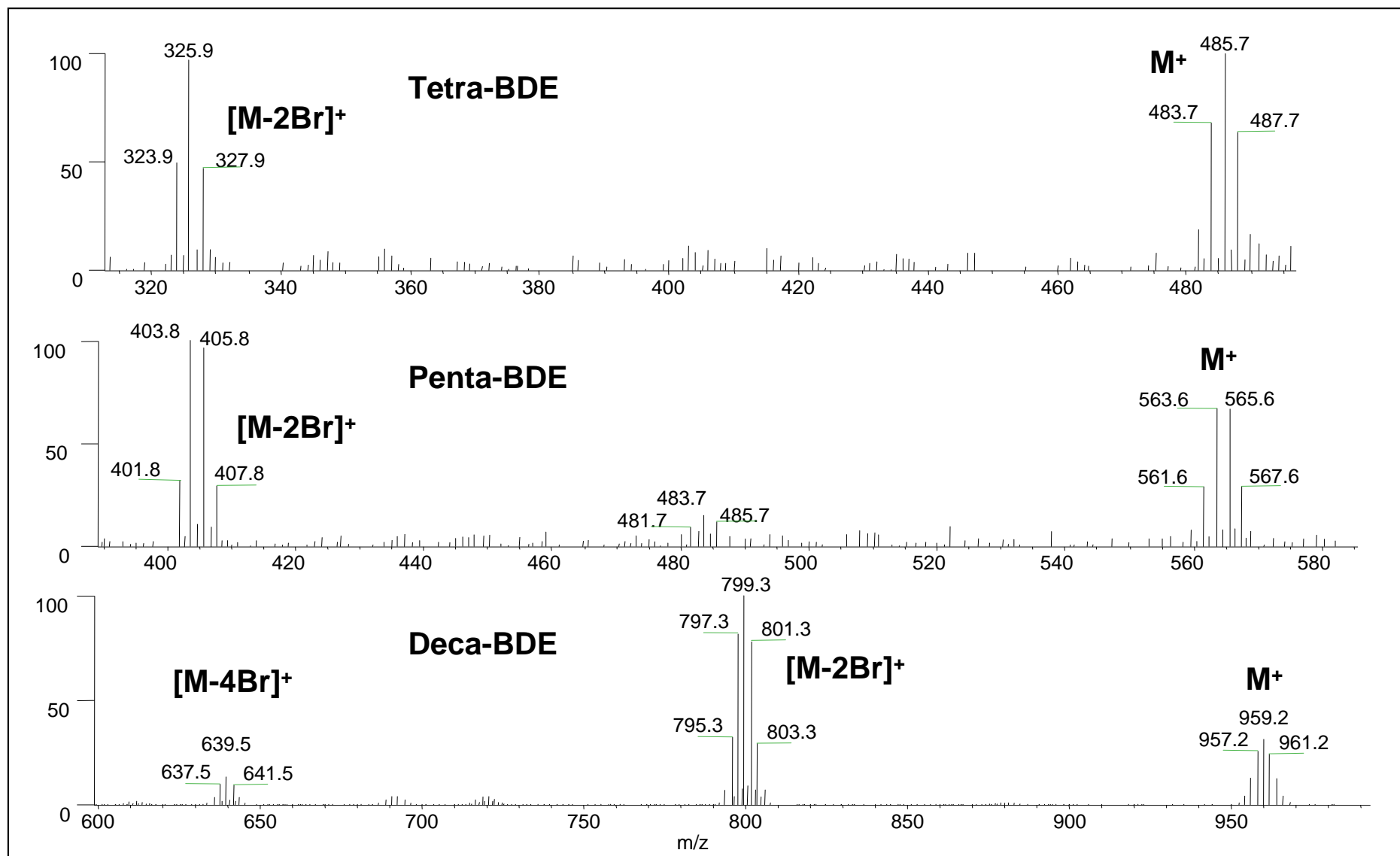
Part 1: Analysis of PBDE

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



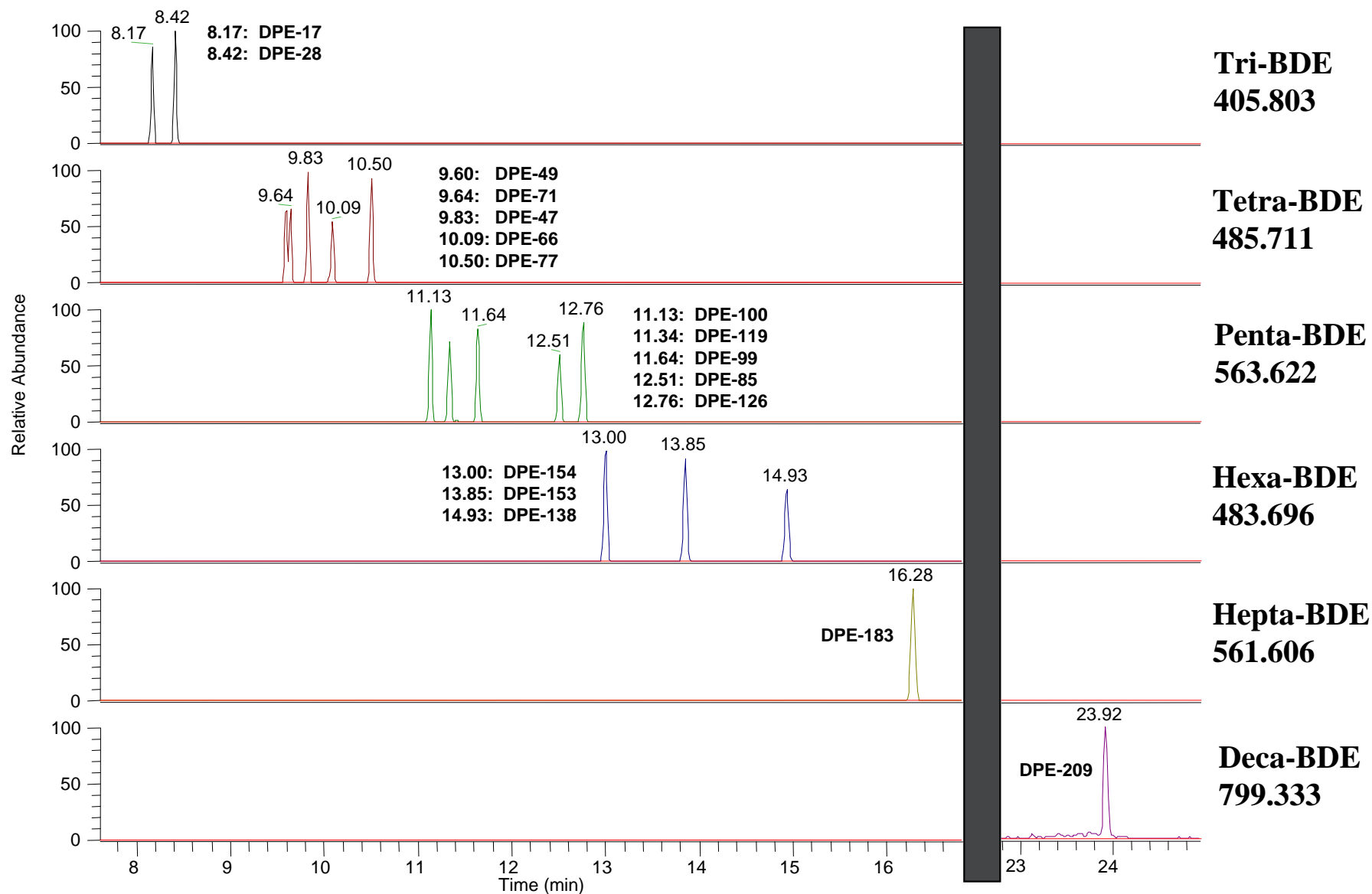
Mass/Intensity List for PBDE

relative intensities of M⁺ versus [M-2Br]⁺

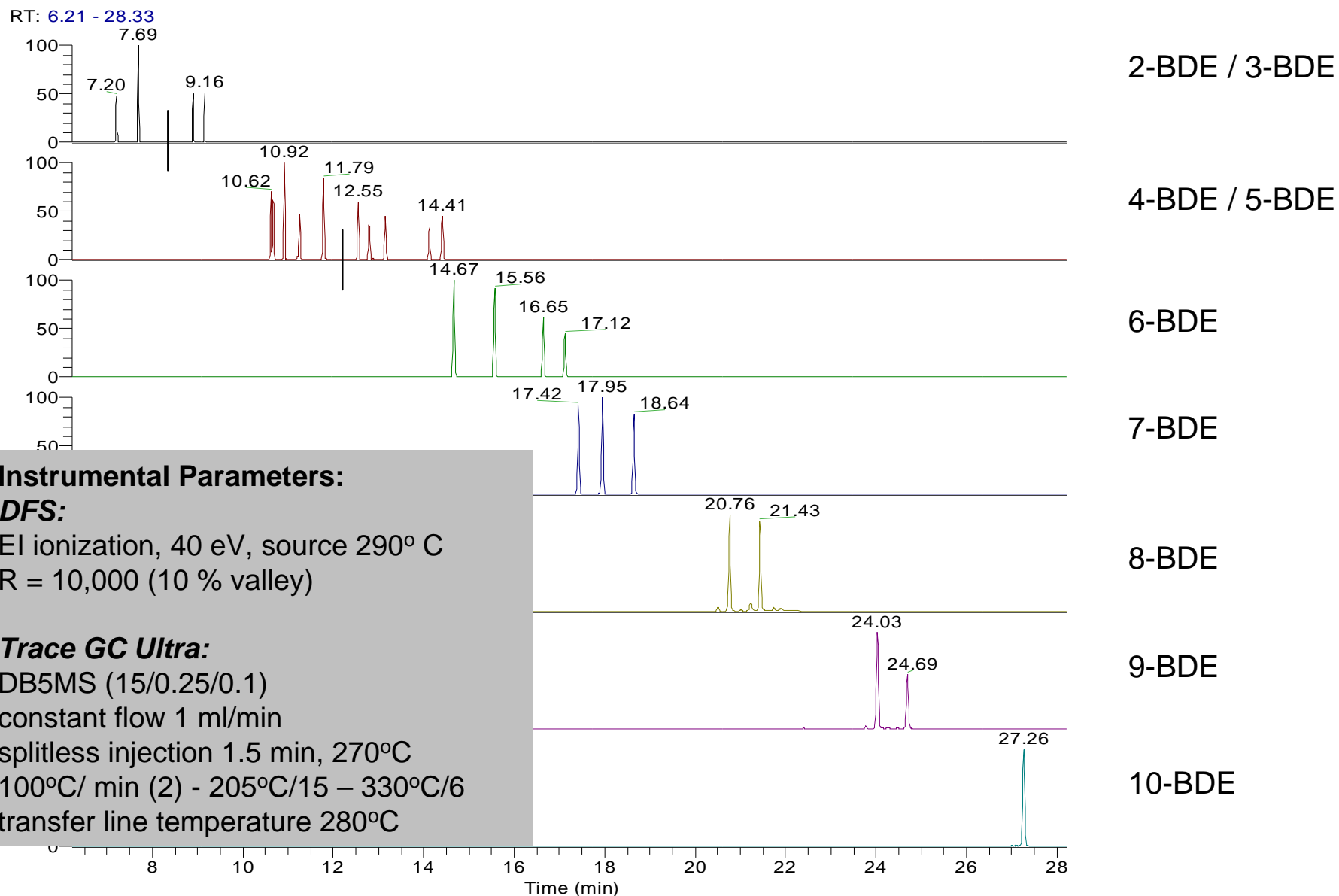
| | # Br | m | | m+2 | | m+4 | | m+6 | | m+8 | | m+10 | | m+12 | | Int. M/M-2Br |
|----------------------|------|---------|-----|---------|-----|---------|-----|---------|-----|---------|-----|---------|-----|---------|----|-----------------|
| [M] ⁺ | 1 | 247,984 | 100 | 249,982 | 98 | | | | | | | | | | | 100 |
| | 2 | 325,894 | 51 | 327,892 | 100 | 329,890 | 49 | | | | | | | | | 100 |
| | 3 | 403,805 | 34 | 405,803 | 100 | 407,801 | 97 | | | | | | | | | 100 |
| | 4 | | | 483,713 | 68 | 485,711 | 100 | 487,709 | 65 | | | | | | | 100 |
| | 5 | | | 561,624 | 51 | 563,622 | 100 | 565,620 | 97 | | | | | | | 85 |
| | 6 | | | | | 641,532 | 76 | 643,530 | 100 | 645,528 | 73 | | | | | 60 |
| | 7 | | | | | 719,443 | 61 | 721,441 | 100 | 723,439 | 97 | | | | | 55 |
| | 8 | | | | | | | 799,351 | 81 | 801,349 | 100 | 803,347 | 78 | | | 50 |
| | 9 | | | | | | | 877,262 | 68 | 879,260 | 100 | 881,258 | 97 | | | 40 |
| | 10 | | | | | | | | | 957,171 | 85 | 959,168 | 100 | 961,166 | 81 | 25 |
| [M-2Br] ⁺ | 1 | | | | | | | | | | | | | | | 0 |
| | 2 | | | | | | | | | | | | | | | 0 |
| | 3 | | | | | | | | | | | | | | | 0 |
| | 4 | 323,879 | 51 | 325,877 | 100 | 327,875 | 49 | | | | | | | | | 95 |
| | 5 | 401,789 | 34 | 403,787 | 100 | 405,785 | 97 | | | | | | | | | 100 |
| | 6 | | | 481,698 | 68 | 483,696 | 100 | 485,694 | 65 | | | | | | | 100 |
| | 7 | | | 559,608 | 51 | 561,606 | 100 | 563,604 | 97 | | | | | | | 100 |
| | 8 | | | | | 639,517 | 76 | 641,515 | 100 | 643,513 | 73 | | | | | 100 |
| | 9 | | | | | 717,427 | 61 | 719,425 | 100 | 721,423 | 97 | | | | | 100 |
| | 10 | | | | | | | 797,336 | 81 | 799,333 | 100 | 801,331 | 78 | | | 100 |

Mass Chromatogram of a 6 window run

Separation by bromination degree on a 15 m column

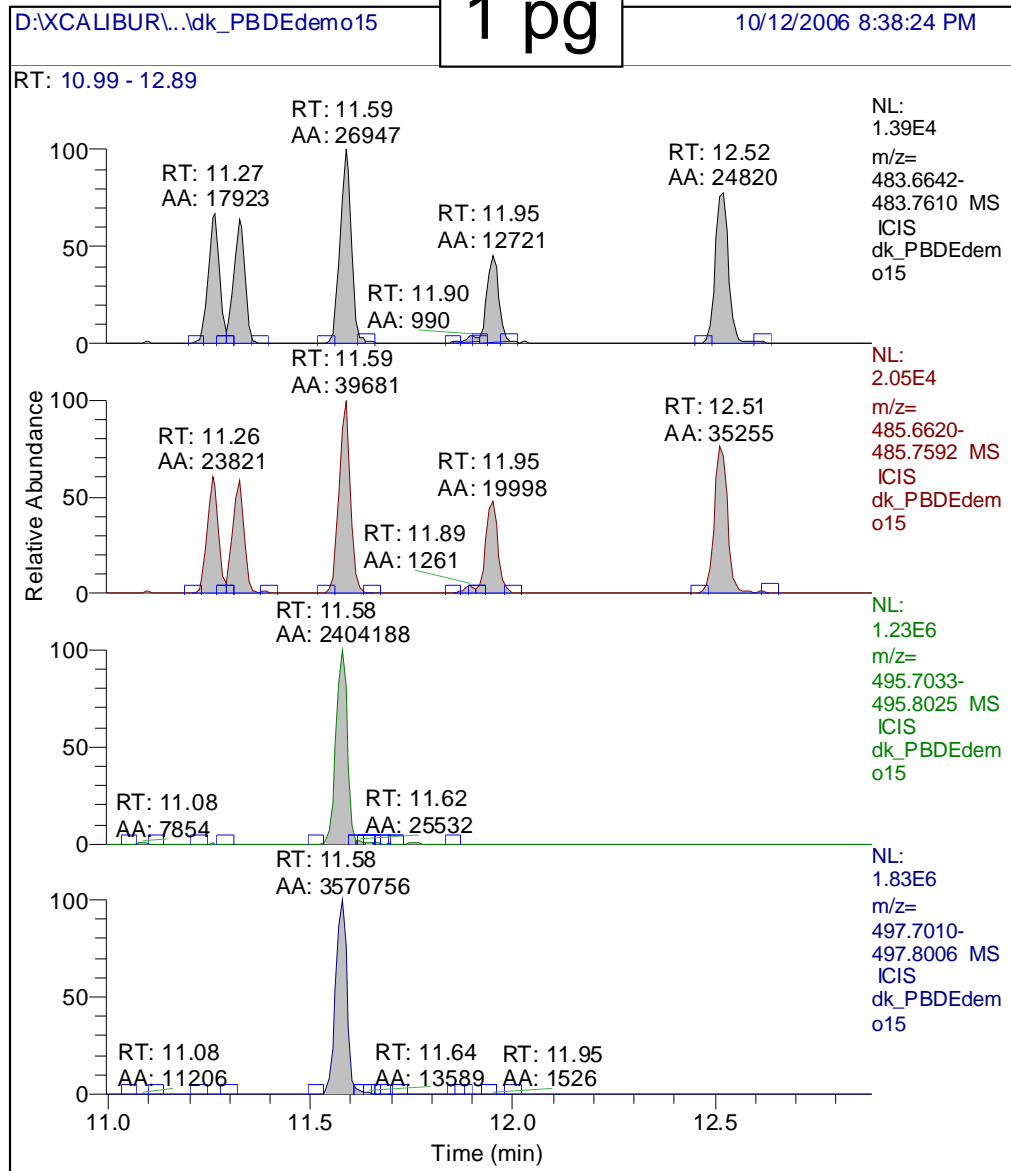


CS3-E: BDE 2-10 brominated on 15 m column / SSL inj.

