RETENTION TIME PROFILING FOR ALL 136 TETRA- THROUGH OCTA-CHLORINATED DIOXINS AND FURANS ON A UNIQUE, LOW-BLEED, THERMALLY-STABLE GAS CHROMATOGRAPHY COLUMN

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Abstract
Individual standards of all 136 tetra- through octa- chlorinated dioxins and furans were used to profile a gas chromatography column with unique selectivity. Fourteen out of seventeen toxic 2378 chlorine substituted congeners were separated from potential interfering congeners. The most significant coelution on the GC column was for 12367 and 12378 penta- chlorinated dioxins, due to the relatively high toxicity of the 12378 congener and the tendency for 12367 to be present in environmental samples.

Introduction
The typical approach to chlorinated dioxin and furan analysis is to determine the 17 tetra- through octa-chlorinated congeners that have 2378 chlorine substitutions because of their toxicity. After what is usually extensive sample preparation, including multiple cleanup steps to remove potential interferences, high resolution gas chromatography – high resolution mass spectrometry (GC-HRMS) is employed to provide the highest sensitivity and selectivity (e.g. EPA Methods 1613, Revision B[1] and/or 8290A[2]). Highly efficient capillary gas chromatography contributes significant selectivity since there are multiple congeners possible for every homolog group, except octa-. For example, there are 22 tetra- chlorinated dioxins (TCDD) and 38 tetra- chlorinated furans (TCDFs). EPA methods specify 60m 5% diphenyl-type columns (e.g. Rtx-5) to be used for the bulk of dioxin and furan work (and for 2378 TCDD specificity) and a cyanopropyl-containing phase (e.g. Rtx-225, Rtx-2330, SP-2331) for TCDF specificity.

Recently in a comprehensive study, Fishman, Martin, and Lamparski[3] compared a wide variety of GC columns for the separation of dioxins and furans across multiple sample types. Not surprisingly they found that none of the columns tested could separate all of the 17 congeners routinely determined from other coeluting congeners. Additionally they came to the conclusion that the current state of the art for unequivocal determination of the 2378 dioxins and furans is a set consisting of "5-type" and cyanopropyl columns, the same set that has been used for many years! A concession was proposed for single column analysis where a laboratory knew the sample type and could separate the congeners that contributed most to overall toxicity. The authors also considered the question of whether it is possible to develop a GC column that could resolve all 2378 congeners and mentioned that one of the hurdles is the unavailability (commercially) of all 136 tetra- through octa- dioxins and furans.

In a landmark 1991 paper by Ryan, Conacher, Panopio, Lau, Hardy, and Masuda[4], all 136 tetra- through octa-chlorinated dioxins and furans were used to characterize nine different GC stationary phases. In this paper, we use the dioxins and furans from that study to record retention time data for a GC column with unique selectivity. We report on the 2378 coelutions encountered under simple linear temperature programming conditions, and we comment on the significance of those coelutions for environmental samples.

Materials and Methods
Standards and Samples
Individual standards of all 49 tetra- through octa- chlorinated dibenzo-p-dioxins and all 87 tetra- through octa-chlorinated dibenzofurans (approximately 25 ng/mL for each congener in nonane) were from Cambridge Isotope Laboratories, Inc. (USA). The full origin details for the materials for these standards are contained in the Ryan paper[4]. Eleven mixes of congeners were prepared for analysis. A Sample Fortification Solution (Cambridge

Isotope Laboratories, Inc.) containing several ^{13}C-labeled chlorinated dioxins (2378, 12378, 123678, 1234678, 12346789) and furans (2378, 12378, 123478, 1234678) was added to each of the mixes prior to analysis to monitor any retention time drift and to use for relative retention time calculations. A fly ash extract was analyzed as a "real world" sample.

GC-HRMS

The 40m x 0.18mm x 0.18µm Rtx-Dioxin2 GC column (Restek Corporation, USA) was installed in an Agilent 6890 GC (USA) connected to a Waters AutoSpec Ultima HRMS (UK). The mass spectrometer was operated at over 10,000 resolving power using electron ionization (40 eV) under selected ion recording conditions. The source temperature was at 280°C. One microliter splitless injections were performed at 280°C. The GC oven was held at 120°C for 1 min, programmed at 10°C/min to 160°C, and then programmed at 4°C/min to 320°C where it was held for 4 min. Helium carrier flow was constant at 1 mL/min.

