CHROMATOGRAPHIC ENHANCEMENT TECHNIQUES FOR THE ANALYSIS OF PERSISTENT ORGANIC POLLUTANTS IN ENVIRONMENTAL SAMPLES

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Introduction

The analysis of Persistent Organic Pollutants (POPs) has changed significantly over the past decade. In the early 1990's, Organochlorine Pesticides, Polychlorinated Biphenyls (PCBs) and Polychlorinated dibenzo-p-dioxins (PCDDs) and Polychlorinated Dibenzofurans (PCDFs) were the main focus.[1] Beginning in the mid 1990's, a shift in the scope of analysis occurred. Laboratories began to analyse other related toxic organic compounds such as Brominated Flame Retardants (BFRs)[2], Polychlorinated Naphthalenes, fluorinated chemicals including Fluorinated Surfactants (FSs) and also began to focus on toxic subgroups. Twelve PCBs [3] and a number PCN congeners were identified as dioxin-like, 22 Toxpahene congeners were identified as toxic, persistent and bioaccumulative. For most laboratories this has created increased demands on sample preparation as well as instrument and analytical capacity. The high cost of analysis for many POPs often limits the number of samples analyzed and the amount of data produced. Each analytical method must be optimized to meet the required data quality objectives for: sensitivity, selectivity, speed (which also relates to analytical capacity) and cost. The optimum method provides maximum sensitivity, selectivity and speed of analysis at the lowest cost. Typically, one or more of these method 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 sacrificed. Due to chromatographic and instrumental limitations, historically, analyte groups have been analyzed in separate fractions or by separate methods. A variety of analytical techniques have been developed over the past few years to speed up

analysis and combine analyte scans. Fast GC [4-8], Dual Column GC analysis[9], analyte specific GC phases[10], comprehensive 2 Dimensional GC (2DGC)[11] and time of flight (TOF) mass spectrometry have been used to reduce analysis times, combine analyte scans and reduce analytical costs without significant losses in sensitivity and selectivity [2,3].

Chromatographic separation either prior to analysis (e.g. open column or HPLC) or during analysis (GC) is required for an accurate determination of analyte concentrations when isomers, congeners or structurally related compounds are analyzed. Oftentimes two or more GC columns are required to separate coeluting isomers. 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]. Fast GC is based on reducing column dimensions while keeping the phase ratio (the ratio of the i.d. of the column to film thickness of the stationary phase) constant, allows column temperature and carrier gas flow rates to be adjusted so that relative retention times of the compounds of interest remain constant from column to column[8-10]. Fast GC techniques can result in a reduction of analysis times of 2 to 5 when compared to conventional wider bore columns[11, 12].

Optimization of stationary phase composition for specific analyte groups can also significantly shorten analytical run times. Many chromatographic runs are extended or longer columns are required to separate critical pairs. Comprehensive 2DGC is a very powerful technique where two independent separations are used in one analysis. This provides significant enhancements in column capacities spreading out chromatographic peaks in 2 dimensions.

Fast chromatographic techniques require fast scanning detectors. For many applications 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. Time-of-flight 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/TOFMS work was performed 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 :m 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^{o}C/min, hold 1 min; injection vol.: 1.0 :L. 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.

The analyte specific column analysis was performed using an HP 6890N GC coupled to a Waters Autospec-Ultima-NT HRMS @ 10,000+ RP. GC Column: 60 m Restek Rtx-Dioxin2, 0.25mm i.d., 0.25Φm film thickness. GC conditions: Injector: 280EC Initial temp 130EC hold 1min, 40EC/min. to 200EC, hold 0min, 3.0EC/min. to 235EC, hold 0 min., 5.0EC/min, to 300EC, hold 5 min.

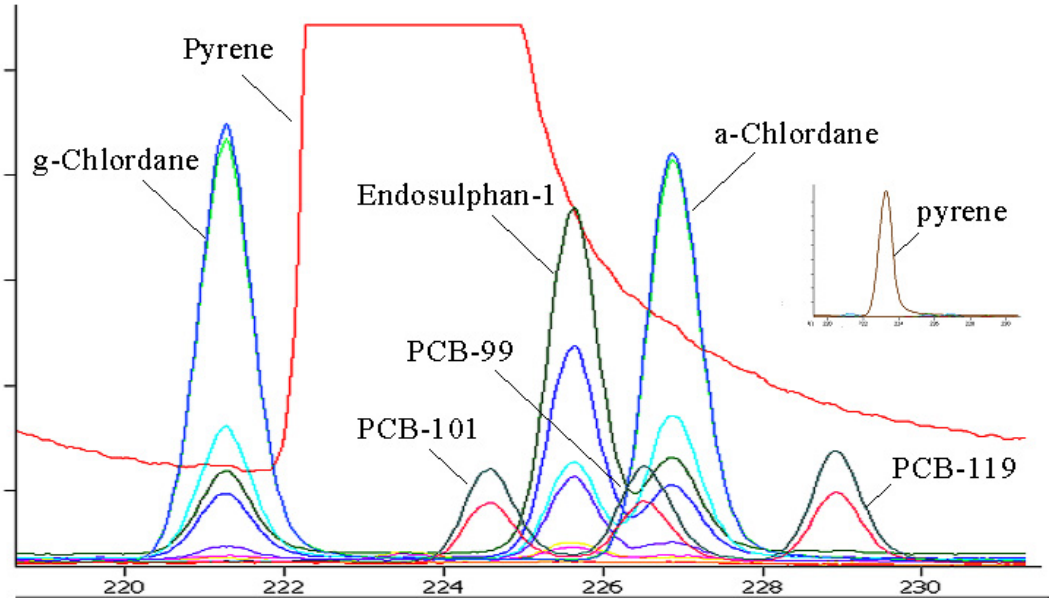
The GC/TofMS work was carried out on a Hewlett Packard 6890N with a split-splitless injector and Leco Quad Jet Modulator coupled to a Leco Pegasus IVD Time-of-Flight mass spectrometric detector operated in full-scan mode (50 spectra/second acquisition). Injector temperature: 250 ^{o}C, source: 225 ^{o}C, carrier gas: He with flow rate 1.5 ml/min; Primary GC column: 50 m, Rtx-1, 0.18 mm id, 0.18 :m film thickness, Secondary column: 5 m, Rtx-PCB, 0.18 mm id, 0.10 :m

