DESIGN OF NEW LOW-BLEED GAS CHROMATOGRAPHIC STATIONARY PHASES AND CAPILLARY COLUMNS

Frank L. Dorman, Gary B. Stidsen, Chris M. English, Rick Morehead, Jack Cochran, Eric J. Reiner, and Karen MacPherson

Restek Corporation
www.restekcorp.com

When is a Low-Bleed Column Important?

- Low concentration GC-MS analyses
  - Full scan
  - SIM
  - High-resolution
- When detector is especially sensitive to bleed
  - ECD
- When bleed directly interferes with quantitation
  - Bleed and analyte share the same ions (rare)

How Do You Make a Low-Bleed Column ?

- Thinner stationary phase thickness
  - Not really purchasing what you think…
- Higher conditioning temperatures
  - Can lead to higher reactivity and phase loss
- Improve deactivation chemistry
  - Phase has more “affinity” for tubing
- Move polymer functionality from side chain to backbone

Considerations for Backbone-modified Polysiloxane Stationary Phases:

- By using a “low-bleed” column, you do not want to give up selectivity.
  - Many “low bleed” columns are dimethyl or phenyl/methyl-type
- Computer Assisted Stationary Phase Design (CASPD) utilized to optimize selectivity of backbone-stabilized polymers for specific applications
  - *Anal. Chem.* **74**(9), 2133-2138 2002.
  - *LC*GC* **18**(9), 928, 2000.
  - *American Laboratory*, **31**(6), 20-26, 1999.

Applications

Rtx-PCB Capillary GC Column

- Proprietary polysiloxane designed for PCB congener separation for GC-MS analysis
- 380 maximum operating temperature in standard high-temperature fused silica tubing
- Standard column dimensions, standard film thickness
- Generally increased retention for aromatics over “5”, “5Sil MS” and “XLB” type phases

Analysis of PCB Congeners – GC-MS

- European congener list:
  - BZ # 28,52,101,118,153,138 and 180 resolved by Cl level or chromatographically from all others
- 1668
  - All 13 congeners resolved
- McFarland and Clarke congeners:
  - 33 of 36 resolved (1 more than best previous column – XLB)
- Aroclor congeners out of 139:
  - 127 resolved (3 more than XLB)
  - 9 pairs are unresolved
- 209 Congeners:
  - 168 resolved (same number but different congeners as XLB)

GC-ToF Analysis

- Pyrethroids analysis
  - Late-eluting pyrethroids can be difficult to keep well resolved on standard columns due to limited maximum operating temperature.
  - Peak width is narrower if the compounds are eluted during the temperature program, not on an isothermal hold
    - Preference is for column with high maximum operating temperature
  - Selectivity must be appropriate to achieve separation between the target compounds which can have similar mass spectra

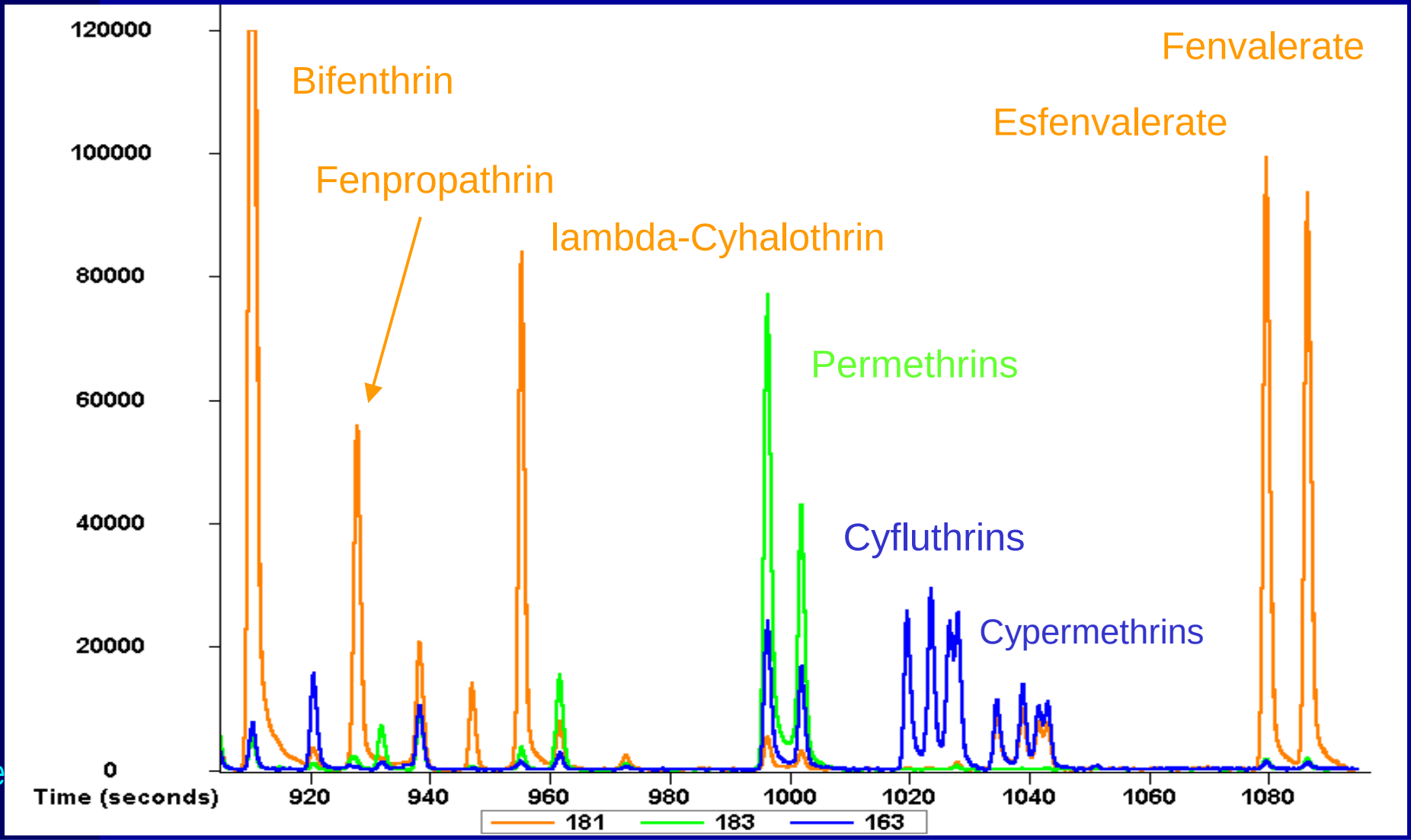
GC Conditions

- Splitless injection
  - One microliter at 250°C
  - 60 sec valve time
  - 30 m x 0.25 mm x 0.25 µm Restek Rtx-PCB column
  - Constant flow helium, 1 mL/minute
- GC oven program
  - 60°C (1 min), 30°/min to 120°, 15°/min to 360° (1 min)
  - Total run time: 20 min