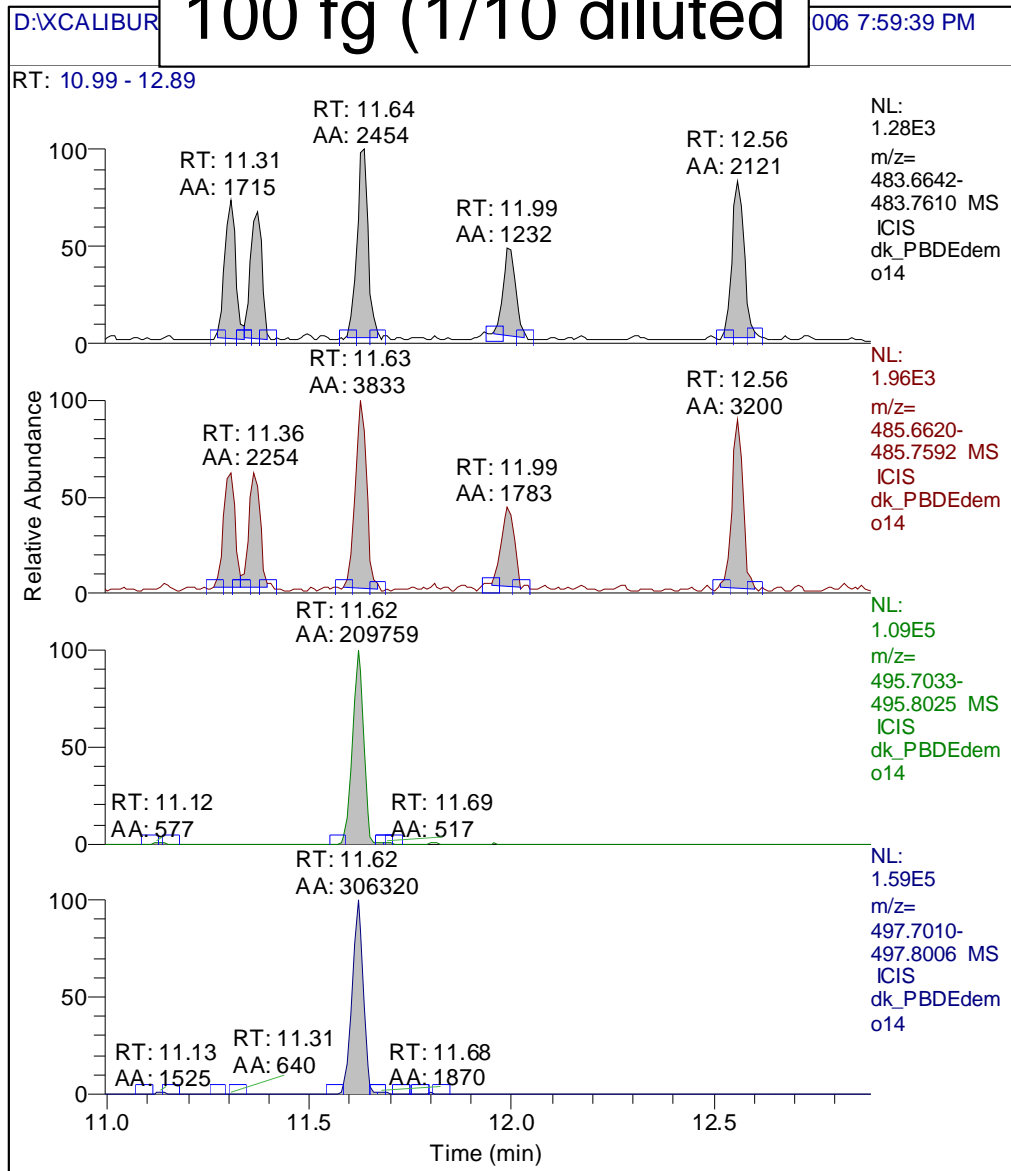


CS3-E: BDE tetra separation on 15 m column / SSL inj.

1 pg



100 fg (1/10 diluted)



DFS – PBDE analysis in low fg range (like PCB, dioxin/furans)

Application Notes on PBDE

Application Note: 30098

Key Words

- Flame Retardants (PBDE)
- HRGC / HRMS
- Multiple Ion Detection (MID)
- Persistent Organic Pollutants (POPs)
- RoHS / WEEE

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

Dirk Krumwiede, Hans-Joachim Hübschmann, Thermo

Introduction

Polybrominated diphenyl ethers (PBDEs) are among most important and widely used flame retardants in variety of different industrial products. They are found worldwide in matrices, moving them into the focus of recent legislation banning certain PBDE congeners. The directive 2003/11/EC prohibits the use of Penta-BDE and Octa-BDE for the member states of the European community^[2].

As a result, analysis of PBDEs has received increased interest due to their known toxicity. Similar to dioxins/furans and PCB's (polychlorinated biphenyl) polybrominated diphenyl ethers exist in a higher number of congeners (209) (Figure 1).

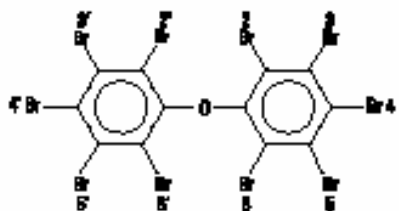
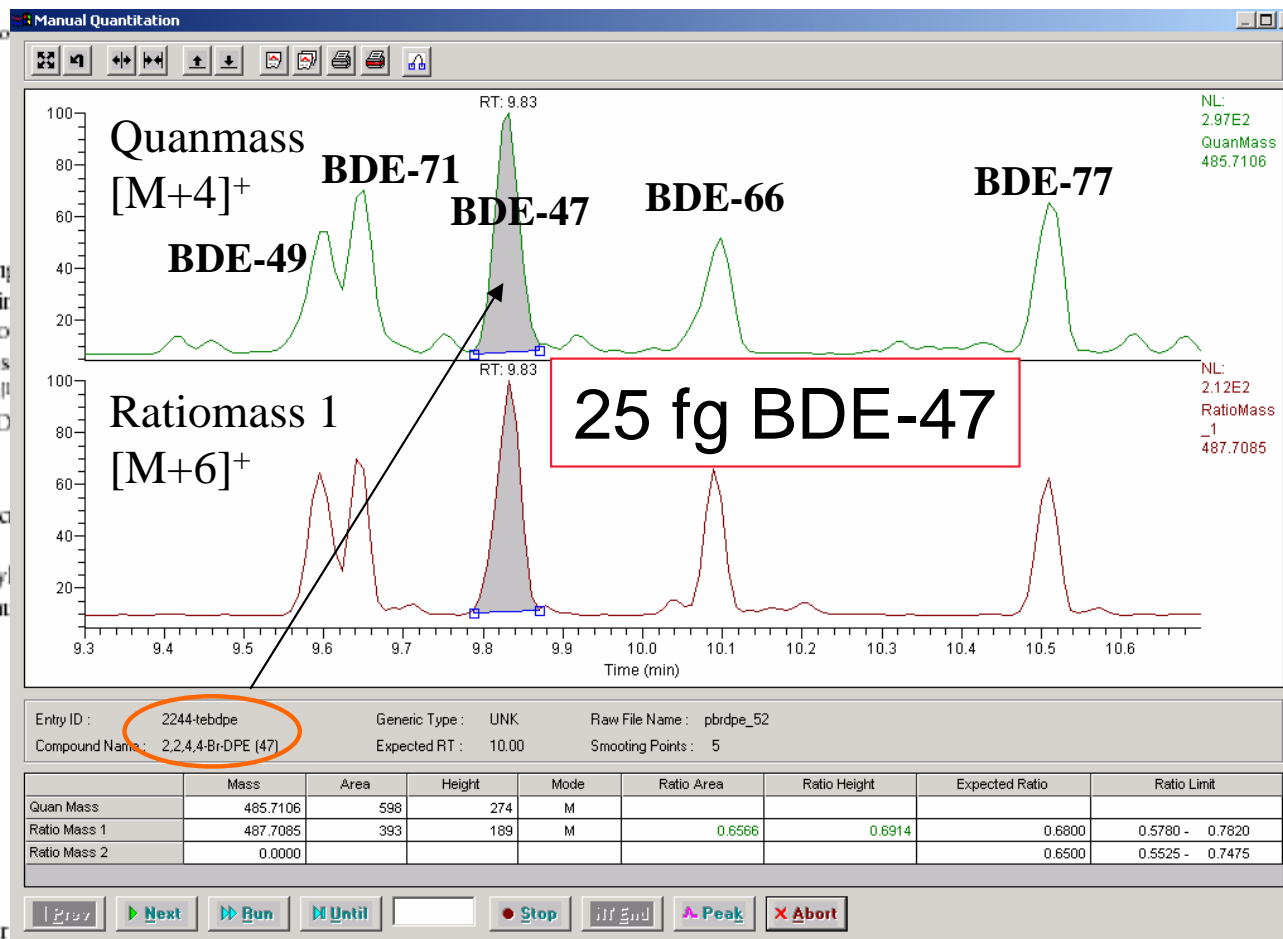


Figure 1: PBDE chemical structure, here Deca-BDE

The most efficient analysis technique by far for three application areas is high resolution GC/MS using isotope dilution technique for quantitation with highest precision and significance.



Transfer line temperature 280 °C

Table 1: GC parameters

Part 2: Optimized analysis for Deca-BDE using SSL and PTV injectors

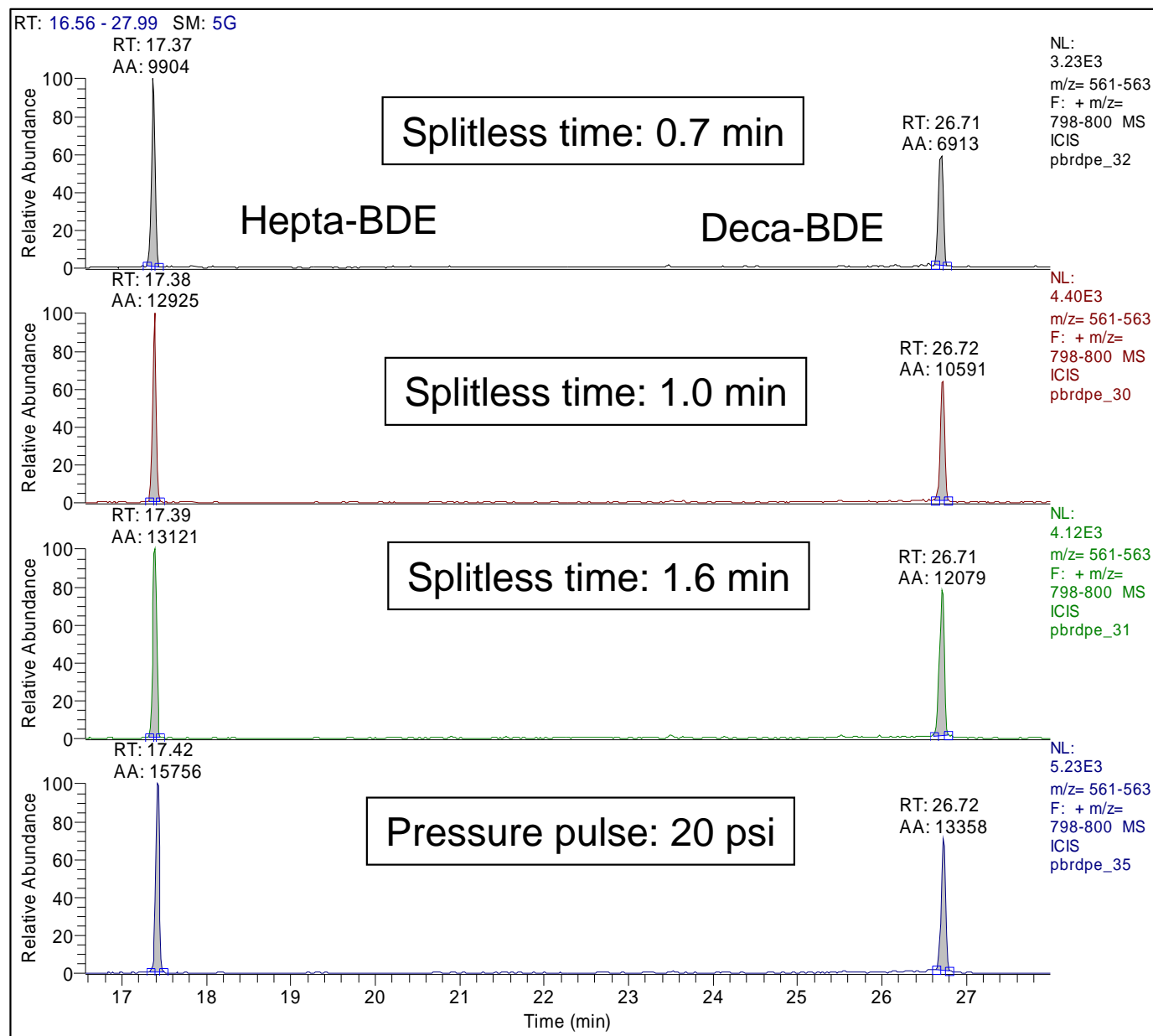
Parameters influencing sensitivity for Deca-BDE:

- 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



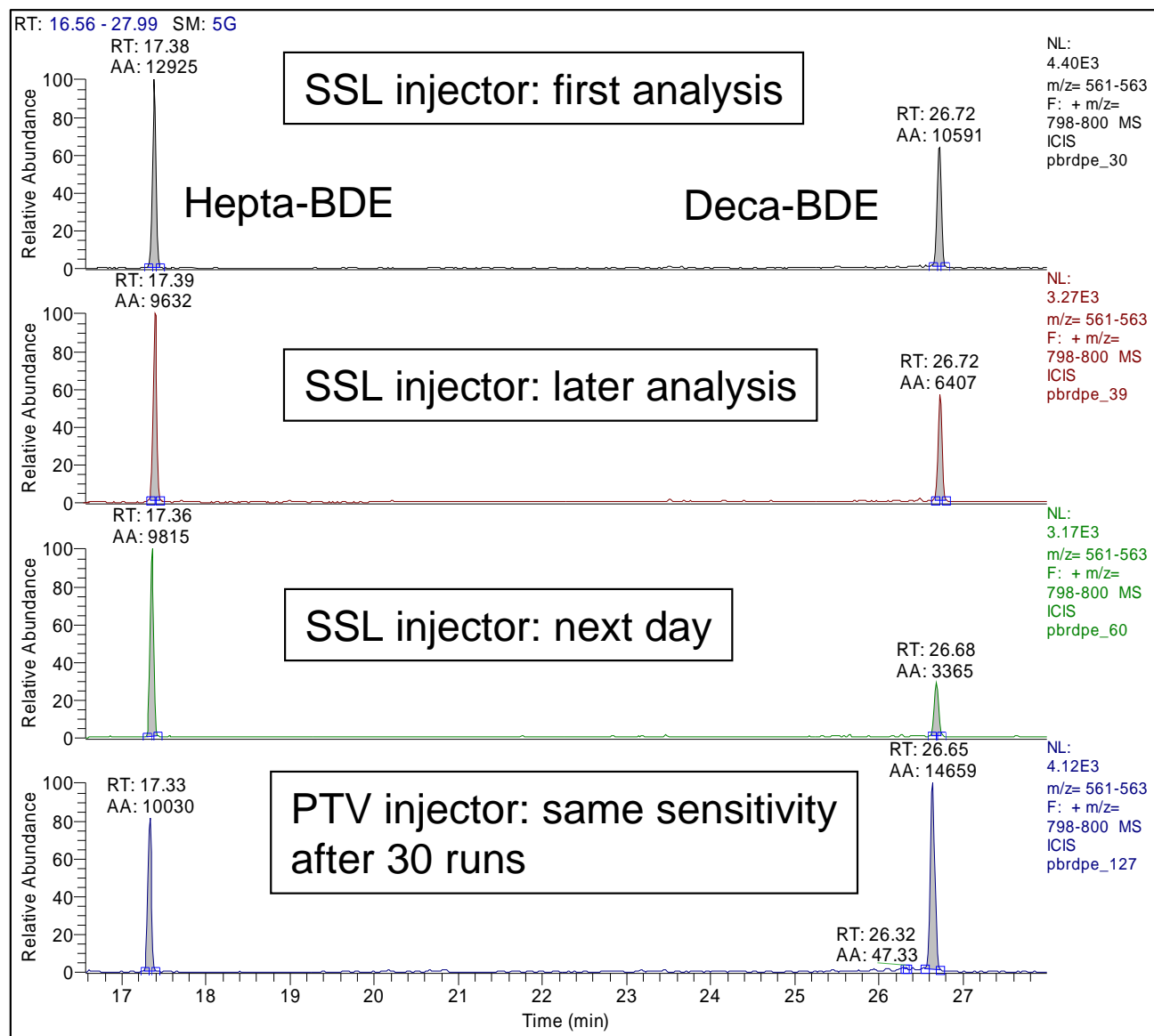
DB5 15 m; 0.25mm;
0.1 um film, 1 ml/min He

SSL injector with
5 mm I.D. liner

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

SSL or PTV for Deca-BDE analysis ?

