Delivering the Right Results

Defining the Retention Times of 209 PCB Congeners Using GCxGC-TOFMS

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INTRODUCTION

Polychlorinated biphenyls (PCBs) are one of the most widely recognized persistent organic environmental pollutants. They are suspected carcinogens and are known to cause liver damage[1]. Because the molecular structures of the 209 PCB congeners are very similar to one another, they have been a challenge to completely separate using one-dimensional gas chromatography. This structural similarity also leads to nearly identical mass spectra for congeners with equivalent numbers of chlorine substitution.

Comprehensive two-dimensional gas chromatography (GCxGC) is a powerful evolution of gas chromatography that allows the user to separate compounds on two different stationary phase chemistries without significantly compromising the separation on either phase. The coupling of a GCxGC system with a LECO Pegasus® 4D Time-of-Flight Mass Spectrometer (TOFMS) produces an instrument with significant improvements in peak capacity, peak detectability, and peak identification capabilities. The work presented here describes the use of a GCxGC-TOFMS instrument to determine the primary retention time (t_r') and secondary retention time (t_r'') for each of the 209 PCB congeners. In addition, a user library with the mass spectra of each congener was created in order to see how successful the ChromaTOF® software would be in appropriately labeling critical pair congeners, which are the most difficult congener pairs to separate.

EXPERIMENTAL

A PCB sample was prepared individually for each of the 209 congeners. The congeners were diluted to 20 µg/mL in isooctane solvent. For the critical pair analyses, small portions of the individual congener samples were combined to make two-congener solutions of roughly 10 µg/mL concentrations.

Comprehensive Two-Dimensional GCxGC-TOFMS (LECO Pegasus 4D)

GC: Agilent 6890 w/ LECO thermal modulator and secondary oven
Injection: 1 µL injection into split/splitless inlet with split ratio varying between 100:1 to 20:1 (see app note for more details)
Carrier: Helium at 1.2 mL/min, corrected constant flow
Primary Column: RTX-PCB, 40 m x 0.18 mm x 0.18 µm
Secondary Column: RTX-17, 1 m x 0.10 mm x 0.10 µm (Restek Corporation, Bellefonte, PA)
Primary Oven: 70°C hold 0.5 min, 10°C/min to 150°C, 1°C/min to 250°C, 4°C/min to 275°C, and final hold 15 min
Secondary Oven: +15°C offset from primary oven
Modulator Offset: 25°C (relative to the secondary oven)
Modulation Period: 4 s (1.10 hot pulse time and 0.90 cold pulse time per stage)
Transfer Line Temp: 280°C

MS: LECO Pegasus 4D
Ion Source Temperature: 200°C
Detector Voltage: 1400 to 1550 V
Electron Energy: -70 eV
Spectral Acquisition Rate: 100 spectra/s
Acquired Mass Range: 45 to 550 m/z

Instrument Control and Data Review:
ChromaTOF 4.22
Microsoft Excel 2007

RESULTS AND DISCUSSION

Retention times in the primary and secondary dimensions were recorded for each of the 209 PCB congeners and can be found in the associated application note in the LECO Applications Library[2]. Figure 1 displays the peak apexes of the congeners in the form of a contour plot organized by the number of chlorine atoms present in the represented PCB congener. In general, the more chlorines that are present, the longer the retention time is in both dimensions. However, there is a great deal of overlap. For instance, the t_r' time span of 2400-3000 seconds contains both 2- and 3-Cl congeners, while the span of 4400-5000 seconds contains 4-, 5-, and 6-Cl congeners. This data was used to create a custom user library containing the spectral information of each congener and was used for further data processing in critical pair analysis using forward searches.

PCB CONGENER RETENTION MAP

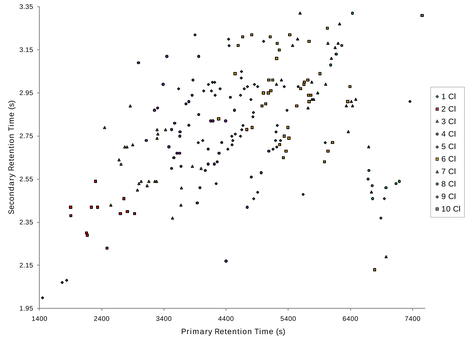

Figure 1. Plotted above are the peak apexes representing the two-dimensional retention times for the 209 PCB congeners. The legend indicates the number of chlorines contained in the represented PCB congener.