Results and Discussion

The 11 standard mixes were designed (based on their reported retention order on a 5% diphenyl 95% dimethylsiloxane GC column) so that the possibility of any coelution between congeners from the same homolog group was eliminated. This approach drastically reduced the overall time needed to do the GC-HRMS analysis versus analyzing individual standards. The GC oven program separated the dioxins: 2378, 123478, 123678, 123789, 1234678, 12346789, and the furans: 2378, 12378, 23478, 123478, 123678, 1234678, 1234789, 12346789. A coelution for a 2378 dioxin was: 12378 and 12367, while coeluting furans were: 123689 and 234678; and 123489 and 123789. **Figures 1 and 2** are fly ash chromatograms for the TCDDs and TCDFs, respectively. 2378 TCDD is separated from other TCDD congeners, including 1237, 1238, and 1239, which can be problematic on 5-type columns. While it could be argued that the separation of 2378 TCDF from 3467 TCDF is insufficient for this fly ash sample **(Figure 2)**, a perpendicular drop integration or peak height measurement (permitted in 8290A) should result in relatively good quantification, and the use of the cyanopropyl containing column can be avoided. It should be noted that the results reported here are from a simple, linear GC oven program, where the primary concern was to fully profile the Rtx-Dioxin2 for all 136 tetra- through octa- chlorinated dioxin and furan elution orders. No attempt at this point was made to optimize the separation. In fact, the Fishman paper[4] illustrates a better 2378 TCDF separation on Rtx-Dioxin2 than shown here (almost baseline), albeit the 3467 TCDF concentration in his example was less than for the fly ash sample in **Figure 2**. Optimized separations for 2378 TCDF on Rtx-Dioxin2 (resulting in accurate quantitative data for reference samples) have been reported elsewhere[5,6].

A separation that needs improvement (seen as a coelution by Fishman[4]) is shown in **Figure 3** for 23478 and 12349 penta- chlorinated furans. These congeners are almost separated in this sample, and with some optimization could likely be baseline resolved. Quantification errors even with the current separation for 23478 furan for samples from this fly ash source would likely be trivial.

The most significant coelution for the Rtx-Dioxin2 is that for 12378 and 12367 penta- chlorinated dioxins due to 12378 having the same relative toxicity (according to the World Health Organization) as 2378 TCDD. Because these congeners can be separated on 5-type GC columns there are data in the literature that can highlight the magnitude of this coelution[7-9]. Depending on the sample (air, soil, sediment, fish, shellfish, crab), 12367 ranged from essentially zero to about the same concentration as 12378. In some cases then, the toxicity of a sample analyzed on Rtx-Dioxin2 could be overestimated due to coelution of 12367 and 12378. For this reason, attempts were made to develop a separation (through manipulation of carrier flow and GC oven program) for 12378 and 12367 penta- chlorinated dioxins. Unfortunately, all efforts failed, so this remains a significant issue for the Rtx-Dioxin2. The same situation exists for the other 2378 hexa- chlorinated furan coelutions on Rtx-Dioxin2, those of 123689 and 234678, and 123489 and 123789. Looking at the congener-specific data[7-9] shows concentrations of 123689 that are approximately 10 times less than 234678 to just over the same concentration (although this estimate may be high due to a coelution of 123469 and 123689[7,8]). The situation is more dramatic for 123489, where it can range from about 5 times less in concentration versus 123789 to about 10 times greater than the 123789 value. In fact, for some samples (soil, sediment, shellfish) the 123489 concentration was remarkably constant at about four times the concentration of 123789[9]. As with the penta-

chlorinated dioxin pair, attempts to alter GC conditions to separate these furans failed.

