Methods of Analysis of Polychlorinated Biphenyl Congeners Using an Application-Specific Capillary GC Column

Frank L. Dorman, Gary B. Stidsen, Chris M. English and Lydia Nolan

Restek Corporation, Bellefonte, PA

Jack Cochran

LECO Corporation, Las Vegas, NV

209 Possible PCB Congeners

- 7 Indicator Congeners (European):
  - 28,52,101,118,138,153,180
  - Indicate presence/absence of PCB's
- 12 Most Toxic Congeners (WHO):
  - 77,81,126,169
  - 105, 114, 118, 123, 156, 157, 167, 189
  - Have Dioxin-like behavior
- 136 Congeners in Aroclor Products
  - Frame, et. al. (Fresenius J. Anal .Chem. 357, 714-22)
  - Add 126, 81, 169, and 209
- 140 Congeners should satisfy almost everyone

Analytical Techniques

- GC-MS
  - Pros:
    - Allows for spectral separation of coeluting congeners of different chlorination level
    - Single column
    - Common instrumentation in many laboratories
  - Cons:
    - Moderate sensitivity

Analytical Techniques

- GC-ECD
  - Pros:
    - High Sensitivity
    - Simplest instrumentation
    - Common instrumentation in many laboratories
  - Cons:
    - Dual column system usually necessary
    - Must have chromatographic separation

Analytical Techniques

- GCxGC-TOFMS (GCxGC – ECD)
  - Pros:
    - Allows for spectral separation of coeluting congeners of different chlorination level
    - Sensitivity improvement –vs- GC-MS
    - Increase in peak capacity for chromatographic separation
  - Cons:
    - Instrumentally more complex
    - Commercial instrumentation only recently available

Which GC Column?

- Identification of all 209 available on many phases
- Rtx-PCB column designed for this analysis
  - Greatest number of resolved congeners
  - High thermal stability
- Rtx-XLB column good for GC-MS
- Rtx-1, 5, CLPesticides, CLPesticides2, 440, Dioxin2, 500, 35, 1301, etc…

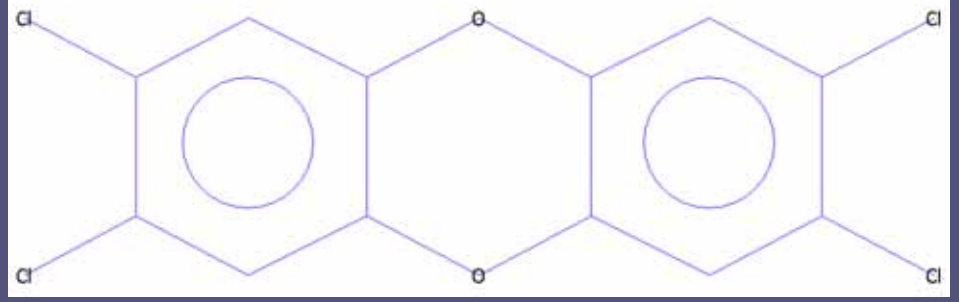
Dioxin-like PCBs

PCB#	Cl#	Cl Pos.		PCB#	Cl#	Cl Pos.
77	4	34-34		156	6	2345-34
81	4	345-4		157	6	234-345
				167	6	245-345
105	5	234-34		169	6	345-345
114	5	2345-4				
118	5	245-34		189	7	2345-345
123	5	345-24				
126	5	345-34				

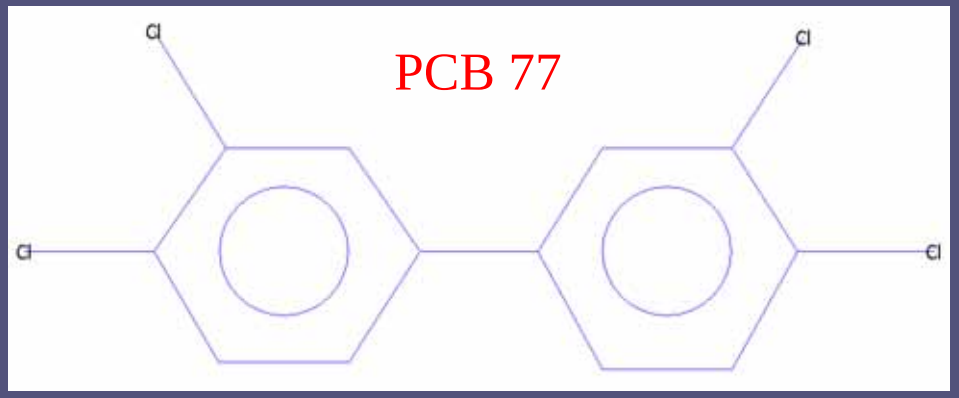
World Health Organization, non- and mono-ortho substituted

Toxicity of PCBs

2378-TCDD

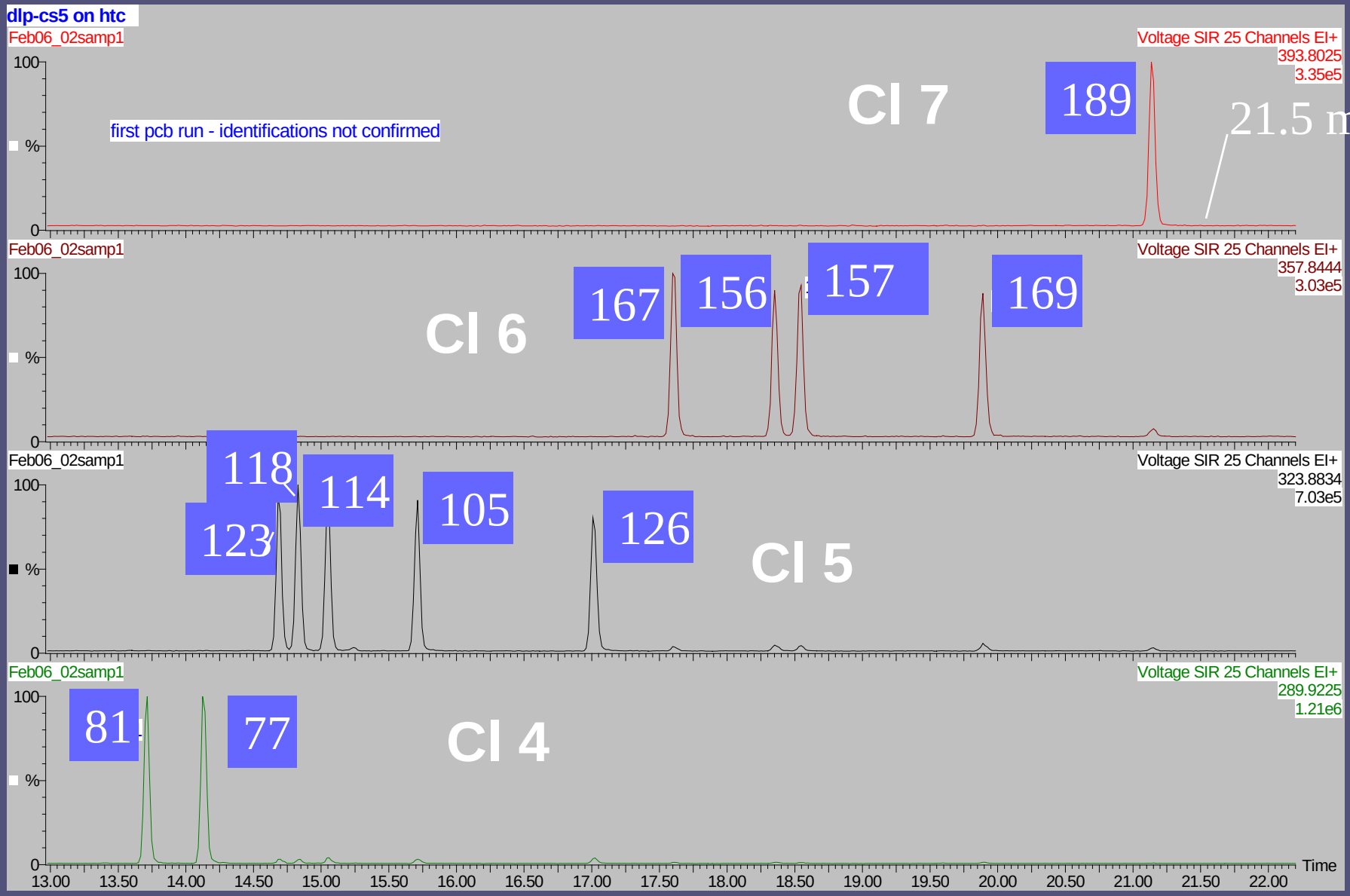


PCB 77

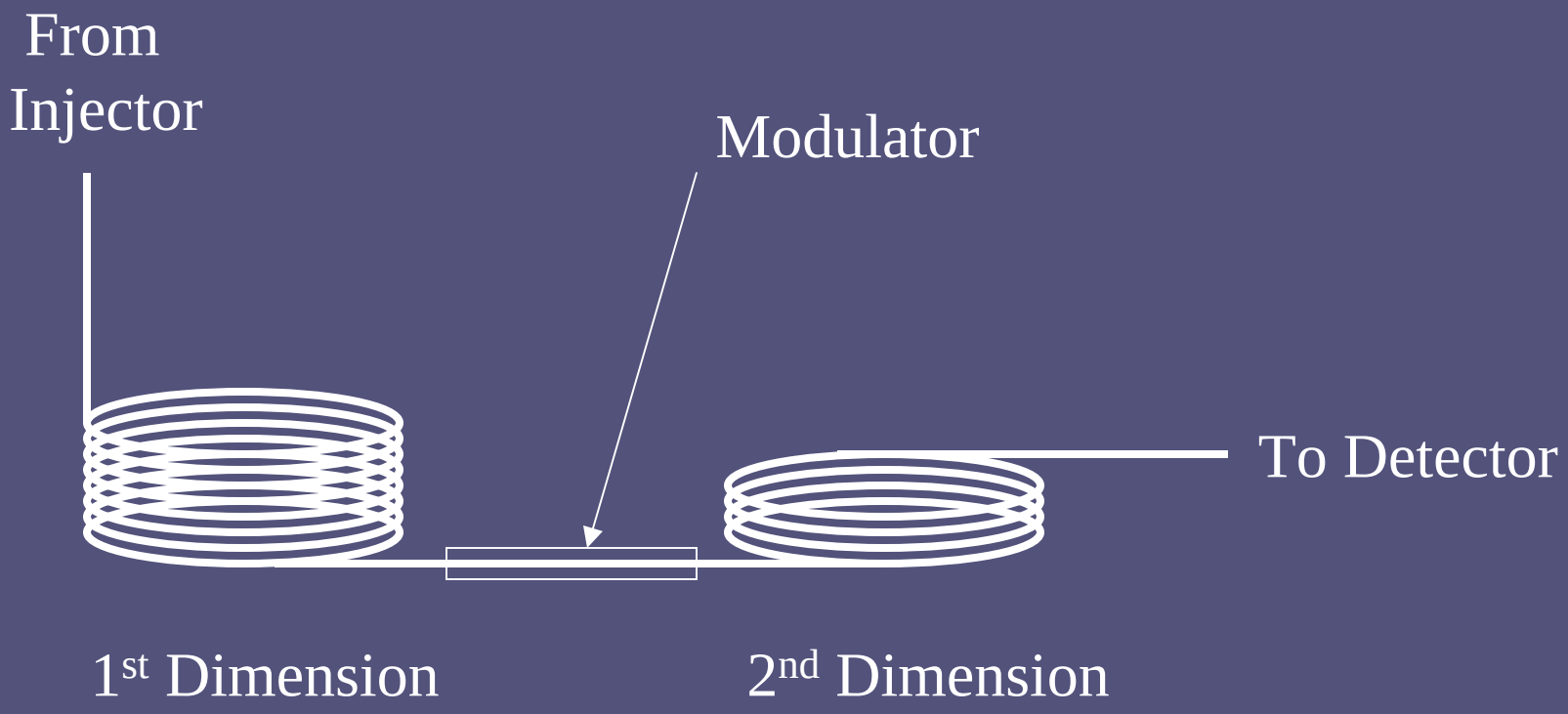


- Planar shape
- Fit in a 3x10Å rectangle
- Chlorines on the corners
- Binds with Ah receptor, like dioxins
- Toxicity Equivalency Factors
  - 2378-TCDD = 1.0
  - PCB 77 = 0.0001
  - PCB 126 = 0.1
  - PCB 169 = 0.01

Toxic PCB Congeners (Carbon Cleanup)



GCxGC Schematic



Two independent separation mechanisms

GCxGC Setup

- Primary column (1st dimension)
  - Longer, wider bore, thicker film
  - Non-polar
- Modulator
  - Thermal in nature
  - Concentrates effluent from primary column
  - “Injects” this effluent onto secondary column
- Secondary column (2nd dimension)
  - Very short, narrow bore, thinner film
  - Polar or selective

GCxGC Column

- Integral column – No press fit
  - 50m x 0.18mm x 0.18µm Rtx-1
  - 5m x 0.18mm x 0.10µm Rtx-PCB
  - Installed so that all 5m of Rtx-PCB was past modulator and in a secondary oven
- Constant flow He at 1.5 mL/min
- Splitless injection
  - 1 µL
  - 250°C
  - Purge time 60 sec