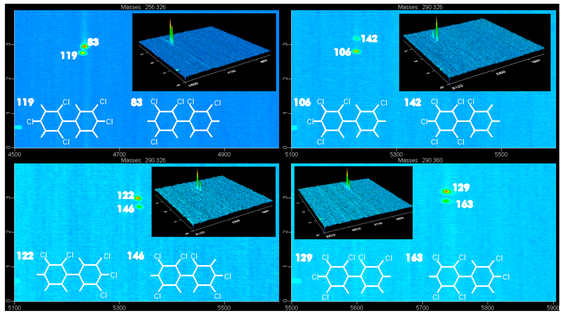

Figure 2. The contour plots of four PCB critical pairs using the summed unique masses of the congeners. The structures of the congeners are displayed at the bottom of each plot labeled by their IUPAC numbering. These examples clearly show the benefit of the second-column separation since they are fully separated in spite of their virtually identical t_r'.

CRITICAL PAIR ANALYSIS

t_r' (s)	Congeners	Cl Content	$\Delta t_r''$ (s)	Test Results	Cl Result
3292	21,33	tri/tri	0.04	24	tri
3524	69,43	tetra/tetra	0.18	64,51	tetra/tetra
3800	59,42	tetra/tetra	0.11	63,52	tetra/tetra
3960	64,40	tetra/tetra	0.27	69,53	tetra/tetra
4112	67,58	tetra/tetra	0.07	55,57	tetra/tetra
4632	119,83	penta/penta	0.19	119,98	penta/penta
4908	120,110	penta/penta	0.49	113,121	penta/penta
5736	163,129	hexa/hexa	0.28	169,134	hexa/hexa
3676	39,75	tri/tetra	0.18	39,57	tri/tetra
5088	77,149	tetra/hexa	0.33	58 (poor sample)	tetra
5212	188,134	hepta/hexa	0.12	190,139	hepta/hexa
5224	106,142	penta/hexa	0.38	112,143	penta/hexa
5336	146,122	hexa/penta	0.23	139,113	hexa/penta
6040	162,174	hexa/hepta	0.5	169 (poor sample)	hexa
6696	199,170	octa/hepta	0.11	199,180	octa/hepta

Table 1. Results for identical t_r' critical pair analyses using the ChromaTOF user library search. The red values in the $\Delta t_r''$ column indicate differences in t_r'' that are below 0.20 s. The test results column indicates the congeners of the top hit, and the Cl result column indicates the number of Cl atoms in the top hit. Correct hits appear in green.

While the top hit for the congeners was typically incorrect, the top hit that was chosen always had the correct number of chlorines present. For the eight critical pairs with identical Cl content, only the tri/tri critical pair was determined to be a single compound after deconvolution. Even the critical pair of 58 and 67, with a $\Delta t_r''$ of 0.07 s, produced two separate peaks in the peak table. The two critical pairs with 'poor sample' labeled in the Test Results column each had one component with S/N too poor to pick up on. However, the large $\Delta t_r''$ indicates easy separation on the second column. Combining the t_r', t_r'', and number of Cl information all assist in identifying the PCB congener.