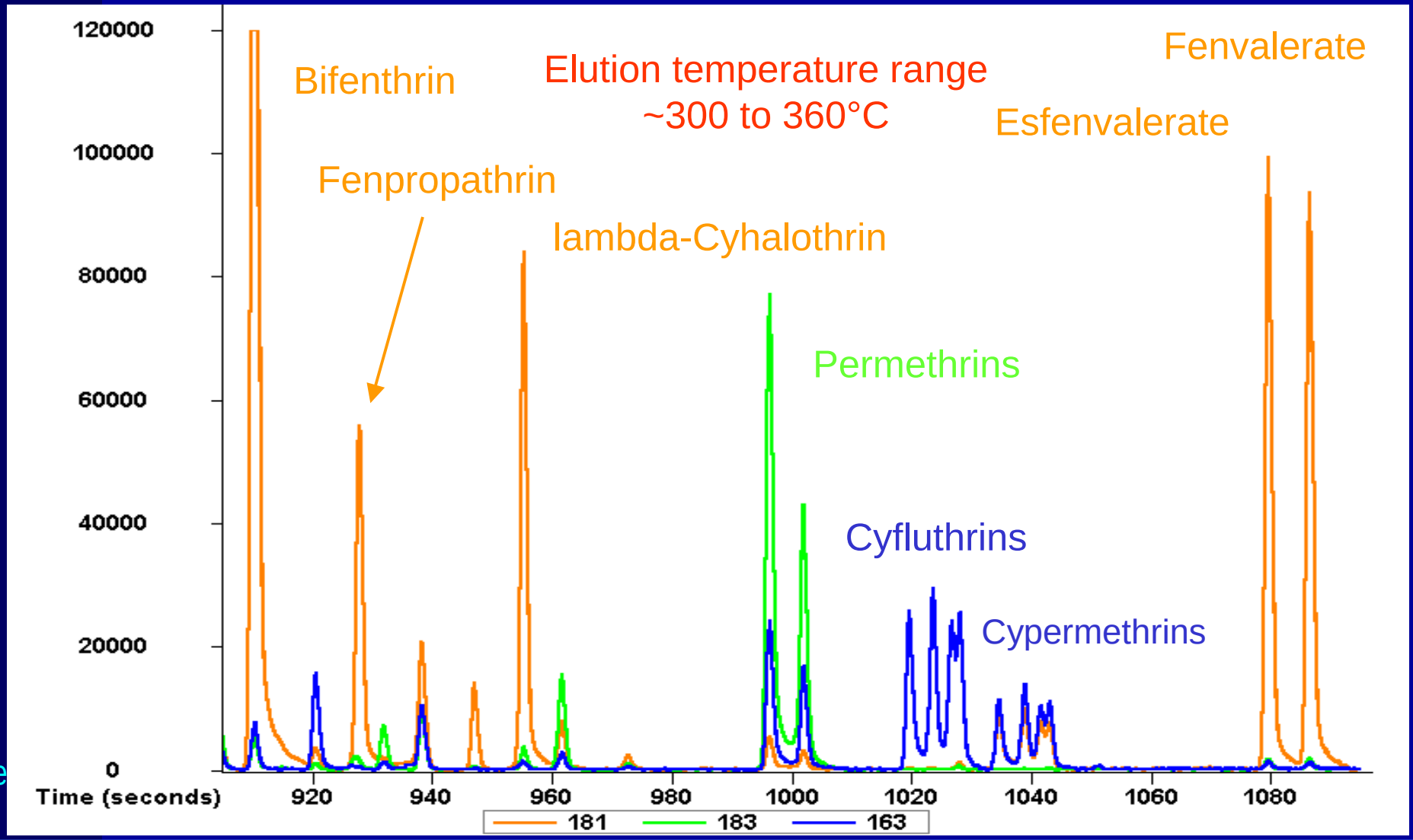
MS Conditions

- Source temperature: 225°C
- Electron ionization: 70 eV
- Stored mass range: 45 to 550 u
- Acquisition rate: 10 spectra/sec

Pyrethroids on Rtx-PCB



Pyrethroids on Rtx-PCB



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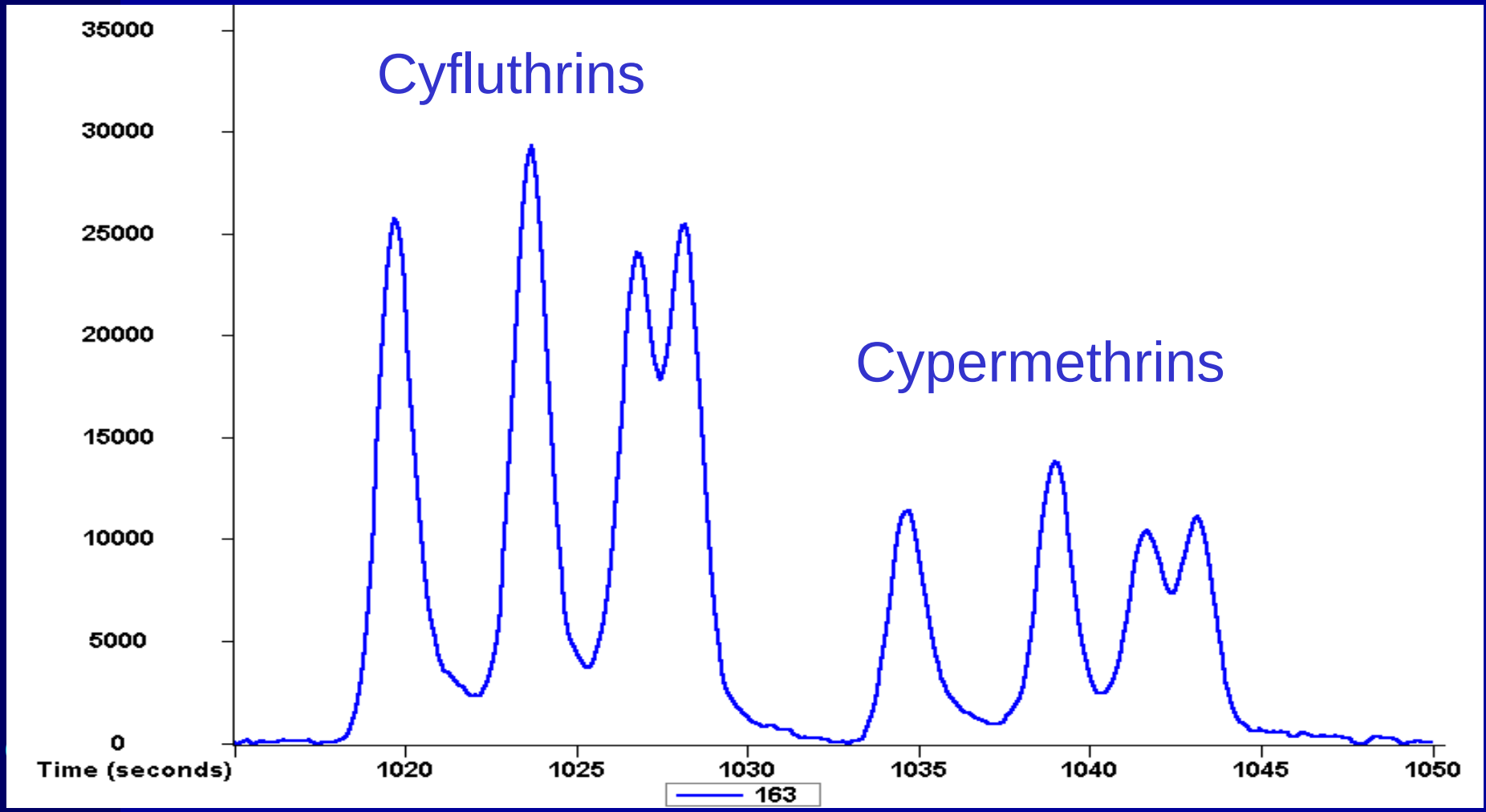
Separation of Cyfluthrins and Cypermethrins on Rtx-PCB



Pyrethroids on Rtx-PCB

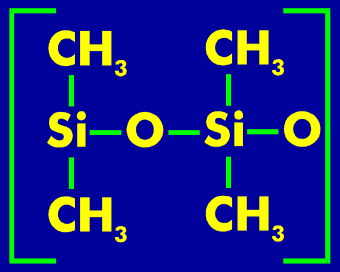
Pesticide	RT sec	RT min	RRT	Elution Temp °C	MW	Formula	CAS#	Ions 1	2	3
Pentachloronitrobenzene	680.5	11.34	1.000	245	293	C6Cl5NO2	82-68-8	237	249	295
Bifenthrin	916.0	15.27	1.346	304	410	C22H22O2ClF3	82657-04-3	165	166	181
Fenpropathrin	933.5	15.56	1.372	308	349	C22H23NO3	39515-41-8	97	181	265
lambda-Cyhalothrin	961.0	16.02	1.412	315			91465-08-6	181	197	208
cis-Permethrin	1002.2	16.70	1.473	326	390	C21H20Cl2O3	54774-45-7	127	163	183
trans-Permethrin	1007.9	16.80	1.481	327	390	C21H20Cl2O3	61949-77-7	127	163	183
Cyfluthrin	1025.5	17.09	1.507	331	433	C22H18Cl2FNO3	68359-37-5	163	206	226
Cyfluthrin	1029.4	17.16	1.513	332	433	C22H18Cl2FNO3	68359-37-5	163	206	226
Cyfluthrin	1032.5	17.21	1.517	333	433	C22H18Cl2FNO3	68359-37-5	163	206	226
Cyfluthrin	1033.9	17.23	1.519	333	433	C22H18Cl2FNO3	68359-37-5	163	206	226
Cypermethrin	1040.5	17.34	1.529	335	415	C22H19Cl2NO3	52315-07-8	163	181	209
Cypermethrin	1044.8	17.41	1.535	336	415	C22H19Cl2NO3	52315-07-8	163	181	209
Cypermethrin	1047.5	17.46	1.539	337	415	C22H19Cl2NO3	52315-07-8	163	181	209
Cypermethrin	1048.9	17.48	1.541	337	415	C22H19Cl2NO3	52315-07-8	163	181	209
Esfenvalerate	1085.6	18.09	1.595	346	419	C25H22ClNO3	66230-04-4	167	225	419
Fenvalerate	1092.5	18.21	1.605	348	419	C25H22ClNO3	51630-58-1	167	225	419

