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METHOD ENHANCEMENT TECHNIQUES FOR THE ANALYSIS OF DIOXIN-LIKE COMPOUNDS IN ENVIRONMENTAL SAMPLES

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Introduction

Polychlorinated dibenzo-p-dioxins (PCDDs) and other related toxic organic compounds have been detected in the environment for decades[1]. The high cost of analysis for this group of compounds oftentimes limits the number of samples analyzed and amount of data produced. Analytical methods are developed to meet required data quality objectives using four main method attributes: sensitivity, selectivity, speed and cost. The optimum method provides maximum sensitivity, selectivity and speed of analysis at the lowest cost. For most methods, one or more of these attributes is compromised to maximize the most critical one. In the case of polychlorinated dioxins, ultimate sensitivity and selectivity is required while both speed (analysis time) and cost are significantly higher than for any other organic analytical method. Over the past few years a number of analytical techniques such as Fast GC, Dual Column GC analysis, analyte specific GC phases and time of flight (TOF) mass spectrometry have been used to reduce analysis times and costs without significant losses in sensitivity and selectivity [2,3].

Chromatographic separation prior to detection is required for an accurate determination of analyte concentrations when isomers, congeners or structurally related compounds are analyzed even if a very selective technique such as high resolution mass spectrometry (HRMS) is used for analyte detection. Complete chromatographic separation of 2,3,7,8 containing congeners is required for PCDD analysis. In order to obtain complete separation of the analytes of interest on a GC column, each analyte passing through the column must interact with the stationary phase to a different degree and therefore spend a different amount of time on the column than other compounds in the sample. The degree to which a specific analyte (peak) is retained on the column by the stationary phase depends upon the column's internal diameter (i.d.), stationary phase composition, film thickness, temperature, carrier gas type and carrier gas flow rate[4-6]. If the phase ratio (ratio of gas-phase to liquid-phase volumes), which is proportional to the ratio of the i.d. of the column to film thickness of the stationary phase is kept constant, the column temperature and carrier gas flow rates can be adjusted so that relative retention times of the compounds of interest remain constant from column to column[8-10]. Fast GC using narrower GC columns and thinner films can produce essentially the same chromatography with analysis times 2 to 5 times shorter than conventional wider bore columns[11, 12].

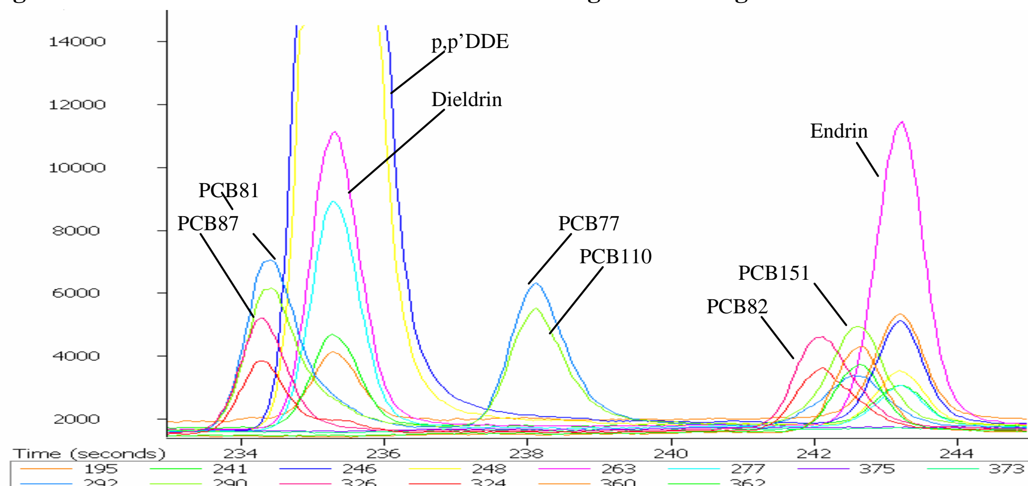
As chromatographic run times are shortened using microbore columns, conventional mass spectrometers (quadrupole and magnetic sector) cannot scan fast enough ($\geq$ 1 second per spectrum) to produce enough data points to accurately define a chromatographic peak. TOF mass spectrometers (TOFMS) can operate at scan speeds of more than 100 spectra per second. This provides a number of advantages over other mass spectrometers such as the capability to deconvolute mass chromatograms for compounds with retention times that differ by more than about 150 ms. If the mass spectrum of the analyte of interest contains at least 1 unique peak (m/z ion), the peaks belonging to that compound can be deconvoluted and subtracted from other coeluting and background compounds.

