Evaluation of the separation capacity of different GC columns for tetra- to octachlorinated PCDD/Fs

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Abstract

Six GC columns: Rt-LC50, Rt-βDEXcst, DB-XLB, SLB-IL61, SLB-IL76, SLB-IL111 were evaluated for their ability to separate all of the 136 tetra- to octachlorinated dibenzo-*p*-dioxins (PCDDs) and dibenzofurans (PCDFs) using gas chromatography- high resolution mass spectrometry (GC-HRMS). The relative performances of those columns were evaluated based on retention time (RT) data and visualized mass chromatograms of the overall separation of the 136 congeners as well as the separation of the 17 most toxic 2,3,7,8-substituted congeners. The results were also compared with those of previous studies. Among the six investigated columns, Supelco SLB-IL61 showed the best separations of 2,3,7,8-congeners and separated 14 congeners, partially separated 1,2,3,7,8-PeCDF and 1,2,3,4,6,7,8-HpCDF, and failed to resolve 1,2,3,4,7,8-HxCDF. It can also completely separated maximum 83 (61%) of the 136 congeners. Thus, it offers better tetra- to octa- CDD/Fs as well as 2,3,7,8-PCDD/Fs separation as compared to other columns evaluated so far. Moreover, this column can be used as a complementary column with any of the following 8 columns: DB-XLB, Rtx-Dioxin2, ZB-5Ums, DB-5ms, ZB-5ms, VF-5ms, CP-Sil 8 CB/MS and VF-Xms, allowing complete separation of all 2,3,7,8-congeners. SLB-IL111 column also has this capability together with a DB-5, HP-5ms, Rtx-5ms, Equity-5, DB-5ms, ZB-5ms, VF-5ms, CP-Sil 8CB/MS or VF-Xm. Finally, any of the three ionic liquid (IL) columns can be used together with a DB-5ms, ZB-5ms, VF-5ms, CP-sil8 CB/MS or VF-Xms column for the same purpose. Separate injections on SLB-IL61 and SLB-IL111 columns can resolve an even more impressive number of tetra- to octa-CDD/F congeners (complete separation of 107 congeners and partial separation of 19 congeners). These columns completely separated all tetrachlorinated dibenzofurans (TeCDFs) except 6 congeners, among which 5 congeners (2,4,6,8-, 1,4,7,8-, 1,2,3,6-, 1,2,4,6- and 1,2,3,4-TeCDF) were separated partially and 1,2,6,9-TeCDF was not separated. Using additional columns, VF-Xms and Dioxin2, four more (two by each column) congeners can also be resolved. Similarly, selected IL columns separated 8 pentachlorinated dibenzofurans (PeCDFs)(1,2,4,7,8-, 1,4,6,7,8-, 1,2,4,7,9-, 1,3,4,6,9-, 1,2,4,6,9-, 1,2,3,4,7-1,2,3,7,8-, and 1,2,3,7,9-PeCDF) partially and failed to separate 2 congeners (1,2,3,6,7-and 1,2,6,7,8-PeCDF). Four of these partially separated and unresolved congeners can be separated on SP-2331. In addition, the SLB-IL columns can also separate all hexachlorinated dibenzofurans (HxCDFs) except 1,2,4,6,8,9-HxCDF (partially separated) which can be resolved by a great number of other columns (incl. Rt-LC50, Rt-βDEXcst, and DB-XLB). Furthermore, the column combination cannot fully separate 6 congeners out of all tetrachlorinated dibenzo-*p*-dioxins (TeCDDs). Among those 6 congeners, four congeners (1,3,6,9-, 1,2,4,7-, 1,2,3,6-, and 1,2,3,4-TeCDD) were partially separated while 2 congeners (1,2,4,6- and 1,2,4,9-TeCDD) were not resolved at all. However, 1,3,6,9-, 1,2,4,9-, and 1,2,3,4- TeCDD can be separated by a LC-50 and 1,2,3,6-TeCDD by a Dioxin2 column. All PeCDDs except 1,2,4,6,7-PeCDD (partially separated) were separated by the SLB-IL columns. This congener can be completely resolved using the Dioxin2 column. All HxCDDs can be completely resolved by the selected SLB-IL columns together with the DB-1 column, which are needed for 1,2,4,6,7,9-, 1,2,4,6,8,9-, 1,2,3,4,6,8-, 1,2,3,6,7,9-, 1,2,3,6,8,9-HxCDD. Principal component analysis (PCA) was applied using the retention order of the PCDD/Fs. This analysis revealed that the positions of the chlorine substituents on the aromatic rings had a large impact on the retention according to polar behavior of columns. The study found, Chlorine substitution in the 4,6-positions of PCDFs and 4,6- or 1,9-positions of PCDDs were correlated with retention on polar or extremely polar columns.

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Introduction

Polychlorinated dibenzo-*p*-dioxins (PCDDs) and dibenzofurans (PCDFs) have common biological mechanism and similar toxic effects, and belong to the class of highly hazardous pollutants, commonly called dioxins (PCDD/Fs).[1-2] They have related planar tricyclic aromatic hydrocarbon structures where two benzene rings are connected through two oxygen bridges in PCDD, and one oxygen and one carbon bridge in PCDF (fig 1).[3-4] PCDD/Fs are formed as unwanted byproducts either in natural or anthropogenic processes. Waste combustion, automobile emissions, medical incineration, industrial combustion processes, pulp industry using chlorine and power generation (coal burning) are identified as major anthropogenic sources, whereas, forest fires is an example of a natural source.[5-7] Since the beginning of the twentieth century, increasing PCDD/F emissions was observed from industrial processes, such as the chloro alkali process and high volume production of organochlorines;[8] but peak environmental concentrations dioxins were observed in the environment about 2 decades ago.[9]

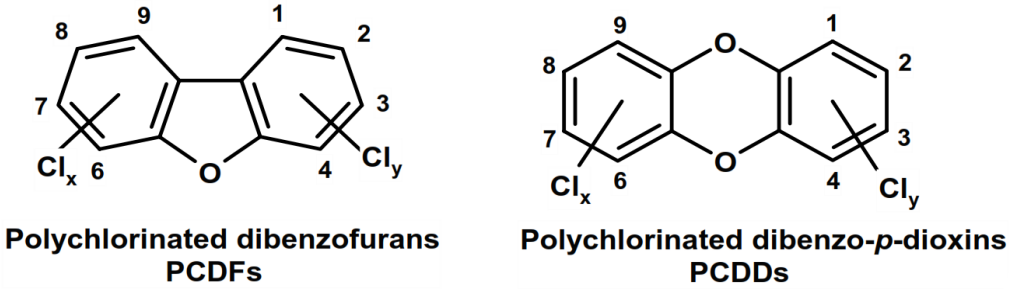


Fig 1: Molecular structure of PCDFs and PCDDs

Since Dioxins are emitted from incineration, their low vapor pressure causes them to associate with particles that can transport them to long distance around the atmosphere and distributed to all compartments of environment.[2] Dioxins are very persistent pollutants. Because of their lipophilic character, they show a high tendency to bio-accumulate in fatty tissues in living organisms. As a consequence, they can enter the food webs and accumulate in top predators, which is a concern for human populations.[3,10] As 90% of the human exposure to dioxins is originating from eating contaminated food, contamination monitoring (analysis) is important to assess the risk.[11]

In PCDD/Fs, there are eight positions which can be substituted by chlorine atoms (Cl). These positions and the degree of chlorination are the basis of the names of the congeners. There are 75 congeners of PCDD and 135 congeners of PCDF (mono to octa) (table 1).[3]

Table 1: Number of PCDD and PCDF congeners according to their homologue[3]

Number of Cl atom	Name	Acronym PCDD/F	Number of PCCD isomers	Number of PCDF isomers
1	Mono	MCDD/F	2	4
2	Di	DCDD/F	10	16
3	Tri	TrCDD/F	14	28
4	Tetra	TeCDD/F	22	38
5	Penta	PeCDD/F	14	28
6	Hexa	HeCDD/F	10	16
7	Hepta	HpCDD/F	2	4
8	Octa	OCDD/F	1	1
Total			75	135

The toxicity of dioxins depends on their ability to bind to the Ah receptor present in the cytoplasm of the cell, which are transported into the cell nucleus and bind to the DNA. As a consequence, they interrupt cell activities; induce cell proliferation and differentiation in many tissues which causes carcinogenicity, reproductive and developmental abnormalities, liver damage, endocrine system disruptions and even death.[12-13] The degree of toxicity of dioxins varies on the number and position of chlorine substituents. Only 7 out of 75 PCDD isomers and 10 out of 135 PCDF isomers exhibits strong toxic effects and have been assigned toxic equivalent factors (TEFs).[3] These 17 congeners are all Cl substituted in the 2, 3, 7, 8-positions. For this reason, dioxins containing four to eight substituted chlorine atoms: 49 PCCDs and 97 PCDDFs (136 congeners) are the most important to separate during chemical analysis.[4-6]

Moreover, EPA health assessment has found some health effects close to average human background body burden level of dioxins;[2] Due to acute toxicity and availability in a low level, less than parts per trillion in the matrix of biological or environmental sample, isomer specific and highly selective and sensitive analytical methods are needed to detect ultra-trace level contamination.[5,14] Consequently, scientists have developed several sensitive analytical methods to detect them. Gas chromatography high-resolution mass spectrometry (GC-HRMS) is the most

common analytical method. In this instrument, HRMS is separating the different homologues and discriminate against non-dioxin background; whereas GC provides the required isomer separation.[7] GC-HRMS can detect very low level (parts per billion) contamination with good accuracy.[15]

Over the last decades, many attempts have been done to improve the GC separation with the ultimate goal to separate all 136 tetra-octa congeners.[15,16] However, no single column has so far even been able to provide complete separation of the seventeen of 2,3,7,8- substituted PCDD/Fs.[17] Therefore, the US EPA suggests to use DB-225 or SP-2330 column as a complementary column to the DB-5 GC column. Moreover, Environment of Canada proposes DB-5 and DB-Dioxin as the complementary column.[18] For this reason, scientists have been trying to resolve all these 17 congeners using one GC column for a long time, and some of them stand out. Ryan et al. have reported the retention profiles of the 136 tetra- to octa-CDD/Fs on nine GC columns, i.e. DB-1, DB-5, DB-17, DB-210, DB-225, CPS-1, SP-2331, CP-Sil 88, SB-Smectic.[7] In recent years, Fishman's research group has published a series of articles, where the separation of the 2,3,7,8-substituted PCDD/Fs were reported for the DB-5, HP-5MS, RTx-5ms, Equity-5, DB-5ms, ZB-5UMS, CP-Sil 8 CB/MS, Rtx-Dioxin2, DB-XLB, DB-225, SP-2331, VF-Xms, and VF-5ms columns. Full congener profiles were also provided for the 136 tetra- to octa-CDD/Fs on the VF-Xms, VF-5ms, DB-5ms, ZB-5ms, Equity-5 and DB-5 columns.[6,9,17] Beside this, two separate studies have reported full congener profile using the Dioxin2 and BPX-DXN columns.[19-20]

In this study, six new GC columns, i.e. Rt-LC50, Rt-βDEXcst, DB-XLB, SLB-IL61, SLB-IL76, and SLB-IL111, were evaluated for their ability to separate the 136 tetra- to octa-CDD/Fs. These have different stationary phase characteristics, and never been reported to use separating the 136 congeners. Several standard mixtures, each containing different PCDD/F homologues, and a Super-Mix with all 136 congeners, were used to obtain unambiguous data and illustrative congener profiles. One column (VF-Xms) was additionally employed for method validation purposes. The separations obtained were compared across the six evaluated GC columns, but also with the aforementioned literature data to find the best column and column combinations. Multivariate data analysis was also employed on the data (based on retention order; RO) to study how the Cl substitution patterns affected the separation on the various columns.

The goal of this project was to find out if a single column can separate all 2,3,7,8-PCDD/F, and to find the best complementary column if the single column approach failed. We also aimed to determine the maximum number of tetra- to octa-CDD/Fs that can be separated using a single column and to figure out if a combination of two or three columns can completely separate all 136 tetra- to octa-CDD/F congeners. Finally, an attempt was done to explain the retention behavior on the various columns was made using multivariate statistics.

Experimental

Standard preparation

Individual standard solutions of the 136 tetra- to octa-CDD/F congeners were obtained from AccuStandard and were used to prepare 38 standard mixtures (in nonane); each individual mixture contains a maximum of one congener of each homologue according to the table 2. The approximate concentration of each congener was 25 ng/mL. Another mix was prepared in the same solvent, which contains all congeners, called Super mix (mix-46). This mixture was concentrated 4 to 5 times and was used to determine the absolute retention time of all congeners. Before the analysis, a solution with ^{13}C-labeled 2,3,7,8-PCDD/Fs was added (in nonane) to each of the 38 mixtures and to the Super mix. The prepared standard was stored in refrigerator when not used.

HRGC-HRMS measurement

All PCDD/F analyses were performed by GC-HRMS using a HP 6890 gas chromatograph (Agilent 6890 Series GC) coupled with a high-resolution mass spectrometer (Waters Micromass Autospec-Ultima). Electron ionization (EI) was used in the positive mode at an electron energy of ca 34 eV and an ionization source temperature of 250°C. Data was collected in SIM mode and the resolution (5%) was about 10000. 2μL of each sample was injected in to the GC inlet system (splitless mode) through an autosampler (CTC / LEAP GC PAL Autosampler). Helium was used as the carrier gas. The GC operation conditions followed the main separation criteria of US EPA method 1613b. Individual optimum temperature programs were developed for the six fused silica columns: Rt-LC50, Rt-βDEXcst, DB-XLB, SLB-IL61, SLB-IL76, and SLB-IL111 to obtain maximum separations of all 136 tetra- to octa-CDD/F congeners.

One extra column: VF-Xms was tested for method validation using the temperature program from Fishman et al.[17]

Table 2: Constituent of individual mixtures and Super mix

	TeCDF	PeCDF	HxCDF	HpCDF	OCDF	TeCDD	PeCDD	HxCDD	HpCDD	OCDD	No of Congeners	Conc per solute (pg/ul)
Mix 1	F01	F39	F67	F83	F87	D01	D23	D37	D47	D49	10	2.5
Mix 2	F02	F40	F68	F84		D02	D24	D38	D48		8	2.5
Mix 3	F03	F41	F69	F85		D03	D25	D39			7	2.5
Mix 4	F04	F42	F70	F86		D04	D26	D40			7	2.5
Mix 5	F05	F43	F71			D05	D27	D41			6	2.5
Mix 6	F06	F44	F72			D06	D28	D42			6	2.5
Mix 7	F07	F45	F73			D07	D29	D43			6	2.5
Mix 8	F08	F46	F74			D08	D30	D44			6	2.5
Mix 9	F09	F47	F75			D09	D31	D45			6	2.5
Mix 10	F10	F48	F76			D10	D32	D46			6	2.5
Mix 11	F11	F49	F77			D11	D33				5	2.5
Mix 12	F12	F50	F78			D12	D34				5	2.5
Mix 13	F13	F51	F79			D13	D35				5	2.5
Mix 14	F14	F52	F80			D14	D36				5	2.5
Mix 15	F15	F53	F81			D15					4	2.5
Mix 16	F16	F54	F82			D16					4	2.5
Mix 17	F17	F55				D17					3	2.5
Mix 18	F18	F56				D18					3	2.5
Mix 19	F19	F57				D19					3	2.5
Mix 20	F20	F58				D20					3	2.5
Mix 21	F21	F59				D21					3	2.5
Mix 22	F22	F60				D22					3	2.5
Mix 23	F23	F61									3	2.5
Mix 24	F24	F62									2	2.5
Mix 25	F25	F63									2	2.5
Mix 26	F26	F64									2	2.5
Mix 27	F27	F65									2	2.5
Mix 28	F28	F66									2	2.5
Mix 29	F29										1	2.5
Mix 30	F30										1	2.5
Mix 31	F31										1	2.5
Mix 32	F32										1	2.5
Mix 33	F33										1	2.5
Mix 34	F34										1	2.5
Mix 35	F35										1	2.5
Mix 36	F36										1	2.5
Mix 37	F37										1	2.5
Mix 38	F38										1	2.5
Mix 46	All TeCDF	All PeCDF	All HxCDF	All HpCDF	OCDF	All TeCDD	All PeCDD	All HxCDD	All HpCDD	OCDD	136	**0.183824**

Information on the individual GC columns and their operation conditions are given in Table 3. The experimental work and data evaluation is summarized in a flow chart (fig 2).

Table 3: Information of GC columns and their Operation condition

Column	Manufacturer	Stationary Phase & Type	Polarity	Column size (m × mm × µm)	Temp program	Mode
LC-50	Restek	Dimethyl (50% liquid crystal), wall coated	Polar	20 × 0.25 ×0.10	120˚C (2min) to 190˚C (30˚C/min) to 260˚C (3˚C/min, 12min)	Constant flow, 1mL/min
β-DEXcst	Restek	14%Cyanoprpoylphenyl/ 86% dimethyl, wall coated	Polar	30 × 0.25 ×0.25	120˚C (2min) to 223˚C (30˚C/min) to 240˚C (1˚C/min, 58min)	constant flow, 1mL/min
DB-XLB	J & W Science	Proprietary, non-bonded	Apolar	60 × 0.18 ×0.18	160˚C (1min) to 230˚C (35˚C/min,15 min) to 260˚C (10˚C/min, 25min) to 310˚C (10˚C/min, 14min)	Constant pressure, 480 Kpa
SLB-IL111	Supelco	Ionic Liquid, non-bonded	Extremely polar	100 × 0.25 ×0.20	120˚C (2min) to 190˚C (30˚C/min, 30 min) to 240˚C (5˚C/min, 15min) to 260˚C (5˚C/min, 20min)	constant flow, 1mL/min
SLB-IL76	Supelco	Ionic Liquid, non-bonded	Highly polar	30 × 0.25 ×0.20	120˚C (2min) to 150˚C (30˚C/min, 10 min) to 190˚C (5˚C/min, 15min) to 260˚C (5˚C/min, 5min)	constant flow, 1mL/min
SLB-IL61	Supelco	Ionic Liquid, non-bonded	Polar	20 × 0.25 ×0.10	120˚C (2min) to 150˚C (30˚C/min, 10 min) to 190˚C (5˚C/min, 15min) to 280˚C (5˚C/min, 5min)	constant flow, 1mL/min

Data analysis and evaluation

During data analysis, the identity of every peak was confirmed using ion ratios of two monitored ions for each homologue. Relative retention times (RRTs) for the individual mixtures and the Super mix were calculated using the retention time (RT) of each congener and the RT of the corresponding ^{13}C-labeled PCDD/F. The RRTs of the individual mixtures were applied to avoid an RT shifting effect to identify the right congeners in the Super mix.