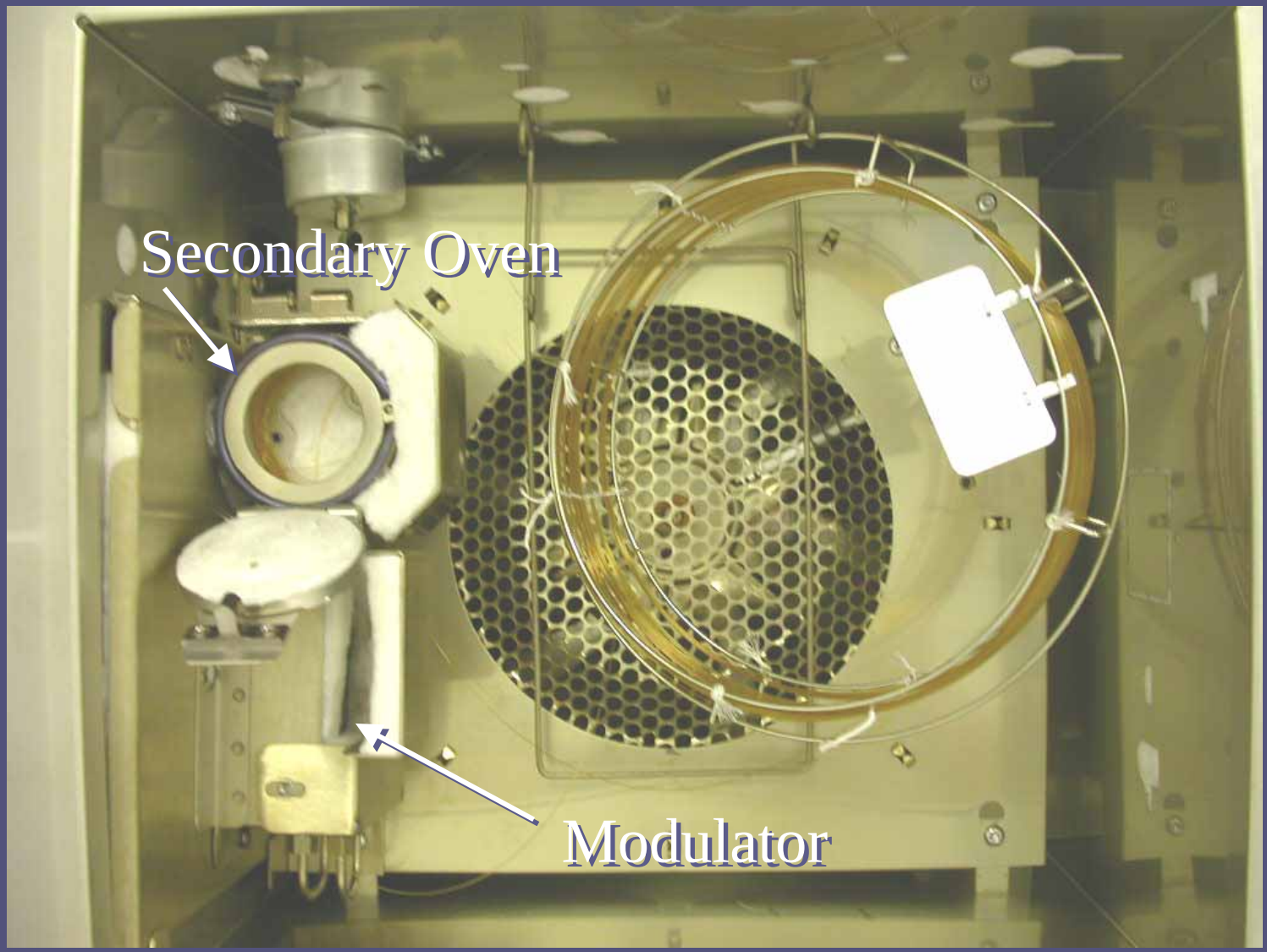
GCxGC Conditions

- Primary oven
  - 120°C (1 min), 5°/min to 330°
- Modulator (quad jet)*
  - Temperature offset: 40°C
  - Modulation time: 2 sec
- Secondary oven
  - 140°C (1 min), 5°/min to 350°

Run time = 43 min

*Built by LECO under license from Zoex Corporation

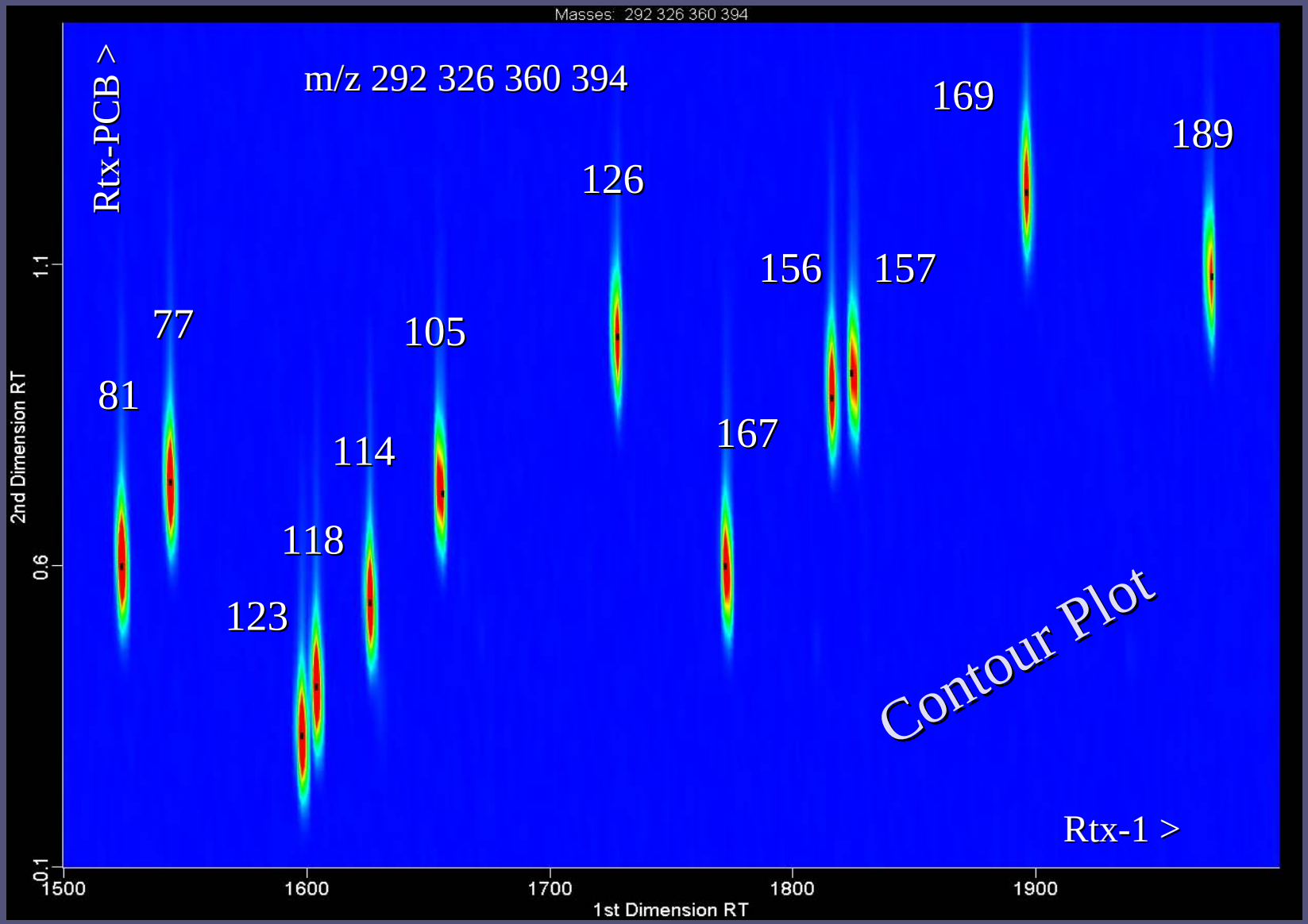
Modulator and Secondary Oven



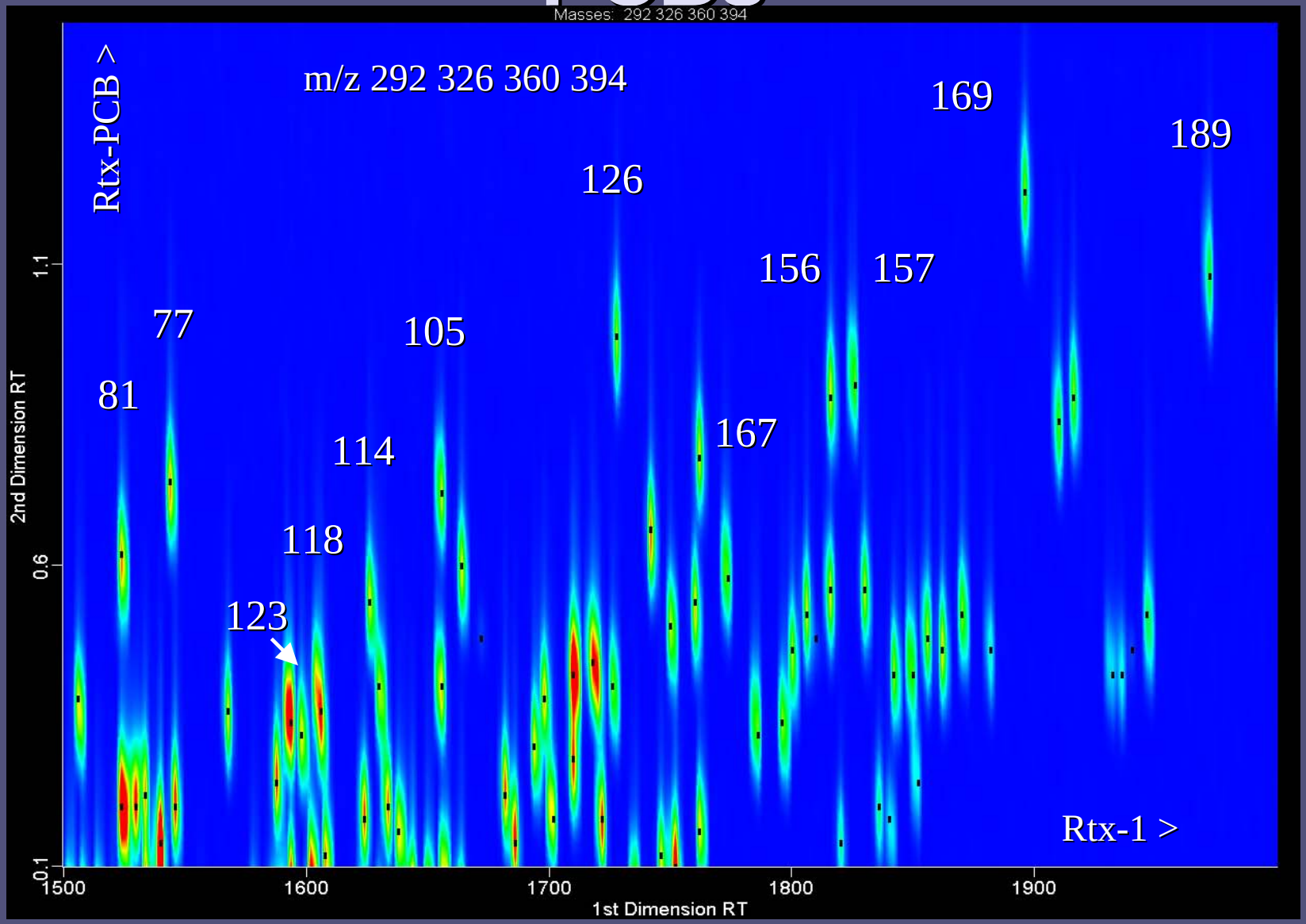
TOFMS Conditions
LECO Pegasus 4D

- Source temperature: 225°C
- Electron ionization: 70 eV
- Stored mass range: 120 to 520 u
- Acquisition rate: 50 spectra/sec