Decrease of sensitivity over time



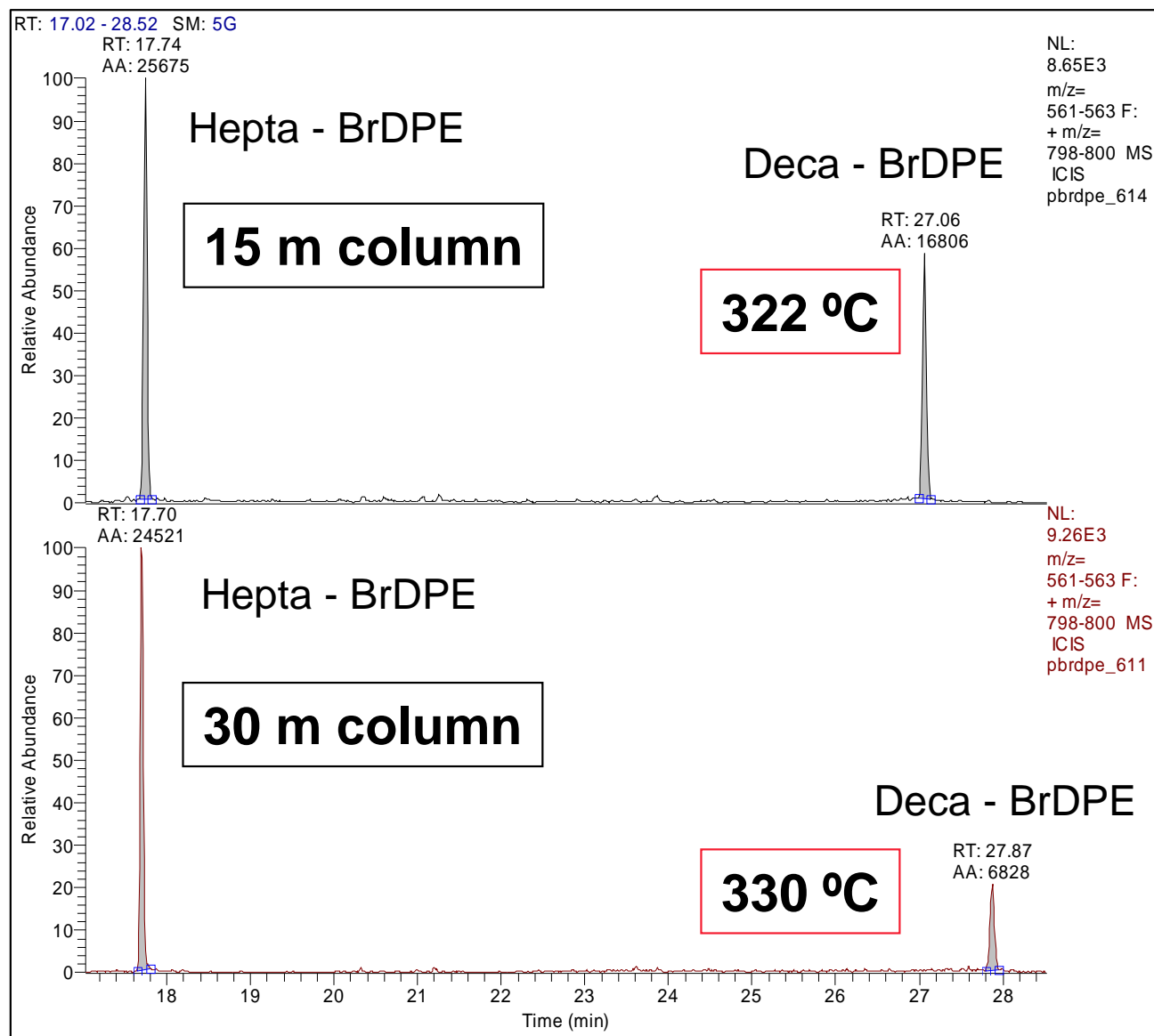
DB5 15 m; 0.25mm;
0.1 um film, 1 ml/min He

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

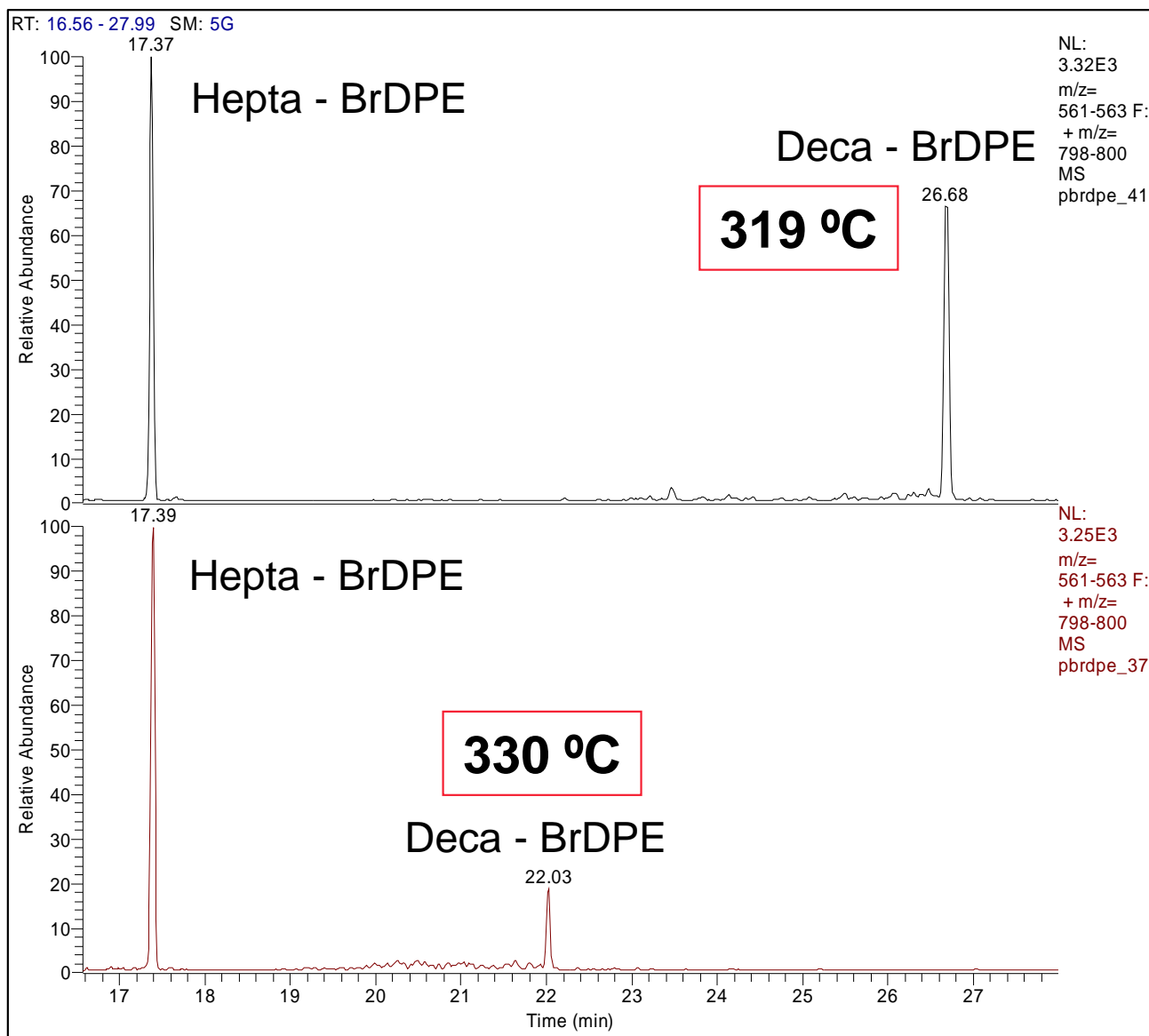


DB5 15 m / 30 m; 0.25mm;
0.1 um film;
Flow: 0.8 ml/min He

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

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

Oven program and Deca-BDE response same 15 m column / two different oven programs

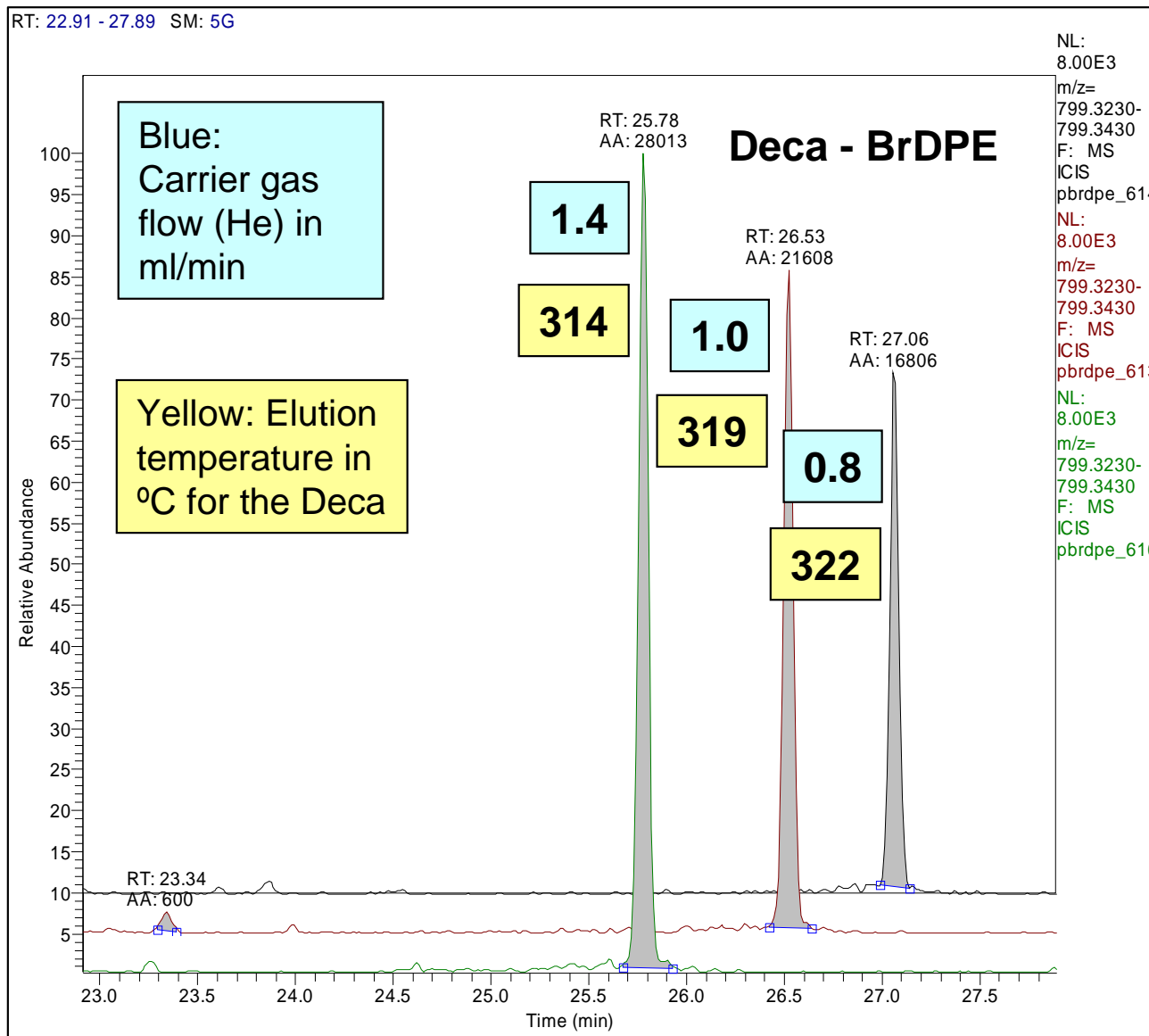


DB5 15 m; 0.25mm;
0.1 um film; 1 ml/min He

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

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

Carrier gas flow and Deca-BDE response



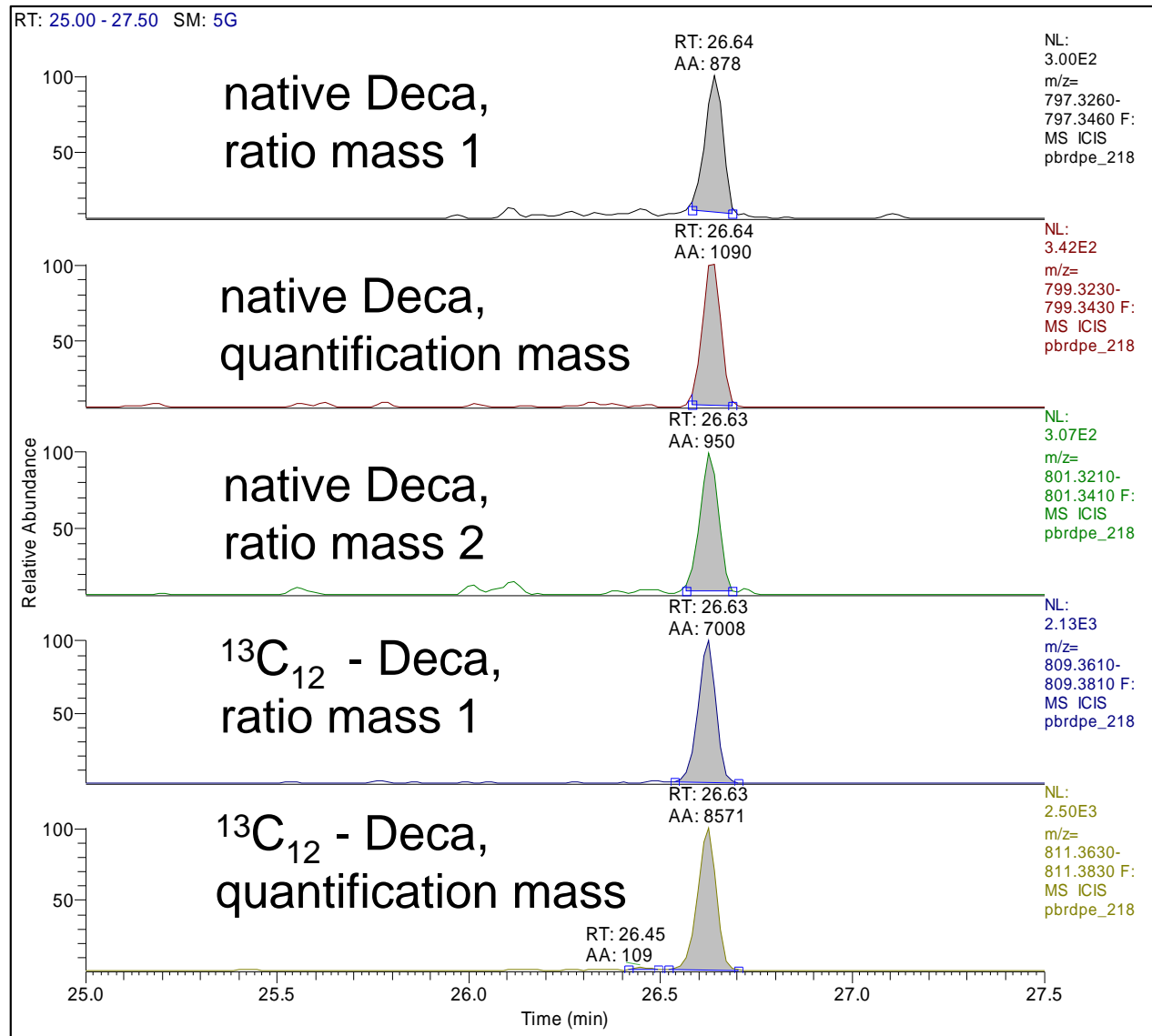
DB5 15 m; 0.25mm;
0.1 um film

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

Optimized analysis for Deca-BDE using SSL and PTV injectors - conclusions

Parameters influencing sensitivity for Deca-BDE:

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

- No significant difference from liner I.D.
- PTV is preferable for Deca-BDE.
- Pressure pulse useful for SSL / PTV
- Assure sufficient splitless time

- oven program
- column length
- column flow

- Rule of thumb: **Elution temperature for Deca-BDE should be as low as possible**

- source temperature
- ionization energy (eV)

