

## PRACTICAL APPLICATIONS OF DUAL COLUMN CHROMATOGRAPHY OF THE ANALYSIS OF DIOXIN-LIKE AND PERSISTENT ORGANIC POLLUTANTS

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### **Introduction**

The significance of toxic congener identification to characterize data is evident by the Toxic Equivalence Factor (TEF) Scheme, adopted in 1988 by NATO<sup>1</sup> and later by the WHO (World Health Organization)<sup>2</sup>. The TEF scheme converts concentrations of Polychlorinated dibenzo-p-dioxins (PCDDs), Polychlorinated dibenzofurans (PCDFs) and Dioxin-like Polychlorinated biphenyls (DLPCBs) into a standard unit of measure, relative to 2,3,7,8-tetrachlorinated dioxin (TCDD). This data reporting format has been exclusively adopted by international regulatory agencies.

Methods to analyze environmental samples for the determination of PCDDs and PCDFs have been published since the early 1970's<sup>3</sup>. Since then, evolution of analytical methods to incorporate Isotope Dilution Quantification techniques enhanced identification and reporting of congener specific data. Commercial standard solutions were also modeled to include congeners identified by NATO and WHO as having toxic significance. In addition, during this time, improvements in Mass Spectrometry (from Low Resolution to High Resolution) provided greater sensitivity (detection limits) and selectivity (resolution) for analytes.

More recently, method development has been driven by advances in Gas Chromatography techniques to reduce analytical run times. Shorter narrower Gas Chromatograph (GC) columns reduce analysis times from 50-80% when used in Fast GCs capable of column head pressures >60psi and temperature ramp rates >75EC/min. This has been demonstrated for a variety of organic analytes<sup>4,5</sup>. Furthermore, current GC designs allow for dual autosampler/injectors and have been shown to facilitate simultaneous analysis, using Fast GC techniques, of different sample extract fractions for the determination of PCDDs/PCDFs and DLPCBs<sup>6</sup>.

During the past 30 years, method developments for the analyses of PCDD/Fs in environmental samples have significantly improved data quality and reduced analysis times. Today the role of congener specific data is becoming more important as a consequence of environmental legislation and regulatory guidelines which are set based on toxic equivalence. Analyte specific phases for PCDD/Fs can provide for improved separation of toxic PCDD/F congeners known to co-elute on conventional liquid phases (5% phenyl and DB225 confirmational) and the potential to apply Fast GC techniques to generate a dual column application for simultaneous analyses and confirmation data for PCDDs and PCDFs.

### Method

All extracts analyzed for this study were prepared using either acid digestion followed by liquid-liquid extraction (biota) or soxhlet extraction (soil/sediment and flyash). Sample extracts were further prepared using a 3 stage cleanup (silica/alumina and carbon) as described elsewhere<sup>7</sup>. All instrument analyses was performed using a Micromass Autospec-Ultima-NT HRMS tuned to a minimum 10,000 resolving power, running MassLynx (V4.0) software. Chromatographic columns were interfaced into the HRMS ion source. Experimental liquid phases used were a 60M RTX-DIOXIN (DX) phase, .25mm i.d. , 0.25µm liquid phase thickness and a 60M RTX-experimental (expt) phase, 0.25mm i.d., 0.25 µm liquid phase thickness. GC experiment conditions were as follows:

		<b>RTX-DX</b>	<b>RTX-expt</b>
Intial Temp:	(°C)	130	130
Hold:	(min.)	1.0	1.0
Ramp1:	(°C/min.)	52	40
Final Temp.:	(°C)	200	200
Hold:	(min.)	10.2	0
Ramp2:	(°C/min.)	2.9	3
Final Temp.:	(°C)	235	235
Hold :	(min.)	10	0
Ramp3:	(°C/min.)	6.9	5
Final Temp.:	(°C)	300	300
Hold:	(min.)	24	20

Gas flow conditions for both columns were set to constant flow at 1.5mL/min.

### Results and Discussion

Chromatographic resolution specifications for 2,3,7,8-TCDD (25% valley with 1237/1238) were easily met on the two experimental columns. Of the 17 toxic congeners, 1,2,3,7,8,9-HxCDD, 1,2,3,4,7,8-HxCDF, 2,3,4,7,8-PCDF and 2,3,7,8-TCDF have known co-elutions on conventional 5% phenyl phase GC columns. The 2,3,7,8-TCDF congener can co-elute with up to 5 other TCDF isomers on this phase<sup>8</sup>.

Table I compares data for 2,3,7,8-TCDF quantified from 3 different flyash extracts run on 3 different liquid phases. The results show that separation of 2378-TCDF is comparable between the confirmation column (DB225) and RTX-DX and both results show great correlation with median data from an interlaboratory study<sup>9</sup>.

Table I-Flyash

	<b>2378-TCDF pg/g</b>			<b>ILS (n&gt;100)</b>
	<b>5% phenyl</b>	<b>DB225</b>	<b>RTX-DX</b>	<b>MEDIAN</b>
<b>Ash A</b>	250	21	30	<b>28</b>
<b>Ash B</b>	2100	300	378	<b>390</b>
<b>Ash C</b>	170	19	28	<b>27</b>

Tables II & III compare data for 2,3,7,8-TCDF and 2,3,7,8-TCDD between the 5%phenyl liquid phase, the DB225 and a different experimental liquid phase. Investigation of this liquid phase also showed results for 2,3,7,8-TCDF which suggest equivalent isomer separation between the DB225 and the experimental phase. In addition data for 2,3,7,8-TCDD is shown as quantified from these liquid phases.

