

Article

Characterization of 9 Gas Chromatography Columns by Linear and Lee Retention Indices for Polychlorinated Biphenyls and Polychlorinated Naphthalenes

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Abstract: Polychlorinated biphenyls (PCBs) and naphthalenes (PCNs) are ubiquitous environmental contaminants with varying degrees of toxicity. There are hundreds of possible congeners with similar chemical characteristics, which make these compounds difficult to isolate in environmental samples. Historically, PCBs and PCNs were identified by using an Aroclor or Halowax mixture instead of the individual compounds, which was impractical because of limited numbers of individual standards. A retention index database was developed with all 209 PCBs and 36 PCNs to help identify these chemicals in environmental and biological matrixes. This study uses linear and Lee retention indices to identify all 209 PCBs and 36 PCNs on nine gas chromatography columns. The most toxic congeners, the 12 dioxin-like PCBs, were compared across all columns to determine which stationary phases gave the best selectivity for those compounds. Column selectivity was also examined to determine columns for confirmatory analyses and GC×GC separations. The Rxi-17SilMS demonstrated the most drastic difference in PCB selectivity and, to a lesser extent, PCNs when compared with the other eight columns and could work as a confirmatory column or as a 2nd dimension column for GC×GC separations.

Keywords: retention indices; GC-MS; polychlorinated biphenyls; polychlorinated naphthalenes; column selectivity

1. Introduction

Polychlorinated biphenyls (PCBs) and polychlorinated naphthalenes (PCNs) are well-known persistent organic pollutants. They were widely used from the 1920s to 1980s as technical mixtures, commonly known as Aroclors in the United States, Clophen in Germany, Kanechlor in Japan [1], while PCNs were produced under the name Halowax or Nibren in the United States and Germany, respectively [2]. PCBs were used for an assortment of purposes, such as dielectric fluids for capacitors and transformers, hydraulic fluids, plasticizers, and solvents [1]. PCNs were mainly used in electrical insulation as a protective covering for wires but were also used for wood preservation, engine oil additives, and capacitors [3,4]. Today, these compounds are listed under Annex A and C in the Stockholm Convention for elimination because of their high toxicity and environmental persistence [5]. While PCBs and PCNs are no longer commercially produced, significant concentrations of these compounds are still found in animals and the environment because of their historical use and resistance to degradation.

The unique physical-chemical properties of PCBs and PCNs such as high melting point, lipid solubility, hydrophobicity, and resistance to degradation by physical or chemical processes lead to their wide adoption as industrial components [1,3] but is also the cause of their environmental persistence and bioaccumulation and toxicity in animals. There are 209 individual PCB congeners and 75 possible PCN congeners. All 209 PCB congeners are commercially available as individual standards, a mixture of components, or as Aroclor standards. PCNs are generally obtained as Halowax oils instead of individual congeners, though, 36 of the possible 75 PCNs are commercially available. 12 PCB congeners are considered highly toxic or dioxin-like by the World Health Organization (WHO) and have been assigned toxic equivalency factors (TEFs) relative to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin [6]. PCNs are expected to have similar toxic effects because of their planar structure like PCBs and dioxins; however, there are no agreed-upon regulatory levels of exposure and research into their toxicity relative to dioxins is ongoing [3,7,8]. Overexposure to PCBs and PCNs is linked to multiple health issues such as chloracne and liver damage in humans and animals [9,10], but both are also believed to be endocrine disruptors and are implicated in certain types of cancers [11,12].

Many animals are affected by PCB and PCN pollution, with some of the highest concentrations found in marine life. PCBs have been found in fish [13,14], whales [15,16], and other marine life [17,18] around the world. Typically, exposure to PCBs and PCNs occurred through the technical mixtures used in the past, as these mixtures would leak out of damaged components or degrade in landfills and eventually find their way into lakes, rivers, and oceans. Since Aroclors and Halowaxes are no longer produced for industrial purposes, the levels of specific PCBs and PCNs associated with those mixtures have started to decline [19]. However, PCBs and PCNs are also produced as byproducts in some industrial processes [20] as well as in fires containing electrical equipment or other common household and office furniture [21]. These alternate sources of contamination often produce different PCB and PCN congeners from those found in the Aroclor and Halowax mixtures [22], for example, PCB 47, PCB 51, and PCB 68 in wood varnish [23]. Aroclors and Halowaxes have been completely characterized and are often used as standards to identify the most prevalent congeners in samples [24,25]; yet, with new sources of contamination arising the methods utilizing these mixtures may be less effective. A more accurate approach would require identifying and monitoring individual congeners unique to each sample being investigated. This would allow for the monitoring of specific congeners; help determine which congeners are most prevalent in any given sample and help determine which PCBs and PCNs are being produced from industrial processes and combustion events.

A retention index database on current GC columns for PCBs and PCNs was developed to identify as many congeners as simply and inexpensively as possible. Kovats [26] was the first to use *n*-alkanes for retention indices as a means of identification, and the *n*-alkanes are still in use as the most popular retention markers. Kovats noticed retention times could vary between instruments and could not be relied on as a sole means of identification, so homologous series of compounds, such as the *n*-alkanes, is used to generate a relative retention value, instead of retention time. These markers bracket the compounds of interest and can be used as a secondary means of identification. The compounds chosen for the homologous series should be uniquely identifiable, share chemical characteristics to the compounds of interest, and respond well to the detector [27]. The compounds are then added to the sample or run separately under the same conditions, and the index of the analytes is calculated. Two retention index markers were used in this study. The *n*-alkanes were used for linear retention indices because they are the most commonly used retention markers, and four PAHs (polycyclic aromatic hydrocarbons), naphthalene, phenanthrene, chrysene, and picene, were used for Lee indices [28]. These PAHs are structurally similar to PCBs and PCNs but do not coelute with any PCBs or PCNs and have distinct mass spectra, which make them easy to identify. There are a variety of publications on linear retention indices and other retention compounds that are utilized for an assortment of analytes [29–32]. Unfortunately, PCBs and PCNs lack a comprehensive retention index system on current commercially available stationary phases. There are many publications on relative retention order and identification of PCBs and PCNs on older column phases [33–35], but using relative

retention markers limits which compounds one can look for in a sample. This study uses retention markers that are similar to the compounds of interest on current stationary phases, which allows for the identification of all target compounds that may be present in a sample.

