DISCUSSION ON THE SEPARATION OF 2378-SUBSTITUTED ISOMERS FROM ALL 136 TETRA- THROUGH OCTA- POLYCHLORINATED DIBENZO-P-DIOXINS AND DIBENZOFURANS ON SI-ARYLENE STATIONARY PHASE.

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Introduction

USEPA methods 1613b[1] and 8290A[2] for the analysis of polychlorinated dibenzo-p-dioxins (PCDD) and polychlorinated dibenzofurans (PCDF) (tetra- through the octa-) have been developed based on the use of a J&W Scientific DB-5 (5% phenyl methyl silicone) GC column with a DB-225 or SP-2330 cyanopropyl column for the confirmation of 2378-TCDF. Out of the 136 PCDD/PCDF congeners there are 17 congeners with chlorines in the 2378-positions that are considered to be of toxicological significance and typically used for the Toxic Equivalence (TEQ) calculations as well as for calculating TM-17 which is the sum of all 17 toxic congeners required for regulatory reporting in the US. In order to establish the "true" TEQ and TM-17 values, an accurate determination of isomer-specific concentrations of all 17 2378-substituted dioxins and furans is required. Previously it has been shown that all 2378-substituted dioxin and furans can be separated from closely eluting isomers using a combination of two sets of non-polar and polar stationary phase columns[3]. One set consists of DB-5 (Agilent HP-5ms, Restek Rtx-5ms, Supelco Equity-5) and DB-225 GC columns and another set would have a combination of DB-5ms (Phenomenex ZB-5ms, Varian VF-5ms) with Supelco SP-2331 (Table 1). It has been shown that Silphenylene Silicon co-polymer (Si-Arylene) based GC columns exhibit superior separation towards 2378-substituted isomers compared to 5% phenyl methyl silicone stationary phase[3,4]. Moreover the TEQ data from VF-5ms suggested that this column has the least amount of co-elution with 2378-substituted isomers[3]. In this study, we are presenting the data for the most widely used GC columns for dioxins and furan analysis in our laboratory, DB-5ms, and VF-5ms, containing Si-Arylene stationary phase. These data are expressed in series of GC mass chromatograms and are considered to be the most comprehensive to date with regard to separation of all 136 PCDD/PCDF isomers and provide a valuable addition to the USEPA methods 1613b[1] and 8290A[2].

Materials and Methods

Standards preparation.

The window standard was prepared by mixing Cambridge Isotope Laboratories (CIL) (Andover, MA, USA) ED-1732-B and EF-1731-B window defining mixtures that contain the first and last eluting isomers for each of the tetra- through hepta- congener groups on DB-5 GC column. In addition, native OCDD and OCDF were added to the above mixture in similar concentrations. All 128 qualitative standards tetra- through hexa- PCDD/PCDF with an approximate concentration of 25 ng/mL for each congener in nonane were obtained from CIL as well. The origin of these standards, the isomer nomenclature assignment, analysis on variety of GC columns including the DB-5 stationary phase, as well as review of previously published reports have been well described by Ryan and co-workers[5]. The 128 qualitative standards were used to prepare 38 individual mixtures (number of TCDF isomers) for the analysis. A solution containing all seventeen 2378-substitured ^{13}C-labeled congeners was prepared "in house" and added into each of 38 individual mixtures prior to the analysis to monitor retention time shift during the GC/HRMS analysis.

GC/HRMS measurements.

All PCDD/PCDF measurements were performed using two double focusing high resolution magnetic sector mass spectrometers: ThermoFinnigan MAT-95S and MAT-95XP. The MAT-95S was equipped with Trace GC 2000 and the MAT-95XP was coupled with Trace GC Ultra. Typical positive electron ionization (EI) conditions were electron energy of 42 eV with the ion source temperature of 270°C and acceleration voltage of 4700 V. Mass spectrometric data were obtained in the selected ion monitoring (SIM) mode at resolution of > 10,000 (10% valley). For the GC analyses three "Series 5" fused-silica capillary columns were used: Equity-5 (60 m × 0.25 mm i.d. × 0.25 μm film) to confirm Ryan et al. original data[5]; DB-5ms (60 m × 0.25 mm i.d. × 0.25 μm film) and VF-5ms (60 m × 0.25 mm i.d. × 0.26 μm film) to obtain all 136 PCDD/PCDF relative retention time data in order to compare the selectivity with respect to each other. DB-5ms was deployed on the Trace GC 2000

running at constant pressure of 36 psi and oven temperature programmed from 160°C (1.5 min hold) to 220°C at 30°C/min and held for 25 min, to 240°C at 5.0°C/min and held for 7 min, to 310°C at 5°C/min and held for 9 min at 310°C. VF-5ms was installed on the Trace GC Ultra running at constant pressure of 42 psi and oven temperature programmed from 160°C (1 min hold) to 215°C at 27.5°C/min and held for 18 min, to 225°C at 5.0°C/min and held for 8 min, to 310°C at 5°C/min and held for 6 min at 310°C. These GC conditions were selected to be close to the USEPA method 1613b[1] and optimized for 2378-TCDD separation (at least 25% valley).