**Table II - 2,3,7,8-TCDF (pg/g)**

	5%phenyl	DB225	RTX-expt	Certified
				Value
<b>WMS-01</b>	78	46	47	<b>52.5± 16</b>
<b>EC-2(DX-1)</b>	88	n/a	37	89 (±44)*
<b>Sediment</b>	37	19	19	
<b>NIST 1974</b>	4.7	n/a	3.3	
<b>Flyash</b>	240	38	32	
<b>Biota-1</b>	1	1.3	0.8	
<b>Biota-2</b>	4.3	4.3	2.2	

**Table III - 2,3,7,8-TCDD (pg/g)**

	5%phenyl	DB225	RTX-expt	Certified
				Value
<b>WMS-01</b>	21	23	14	<b>17.7±5.6</b>
<b>EC-2 (DX-1)</b>	240	n/a	284	263(±53)
<b>Sediment</b>	8.5	8.1	9	
<b>NIST 1974</b>	nd	nd	nd	
<b>Flyash</b>	5.6	6.7	4.4	
<b>Biota-1</b>	nd	nd	nd	
<b>Biota-2</b>	nd	nd	nd	

**Table IV - 1,2,3,4,7,8-HxCDF (pg/g)**

	5%phenyl	RTX-expt	Certified
			Value
<b>WMS-01</b>	80	50	67±24
<b>Sediment</b>	290	210	
<b>Flyash</b>	570	200	
<b>EC-2 (DX-1)</b>	780	630	714 (±276)*

**Table V - 1,2,3,7,8,9-HxCDD (pg/g)**

	5%phenyl	RTX-expt	Certified
			Value
<b>WMS-01</b>	27	29	17.3±8
<b>Sediment</b>	5	6.3	
<b>Flyash</b>	40	38	
<b>EC-2 (DX-1)</b>	65	38	53 (±24)*

Tables IV, and V report values for 1,2,3,4,7,8-HxCDF and 1,2,3,7,8,9-HxCDD, respectively, as calculated in a variety of environmental sample extracts analyzed on 3 different liquid phases. All data values show excellent correlation with certified values and/or values obtained using DB225.

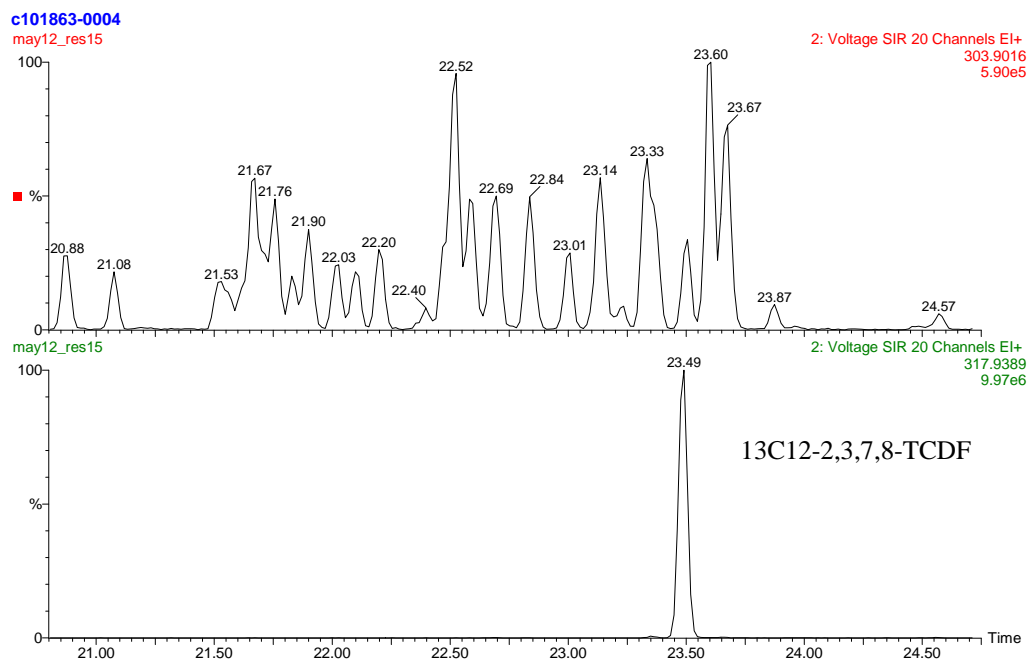
The top chromatogram in Figure 1 shows separation of 2,3,7,8-TCDF isomers in a flyash extract on the RTX-expt2 column. The <sup>13</sup>C<sub>12</sub>-2,3,7,8-TCDF ion channel is also included as a marker for the native analogue. Separation of the 2,3,7,8-TCDF from adjacent eluting isomers is close to 100% resolution.

### Conclusions

The experimental liquid phases investigated in this study show improved or equivalent separation of 2,3,7,8-substituted (toxic) PCDD/F congeners relative to conventional 5%phenyl e.g. DB5, RT-5 and confirmation (DB225) liquid phases. Thermal stability of these experimental liquid phases was compatible with to 5% phenyl liquid phases (≥300°C) and much higher than conventional DB225 phase (240°C).

The use of these columns in standard (0.25) and/or microbore (0.18, -0.10) i.d.'s would allow for a PCDD/F confirmation column to be installed into a HRMS allowing for analysis and confirmation to be carried out simultaneously or without column changeover.

Figure I – TCDF ion channel for Flyash extract as separated on RTX-expt liquid phase.



## References

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