Linear and Lee retention markers were used to analyze PCBs and PCNs on nine GC stationary phases to develop a database of retention indices. This retention index database contains values for all 209 PCBs and the 36 PCNs that are commercially available. The retention indices were collected on 9 GC columns: Rtx-1MS, Rtx-5MS, Rtx-200, Rtx-Dioxin2, Rxi-5MS, Rxi-5SilMS, Rxi-17SilMS, Rxi-XLB, and HT8. These columns are uniquely selective for different analytes and were chosen as they are the most commonly used for the analysis of persistent organic pollutants [36–38]. These retention indices can be used in many ways, from defining elution order to the identification of targets on the specific columns used in this work. This data can also aid in determining which congeners interfere with the quantification of targets on the profiled columns. This database will help inform which column sets or appropriate standards are needed to quantify the true value of the target or which column may resolve the coelution. Finally, this database can be used to save method development and time by finding the optimal column for confirmatory analyses and possible 2nd dimension columns for comprehensive 2-dimensional GC (GC×GC).

2. Materials and Methods

2.1. Standards and Reagents

All 209 PCBs were obtained from AccuStandard (AccuStandard, New Haven, CT, USA) in the form of 9 PCB congener mixes (Cat#: C-CS-01 through 09). 36 individual PCNs were obtained from Cambridge Isotope Labs (Cambridge Isotope Labs, Tewksbury, MA, USA). Naphthalene (Cat#: H-152S), phenanthrene (Cat#: H-122S), chrysene (Cat#: H-115S), and picene (Cat#: H-184S), were purchased from AccuStandard for use as Lee retention index markers. *n*-Alkanes were purchased as a standard mix, MA aliphatic hydrocarbon standard (Cat#: 31459), from Restek (Restek, Bellefonte, PA, USA) for use as linear retention index markers. Toluene (Cat#: 9351-03), obtained from J.T. Baker-Avantor (Avantor, Radnor, PA, USA), was used to prepare the PCNs, while isooctane (Cat#: O296-4) from Fisher Scientific (Fisher Scientific, Hampton, NH, USA) was used to prepare the PCBs.

The 9 PCB congener mixes and PCNs were diluted in isooctane and toluene, respectively, to 100 pg/μL. The 36 individual PCN congeners were aliquoted into five separate mixtures. Consulting earlier work by Jarnberg [36], Lei [39], and Lega [14], PCNs solutions were made that provided the best resolution between congeners. The 9 PCB congener mixtures sold by AccuStandard are already optimized to avoid coelutions [25]. PCN congeners with the same degree of chlorination were added to separate mixtures, which made unambiguous identification of each congener with minimum GC experiments possible. The *n*-Alkanes and PAHs markers were injected with the PCBs and PCNs in the same analytical separation. The supplemental information, Excel sheet Supplemental Information: Coelutions Congeners, includes the mixtures for each group of PCBs and PCNs.

2.2. Calculation of Retention Indices

Calculations for linear and Lee retention indices were completed using Equations (1) and (2), respectively:

$$LI^T = 100n \left(\frac{t_R - t_{R(C_z)}}{t_{R(C_{z+n})} - t_{R(C_z)}} \right) + 100z \quad (1)$$

Equation (1) [27,30] calculates the linear retention index (LI^T) from the *n*-alkane retention markers, where n is the difference in carbon number between the two bracketing alkanes, t_R is the retention time of the target compound, $t_{R(C_z)}$ is the retention time of the alkane that elutes before the target compound, $t_{R(C_{z+n})}$ is the retention time of the next eluting alkane, and z is the carbon number of the previous alkane.

$$LeeI^T = 100 \left(\frac{t_R - t_{R(C_z)}}{t_{R(C_{z+1})} - t_{R(C_z)}} \right) + 100z \quad (2)$$

Equation (2) [28] calculates the temperature-programmed Lee indices ($LeeI^T$), from the PAH retention markers, where t_R is the retention time of the target compound, $t_{R(C_z)}$ is the retention time of the PAH that elutes before the compound, $t_{R(C_{z+1})}$ is the retention time of the next eluting PAH, and z is the ring number for the previous PAH.

2.3. GC Columns and Physical Dimensions

Restek (Bellefonte, PA, USA) supplied all the gas chromatographic columns used in this study, including the HT8, which was purchased through Trajan Scientific (Austin, TX, USA). Table 1 lists all columns and dimensions. Rtx and Rxi series columns were used throughout this study. The Rtx and Rxi designation refers to a difference in column deactivation technique or percent composition as per the manufacture's information. Columns with the same phase-type, such as Rtx-5MS or Rxi-5MS, will have the same percent substitution of phenyl in the stationary phase. However, any differences in the manufacturing of these columns may contribute to changes in the retention behavior of closely eluting molecules. A discussion of these effects can be found in previous work by Stultz and Dorman [40].

Table 1. GC column characteristics: information on column dimensions and type of stationary phase used for the development of retention indices for PCBs and PCNs.

Column	Dimension	Phase
Rtx-1MS	60 m × 0.18 mm × 0.10 μm	100% polydimethyl siloxane
Rtx-Dioxin2	60 m × 0.18 mm × 0.10 μm	Restek proprietary phase
Rtx-200	60 m × 0.25 mm × 0.25 μm	Trifluoropropylmethyl polysiloxane
Rtx-5MS	60 m × 0.18 mm × 0.10 μm	5% diphenyl 95% polydimethyl siloxane
Rxi-5MS	60 m × 0.18 mm × 0.10 μm	5% diphenyl 95% polydimethyl siloxane
Rxi-5SilMS	60 m × 0.18 mm × 0.10 μm	1,4-bis(dimethylsiloxy)phenylene polydimethyl siloxane
Rxi-XLB	60 m × 0.18 mm × 0.10 μm	Restek proprietary phase
Rxi-17SilMS	60 m × 0.25 mm × 0.25 μm	(50%-phenyl)-methylpolysiloxane
HT8	60 m × 0.18 mm × 0.10 μm	8% phenyl polycarborane-siloxane