Rtx-500 Capillary GC Column

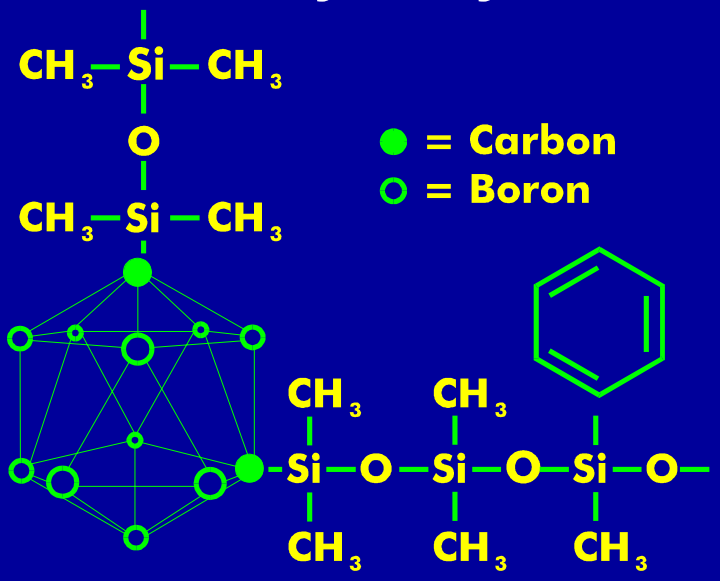
- Carborane-stabilized stationary phase
  - Maximum temperature of 380 C in “standard high-temperature” tubing
  - Maximum temperature of 440 C in passivated metal columns (Mxt-500)
- Extremely low bleed levels
  - Surpasses phenyl/methyl phases, and silphenylene stationary phases
- Common dimensions available

Carborane-polysiloxane Stationary Phase

Dimethyl Polysiloxane



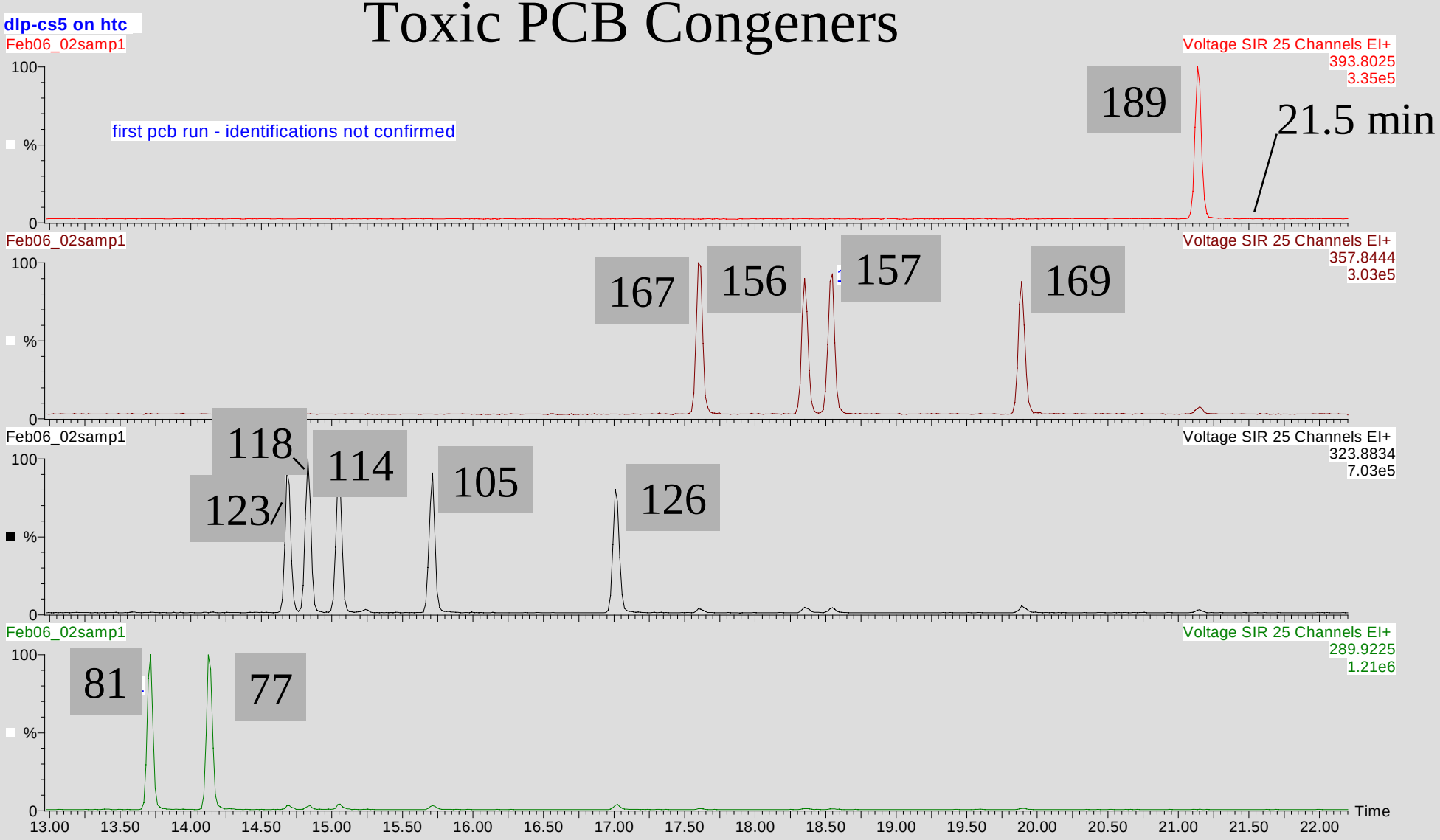
Carborane Dimethyl Polysiloxane



Analysis of PCB Congeners by GC-MS

- Toxic PCB's by USEPA method 1668 commonly run on "octyl" phases
  - Very high background from bleed decreases sensitivity
  - Phase loss can cause retention order changes
  - "5" phases can have coelution issues
    - 118/123
    - 156/157
  - Rtx-500 Column can improve upon these…

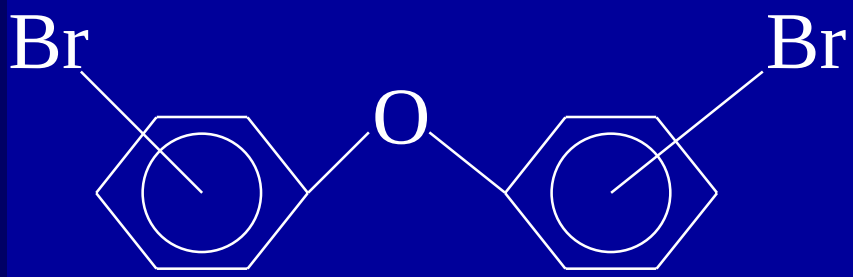
Toxic PCB Congeners



Analysis of Siloxanes in the Environment

- Rare case, but becoming important
- Since most all GC phases are siloxanes, bleed ions directly interfere with quantitation ions for GC-MS analyses
- Rtx-500 Carborane-based capillary column does not have any siloxane character in its bleed spectrum
- Rtx-500 also useful for PBDE flame retardant analyses (Pittcon 2003 Workshop)

Polybromonated Diphenylethers



Br 1-10

- Named similarly to PCB congeners (BDE 1-209)