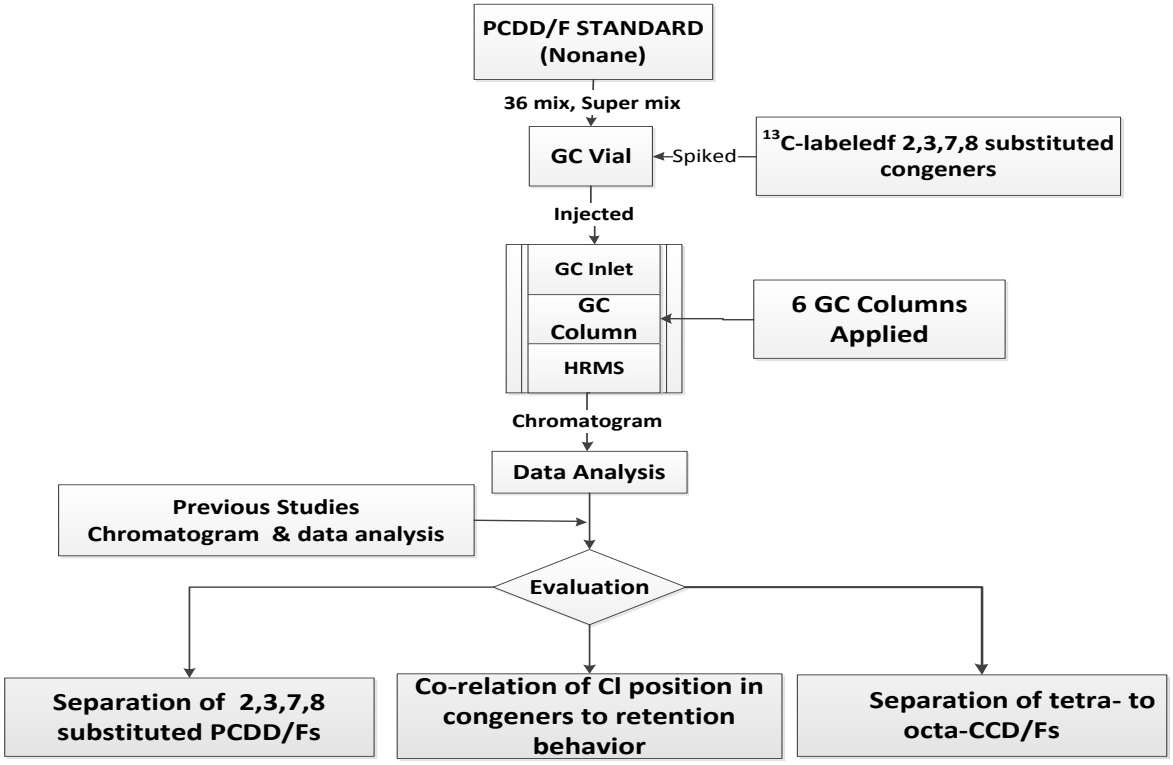


Fig 2: Experimental Flow chart

In the acquired chromatogram, the individual congener separations were evaluated by classifying the peaks into four categories based on the height of the peak valley. The first one was 'Complete separation' (++) where the peak valley was less than 10% above the baseline. The second and third are 'Partial separation' (+-) and 'Poor separation' (+--), where the peak valley were at 10-50% and 50-90%, respectively; and the last one was 'No separation' (--). The acquired data were compared with all previous studies of PCDD/Fs to evaluate the relative performance of the different columns for separating the seventeen 2,3,7,8-PCDD/Fs and the 136 tetra- to octa-CDD/Fs, respectively. The RRT data was also transformed to retention order data (ROs) and evaluated using principal component analysis (PCA) to find associations between the Cl substituent positions and the retention on the different GC columns. In this approach, two plots were obtained. The score plot illustrated the relationships between the columns (observations) while loading plot shows the contributions of the various congeners (variables) to the distribution of the columns on the score plot.

Result and discussion

Different isomer resolution and retention order was obtained on every GC column. It was also found that the ^{13}C-labeled reference standards eluted slightly faster than the corresponding unlabeled compound, which is in agreement with previous findings.[9] Some other observations were also noted during the runs (Table 4). Tailing peaks were observed for the LC-50 column, probably due to poor column deactivation. Retention time shifts were observed for β-DEXcst, DB-XLB, SLB-IL111. This might be the results of inlet leakage or loss of stationary phase (likely the reason for the decreasing retention times on the β-DEXcst and SLB-IL111 columns). Extra peaks (low intense) were observed using SLB-IL76 and SLB-IL61 indicating analyte degradation. As the main aim of this study was PCDD/Fs separation, not quantification, the data was still useful. Moreover, VF-Xms chromatograms were similar to those in the reference article, which shows the cited separations are repeatable.

Table 4: Observations for different GC column during run

Column Name	Bleed	RT Shifting	Tailing or Fronting	Degradation
LC-50	Low	No	Yes, Tailing	No
β-DEXcst	Low	Yes, Decreasing	No	No
DB-XLB	Low	Yes, Random	No	No
SLB-IL111	High	Yes, Decreasing	No	No
SLB-IL76	Medium	No	No	Degradation
SLB-IL61	Low	No	No	Low degradation

Separation of 2,3,7,8 substituted PCDD/Fs

All applied columns showed some potential to separate 2,3,7,8-congeners (fig 3 and Table 5). Among them, SLB-IL61 provided (included partial separation) separation of all congeners except 1,2,3,4,7,8-HxCDF; where 1,2,3,7,8-PeCDF was partially (+-) and 1,2,3,4,6,7,8-HpCDF was poorly (+--) separated. The two other ionic liquid columns, SLB-IL111 and SLB-IL76, performed slightly worse with 14 and 13 (partially) separated congeners, respectively. SLB-IL111 failed to resolve 2,3,7,8-TeCDD, 1,2,3,7,8-PeCDD, and 1,2,3,7,8-PeCDF, and provided poor resolution for 1,2,3,6,7,8-HxCDF. Similarly, 1,2,3,7,8-PeCDD, 2,3,7,8-TeCDF, 1,2,3,7,8-PeCDF, and 1,2,3,4,7,8-HxCDF were not resolved and 1,2,3,4,6,7,8-HpCDF was poorly resolved by SLB-IL61.

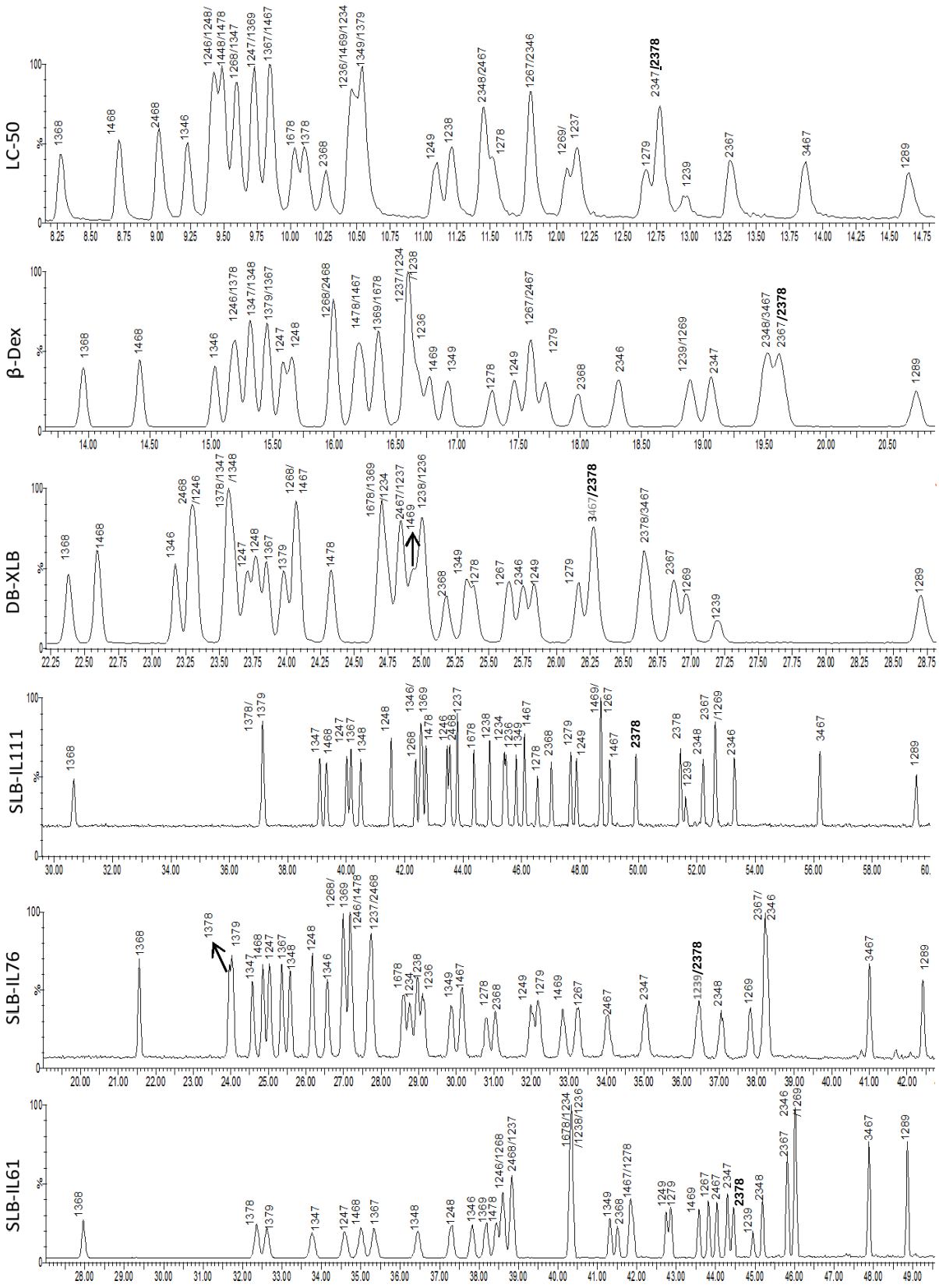


Fig 3.1: Chromatograms of TeCDFs from LC-50, β-Dex, DB-XLB, SLB-IL111, SLB-IL76, SLB-IL61 columns

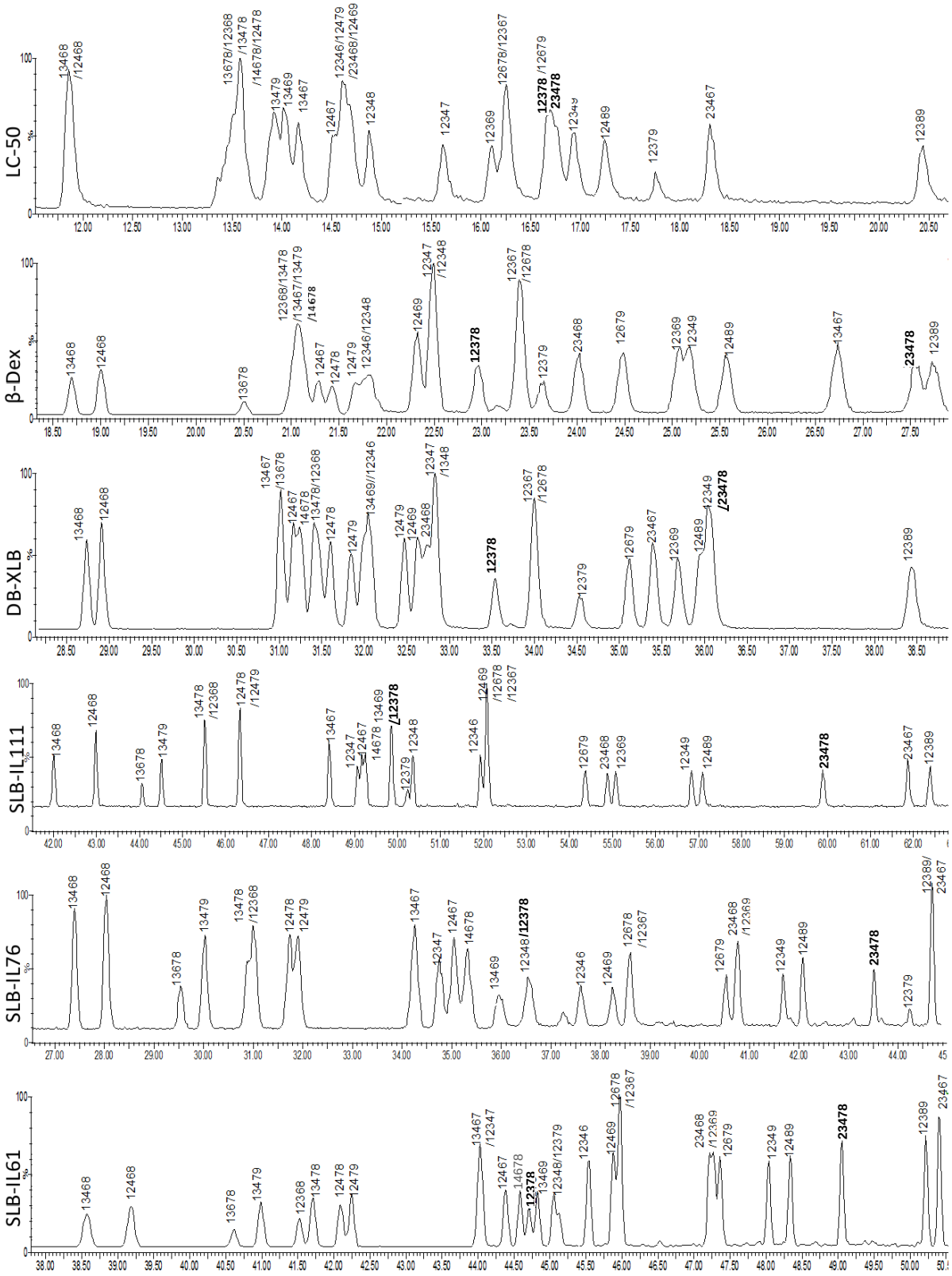


Fig 3.2: Chromatograms of PeCDFs from LC-50, β-Dex, DB-XLB, SLB-IL111, SLB-IL76, SLB-IL61 columns

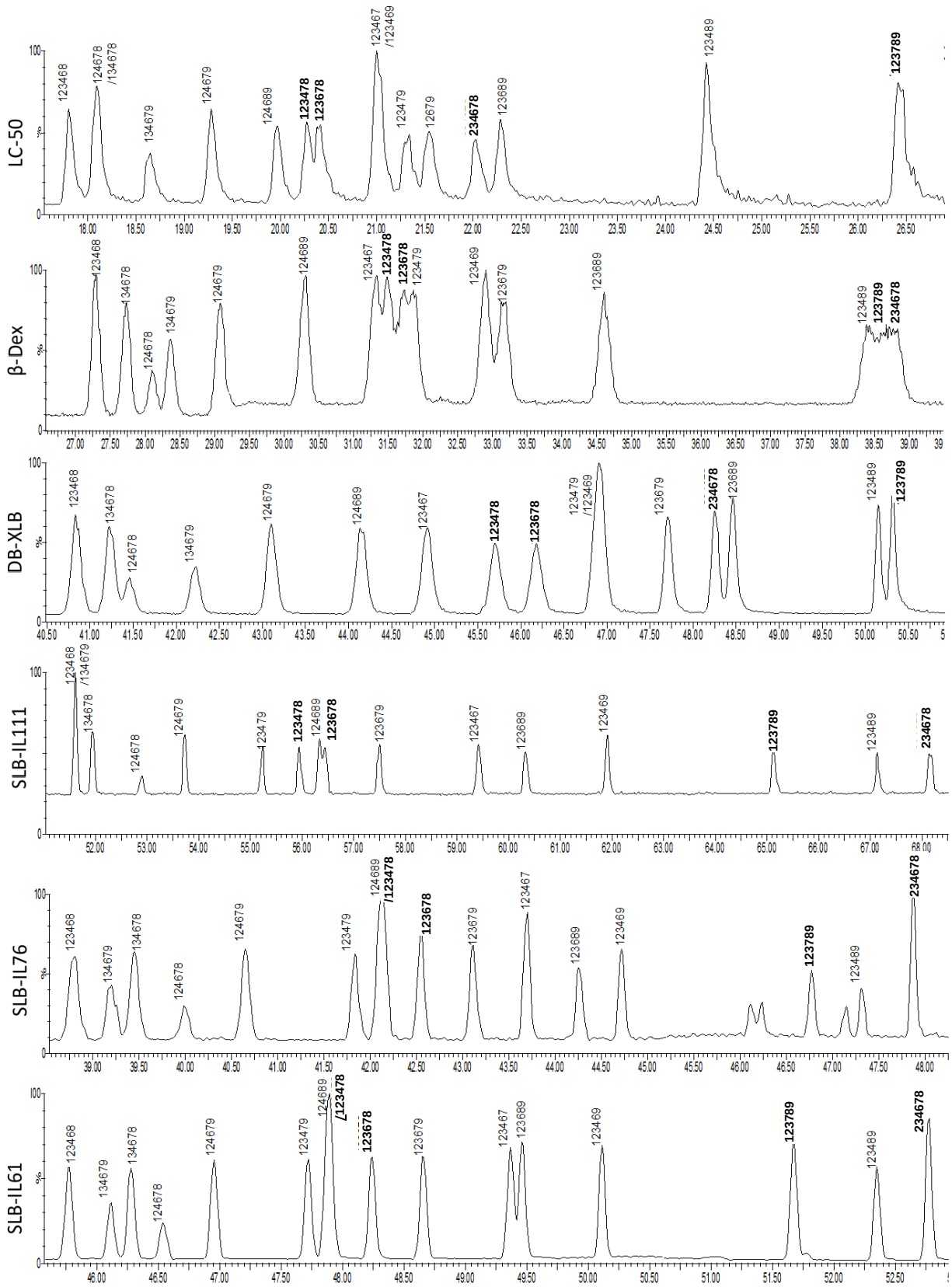


Fig 3.3: Chromatograms of HxCDFs from LC-50, β-Dex, DB-XLB, SLB-IL111, SLB-IL76, SLB-IL61 columns

