Using Classifications and Mass Spectral Filtering to Process and View GCxGC-TOFMS Data for Environmental Samples

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Introduction

A relatively new way to solve separation problems for complex environmental samples is to use comprehensive two-dimensional GC (GCxGC). GCxGC increases peak capacity by applying two independent separations to a sample in one analysis. Typically, GCxGC involves a serial column configuration (employing orthogonal phases) separated by a thermal modulator.

Due to modulation, most GCxGC peaks are on the order of 50 to 250 ms wide, requiring a fast detector. When MS is used, only time-of-flight (TOF) has the necessary acquisition rates (hundreds of spectra/sec). The ability of the thermal modulator to narrow peaks (thereby increasing their height) prior to their detection also affords the ability to increase TOFMS sensitivity.

GCxGC-TOFMS offers the possibility of handling complex samples, but the data generated is itself complex. This makes data processing routines very important if the full potential of GCxGC-TOFMS is to be realized. One of the ways that data can be processed is by taking advantage of the structure of GCxGC chromatograms; certain compounds elute in certain areas of the contour plot under the right conditions, and can be grouped. Another way is to employ mass spectral filtering, which can be done in conjunction with grouping, or geographical classification of compounds[1,2]. In this work, Classifications and Scripting (mass spectral filtering) software was employed to process polychlorinated biphenyls (PCBs) data generated using GCxGC-TOFMS. In addition, mass spectral filtering can be used to selectively view certain compounds, or groups of compounds, in a contour plot.

Materials and Methods

PCB standard solutions and dilutions of Aroclors were obtained from AccuStandard (New Haven, CT, USA). Separations were carried out using a LECO Pegasus 4D GCxGC-TOFMS that has a quad-jet, dual-stage modulator (St. Joseph, MI, USA). The GCxGC column configuration was from Restek Corporation (Bellefonte, PA, USA). The integral column, a 50m x 0.18mm x 0.18µm Rtx-1 x 10m x 0.10mm x 0.1µm Rtx-PCB, was installed so that 4m of the Rtx-PCB resided in a secondary oven past the modulator. No press fit connection is necessary for this type of column. A splitless injection at 250°C, with a purge time of 60 sec, was used for each analysis. The primary oven was programmed as follows: 70°C (1 min), 50°/min to 120°, 8°/min to 340° (0.5 min). The modulator temperature offset was 40°C. The second dimension separation time (modulation time) was set to 2 sec, with hot pulse time and cool time between stages at 0.5 sec each. The secondary oven program was: 90°C (1 min), 50°/min to 120°, 8°/min to 340° (3 min). Helium carrier gas was a constant 1.5 mL/min. Total run time was 30 min. Electron ionization at 70eV was used for TOFMS with a source temperature of 225°C, a data acquisition rate of 100 spectra/sec, and a stored mass range of 120 to 520u.

Data was processed with LECO ChromaTOF software, which includes the Classifications and Scripting functionality.

Results and Discussion

Figure 1 shows a contour plot for a mix of PCBs with Classifications of the various chlorination levels shown as ovals. Inevitably, there will be chromatographic overlap of PCB congeners for the various chlorination levels and a Peak Table generated from the Classifications scheme will reflect that by showing individual PCBs belonging to several chlorination classes when obviously they should only belong to one (**Figure 2**). In addition, compounds that

were located by an automatic peak find routine and fall into the geographic space for the PCB classes will also be noted in the Peak Table, even though they may not be PCBs.