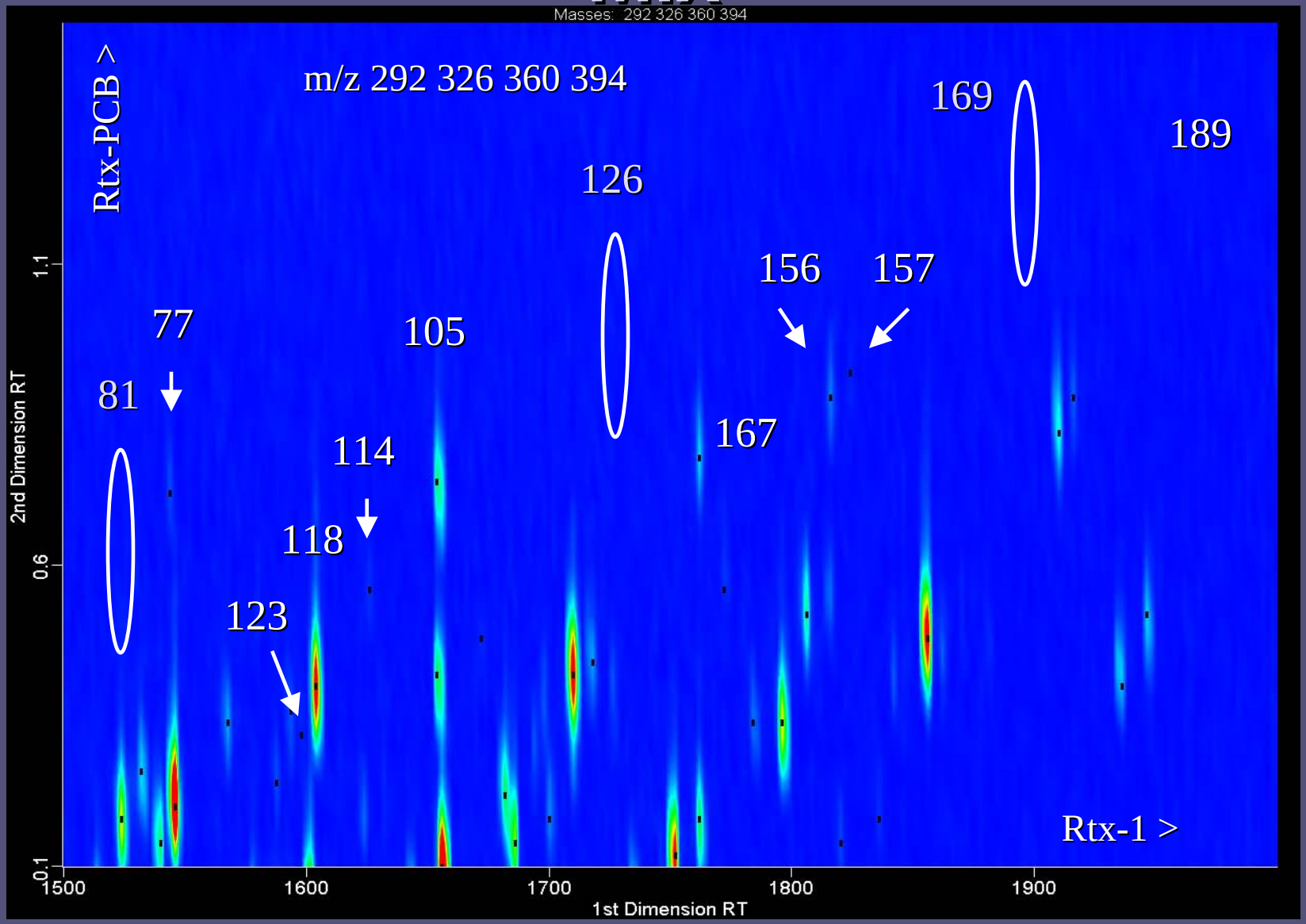
Dioxin-like PCBs



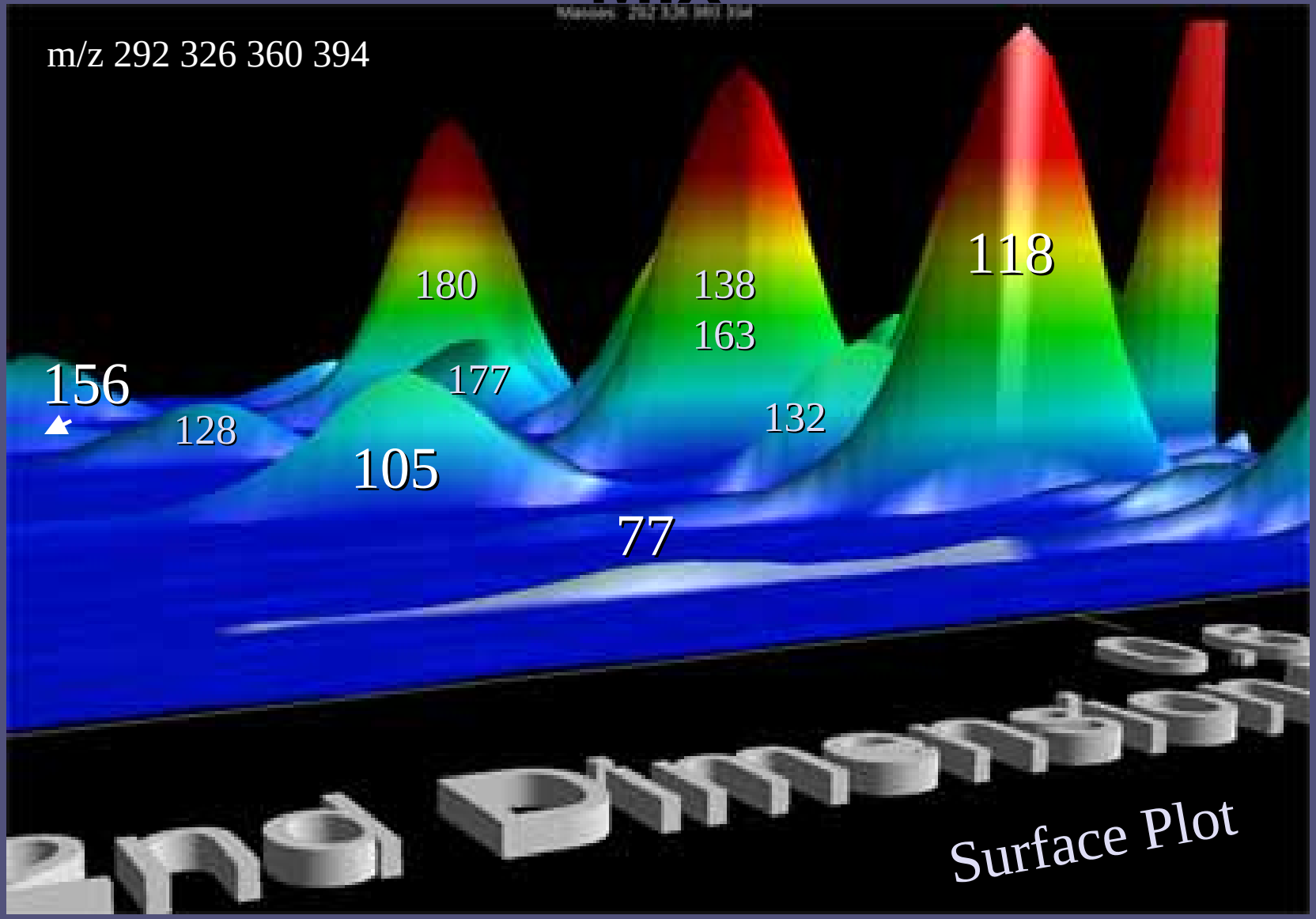
Dioxin-like PCBs vs. Other PCBs



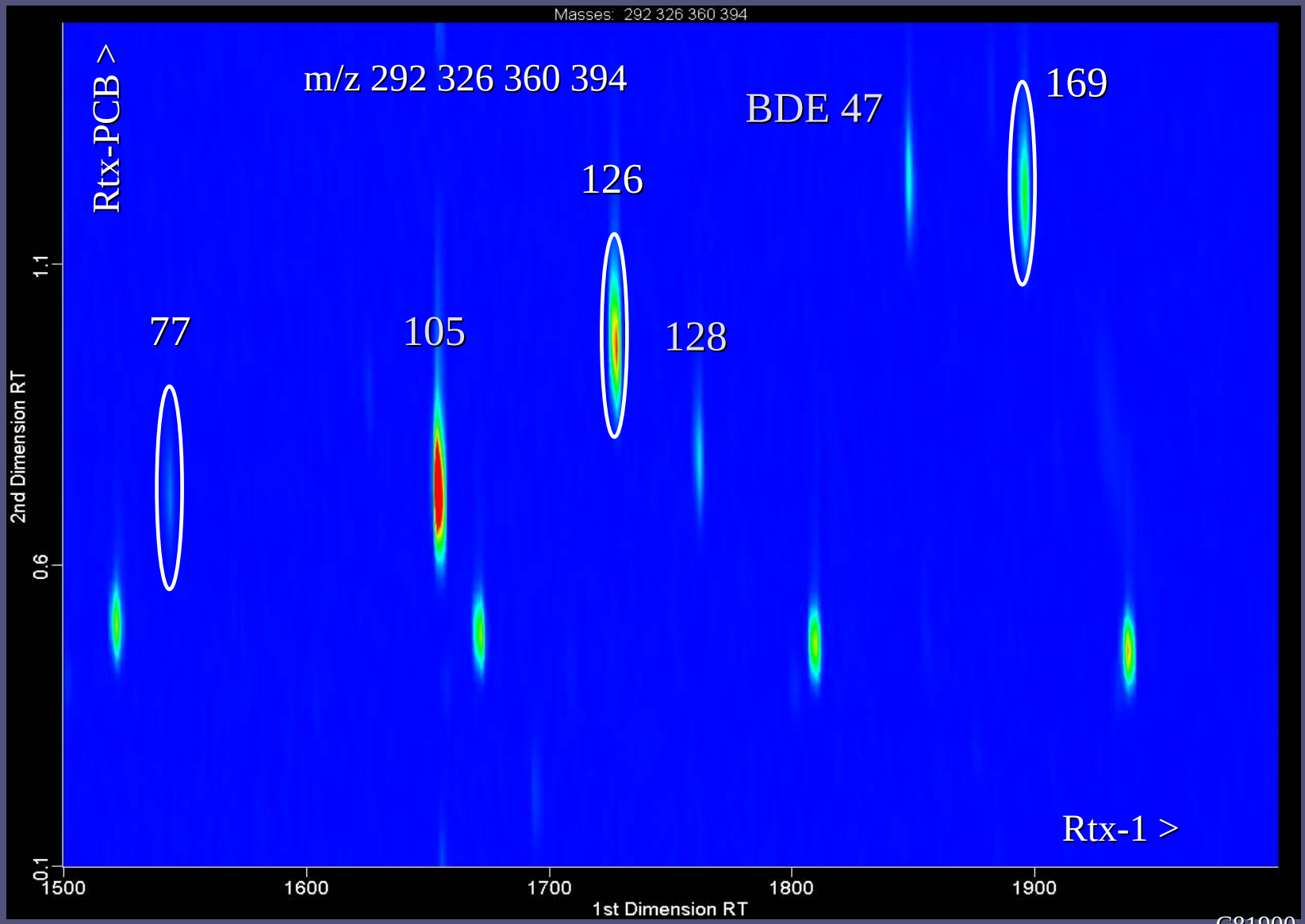
Dioxin-like PCBs in an Aroclor Mix



Dioxin-like PCBs in an Aroclor Mix

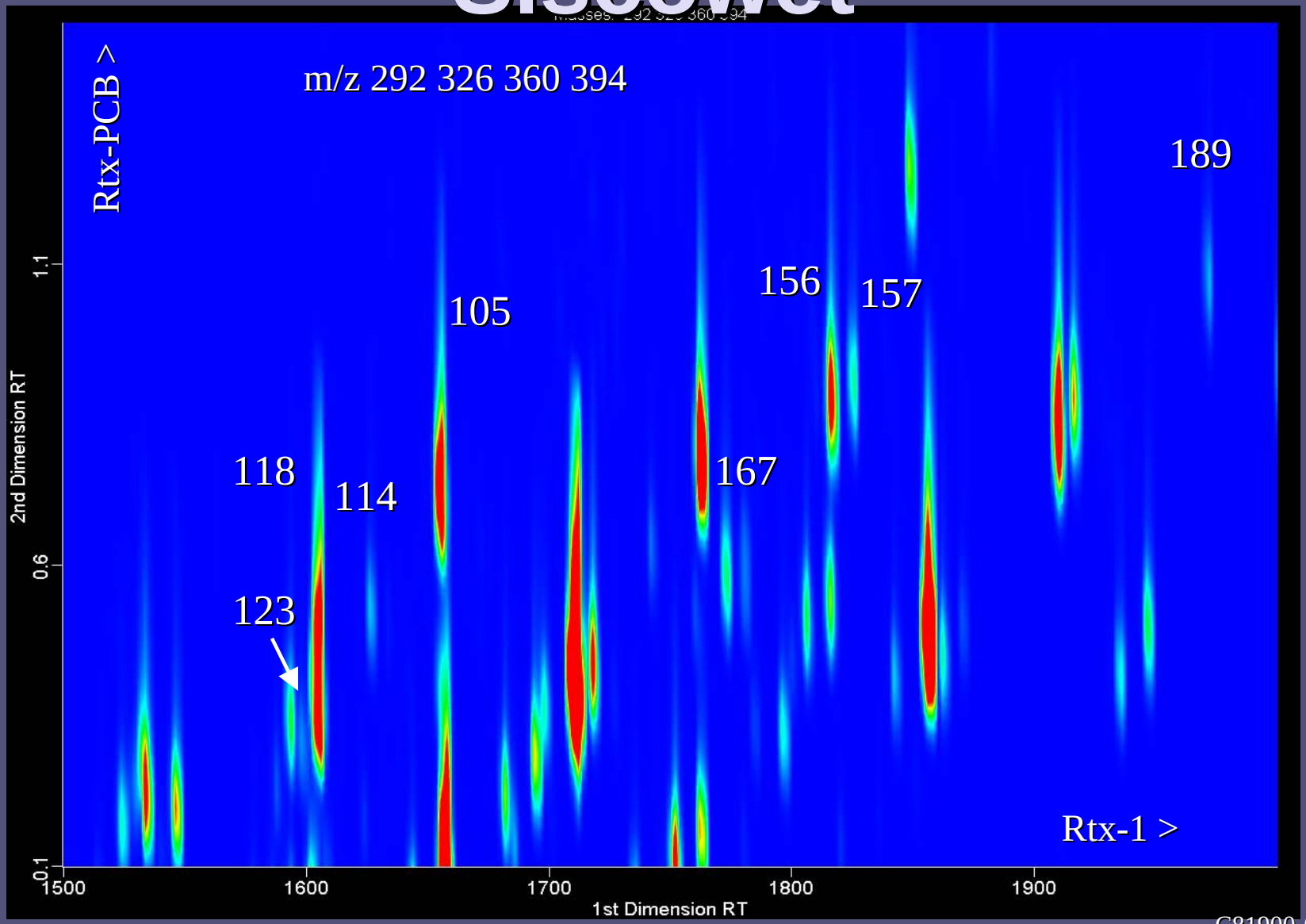


Non-ortho PCBs in Siscowet



C81900-0019

Mono-ortho PCBs in Siscowet



C81900-0019

Non-ortho PCBs in Siscowet

PCB		HR	TOF		HR	TOF		HR	TOF
77		170	170		140	150		640	630
81		22	24		30	31		83	84
126		610	570		1200	1100		1700	1400
169		250	270		550	490		470	440

- Results in pg/g for three different Siscowet from Lake Superior
- Based on extracted amount and final extract volume:
  - 20 pg/g = ~2.5 pg/µL
  - 200 pg/g = ~25 pg/µL

C81900-0018,19,20

Conclusions

- GCxGC-TOFMS is a viable way to determine dioxin-like PCBs in fish
  - Sensitive: modulation enhances detection
  - Selective: 2nd dimension separations
- Full mass spectra provide powerful confirmations
- Peak find and spectral deconvolution enable location, identification, and quantification (?) of other environmentally significant compounds