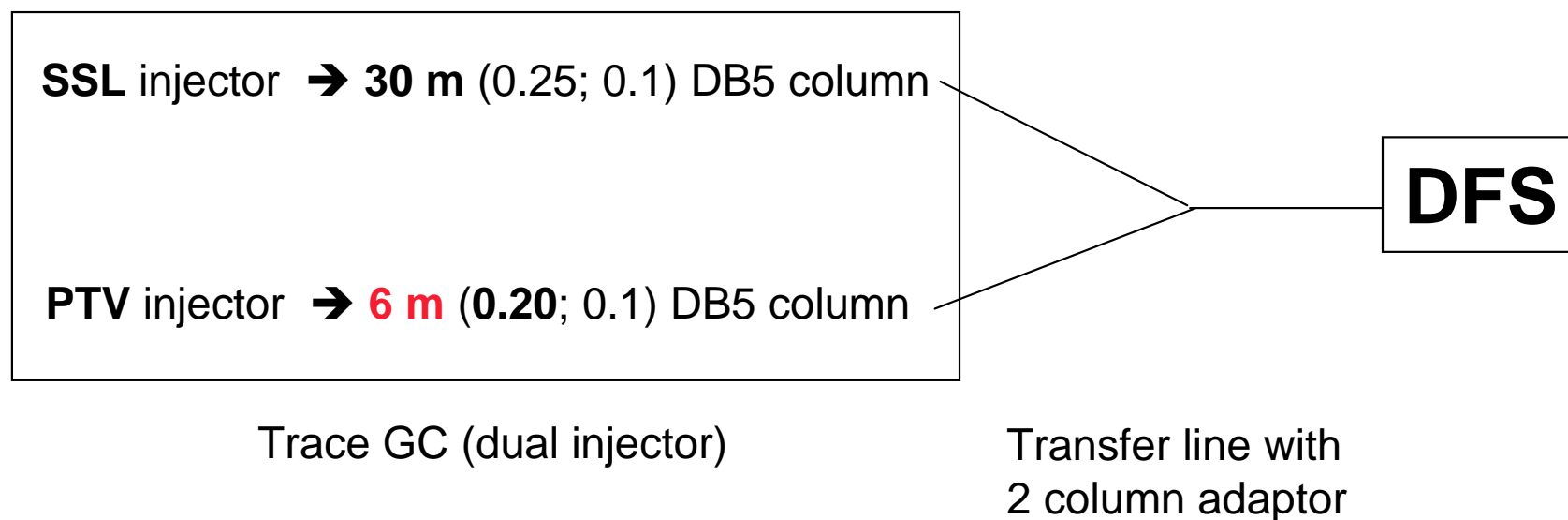
- 270 – 280 °C
- same as for dioxins (35 / 40 eV)

Part 3: A dual column setup for PBDE analysis combining sensitivity and separation efficiency

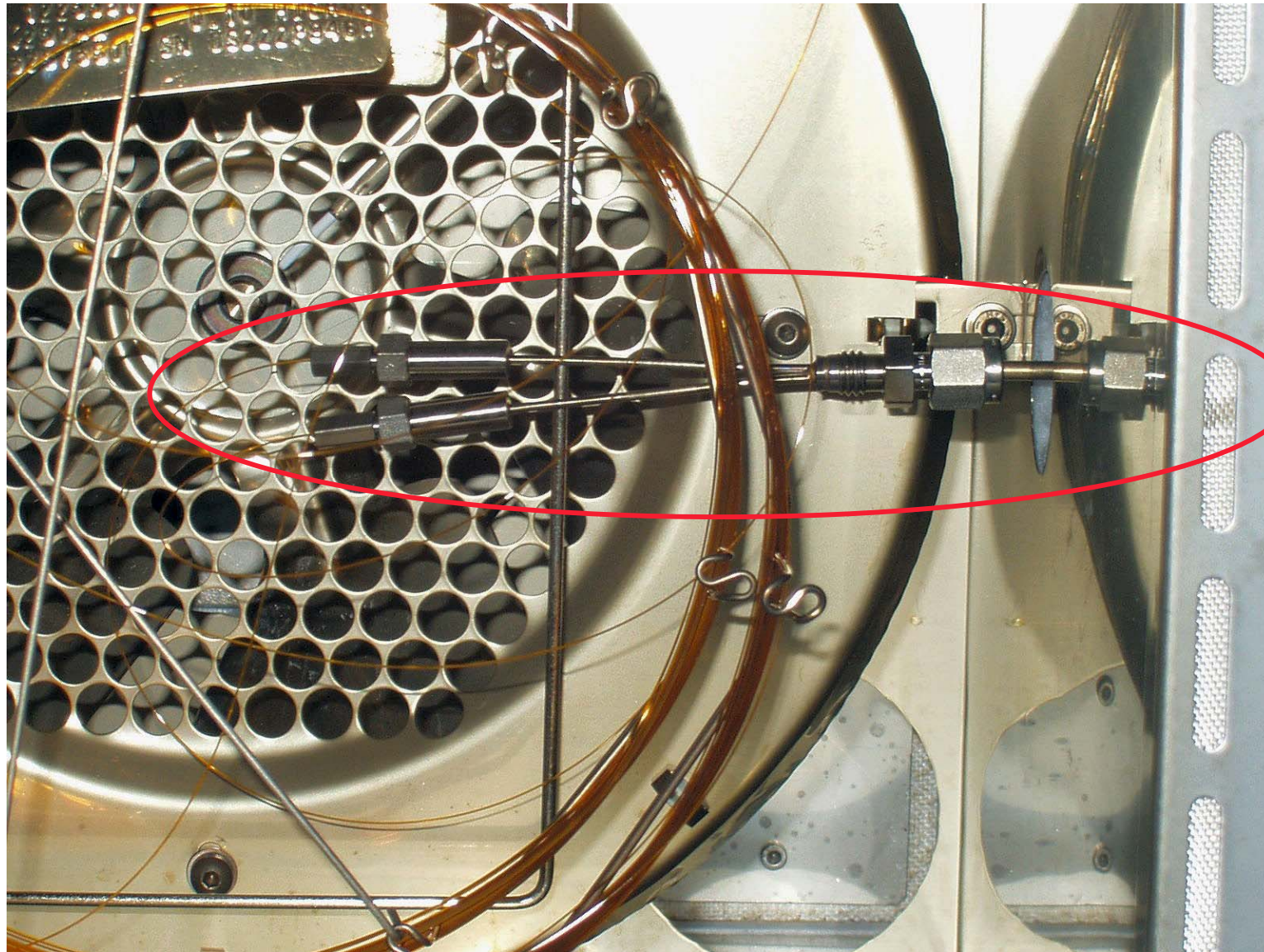
Following some minor adaptations of source and transferline parts two columns were installed into the same GC (two injectors) and source using a Y-shaped dual column adaptor.

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

For PBDE:

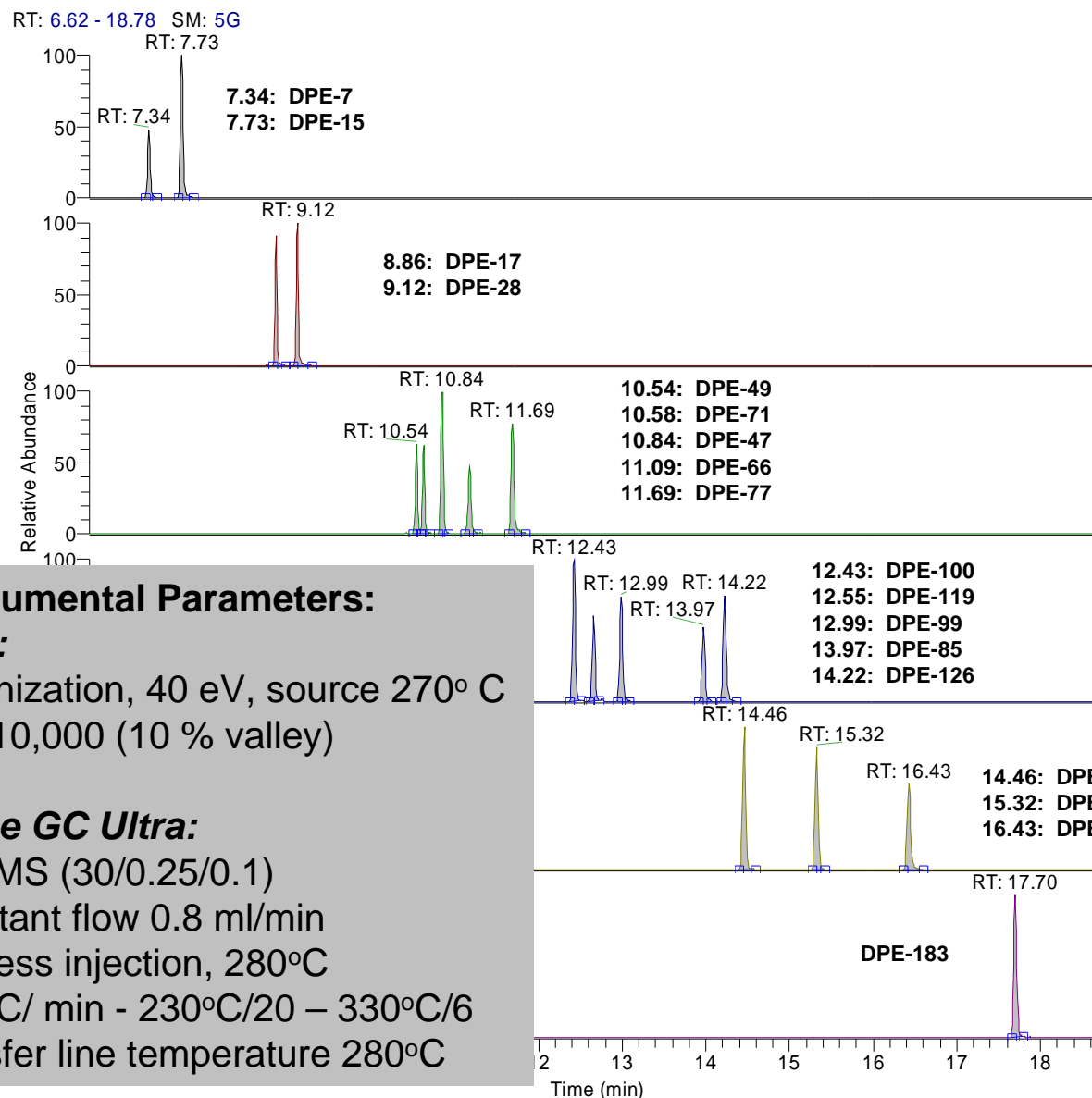


Dual column setup for PBDE analysis



Typical chromatogram

Separation on DB5 (here 30 m) by bromination degree



Di-BDE
327.892

Tri-BDE
405.803

Tetra-BDE
485.711

Penta-BDE
563.622

Hexa-BDE
483.696

Hepta-BDE
561.606

Instrumental Parameters:

DFS:

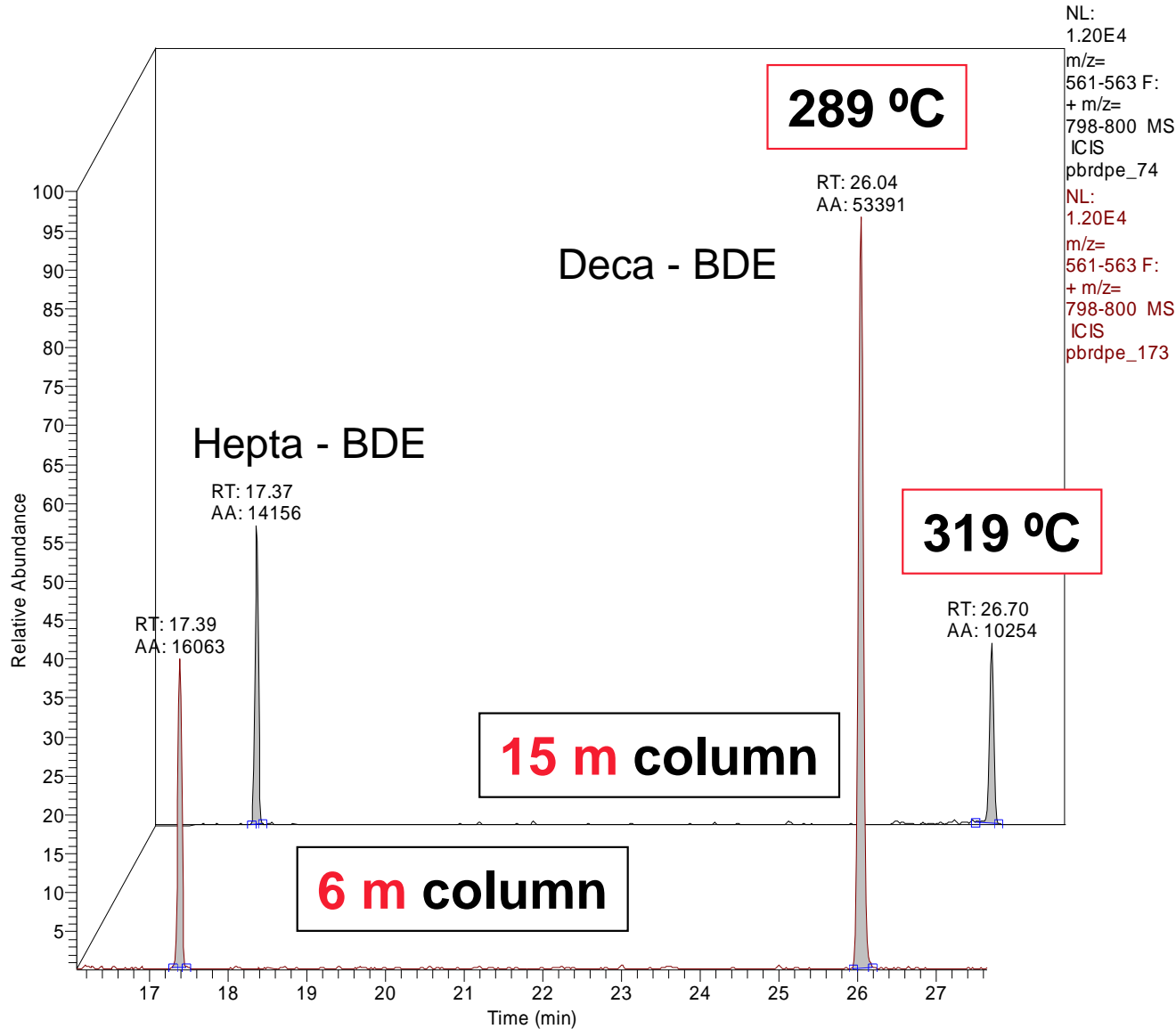
El ionization, 40 eV, source 270° C
R = 10,000 (10 % valley)

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

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

RT: 16.08 - 27.64 SM: 5G



NL: 1.20E4
m/z= 561-563 F:
+ m/z= 798-800 MS
ICIS
pbrdpe_74
NL: 1.20E4
m/z= 561-563 F:
+ m/z= 798-800 MS
ICIS
pbrdpe_173

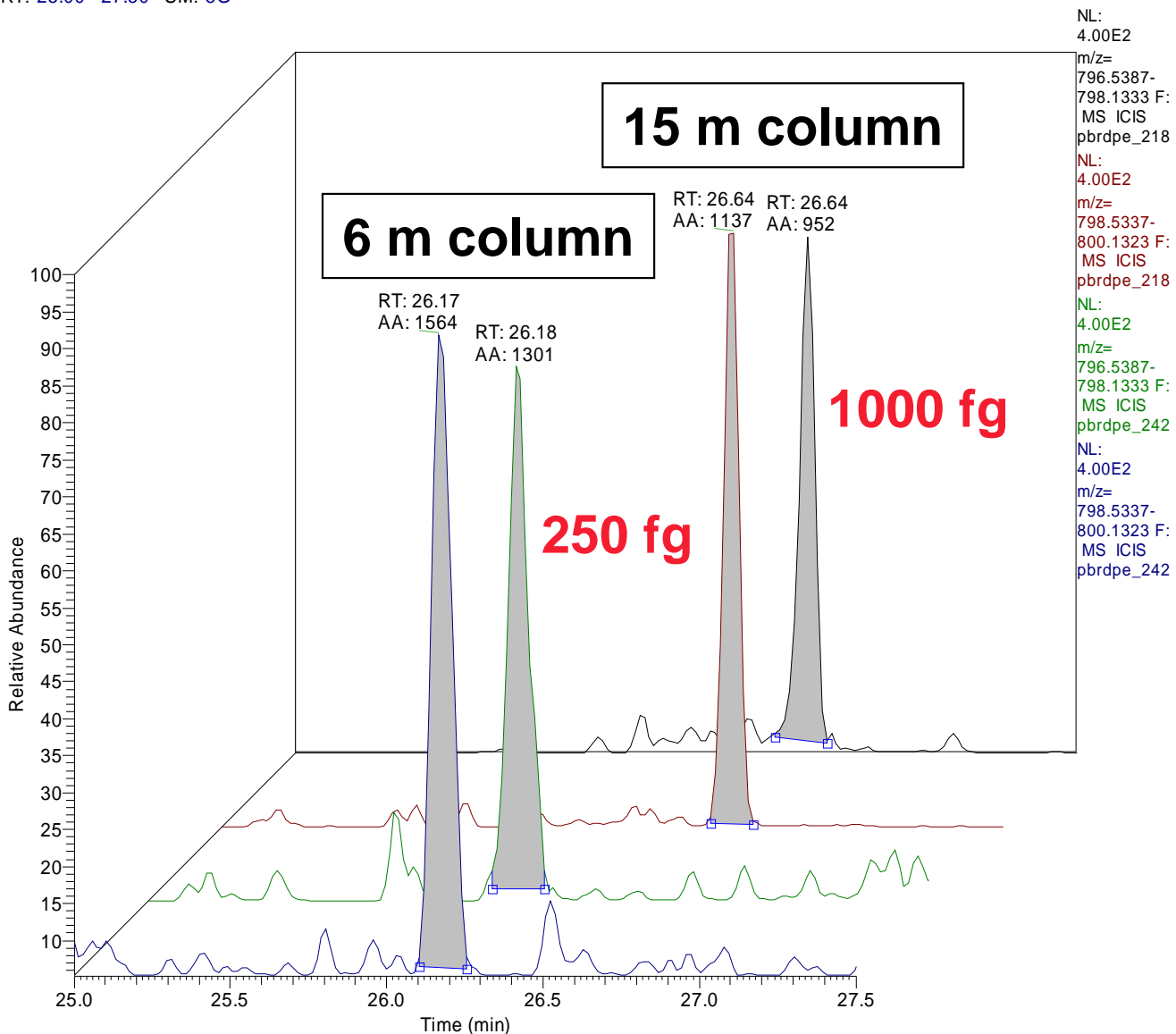
DB5 15 m; 0.25mm; 0.1 um
DB5 6 m; 0.2mm; 0.1 um