For the first time since 1991, all 136 tetra- through octa- chlorinated dioxins and furans have been used to characterize a GC column. Even with the coelutions mentioned in this paper the Rtx-Dioxin2 can be considered a good column for dioxin and furan analysis, especially because of its specificity for TCDD and TCDF. The availability of standards will allow profiling of additional GC columns/phases, including any new ones specifically manufactured for single column dioxin and furan analysis.

References

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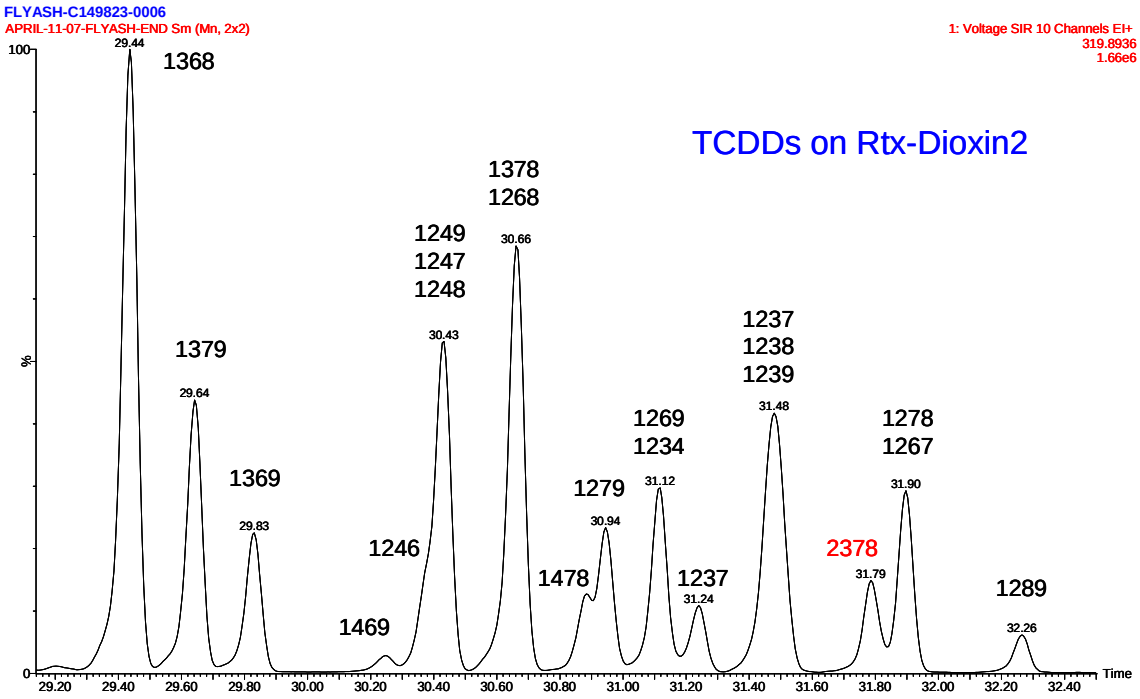

Figure 1. GC-HRMS chromatogram of tetra- chlorinated dioxins in fly ash on Rtx-Dioxin2.