GC-TOFMS Analysis of Indicator Polychlorinated Biphenyls

Experiment

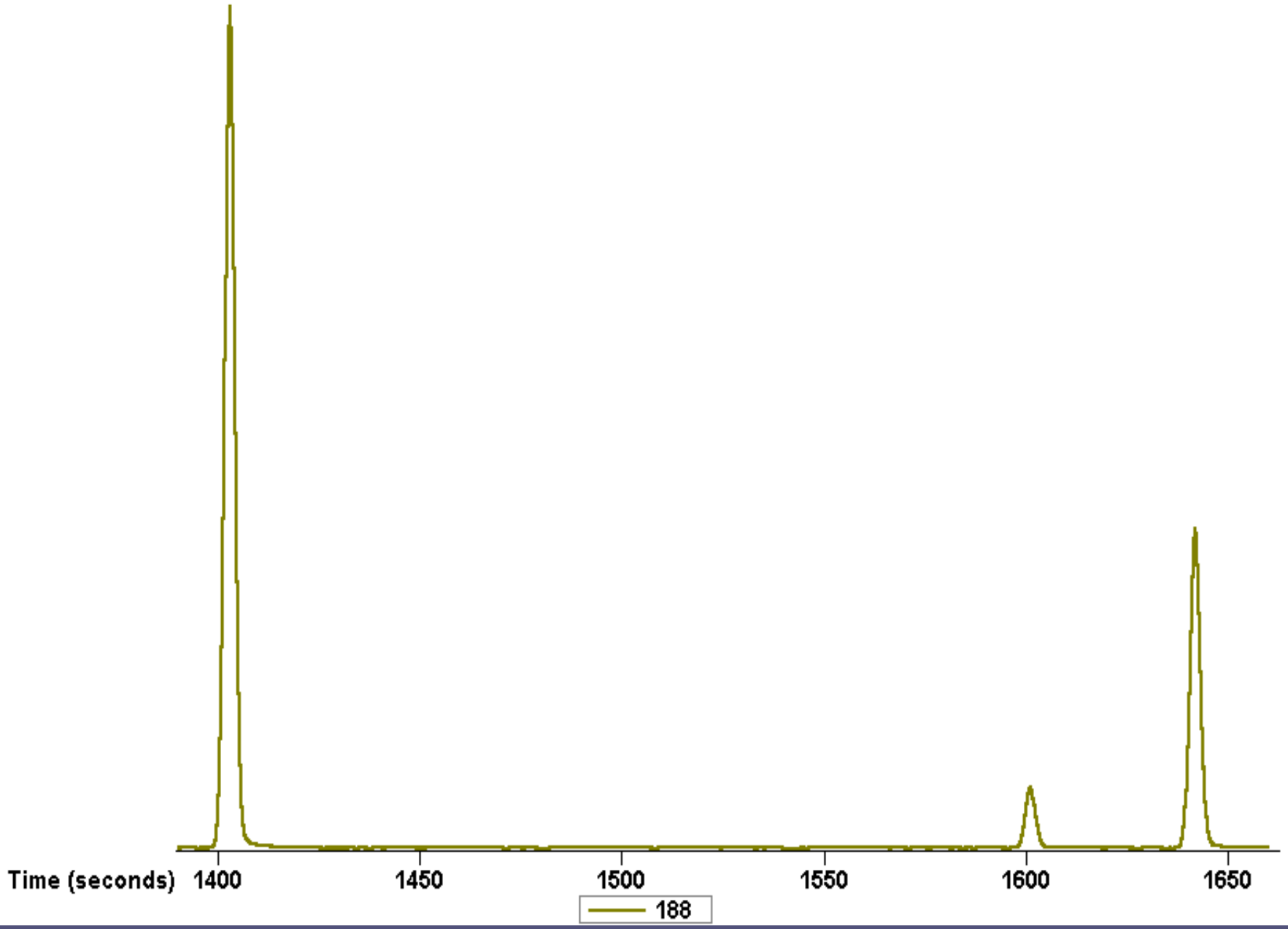
- Determine retention times for all 209 PCBs on a new low-bleed capillary column
  - Analyze individual congener standards
  - Hexachlorobenzene for relative retention times
- Analyze mix of Aroclors 1221:1242:1254:1262
  - 1:2:2:2 ratio
  - Show chromatogram with congeners labeled
- GC-MS results obtained using a time-of-flight mass spectrometer to allow for possible spectral deconvolution

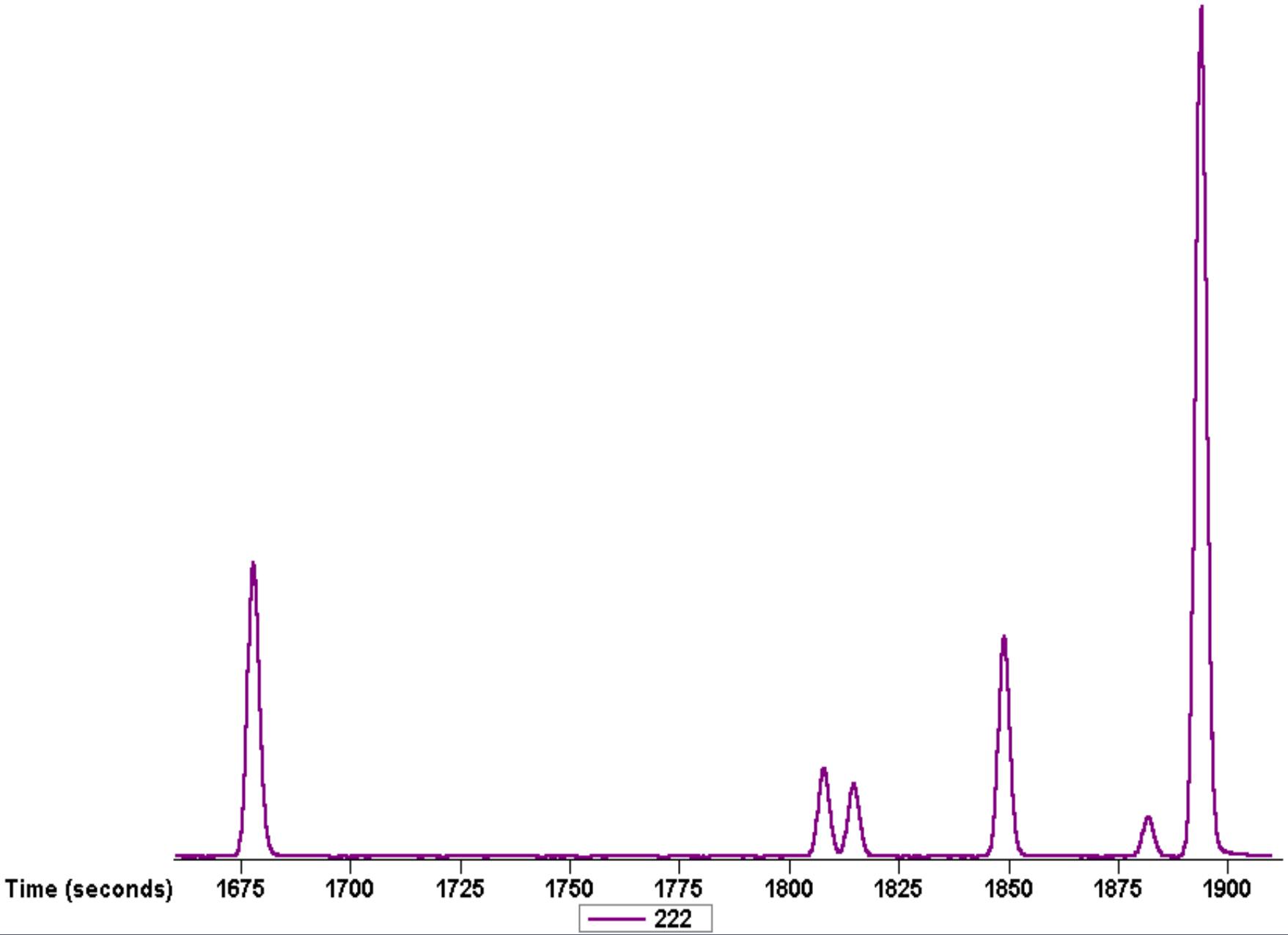
GC-TOFMS Conditions

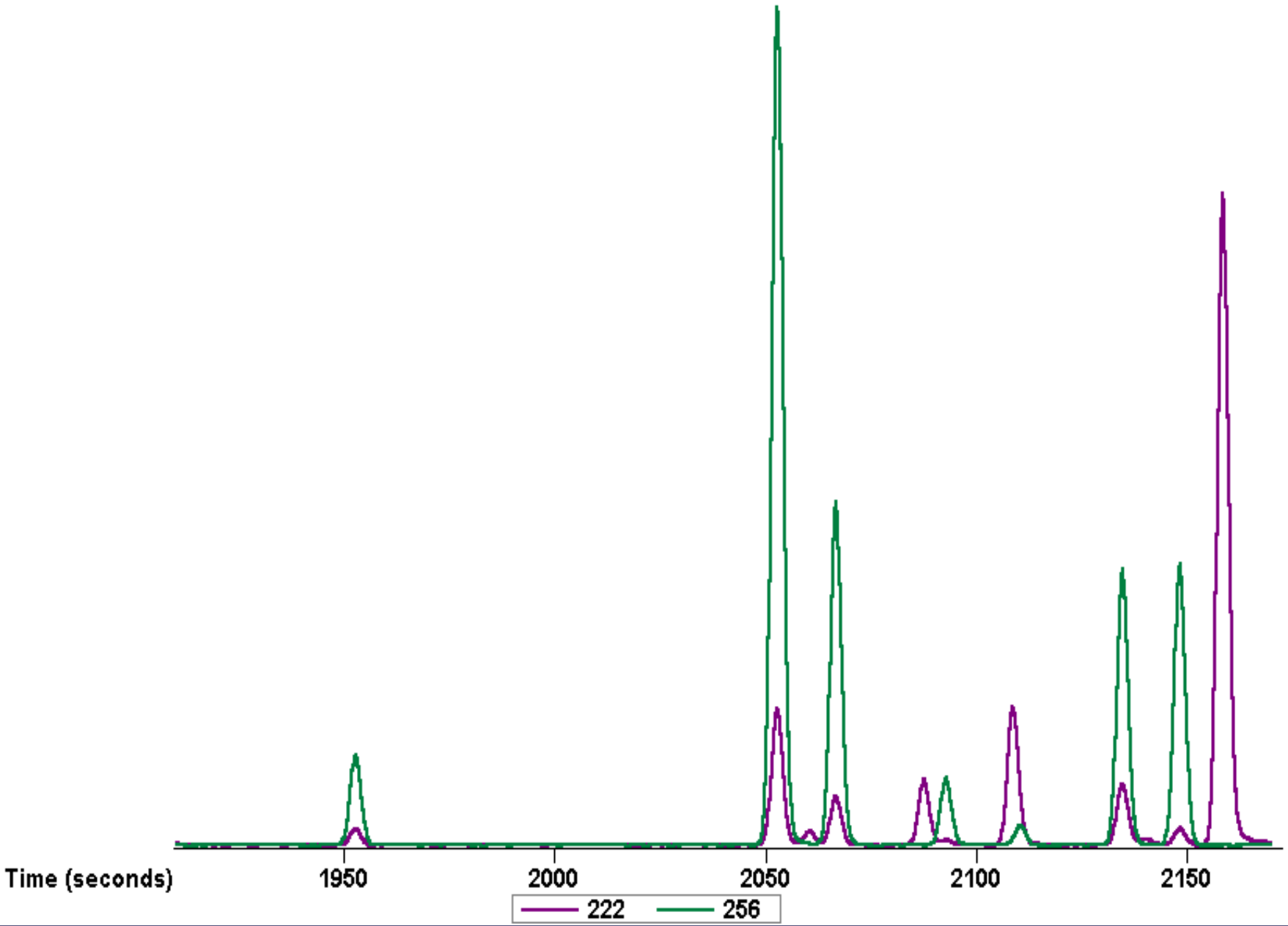
- Gas Chromatography
  - One microliter splitless 250°C, 60 sec valve
  - 60 m x 0.18 mm x 0.18 µm Restek Rtx-PCB column
    - Constant flow helium, 1.5 mL/minute
    - 70°C (1 min), 50°/min to 120°, 3°/min to 360° (1 min)
- Mass Spectrometry LECO Pegasus III
  - Source temperature: 225°C
  - Electron ionization: 70 eV
  - Stored mass range: 120 to 520 u
  - Acquisition rate: 5 spectra/sec