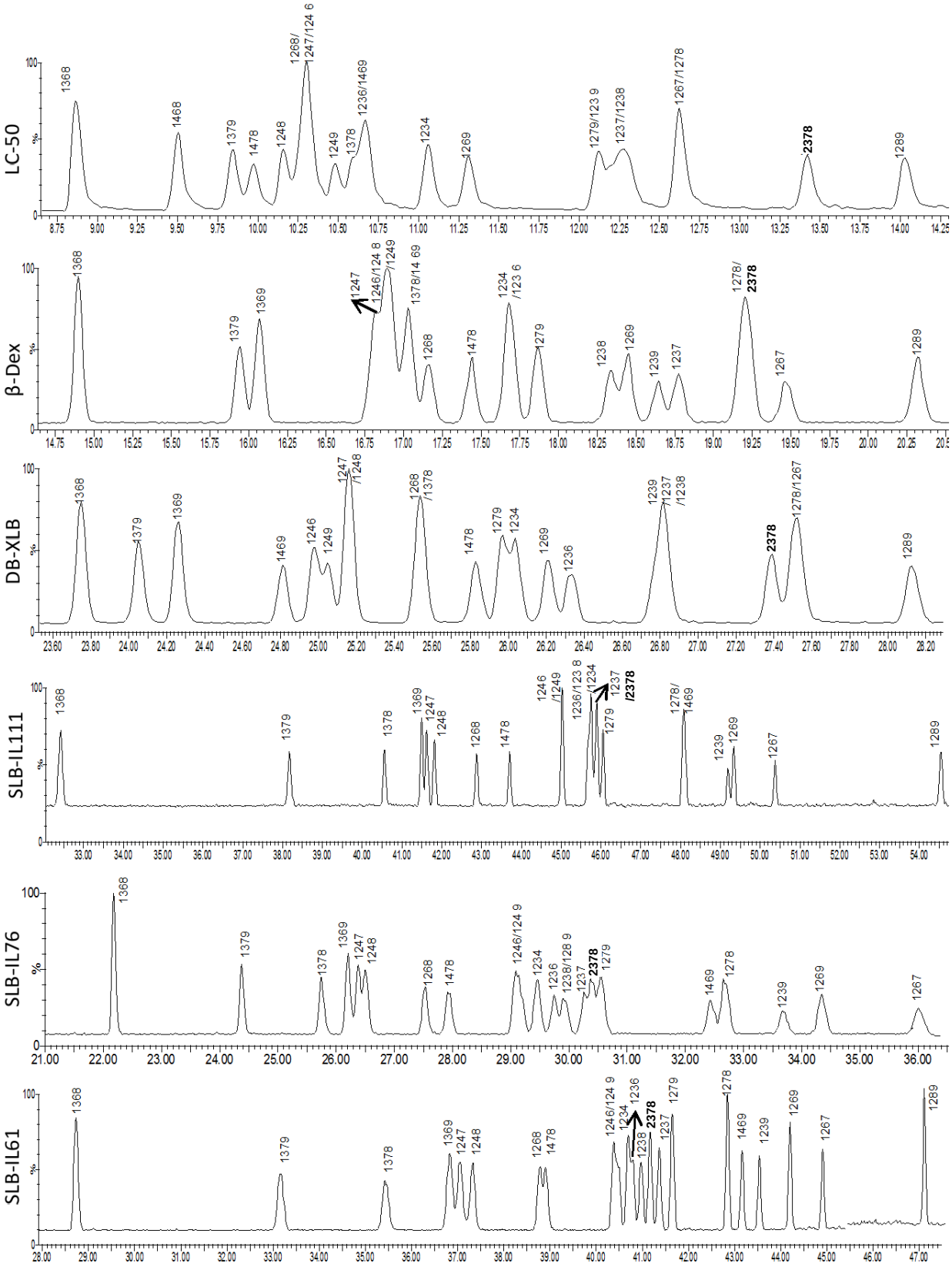


Fig 3.4: Chromatograms of TeCDDs from LC-50, β-Dex, DB-XLB, SLB-IL111, SLB-IL76, SLB-IL61 columns

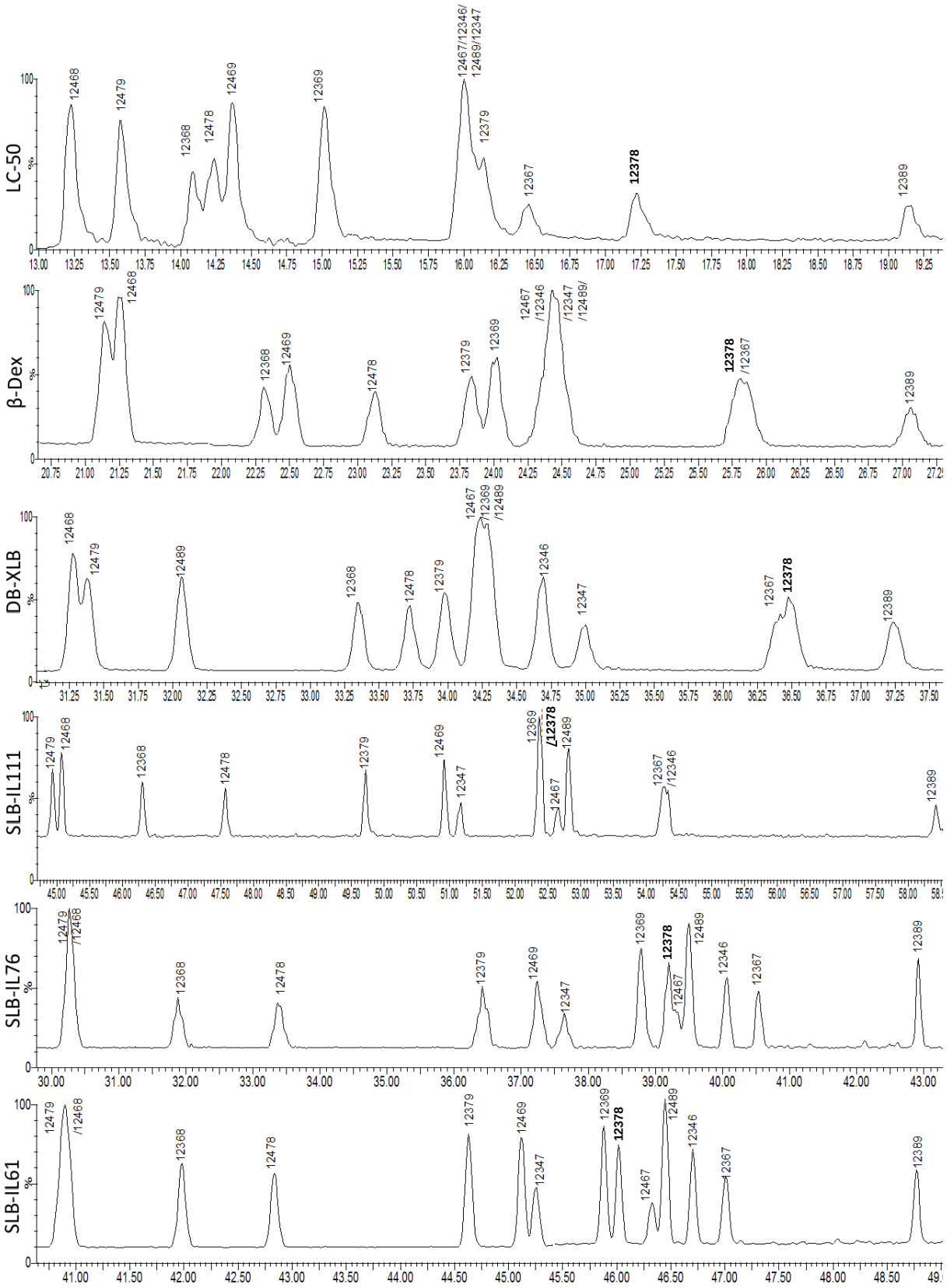


Fig 3.5: Chromatograms of PeCDDs from LC-50, β-Dex, DB-XLB, SLB-IL111, SLB-IL76, SLB-IL61 columns

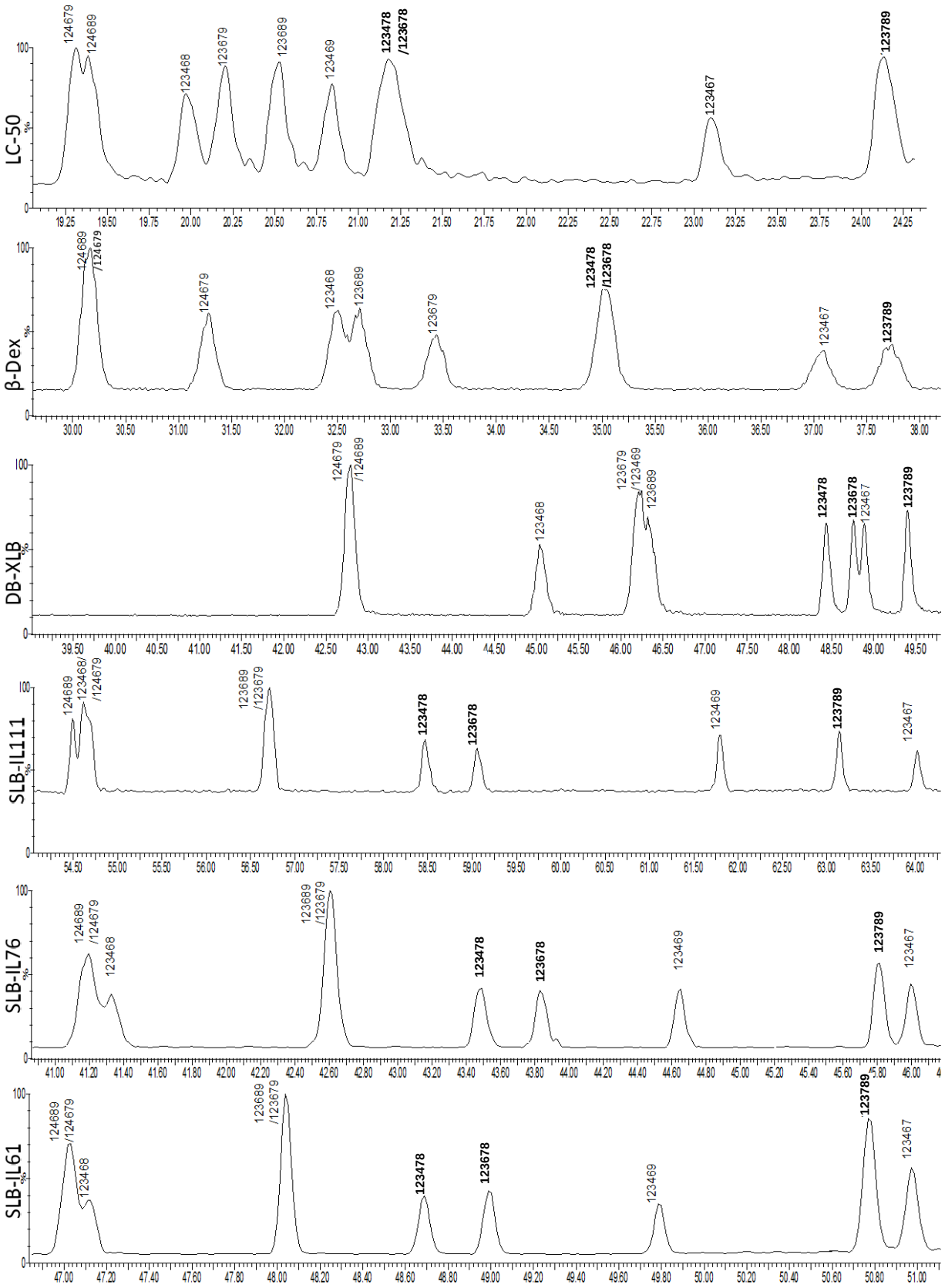


Fig 3.6: Chromatograms of HxCDDs from LC-50, β-Dex, DB-XLB, SLB-IL111, SLB-IL76, SLB-IL61 columns

DB-XLB column was the second best performing column with 15 congeners (included partial separation) separated, but it failed on two important congeners, 2,3,7,8-TeCDF and 2,3,4,7,8-PeCDF, and provided partial separation (+-) for 2,3,7,8-TeCDD, 1,2,3,6,7,8-HxCDD, 2,3,4,6,7,8-HxCDF, and 1,2,3,7,8,9-HxCDF, and poor separation (+--) for 12378-PeCDD.

Both of Rt-LC-50 and Rt-βDEXcst columns performed the worst and separated (included partial separation) of 12 congeners. The LC-50 failed on 1,2,3,4,7,8-HxCDD, 1,2,3,6,7,8-HxCDD, 2,3,7,8-TeCDF, 1,2,3,7,8-PeCDF, and 2,3,4,7,8-PeCDF and provided partial separation (+-) of 1,2,3,4,7,8-HxCDF and 1,2,3,6,7,8-HxCDF. On the other hand, Rt-βDEXcst failed on 2,3,7,8-TeCDD, 1,2,3,7,8-PeCDD, 1,2,3,4,7,8-HxCDD, 1,2,3,6,7,8-HxCDD, and 2,3,7,8-TeCDF, provided partial separation (+-) of 2,3,4,7,8-PeCDF, and poor separation (+--) of all HxCDF.

In previous study, VF-Xms was reported as better than any other column available in market to separate 2,3,7,8 substituted congeners. Comparing the evaluation criteria of peak separation to previous studies, our current study was more specified to evaluate the peak separation capacity of investigated columns. In the comparison, the separation on SLB-IL61 was found superior to DB-5ms, VF-5ms and VF-Xms (Table 5). Furthermore, SLB-IL61 is complementary to DB-XLB, Rtx-Dioxin2, ZB-5Ums, DB-5ms, ZB-5ms, VF-5ms, CP-Sil 8 CB/MS and VF-Xms. Any of these 8 column combinations provide complete separation of all 2,3,7,8-substituted PCDD/Fs. The SLB-IL111 column can also be used to obtain a full separation if combined with DB-5, HP-5ms, Rtx-5ms, Equity-5, DB-5ms, ZB-5ms, VF-5ms, CP-Sil 8CB/MS or VF-Xm (total 9 columns) as a complementary column. Finally, the test showed that any of the 3 SLB-IL columns can be combined with DB-5ms, ZB-5ms, VF-5ms, CP-sil8 CB/MS, or VF-Xms to resolve all 17 congeners. This result showed that a satisfactory number of columns are available complementary to IL columns,

In selecting a feasible column combination for the separation of 2,3,7,8-PCDD/Fs, it is important not only to evaluate the separation, but also some other factors like column lifetime and column bleed, those affect the limit of detection (IL-111 column showed high bleed and degradation of highly chlorinated congeners seems to occur on the IL-61 and IL-76 columns). It is therefore advisable to quantify as many congeners as possible using one of the stable low bleed columns (e.g. VF-Xms) and use the polar (e.g. IL-111) column for the complementary separations.

Table 5: Comparison of separation capacity of 2,3,7,8 substituted Cl of PCDD/Fs

References	Own Analysis						Fishman et al. (17)							
Column→ / Congeners↓	SLB-IL111	SLB-IL76	SLB-IL61	LC50	βDEXcst	DB-XLB	Rtx-Dioxin2	ZB-5UMS	DB-5, HP-5MS, Rtx-5MS, Equity-5	DB-225	DB-5MS ,ZB-5MS	SP-2331	VF-5ms, CP-Sil 8 CB/MS	VF-Xms
2378-TeCDD	--	+--	++	++	--	+-	++	++	++	+-	++	+-	++	++
12378-PeCDD	--	--	++	++	--	+--	--	--	++	--	++	--	++	++
123478-HxCDD	++	++	++	--	--	++	++	++	++	++	++	++	++	++
123678-HxCDD	++	++	++	--	--	+-	++	++	++	++	++	++	++	++
123789-HxCDD	++	++	++	++	++	++	++	++	--	++	++	++	++	++
1234678-HpCDD	++	++	++	++	++	++	++	++	++	++	++	++	++	++
OCDD	++	++	++	++	++	++	++	++	++	++	++	++	++	++
2378-TeCDF	++	--	++	--	--	--	++	++	--	++	++	+-	++	++
12378-PeCDF	--	--	+-	--	++	++	++	++	++	--	++	--	++	++
23478-PeCDF	++	++	++	--	+-	--	--	--	--	++	--	++	--	--
123478-HxCDF	++	--	--	+-	+--	++	++	++	--	++	++	--	++	++
123678-HxCDF	+--	++	++	+-	+--	++	++	++	++	--	++	++	++	++
234678-HxCDF	++	++	++	++	+--	+-	--	--	++	--	--	++	--	+-
123789-HxCDF	++	++	++	++	+--	+-	--	--	++	++	--	++	+-	+-
1234678-HpCDF	++	+-	+--	++	++	++	++	++	++	++	++	++	++	++
1234789-HpCDF	++	++	++	++	++	++	++	++	++	++	++	++	+-	++
OCDF	++	++	++	++	++	++	++	++	++	++	++	++	++	++

Separation of the 136 tetra- to octa-chlorinated PCDD/Fs

Since no single column, or dual column combinations (Appendix-1) have been reported to separate all 136 congeners (tetra-octa), this study evaluated the possibilities to achieve such a separation. From the previously studied chromatograms, it was difficult to accurately differentiate between partially separated (+-) and poorly separated (+--) congeners. Therefore, complete separation (++) data were first considered as a basis for the performance evaluation.

The possibility of single column separation was evaluated first (Appendix-1.7) and it was found that the ionic liquid performed best (Table 6). The SLB-IL61 separated 83 (61%) out of the 136 congeners In the same way, IL-111 and IL-76 separating 81 (59.56%) and 69 (50.74%) congeners, respectively. Each of the remaining tested columns separated less than 48 (35%) congeners. However, the commercially available column, Dioxin2 shows the best performance

among the previously studied columns (Appendix-1.7), and separated 71 (52%) congeners among all tetra- to octa- CDD/Fs, which performs close to the SLB-IL76 and less than the other two IL-columns.