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)

RT: 25.00 - 27.50 SM: 5G

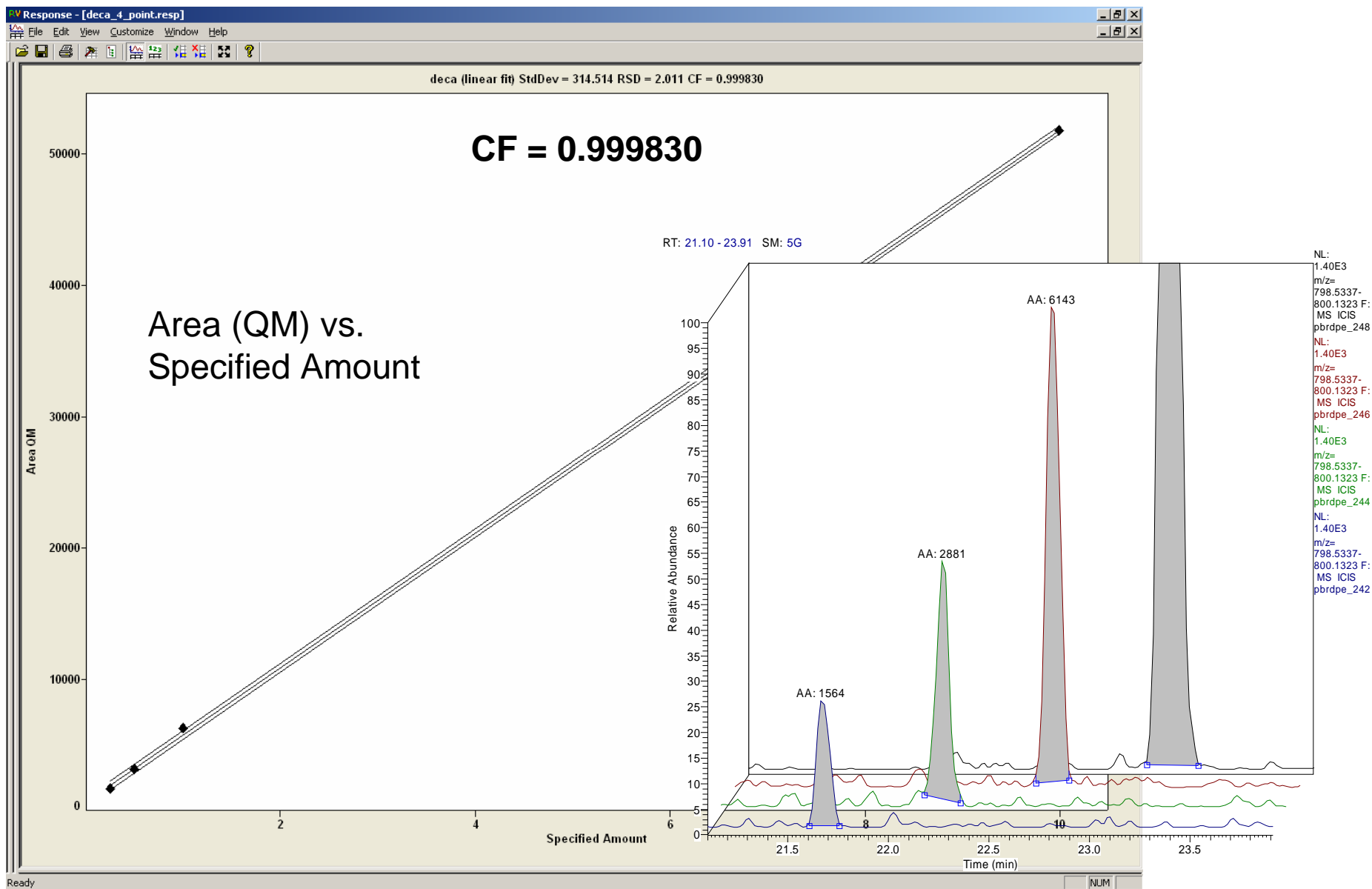


DB5 15 m; 0.25mm; 0.1 um
DB5 6 m; 0.2mm; 0.1 um

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

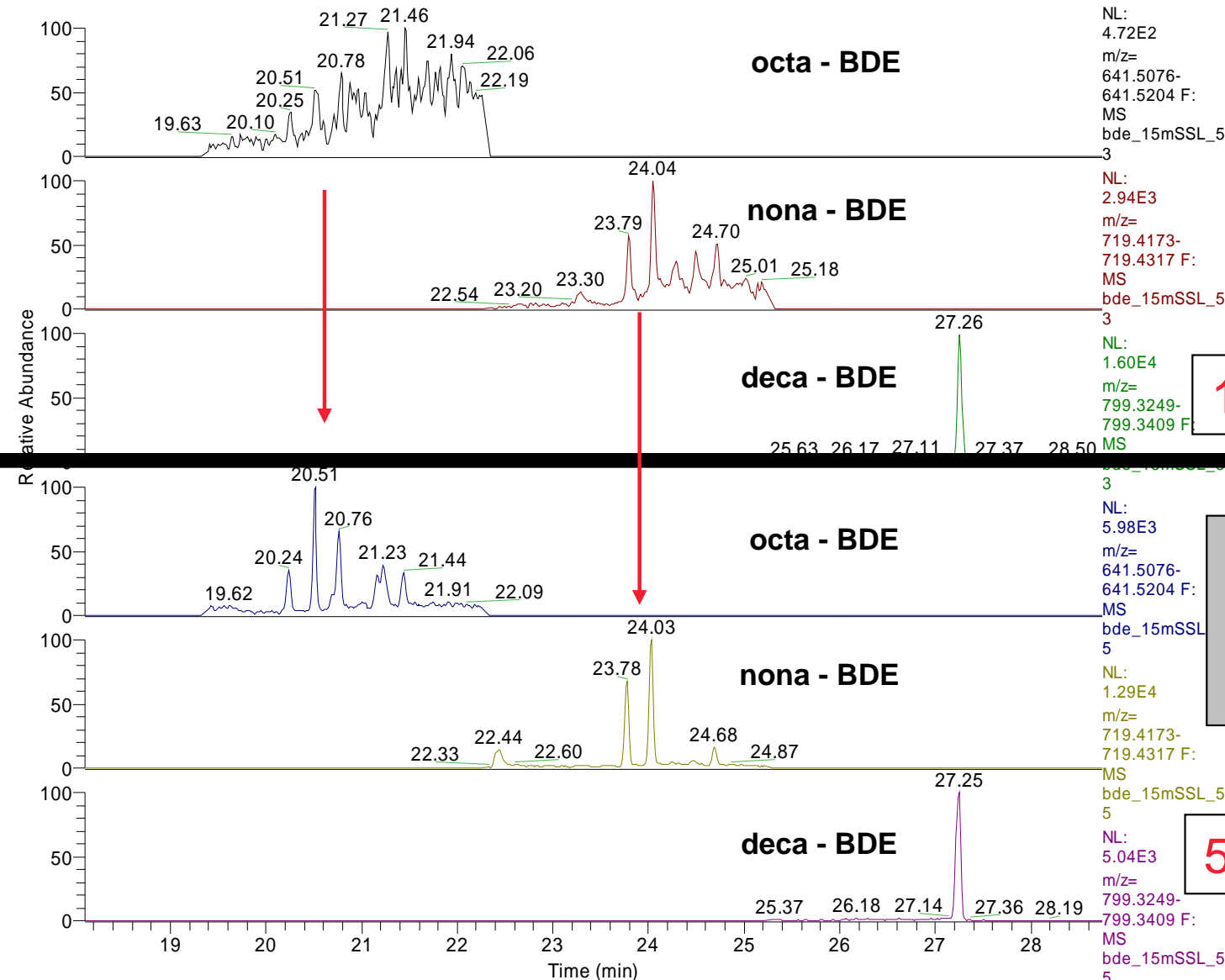


Thermal degradation products of Deca-BDE

100 pg native only BDE-209 was injected

D:\XCALIBUR1...\bde_15mSSL_53 5/12/2005 6:43:20 PM 100 pg/ul deca nat., ists 290 C, inj 250 C, surge
DB5 MS 15*0.25 (0.1), 100 (2)-15-205-6-330(5), SSL splitless, 40 eV, emult 1.78 KV, ists 290 C

RT: 18.10 - 28.74



Optimized splitless injection

for minimized thermal decomposition.

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

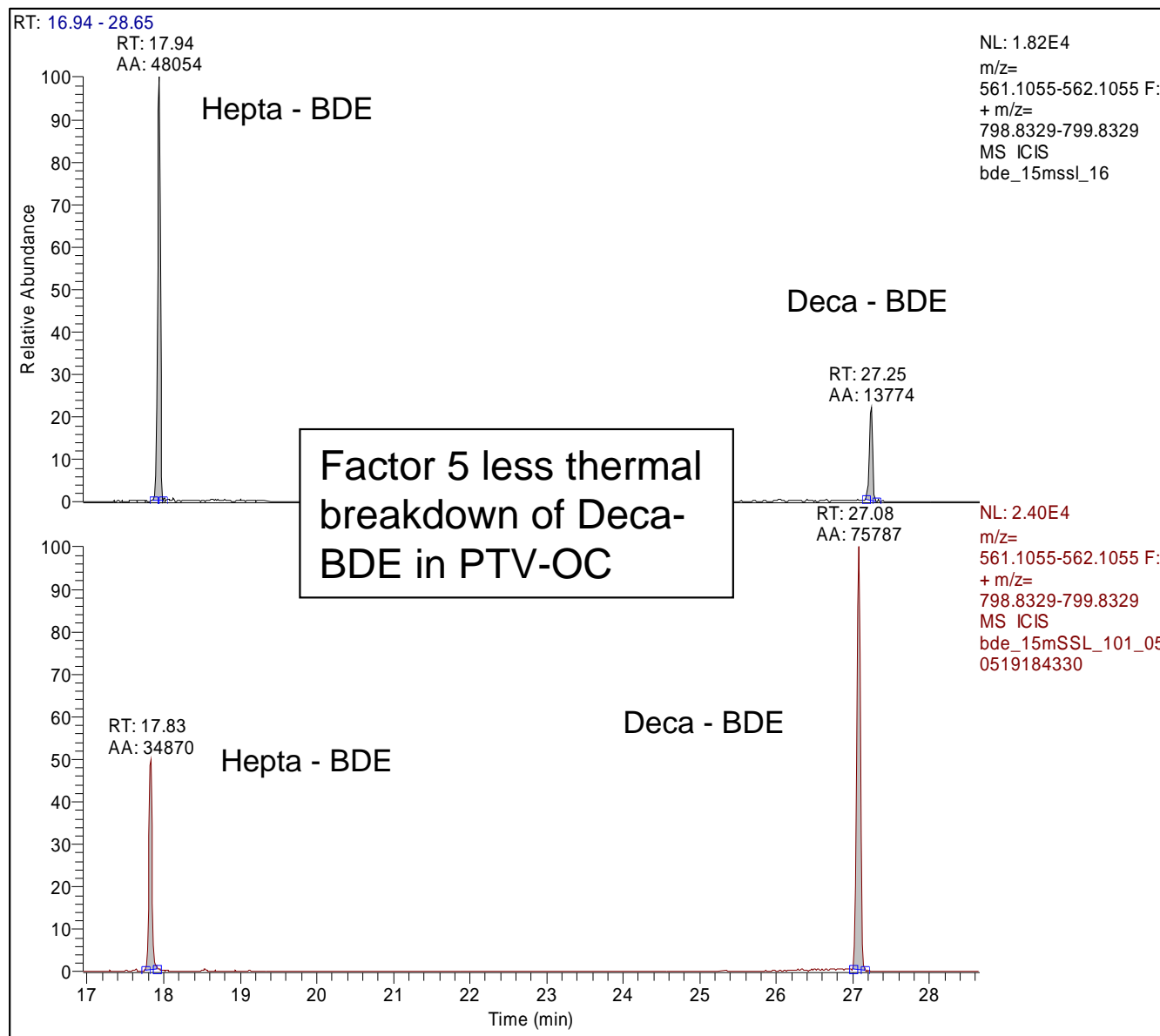
1.6 E4 deca peak height

Enforced / increased thermal decomposition

Injector temp.: 310 °C

5.04 E3 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 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



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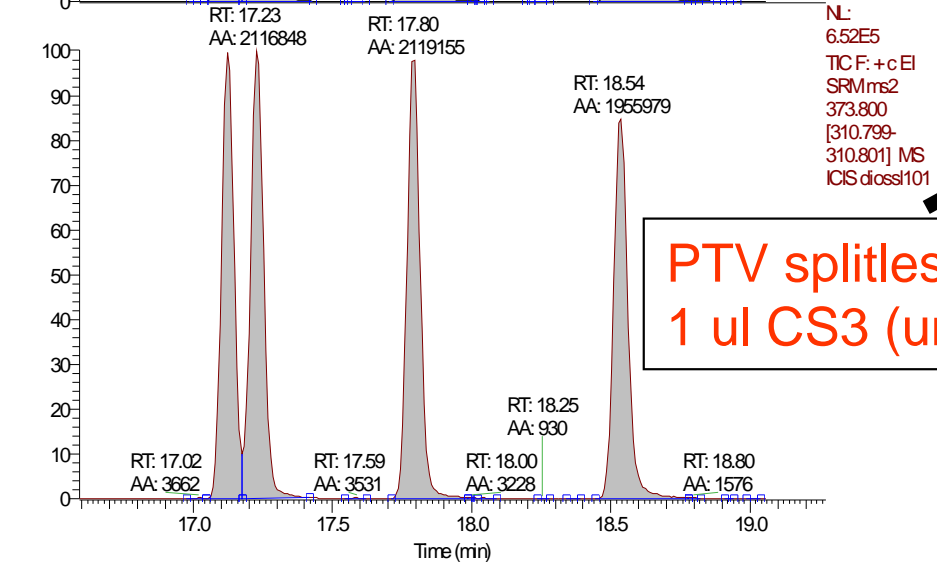
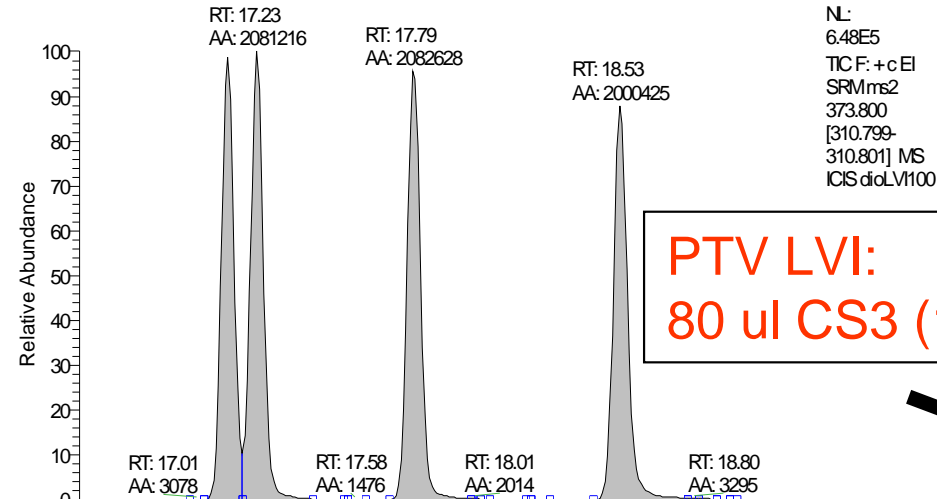
PTV LVI for POPs analysis