Results and Discussion

It has been shown that Equity-5 GC column has selectivity towards dioxin and furan separation identical to DB-5 GC column[3,4], therefore it is analytically interchangeable and was used to confirm the retention order of all 136 PCDD/PCDF standards based upon Ryan et al. original data obtained on DB-5 (30 m × 0.32 mm i.d. × 0.25 μm film) GC column[5]. Considering the differences in the GC column dimensions, it was found that the retention time order data agreed well to each other with two exceptions: 12678-PnCDF co-elutes with 12367-PnCDF instead of 12379-PnCDF; and 12489-PnCDF co-elutes with 23467-PnCDF instead of 23478-, 12679-, and 12369-PnCDF as described by Ryan and co-workers[5]. In addition it was confirmed in our laboratory that the DB-5ms retention time order data for TCDF, PnCDF and HxCDF agreed well with CIL Material Specification[6].

The relative separation performance between DB-5ms and VF-5ms GC columns have had been compared based upon mass chromatograms utilizing a "visualization approach"[3]. As anticipated, a DB-5ms GC column shows exactly the same PCDD/PCDF retention time order compared to a VF-5ms, however it exhibits a somewhat different degree of separation towards dioxins and especially furans; e.g., some isomers co-eluting on DB-5ms GC column were found to have some degree of separation on VF-5ms column as shown on Figures 1-3.

Table 1. Isomeric specific separation of 2378-substituted PCDD/PCDF on variety GC columns.

PCDD/PCDF	DB-5, HP-5MS Rtx-5MS,Equity-5	DB-225	DB-5MS ZB-5MS	SP-2331	VF-5ms CP-Sil 8 CB/MS
2,3,7,8-TCDD	+ +	+ -	+ +	+ -	+ +
1,2,3,7,8-PnCDD	+ +	- -	+ +	- -	+ +
1,2,3,4,7,8-HxCDD	+ +	+ +	+ +	+ +	+ +
1,2,3,6,7,8-HxCDD	+ +	+ +	+ +	+ +	+ +
1,2,3,7,8,9-HxCDD	- -	+ +	+ +	+ +	+ +
1,2,3,4,6,7,8-HpCDD	+ +	+ +	+ +	+ +	+ +
OCDD	+ +	+ +	+ +	+ +	+ +
2,3,7,8-TCDF	- -	+ +	+ +	+ -	+ +
1,2,3,7,8-PnCDF	+ +	- -	+ +	- -	+ +
2,3,4,7,8-PnCDF	- -	+ +	- -	+ +	+ -
1,2,3,4,7,8-HxCDF	- -	+ +	+ +	- -	+ +
1,2,3,6,7,8-HxCDF	+ +	- -	+ +	+ +	+ +
2,3,4,6,7,8-HxCDF	+ +	- -	- -	+ +	- -
1,2,3,7,8,9-HxCDF	+ +	+ +	- -	+ +	+ -
1,2,3,4,6,7,8-HpCDF	+ +	+ +	+ +	+ +	+ +
1,2,3,4,7,8,9-HpCDF	+ +	+ +	+ +	+ +	+ +
OCDF	+ +	+ +	+ +	+ +	+ +

++ : Baseline separation exists or can be achieved by adjusting the GC conditions;
+ - : Quantifiable result exists (separation that allows the peak area measurement) or can be achieved by adjusting the GC conditions;
- - : Interference present (pure co-elution).

As described in the literature[3,4] both DB-5ms and VF-5ms GC columns showed satisfactory separation towards all 2378-substituted PCDD and these data have been confirmed in this study using CIL qualitative standards. As predicted based upon our dual column analysis data[3] 2378-TCDF exhibits baseline separation on a VF-5ms GC

column from other closely eluted isomers and DB-5ms yields quantifiable results (Figure 1). Therefore there is no need to confirm 2378-TCDF on a complimentary GC column when strictly following the USEPA methods 1613b[1] and 8290A[2]. Unfortunately, these methods give no guidance for the determination of accurate concentration values for penta- and hexa-isomers where interferences are present, even though the majority of the TEQ may come from the higher chlorinated dioxins and furans depending on the sample matrix[3].