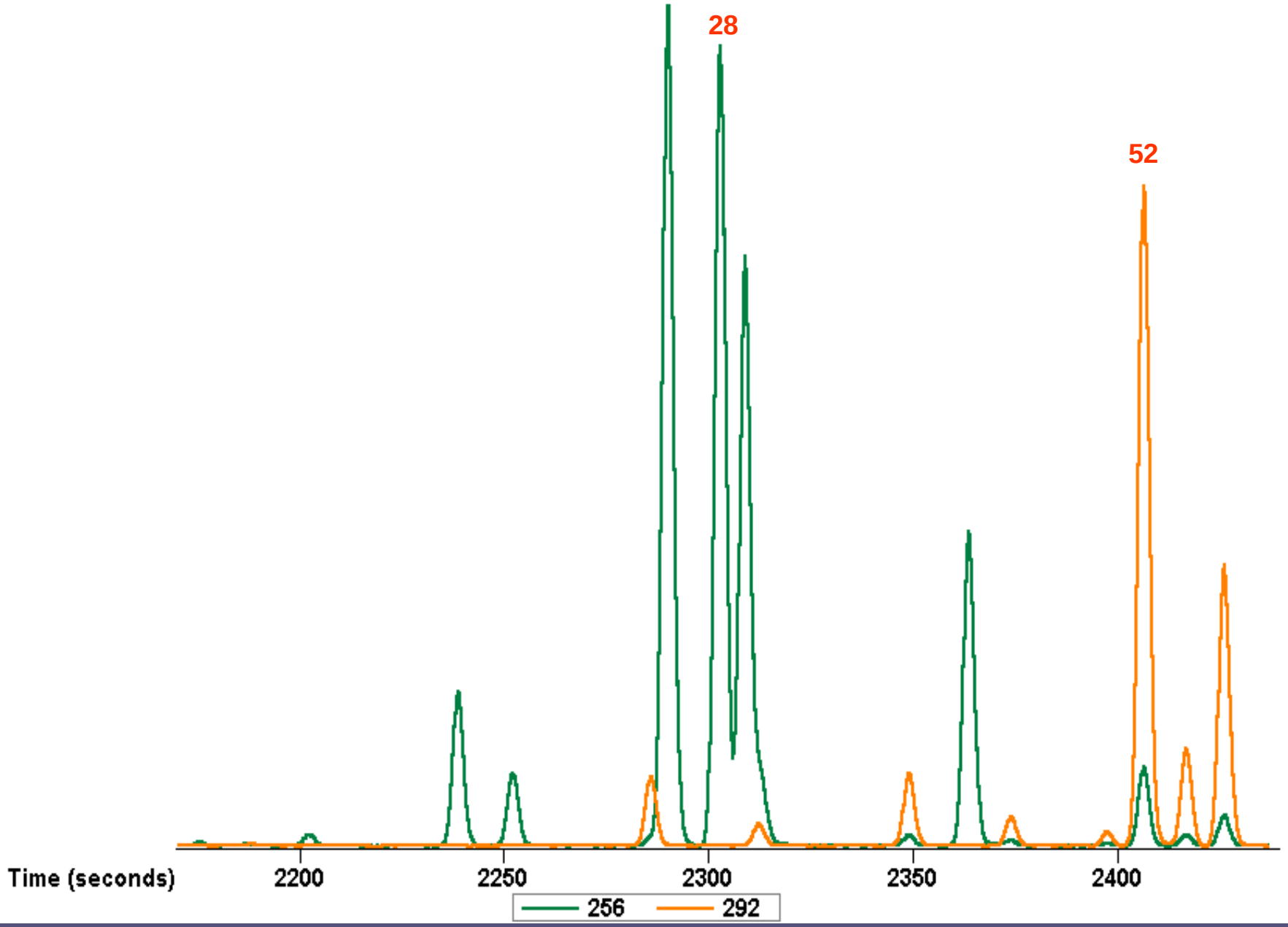
Aroclor Mix Chromatogram

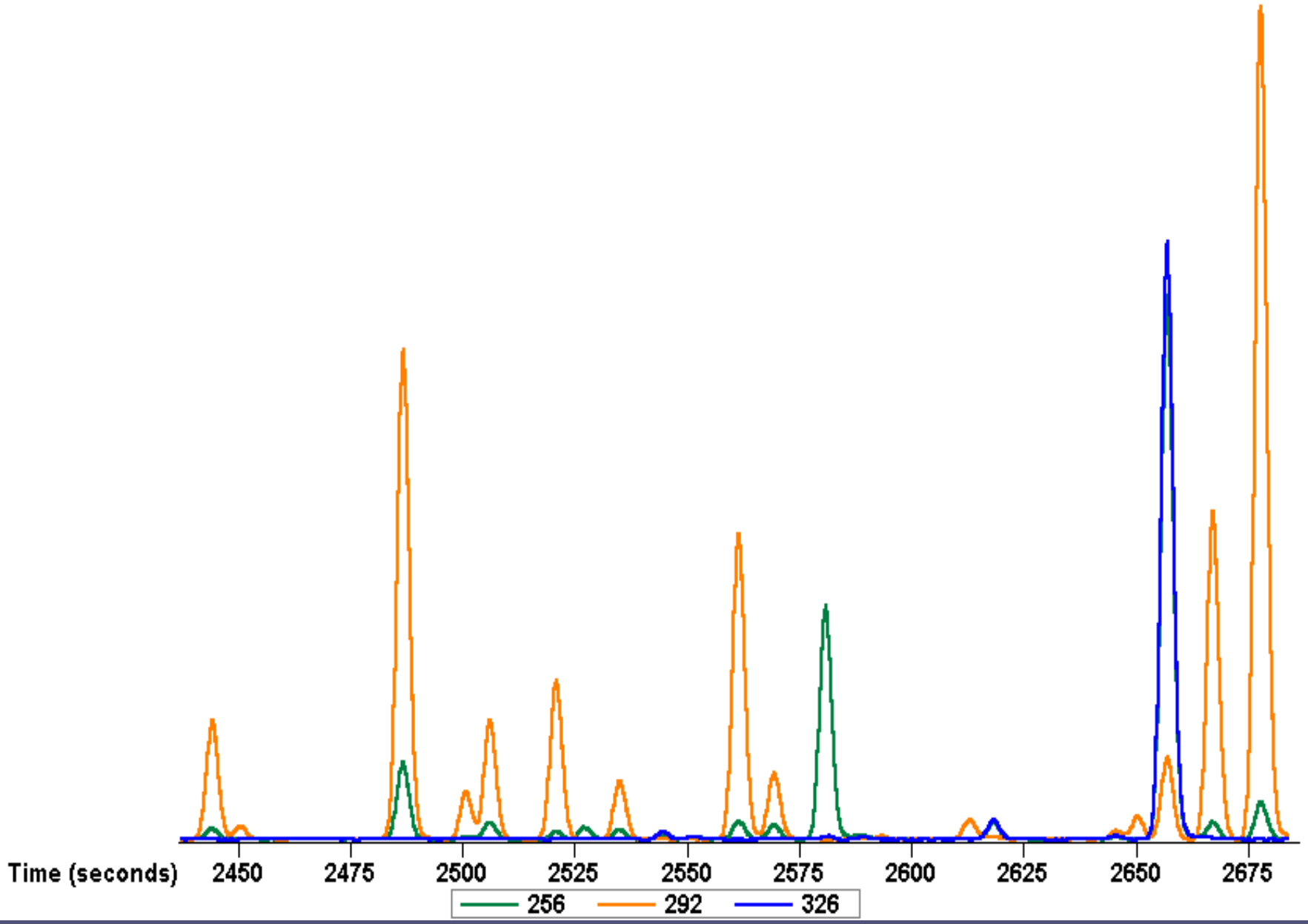
- Overlaid ion chromatograms are plotted
  - For example, hexachlorobiphenyl is 360
  - Peaks are labeled with congener numbers
    - Text size and format is only for space and not meant to indicate anything about PCBs, except:
    - European indicator congeners are in red
- Chromatograms are not to one scale
  - Each panel is adjusted so most of the PCB peaks can be seen