80 ul dioxins/furans with PTV LVI

Recovery and peakshape

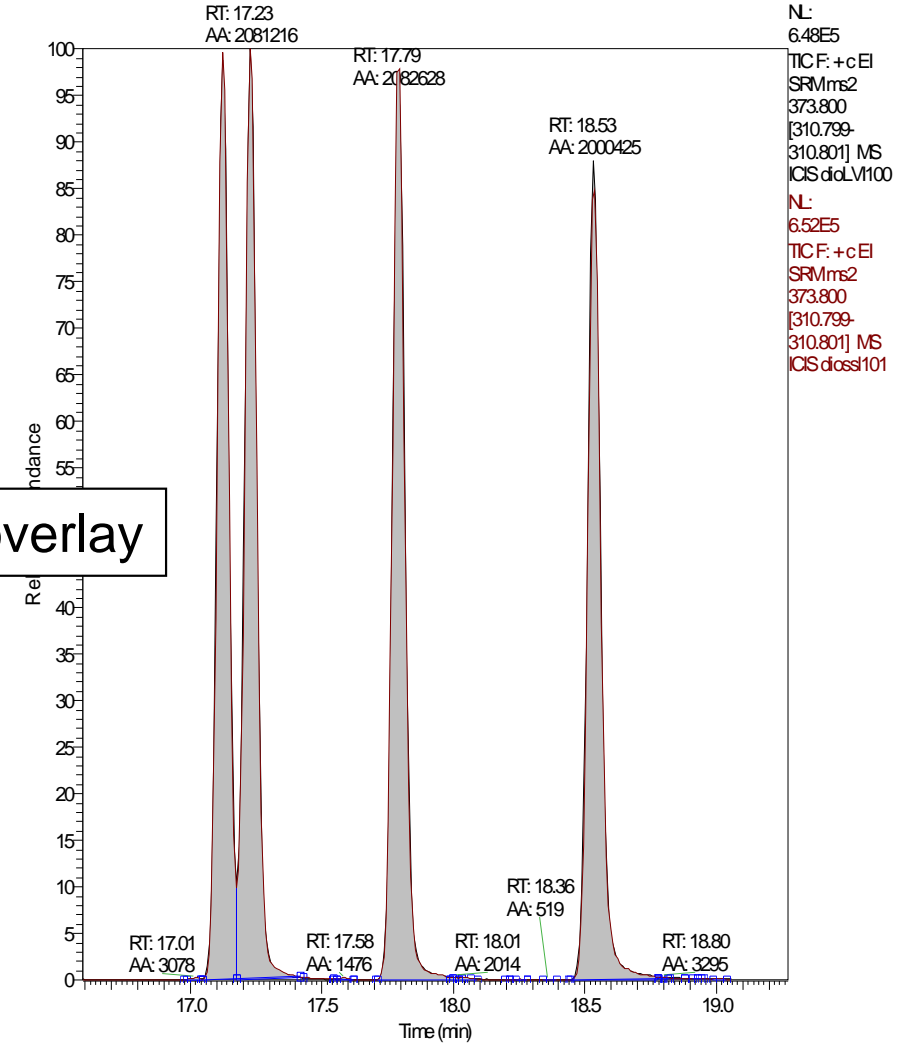
C:\Calibur\dioxLM100 4/18/2008 11:08:34 AM LV: 80 ul CS3 (1/80), PTV100(0.2), 50 ml, 20 ul/sec, PTV/LVI installed
 closed EI vol., 30 m TR5MS (0.25), 140(2)-, tune last, LV metall liner with wool, 100 ul syringe with side hole

RT: 16.59 - 19.27



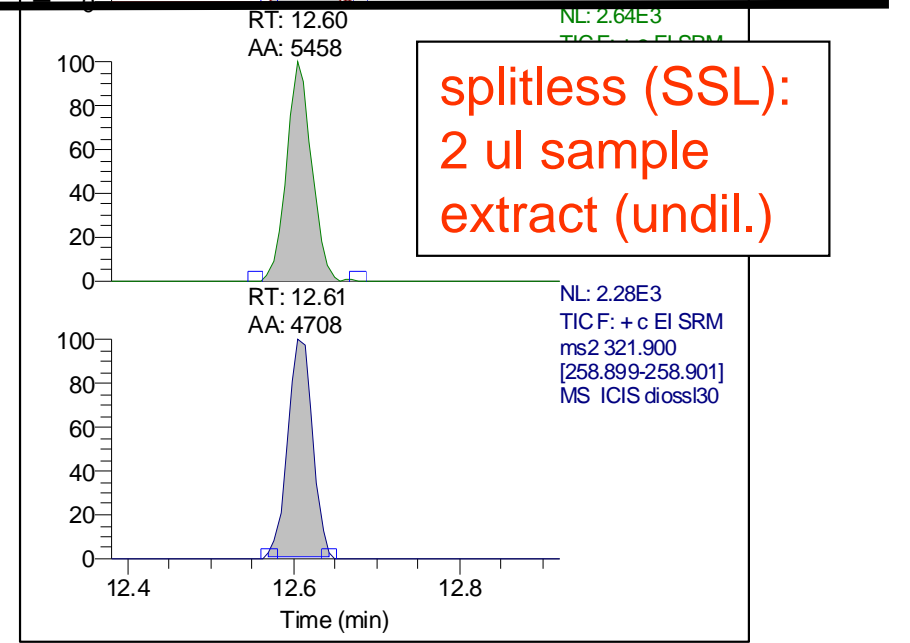
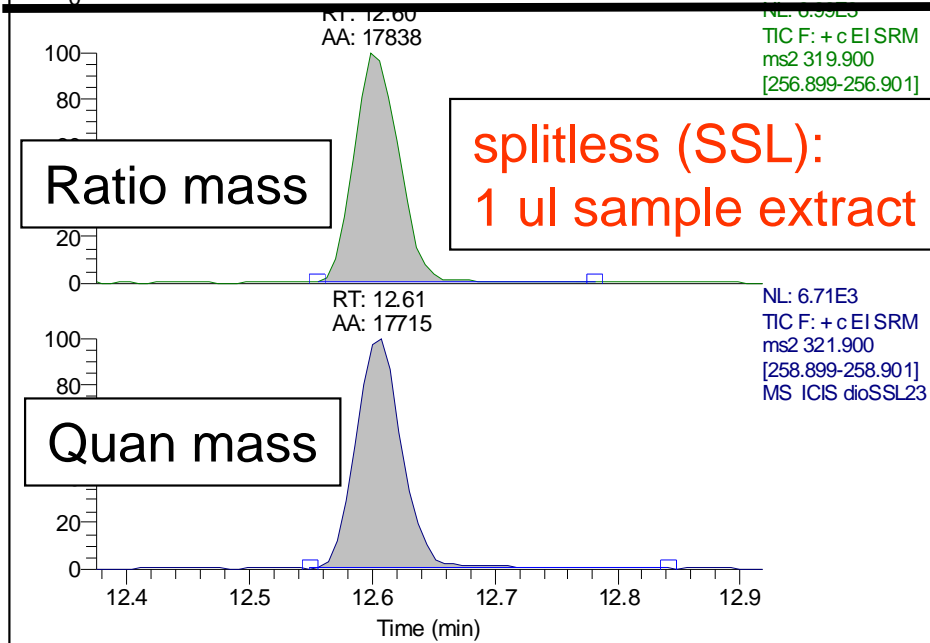
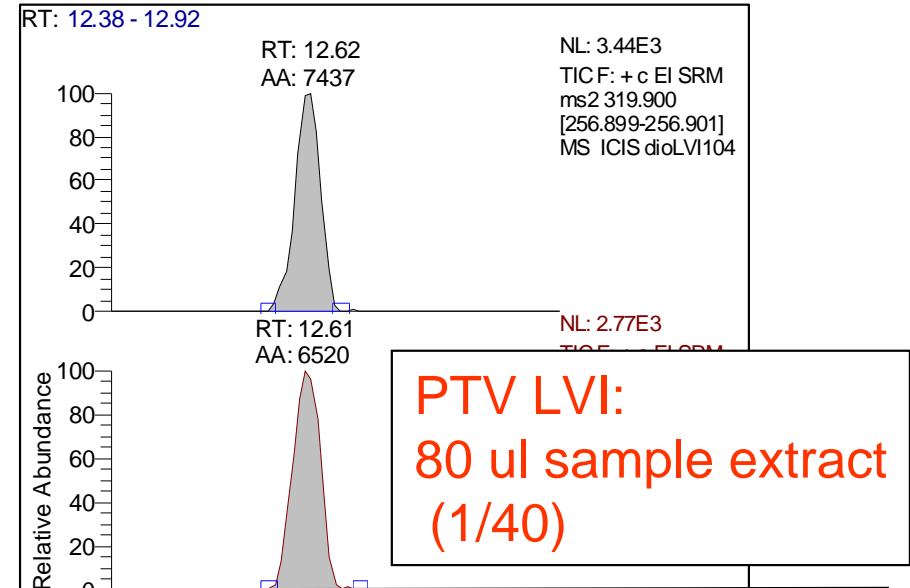
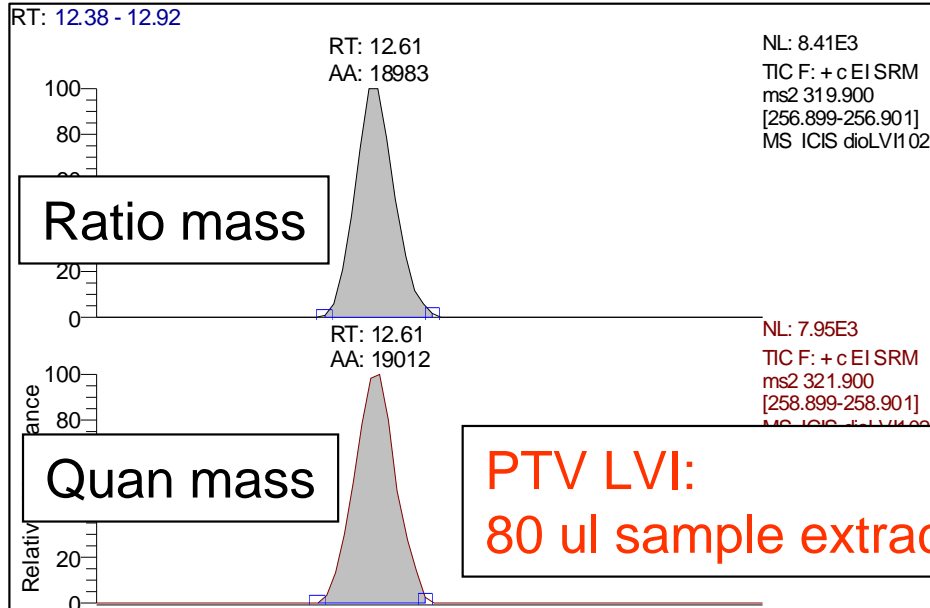
C:\Calibur\dioxLM100 4/18/2008 11:08:34 AM LV: 80 ul CS3 (1/80), PTV100(0.2), 50 ml, 20 ul/sec, PTV/LVI installed
 closed EI vol., 30 m TR5MS (0.25), 140(2)-, tune last, LV metall liner with wool, 100 ul syringe with side hole

RT: 16.59 - 19.27



80 ul dioxins/furans PTV LVI on samples

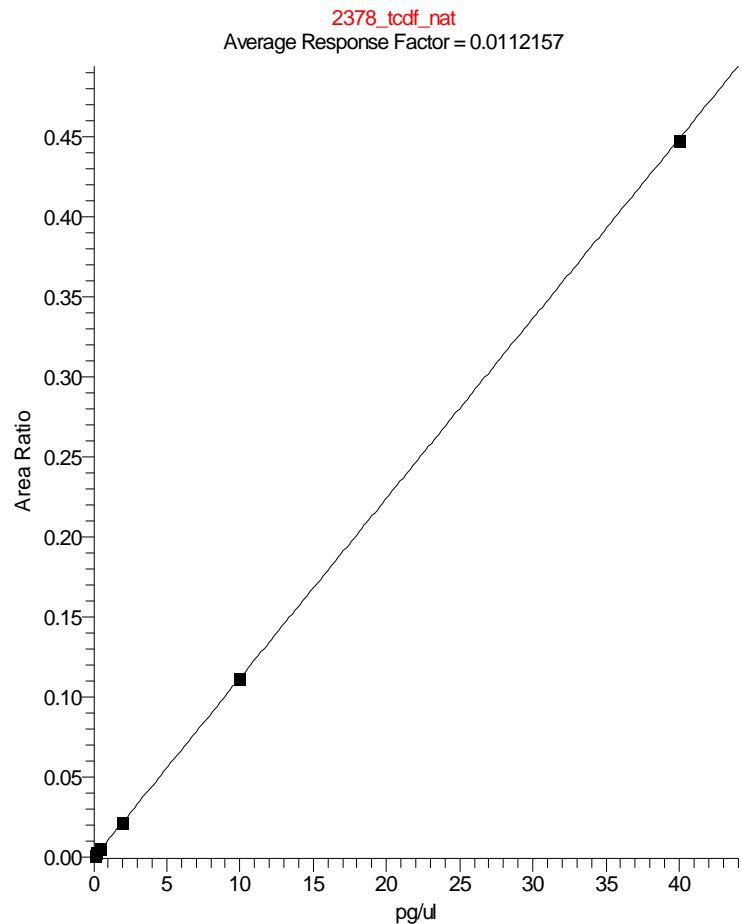
peregrine falcon egg extract / eal extract - tcdd



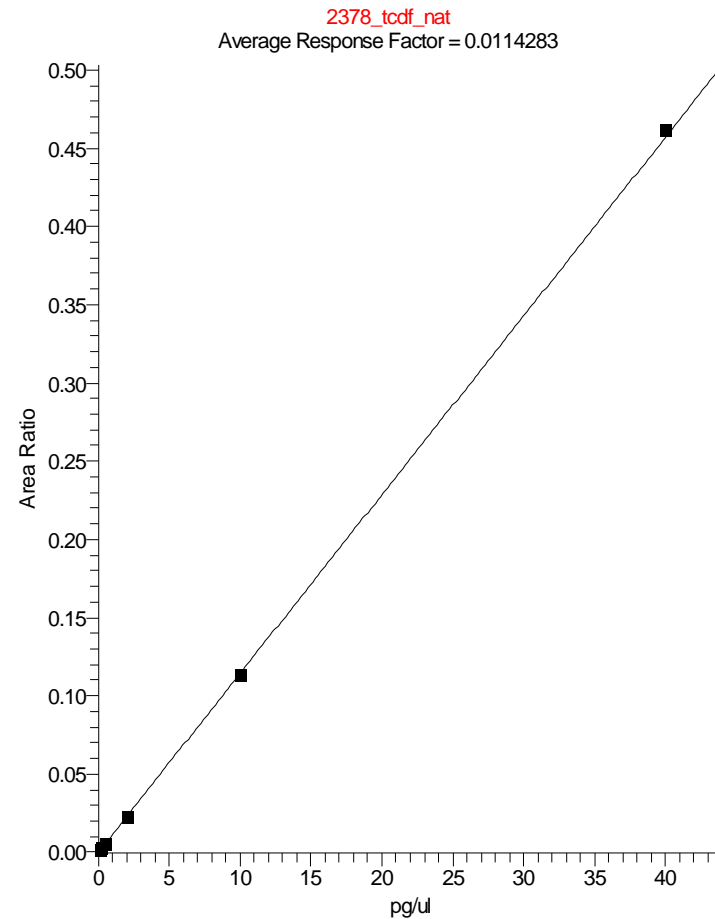
80 ul and 1 ul calibration curve: tcdf

average response EPA 1613 standards: CSL, CS 0.5, CS1 – CS4

80 ul calibration curve



1 ul calibration curve





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DFS dual data acquisition

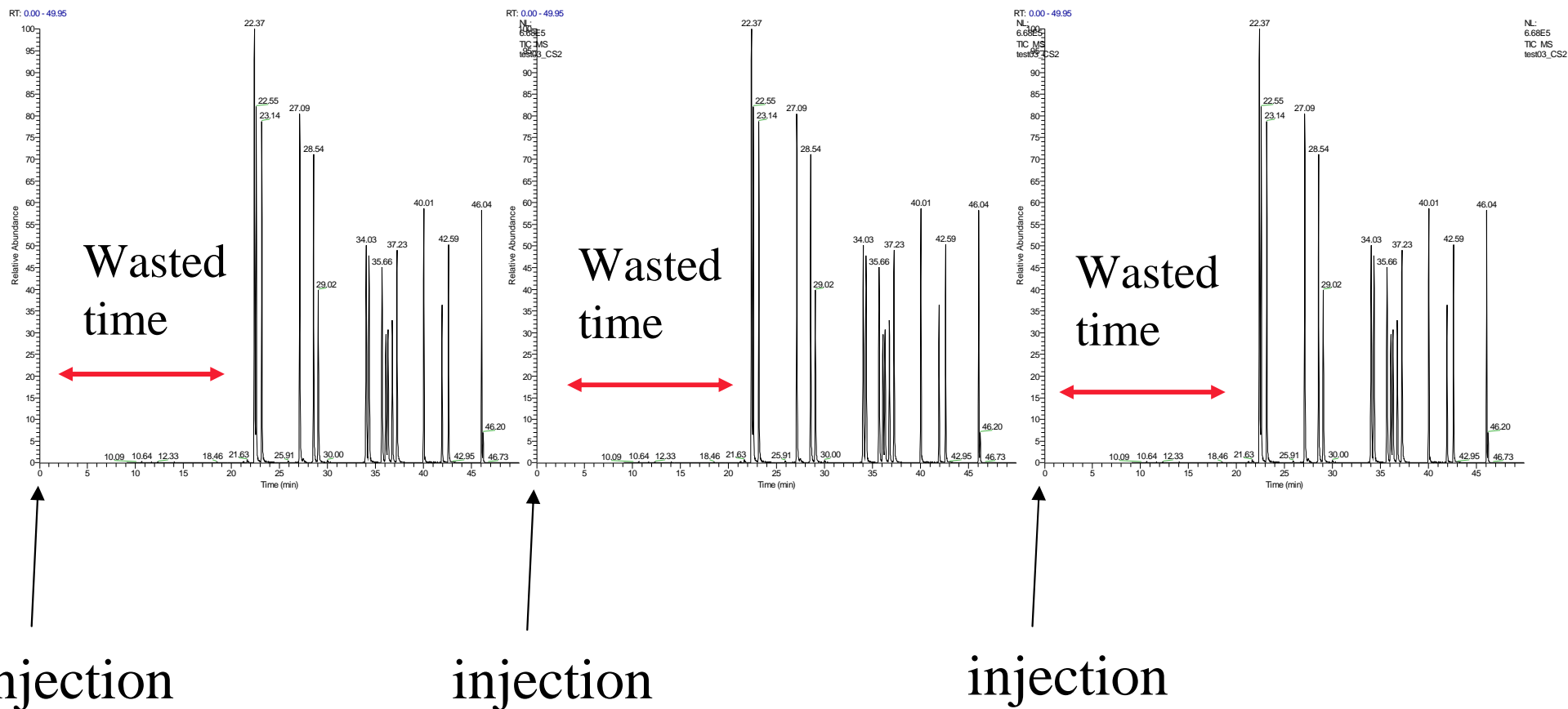
DFS – Dual data option up to two fold increased sample throughput