  J. High Resolut Chromatogr **15**:260

- Human exposure via the food chain is 0.2 – 0.7 mg/day

  Organohalogen Compounds **35**:411

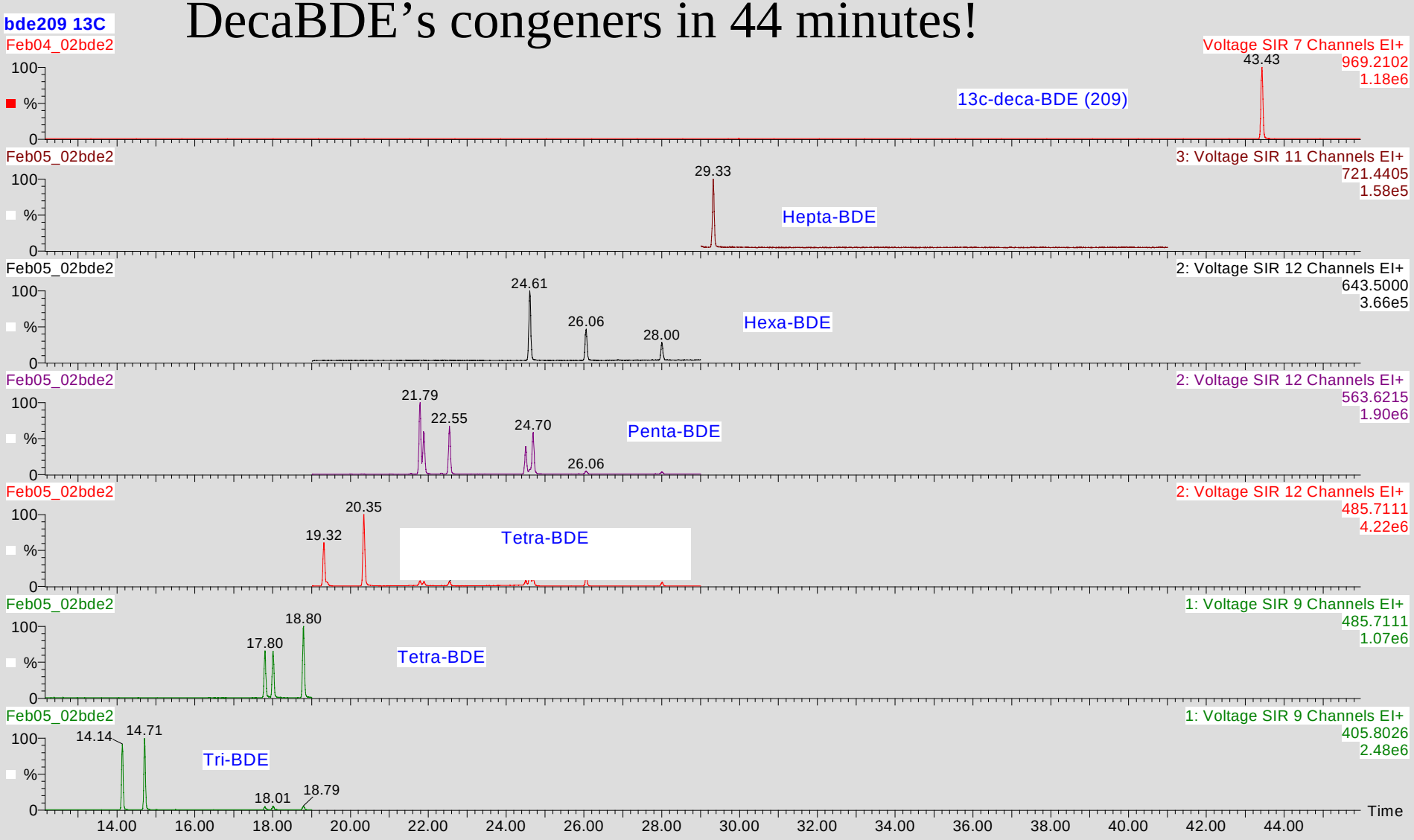
- Listed as Endocrine Disruptors

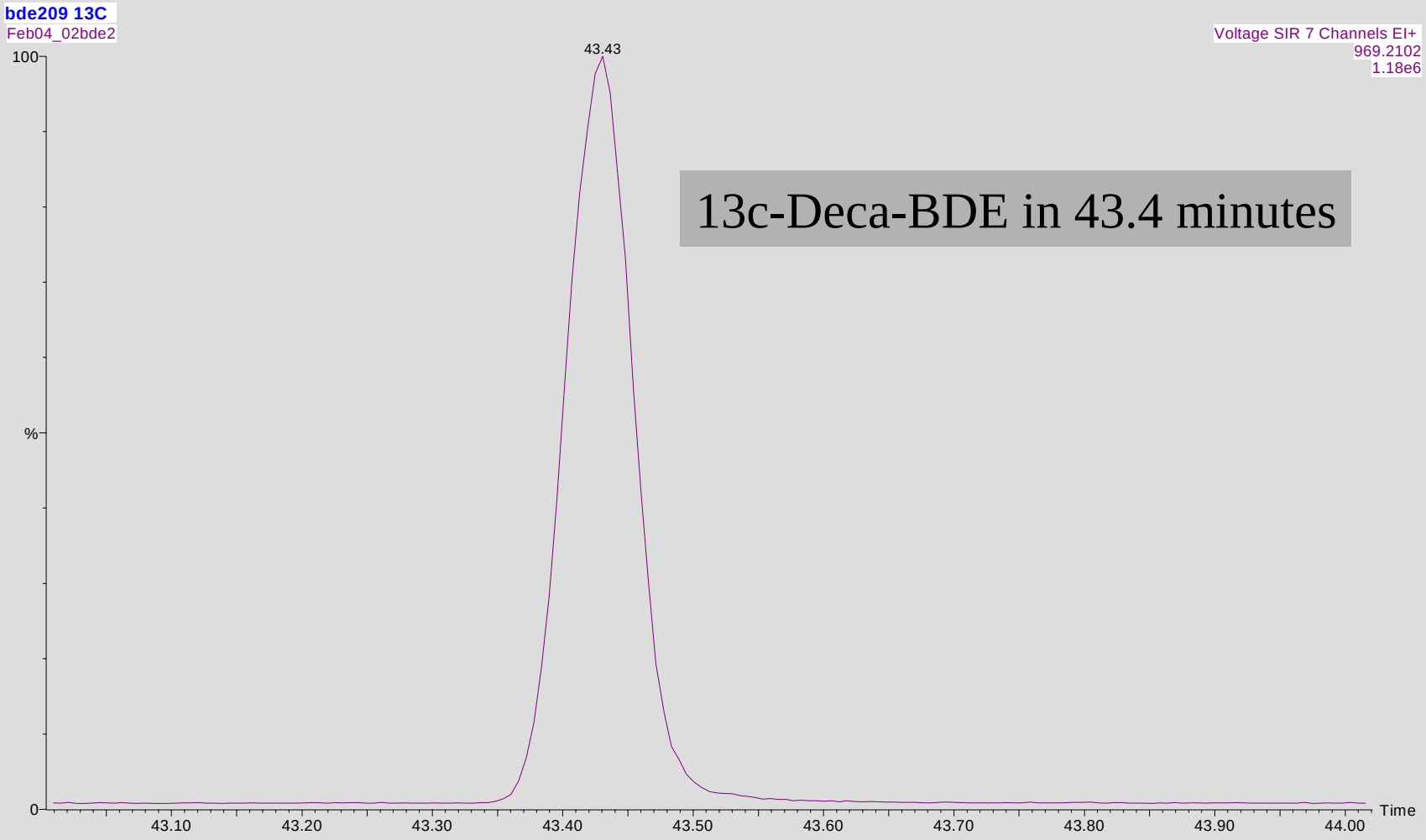
  Environ Health Perspect **101**:378

Wellington Laboratories BDE Mix-C

4-bromodiphenyl ether (3)	250	141,115
2,4-Dibromodiphenyl ether (7)	168	328,139
4,4'-dibromodiphenyl ether (15)	328	168,221
2,2',4-tribromodiphenyl ether (17)	248	408,406
2,4,4'-tribromodiphenyl ether (28)	406	248,246
2,2',4,5'-tetrabromodiphenyl ether (49)	326	486,328,324
2,3',4',6-tetrabromodiphenyl ether (71)	326	486,324,328
2,2',4,4'-tetrabromodiphenyl ether (47)	326	486,328,324
2,3',4,4'-tetrabromodiphenyl ether (66)	326	486,328,324
3,3',4,4'-tetrabromodiphenyl ether (77)	326	486,328,324
2,2',4,4',6-pentabromodiphenyl ether (100)	406	564,566
2,3',4,4',6-pentabromodiphenyl ether (119)	404	406,564
2,2',4,4',5-pentabromodiphenyl ether (99)	406	564,566
2,2',3,4,4'-pentabromodiphenyl ether (85)	406	564,566
3,3',4,4',5-pentabromodiphenyl ether (126)	566	564,568,406
2,2',4,4',5,6'-hexabromodiphenyl ether (154)	484	644,486
2,2',4,4',5,5'-hexabromodiphenyl ether (153)	644	484,486,482
2,2',3,4,4',5'-hexabromodiphenyl ether (138)	642	484
2,2',3,4,4',5',6-heptabromodiphenyl ether (183)	722	564
decabromodiphenyl ether (209)	956	

Baseline separation of Tri, Tetra, Penta, Hexa, Hepta, and DecaBDE's congeners in 44 minutes!





13c-Deca-BDE in 43.4 minutes

BROMINATED FLAME RETARDANT GC PROGRAM

INSTRUMENT CONFIGURATION

Micromass Autospec-UltimaNT (High Resolution Mass Spectro

Source Temperature = **300°C**

GC CONDITIONS (HP 6890 +)

Constant flow @ **1.5mL/min**

Injector Temp. **300°C**

Temp. Ramp	**Temp.**	**Hold Time**
Start Temp.	100	1 min
10	110	0.64
80	180	0
5	350	23