If a Script, or mass spectral filter, is employed to work in conjunction with the geographic location of a compound in the GCxGC data (contour plot), then the Classification of a PCB can be specific. An example of a ChromaTOF Script (uses Microsoft Visual Basic Scripting Edition) for tetrachlorobiphenyl is shown below. Rank(1) defines the base peak of the mass spectrum, and Abundance() is the intensity of the m/z ion in the spectrum based on a normalization of the base peak to 999. Essentially what the Script says is that the base peak must be 292 (a molecular ion of tetrachlorobiphenyl) or 222 (from the loss of Cl_2) and that the abundance (in the deconvoluted mass spectrum) of 292 and 222 must be greater than 500. Similar Scripts were written for other homolog groups of the PCBs. A Peak Table using PCB elution area and Scripts together resulted in no overlap of the Classifications (**Figure 3**). Scripts can include arithmetic, comparison, and logical operators, and/or conditional statements (e.g. If… Then…Else) and/or loops.

```
FUNCTION Cl4PCBs()

Cl4PCBs = (Rank(1) = 292 OR Rank(1) = 220) AND Abundance(292)>500 AND Abundance(220)>500

END FUNCTION
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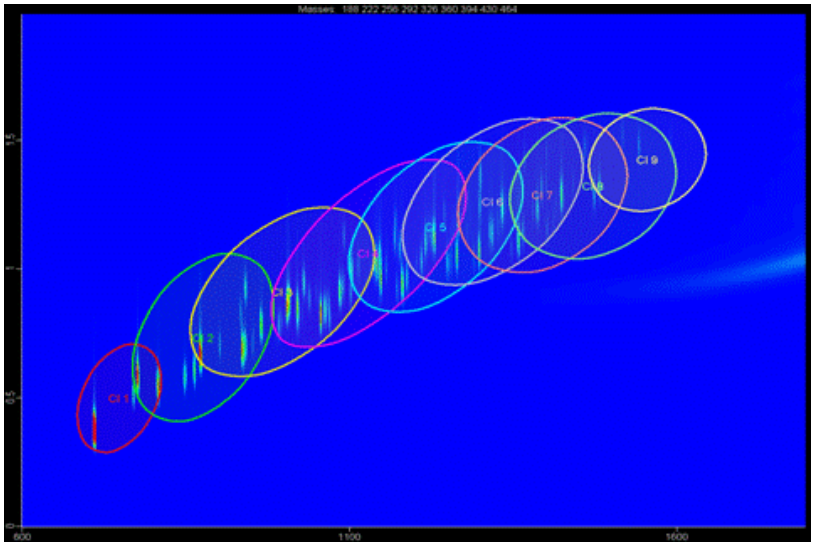

Figure 1. Contour plot of PCBs with Classifications (ovals) for the various chlorination levels. Note the chromatographic overlap.

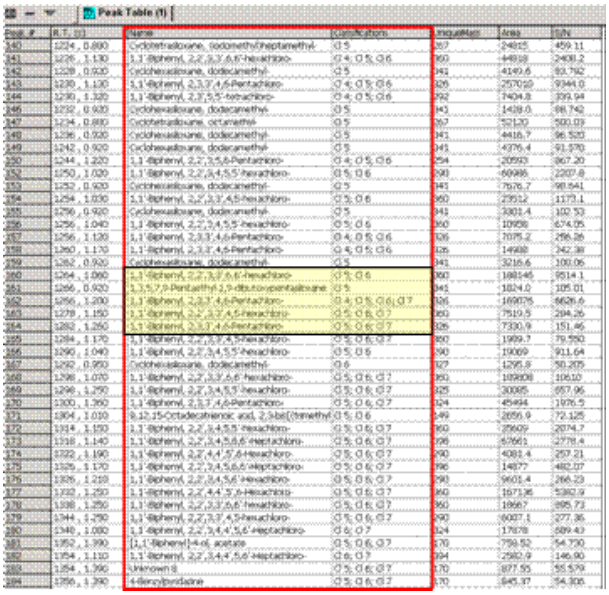

Figure 2. Peak Table with Classifications (PCB chlorination level) based on geographic location of compounds alone. Note the assignment to more than one class for some PCBs and the labeling of compounds other than PCBs in the classes.

Peak Table (1)

Peak #	R.T. (s)	Name	Classifications	UniqueMass	Area	S/N
133	1210 , 1.030	1,1'-Biphenyl, 2,2',3,5,6-Pentachloro-	Cl 5	326	56197	2208.7
135	1216 , 1.110	1,1'-Biphenyl, 2,2',3,5,6-Pentachloro-	Cl 5	254	91817	3731.4
137	1220 , 1.130	1,1'-Biphenyl, 2,3,3',4,6-Pentachloro-	Cl 5	326	6617.0	278.32
139	1222 , 1.140	1,1'-Biphenyl, 2,2',3,5,6-Pentachloro-	Cl 5	326	27655	1283.4
141	1226 , 1.130	1,1'-Biphenyl, 2,2',3,3',6,6'-hexachloro-	Cl 6	360	44818	2408.2
143	1230 , 1.130	1,1'-Biphenyl, 2,3,3',4,6-Pentachloro-	Cl 5	326	257010	9344.0
144	1230 , 1.320	1,1'-Biphenyl, 2,3',5,5'-tetrachloro-	Cl 4	292	7404.8	339.94
150	1244 , 1.220	1,1'-Biphenyl, 2,2',3,5,6-Pentachloro-	Cl 5	254	20593	867.20
152	1250 , 1.020	1,1'-Biphenyl, 2,2',3,4,5,5'-hexachloro-	Cl 6	290	68986	2207.8
154	1254 , 1.030	1,1'-Biphenyl, 2,2',3,3',4,5-hexachloro-	Cl 6	360	23512	1173.1
156	1256 , 1.040	1,1'-Biphenyl, 2,2',3,4,5,5'-hexachloro-	Cl 6	360	10958	674.05
157	1256 , 1.120	1,1'-Biphenyl, 2,3,3',4,6-Pentachloro-	Cl 5	326	7075.2	256.26
158	1260 , 1.170	1,1'-Biphenyl, 2,3,3',4,6-Pentachloro-	Cl 5	326	14988	342.38
160	1264 , 1.060	1,1'-Biphenyl, 2,2',3,3',6,6'-hexachloro-	Cl 6	360	188146	9514.1
162	1266 , 1.200	1,1'-Biphenyl, 2,3,3',4,6-Pentachloro-	Cl 5	326	169076	6626.6
163	1278 , 1.150	1,1'-Biphenyl, 2,2',3,3',4,5-hexachloro-	Cl 6	360	7519.5	284.26