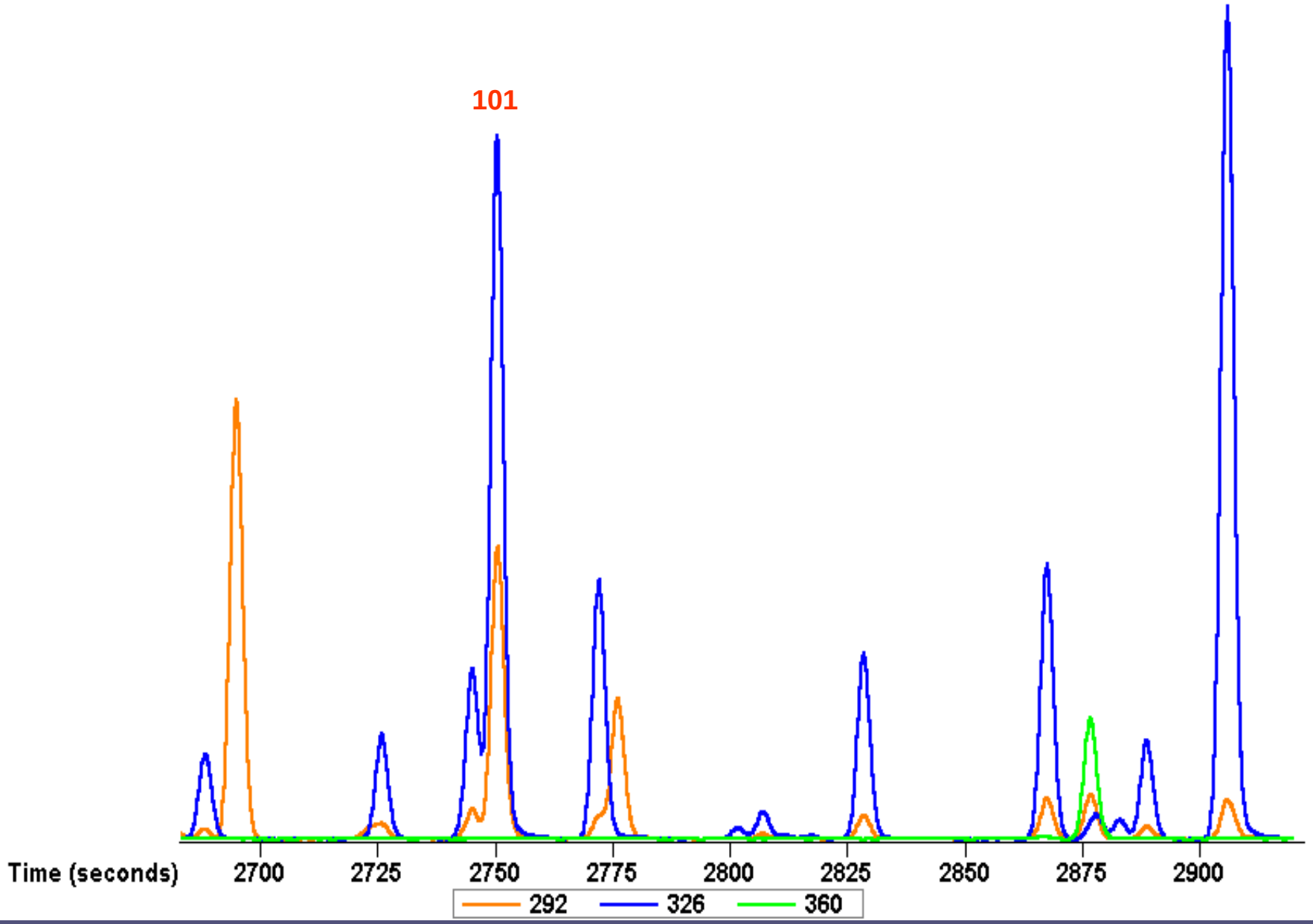

Time (seconds)
1400
1450
1500
1550
1600
1650
188

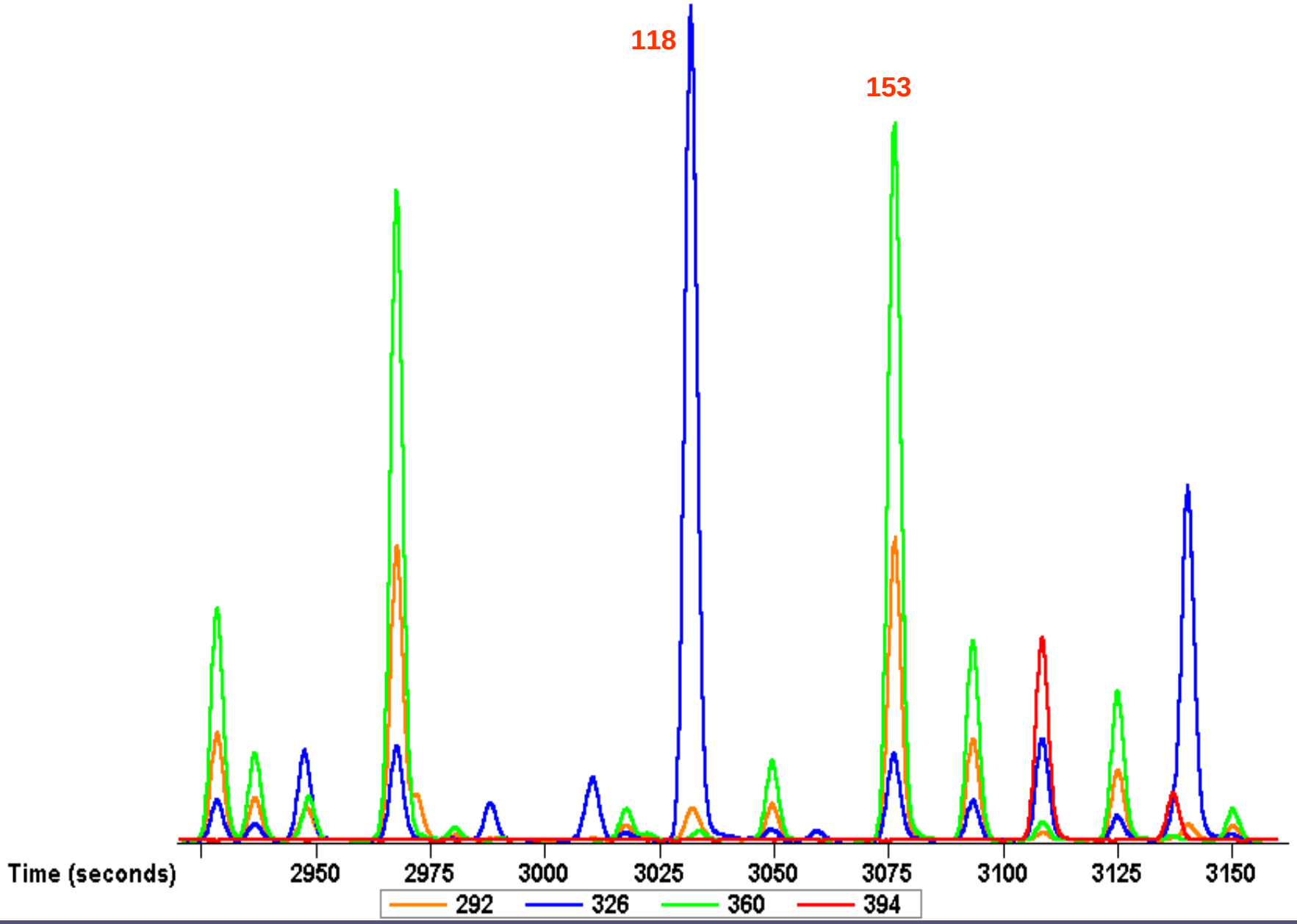

Time (seconds)
1675
1700
1725
1750
1775
1800
1825
1850
1875
1900
222

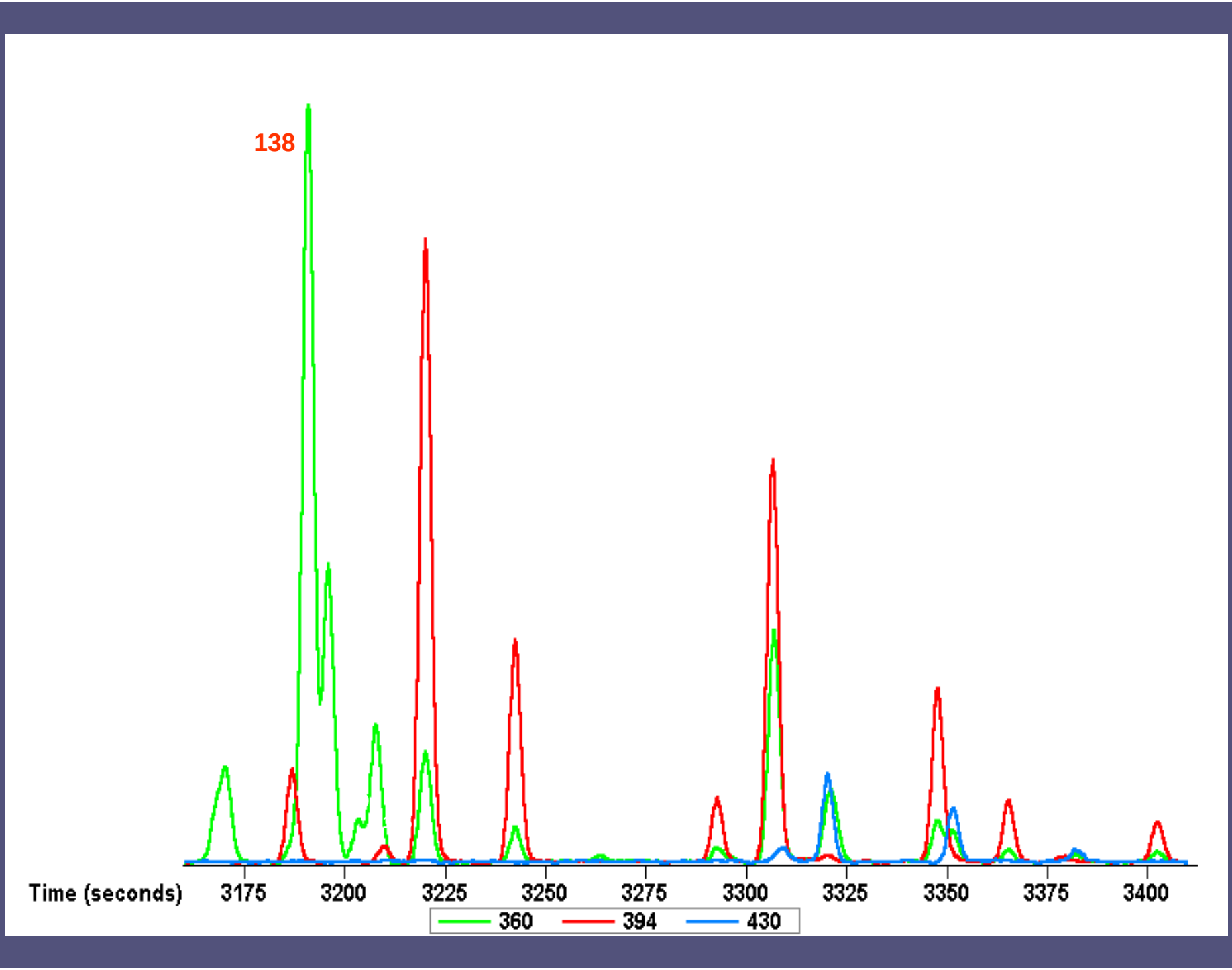

Time (seconds)
1950
2000
2050
2100
2150
222
256

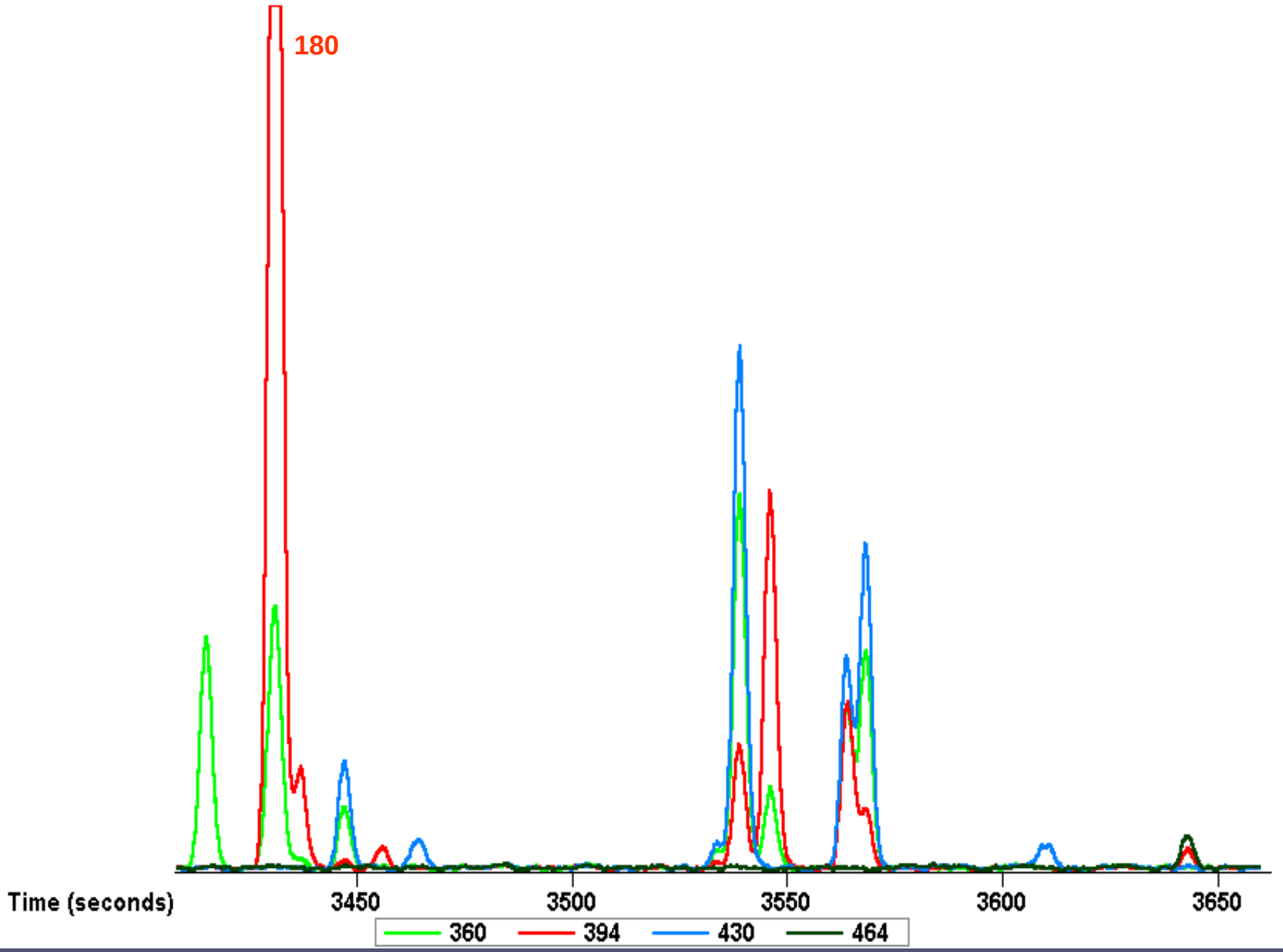

28
52
Time (seconds)
2200
2250
2300
2350
2400
256
292

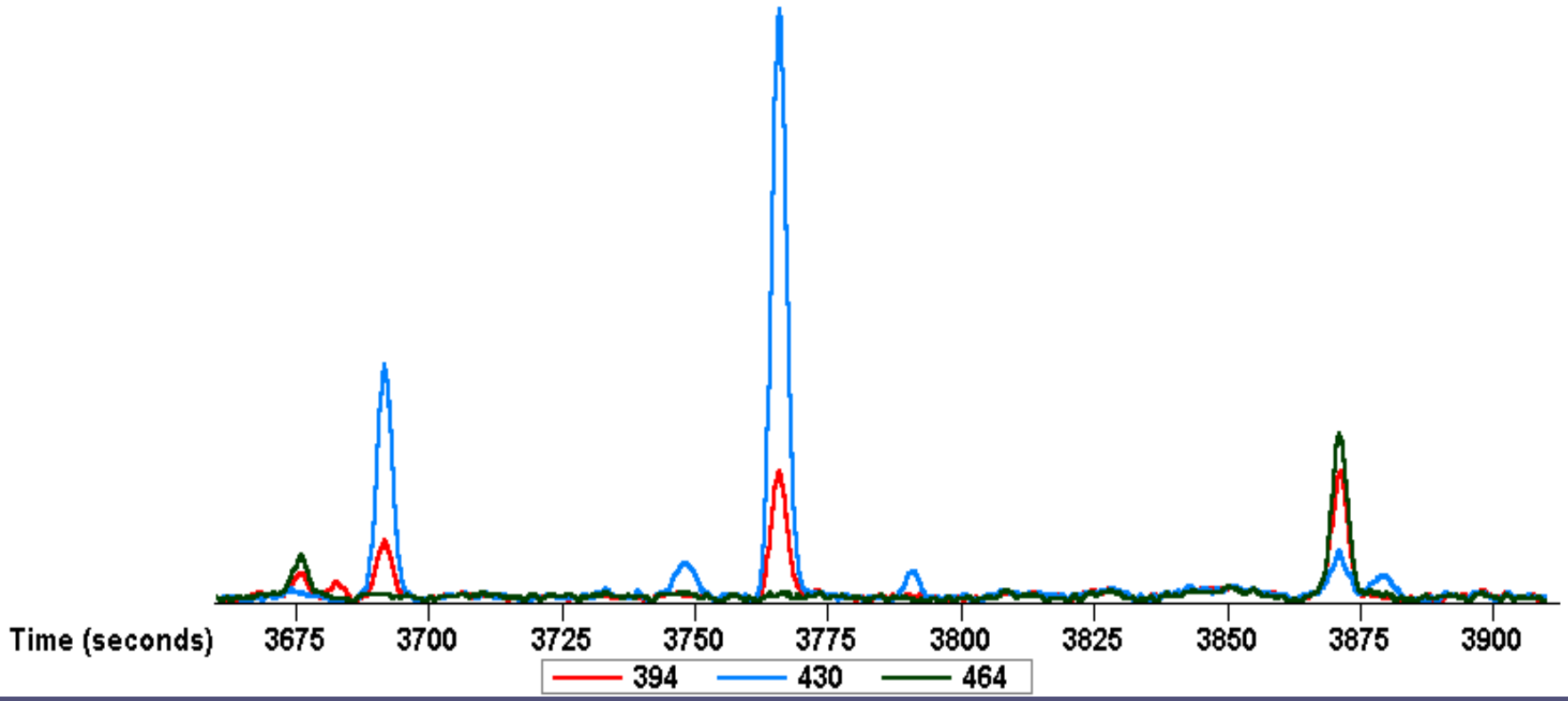

Time (seconds)
2450
2475
2500
2525
2550
2575
2600
2625
2650
2675
256
292
326


101
Time (seconds)
2700
2725
2750
2775
2800
2825
2850
2875
2900
292
326
360


118
153
Time (seconds)
2950
2975
3000
3025
3050
3075
3100
3125
3150
292
326
360
394