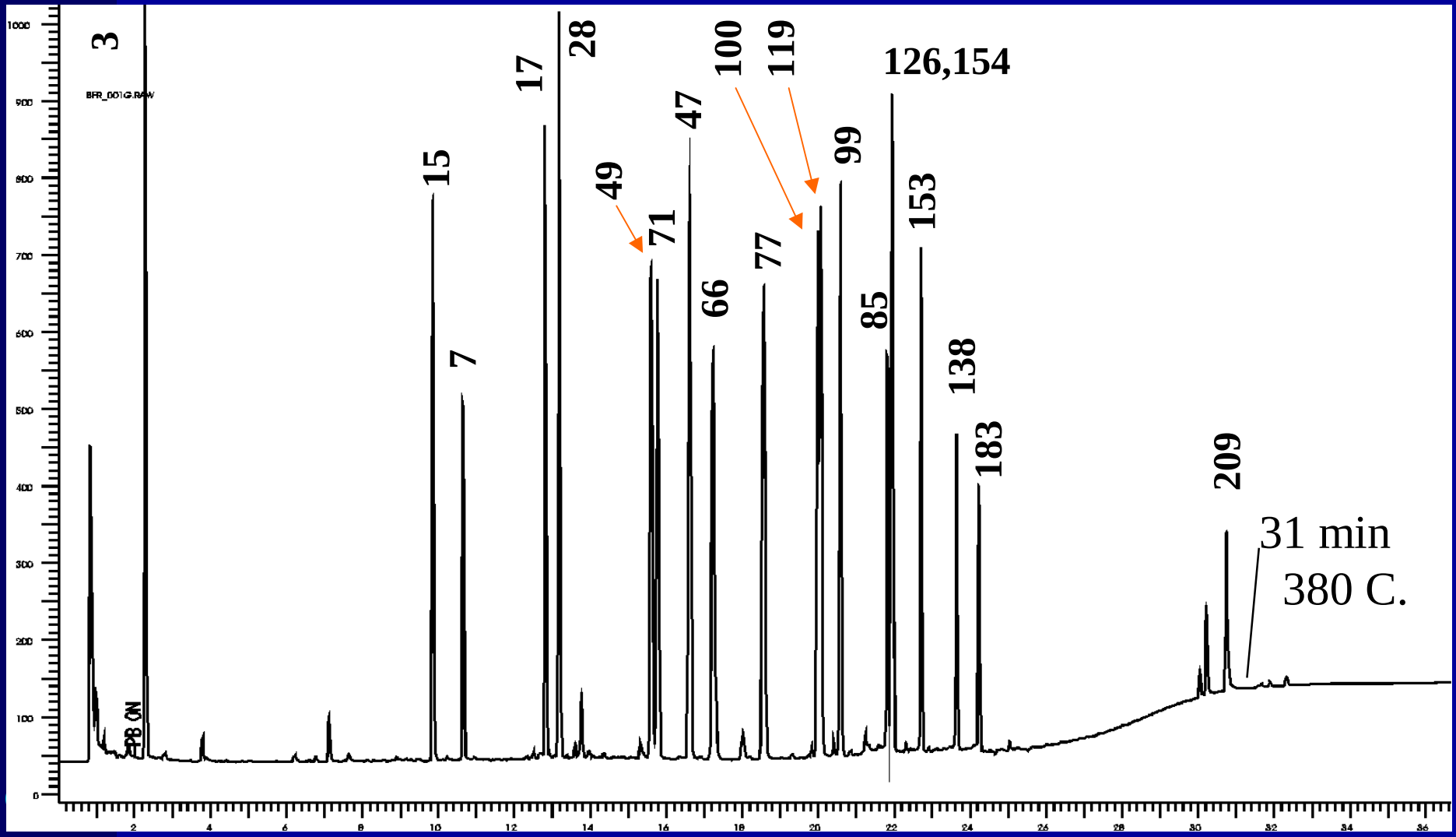
TOTAL RUN TIME = 60.51

**NB: DecaBDE (last elutor) elutes at ~43 min.

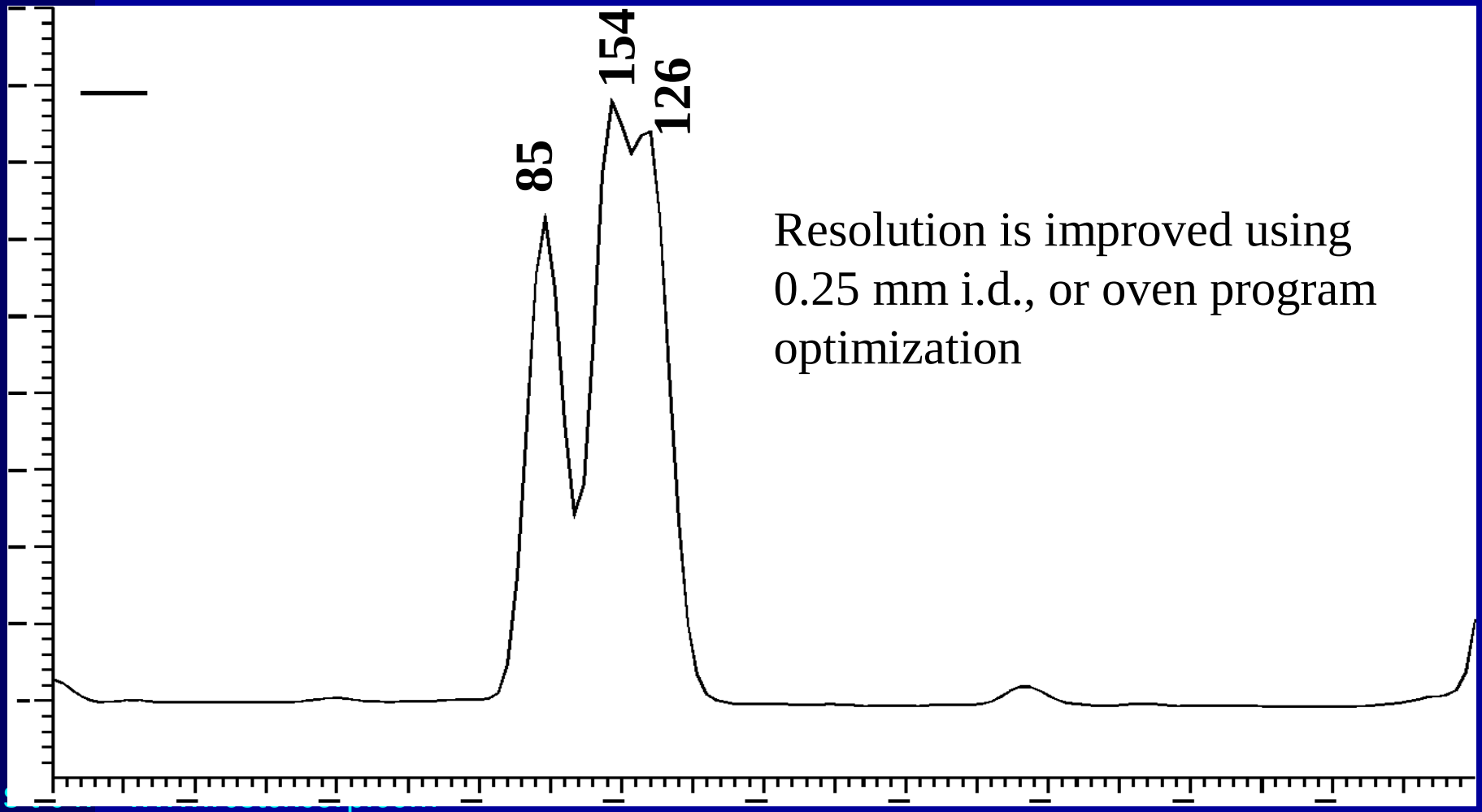
GC-ECD Analysis

- Electron capture detector is common to many laboratories
- Compounds have excellent response by ECD
- Higher flow rates may allow for more rapid separation using larger-diameter columns
- Instrumentation less expensive than HRMS
- Instrumentation is also field portable