2.4. Instrument Parameters

A Waters Xevo TQ-S triple quadrupole mass spectrometer (Waters Corporation, Milford, MA, USA) coupled to an Agilent 7890A gas chromatograph, and Agilent 7693 autosampler (Agilent Technologies, Santa Clara, CA, USA) was used for column characterization. Helium carrier gas was used at a flow rate of 1.2 mL/min with a split ratio of 10:1. The injector temperature was kept at 290 °C and a 4.0 mm Split/Splitless Skyliner with a deactivated glass wool plug (Restek, Bellefonte, PA, USA) was used. A 1.0-μL injection volume was used with these conditions. A slow and fast temperature program was used for column characterization: the first, the "slow program," began with an initial temperature of 50 °C for 1 min, 5 °C/min to 325 °C hold for 6 min and the second temperature program, the "fast program," had a starting temperature of 75 °C for 1 min, 10 °C/min to 335 °C hold for 8 min.

The Waters triple quadrupole mass spectrometer has an atmospheric pressure chemical ionization source, usually referred to as APCI; however, to remain consistent with Waters terminology and the description of their instrumentation, the term APGC will be used throughout this work. Data were recorded using selected ion recording (SIR) and scan mode of the molecular ion peak. Charge transfer ionization was promoted in the source by operating under dry conditions. The APGC source was maintained at 150 °C. Corona voltage was 20 μA for the first 8 min of the analysis and then maintained at 2.0 μA until the end of the analysis. A 30 V cone voltage was used for all compounds. Cone gas flow was maintained at a flow rate of 230 L/h, except for the first 8 min of the analysis when it was off. An NM32LA nitrogen generator from Peak Scientific (Billerica, MA, USA) supplied the auxiliary nitrogen gas at a flow rate of 400 L/h. The collision cell used argon at a flow rate of 0.18 mL/min.

An 85-cm deactivated silica capillary column with an internal diameter of 0.18 mm (Restek, Bellefonte, PA, USA) was used as a transfer line and kept at 300 °C for the entirety of the separation.

3. Results and Discussion

3.1. *n*-Alkanes vs. PAH Retention Markers

Linear and Lee retention indices were used in this study to offer a greater range of selectivity for retention markers and provide an alternative to historically popular relative retention indices, which require the use of spiked target compounds as retention markers [41]. Relative retention markers were often used with an electron capture detector (ECD), which is not suitable for the detection of *n*-alkanes or PAHs [24]. The *n*-alkane and PAH retention markers are meant to be used with a mass spectrometer. Ideally, for the analysis of PCBs and PCNs, an APGC triple quadrupole instrument (APGC MS/MS) would be used. APGC MS/MS is a soft ionization technique that produces more molecular ion than electron ionization, which provides greater sensitivity. The MS/MS capabilities provide more selectivity and help avoid misidentification with other isobaric compounds [42]. However, while APGC MS/MS offers many advantages for PCB and PCN analysis, traditional electron ionization instruments can be just as capable and are still compatible with the retention index method discussed throughout this work.

n-Alkanes are the most popular compounds used for retention indices, but they are not chemically or structurally similar to PCBs or PCNs. The four PAHs used in this work, naphthalene, phenanthrene, chrysene, and picene, are more chemically similar in their interactions with the stationary phase and similar in structure to the target analytes than the *n*-alkanes. The inclusion of both types of retention markers in one database allows others to choose the best markers for their specific needs and separations.

This study provided a secondary opportunity to compare the phase selectivity differences or differences in retention between analytes and retention marker compounds, in this case, *n*-alkanes and PAHs. The *n*-alkanes primarily interact with the column stationary phase through dispersion forces, while PAHs can interact through π - π interactions on phenyl substituted columns. These differences were visualized by graphing the retention indices for each column against each other. For example, Figure 1 is the Lee indices of the 12 dioxin-like PCBs from the Rtx-1MS plotted on the *x*-axis, and the Lee indices from the Rxi-17SilMS plotted on the *y*-axis. Figure 2 is the same plot as Figure 1 but uses linear retention indices instead of Lee indices. Data from the slow program was used for all figures comparing columns and congener selectivity. The Supplementary materials contains all the data for the fast program.

The *n*-alkane and PAH retention markers show different interactions in the same phase, as can be seen in Figures 1 and 2. The blue points represent the dioxin-like PCBs, and the dotted blue line is the line of best fit for this data. If all the points fell on the line of best fit, the two columns would have no difference in selectivity. However, points fall both above and below this line, which indicates that a particular compound's affinity for a specific phase. The points that lie above the line of best fit are more selective in the *y*-axis phase, the Rxi-17SilMS, and the points below the line are more selective in the *x*-axis phase, the Rtx-1MS. The solid orange line is the plot of $y = x$. If the dotted line falls below the solid line, then the retention markers are more retained on the *y*-axis phase, which decreases the observed retention index. The Lee indices, plotted in Figure 1, display this trend. The primary form of retention for the PAHs is π - π interactions between aromatic rings, which is the same form of retention for PCBs and PCNs. The Rxi-17SilMS is a 50% substituted phenyl phase that will interact strongly with these compounds increasing their retention. The increase in PAH retention is commensurate with the increase in PCB and PCN retention, which will then decrease the calculated retention index of the target compounds relative to the linear retention indices. The linear indices display the opposite trend in Figure 2. The *n*-alkanes are not as well retained on the 17SilMS phase as the target compounds. PCBs and PCNs will interact with the stationary phase through π - π interactions, which are stronger than the *n*-alkanes' dispersion forces, which increases the calculated retention index because the PCBs and PCNs are retained longer relative to the *n*-alkanes.