Table 6: Percent of completely separated tetra- to octa- CDD/Fs on different columns

	SLB-IL111	SLB-IL76	SLB-IL61	LC50	βDEXcst	DB-XLB
TeCDFs	60.53	39.47	60.53	21.05	23.68	18.42
PeCDFs	46.43	39.29	46.43	17.86	28.57	28.57
HxCDFs	75.00	87.50	75.00	62.50	43.75	50.00
HpCDFs	100.00	50.00	50.00	100.00	100.00	100.00
OCDFs	100.00	100.00	100.00	100.00	100.00	0.00
TeCDDs	50.00	40.91	63.64	31.82	27.27	27.27
PeCDDs	64.29	64.29	71.43	35.71	14.29	42.86
HxCDDs	50.00	50.00	50.00	40.00	40.00	30.00
HpDDs	100.00	100.00	100.00	100.00	100.00	100.00
OCDDs	100.00	100.00	100.00	100.00	100.00	100.00
Total	59.56	50.74	61.03	34.56	32.35	33.09

In this present investigation, no single column was found that can resolve all the 136 congeners. Therefore, complementary columns were considered to maximize the separation. Simultaneous evaluation of the possibilities of additional columns was conducted as an attempt to resolve the remaining congeners. For this purpose, SLB-IL111 was chosen as the primary complementary column of SLBIL61 because of their different retention preferences. The two SLB-IL (111 and 61) columns together resolved all TeCDFs except six congeners (Table 7.1) among which, five (2,4,6,8-, 1,4,7,8-, 1,2,3,6-, 1,2,4,6- and 1,2,3,4-TeCDF) were partially separated and 1,2,6,9-TeCDF was not separated at all. However, 2,4,6,8- and 1,2,6,9-TeCDF can be resolved on VF-Xms and 1,4,7,8- and 1,2,3,6-TeCDF on Dioxin-2. For the remaining two congeners (1,2,4,6- and 1,2,3,4-TeCDF), commercially columns are not available nowadays (CPS-1 and Smectic are no longer in the market). Subsequently, all PeCDF can be resolved except ten congeners (Table 7.2), eight of which are partially (+-) or poorly (+--) separated (1,2,4,7,8-, 1,4,6,7,8-, 1,2,4,7,9-, 1,3,4,6,9-, 1,2,4,6,9-, 1,2,3,4,7- 1,2,3,7,8-, and 1,2,3,7,9- PeCDF)

and only two were unresolved (1,2,3,6,7-and 1,2,6,7,8-PeCDF). Of the ten congeners those were not completely resolved, four can be separated using SP-2331 (1,2,4,7,8-, 1,3,4,6,9-, 1,2,3,6,7- and 1,2,3,7,9-PeCDF). 1,4,6,7,8- and 1,2,4,7,9-PeCDF can only be separated by CPS-1 and Smectic (those are not on the market). The remaining unresolved congeners**,** 1,2,3,4,7-, 1,2,4,6,9-, 1,2,3,7,8-, 1,2,6,7,8-PeCDF cannot be separated on any column. All HxCDFs were separated on the two IL columns except 1,2,4,6,8,9-HxCDF, which was partially resolved (Table 7.3). This congener was completely separated by a great number of other columns (incl. Rt-LC50, Rt-βDEXcst, and DB-XLB).

Furthermore, all TeCDDs were separated using SLB-IL111 and SLB-IL61 together except six congeners (1,3,6,9-, 1,2,4,7-, 1,2,4,6-, 1,2,4,9-, 1,2,3,6- and 1,2,3,4-TeCDD) (Table 7.4). Three of these (1,3,6,9-, 1,2,4,9- and 1,2,3,4-TeCDD) can be resolved by LC-50 and one (1,2,3,6-TeCDD) by Dioxin2. For the remaining two separations, there are no commercially available columns. However, 1,3,6,9-, 1,2,4,7-, 1,2,3,6- and 1,2,3,4-TeCDF were partially or poorly separated (+- or +--) by the selected IL columns. Except 12467-PeCDD (partially resolved), all PeCDDs were completely separated by the two IL columns (Table 7.5), and this congener can be completely resolved using the Dioxin2 column. Five of the ten HxCDDs, HpCDD and OCDD were completely resolved by the IL columns. The remaining HxCDDs (1,2,4,6,7,9-, 1,2,4,6,8,9-, 1,2,3,4,6,8-, 1,2,3,6,7,9-, and 1,2,3,6,8,9-HxCDD) were all separated by the DB-1 column (Table 7.6). Moreover, All HpCDD/Fs and OcDD/F can be resolved by any of the SLB-IL columns. Among which, two HpCDFs were poorly separated by SLB-IL61.

In conclusion, the evaluation of the SLB-IL 61 and SLB-IL 111 data showed that 107 of the tetra- to octa- CDD/F congeners can be completely separated and 19 congeners can also be partially resolved from the remaining congeners. No third column can be specified for separating all remaining unresolved congeners. The choice of additional complementary column(s) to use besides the two IL-columns, thus has to be based on the problem at hand.

Table 7.1: Separation Capacity of SLB-IL, LC-50, βDEXcst, DB-XLB Columns for TeCDF and complementary option of columns for unresolved congeners by SLB-IL 111 and SLB-IL 61

Congener	SLB-IL111	SLB-IL76	SLB-IL61	LC50	βDEXcst	DB-XLB	Additional column(s) to do complete separation for unresolved congeners after SLB-IL111 and SLB-IL61 complementary
1368-TeCDF	++	++	++	++	++	++	
1468-TeCDF	++	-+	++	++	++	++	
2468-TeCDF	+-	--	--	++	--	--	Vf-Xms, VF-5ms, DB-5ms, Equity-5, DB-5, DB-225, Sil-88,BPX-DXN
1247-TeCDF	+-	-+	++	--	+--	+--	
1347-TeCDF	++	++	++	--	--	--	
1378-TeCDF	--	+--	++	+--	--	--	
1346-TeCDF	--	++	++	++	++	++	
1246-TeCDF	+-	--	--	--	--	--	CPS-1, Smectic
1348-TeCDF	++	++	++	--	--	--	
1367-TeCDF	+-	++	++	--	--	+--	
1248-TeCDF	++	++	++	--	+--	+--	
1379-TeCDF	--	+--	++	--	--	+--	
1268-TeCDF	++	--	--	--	--	--	
1467-TeCDF	++	++	--	--	--	--	
1478-TeCDF	+-	--	+--	--	--	++	DB_XLB, Dioxin-2
2368-TeCDF	++	+-	++	++	++	++	
1237-TeCDF	++	--	--	--	--	--	
1369-TeCDF	--	--	++	--	--	--	
2467-TeCDF	++	++	++	--	--	--	
1469-TeCDF	--	++	++	--	+-	+--	
1238-TeCDF	++	+-	--	+-	--	--	
1236-TeCDF	+--	+--	--	--	--	--	Dioxin-2
1678-TeCDF	++	+-	--	+--	--	--	
1234-TeCDF	+--	+-	--	--	--	--	No good complement
1278-TeCDF	++	+-	--	--	++	+--	
1349-TeCDF	++	+-	++	--	++	+--	
1267-TeCDF	--	++	++	--	--	+-	
2347-TeCDF	++	++	++	--	++	--	
2348-TeCDF	++	++	++	--	--	--	
1249-TeCDF	++	+-	+--	+-	+-	+--	
1279-TeCDF	++	+-	+-	+--	+-	+-	
2346-TeCDF	++	--	--	--	++	+--	
2378-TeCDF	++	--	++	--	--	--	
2367-TeCDF	--	--	++	++	--	+-	
1269-TeCDF	--	++	--	--	--	+--	VF-Xms, VF-5ms, DB-5ms, DB-17,SP-2331, Sill88, BPX-DXN
3467-TeCDF	++	++	++	++	--	--	
1239-TeCDF	++	--	++	+-	--	++	
1289-TeCDF	++	++	++	++	++	++	

Table 7.2: Separation Capacity of SLB-IL, LC-50, βDEXcst, DB-XLB Columns for PeCDFs and complementary column options for unresolved congeners by SLB-IL 111 and SLB-IL 61.

Congener	SLB-IL111	SLB-IL76	SLB-IL61	LC50	βDEXcst	DB-XLB	Additional column(s) to do complete separation for unresolved congeners after SLB-IL111 and SLB-IL61 complementary
13468-PeCDF	++	++	++	--	++	++	
12468-PeCDF	++	++	++	--	++	++	
13678-PeCDF	++	++	++	--	++	--	
13467-PeCDF	++	++	--	+-	--	--	
12368-PeCDF	--	--	++	--	--	--	
13478-PeCDF	--	--	++	--	--	--	
12478-PeCDF	--	+--	+-	--	+-	+-	SP-2331, CPS-1, Sil88
12467-PeCDF	+--	+-	++	+--	+-	+--	
13479-PeCDF	++	++	++	+--	--	+-	
14678-PeCDF	+--	+-	+-	--	--	+--	CPS-1, Smectic
12479-PeCDF	--	+--	+-	--	+--	+-	CPS-1
13469-PeCDF	--	++	+-	+--	--	--	SP-2331, CPS-1, Sil88, Smectic
23468-PeCDF	++	--	+--	--	++	+--	
12469-PeCDF	--	++	+--	--	+-	+--	Dioxin2
12346-PeCDF	+-	++	++	--	--	--	
12347-PeCDF	+--	+-	--	++	--	--	Smectic
12348-PeCDF	++	--	+--	+-	--	--	
12378-PeCDF	--	--	+-	--	++	++	VF-Xms, VF-5ms, DB-5ms, Smectic
12367-PeCDF	--	--	--	--	--	--	Equity-5,SP-2331, Sil88
12678-PeCDF	--	--	--	--	--	--	Equity-5, DB-17, DB-225, CPS-1, Smectic
12379-PeCDF	+-	++	+--	++	++	++	VF-Xms, VF-5ms, DB-5ms, DB-17, SP-2331, CPS-1, Sil88, Smetic, BPX-DXN, Dioxin2
23478-PeCDF	++	++	++	--	+-	--	
12679-PeCDF	++	+-	+-	--	++	++	
23467-PeCDF	++	--	++	++	++	++	
12369-PeCDF	++	--	+--	+-	+--	++	
12489-PeCDF	++	++	++	++	++	+--	
12349-PeCDF	++	++	++	+-	+--	--	
12389-PeCDF	++	--	++	++	+-	++	

Table 7.2: Separation Capacity of SLB-IL,LC-50, βDEXcst, DB-XLB Columns for PeCDF and complementary option of columns for unresolved congeners by SLB-IL 111 and SLB-IL 61.

Congener	SLB-IL111	SLB-IL76	SLB-IL61	LC50	βDEXcst	DB-XLB	Additional column(s) to do complete separation for unresolved congeners after SLB-IL111 and SLB-IL61 complementary
123468-HxCDF	--	++	++	++	++	++	
134678-HxCDF	++	++	++	--	++	+-	
124678-HxCDF	++	++	++	--	++	+-	
134679-HxCDF	--	++	++	++	++	++	
124679-HxCDF	++	++	++	++	++	++	
124689-HxCDF	+--	--	--	++	++	++	VF-Xms, DB-5ms, Equity-5, DB-5, DB-210, SP-2331, Smectic, BPX-DXN, Dioxin2
123467-HxCDF	++	++	+-	--	+--	++	
123478-HxCDF	++	--	--	+-	+--	++	
123678-HxCDF	+--	++	++	+-	+--	++	
123479-HxCDF	++	++	++	++	+--	--	
123469-HxCDF	++	++	++	--	+-	--	
123679-HxCDF	++	++	++	++	+-	++	
234678-HxCDF	++	++	++	++	+--	+-	
123689-HxCDF	++	++	+-	++	++	+-	
123789-HxCDF	++	++	++	++	+--	+-	
123489-HxCDF	++	++	++	++	+--	+-	

Table 7.4: Separation Capacity of SLB-IL, LC-50, βDEXcst, DB-XLB Columns for TeCDDs and complementary column options for unresolved congeners by SLB-IL 111 and SLB-IL 61.

Congener	SLB-IL111	SLB-IL76	SLB-IL61	LC50	βDEXcst	DB-XLB	Additional column(s) to do complete separation for unresolved congeners after SLB-IL111 and SLB-IL61 complementary
1368-TeCDD	++	++	++	++	++	++	
1379-TeCDD	++	++	++	+-	+ -	++	
1369-TeCDD	+-	+-	+-	++	++	++	VF-Xms, VF-5ms, DB-5ms, Equity-5, DB-1,DB-5, DB-17, DB-210,DB-225, Smectic, BPX-DXN, Dioxin2
1469-TeCDD	--	+-	++	--	--	++	
1247-TeCDD	+-	+--	+-	--	+--	--	No good complement
1248-TeCDD	++	+--	++	+-	--	--	
1378-TeCDD	++	++	++	--	--	--	
1246-TeCDD	--	--	--	--	--	+--	Partially separated on Smectic
1249-TeCDD	--	--	--	++	--	+--	
1268-TeCDD	++	++	+--	--	+-	--	
1478-TeCDD	++	++	+--	+-	++	++	
1279-TeCDD	++	+--	++	+--	++	+--	
1269-TeCDD	++	++	++	++	+-	+-	
1236-TeCDD	--	+-	+--	--	--	+-	Dioxin-2
1237-TeCDD	--	+--	++	--	+-	--	
1234-TeCDD	--	++	+--	++	--	+--	Smectic
1238-TeCDD	--	--	++	--	+--	--	
2378-TeCDD	--	+--	++	++	--	+-	
1239-TeCDD	++	++	++	--	+-	--	
1278-TeCDD	--	+-	++	--	--	--	
1267-TeCDD	++	++	++	--	++	--	
1289-TeCDD	++	--	++	++	++	++	

Table 7.5: Separation Capacity of SLB-IL, LC-50, βDEXcst, DB-XLB Columns for PeCDDs and complementary column options for unresolved congeners by SLB-IL 111 and SLB-IL 61.

Congener	SLB-IL111	SLB-IL76	SLB-IL61	LC50	βDEXcst	DB-XLB	Other column options to do complete separation for unresolved congeners by SLB-IL111 and SLB-IL61 complementary
12468-PeCDD	++	--	--	++	+-	+-	
12479-PeCDD	++	--	--	++	+--	+--	
12469-PeCDD	++	++	++	+-	+-	++	
12368-PeCDD	++	++	++	+-	+-	++	
12478-PeCDD	++	++	++	+-	++	++	
12379-PeCDD	++	++	++	+--	+-	+-	
12369-PeCDD	--	++	++	++	+-	--	
12467-PeCDD	+-	--	+-	--	--	--	Dioxin2
12489-PeCDD	++	+-	++	--	--	+-	
12347-PeCDD	++	++	+-	--	--	++	
12346-PeCDD	--	++	++	--	--	++	
12378-PeCDD	--	--	++	++	--	+--	
12367-PeCDD	--	++	++	+--	--	+--	
12389-PeCDD	++	++	++	++	++	++	

Table 7.6: Separation Capacity of SLB-IL, LC-50, βDEXcst, DB-XLB Columns for HxCDDs and complementary column options for unresolved congeners by SLB-IL 111 and SLB-IL 61.

Congener	SLB-IL111	SLB-IL76	SLB-IL61	LC50	βDEXcst	DB-XLB	Additional column(s) to do complete separation for unresolved congeners after SLB-IL111 and SLB-IL61 complementary
124679-HxCDD	--	--	--	+--	--	--	DB-1
124689-HxCDD	+--	--	--	+--	--	--	DB-1
123468-HxCDD	--	+--	+--	+-	++	++	VF-Xms, VF-5ms, DB-5ms, Equity-5, DB-1, DB-5, DB-17, DB-210, DB-225, BPX-DXN, Dioxin2
123679-HxCDD	--	--	--	+-	+--	--	DB-1
123689-HxCDD	--	--	--	++	+--	+--	DB-1
123469-HxCDD	++	++	++	++	++	--	
123478-HxCDD	++	++	++	--	--	++	
123678-HxCDD	++	++	++	--	--	+-	
123467-HxCDD	++	++	++	++	++	+-	
123789-HxCDD	++	++	++	++	++	++	

Influence of the Cl substituent positions on the retention

ROs of 22 GC columns and their polar behaviors were applied for PCA approach (Appendix-2). The PCA showed that the stationary phase interaction of different columns (based on polar behavior), and thus retention, depend on the Cl substituent positions. In this study, two groupings in all score plot, representing polar (polar to extreme polar range) and non-polar columns (non polar to medium polar range) (Fig 4). The loading plots explain the contribution of the various congeners to the distribution (and clustering) in the score plot.

For PCDFs analysis, LC-smectic, LC-50 and DB-17 showed different selectivity from the other columns. Two of these (LC-Smectic and LC-50) offer a different mode of separation, as they are shape selective. Separate models were made in which these three columns were excluded. In these refined models, 83%, 70%, 88% of the variation for the TeCDFs, PeCDFs and HxCDFs can explained, respectively.

In the PCDDs model, ROs of LC-Smectec, LC-50, DB-17 and SLB-IL76 column were offering different selectivity for TeCDDs; and the two LC columns and DB-17, and LC-Smectic, β-DEXcst showed different selectivity for PeCDDs and HxCDDs, respectively. Reduced models were developed, as for the PCDDs, and they were able to explain 90%, 94%, 78% of the variation for the TeCDDs, PeCDDs and HxCDDs congeners, respectively.