Wellington Laboratories BDE Mix-C

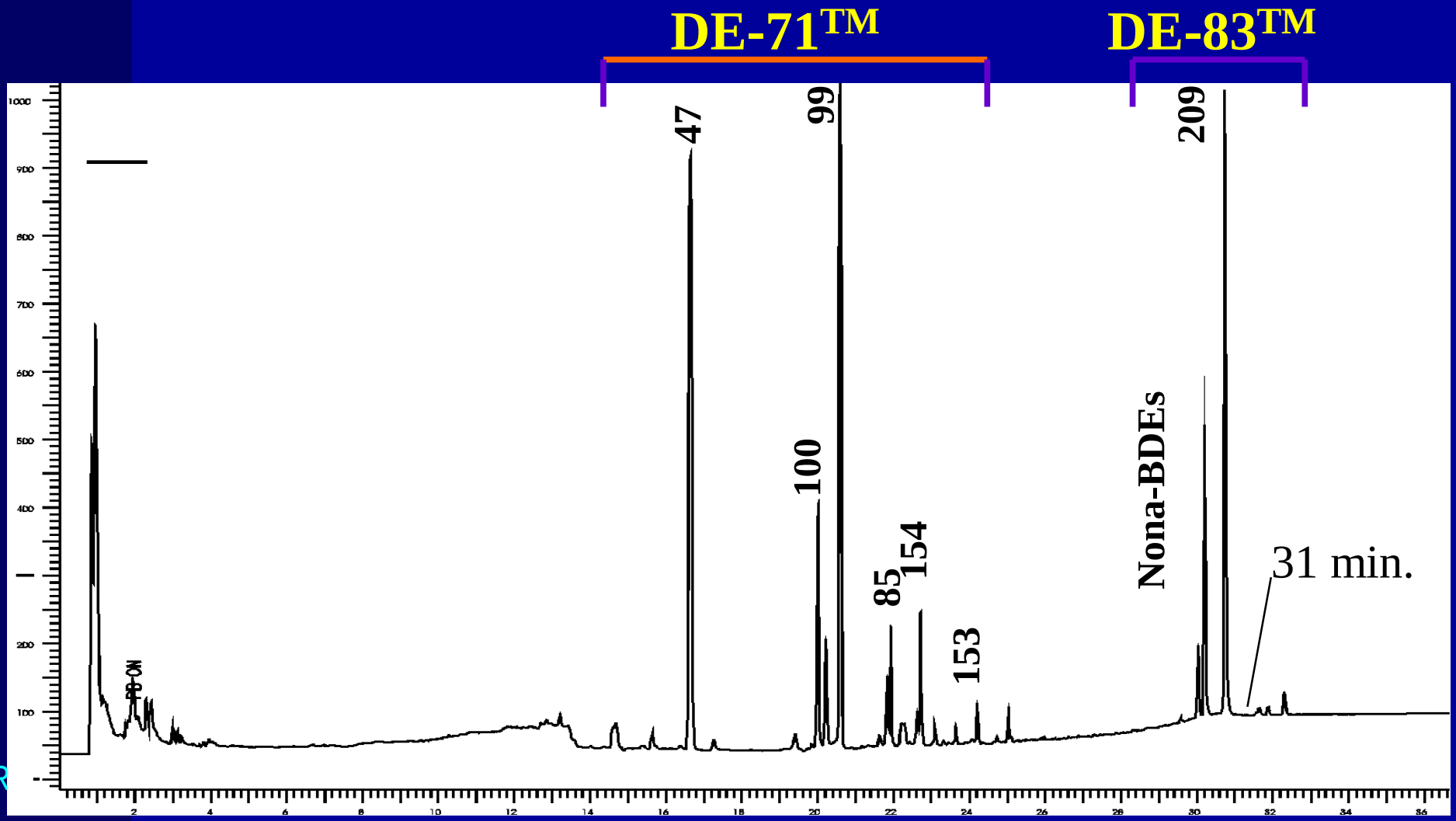


0.53 mm i.d. column partially resolves BDE 154 and 126 under these conditions

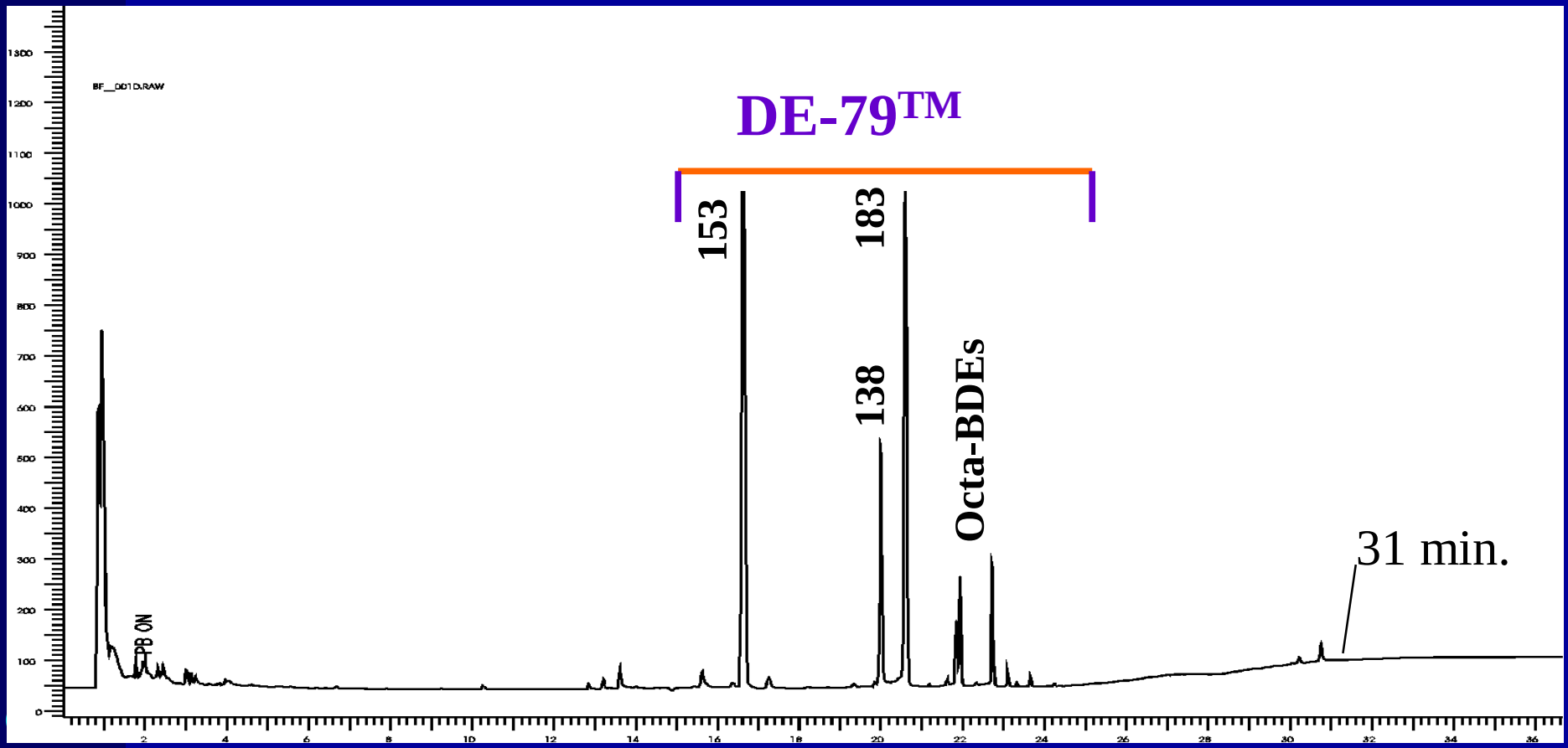


Resolution is improved using 0.25 mm i.d., or oven program optimization

Commercially Available PBDE Mixes



Commercially Available PBDE Mixes



Conditions

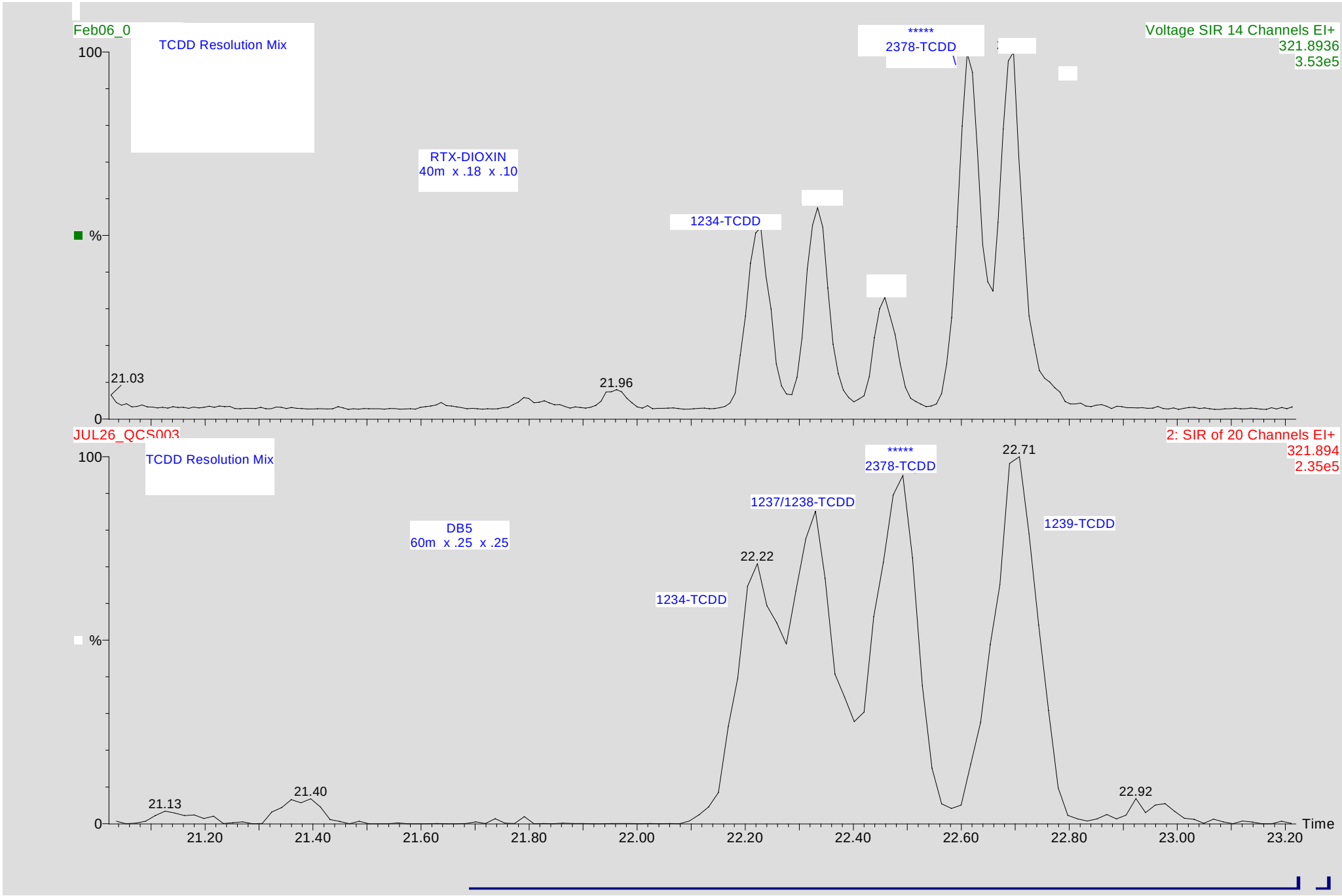
Column: Rtx-500 30m x 0.53mm x 0.15 Ser# 233548
Flows: Hydrogen 7.69ml/min (66.7cm/sec) @ 100°C
Dead Time: MeCl2 Headspace 0.76min @ 100°C
Oven: 100(1) 15/260(5) 15/380 (15) ~ 40 min runtime
Standards: Wellington Laboratory BDE-Mix C
1ul injection 30pg on column.
Injection: Drilled Press-Tight Uniliner.