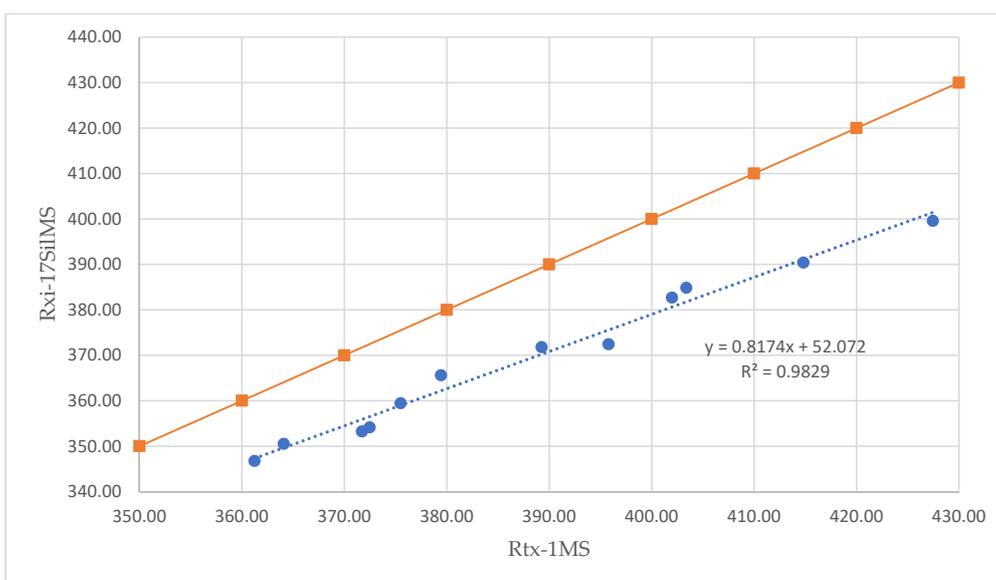


Figure 1. Lee Indices of Dioxin-Like PCBs on Rxi-17SiIMS vs. Rtx-1MS. Figure 1. This is the plot of Lee indices for the 12-toxic dioxin-like PCBs on the Rtx-1MS and Rxi-17SiIMS. The dotted blue line is the line of best fit through the plotted indices. If all the blue points, the 12 PCB congeners, were perfectly linear, there would be no selectivity difference between the two columns. The solid orange line is the plot of $y = x$, which shows where the points and line of best should fall if the columns had equal selectivity for the PCB congeners. Lee indices fall below the line $y = x$, indicating they are more retained by the Rxi-17SiIMS phase. The Lee indices will show a decrease in the retention index as compared to the linear indices.

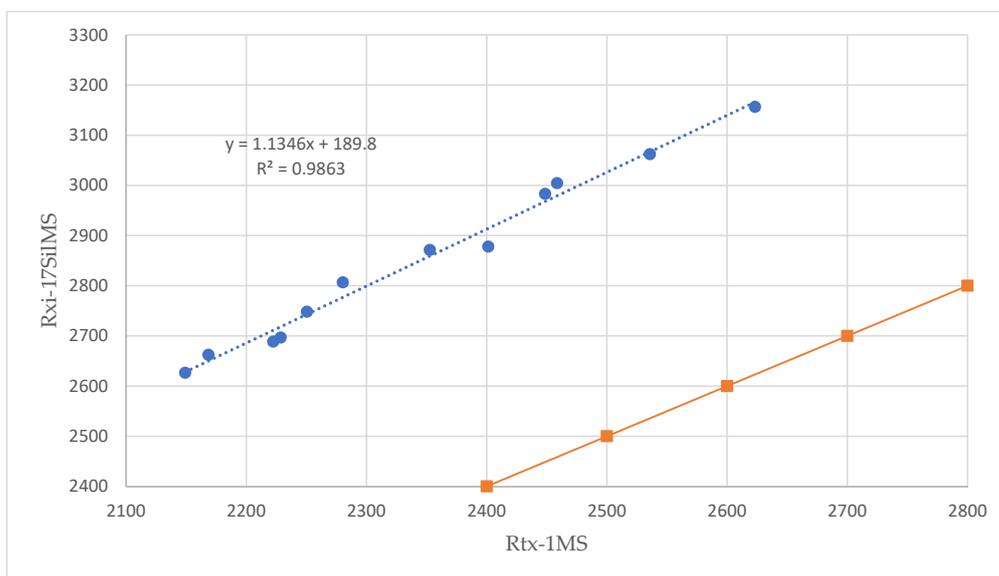


Figure 2. Linear Indices of Dioxin-Like PCBs on Rxi-17SiIMS vs. Rtx-1MS. Figure 2. This is the plot of Linear indices for the 12-toxic dioxin-like PCBs on the Rtx-1MS and Rxi-17SiIMS. The dotted blue line is the line of best fit through the plotted indices. If all the blue points were perfectly linear, there would be no selectivity difference between the two columns. The solid orange line is the plot of $y = x$, which shows where the points and line of best fit should fall if the columns had equal selectivity for the PCB congeners. Linear indices fall above the line $y = x$, indicating they are less retained by the Rxi-17SiIMS and relatively more retained on the Rtx-1MS. The linear indices will show an increase in overall retention index as compared to the Lee indices because of their weaker relative retention.

3.2. Critical Congener Resolution

Dioxin-like PCBs are considered the most toxic congeners because they exhibit dioxin-like characteristics such as a planar conformation and the ability to interact with the aryl hydrocarbon receptor in humans [43]. The goal of many PCBs analyses is to identify and quantify these 12 compounds in any given sample as well as to characterize the total PCB content. While determining the total amount of PCBs in a sample may be possible, it is often difficult to identify individual congeners due to coelutions. Resolution between congeners and identification of target compounds is made possible by examining the linear and Lee indices collected. Table 2 lists some of the penta-substituted compounds on the Rtx-1MS column used in this work. The retention indices in this table help identify coelutions and can help determine a retention index resolution between congeners. To replicate these retention indices and identify coelutions, the exact parameters as those listed in Section 2.4 must be used. If the experimental conditions are modified, the retention indices generated will not match those reported here [38]. The elution order and coelution data in the following tables are still effective and beneficial for designing a successful separation even if the conditions are modified. The supplemental information, Supplemental Information: Coelutions, contains the rest of the congeners characterized on the Rtx-1MS as well as the other columns profiled.

Table 2. Selected penta-substituted PCB coelutions on the Rtx-1MS.