t_{r1}' (s)	t_{r2}' (s)	Congeners	Cl Content	Δtr'' (s)	Test Results	Cl Result
1900	1904	4,10	di/di	0.04	9	di
3284	3292	28,33	tri/tri	0.20	35,31	tri/tri
3652	3656	47,65	tetra/tetra	0.08	62,51	tetra/tetra
3656	3660	65,62	tetra/tetra	0.02	69,63	tetra/tetra
3676	3684	39,38	tri/tri	0.08	39,24	tri/tri
4216	4224	88,95	penta/penta	0.06	89,90	penta/penta
4392	4400	55,80	tetra/tetra	0.65	63,80	tetra/tetra
4440	4448	89,84	penta/penta	0.03	89	penta
4492	4496	90,101	penta/penta	0.04	95	penta
4644	4648	125,86	penta/penta	0.03	96,125,90	penta/penta/penta
4648	4652	86,112	penta/penta	0.27	99,90	penta/penta
4812	4820	136,154	hexa/hexa	0.43	145,148	hexa/hexa
4832	4840	117,115	penta/penta	0.02	121,106,110	penta/penta/penta
4840	4844	115,111	penta/penta	0.40	107,104	penta/penta
5080	5088	147,149	hexa/hexa	0.06	139	hexa
5196	5204	107,123	penta/penta	0.04	125,125	penta/penta
5216	5224	109,106	penta/penta	0.10	125,110	penta/penta
5256	5264	131,133	hexa/hexa	0.44	159,133	hexa/hexa
5652	5660	130,164	hexa/hexa	0.01	148,143,163	hexa/hexa/hexa
5728	5736	160,163	hexa/hexa	0.03	156,166,152	hexa/hexa/hexa
5728	5736	160,129	hexa/hexa	0.25		
6172	6176	201,204	octa/octa	0.00		
6748	6756	196,203	octa/octa	0.06		

Table 2. Critical pair analyses where the t_r' apex is within 1 or 2 modulation periods of one another using the ChromaTOF user library search. The red values in the $\Delta t_r''$ column indicates differences in t_r'' that are below 0.20 s. The test results column indicates the congeners of the top hit, and the Cl result column indicates the number of Cl atoms in the top hit. Correct hits appear in green.

Unfortunately, there were four cases where the software picked out three peaks where only two existed. In these four cases, the $\Delta t_r''$ was 0.03 s or less. There were also four cases where the software picked out only one peak where two existed—these situations also occurred when $\Delta t_r''$ was 0.06 s or less. Once more, however, the top hits contained the correct number of chlorines even though most congener assignments were incorrect, primarily due to the similarity of the isomers' EI spectra. However, more in-depth processing can achieve improved results, as shown in the next column.

USE OF ION RATIOS TO IMPROVE HIT RESULTS

The frequent mislabeling of congeners led to the possibility of using ChromaTOF's Ion Ratio Calculation as part of a Classification Method to get better matches. To demonstrate this feature, critical pair 43/69 was used. As shown in Table 1, congener 69 was mislabeled as 64. In the generated User Library, the ion ratios between masses 150 and 292 were approximately 0.455 for congener 64 and 0.380 for congener 69, which was sufficiently different to warrant their use in identification. Without using the Ion Ratio, the similarity for congener 64 was 865 and for congener 69 was 854. Reprocessing the data with the Ion Ratio changed the similarity of congener 69 to 886, allowing it to take the top hit position.

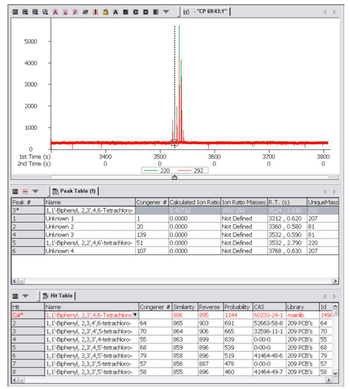

Figure 3. The chromatogram of the critical pair congeners 43 (2,2',3,5) and 69 (2,3',4,6) with associated Peak Table and Hit Table. The use of Calculated Ion Ratio increased the similarity of the congener 69 to 886, allowing it to take the top hit slot.

CONCLUSIONS

The experiments described in this presentation demonstrate the use of the LECO Pegasus 4D (GCxGC-TOFMS) with ChromaTOF software for defining the retention times of the 209 PCB congeners. The study resulted in valuable t_r' and t_r'' information for the identification of PCBs present in a sample. Based on t_r information, only one, two, or rarely three possible congeners will be contenders for the correct congener number. While the mass spectra alone did not usually provide the correct congener in the top hit, combining the t_r and Ion Ratio information from a Classification Method leads to confident congener identification.

REFERENCES

[1]Safe, S. *Polychlorinated biphenyls (PCBs) and polybrominated biphenyls (PBBs): biochemistry, toxicology, and mechanism of action.* Crit. Revi. Toxicol., 1984, 13 (4), 319-395.

[2]http://www.leco.com/resources/application_note_subs/pdf/separation_science/-380.pdf