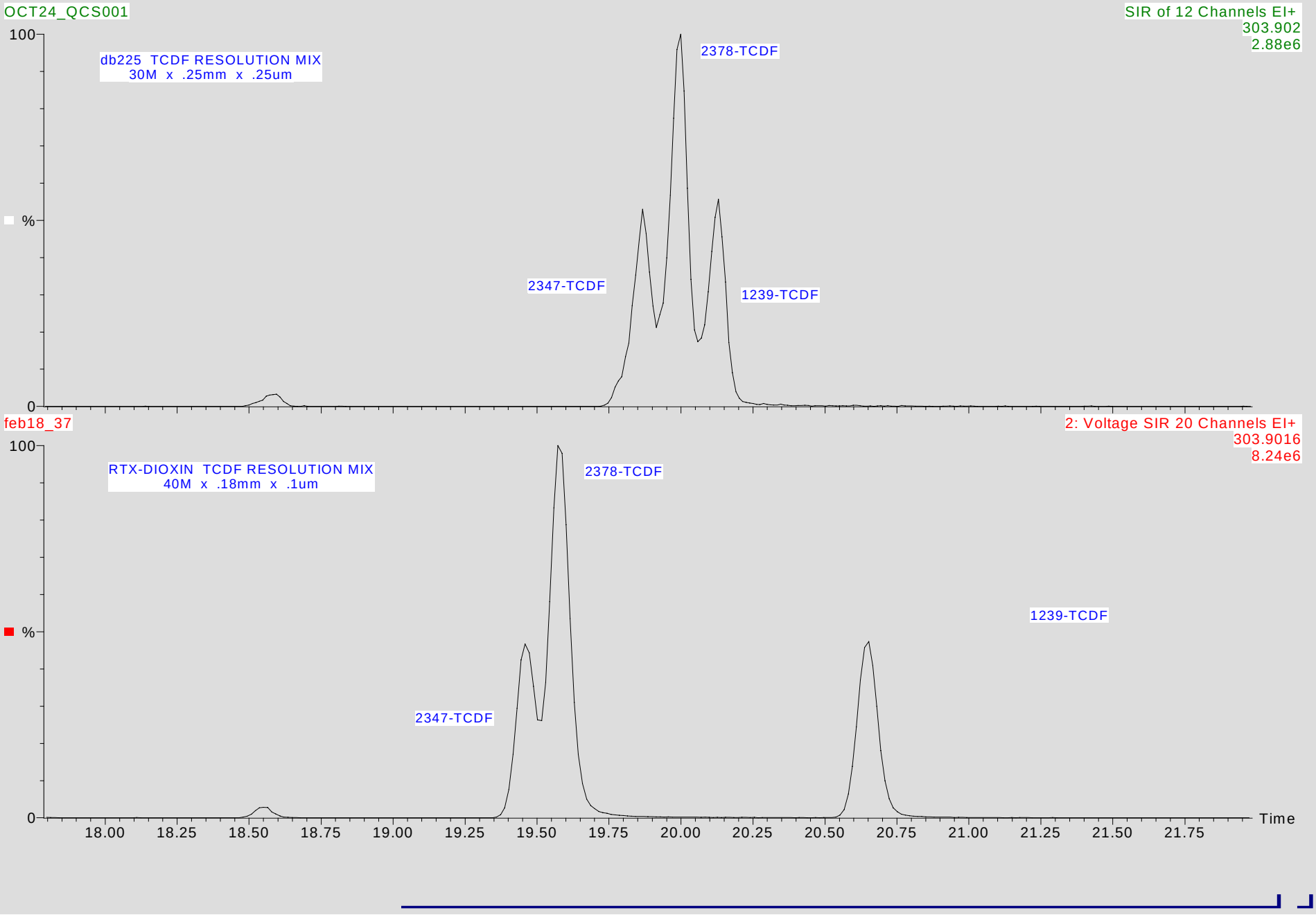
Rtx-Dioxin Capillary Column

- Proprietary polysiloxane designed for replacement of “5”-type columns as primary, or high-cyano secondary columns for toxic dioxin and furan analysis by GC-HRMS
- 380 maximum operating temperature in standard high-temperature fused silica tubing

Dioxin and Furan Analysis

- Dual column method
  - Usually 5% diphenyl column and a high-cyano column (eg Rtx-225)
  - Cyano columns have poorer lifetimes and lower maximum operating temperatures
  - 5% diphenyl phases do not have the selectivity to accurately quantitate most samples
    - USEPA requires 2,3,7,8-tcdf to be confirmed on a X-225
- Desirable to have both columns in the same oven, and to improve the separation of the “5”


Feb06_0
TCDD Resolution Mix
RTX-DIOXIN
40m x .18 x .10
Voltage SIR 14 Channels EI+
321.8936
3.53e5
*****
2378-TCDD
1234-TCDD
21.03
21.96
100
%
0
JUL26_QCS003
TCDD Resolution Mix
DB5
60m x .25 x .25
2: SIR of 20 Channels EI+
321.894
2.35e5
*****
2378-TCDD
1237/1238-TCDD
1239-TCDD
1234-TCDD
22.22
22.71
21.13
21.40
22.92
Time
21.20
21.40
21.60
21.80
22.00
22.20
22.40
22.60
22.80
23.00
23.20


OCT24_QCS001
SIR of 12 Channels EI+
303.902
2.88e6
100
db225 TCDF RESOLUTION MIX
30M x .25mm x .25um
2378-TCDF
%
2347-TCDF
1239-TCDF
0
feb18_37
2: Voltage SIR 20 Channels EI+
303.9016
8.24e6
100
RTX-DIOXIN TCDF RESOLUTION MIX
40M x .18mm x .1um
2378-TCDF
%
1239-TCDF
2347-TCDF
0
Time
18.00
18.25
18.50
18.75
19.00
19.25
19.50
19.75
20.00
20.25
20.50
20.75
21.00
21.25
21.50
21.75

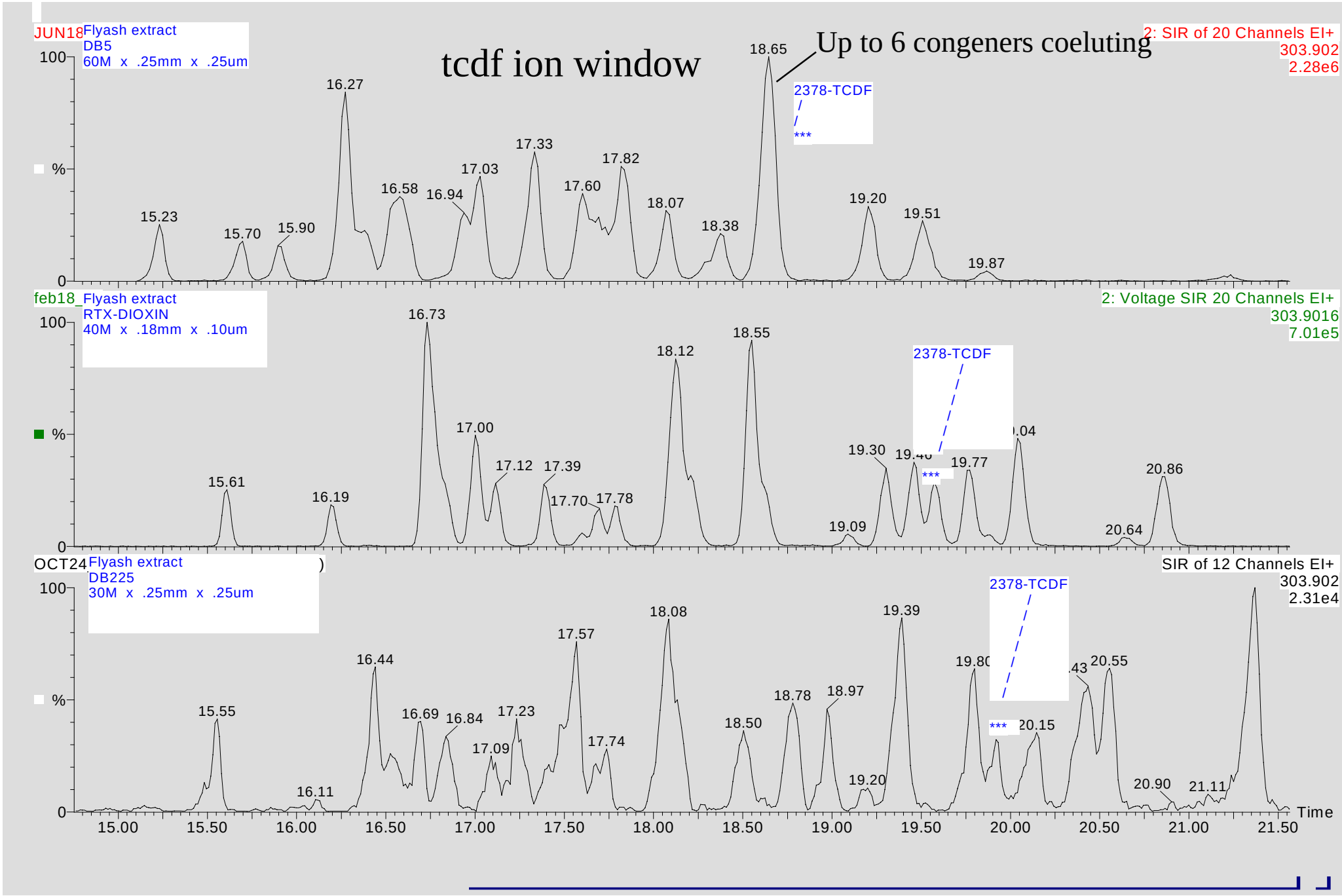
Fly Ash Round Robin Samples
>110 laboratories participating