- 2 GCs attached to 1 DFS
- 1 extra wide Autosampler covering both GC's



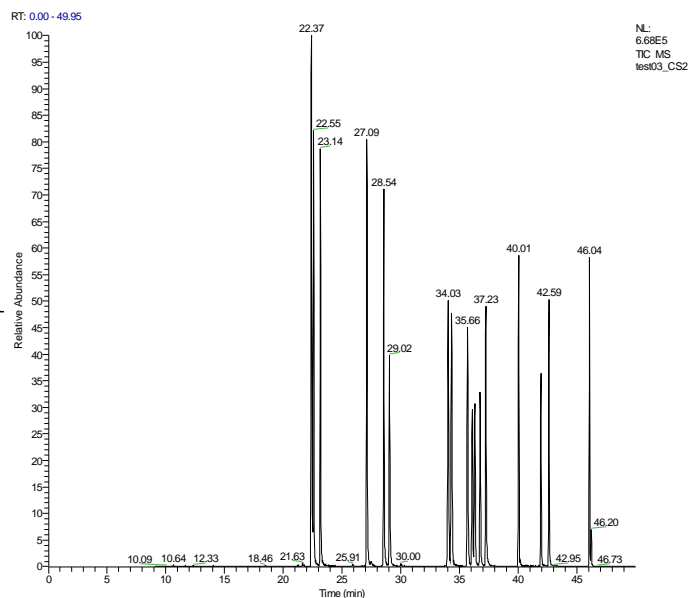
The standard way to inject samples single GC system: sequence of 3 injections

One
GC

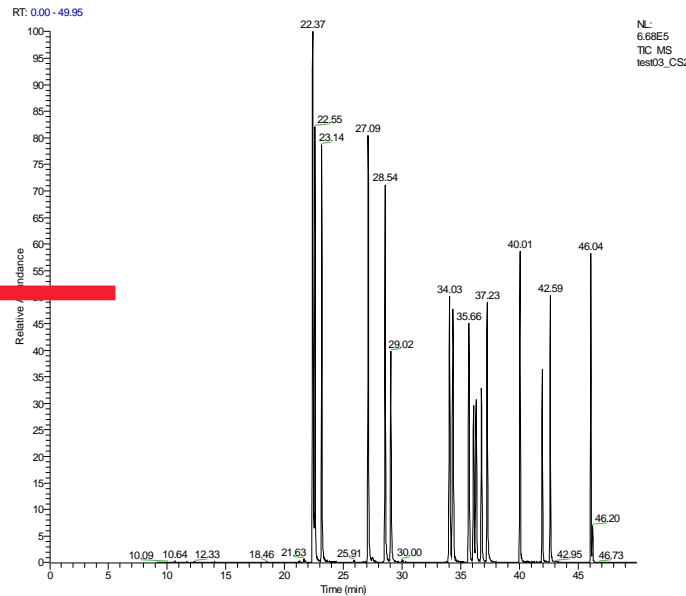


Dual GC system the standard way to inject samples same time for 3 injections as on 1 GC system

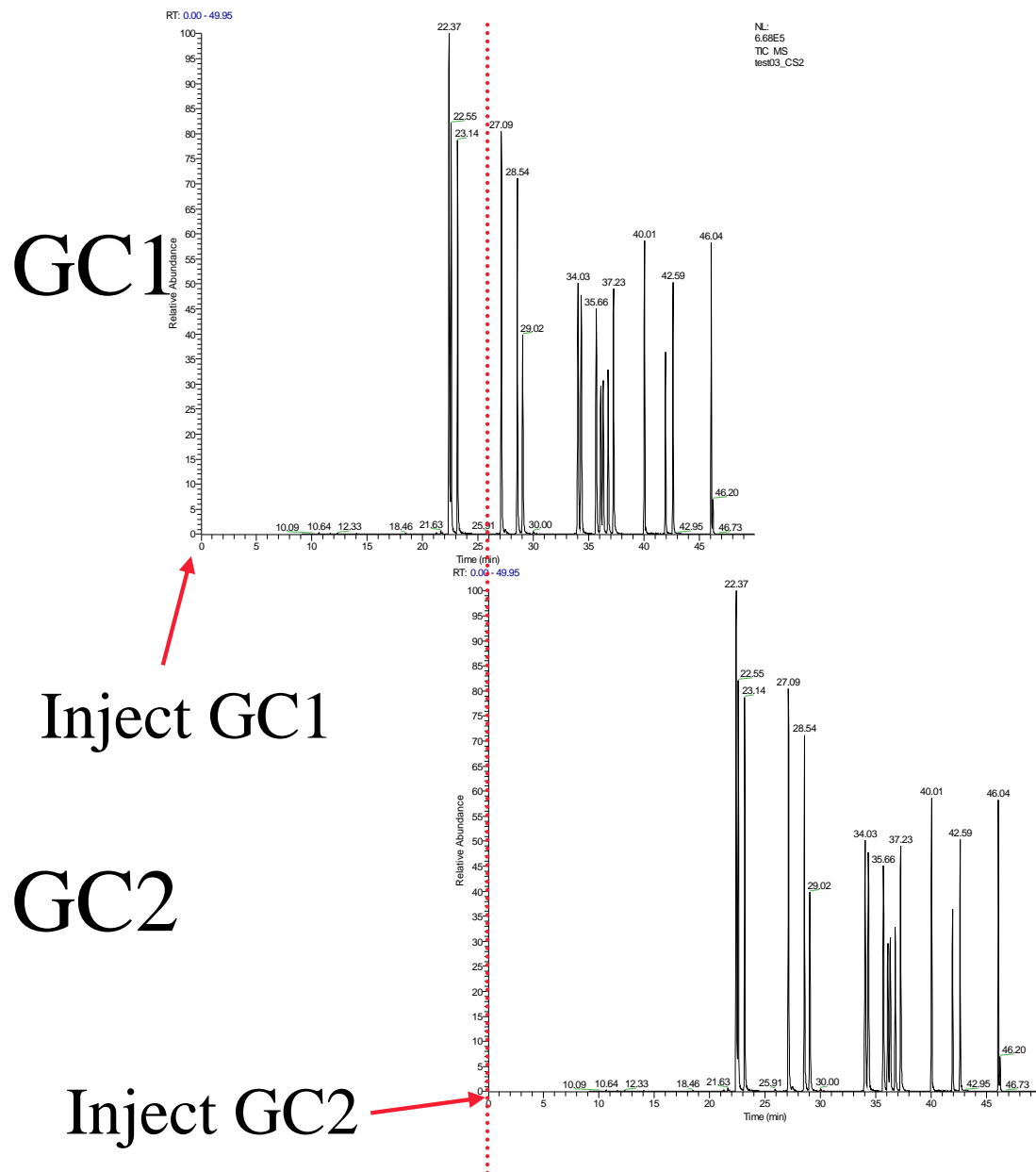
GC1



GC2



Dual Data acquisition – dual GC system how to achieve higher throughput



➔ Staggered injection !!:

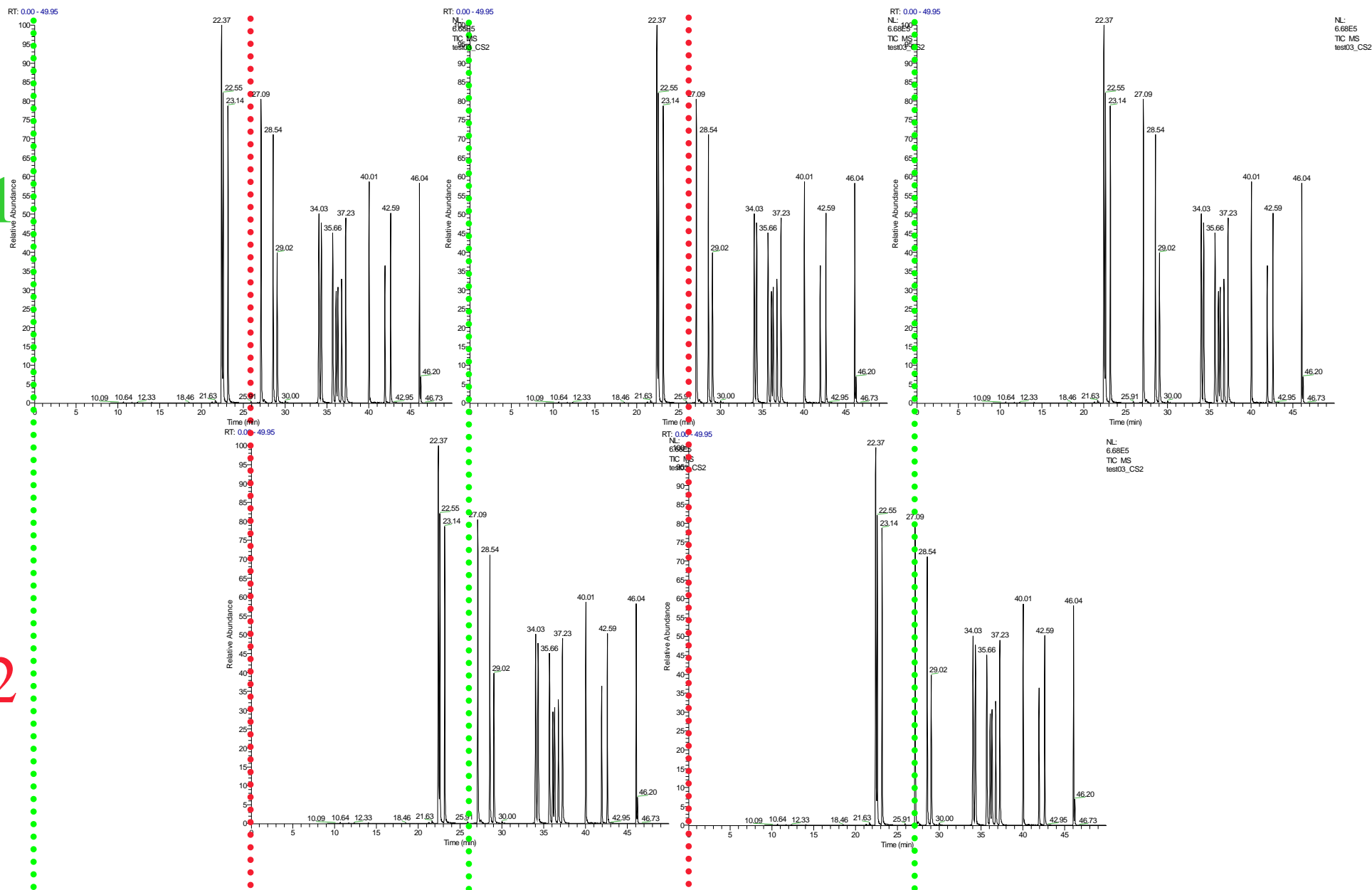
Inject next sample on GC2 already during elution of peaks on GC 1