PCB Number	Retention Time	Lee Index	Linear Index
117	39.11	361.41	2150.29
87	39.13	361.49	2151.29
111	39.17	362.00	2154.29
115	39.19	362.00	2154.73
85	39.26	362.67	2158.86
116	39.27	362.84	2160.00
120	39.38	363.76	2166.29
110	39.48	364.43	2171.35
82	39.83	367.37	2191.40
124	40.16	370.22	2211.21
108	40.23	370.89	2216.20
107	40.25	370.89	2216.82
123	40.34	371.73	2222.43
118	40.44	372.48	2228.66
106	40.46	372.82	2230.53
114	40.79	375.50	2250.47
122	40.86	376.09	2254.83
105	41.27	379.45	2280.37
127	41.41	380.70	2289.10
126	42.43	389.26	2352.65

Table 2 is the retention order for a subset of the penta-substituted PCBs. The red numbers indicate a dioxin-like PCB, and the compounds bordered in blue will not be fully resolved from the nearest-eluting congener.

PCNs share many of the same characteristics as PCBs and are believed to be of similar toxicity [44]. While specific PCN congeners have yet to be given TEF values, it is still beneficial to identify individual congeners in a specific sample. Identifying individual compounds will allow for the study of specific congeners and, hopefully, lead to a better understanding of the overall toxicity of PCNs and which

congeners are most prevalent in environmental samples. As with PCBs, PCNs often coelute, making the identification of individual compounds difficult. The retention indices established in this study can help identify coelutions for half the possible PCN congeners. Table 3 lists the coelutions for all hexachlorinated naphthalenes on the Rtx-1MS. The supplemental information, Supplemental Information: Coelutions, contains all compounds characterized on the Rtx-1MS and the other columns profiled in this work.

Table 3. Hexachlorinated naphthalene coelutions on the Rtx-1MS.

Hexachlorinated Naphthalenes	Retention Time (mins)	Lee Index	Linear Index
1,2,3,4,6,7-hexachloro	41.79	383.92	2310.56
1,2,3,5,6,7-hexachloro	41.81	384.09	2311.80
1,2,3,4,5,7-hexachloro	42.20	387.35	2336.65
1,2,3,5,6,8-hexachloro	42.20	387.35	2336.65
1,2,3,5,7,8-hexachloro	42.35	388.61	2345.34
1,2,4,5,7,8-hexachloro	42.44	389.36	2350.93
1,2,4,5,6,8-hexachloro	42.45	389.45	2351.55
1,2,3,4,5,6-hexachloro	43.10	394.89	2392.55
1,2,3,4,5,8-hexachloro	43.37	397.15	2410.10
1,2,3,6,7,8-hexachloro	43.47	397.99	2416.22

Table 3 lists the retention order for all hexachlorinated naphthalenes. The compounds enclosed in blue boxes will not be fully resolved from the next closest congener.

The compounds outlined in blue in Tables 2 and 3 will coelute to varying degrees. Three conditions were chosen to define the coelutions in Tables 2 and 3. The conditions were defined after measuring the average base and half max peak width of the analytes. A retention index difference for peaks that did not overlap at the base of the peak was calculated, and this value, in conjunction with retention time, was used to define the three conditions. The retention time is not essential to determine the index resolution between peaks or if the peaks will coelute; however, it is useful if there are slight inconsistencies between the Lee and linear indices. The conditions used to determine coelutions were experimentally determined and are as follows: First, the linear index of two congeners must have a difference greater than 10 index units; second, the Lee index between two congeners must have a difference greater than one index unit; third, the retention time between congeners must be greater than 7 s. The conditions described above apply to most coelutions between congeners, but band broadening can change how these are applied. Analyzing these congeners under different experimental settings, with a different liner, or any deficiencies in the injector or column may affect how the coelution conditions are used. The coelution conditions could be narrowed or coelutions observed in the data from this study may not occur if very narrow band widths are observed. However, if wider peaks are observed, then the coelution conditions may need to be adjusted for the wider bands by increasing the difference between index units.

Generally, it is desirable to identify dioxin-like congeners from other interfering compounds. This can be accomplished using this retention index system. Table 4 is the list of interfering compounds for select dioxin-like PCBs on each column, as characterized in this study. Information on coelutions for these compounds is available in other formats [34,36] but, the tables presented here can be used as a quick reference to identify any congeners that may interfere with a dioxin-like compound on the phases profiled in this study. Many of the columns profiled share similar coelutions, which means the columns have similar selectivity mechanisms, or the congeners show minute to no differences in the stationary phase's selectivity. A full table for all compounds can be found in the supplemental information.

Table 4. Interfering compounds for Dioxin-Like PCBs.

Column	PCB 105	PCB 114	PCB 118	PCB 123	PCB 126	PCB 156	PCB 157	PCB 167
Rtx-1MS		122	106 123	118 108 107				
Dioxin2	127	122	106	107 108 124		157	156	
Rtx-200	127			107 106				128
Rtx-5MS	127	122	123 106	118 108 107 106				128
Rxi-5MS	127	122	123 106	108 107 106 118				128
Rxi-5SilMS	127	122	123 106	124 108 107 106 118				128
Rxi-17SilMS		122	123 106	118 107 108				129
Rxi-XLB		122	106	106 107 108		157	156	
SGE-HT8	127		106	107 108 124 106				128

Table 4 lists a subset of the dioxin-like PCBs with interfering congeners on each of the nine columns profiled in this study. The full table can be found in the supplemental information.

3.3. Column Selectivity

As discussed in Section 3.1, the retention indices plotted in Figures 1 and 2 provide insights into the degree of column selectivity, without the plot of $y = x$. The greater the difference in selectivity between the columns is described by the amount of scattering from the line of best fit, the dotted line. The R^2 value provides a convenient measure of column selectivity; as the difference between 1 and R^2 increases, the selectivity difference between the two phases increases. The following column comparisons provide information on which phases select for specific congeners and which columns may have the greatest potential for confirmation columns or GC×GC separations. While these visualizations allow for an overall comparison of selectivity, target compounds that coelute will need to be assessed by their retention indices as one column may separate target compounds better than others.