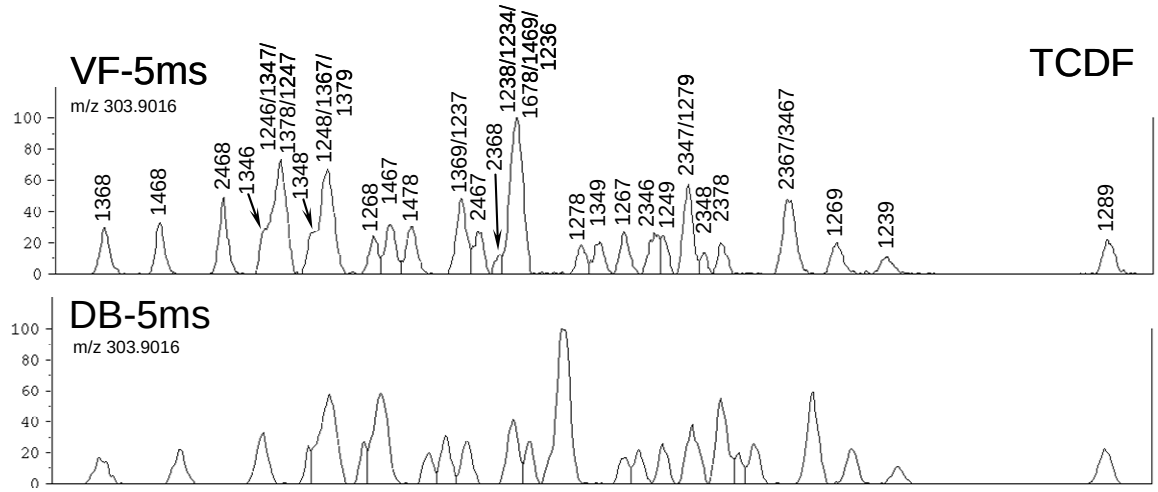

Figure 1. TCDF mass chromatograms showing the difference in separation between DB-5ms and VF-5ms GC columns.

The most challenging task in the entire PCDD/PCDF analysis with respect to true TEQ determination is the separation of 23478-PnCDF from other closely eluting isomers, 12369- and 12489-PnCDF. Our data indicate, again, as it was predicted[3,4], that the VF-5ms produces the least amount of interference, where the DB-5ms exhibits pure co-elution with 12489-PnCDF. Both GC columns of interest demonstrate either baseline or near baseline separation of 123478-, 123678-HxCDF, however they could not resolve 234678- from 123689-HxCDF. A unique feature observed for VF-5ms column is that it has some degree of separation between 123789- and 123489-HxCDF. Our internal laboratory data demonstrate that this particular separation can be improved better than 50% valley between two peaks at the cost of significantly increasing the analysis time depending upon the manufacture's GC column actual plate number.

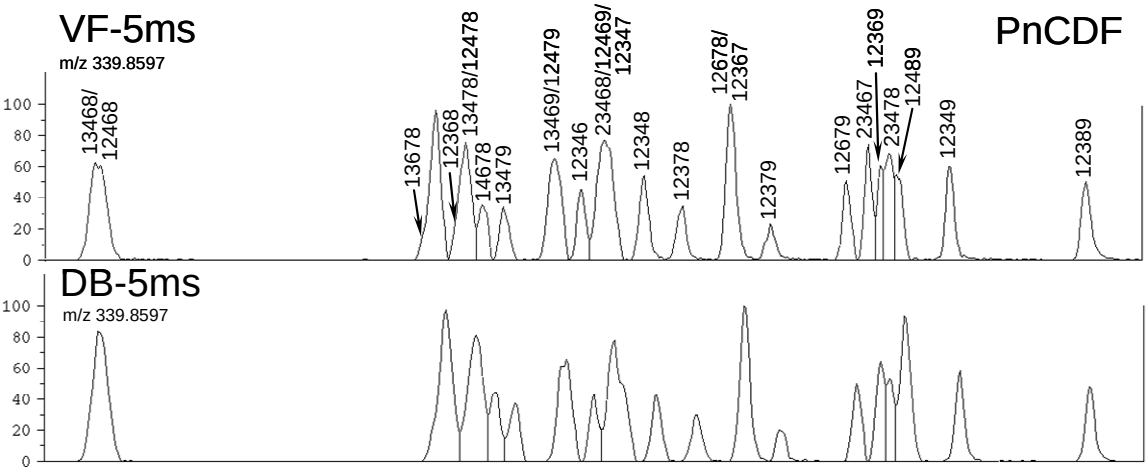

Figure 2. PnCDF mass chromatograms showing the difference in separation between DB-5ms and VF-5ms GC columns.