Red dotted line:
Injection time on GC2

Dual Data acquisition – dual GC system

5 samples injected in the same time as before 3

GC1

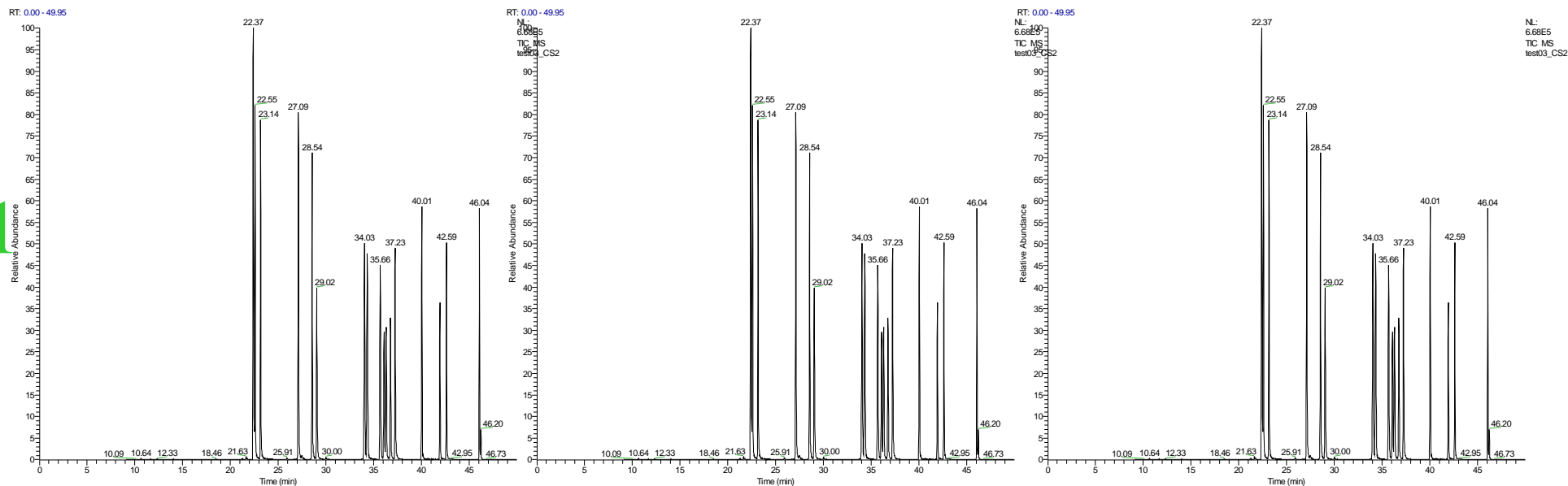


GC2

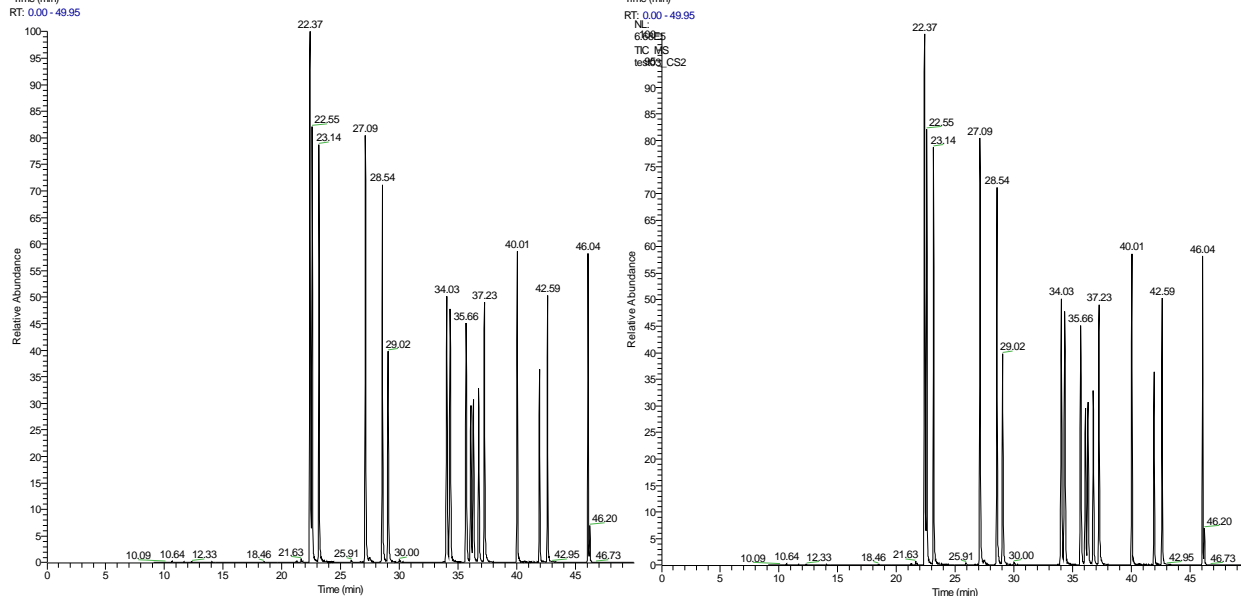
Dual Data acquisition – dual GC system

5 samples injected in the same time as before 3

GC1



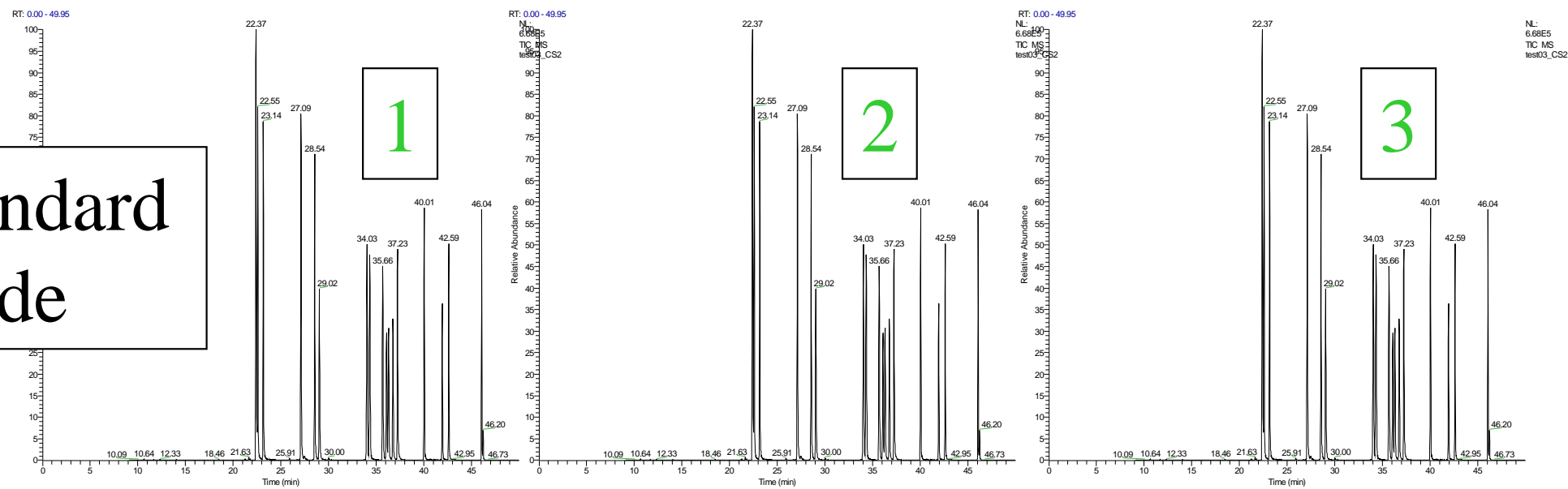
GC2



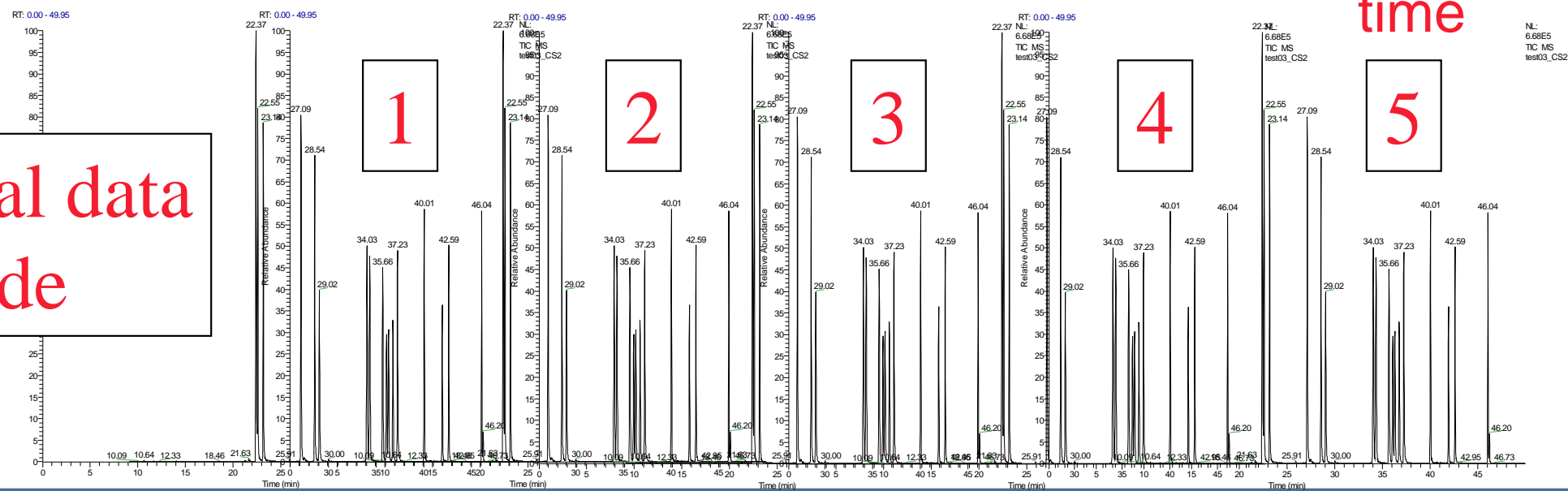
Dual Data acquisition – dual GC system

5 samples injected in the same time as before 3

Standard mode



Dual data mode





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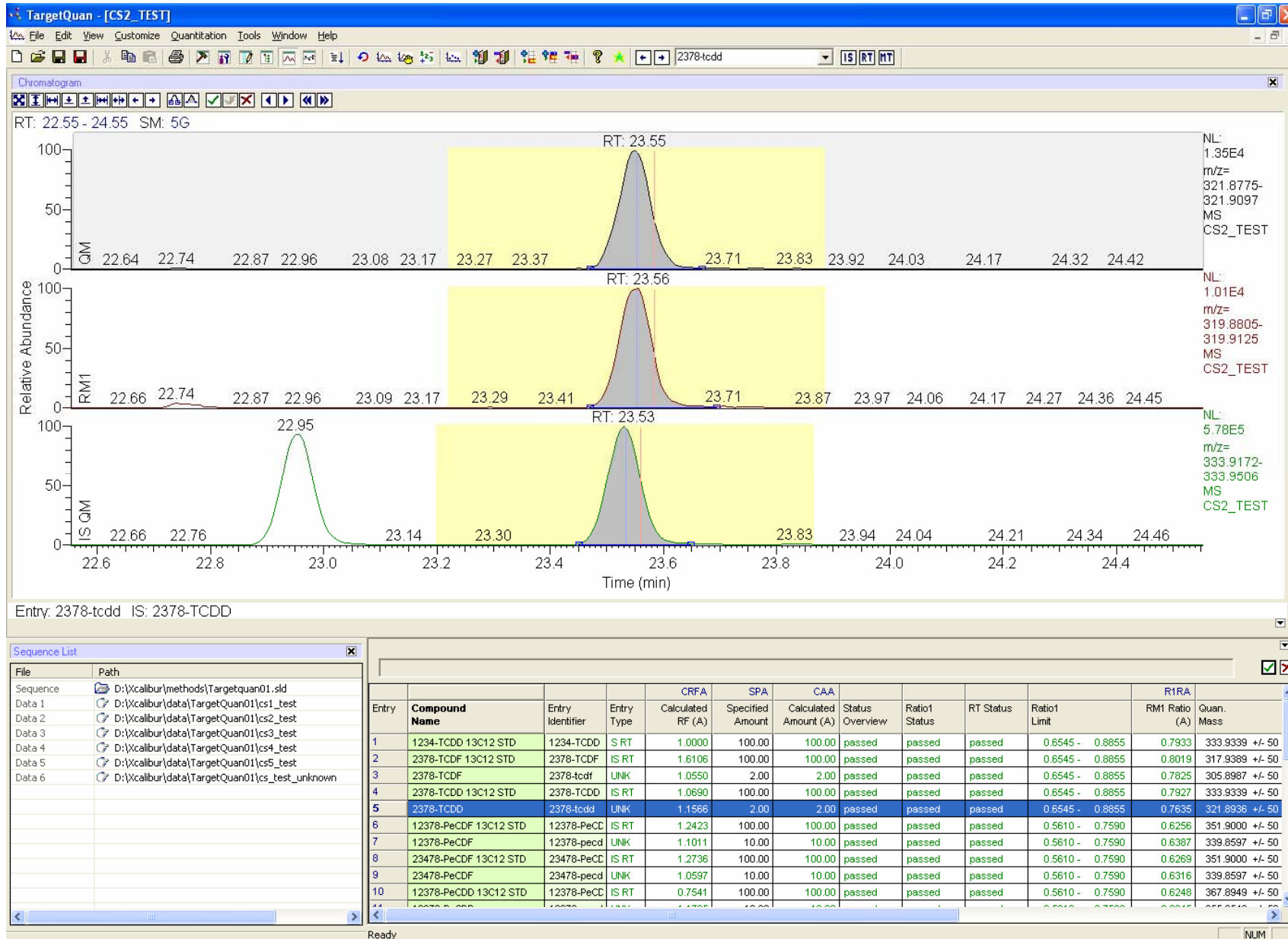
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Targetquan: new Quantification software

TargetQuan – New Quantification Software package



TargetQuan – The New Peak Browser

The screenshot displays the TargetQuan software interface. On the left, three chromatograms are shown with their respective peak data:

- Chromatogram 1 (QIM):** Peak at RT: 23.56, NL: 5.56E4, m/z= 321.9, 321.9 MS, cs3_test.
- Chromatogram 2 (RMI):** Peak at RT: 23.56, NL: 4.56E4, m/z= 319.9, 319.9 MS, cs3_test.
- Chromatogram 3 (IS QIM):** Peaks at RT: 22.96 and 23.54, NL: 4.86E5, m/z= 333.9, 334.0 MS, cs3_test.

At the bottom left, a table shows peak integration results:

| | Area | Height | Integra... | Ratio Area | Ratio Height | Ratio Limit | Expect |
|--------------|------------|-----------|------------|------------|--------------|-----------------|--------|
| Quan Mass | 220297.590 | 55538.157 | A | | | | |
| Ratio Mass 1 | 172435.926 | 45419.885 | A | 0.783 | 0.818 | 0.6545 - 0.8855 | 0.77 |
| Ratio Mass 2 | 0.000 | 0.000 | --- | 0.000 | 0.000 | 0.6545 - 0.8855 | 0.77 |

In the center, a callout box states: **By Analysis One Compound Sequentially Selected Files**.

On the right, a 'Sequence List' and a 'Compound List' table are visible. The 'Compound List' table is as follows:

| Entry | Compound Name | Entry Identifier | Quan. Mass | OM1 |
|-------|----------------------|------------------|---------------------|-----|
| 1 | 1234-TCDD 13C12 STD | 1234-TCDD | 333.9339 +/- 50 ppm | |
| 2 | 2378-TCDF 13C12 STD | 2378-TCDF | 317.9389 +/- 50 ppm | |
| 3 | 2378-TCDF | 2378-tcdf | 305.8987 +/- 50 ppm | |
| 4 | 2378-TCDD 13C12 STD | 2378-TCDD | 333.9339 +/- 50 ppm | |
| 5 | 2378-TCDD | 2378-tcdd | 321.8936 +/- 50 ppm | |
| 6 | 12378-PeCDF 13C12 ST | 12378-PeCDF | 351.9000 +/- 50 ppm | |
| 7 | 12378-PeCDF | 12378-pecdf | 339.8597 +/- 50 ppm | |
| 8 | 23478-PeCDF 13C12 ST | 23478-PeCDF | 351.9000 +/- 50 ppm | |
| 9 | 23478-PeCDF | 23478-pecdf | 339.8597 +/- 50 ppm | |
| 10 | 12378-PeCDD 13C12 ST | 12378-PeCDD | 351.9000 +/- 50 ppm | |
| 11 | 12378-PeCDD | 12378-pe | | |
| 12 | 123789-HxCDD 13C12 S | 123789-H | | |
| 13 | 123478-HxCDF 13C12 S | 123478-H | | |
| 14 | 123478-HxCDF | 123478-H | | |
| 15 | 123678-HxCDF 13C12 S | 123678-H | | |
| 16 | 123678-HxCDF | 123678-H | | |
| 17 | 234678-HxCDF 13C12 S | 234678-H | | |
| 18 | 234678-HxCDF | 234678-H | | |
| 19 | 123789-HxCDF 13C12 S | 123789-H | | |
| 20 | 123789-HxCDF | 123789-H | | |
| 21 | 123478-HxCDD 13C12 S | 123478-H | | |
| 22 | 123478-HxCDD | 123478-H | | |
| 23 | 123678-HxCDD 13C12 S | 123678-HxCDD | 401.8559 +/- 50 ppm | |
| 24 | 123678-HxCDD | 123678-hxcdd | 389.8157 +/- 50 ppm | |
| 25 | 123789-HxCDD | 123789-hxcdd | 389.8157 +/- 50 ppm | |
| 26 | 1234678-HpCDF 13C12 | 1234678-HpCDF | 419.8220 +/- 50 ppm | |
| 27 | 1234678-HpCDF | 1234678-hpcdf | 407.7818 +/- 50 ppm | |
| 28 | 1234789-HpCDF 13C12 | 1234789-HpCDF | 419.8220 +/- 50 ppm | |
| 29 | 1234789-HpCDF | 1234789-hpcdf | 407.7818 +/- 50 ppm | |
| 30 | 1234678-HpCDD 13C12 | 1234678-HpCDD | 435.8169 +/- 50 ppm | |

At the bottom right, another callout box states: **By Compound One Analysis Sequentially Selected Entry**.

A yellow box at the bottom center contains the text: **Xcalibur Analysis Sequence:**

1. Loads Sequence Data Files
2. For Calibrations and Unknowns

TargetQuan – The New Screen Layout

The screenshot displays the TargetQuan software interface. On the left, the 'Parameter Overview' window shows various parameters and their values. Below it, the 'Spreadsheet Layout' window allows users to customize the data table. A callout box points to the 'Spreadsheet Individual Layout' options, which include checkboxes for 'Compound Name', 'Entry Identifier', 'Entry Type', 'Entry Type Name', 'Group', 'Int. Std. Identifier', 'Ret. Time Std. Identifier', 'Specified Amount', 'TEF', 'Specified RF (A)', 'Specified RF (H)', 'Peak Parameters', 'Identification', 'Specified RT [min]', 'Expected RT', 'RT Window [min]', 'RT View Width [min]', 'Limits', 'Ratio1 Limit', 'Ratio2 Limit', and 'RT Limit [min]'. The 'Compound Name', 'Entry Identifier', 'Quan. Mass', and 'Ratio Mass 1' options are circled in red.

The main data table shows the following columns: Entry, Compound Name, Entry Identifier, Quan. Mass, Ratio Mass 1, Specified Amount, RM1 Time Limit [min], and Ratio1 Limit. The table contains 27 rows of data, including entries for 2378-TCDF, 2378-TCDD, 23478-PeCDF, 12378-PeCDF, 12378-PeCDD, 123478-HxCDF, 123678-HxCDF, 123789-HxCDF, 123478-HxCDD, 123678-HxCDD, 123789-HxCDD, 123478-HxCDD, 123678-HxCDD, 123789-HxCDD, 123478-HpCDF, and 123789-HpCDF.

Customized Windows:

1. Any Combination Possible
2. Store as Screen Layout
3. Fast Switching Chro ↔ Spreadsheet

TargetQuan – The ISTD Dependency Tree

The screenshot displays the TargetQuan interface with the 'Entry Dependencies' window open. The tree structure is as follows:

- cs3_test
 - 1234-TCDD
 - 2378-TCDF (Natives)
 - 2378-tcdf
 - 2378-TCDD (ISTD)
 - 2378-tcdd
 - 12378-PeCDF
 - 12378-pecdf
 - 12378-PeCDD (Surrogates)
 - 12378-pecdd
 - 123789-HxCDD
 - ocdf
 - ocdd

Callouts in the image identify 'Natives' (2378-TCDF), 'ISTD' (2378-TCDD), and 'Surrogates' (12378-PeCDD).

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