Np= Non polar, Lp= Low polar, FP= fairly polar, Mp = Moderately polar, P= Polar, Exp= Extremely polar

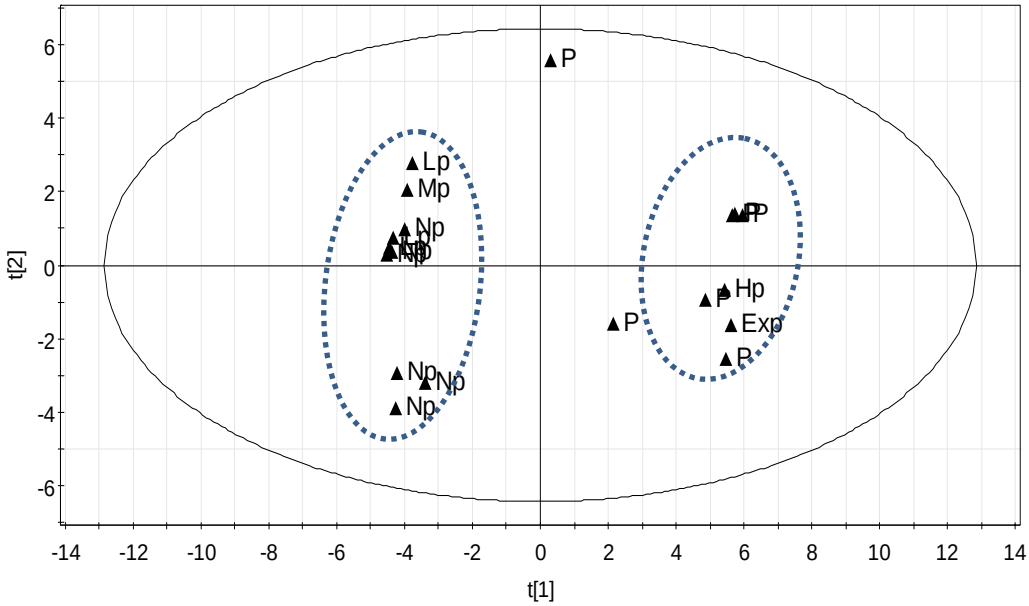


Fig 41: Score plot of TeCDFs

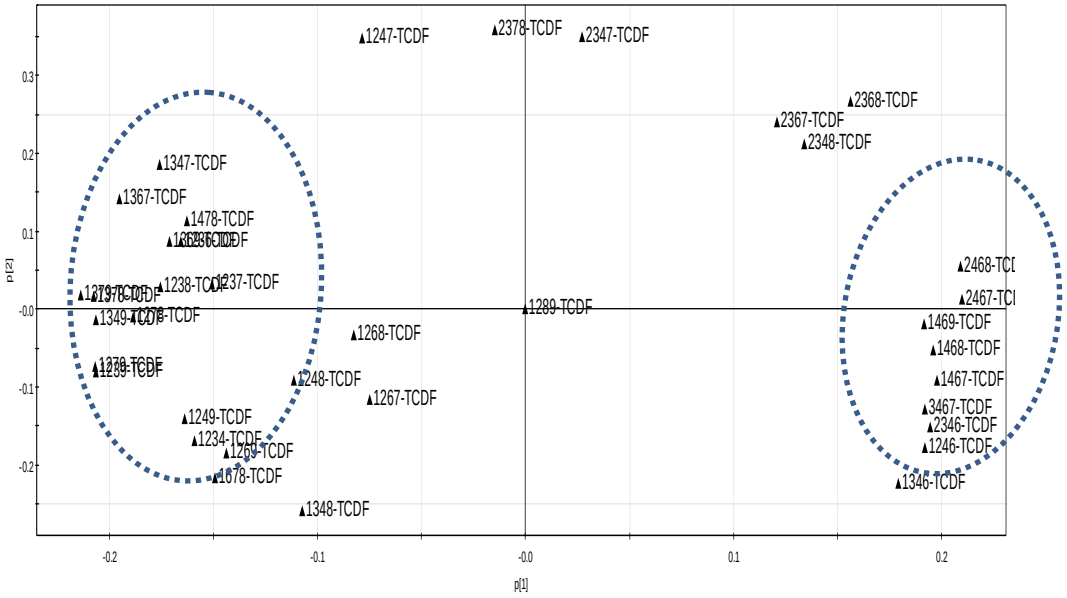


Fig 4.2: Loading plot of TeCDFs

Np= Non polar, Lp= Low polar, FP= fairly polar, Mp = Moderately polar, P= Polar, Exp= Extremely polar

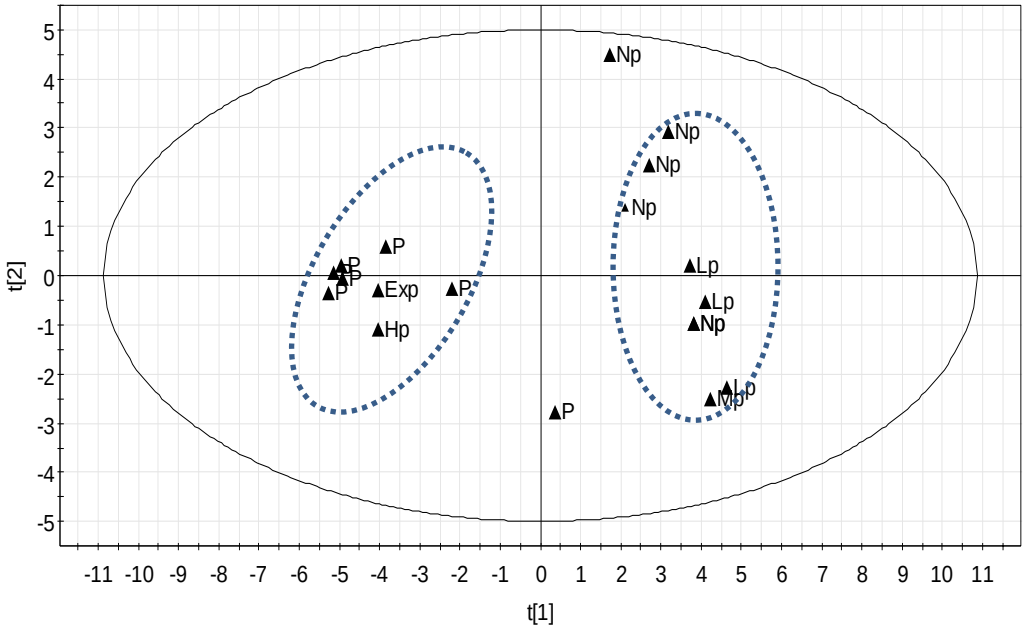


Fig 4.3: Score plot of PeCDFs

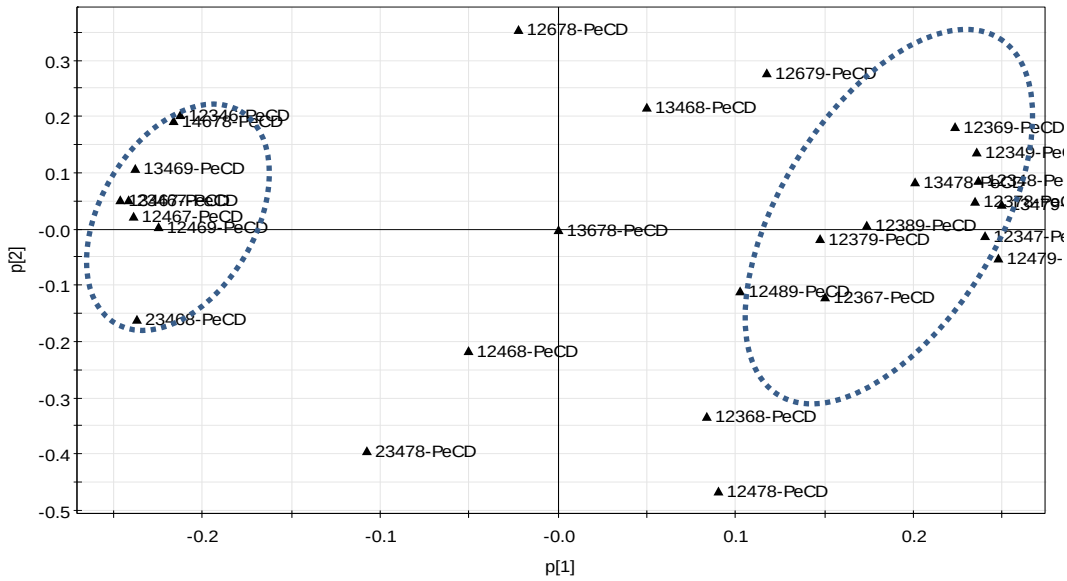


Fig 4.4: Loading plot of PeCDFs

Np= Non polar, Lp= Low polar, FP= fairly polar, Mp = Moderately polar, P= Polar, Exp= Extremely polar

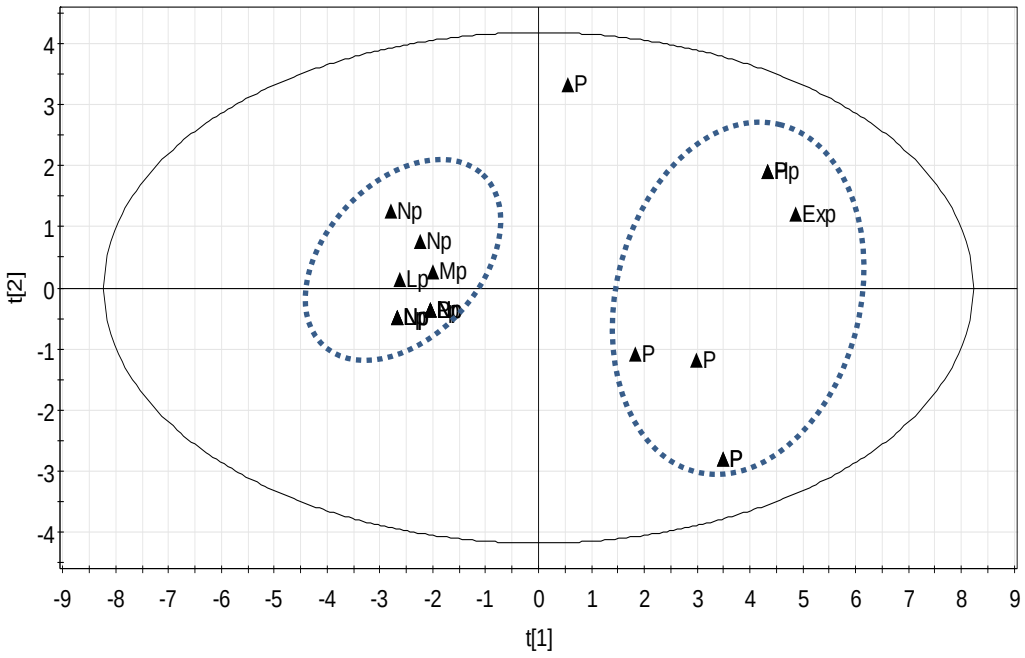


Fig 4.5: Score plot of HxCDFs

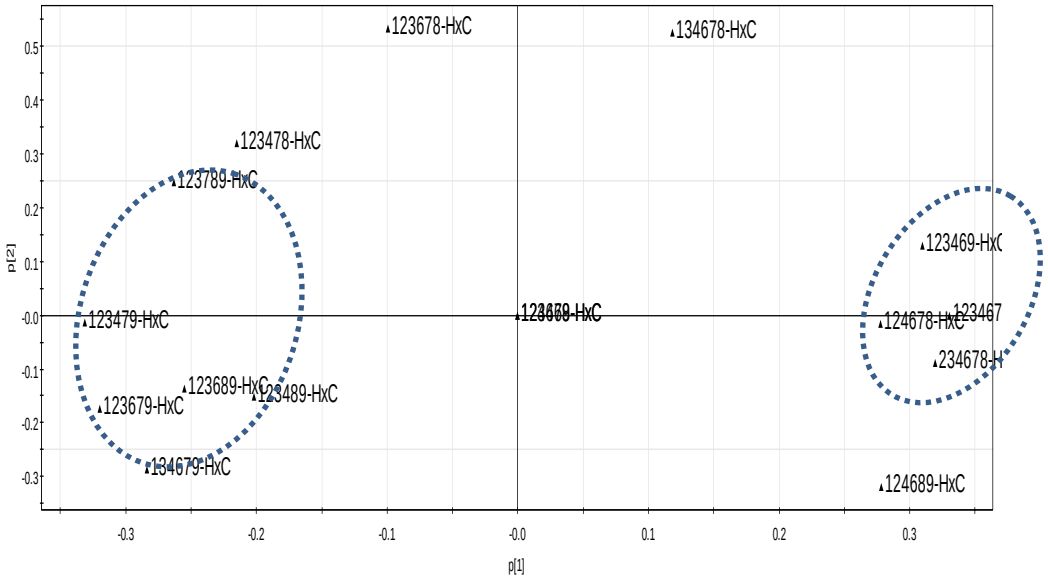


Fig 4.6: Loading plot of HxCDFs

Np= Non polar, Lp= Low polar, FP= fairly polar, Mp = Moderately polar, P= Polar, Exp= Extremely polar

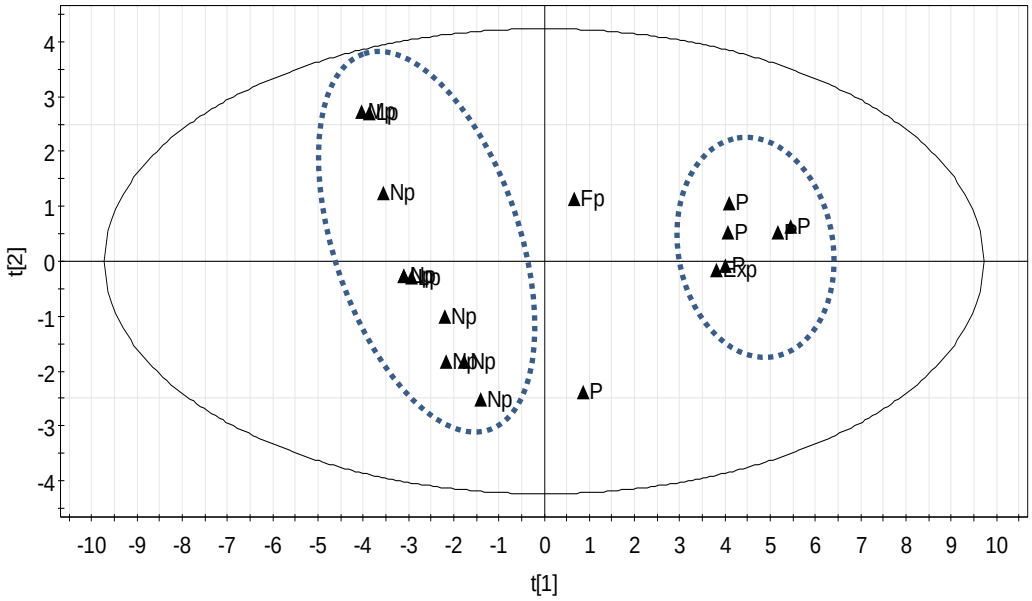


Fig 4.7.: Score plot of TeCDDs

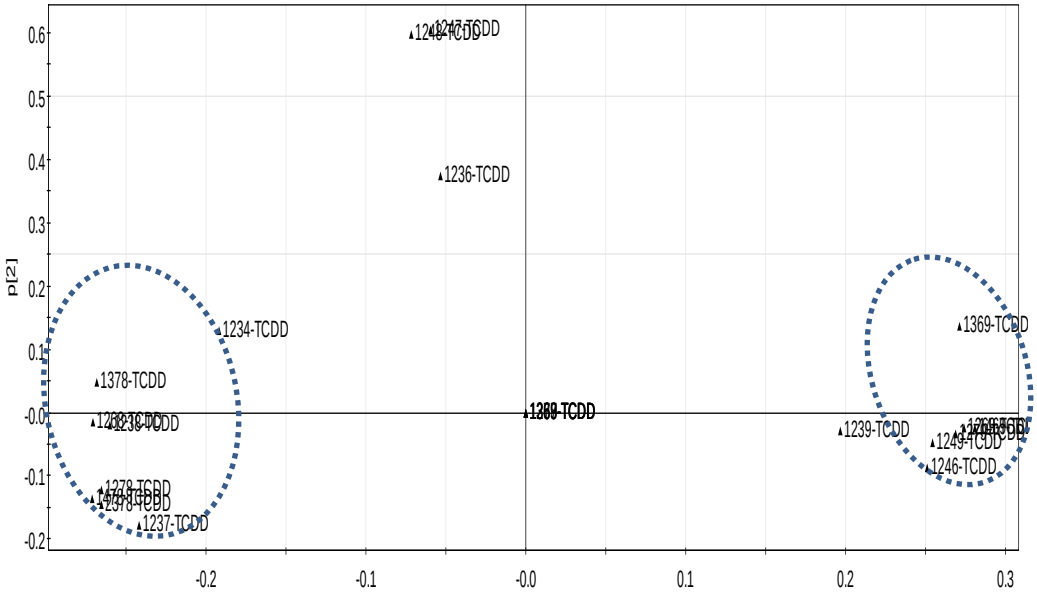


Fig 4.8: Loading plot of TeCDDs

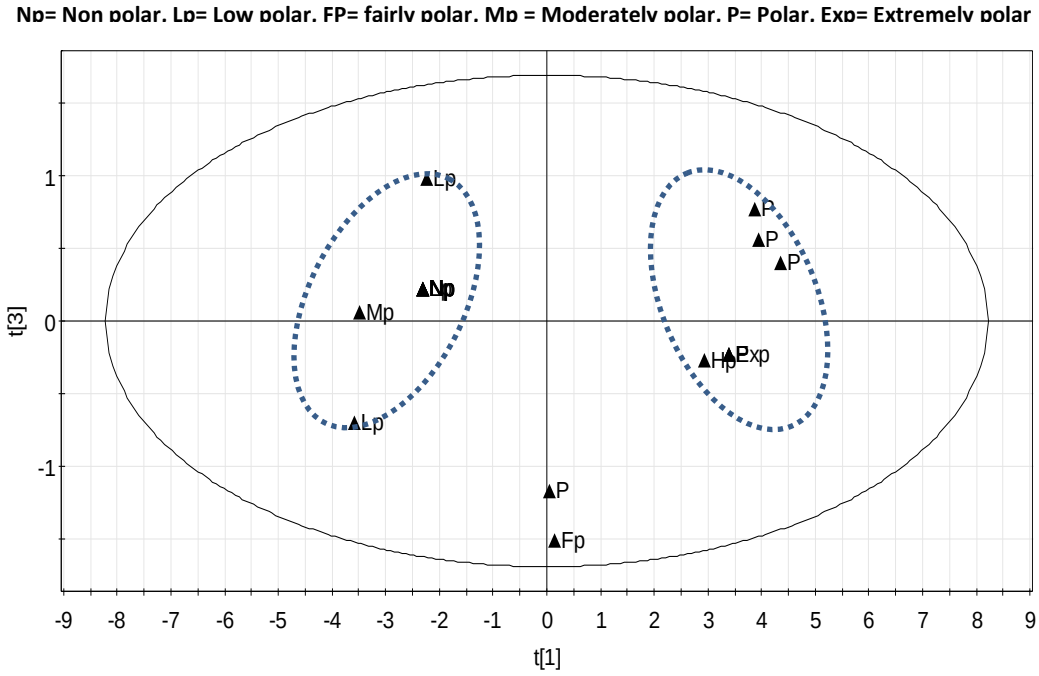


Fig 4.9: Score plot of PeCDDs

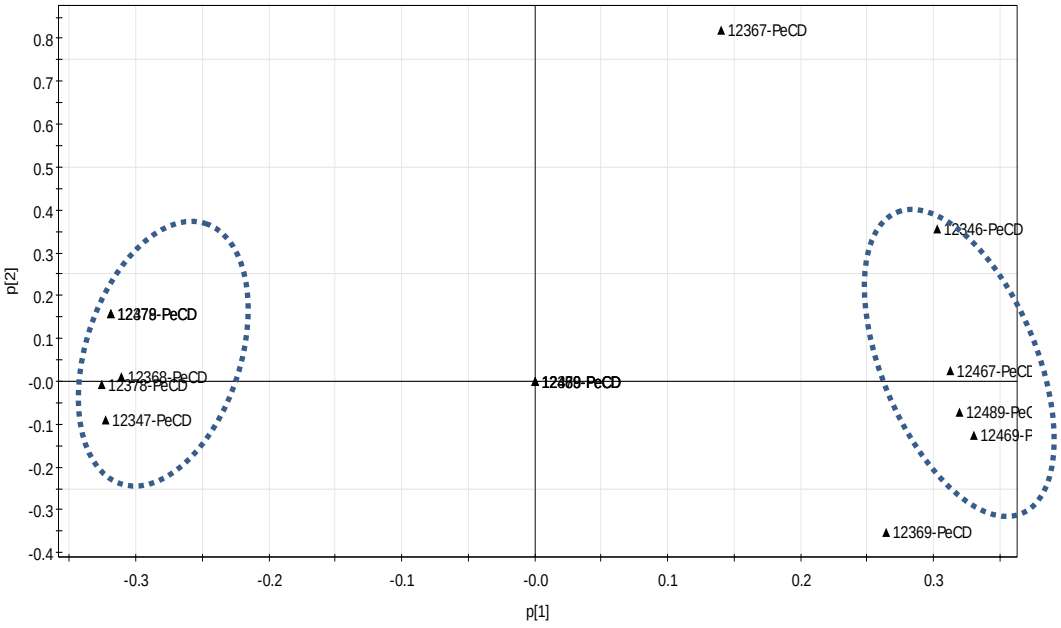


Fig 4.10: Loading plot of PeCDDs

Np= Non polar, Lp= Low polar, FP= fairly polar, Mp = Moderately polar, P= Polar, Exp= Extremely polar