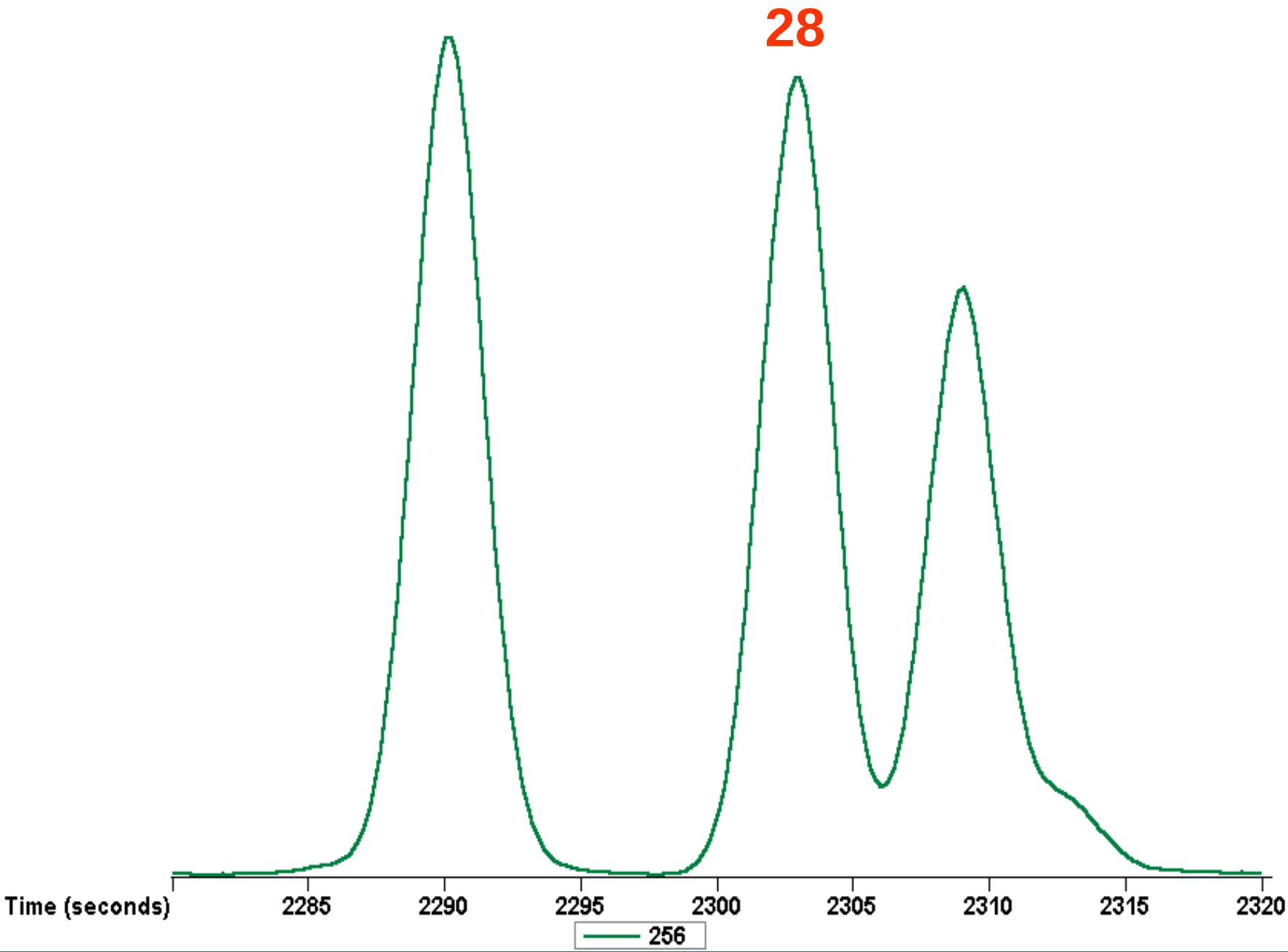

138
Time (seconds)
3175
3200
3225
3250
3275
3300
3325
3350
3375
3400
360
394
430

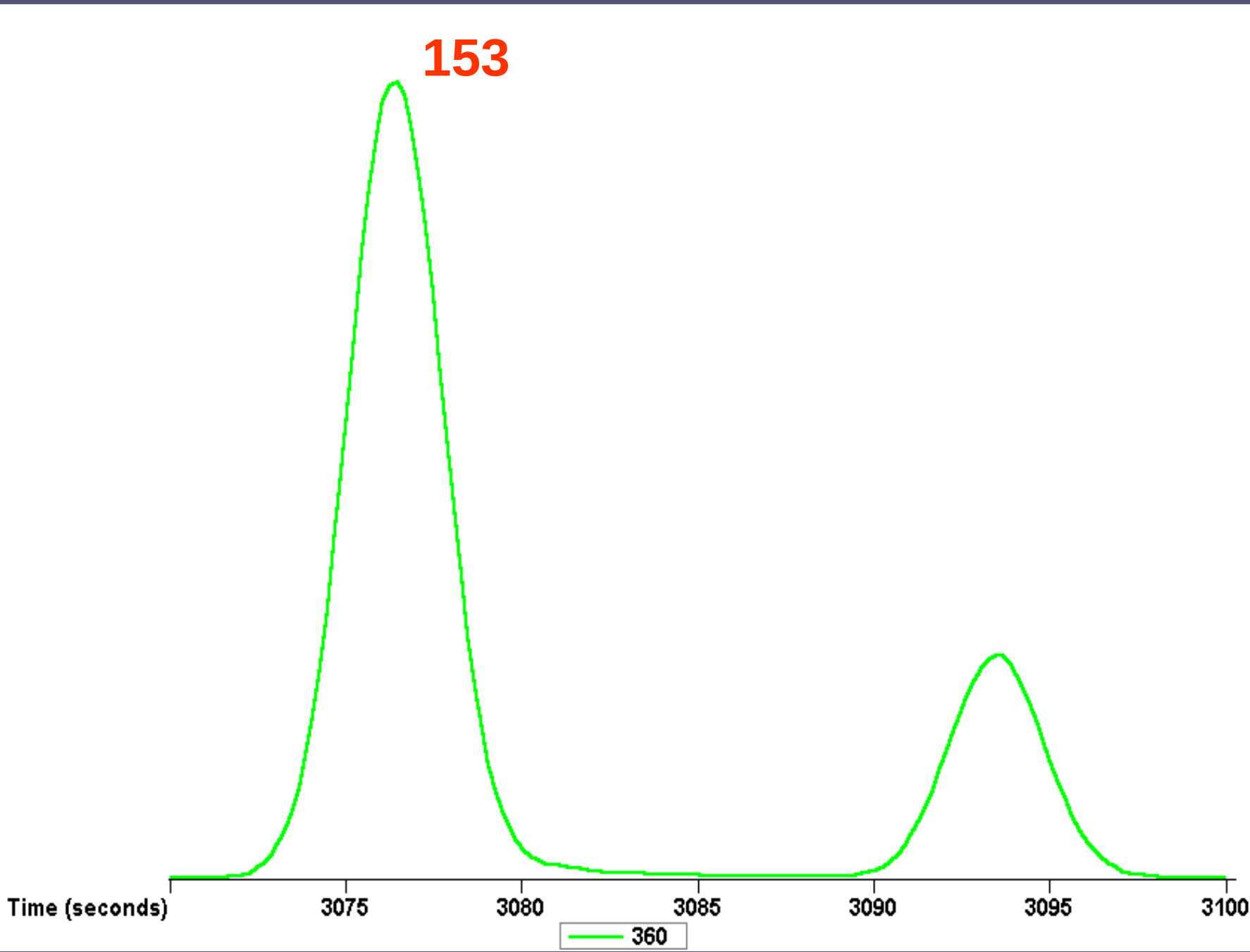

180
Time (seconds)
3450
3500
3550
3600
3650
360
394
430
464

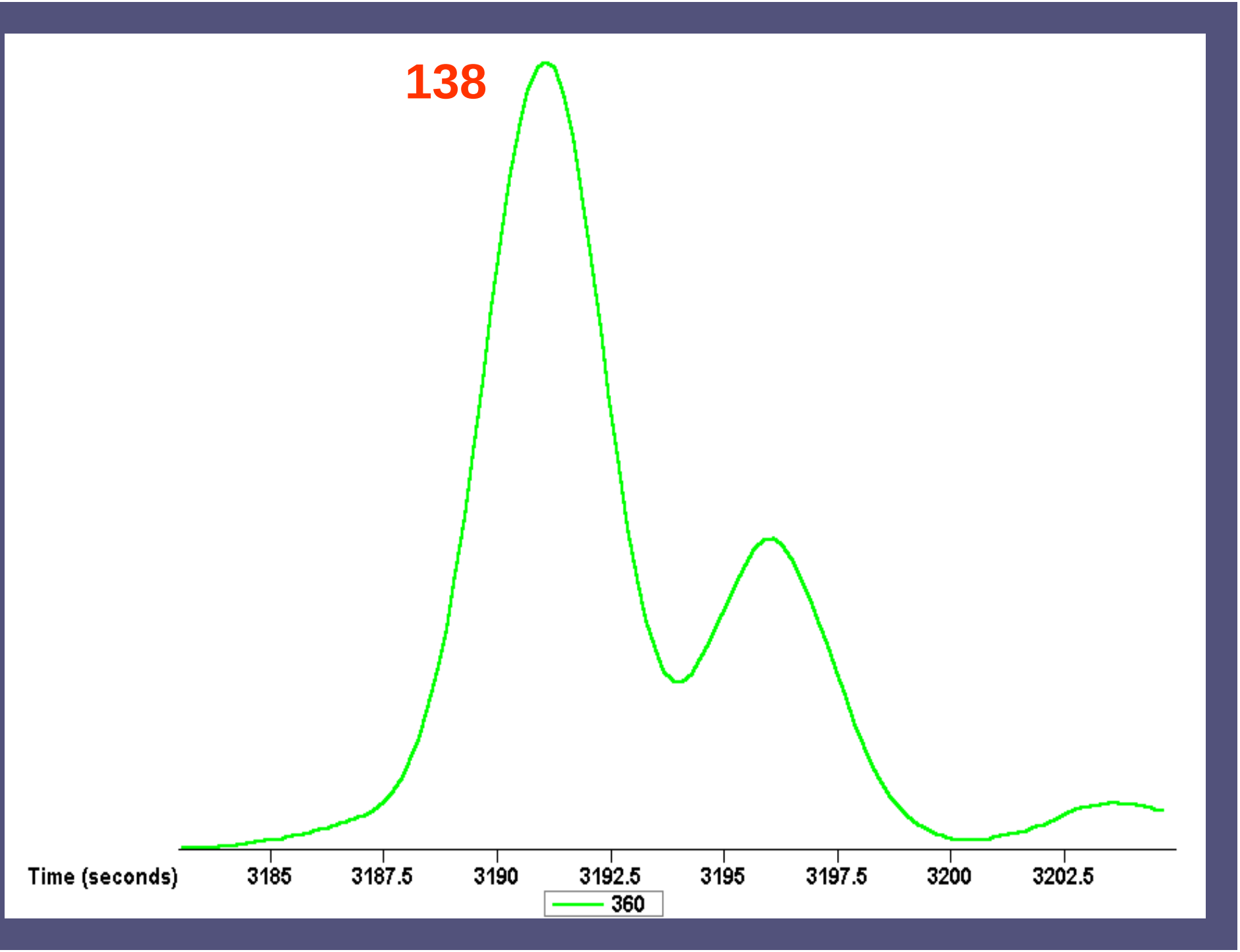

Time (seconds)
3675
3700
3725
3750
3775
3800
3825
3850
3875
3900
394
430
464

Important Congener Separations

- European indicator PCBs
- May coelute on 5% phenyl methylsilicone and other column phases


28
Time (seconds)
2285
2290
2295
2300
2305
2310
2315
2320
256


153
Time (seconds)
3075
3080
3085
3090
3095
3100
360


138
Time (seconds)
3185
3187.5
3190
3192.5
3195
3197.5
3200
3202.5
360

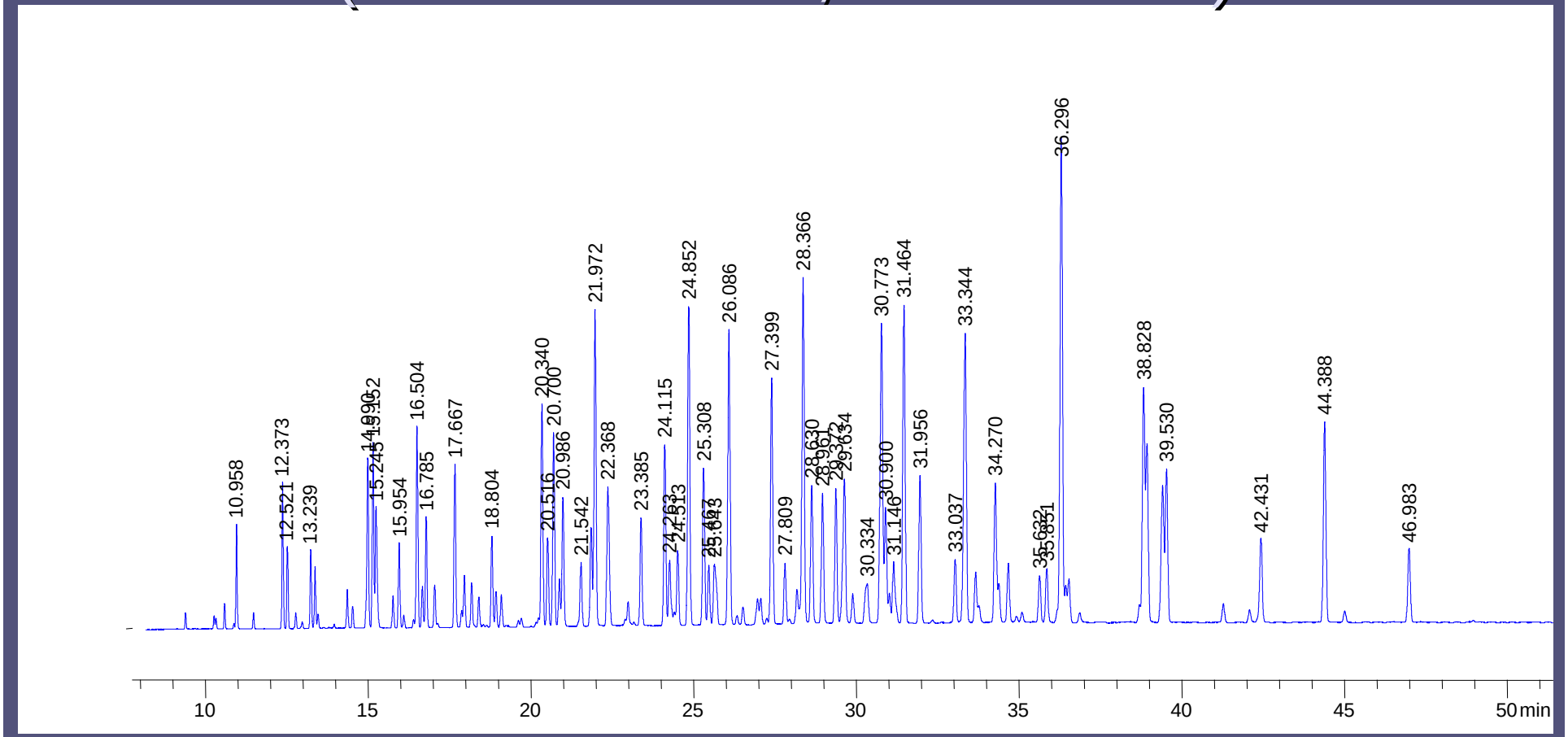
Conclusions

- Greater than 140 Aroclor PCBs can be determined with the new column and mass spectrometry
- The seven European indicator PCBs can be determined individually
- Spectral deconvolution available with TOFMS allows qualitative identification for coeluting PCBs

GC-ECD Analysis

- Columns should be “complementary”
  - As many congeners resolved as possible
  - Different coelutions (nothing will resolve all 140 environmentally-significant congeners)
  - Similar thermal properties
  - Operation under same temperature and pressure programs