In general, the Rxi-17SilMS paired with any column displays the greatest selectivity difference. The Rxi-17SilMS plotted against the Rtx-1MS, as shown in Figure 1, gives the lowest R^2 value for any of the column sets. This trend is true for PCBs and PCNs; the Rxi-17SilMS phase offers the greatest selectivity difference when paired with the other eight columns. This suggests that the Rxi-17SilMS phase would make the best 2nd dimension GC×GC column for most PCB and PCN separations.

3.3.1. Column Selectivity PCBs

There are a number of publications that report retention order and relative retention indices for PCBs on a diverse range of stationary phases. Most notably, Frame et al. published full retention orders for all PCB congeners on 20 different stationary phases [25]. However, some of those phases are no longer in use, and formulations for others may have changed, such as the HT8 column, which was recently re-released by Trajan with a new formulation [45]. There is also the omission of a trifluoropropyl or “200” type column from Frame’s list, and to the best of the authors’ knowledge, this type of column has never been fully characterized with all 209 PCB congeners until this study. This study also provided the opportunity to characterize a relatively new column for persistent organic pollutant analysis: the Rtx-Dioxin2. As PCBs are often found and analyzed along with dioxins, a full characterization of this popular dioxin column is beneficial for future analyses. Full elution order tables for the Rtx-200 and Rtx-Dioxin2 can be found in the supplemental information.

As previously mentioned, it is possible to visualize the selectivity differences between columns by plotting the retention indices from one column against another. Figure 3 is the plot of Lee indices for the 12 dioxin-like PCBs on the Rxi-17SilMS and the Rtx-Dioxin2. The R^2 value for the line of best fit is 0.988, which suggests slightly different selectivity mechanisms. The selectivity plots shown in Figures 1–3 provide an adequate indication of which columns offer the greatest selectivity differences for PCBs and PCNs, but quantifying the differences between congeners is difficult. Figure 4 plots the congeners selectivity in one phase over the other, in this case, Rtx-Dioxin2 and Rxi-17SilMS, and quantifies this preference. The magnitude of the bar in Figure 4 is the distance from the line of best fit to the individual congener. The x -axis stationary phase, Rtx-Dioxin2, is more selective for the congeners under the line of best fit, and the y -axis phase, Rxi-17SilMS, is more selective for congeners above the line of best fit. The actual numerical value of the distance from the line of best fit to the congener has no relation to the retention index. However, the magnitude of the value relative to the other congeners shows which phase is more selective for specific congeners.

Figure 4 shows a distinct pattern that is repeated for all columns plotted with the Rxi-17SilMS, where four congeners show strong selectivity for the Rxi-17SilMS phase: PCB 105, PCB 114, PCB 156, and PCB 157. These four PCBs are always strongly retained in the Rxi-17SilMS phase, no matter which column the Rxi-17SilMS is compared to. It is interesting to note that these four PCBs are ortho-substituted, but the other ortho-substituted PCBs, PCB 189, PCB 167, PCB 123, and PCB 118, are never more retained in the Rxi-17SilMS phase over another column. While the exact mechanism for the selectivity difference between these compounds on the Rxi-17SilMS is unknown, this data provide insights into the selectivity of individual PCBs that can be used to optimize separations of difficult to resolve congeners. Similar patterns of congeners that prefer a single phase over all the other phases may also be observed when comparing other columns from this study that could aid in separating particular congener pairs.

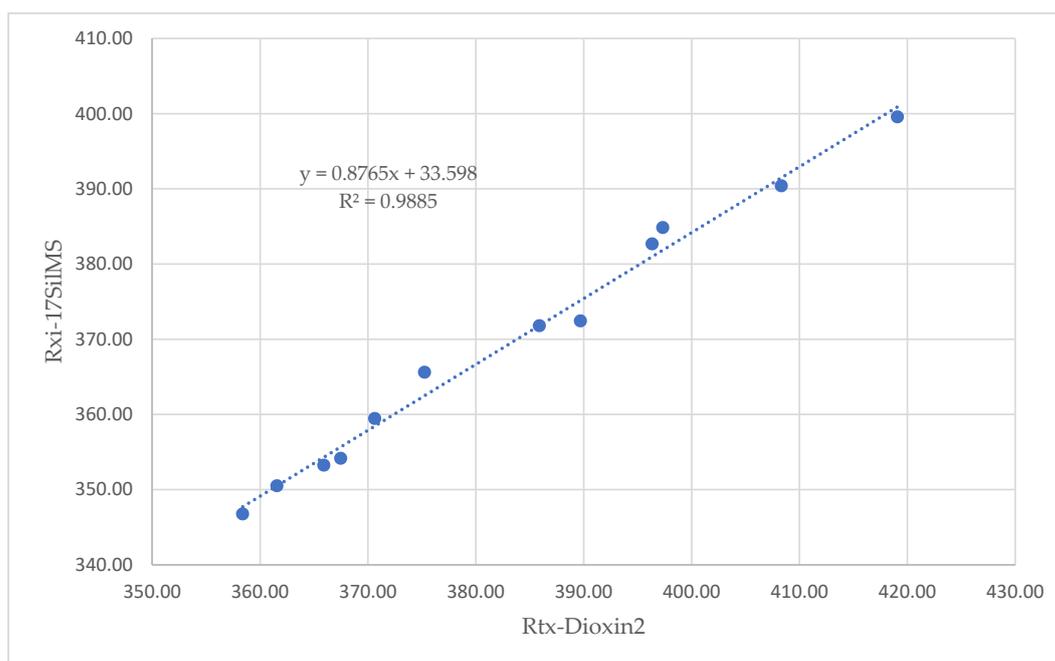


Figure 3. Lee Indices of Dioxin-Like PCBs on Rxi-17SiIMS vs. Rtx-Dioxin2. Figure 3. This is the plot of the selectivity difference for dioxin-like PCBs on the Rxi-17SiIMS and Rtx-Dioxin2. The line of best fit and R^2 value indicates the degree of selectivity difference in this column pairing. The greater the deviation from the line, the lower the R^2 value, and the greater the selectivity differences between the two columns. If a point falls below the line of best fit, the Rtx-Dioxin2 is more selective for that compound, and if a point lies above the line of best fit, the Rxi-17SiIMS is more selective for that compound.

