

Characterization of the Rtx-Dioxin2 for PCB Analysis by Kovats and Lee Retention Indices

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Introduction

Polychlorinated dibenzo-*p*-dioxins (PCDD) and dibenzofurans (PCDF) have been important compounds to analytical chemists for decades, with the first publications describing analytical techniques for their identification appearing in the 1970s.¹⁻³ The international scientific community has acknowledged that these compounds are known to be detrimental to and accumulate in the environment and animals.⁴⁻⁷ Of the 210 chlorinated PCDD and PCDF congeners, 2,3,7,8-TCDD is considered the most toxic and only 17 other congeners have been given Toxic Equivalency Factors (TEFs) relative to 2,3,7,8-TCDD by the World Health Organization.⁸ These 17 congeners are the only compounds routinely monitored for in environmental methodologies such as EPA 1613. However, the combustion processes that form dioxins and furans often form other halogenated compounds, such as polychlorinated biphenyls (PCBs), that can be potentially toxic as well.⁹ The mixture of these compounds in environmental samples poses a daunting analytical challenge for chromatographers. Many of the analytes of interest, which includes dioxins, furans, and PCBs, can all be found at trace levels in soil and sediment, tissue, and serum. Due to the trace levels of analytes, dioxin and PCB analysis has traditionally been performed using gas chromatography coupled to high resolution mass spectrometry (GC-HRMS), which allows for the resolution of target compounds from other halogenated pollutants. Many other instruments, tandem mass spectrometers, high-resolution time of flight mass spectrometers, and two dimensional GCs, are now available that can perform trace dioxin and PCB analysis with comparable results to the GC-HRMS.¹⁰ While the detection of these compounds has become more routine with advanced instrumentation, the chromatographic separation of similar halogenated analytes, like PCBs and dioxins, is still quite challenging.

The column of choice for GC-HRMS analysis of dioxins and PCBs is usually a variation on a 5% diphenyl poly(dimethylsiloxane), such as a DB-5 or DB-5MS column. PCBs have been extensively characterized by Frame on over 20 different stationary phases including the DB-5MS.¹¹ However, the Rtx-Dioxin2 is a dioxin specific column that resolves some dioxin congener coelutions that occur with a DB-5.¹² The Rtx-Dioxin2 has been well characterized for dioxin analysis, but has thus far been underutilized for PCB analysis. Since the clean-up procedure for dioxin analysis often includes PCBs in the same fraction as dioxins, it would be advantageous to know where possible coelutions might occur and where more toxic PCBs elute during the analysis. Characterization of the Rtx-Dioxin2 for PCB analysis was completed using the Kovats retention index utilizing *n*-alkanes and the Lee retention index, which utilizes polyaromatic hydrocarbons (PAH).¹³ The use of these two retention index systems allows for the complete characterization of all 209 PCB congeners on the Rtx-Dioxin 2.

Materials and Methods

The nine PCB mixes used in this analysis were obtained from AccuStandard (AccuStandard, Inc., New Haven CT) and are the same standards used by Frame in his evaluation of 24 stationary phases.¹¹ Five polyaromatic hydrocarbons, Naphthalene, Phenanthrene, Chrysene, and Picene were also purchased from AccuStandard for use as Lee retention index markers.¹³ MA aliphatic hydrocarbon standard was obtained from Restek (Restek, Bellefonte, PA) and used for the Kovats retention index markers.

PCB analysis was performed with an Agilent 7000C triple quadrupole mass spectrometer coupled to an Agilent 7890A gas chromatograph, and Agilent 7693 autosampler (Agilent Technologies, Santa Clara, CA). A 60 m × 0.18 mm × 0.10 μm Rtx Dioxin-2 (Restek, Bellefonte, PA) column was used for the analysis. Helium carrier gas was used, and the GC was operated at a split ratio of 10:1 with a flow rate of 1.2 mL/min. The injector was maintained at a temperature of 290 °C and utilized a 4.0 mm Split/Splitless Skyliner with wool plug (Restek, Bellefonte, PA). Samples were injected under these conditions at a volume of 1.0 μL. Two oven programs were used for this analysis the first started with an initial temperature of 50°C for 1min, 5°C/min to 300°C hold for 10mins. The second oven program started with an initial temperature of 100°C for 1min, 10°C/min to 315°C hold for 2mins. The 7000C source was set to 35 eV and maintained at a 300 °C. Nitrogen was supplied for the collision gas at a flow rate of 2.25 mL/min. A helium quench gas was also used in the collision cell and maintained at 1.25 mL/min.

Results and Discussion

The Rtx-Dioxin2 showed similar selectivity for PCBs as the DB-5MS and Rtx-5 stationary phases characterized by Frame.¹¹ The Rtx-Dioxin2 was able to resolve most of the PCBs in each mix with only minor coelutions that occurred between congener classes. Only one non-fully resolved pair was observed in the hexachloro biphenyls between PCB 140 and 143. However, they were resolved enough to assign each congener a retention time and retention index. With several exceptions, the Rtx-Dioxin2 gave the same retention order as the Rtx-5 and DB-5MS for all 209 PCBs. Kovats and Lee retention indices were calculated using equation 1:

$$I = 100 \frac{T_{RS} - T_{RZ}}{T_{RZ+1} - T_{RZ}} + 100Z \quad (1)$$

Where T_{RS} is the retention time of the substance for which the retention index is to be determined, T_{RZ} and T_{RZ+1} are the retention times for the n-alkane or PAH that bracket the substance of interest, and Z is the number of carbon atoms in the n-alkane chain or number of rings in the PAH that elutes just prior to the substance of interest. Retention indices for PCB mix are given in table 1.

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Table 1: PCB Mix 1

Compound (IUP AC)	Cl Pos.	RT	Lee RI	Kovats RI
1	2	12.26	259.65	1575.84
2	3	13.23	271.21	1659.64
3	4	13.37	272.83	1672.47
4	2-2	13.71	276.66	1702.71
9	25	14.31	283.21	1754.56
6	2-3	14.55	285.70	1774.28
8	2-4	14.73	287.59	1789.20
19	26-2	15.10	291.33	1822.73
18	25-2	15.59	296.16	1868.82
16	23-2	16.02	300.62	1909.64
25	24-3	16.51	309.27	1958.69
28	24-4	16.72	312.76	1978.50
22	23-4	17.07	318.68	2014.09
52	25-25	17.33	322.97	2042.40

44	23-25	17.75	329.92	2088.24
71	26-34	17.94	332.98	2108.44
67	245-3	18.34	339.28	2150.04
74	245-4	18.57	342.82	2173.36
66	24-34	18.71	344.93	2187.33
56	23-34	19.03	349.83	2223.56
99	245-24	19.17	351.94	2240.28
87	234-25	19.63	358.70	2293.85
110	236-34	19.81	361.30	2314.41
82	234-23	20.05	364.68	2341.23
147	2356-24	20.11	365.64	2348.78
146	235-245	20.58	372.16	2400.51
153	245-245	20.72	374.08	2418.46
179	2356-236	20.86	376.02	2436.54
138	234-245	21.27	381.56	2488.18
187	2356-245	21.45	383.99	2510.77
174	2345-236	21.88	389.69	2563.93
177	2356-234	22.03	391.64	2582.15
173	23456-23	22.24	394.37	2608.36
180	2345-245	22.50	397.73	2643.01
199	2345-2356	23.07	404.80	2715.85
203	23458-245	23.23	406.83	2736.35
195	23456-234	23.86	414.54	2814.27
194	2345-2345	24.34	420.29	2869.42
206	23458-2345	25.03	428.34	2946.65