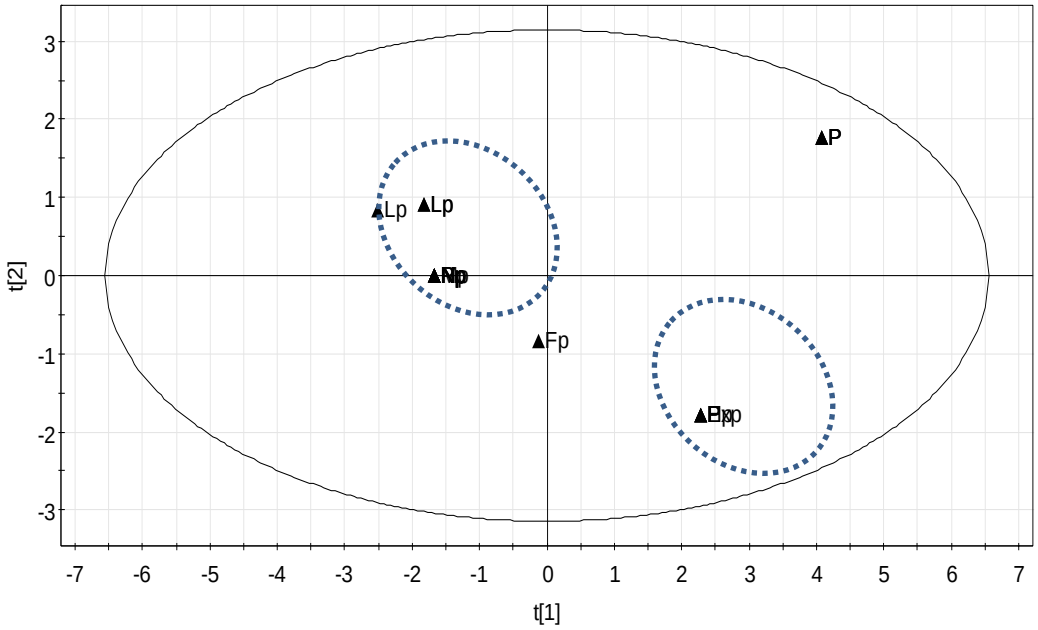


Fig 4.11: Score plot of HxCDDs

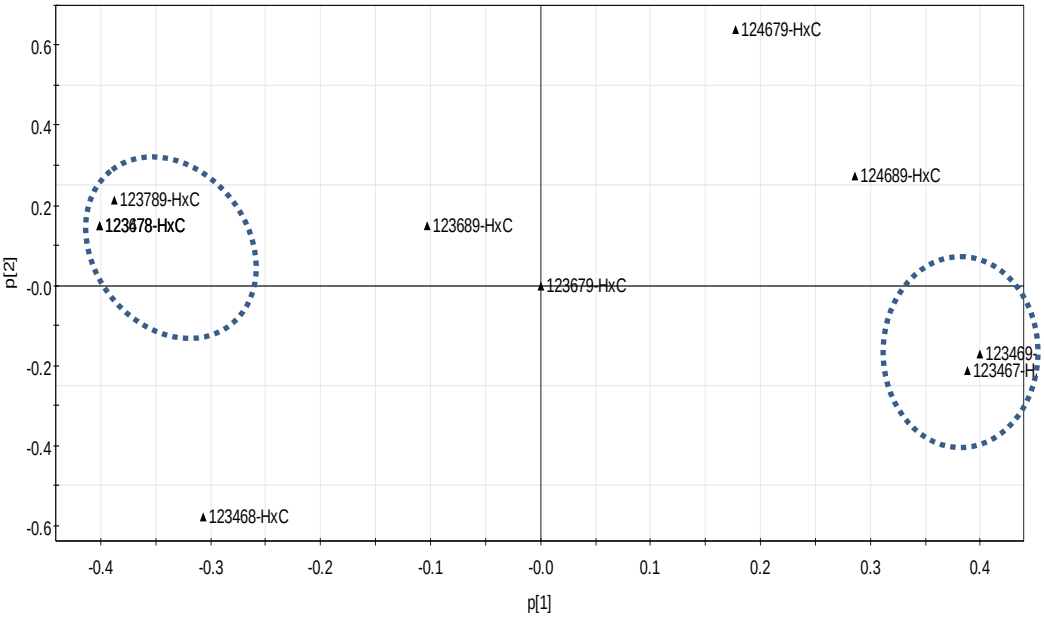
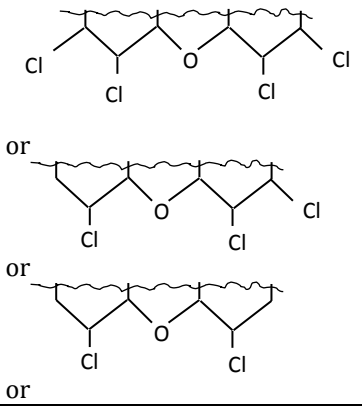
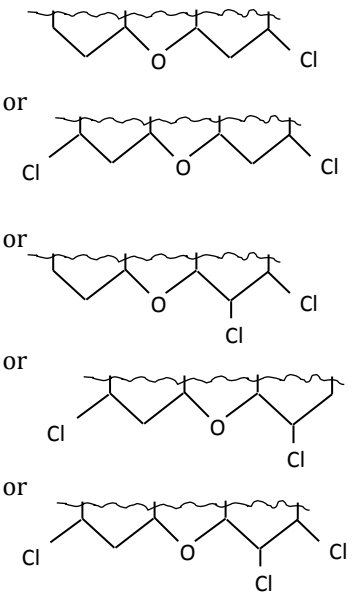


Fig 4.12: Loading plot of HxCDDs

Table 8: Positions of substituted Cl in PCDD/F according to stationary phase polarity of Column

Column behavior	Homologue	Substituted Cl position in		Common structure
		PCDF	PCDD	
Extremely Polar and Polar	TeCDD/F	1346,2346,1467,2467,1246,1468,1469,2468,3467	1239,1246,1249,1279,1269,1469,1369	
	PeCDD/F	12346,14678,13469,13467,23467,12467,12469,23468,	12489,12469,12369,12346,12467	
	HxCDD/F	123689,124678,124679,134678,134679,234678	123469,123467	
Non Polar and Medium Polar	TeCDD/F	1347,1367,1478,1369,1236,1379,1378,1238,1237,1348,1678,1269,1234,1249,1267,1248,1268	1234,1478,1238,2378,1237,1268	
	PeCDD/F	12389,12369,12349,13478,12348,12378,12379,12347,12379	12347,12378,12368,12478,12379	
	HxCDD/F	123479,123689,123489,123679,123478,123789	123478,123678, 123689(not accepted)	

In this analysis, the group of congeners, whose retention were correlated to the extreme polar and polar columns, had Cl substituents located in 4,6 positions of PCDFs and 1,9 or 4,6 positions of PCDDs. Similarly, the remaining congeners, containing Cl substituents in other positions in the aromatic ring were correlated to the non-polar and medium polar columns (Table 8). As GC column follows 'Like dissolve like' theory for stationary phase interaction with solute, the polarity of solute corresponds to the polarity of the stationary phase. Therefore, a polar substance will interact with a polar stationary phase and vice versa. In a nonpolar

column, the separations depend on the vapor pressure of the solutes. Due to the narrow boiling point range of the PCDD/F isomers, non-polar columns have limited separation potential, and additional stationary phase interactions (besides dispersive forces) are beneficial for separation of isomers. Cl and O induces negative electrostatic potential (-δ) in PCDD/Fs molecules. The stationary phase of polar column contains dipoles that can interact with the Cls and O rich regions of PCDD/F through dipole-induced dipole interactions. When these atoms are located in the peri positions (4, 6 or 1,9 position) maximum interaction may occur. There are however exceptions and further studies are needed to fully characterize and ultimately explain the structure-retention relationships.

Conclusion

This is a qualitative approach which achieved significant information about six different columns for separating all tetra- to octa- CDD/Fs. In this evaluation study, SLB-IL61 was shown to have superior separation capacity compared to other GC columns for 2,3,7,8 congeners and for tetra-octa CDD/Fs, in general. IL-61 and IL-111 can be used with large number of complementary columns to separate all 2,3,7,8 congeners. Furthermore, the SLB-IL 61 and SLB-IL 111 columns have different selectivity and can together resolve a great number of tetra- to octa- CDD/Fs. Only a handful of congeners were not separated or partially separated by the two columns. Analysis of structure retention relationships, i.e. how different Cl substituents in PCDD/Fs interact with the stationary phase, may help researchers to design a more fine tuned stationary phase. This may lead to the full separation of all 136 tetra- to octa- CDD/Fs

References

1. Singh, S. B., Kulshrestha ,G.; (1997) Gas chromatographic analysis of polychlorinated dibenzo-p-dioxins and dibenzofurans; J. Chromatogr. A. 774 (1–2): 97-109.
2. Haglund, P., Korytar, P. Danielsson, C., Diaz, J.,Wiberg, K., Leonards, P., Brinkman, U. A.,de Boer, J.; (2008) GC×GC-ECD: a promising method for the determination of dioxins and dioxin-like PCBs in food and feed; Anal. Bioanal. Chem. 390(7): 1815-1827.
3. Danielsson, C.; (2007) Trace analysis of dioxins and dioxin-like PCBs using comprehensive two-dimensional gas chromatography with electron capture detection; Doctoral Dissertation, Umeå University; ISBN: 91-7264-234-3.
4. Environment Australia; (1999) Incineration and Dioxins: Review of Formation Processes; consultancy report prepared by Environmental and Safety Services for Environment Australia, Commonwealth Department of the Environment and Heritage, Canberra.
5. Marklund, S.; (1990) Dioxin Emissions and Environmental imissions – a study of polychlorinater dibenzodioxins and dibenzofurans in combustion process; Umeå University; ISBN:91-7174-496-7
6. Fishman, V. N. , Martin, G. D. , Lamparski, L. L. ; (2004) Comparison of Series 5 gas chromatography column performances from a variety of manufacturers for separation of chlorinated dibenzo-p-dioxins and dibenzofurans using high-resolution mass spectrometry; J. Chromatogr. A. 1057:151–161
7. Ryan, O. J., Conacher, H. B. S., Panopio, L. G., Lau B. P. Y., Hardy, J. A.; (1991) Gas chromatographic separations of all 136 tetra- to octa-polychlorinated dibenzo-pdioxins and polychlorinated dibenzofurans on nine different stationary phases; J. Chromatogr. 541:131-183
8. Roland Weber, Mats Tysklind and Caroline Gaus , Dioxin- Contemporay and Future Challenge of Historical Legacy , Env Sci Pollut Res 15(2) 96-100m(2008)
9. Fishman, V. N., Martin, G. D., Lamparski, L. L.;(2007) Comparison of a variety of gas chromatographic columns with different polarities for the separation of chlorinated dibenzo-*p*-dioxins and dibenzofurans by high-resolution mass spectrometry; J. Chromatogr. A. 1139: 285–300

10. Malavia, J., Abalos, A., Santos F.J., Abad, E., Rivera, J., Galceran, M.T.; (2007) Analysis of polychlorinater dibenzodioxins and dibenzofurans and dioxin-like polychlorinated biphenyls in vegetable oil sample by gas chromatography-ion trap tandem mass spectrometry; J. Chroatogr. A. 1149: 321-332
11. Dioxins and Furans (accessed on: 01.03.2013) http://www.epa.gov/osw/hazard/wastemin/minimize/factshts/dioxfura.pdf
12. California Depart ment of Health Services; (2003) Dioxins Technical Information for California Health Officials; (Accessed: 01.03.2013) http://www.ehib.org/papers/Dioxin.pdf
13. Gatehouse, R.; (2004) Ecological Risk Assessment of Dioxins in Australia; National Dioxins Program Technical Report No. 11, Australian Government Department of the Environment and Heritage, Canberra.
14. Srogi, K.; (2007) MiniiReview:Overview of analytical Method for Dioxin Analysis; Anal. Let. 40:1647-1671
15. Santos, F.J., Galceran, M. T.; (2003) Modern development in gas Chromatography- mass spectrometry based environmental analysis; J. Chromatogr. A. 1000: 125-151.
16. *Poole, C.; (2012) Gas Chromatography;1st edition;* Elsevier Inc.
17. Fishman, V. N., Martin, G. D., Wilken, M.; (2011) Retention time profiling of all 136 tetra- through octa- chlorinated dibenzo-p-dioxins and dibenzofurans on a variety of Si-Arylene gas chromatographic stationary phases; Chemosp. 84: 913–922
18. Abad, E., Caixach, J., Rivera, J. ; (1997) Application of DB-5ms gas chromatography column for the complete assignment of 2,3,7,8-substituted polychlorodibenzo-p- dioxins and polychlorodibenzofurans in samples from municipal waste incinerator emissions; J. Chromatogr. A. 786:125-134
19. Cochran, J.; Characterizing All 136 Tetra- to Octachlorinated Dioxins and Furans Using the Rtx®-Dioxin2 Column; (Online: Accessed 01.03.2013)http://www.restek.com/Technical-Resources/Technical-Library/Environmental/env_A030
20. Difeo, Jr. D.; (2007) The analysis of Dioxin compounds using selective dioxin capillary column, (online: Accessed 01.03.2013) http://www.sge.com/uploads/a5/07/a5075d83a5c485cafc825f3b9ef09213/TP-0093-C.pdf

Appendix

Appendix-1.1: Separation Capacity of different columns for TeCDF

References	Own Experiment						Fishman et al. (17)				Ryan et al. (7)									(20)	(19)
Congener	SLB-IL111	SLB-IL76	SLB-IL61	LC50	βDEXcst	DB-XLB	Vf-Xms	VF-5ms	DB-5ms	Equity-5	DB-1	DB-5	DB-17	DB-210	DB-225	SP-2331	CPS-1	Sil88	Smectic	BPX-DXN	Dioxin
1368-TeCDF	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	+-	++	++
1468-TeCDF	++	+-	++	++	++	++	++	++	++	++	++	++	--	+-	--	++	--	++	+-	++	++
2468-TeCDF	+-	--	--	++	--	--	++	++	++	++	+-	++	--	+-	++	--	--	++	+-	++	--
1247-TeCDF	+-	-+	++	--	+-	+-	--	--	--	--	--	--	+-	--	--	--	--	--	--	--	--
1347-TeCDF	++	++	++	--	--	--	--	--	--	--	--	--	--	+-	--	++	++	++	++	--	--
1378-TeCDF	--	+-	++	+-	--	--	--	--	--	--	--	--	--	--	--	--	--	--	++	--	--
1346-TeCDF	--	++	++	++	++	++	+-	--	+-	+-	--	--	++	--	++	--	--	--	++	--	--
1246-TeCDF	+-	--	--	--	--	--	--	--	--	+-	--	--	--	--	--	--	++	--	++	--	--
1348-TeCDF	++	++	++	--	--	--	+-	--	+-	--	--	--	--	--	++	++	++	++	++	--	--
1367-TeCDF	+-	++	++	--	--	+-	--	--	--	--	--	--	+-	+-	++	--	++	--	+-	--	--
1248-TeCDF	++	++	++	--	+-	+-	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
1379-TeCDF	--	+-	++	--	--	+-	--	--	--	--	--	--	++	--	--	--	--	--	++	--	--
1268-TeCDF	++	--	--	--	--	--	+-	+-	+-	--	+-	--	--	--	--	--	--	--	--	+-	++
1467-TeCDF	++	++	--	--	--	--	+-	+-	+-	--	--	--	--	--	--	--	--	--	--	+-	++
1478-TeCDF	+-	--	+-	--	--	++	+-	+-	+-	--	--	--	--	--	--	--	--	--	--	+-	++
2368-TeCDF	++	+-	++	++	++	++	--	+-	--	--	--	+-	--	--	++	+-	++	++	--	--	++
1237-TeCDF	++	--	--	--	--	--	--	--	--	--	--	--	--	+-	--	--	--	--	++	--	++
1369-TeCDF	--	--	++	--	--	--	--	--	--	--	--	--	++	--	+-	--	--	--	+-	--	++
2467-TeCDF	++	++	++	--	--	--	--	+-	+-	--	--	--	+-	--	++	++	++	++	--	--	--
1469-TeCDF	--	++	++	--	+-	+-	--	--	--	--	--	--	--	--	--	+-	--	+-	--	--	--
1238-TeCDF	++	+-	--	+-	--	--	--	--	--	--	--	--	++	--	--	--	--	--	++	--	--
1236-TeCDF	+-	+-	--	--	--	--	--	--	--	--	--	--	--	+-	--	--	--	--	--	--	++
1678-TeCDF	++	+-	--	+-	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
1234-TeCDF	+-	+-	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
1278-TeCDF	++	+-	--	--	++	+-	++	+-	+-	++	++	+-	--	+-	+-	++	++	++	--	++	--
1349-TeCDF	++	+-	++	--	++	+-	--	+-	+-	+-	--	--	+-	+-	+-	++	++	++	--	+-	--
1267-TeCDF	--	++	++	--	--	+-	--	++	++	+-	--	--	--	++	--	--	--	--	--	+-	++
2347-TeCDF	++	++	++	--	++	--	--	--	--	--	--	--	--	--	++	+-	+-	++	++	--	++
2348-TeCDF	++	++	++	--	--	--	--	+-	+-	--	--	--	+-	--	++	+-	--	+-	--	--	++
1249-TeCDF	++	+-	+-	+-	+-	+-	++	+-	--	--	--	--	++	++	++	+-	--	+-	+-	--	++
1279-TeCDF	++	+-	+-	+-	+-	+-	--	--	--	--	--	--	--	--	--	--	--	--	++	--	++
2346-TeCDF	++	--	--	--	++	+-	--	+-	--	--	--	--	--	--	+-	++	++	++	+-	+-	++
2378-TeCDF	++	--	++	--	--	--	++	++	+-	--	--	--	++	+-	--	+-	--	+-	--	+-	++
2367-TeCDF	--	--	++	++	--	+-	--	--	--	++	++	++	++	++	+-	++	++	++	--	--	--
1269-TeCDF	--	++	--	--	--	+-	++	++	++	--	--	--	++	--	+-	++	+-	++	--	++	--
3467-TeCDF	++	++	++	++	--	--	--	--	--	--	--	--	+-	++	++	++	++	++	++	--	++
1239-TeCDF	++	--	++	+-	--	++	++	++	++	++	++	+-	+-	--	--	+-	++	++	--	++	++
1289-TeCDF	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++