Methods and Materials
The GC/FOTMS work was carried out on a Hewlett Packard 6890 with a split-splitless injector coupled to a Leco Pegasus II Time-of-Flight mass spectrometric detector operated in full-scan mode (20 spectra/second acquisition). Injector temperature: 275 ^{o}C, transfer line: 300 ^{o}C, source: 250 ^{o}C, carrier gas: He; GC column: 10 m, DB-5, 0.18 mm id, 0.18 µm film thickness with a 1m deactivated fused silica retention gap**,** constant pressure mode (20.6 psi.). Initial temp: 75 ^{o}C, hold 0.16 min, ramp to 125 ^{o}C at 94 ^{o}C/min, ramp to 300 ^{o}C at 31°C/min, hold 1 min; injection vol.: 1.0 µL. Total run time: 7.5 minutes.

The dual column analysis used to analyse PCDDs, PCDFs and Dioxin-like PCBs in a single analysis was performed using an HP 6890+ GC coupled to a Micromass Autospec-Ultima-NT HRMS @ 10,000+ RP. Two columns, a 20M Restek Rtx-5, 0.1 mm i.d., 0.1Φm film thickness and a 40M Restek Rtx-5, 0.18mm, 0.18Φm were installed in parallel into the HRMS ion source. The GC conditions were as follows: 40M (front injector - 280EC) Rtx-5, 0.18mm, 0.18Φm, 380kPa, (constant pressure) and 20M (back injector - 280EC, Rtx5, 0.1mm, 0.1Φm, 610 kPa, (constant pressure). The GC program was as follows: Initial temp 130EC hold 1min, 52EC/min. to 200EC hold 0min, 2.9EC/min. to 235EC hold 10.2 min., 6.9EC/min, to 300EC, hold 3.5 min.

Results and Discussion
One of the most difficult problems to resolve in analytical chemistry is obtaining the required selectivity with sufficient sensitivity to detect the analytes of interest. Analytical methods for the analysis of polychlorinated dioxins and furans require the seventeen 2,3,7,8 containing toxic congeners to be separated from the remaining 193 congeners. The difference in toxicity of closely eluting congeners can differ by up to 6 orders of magnitude. This is also the case for dioxin-like PCBs such as the coeluting PCB77 / PCB110 and PCB 81 / PCB 87 pairs. These compounds are

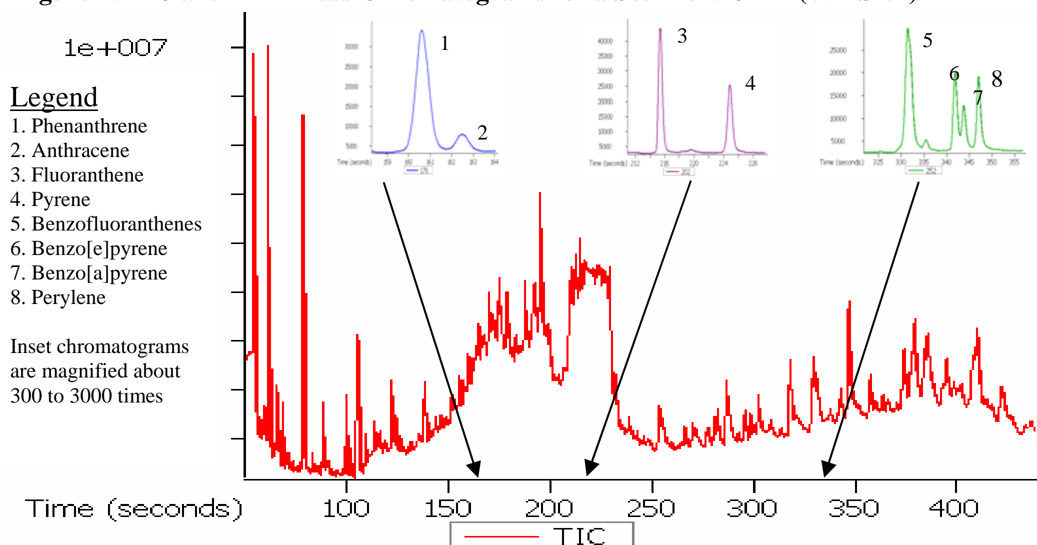
Figure 1:Deconvoluted GC/TOFMS Mass Chromatogram of a Vegetation Reference