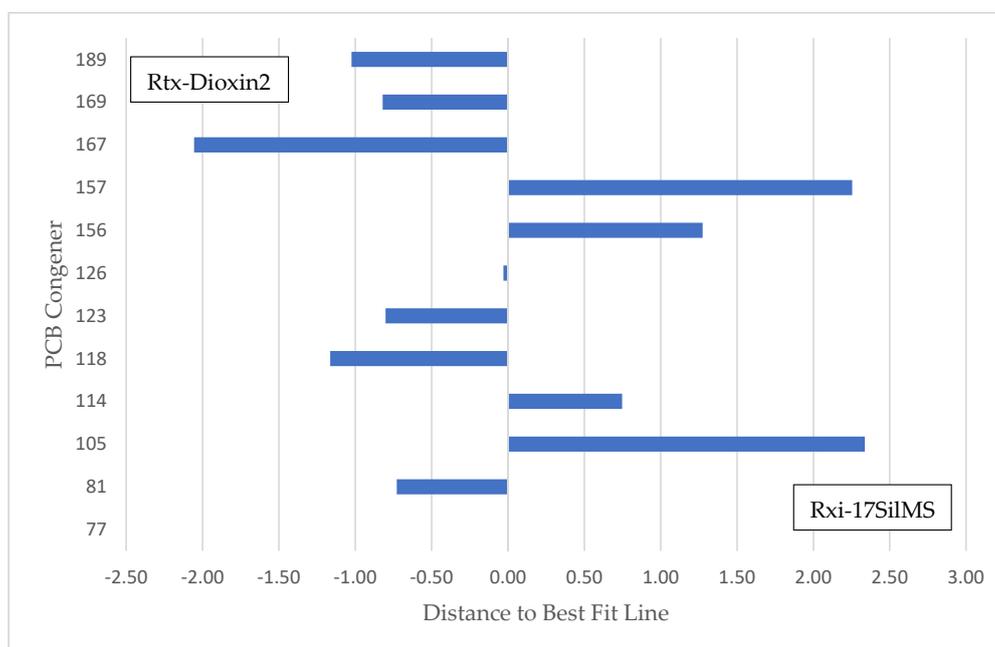


Figure 4. Distance to Line of Best Fit for Rxi-17SiIMS vs. Rtx-Dioxin2: Dioxin-Like PCBs. This is the plot of the distance from the best fit line for each dioxin-like PCB from Figure 3. If the point in Figure 3 lies above the line of best fit, the Rxi-17SiIMS is the more selective phase for that compound, which produces a positive value on the chart. If the point lies below the line of best fit, then the Rtx-Dioxin2 is the more selective phase, which produces a negative value on the chart. The magnitude of the bar indicates how selective the phase is for that compound over the other phase.

3.3.2. Column Selectivity PCNs

Column selectivity for PCNs is much less variable than PCBs. The lowest R^2 value achieved for any of the column pairings was 0.992 for the Rxi-17SilMS vs. the Rtx-1MS. As seen in the comparison of Lee indices in Figure 5, PCNs almost fall exactly on the line of best fit. This trend is seen for every column comparison. It is difficult to effectively separate individual PCN congeners on any given column, however using comparisons like those in Figure 6, it is possible to identify which phases could be used as confirmation columns or for GC×GC separations. For example, no single phase shows the same selectivity for all hexa-substituted congeners. There are four congeners, PCN 72, PCN 71, PCN 65, and PCN 63, that display greater selectivity for the Rxi-17SilMS phase over all other phases. However, the five other hexa-substituted congeners are more selective in the other phase over the Rxi-17SilMS. The only deviation in this pattern is observed when comparing the Rxi-17SilMS and the Rtx-200 for PCN 70. The Rxi-17SilMS is always more selective for PCN 70 except when compared to the Rtx-200, which shows greater selectivity for the particular congener.

Because of the lack of variability in column selectivity for PCN congeners, the retention indices collected are useful for identifying individual compounds on any column characterized in this study. This data will also help in choosing a confirmation column or 2nd dimension GC×GC column for the analysis of PCNs, which will help mitigate time intensive method development.

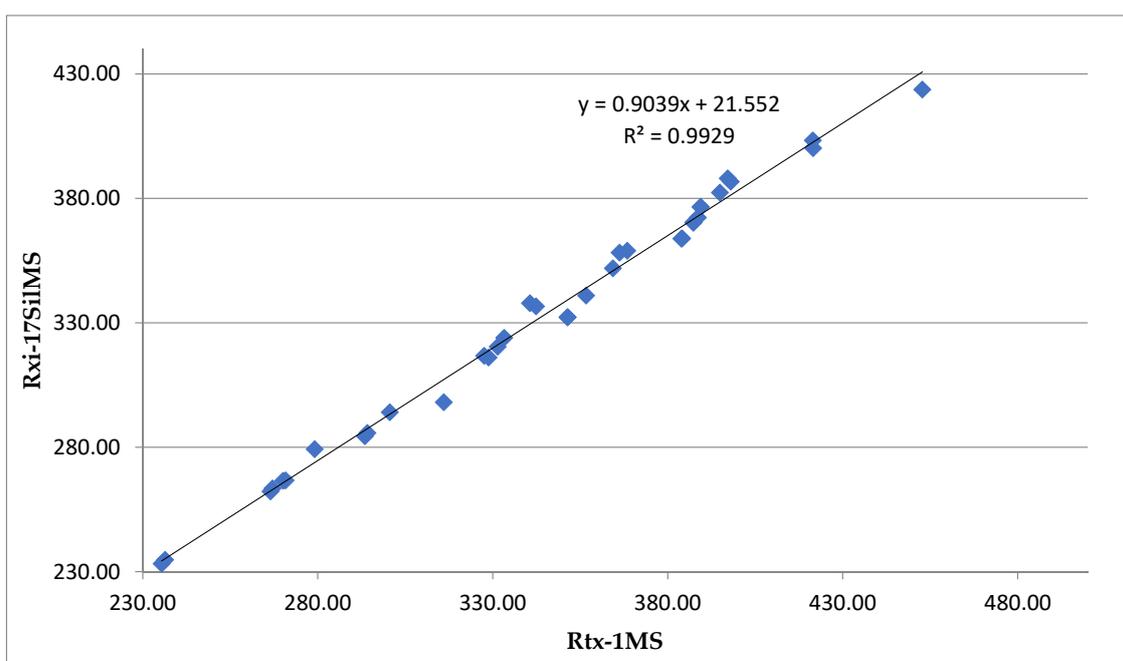


Figure 5. Lee indices of 35 PCNs on Rxi-17SilMS vs. Rtx-1MS. This is the plot of 36 PCNs on the Rxi-17SilMS vs. the Rtx-1MS. The line of best fit and R^2 value indicates the degree of selectivity difference for these two columns. Of the nine columns compared, this pairing showed the greatest deviation from the line of best fit, which gave the lowest R^2 value. The lower value indicates that these columns show the greatest difference in selectivity for PCN congeners.