Appendix-1.2: Separation Capacity of different columns for PeCDF

References	Own Experiment						Fishman et al. (17)				Ryan et al. (7)									(20)	(19)
Congener	SLB-IL111	SLB-IL76	SLB-IL61	LC50	βDEXcst	DB-XLB	Vf-Xms	VF-5ms	DB-5ms	Equity-5	DB-1	DB-5	DB-17	DB-210	DB-225	SP-2331	CPS-1	Sil88	Smectic	BPX-DXN	Dioxin
13468-PeCDF	++	++	++	--	++	++	--	--	--	--	--	--	--	+-	++	++	++	++	+-	--	++
12468-PeCDF	++	++	++	--	++	++	--	--	--	--	--	--	--	+-	++	++	++	++	+-	--	++
13678-PeCDF	++	++	++	--	++	--	--	--	--	++	--	+-	++	--	++	++	++	++	+-	+-	--
13467-PeCDF	++	++	--	+-	--	--	--	--	--	--	--	--	+-	--	--	--	++	--	--	--	--
12368-PeCDF	--	--	++	--	--	--	--	--	--	--	--	--	--	+-	--	--	--	--	+-	--	--
13478-PeCDF	--	--	++	--	--	--	--	--	--	--	--	--	+-	--	--	--	--	--	+-	--	--
12478-PeCDF	--	+--	+-	--	+-	+-	--	--	--	--	--	--	--	--	+-	++	++	++	+-	--	--
12467-PeCDF	+--	+-	++	+--	+-	+--	--	--	--	--	--	--	+-	--	+-	++	+-	+-	+-	--	--
13479-PeCDF	++	++	++	+--	--	+-	++	++	+-	--	--	--	+-	--	+-	++	++	++	--	++	++
14678-PeCDF	+--	+-	+-	--	--	+--	--	+-	+-	--	--	--	+-	--	+-	--	++	+-	++	--	--
12479-PeCDF	--	+--	+-	--	+--	+-	--	--	--	+-	--	--	+-	--	--	--	++	--	+-	--	--
13469-PeCDF	--	++	+-	+--	--	--	+-	--	--	+-	--	--	+-	--	--	++	++	++	++	--	++
23468-PeCDF	++	--	+--	--	++	+--	+-	--	--	--	+-	--	+-	+-	+-	++	++	++	++	--	--
12469-PeCDF	--	++	+--	--	+-	+--	--	--	--	+-	--	--	+-	+-	+-	--	--	--	--	--	++
12346-PeCDF	+-	++	++	--	--	--	--	+-	++	--	--	--	--	+-	--	++	--	++	+-	--	--
12347-PeCDF	+--	+-	--	++	--	--	--	--	--	+-	--	--	+-	--	+-	--	+-	+-	++	--	--
12348-PeCDF	++	--	+--	+-	--	--	++	++	++	+-	+-	+-	+-	+-	--	--	+-	--	++	++	--
12378-PeCDF	--	--	+-	--	++	++	++	++	++	+-	+-	+-	--	--	--	--	--	--	++	++	++
12367-PeCDF	--	--	--	--	--	--	--	--	--	++	++	+-	+-	--	+-	++	--	++	--	--	--
12678-PeCDF	--	--	--	--	--	--	--	--	--	++	--	--	++	--	++	--	++	--	++	--	--
12379-PeCDF	+-	++	+--	++	++	++	++	++	++	--	--	--	++	+-	+-	++	++	+-	++	++	++
23478-PeCDF	++	++	++	--	+-	--	--	+-	--	--	--	--	++	++	++	++	+-	++	++	--	++
12679-PeCDF	++	+-	+-	--	++	++	--	++	++	--	--	--	--	--	--	++	++	++	++	--	++
23467-PeCDF	++	--	++	++	++	++	--	+-	+-	--	--	+-	--	++	--	++	++	++	++	--	--
12369-PeCDF	++	--	+--	+-	+--	++	--	+-	+-	--	--	--	++	--	--	++	++	++	++	--	--
12489-PeCDF	++	++	++	++	++	+--	+-	+-	--	--	--	--	--	++	--	++	++	++	+-	+-	--
12349-PeCDF	++	++	++	+-	+--	--	++	++	++	++	++	++	--	++	--	++	++	++	++	++	++
12389-PeCDF	++	--	++	++	+-	++	++	++	++	++	++	++	++	++	--	++	+-	++	++	++	++

Appendix-1.3: Separation Capacity of different columns for HxCDD/F

References	Own Experiment						Fishman et al. (17)				Ryan et al. (7)									(20)	(19)
Congener	SLB-IL111	SLB-IL76	SLB-IL61	LC50	βDEXcst	DB-XLB	Vf-Xms	VF-5ms	DB-5ms	Equity-5	DB-1	DB-5	DB-17	DB-210	DB-225	SP-2331	CPS-1	Sil88	Smectic	BPX-DXN	Dioxin
123468-HxCDF	--	++	++	++	++	++	++	++	++	++	++	++	++	+-	++	++	++	++	++	++	++
134678-HxCDF	++	++	++	--	++	+-	--	--	--	--	++	--	--	--	+-	+-	--	--	--	--	--
124678-HxCDF	++	++	++	--	++	+-	--	--	--	--	++	--	--	--	--	++	++	++	--	--	--
134679-HxCDF	--	++	++	++	++	++	++	++	++	++	++	++	++	+-	--	+-	--	--	++	++	++
124679-HxCDF	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
124689-HxCDF	+-	--	--	++	++	++	++	++	++	++	--	++	+-	++	--	++	--	--	++	++	++
123467-HxCDF	++	++	+-	--	+-	++	++	++	++	--	--	--	--	--	++	++	++	--	+-	++	++
123478-HxCDF	++	--	--	+-	+-	++	++	++	++	--	+-	--	+-	--	+-	--	--	--	++	++	++
123678-HxCDF	+-	++	++	+-	+-	++	++	++	++	+-	+-	+-	--	+-	--	+-	++	++	++	++	++
123479-HxCDF	++	++	++	++	+-	--	++	++	++	+-	--	+-	++	--	--	--	--	--	+-	++	++
123469-HxCDF	++	++	++	--	+-	--	++	++	++	+-	--	--	+-	+-	--	--	--	--	++	++	++
123679-HxCDF	++	++	++	++	+-	++	++	++	++	+-	--	--	+-	+-	++	++	++	++	++	++	++
234678-HxCDF	++	++	++	++	+-	+-	+-	--	--	+-	--	+-	+-	++	--	++	++	++	--	--	--
123689-HxCDF	++	++	+-	++	++	+-	+-	--	--	+-	--	+-	+-	++	--	--	--	--	--	--	--
123789-HxCDF	++	++	++	++	+-	+-	+-	--	--	+-	+-	+-	++	--	++	++	++	++	++	--	--
123489-HxCDF	++	++	++	++	+-	+-	+-	--	--	+-	+-	+-	++	--	--	++	++	++	++	--	--

Appendix-1.4: Separation Capacity of different columns for TeCDD

References	Own Experiment						Fishman et al. (17)				Ryan et al. (7)									(20)	(19)
Congener	SLB-IL111	SLB-IL76	SLB-IL61	LC50	βDEXcst	DB-XLB	Vf-Xms	VF-5ms	DB-5ms	Equity-5	DB-1	DB-5	DB-17	DB-210	DB-225	SP-2331	CPS-1	Sil88	Smectic	BPX-DXN	Dioxin
1368-TeCDD	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
1379-TeCDD	++	++	++	+-	+ -	++	++	++	++	++	++	++	++	++	++	++	++	++	--	++	++
1369-TeCDD	+-	+-	+-	++	++	++	++	++	++	++	++	++	++	++	++	--	+-	--	++	++	++
1469-TeCDD	--	+-	++	--	--	++	+-	+-	++	--	+-	--	--	+-	++	--	++	++	+-	--	++
1247-TeCDD	+-	+--	+-	--	+--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
1248-TeCDD	++	+--	++	+-	--	--	--	--	--	--	--	--	--	--	--	--	--	--	+-	--	--
1378-TeCDD	++	++	++	--	--	--	+-	+-	+-	--	--	--	+-	+-	++	++	++	++	++	--	--
1246-TeCDD	--	--	--	--	--	+--	--	--	--	--	--	--	--	--	--	--	--	--	+-	--	--
1249-TeCDD	--	--	--	++	--	+--	--	--	--	--	--	--	--	--	--	--	--	--	+-	--	--
1268-TeCDD	++	++	+--	--	+-	--	++	++	++	+-	+-	--	--	+-	++	++	++	++	--	--	--
1478-TeCDD	++	++	+--	+-	++	++	++	++	++	+-	+-	--	--	++	+-	++	++	+-	++	++	++
1279-TeCDD	++	+--	++	+--	++	+--	++	++	++	++	++	++	+-	--	--	--	+-	--	++	++	++
1269-TeCDD	++	++	++	++	+-	+-	--	--	--	--	++	--	--	--	+-	++	++	++	++	--	--
1236-TeCDD	--	+-	+--	--	--	+-	+-	+-	+-	--	+-	--	+-	--	+-	--	+-	--	--	+-	++
1237-TeCDD	--	+--	++	--	+-	--	--	--	--	--	--	--	--	--	--	+-	--	+-	+-	--	--
1234-TeCDD	--	++	+--	++	--	+--	--	--	--	--	--	--	--	+-	--	--	--	--	++	--	--
1238-TeCDD	--	--	++	--	+--	--	--	--	--	--	--	--	--	--	--	--	--	--	++	--	--
2378-TeCDD	--	+--	++	++	--	+-	++	++	++	++	+-	--	--	--	+-	++	--	+-	++	+-	++
1239-TeCDD	++	++	++	--	+-	--	++	+-	++	++	+-	+-	--	--	+-	++	++	++	++	+-	--
1278-TeCDD	--	+-	++	--	--	--	++	++	++	++	+-	++	--	--	+-	--	++	++	+-	++	--
1267-TeCDD	++	++	++	--	++	--	++	++	++	++	+-	++	++	+-	++	++	++	++	+-	++	--
1289-TeCDD	++	--	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	+-	++

Appendix-1.5: Separation Capacity of different columns for PeCDD

References	Own Experiment						Fishman et al. (17)				Ryan et al. (7)									(20)	(19)
Congener	SLB-IL111	SLB-IL76	SLB-IL61	LC50	βDEXcst	DB-XLB	Vf-Xms	VF-5ms	DB-5ms	Equity-5	DB-1	DB-5	DB-17	DB-210	DB-225	SP-2331	CPS-1	Sil88	Smectic	BPX-DXN	Dioxin
12468-PeCDD	++	--	--	++	+-	+-	--	--	--	--	--	--	--	--	--	--	--	--	++	--	--
12479-PeCDD	++	--	--	++	+--	+--	--	--	--	--	--	--	--	--	--	--	--	--	++	--	--
12469-PeCDD	++	++	++	+-	+-	++	++	++	++	++	++	++	--	+-	++	--	++	--	++	++	++
12368-PeCDD	++	++	++	+-	+-	++	++	++	++	++	+-	++	++	++	++	++	++	++	+-	++	++
12478-PeCDD	++	++	++	+-	++	++	+-	+-	+-	++	+-	+-	--	+-	++	++	++	++	+-	++	++
12379-PeCDD	++	++	++	+-	+--	+-	+-	+-	+-	++	++	+-	+-	++	++	++	++	++	++	++	++
12369-PeCDD	--	++	++	++	+-	--	--	--	--	+-	--	+-	++	--	--	++	--	++	+-	--	--
12467-PeCDD	+-	--	+-	--	--	--	--	--	--	--	--	--	--	--	--	+-	--	+-	+-	--	++
12489-PeCDD	++	+-	++	--	--	+-	--	+-	+-	--	--	--	--	--	--	+-	+-	+-	++	--	--
12347-PeCDD	++	++	+-	--	--	++	--	--	--	++	+-	+-	++	--	++	--	++	--	++	--	++
12346-PeCDD	--	++	++	--	--	++	--	--	--	++	+-	+-	+-	+-	++	+-	++	+-	++	--	++
12378-PeCDD	--	--	++	++	--	+--	++	++	+-	++	+-	++	+-	+-	--	++	++	++	++	+-	--
12367-PeCDD	--	++	++	+--	--	+--	++	++	+-	++	+-	++	++	+-	++	+-	++	+-	++	+-	--
12389-PeCDD	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++

Appendix-1.6: Separation Capacity of different columns for HxCDD

References	Own Experiment						Fishman et al. (17)				Ryan et al. (7)									(20)	(19)
Congener	SLB-IL111	SLB-IL76	SLB-IL61	LC50	βDEXcst	DB-XLB	Vf-Xms	VF-5ms	DB-5ms	Equity-5	DB-1	DB-5	DB-17	DB-210	DB-225	SP-2331	CPS-1	Sil88	Smectic	BPX-DXN	Dioxin
124679-HxCDD	--	--	--	+-	--	--	--	--	--	--	++	--	--	--	--	--	--	--	++	--	--
124689-HxCDD	+-	--	--	+-	--	--	--	--	--	--	++	--	--	--	--	--	--	--	+-	--	--
123468-HxCDD	--	+-	+-	+-	++	++	++	++	++	++	++	++	++	++	++	--	--	--	+-	++	++
123679-HxCDD	--	--	--	+-	+-	--	--	--	--	--	++	--	--	--	--	--	--	--	+-	--	--
123689-HxCDD	--	--	--	++	+-	+-	--	--	--	--	++	--	--	--	--	--	--	--	+-	--	--
123469-HxCDD	++	++	++	++	++	--	++	++	+-	++	++	+-	++	++	++	++	++	++	+-	++	++
123478-HxCDD	++	++	++	--	--	++	++	++	+-	+-	++	+-	++	++	++	++	++	++	--	++	++
123678-HxCDD	++	++	++	--	--	+-	++	++	+-	+-	+-	+-	++	++	++	++	++	++	--	++	++
123467-HxCDD	++	++	++	++	++	+-	++	+-	+-	--	+-	--	++	++	++	++	++	++	++	++	++
123789-HxCDD	++	++	++	++	++	++	++	+-	+-	--	++	--	++	++	++	++	++	++	++	++	++

Appendix-1.7: Maximum Separation Capacity of different columns for PCDD/Fs

References	Own Experiment						Fishman et al. (17)				Ryan et al. (7)									(20)	(19)
Congener	SLB-IL111	SLB-IL76	SLB-IL61	LC50	βDEXcst	DB-XLB	Vf-Xms	VF-5ms	DB-5ms	Equity-5	DB-1	DB-5	DB-17	DB-210	DB-225	SP-2331	CPS-1	Sil88	Smectic	BPX-DXN	Dioxin
TeCDFs	23	15	23	8	9	7	9	8	7	7	6	5	10	6	12	12	14	16	12	7	19
PeCDFs	13	11	13	5	8	8	6	7	7	5	3	2	6	5	5	18	16	16	13	6	11
HxCDFs	12	14	12	10	7	8	10	10	10	4	5	4	5	4	5	9	9	8	10	10	10
HpCDFs	4	2	2	4	4	4	4	4	4	4	Information not available									4	4
OCDF	1	1	1	1	1	1	1	1	1	1	Information not available									1	1
TeCDDs	11	9	14	7	6	6	11	10	12	9	6	7	5	5	8	10	11	10	11	7	9
PeCDDs	9	9	10	5	2	6	5	5	3	9	3	5	5	3	8	6	9	6	10	5	8
HxCDDs	5	5	5	4	4	3	6	4	1	2	8	1	6	6	6	5	5	5	3	6	6
HpCDDs	2	2	2	2	2	2	2	2	2	2	Information not available									2	2
OCDD	1	1	1	1	1	1	1	1	1	1	Information not available									1	1
Total	81	69	83	47	44	46	55	52	48	44										49	71