typically analysed using GC/HRMS. Unfortunately, GC/HRMS (at 10,000 resolving power) must be operated in the single ion monitoring (SIM) mode to meet sensitivity requirements. In order to resolve the above coeluting PCB pairs, sample extracts must be reanalyzed on a different chromatographic column phase or by full scan mass spectrometry where there is a significant

difference in mass spectra. Figure 1 shows a chromatographic segment from the analysis of a reference material containing PCBs, PAH and OC pesticides. This chromatogram shows that 6 PCBs and 3 OC pesticides (9 compounds in total) can be separated and quantified within 10 seconds using GC/TOFMS. Both PCB 81 / 87 and PCB 77 / 110 pairs are resolved. Figure 2 shows the total GC/TOFMS ion chromatogram (TIC) of a sediment reference material and 3 magnified (300 to 3000 times) mass chromatographic segments for a number of PAH. Phenanthrene and Anthracene are resolved (first inset chromatogram) using this method despite the highly complex and concentrated background. Unfortunately, the Benzofluoranthenes cannot be completely resolved using a 5% phenyl chromatographic phase. Their mass spectra are essentially identical and therefore deconvolution as shown in figure 1 for the PCBs is not possible. The benzofluoranthenes (B[b]F, B[j]F and B[k]F) can all be resolved on a 50% phenyl phase.

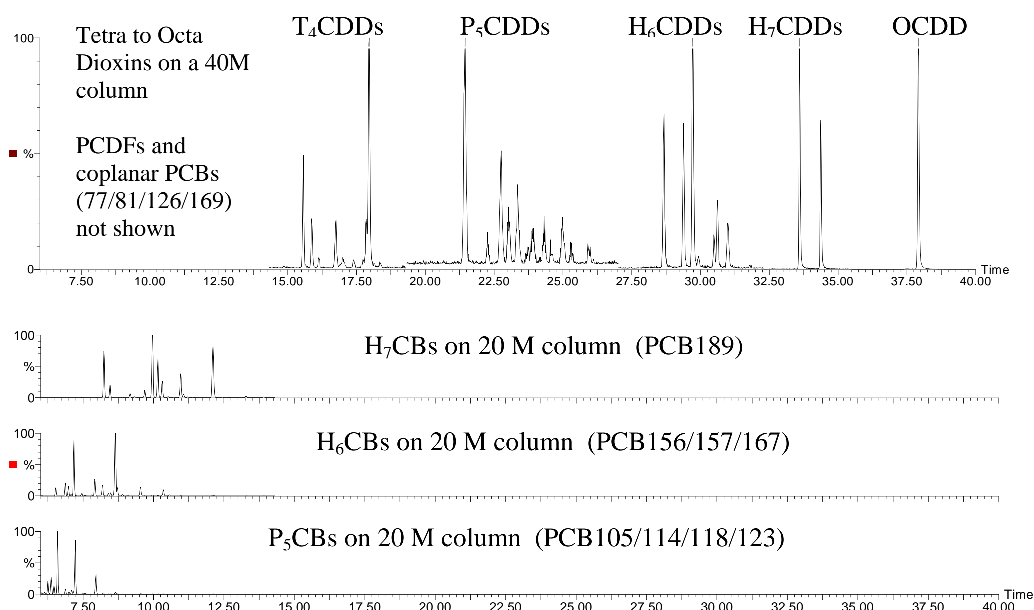
Figure 2: TIC and PAH Mass Chromatograms for a Sediment CRM (WMS-01)