164	1282 , 1.260	1,1'-Biphenyl, 2,3,3',4,6-Pentachloro-	Cl 5	326	7330.9	151.46
165	1284 , 1.170	1,1'-Biphenyl, 2,2',3,3',4,5-hexachloro-	Cl 6	360	1989.7	79.550
166	1290 , 1.040	1,1'-Biphenyl, 2,2',3,4,5,5'-hexachloro-	Cl 6	290	19069	911.64
168	1298 , 1.070	1,1'-Biphenyl, 2,2',3,3',6,6'-hexachloro-	Cl 6	360	189808	10610
169	1298 , 1.250	1,1'-Biphenyl, 2,2',3,4,5,5'-hexachloro-	Cl 6	325	30085	657.96
170	1300 , 1.360	1,1'-Biphenyl, 2,3,3',4,6-Pentachloro-	Cl 5	324	45494	1976.5
172	1314 , 1.150	1,1'-Biphenyl, 2,2',3,4,5,5'-hexachloro-	Cl 6	360	35609	2074.7
173	1318 , 1.140	1,1'-Biphenyl, 2,2',3,4,5,6,6'-Heptachloro-	Cl 7	396	67661	2778.4
174	1322 , 1.190	1,1'-Biphenyl, 2,2',4,4',5',6-Hexachloro-	Cl 6	290	4081.4	257.21
175	1326 , 1.170	1,1'-Biphenyl, 2,2',3,4,5,6,6'-Heptachloro-	Cl 7	396	14877	482.07
176	1326 , 1.210	1,1'-Biphenyl, 2,2',3,4,5,6'-Hexachloro-	Cl 6	290	9601.4	266.23
177	1332 , 1.250	1,1'-Biphenyl, 2,2',4,4',5',6-Hexachloro-	Cl 6	360	167136	5382.9
178	1338 , 1.250	1,1'-Biphenyl, 2,2',3,3',6,6'-hexachloro-	Cl 6	360	18667	895.73
179	1344 , 1.250	1,1'-Biphenyl, 2,2',3,3',4,5-hexachloro-	Cl 6	290	6007.1	277.36
180	1348 , 1.080	1,1'-Biphenyl, 2,2',3,4,4',5,6'-Heptachloro-	Cl 7	324	17878	689.43
182	1354 , 1.110	1,1'-Biphenyl, 2,2',3,4,4',5,6'-Heptachloro-	Cl 7	394	2582.9	146.90
185	1358 , 1.110	1,1'-Biphenyl, 2,2',3,4,4',5,6'-Heptachloro-	Cl 7	394	127730	6452.0
186	1364 , 1.140	1,1'-Biphenyl, 2,2',3,4,4',5,6'-Heptachloro-	Cl 7	324	39858	1682.7
187	1366 , 1.440	1,1'-Biphenyl, 2,2',3,3',4,5-hexachloro-	Cl 6	360	17823	548.89
188	1372 , 1.300	1,1'-Biphenyl, 2,3,3',4,5,6-hexachloro-	Cl 6	360	4063.9	247.13
190	1380 , 1.230	1,1'-Biphenyl, 2,2',3,4,5,6,6'-Heptachloro-	Cl 7	394	12349	636.40
191	1386 , 1.240	1,1'-Biphenyl, 2,2',3,4,4',5,6'-Heptachloro-	Cl 7	396	80408	2477.7
194	1394 , 1.310	1,1'-Biphenyl, 2,2',3,4,4',5,6'-Heptachloro-	Cl 7	324	34847	1515.7
196	1400 , 1.330	1,1'-Biphenyl, 2,2',3,4,4',5,6'-Heptachloro-	Cl 7	324	12106	547.83
198	1400 , 1.450	1,1'-Biphenyl, 2,2',3,3',6,6'-hexachloro-	Cl 6	360	13204	551.54
203	1406 , 1.480	1,1'-Biphenyl, 2,3,3',4,4',5-hexachloro-	Cl 6	360	2348.0	126.75
207	1414 , 1.260	1,1'-Biphenyl, 2,2',3,4,4',5,6'-Heptachloro-	Cl 7	324	8256.6	240.77
213	1424 , 1.290	1,1'-Biphenyl, 2,2',3,4,4',5,6'-Heptachloro-	Cl 7	324	146574	6450.4
215	1428 , 1.280	1,1'-Biphenyl, 2,3,3',4,5,5',6-heptachloro-	Cl 7	396	6346.6	373.88
238	1460 , 1.460	1,1'-Biphenyl, 2,2',3,4,4',5,6'-Heptachloro-	Cl 7	394	31706	884.06
242	1464 , 1.480	1,1'-Biphenyl, 2,2',3,4,5,6,6'-Heptachloro-	Cl 7	394	10188	467.22

Figure 3. Peak Table showing Classifications for PCBs generated from GCxGC elution area and mass spectral filters (Scripts). All PCBs were properly assigned to class.

Conclusions

Classifications and Scripting (mass spectral filters) are powerful mechanisms for processing GCxGC-TOFMS data, and are also helpful for visual data review.

References

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