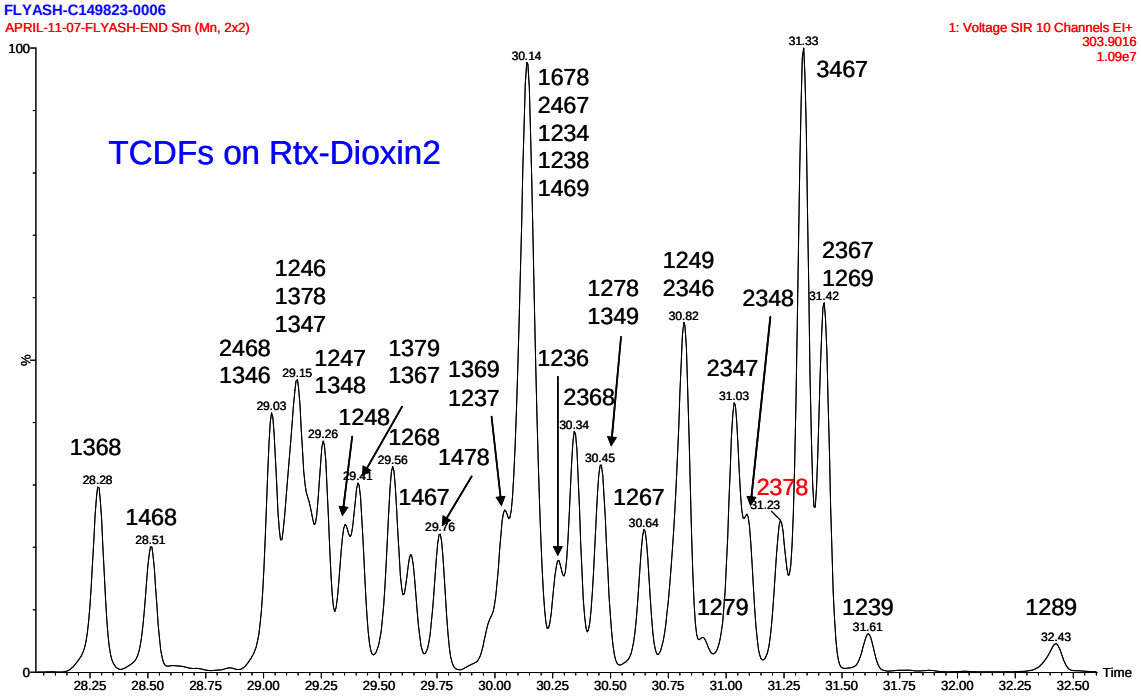

Figure 2. GC-HRMS chromatogram of tetra- chlorinated furans in fly ash on Rtx-Dioxin2.

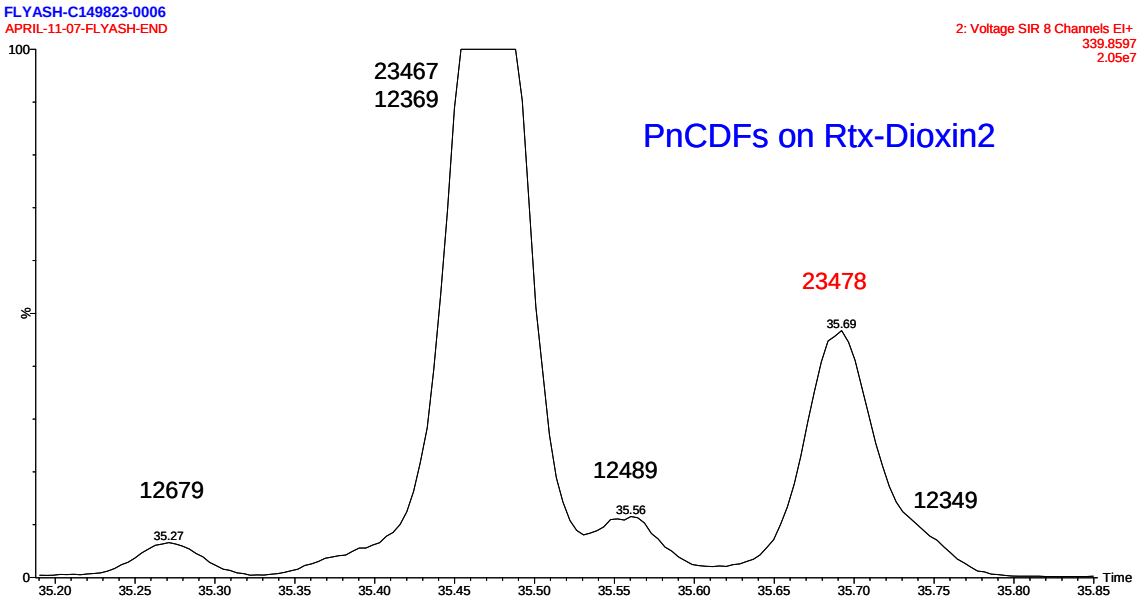

Figure 3. GC-HRMS chromatogram of select penta- chlorinated furans in fly ash on Rtx-Dioxin2.