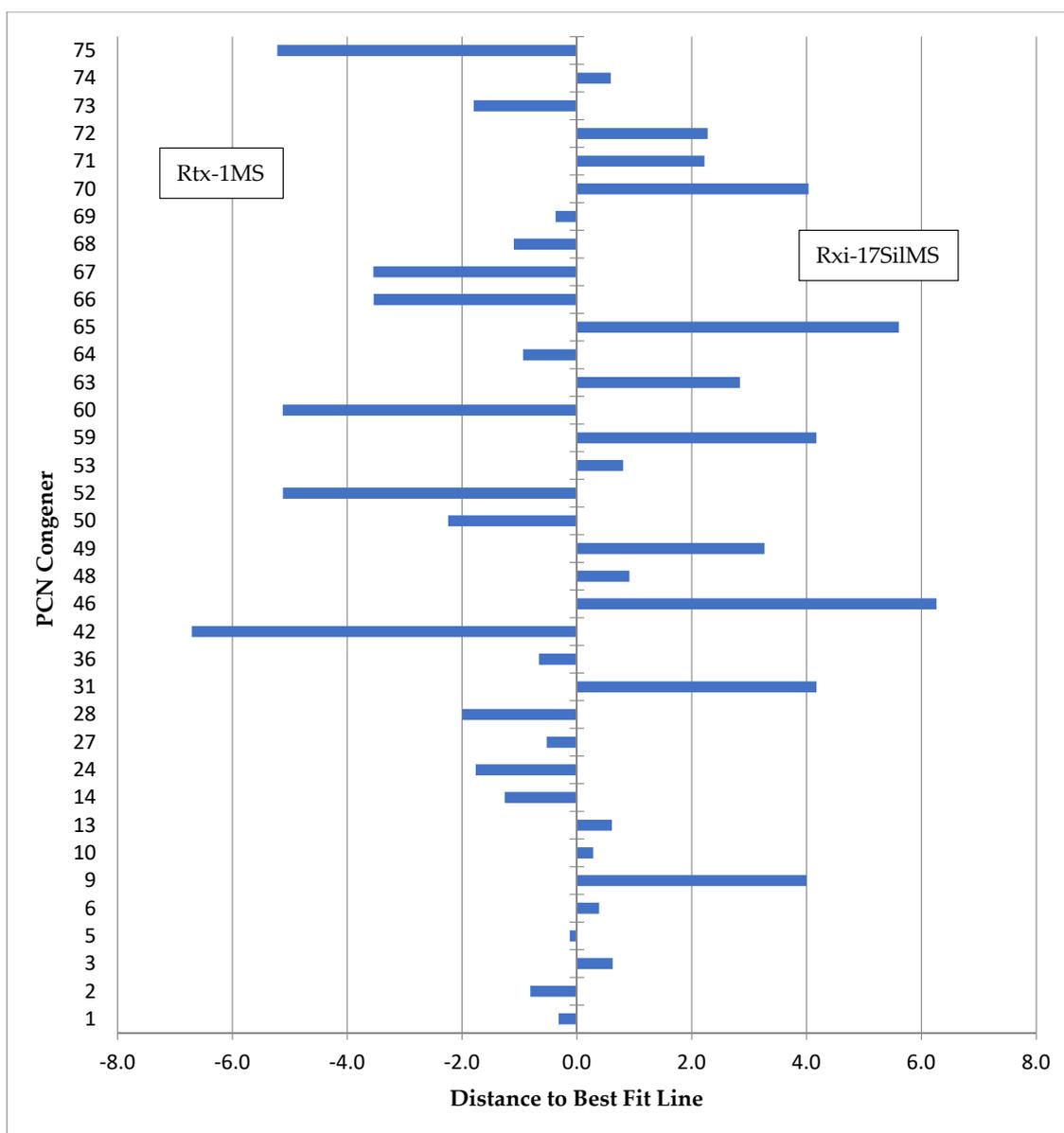


Figure 6. Distance to Line of Best Fit for Rxi-17SiIMS vs. Rtx-1MS: PCNs. This is the distance for each PCN congener from the line of best fit from Figure 5. This same pattern can be seen for every column compared to the Rxi-17SiIMS with only minor deviations, which indicates that PCN congeners are chemically similar. If the point in Figure 5 falls above the line of best fit, then the Rxi-17SiIMS is the more selective phase for that compound, which produces a positive value on the chart. If the point in Figure 5 falls below the line of best fit, the Rtx-1MS is the more selective phase, which produces a negative value on the chart. The magnitude of the bar indicates how selective the phase is for that compound over the other phase.

4. Conclusions

This study provides an alternate means of identification for PCB and PCN congeners using linear and Lee retention indices. Since individual congeners were profiled instead of Aroclor or Halowax mixtures, this retention index system can offer a greater degree of confidence in identifying specific congeners and their elution orders. The use of relative retention times with labeled standards of the target compounds was avoided, and this method is intended to be used with a mass spectrometer instead of an ECD. Finally, this work characterized nine current GC stationary phases for PCB and PCN analysis that offers a simple and updated database of retention indices with tabulated coelutions

for each column characterized. The comparisons made between columns and comparisons of column selectivity can help inform column choice for confirmation analyses and GC×GC separations.

Supplementary Materials: The following are available online at <http://www.mdpi.com/2297-8739/7/3/38/s1>, All selectivity graphs for all PCBs, and PCNs on all nine columns used in this study as well as full elution tables for all compounds on each column.

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