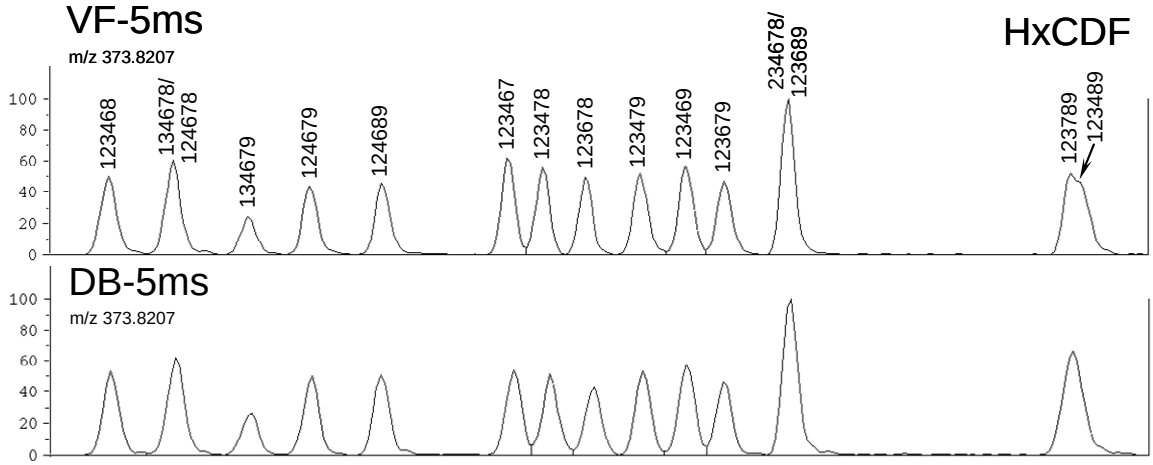

Figure 3. HxCDF mass chromatograms showing the difference in separation between DB-5ms and VF-5ms GC columns.

These discrepancies towards PCDD/PCDF separation on DB-5ms versus VF-5ms GC columns could be explained either in the slightly different composition of the stationary phases (amount/composition of Si-Arylene co-polymer to match DB-5 polarity), film thickness, and/or some variation in manufacturing process. Based upon data reported here we strongly believe that the development of a single GC column for the separation of 16 2378-substitured dioxins and furans (except 234678-HxCDF) is feasible by either further "tuning" the Si-Arylene stationary phase or perhaps even by increasing the film thickness. We think that this approach would be economically more reasonable compared to the alternative of creating either the instrumentation with dual GC ovens[7] that are equipped with the columns of different polarities or using a comprehensive GC × GC methodology[8,9].

Having one specific GC column developed to separate all 2378-substituted PCDD/PCDF will significantly improve the data quality of the USEPA methods[1,2] and at the same time will lead to a more cost efficient (more samples for the same cost) operation. Furthermore it would make the data obtained by USEPA methods[1,2] more comparable with other state-of-the-art analytical methods and better suitable for the Principal Component Analysis (PCA) or similar type of data assessment and allow a better judgment about the source of dioxins and furans in a particular sample.

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References

[1] Method 1613 Revision B, Tetra – Through Octa – Chlorinated Dioxins and Furans by Isotope Dilution HRGC/HRMS, EPA, October 1994, Washington, D.C.
[2] SW-846 8290A, Polychlorinated Dibenzodioxins and Polychlorinated Dibenzofurans by High-Resolution Gas Chromatography/High-Resolution Mass Spectrometry, EPA, February 2007, Washington, D.C.
[3] Fishman VN, Martin GD, Lamparski, LL, J. Chromatogr. A 1139 (2007) 285-300.
[4] Fishman VN, Martin GD, Lamparski, LL, J. Chromatogr. A 1057 (2004) 151-161.
[5] Ryan JJ, Conacher HBS, Panopio LG, Lau BP-Y, Hardy JA, J. Chromatogr. 541 (1991) 131-183.
[6] Material Specification, Cambridge Isotope Laboratories (Andover, MA, USA).
[7] Moche W, Thanner G, in R. Hites (Ed.), Modification of MAT-90 for Use with Two Gas Chromatographs (Organohalogen Compounds Vol. 31) Proceedings of the 17th International Symposium on the Chlorinated Dioxins and Related Compounds, Indianapolis, Indiana, USA, 1997.
[8] Focant J-F, Reiner EJ, MacPherson K, Kolic T, Sjodin A, Patterson Jr. DG, Reese SL, Dorman FL, Cochran J, *Talanta* 63 (2004) 1231.
[9] Korytar P, Danielsson C, Leonards PEG, Haglund P, De Boer J, Brinkman UATh, *J. Chromatogr. A.* 1038 (2004) 189.