Analysis times are at least doubled when confirmation on a secondary column is required. If only one instrument is available for analysis, column change over can significantly increase sample turnaround times due to increased instrument downtime from column switching. Microbore GC columns have much smaller gas loads than conventional 0.25 mm id and 0.32 mm id GC columns. Inserting both a 0.10 mm id and 0.18 mm.id GC column into the mass spectrometer ion source can result in a carrier gas load that is not significantly different from that of a single 0.25 mm id column. If both columns are the same phase or are temperature compatible, the analysis of split sample extracts or the analysis and confirmation of a single extract can be completed in a single analytical run. Figure 3 shows the analysis of the dioxin fraction (seventeen 2,3,7,8-substituted dioxin/furan congeners and 4 coplanar WHO PCBs obtained by reverse elution on activated carbon) on a 40M 0.18mm id, 0.18 µm DB-5 column and mono-ortho WHO PCB fraction (8 congeners – obtained during sample preparation by forward elution on activated carbon) on a 20M 0.10mm id, 0.10 µm DB-5 column. Dual column analysis is an alternative approach to peak deconvolution using GC/TOFMS shown above where PCB81 and PCB77 are in the dioxin fraction and PCB110 and PCB 87 are in the mono-ortho fraction. Alternatively, if both columns are thermally compatible, the analysis and confirmation can be completed in the same analytical run (e.g. 2,3,7,8-TCDF). Both DB-5 ms and RTX-500 phases[13] have demonstrated the ability to

chromatographically separate 2,3,7,8-containing congeners from non 2,3,7,8-containing congeners.
Several method enhancement techniques have been utilized to achieve the required selectivity, sensitivity and analytical time savings in the analysis of dioxin-like compounds. Using GC/TOFMS, structurally similar coeluting compounds were deconvoluted; various types of analytes (including dioxin-like PCBs, organochlorine pesticides and PAH) were analysed simultaneously; and enhanced sensitivity and selectivity for target compounds were achieved despite highly complex environmental matrix backgrounds. As an alternative to peak deconvolution, dual-column GC/HRMS analysis can be used to fully resolve and quantify coplanar and mono-ortho dioxin-like PCBs from separate fractions within the same analytical run.

Figure 3: GC/HRMS Dual Column Analysis of Dioxins and WHO PCBs in NIST 1944



1 Norstrom, R.J., Clark, T.P. and Weseloh, D.W., In Hazarous Contaminants in Ontario: Human Environmental Effects, (1985) 88-86.
2. MacPherson K.A, Brunato R, Chen,T, Bogard, MA and Reiner E.J, . Organohalogen Compounds, (1999) **40,** 19.
3. Dimandja M-J, Grainger J, and Patterson DG Jr, Organohalogen Compounds, (1999) **40,** 23
4. Reed,G.L., Clark-Baker,K. and McNair;H.M., *J. Chrom. Sci*., (1999), **37**, 300.
5. van Ysacker,P.G., Janssen,H.G., Snijders,H.M.J., Cramers,C.A., (1995) *J. High Resol. Chromatogr*., **18**, 397.
6. Dorman;F., The Advantage - Restek, Winter, (1998), 1.
7. Giddings;J.C., *Anal. Chem*., (1962) **34** (3), 314.
8. Hinshaw,J.V.; LC-GC, (1995)**13** (12), 994, (1995).
9. Blumberg;L.M., *J. High Resol. Chromatogr*., (1997), **20**, 597.
10. Blumberg;L.M., *J. High Resol. Chromatogr*., (1999), **20**, 67.
11.van Es,A, Janssen,J., Cramers,C.and Rijks;J, (1988), *J. High Resol Chromatogr.& Chromatogr. Communications*, **11**, 852.
12. Sellers,K., The Advantage - Restek, Winter (2000), 13.

13. MacPherson,K.A., Kolic,T.M., Reiner,E.J. and Dorman,F., (2003) Organohalogen Compounds (in press).