Rtx-PCB
(40m x 0.18mm, 0.18um film)

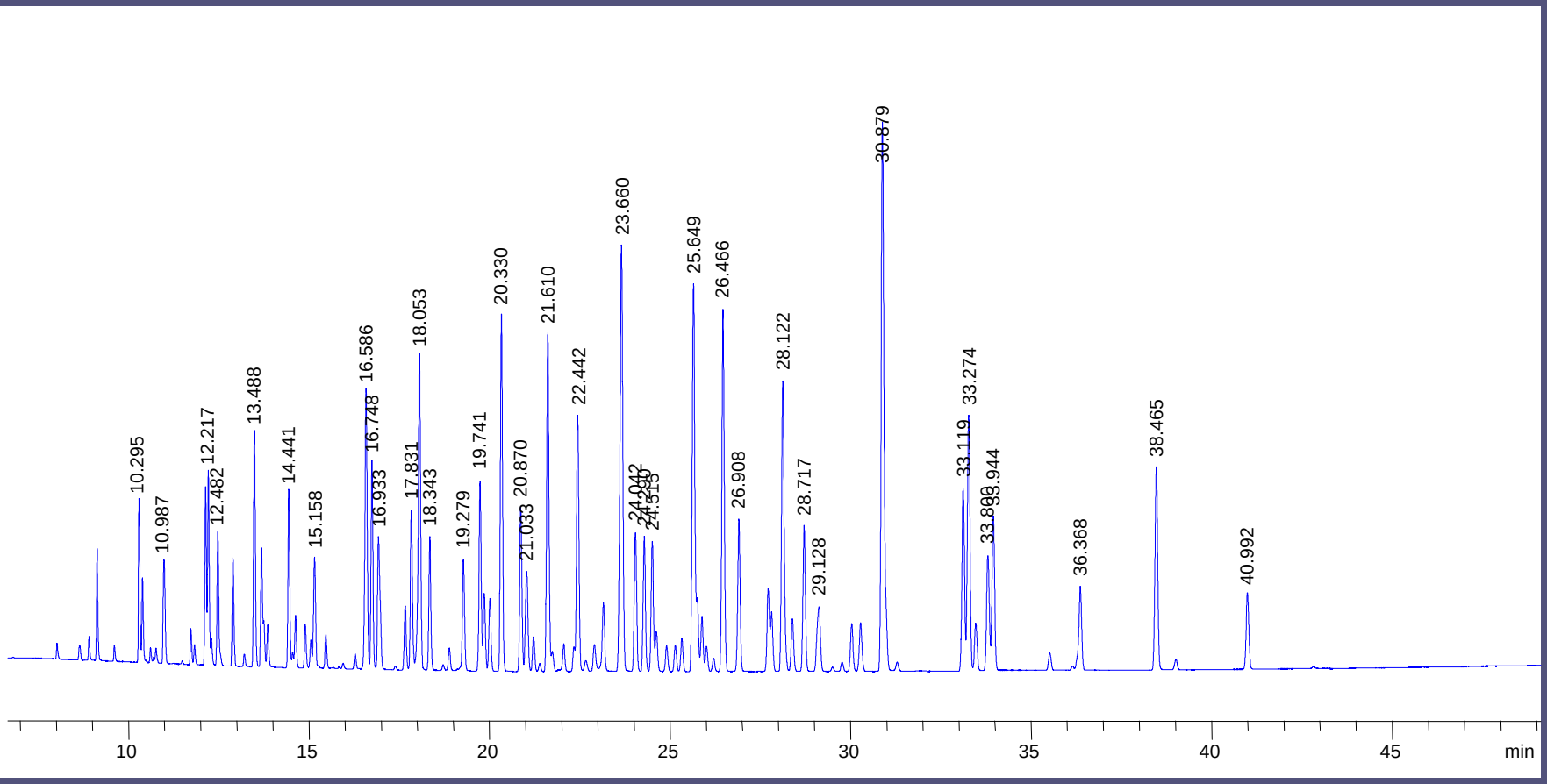


300ppb Aroclor 1242, 1254, 1262

100°C(1 min) 20°C/min 200°C(0 min)

2°C/min 320°C(1 min)

Rtx-440
(40m x 0.18mm, 0.18um film)



300ppb Aroclor 1242, 1254, 1262
100°C(1 min) 20°C/min 200°C(0 min)
2°C/min 320°C(1 min)

Quantitation of Congeners

Rule#1: If compound elutes as single on both columns report lower value

	Rtx-PCB	Rtx-440
Peak	PCB 28	PCB 28

Quantitation of Congeners

Rule#2: If compound elutes as single on one column and multiple congeners on the second report the single column result if confirms on second column

	Rtx-PCB	Rtx-440
Peak	PCB 53 + 31	PCB 31

Quantitation of Congeners

Rule #3: If there are complicating coelutions on both columns calculate the results using normalized responses (ex. PCB 136)

	Rtx-PCB	Rtx-440
Peak	PCB 136 + 117	PCB 115 + 136
	PCB 115	

Quantitation of Congeners

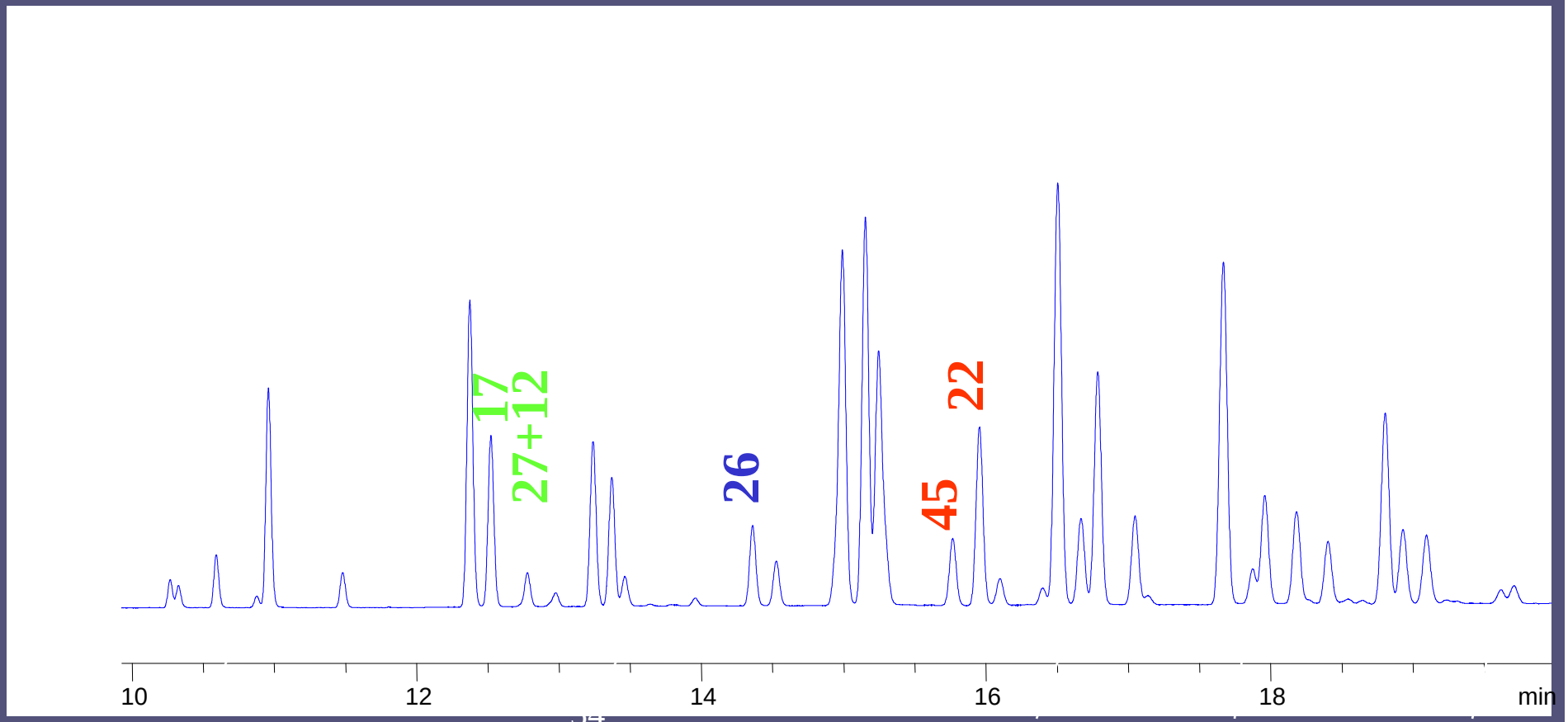
Rule #3 continued:

PCB 136 Concentration =

$$\left([\text{PCB } 115+136] \times \frac{(RF115 + RF136)}{2 \times RF115} - [\text{PCB } 115]\right)\frac{RF115}{RF136}$$

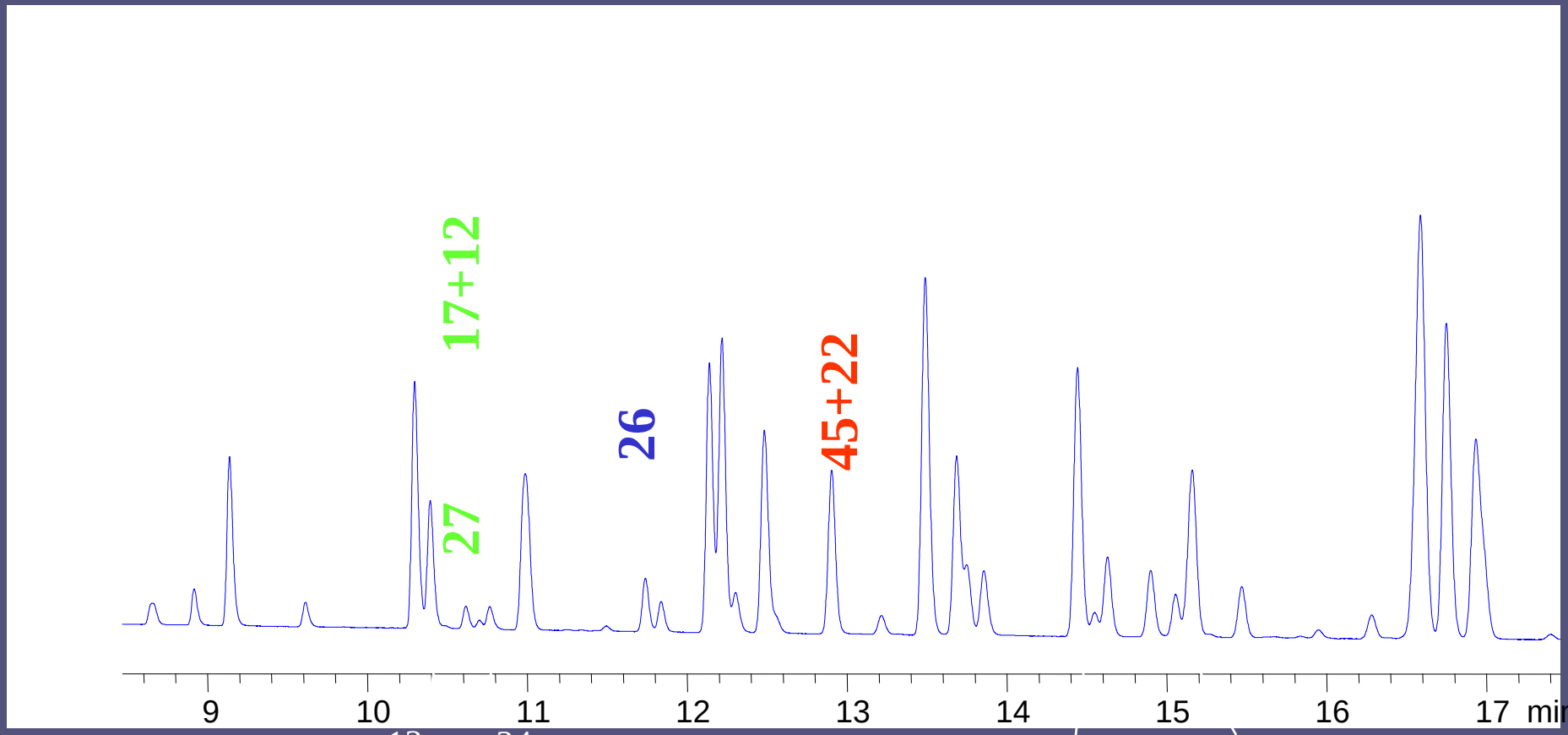
RF = response factor of congener on ECD

Rtx-PCB
(40m x 0.18mm, 0.18um film)



54 48 59 67

Rtx-440
(40m x 0.18mm, 0.18um film)



PCB Congener Separation

				50	72	19		141
Rtx-440		Rtx-PCB			Case			
PCB#	s or m	PCB#	s or m	1	2	3		
1	s	1	s	1				
3	s	3	s	1				
4	m	4	m			1	4+10 reported together	
5	m	5	s		1			
6	s	6	s	1				
7	m	7	s		1			
8	m	8	s		1			
9	m	9	s		1			
10	m	10	m			1	4+10 reported together	
12	m	12	m			1	ok	
13	s	13	m		1			
15	s	15	s	1				
16	m	16	s		1			
17	m	17	s		1			
18	s	18	s	1				
19	s	19	s	1				
20	m	20	m			1	33+20 reported together	
22	m	22	s		1			

GC-ECD Summary

- Rtx-PCB and Rtx-440 Combination
  - Quantification of the 141 PCB congeners possible using the 3-case quantification system
  - High thermal stability
  - Good choice for dual column operation

Overall Conclusions

- GC-MS analysis with Rtx-PCB column
  - European indicator unbiased
  - WHO-12 with carbon cleanup
- GCxGC-TOFMS
  - TOFMS has acquisition speed necessary to characterize the narrow peaks
  - WHO-12 resolved from non-toxic without carbon cleanup
- Dual-Column GC-ECD
  - Also successful, with high sensitivity
  - All congeners of significance can be quantified