Appendix-2.1: RO of different columns for TeCDF

	Non polar(Np)	Non polar (Np)	Fairly polar (Fp)	Polar (P)	Polar (P)	Polar (P)	Polar (P)	Polar (p)	Polar (p)	Non polar (Np)	Non polar (Np)	Non polar (Np)	Low polar (Lp)	Low polar (Lp)	Polar (Lp)	Polar (P)	Polar (P)	High polar (Hp)	Extremely polar (Exp)	Medium polar (Mp)	Non polar(Np)	Low polar (Lp)
	DB-1	DB-5	DB-17	DB-210	DB-225	CPS-1	SP-2331	CP-Sil 88	LC-Smectic	Equity-5	DB-5ms	VF-5ms	VF-Xms	DB-XLB	βDEXcst	LC-50	SLBIL61	SLBIL76	SLBIL111	Dioxin	BPX-DXN	5Sil MS
1368-TeCDF	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
1468-TeCDF	2	2	2	2	6	5	5	5	2	2	2	2	2	2	2	2	6	5	5	2	2	2
2468-TeCDF	3	3	3	12	16	21	18	21	4	3	3	3	3	4	12	3	15	15	15	3	3	3
1247-TeCDF	4	4	6	4	5	6	6	6	15	4	8	8	8	9	9	11	5	6	6	8	9	8
1347-TeCDF	5	5	5	3	4	4	4	4	13	5	6	6	6	6	6	9	4	4	4	7	6	6
1378-TeCDF	6	6	4	5	2	2	2	2	22	6	7	7	7	7	4	16	2	2	2	6	8	7
1346-TeCDF	7	7	9	8	9	9	9	9	3	7	4	4	4	3	3	4	10	10	11	4	5	4
1246-TeCDF	8	8	12	10	11	11	11	12	5	8	5	5	5	5	5	5	13	13	14	5	4	5
1348-TeCDF	9	10	11	9	8	8	8	8	12	10	9	9	9	8	7	6	8	8	8	9	7	9
1367-TeCDF	10	9	7	7	7	7	7	7	18	9	11	11	11	11	10	13	7	7	7	12	11	10
1248-TeCDF	11	12	10	11	10	10	10	10	6	12	10	10	10	10	11	7	9	9	9	10	10	11
1379-TeCDF	12	11	8	6	3	3	3	3	23	11	12	12	12	12	8	21	3	3	3	11	12	12
1268-TeCDF	13	13	16	15	13	13	12	11	10	13	13	13	13	13	13	10	14	11	10	13	13	13
1467-TeCDF	14	14	17	18	18	19	20	19	9	14	14	14	14	14	14	14	23	22	22	14	14	14
1478-TeCDF	15	15	13	14	14	15	13	14	7	15	15	15	15	15	15	8	12	14	13	15	15	15
2368-TeCDF	16	18	15	23	24	28	28	28	21	18	19	19	19	24	29	17	22	24	24	24	20	19
1237-TeCDF	17	17	14	16	12	12	15	13	35	17	17	17	17	19	18	30	16	16	16	17	16	17
1369-TeCDF	18	16	18	13	15	14	14	15	8	16	16	16	16	16	16	12	11	12	12	16	17	16
2467-TeCDF	19	19	24	29	29	29	29	29	26	19	18	18	18	20	26	25	29	29	29	19	18	18
1469-TeCDF	20	22	27	24	27	26	26	26	14	22	22	23	24	23	22	19	27	27	27	22	23	22
1238-TeCDF	21	20	19	20	17	18	19	18	25	20	20	20	21	21	19	24	17	19	18	21	19	20
1236-TeCDF	22	21	21	19	21	20	21	20	20	21	24	24	23	22	20	18	18	20	20	23	22	24
1678-TeCDF	23	23	20	21	20	17	16	16	11	23	23	21	20	17	17	15	19	17	17	18	24	23
1234-TeCDF	24	24	22	17	19	16	17	17	17	24	21	22	22	18	21	20	20	18	19	20	21	21
1278-TeCDF	25	25	23	25	22	23	23	23	29	25	25	25	25	26	24	26	24	23	23	25	25	25
1349-TeCDF	26	26	29	22	23	22	22	22	16	26	26	26	26	25	23	22	21	21	21	26	26	26
1267-TeCDF	27	27	31	26	26	25	24	25	31	27	27	27	27	27	27	28	28	28	28	27	27	27
2347-TeCDF	28	30	26	30	30	31	31	31	33	30	30	30	30	32	33	34	30	30	30	31	30	31
2348-TeCDF	29	28	28	31	33	34	34	34	28	33	32	32	32	31	34	27	33	33	33	32	32	32
1249-TeCDF	30	32	33	27	28	27	27	27	19	29	29	29	29	29	25	23	25	25	26	28	29	29
1279-TeCDF	31	33	30	28	25	24	25	24	32	31	31	31	31	30	28	32	26	26	25	30	31	30
2346-TeCDF	32	31	32	35	35	35	35	35	24	32	28	28	28	28	30	29	35	35	36	29	28	28
2378-TeCDF	33	29	25	32	31	33	33	33	37	28	33	33	33	33	36	33	31	31	31	33	33	33
2367-TeCDF	34	34	34	36	36	36	36	36	38	34	34	34	35	35	37	36	34	36	34	35	34	34
1269-TeCDF	35	36	37	33	34	32	32	32	27	36	36	36	36	36	31	31	36	34	35	36	36	36
3467-TeCDF	36	35	35	37	37	37	37	37	34	35	35	35	34	34	35	37	37	37	37	34	35	35
1239-TeCDF	37	37	36	34	32	30	30	30	30	37	37	37	37	37	32	35	32	32	32	37	37	37
1289-TeCDF	38	38	38	38	38	38	38	38	36	38	38	38	38	38	38	38	38	38	38	38	38	38

Appendix-2.2: RO of different columns for PeCDF

	Non polar(Np)	Non polar (Np)	Fairly polar (Fp)	Polar (P)	Polar (P)	Polar (P)	Polar (P)	Polar (p)	Polar (p)	Non polar (Np)	Non polar (Np)	Non polar (Np)	Low polar (Lp)	Low polar (Lp)	Polar (Lp)	Polar (P)	Polar (P)	High polar (Hp)	Extremely polar (Ep)	Medium polar (Mp)	Non polar(Np)	Low polar (Lp)
	DB-1	DB-5	DB-17	DB-210	DB-225	CPS-1	SP-2331	CP-Sil 88	LC-Smectic	Equity-5	DB-5ms	VF-5ms	VF-Xms	DB-XLB	βDEXcst	LC-50	SLBIL61	SLBIL76	SLBIL111	Dioxin	BPX-DXN	5Sil MS
13468-PeCDF	1	1	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	1
12468-PeCDF	2	2	1	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	1	2
13678-PeCDF	3	3	3	3	3	3	3	3	5	3	3	3	3	3	3	3	3	3	3	3	3	3
13467-PeCDF	4	6	7	8	8	9	9	9	10	5	4	4	4	4	5	10	9	9	9	4	6	4
12368-PeCDF	5	4	4	5	5	5	5	6	6	4	6	6	6	7	4	4	5	5	5	8	4	6
13478-PeCDF	6	7	6	6	6	6	6	5	8	8	7	7	7	8	6	6	6	6	6	7	8	8
12478-PeCDF	7	5	5	7	7	7	7	7	7	7	8	8	8	9	10	5	7	7	7	9	7	7
12467-PeCDF	8	8	9	9	10	10	10	10	13	6	5	5	5	5	9	11	11	11	11	5	5	5
13479-PeCDF	9	9	8	4	4	4	4	4	11	9	10	10	10	10	7	8	4	4	4	10	10	10
14678-PeCDF	10	10	10	11	12	12	11	12	3	10	9	9	9	6	8	7	12	12	12	6	9	9
12479-PeCDF	11	11	11	10	9	8	8	8	14	11	12	12	12	13	11	12	8	8	8	12	11	12
13469-PeCDF	12	12	14	13	13	13	13	13	4	12	11	11	11	11	12	9	14	13	13	11	12	11
23468-PeCDF	13	13	13	21	21	23	23	23	15	16	14	14	14	15	21	15	21	21	22	15	14	14
12469-PeCDF	14	14	18	16	19	19	19	19	9	14	15	15	15	14	14	13	18	17	18	14	16	15
12346-PeCDF	15	16	15	14	16	16	16	16	12	15	13	13	13	12	13	14	17	16	17	13	13	13
12347-PeCDF	16	15	12	12	11	11	12	11	20	13	16	16	16	16	15	17	10	10	10	16	15	16
12348-PeCDF	17	17	17	15	15	14	14	14	16	17	17	17	17	17	16	16	15	14	16	17	17	17
12378-PeCDF	18	18	16	17	14	15	15	15	25	18	18	18	18	18	17	21	13	15	14	18	18	18
12367-PeCDF	19	19	19	18	18	18	18	18	23	20	20	20	20	20	19	20	20	19	20	20	19	19
12678-PeCDF	20	20	21	19	20	20	20	20	17	19	19	19	19	19	18	19	19	18	19	19	20	20
12379-PeCDF	21	21	20	20	17	17	17	17	27	21	21	21	21	21	20	26	16	26	15	21	21	21
23478-PeCDF	22	22	22	26	26	26	26	26	24	23	25	25	24	26	27	22	26	25	26	26	25	25
12679-PeCDF	23	24	24	22	22	21	21	21	21	22	22	22	22	22	22	23	23	20	21	22	22	22
23467-PeCDF	24	26	25	27	27	28	28	28	26	26	23	23	23	23	26	27	28	27	27	23	23	23
12369-PeCDF	25	25	23	23	23	22	22	22	18	24	24	24	25	24	23	18	22	22	23	24	24	24
12489-PeCDF	26	23	26	25	25	25	25	25	22	25	26	26	26	25	25	25	25	24	25	25	26	26
12349-PeCDF	27	27	27	24	24	24	24	24	19	27	27	27	27	27	24	24	24	23	24	27	27	27
12389-PeCDF	28	28	28	28	28	27	27	27	28	28	28	28	28	28	28	28	27	28	28	28	28	28

Appendix-2.3: RO of different columns for PeCDF

	Non polar(Np)	Non polar (Np)	Fairly polar (Fp)	Polar (P)	Polar (P)	Polar (P)	Polar (P)	Polar (p)	Polar (p)	Non polar (Np)	Non polar (Np)	Non polar (Np)	Low polar (Lp)	Low polar (Lp)	Polar (Lp)	Polar (P)	Polar (P)	High polar (Hp)	Extremely polar (Exp)	Medium polar (Mp)	Non polar(Np)	Low polar (Lp)
	DB-1	DB-5	DB-17	DB-210	DB-225	CPS-1	SP-2331	CP-Sil 88	LC-Smectic	Equity-5	DB-5ms	VF-5ms	VF-Xms	DB-XLB	βDEXcst	LC-50	SLBIL61	SLBIL76	SLBIL111	Dioxin	BPX-DXN	5Sil MS
12389-PeCDF	28	28	28	28	28	27	27	27	28	28	28	28	28	28	28	28	27	28	28	28	28	28
123468-HxCDF	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
134678-HxCDF	2	2	3	3	2	2	2	2	3	2	3	2	2	2	2	2	3	3	3	2	3	2
124678-HxCDF	3	3	2	4	3	4	4	4	2	3	2	3	3	3	3	3	4	4	4	3	2	3
134679-HxCDF	4	4	4	2	4	3	3	3	4	4	4	4	4	4	4	4	2	2	2	4	4	4
124679-HxCDF	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
124689-HxCDF	6	6	6	6	7	8	9	9	6	6	6	6	6	6	6	6	7	7	8	6	6	6
123467-HxCDF	7	7	8	8	10	10	10	10	10	7	7	7	7	7	7	9	11	11	11	7	7	7
123478-HxCDF	8	8	7	7	6	6	6	6	8	8	8	8	8	8	8	7	8	8	7	8	8	8
123678-HxCDF	9	9	9	10	9	9	8	8	9	9	9	9	9	9	9	8	9	9	9	9	9	9
123479-HxCDF	10	10	10	9	8	7	7	7	11	10	10	10	10	10	10	11	6	6	6	10	10	10
123469-HxCDF	11	11	12	12	13	12	12	12	7	11	11	11	11	11	11	10	13	13	13	11	11	11
123679-HxCDF	12	12	11	11	11	11	11	11	12	12	12	12	12	12	12	12	10	10	10	12	12	12
234678-HxCDF	13	14	14	14	15	16	16	16	13	14	14	13	13	13	14	13	16	16	16	14	13	14
123689-HxCDF	14	13	13	13	12	13	13	13	14	13	13	14	14	14	13	14	12	12	12	13	14	13
123789-HxCDF	15	15	15	16	14	14	14	14	16	15	15	15	15	16	15	16	14	14	14	16	16	15
123489-HxCDF	16	16	16	15	16	15	15	15	15	16	16	16	16	15	16	15	15	15	15	15	15	16

Appendix-2.4: RO of different columns for TeCDD

	Non polar (Np)	Fairly polar (Fp)	Polar (P)	Polar (P)	Polar (P)	Polar (P)	Polar (p)	Polar (p)	Non polar (Np)	Non polar (Np)	Non polar (Np)	Low polar (Lp)	Low polar (Lp)	Polar (Lp)	Polar (P)	Polar (P)	High polar (Hp)	Extremely polar (Exp)	Medium polar (Mp)	Non polar(Np)	Low polar (Lp)	Non polar (Np)
	DB-1	DB-5	DB-17	DB-210	DB-225	CPS-1	SP-2331	CP-Sil 88	LC-Smectic	Equity-5	DB-5ms	VF-5ms	VF-Xms	DB-XLB	βDEXcst	LC-50	SLBIL61	SLBIL76	SLBIL111	Dioxin	BPX-DXN	5Sil MS
1368-TeCDD	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
1379-TeCDD	2	2	2	2	2	2	2	2	8	2	2	2	2	2	2	3	2	2	2	2	2	2
1369-TeCDD	3	3	3	3	4	4	4	4	2	3	3	3	3	3	3	2	4	4	4	3	3	3
1469-TeCDD	4	7	11	12	17	17	17	18	7	7	4	4	4	4	8	10	18	18	17	4	6	4
1247-TeCDD	5	4	5	4	5	5	5	5	11	4	6	5	5	7	4	6	5	5	5	7	4	5
1248-TeCDD	6	5	6	5	6	6	6	6	6	6	7	6	6	8	5	5	6	6	6	8	5	6
1378-TeCDD	7	6	4	6	3	3	3	3	14	5	9	9	9	9	9	11	3	3	3	9	7	9
1246-TeCDD	8	8	9	8	10	9	12	12	4	8	5	7	7	5	6	7	9	9	9	5	8	7
1249-TeCDD	9	9	10	9	11	11	13	13	5	9	8	8	8	6	7	9	10	10	10	6	9	8
1268-TeCDD	10	10	8	7	7	7	7	7	9	10	10	10	10	10	10	8	7	7	7	10	10	10
1478-TeCDD	11	11	7	11	8	8	8	8	3	11	11	11	11	11	11	4	8	8	8	11	11	11
1279-TeCDD	12	12	13	15	15	16	16	16	16	12	12	12	12	12	14	15	16	17	16	12	12	12
1269-TeCDD	13	15	19	18	20	20	20	20	13	13	13	13	14	14	16	14	20	21	20	13	14	13
1236-TeCDD	14	14	17	13	16	15	15	15	10	14	15	15	15	15	13	12	12	12	12	15	15	15
1237-TeCDD	15	16	14	14	12	13	10	10	20	16	16	16	16	16	18	16	15	15	14	16	16	16
1234-TeCDD	16	13	16	10	14	10	11	11	12	15	14	14	13	13	12	13	11	11	11	14	13	14
1238-TeCDD	17	17	15	16	13	14	14	14	17	17	17	17	17	17	15	17	13	13	13	17	17	17
2378-TeCDD	18	18	12	17	9	12	9	9	22	18	19	19	19	19	19	21	14	16	15	19	19	19
1239-TeCDD	19	19	18	19	19	19	19	19	15	19	18	18	18	18	17	18	19	20	19	18	18	18
1278-TeCDD	20	20	20	20	18	18	18	17	18	20	20	20	20	20	20	19	17	19	18	20	20	20
1267-TeCDD	21	21	21	21	21	21	21	21	19	21	21	21	21	21	21	20	21	22	21	21	21	21
1289-TeCDD	22	22	22	22	22	22	22	22	21	22	22	22	22	22	22	22	22	14	22	22	22	22

Appendix-2.5: RO of different columns for PeCDF

	Non polar (Np)	Fairly polar (Fp)	Polar (P)	Polar (P)	Polar (P)	Polar (P)	Polar (p)	Polar (p)	Non polar (Np)	Non polar (Np)	Non polar (Np)	Low polar (Lp)	Low polar (Lp)	Polar (Lp)	Polar (P)	Polar (P)	High polar (Hp)	Extremely polar (Exp)	Medium polar (Mp)	Non polar(Np)	Low polar (Lp)	Non polar (Np)
	DB-1	DB-5	DB-17	DB-210	DB-225	CPS-1	SP-2331	CP-Sil 88	LC-Smectic	Equity-5	DB-5ms	VF-5ms	VF-Xms	DB-XLB	βDEXcst	LC-50	SLBIL61	SLBIL76	SLBIL111	Dioxin	BPX-DXN	5Sil MS
12468-PeCDD	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	1	1	1	1	1	1	1
12479-PeCDD	2	2	2	2	2	2	2	2	2	2	2	2	2	2	1	2	2	2	2	2	2	2
12469-PeCDD	3	3	4	4	6	6	6	7	3	3	3	3	3	3	4	5	6	6	6	3	3	3
12368-PeCDD	4	4	3	3	3	3	3	3	4	4	4	4	4	4	3	3	3	3	3	4	4	4
12478-PeCDD	5	5	5	5	4	4	4	4	5	5	5	5	5	5	5	4	4	4	4	5	5	5
12379-PeCDD	6	6	6	6	5	5	5	5	12	6	6	6	6	6	6	11	5	5	5	6	6	6
12369-PeCDD	7	7	7	8	8	9	9	9	6	7	7	7	8	7	7	6	8	8	8	8	7	7
12467-PeCDD	8	8	9	9	10	11	10	10	9	8	8	8	7	8	8	7	10	9	10	7	8	8
12489-PeCDD	9	9	10	10	11	10	11	11	8	9	9	9	9	9	9	9	11	11	11	9	9	9
12347-PeCDD	10	10	8	7	7	7	7	6	10	10	10	10	10	11	10	10	7	7	7	11	10	10
12346-PeCDD	11	11	12	11	12	12	12	12	7	11	11	11	11	10	11	8	12	12	12	10	11	11
12378-PeCDD	12	12	11	12	9	8	8	8	13	12	12	12	12	13	12	13	9	10	9	13	12	12
12367-PeCDD	13	13	13	13	13	13	13	13	11	13	13	13	13	12	13	12	13	13	13	12	13	13
12389-PeCDD	14	14	14	14	14	14	14	14	14	14	14	14	14	14	14	14	14	14	14	14	14	14

Appendix-2.6: RO of different columns for HxCDF

	Non polar (Np)	Fairly polar (Fp)	Polar (P)	Polar (P)	Polar (P)	Polar (P)	Polar (p)	Polar (p)	Non polar (Np)	Non polar (Np)	Non polar (Np)	Low polar (Lp)	Low polar (Lp)	Polar (Lp)	Polar (P)	Polar (P)	High polar (Hp)	Extremely polar (Exp)	Medium polar (Mp)	Non polar(Np)	Low polar (Lp)	Non polar (Np)
	DB-1	DB-5	DB-17	DB-210	DB-225	CPS-1	SP-2331	CP-Sil 88	LC-Smectic	Equity-5	DB-5ms	VF-5ms	VF-Xms	DB-XLB	βDEXcst	LC-50	SLBIL61	SLBIL76	SLBIL111	Dioxin	BPX-DXN	5Sil MS
124679-HxCDD	1	1	1	1	1	2	2	2	1	1	1	1	2	1	1	1	1	1	1	1	1	2
124689-HxCDD	2	2	2	2	2	3	3	3	2	2	2	2	1	2	2	2	2	2	2	2	2	1
123468-HxCDD	3	3	3	3	3	1	1	1	4	3	3	3	3	3	3	3	3	3	3	3	3	3
123679-HxCDD	4	4	4	4	4	4	4	4	5	4	4	4	4	4	5	4	4	4	4	4	4	4
123689-HxCDD	5	5	5	5	5	5	5	5	6	5	5	5	5	6	4	5	5	5	5	5	5	5
123469-HxCDD	6	6	6	6	8	8	8	8	3	6	6	6	6	5	6	6	8	8	8	6	6	6
123478-HxCDD	7	7	7	7	6	6	6	6	7	7	7	7	7	7	7	7	6	6	6	7	7	7
123678-HxCDD	8	8	8	8	7	7	7	7	8	8	8	8	8	8	8	8	7	7	7	8	8	8
123467-HxCDD	9	9	10	9	10	10	10	10	9	9	9	9	9	9	9	9	10	10	10	9	9	9

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