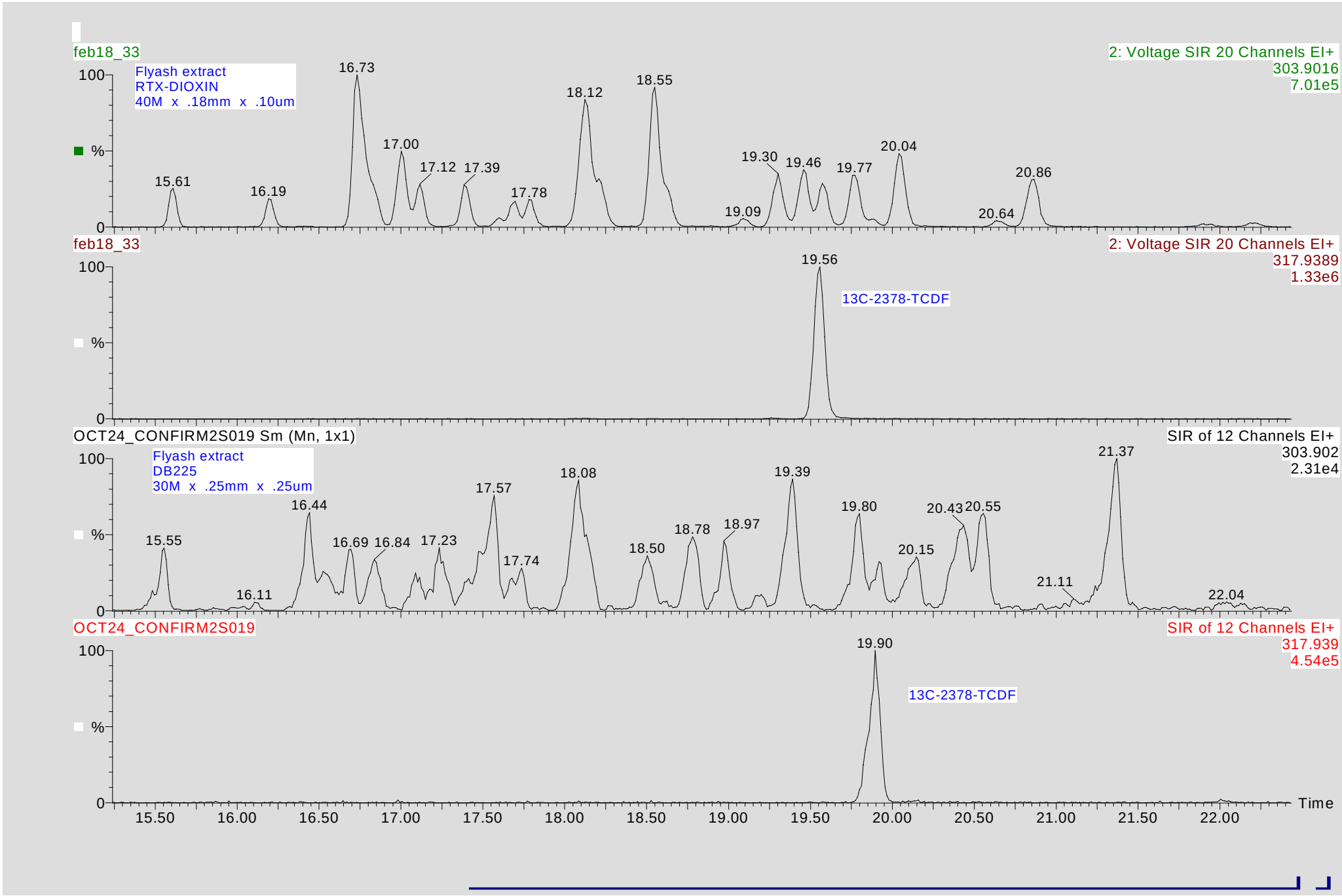
	DB-5	DB-225	RTX-DIOXIN	MEDIAN	MEAN
Ash A	250	21	30	**28**	32
Ash B	2100	300	378	**390**	390
Ash C	170	19	28	**27**	32
	All results reported as pg/g				

Median and Mean agreement gives good confidence in “true” value

Rtx-Dioxin Conditions

Micromass Altima High Resolution GC-MS				
Rtx-Dioxin 40m x .18 x .1				
Initial Temp 130 C				
	Time	Rate C/min	Temp	
	0	52	200	
	10.2	2.9	235	
	10	6.9	300	
	24			
Constant Pressure of 1.2 mL/min				
Injector Temp = 270C				


tcdf ion window
Up to 6 congeners coeluting
JUN18
Flyash extract
DB5
60M x .25mm x .25um
2: SIR of 20 Channels EI+
303.902
2.28e6
2378-TCDF
***
15.23
15.70
15.90
16.27
16.58
16.94
17.03
17.33
17.60
17.82
18.07
18.38
18.65
19.20
19.51
19.87
feb18_Flyash extract
RTX-DIOXIN
40M x .18mm x .10um
2: Voltage SIR 20 Channels EI+
303.9016
7.01e5
2378-TCDF
***
15.61
16.19
16.73
17.00
17.12
17.39
17.70
17.78
18.12
18.55
19.09
19.30
19.77
20.64
20.86
OCT24
Flyash extract
DB225
30M x .25mm x .25um
SIR of 12 Channels EI+
303.902
2.31e4
2378-TCDF
***
15.55
16.11
16.44
16.69
16.84
17.09
17.23
17.57
17.74
18.08
18.50
18.78
18.97
19.20
19.39
19.80
20.15
20.55
20.90
21.11
%
Time
15.00
15.50
16.00
16.50
17.00
17.50
18.00
18.50
19.00
19.50
20.00
20.50
21.00
21.50


feb18_33
Flyash extract
RTX-DIOXIN
40M x .18mm x .10um
2: Voltage SIR 20 Channels EI+
303.9016
7.01e5
15.61
16.19
16.73
17.00
17.12
17.39
17.78
18.12
18.55
19.09
19.30
19.46
19.77
20.04
20.64
20.86
feb18_33
2: Voltage SIR 20 Channels EI+
317.9389
1.33e6
19.56
13C-2378-TCDF
OCT24_CONFIRM2S019 Sm (Mn, 1x1)
Flyash extract
DB225
30M x .25mm x .25um
SIR of 12 Channels EI+
303.902
2.31e4
15.55
16.11
16.44
16.69
16.84
17.23
17.57
17.74
18.08
18.50
18.78
18.97
19.39
19.80
20.15
20.43
20.55
21.11
21.37
22.04
OCT24_CONFIRM2S019
SIR of 12 Channels EI+
317.939
4.54e5
19.90
13C-2378-TCDF
100
%
0
Time
15.50
16.00
16.50
17.00
17.50
18.00
18.50
19.00
19.50
20.00
20.50
21.00
21.50
22.00

Rtx-Dioxin Column

- Replaces a 5% diphenyl column for high-resolution dioxin and furan analyses
  - Improves sensitivity due to lower bleed levels
  - Improves separation of many congeners
- May replace high-cyano columns as confirmation column to the 5% diphenyl
  - All but 2 congeners were within 10% of the fly ash median values
    - These 2 can be quantitated using the 5% diphenyl column
  - May eliminate the need for –2330, -2331, -225 confirmation columns
  - Final characterization currently underway

Low-Bleed Columns Summary:

- Rtx-XLB
  - Low bleed column for pesticides and PCB Congeners, similar to DB-XLB
- Rtx-PCB
  - Low bleed column for pesticides and PCB Congeners, resolves a few more congeners than XLB phases
  - Unbiased GC-MS results for European PCB congener method
- Rtx-500
  - Carborane-based column, exceptionally high thermal stability
- Rtx-Dioxin
  - Improvement over the 5% diphenyl columns, and possible replacement for high-cyano confirmation phases

Acknowledgements

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- Dr. Eric Reiner and Karen MacPherson of the Ontario Ministry of the Environment – Etobicoke, Ontario provided the HRMS analyses, and guidance.
- Jack Cochran of LECO Corporation – Pesticide and PCB analyses