film thickness installed after modulator, Modulation time: 2 Sec. Temperature offset 40 °C, Primary oven: Initial temp: 120 °C, hold 1.0 min, ramp to 330 °C at 5 °C/min, Secondary oven Initial temp: 140 °C, hold 1.0 min, ramp to 350 °C at 5 °C/min, Injection vol.: 1.0 :L. Total run time: 43 minutes.

Results and Discussion

Increasing instrument capacity can be accomplished by using selective detection to combine analytical runs of different analyte groups. Historically, PCBs, OC pesticides and PAH have been run separately. Figure 1 shows a section of the deconvoluted mass chromatogram of a vegetation reference material as analyzed by Fast GC-TofMS. The total runtime is 7.3 min in length. Over one hundred PCBs, OC pesticides and PAH can be separated and quantified simultaneously. Note that even when some compounds (see pyrene inset) are present at concentrations significantly higher than others, detection and quantification are still possible.

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



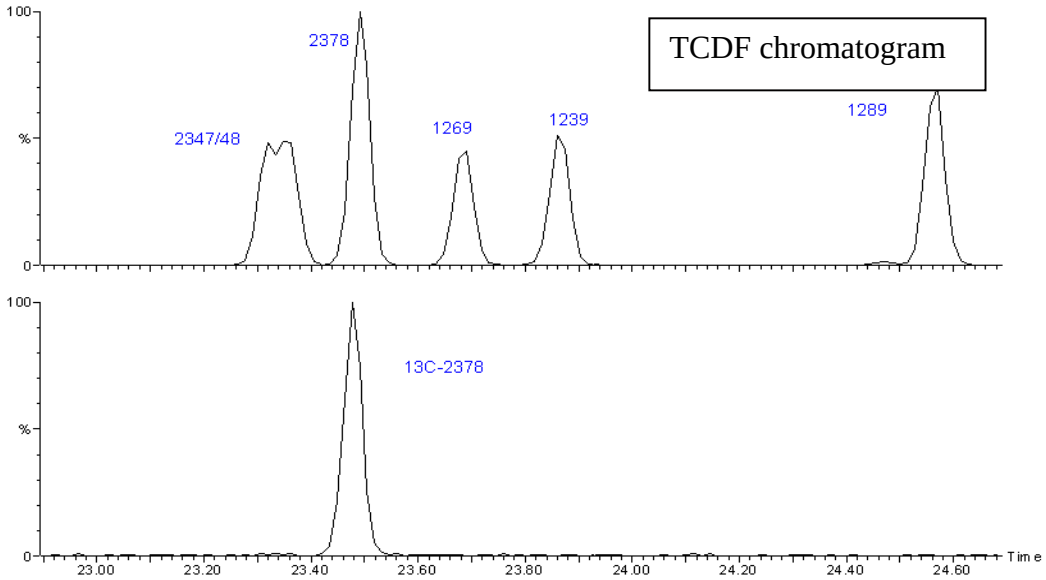
Analyte specific columns enable reduction in coelutions and analytical run times. Table 1 shows data for 5% Phenyl (DB-5), 50% cyanopropyl (DB-225 - the corresponding confirmation column), Rtx-Dioxin2 columns and the certified value for WMF-01 and the mean values for three intercalibration samples. Note that results on the Rtx-Dioxin2 column are in excellent agreement with the certified / mean values and the DB-225 confirmation column.

Table 1: Data comparison for 2,3,7,8-TCDF for various samples (in pg/g)

	5% Phenyl	DB-225	Rtx-Dioxin2	Certified / Mean Value
WMF-01	78	46	47	53
Sediment	37	19	19	23
Flyash	250	40	32	28
Biota	4.3	4.3	2.2	1.5

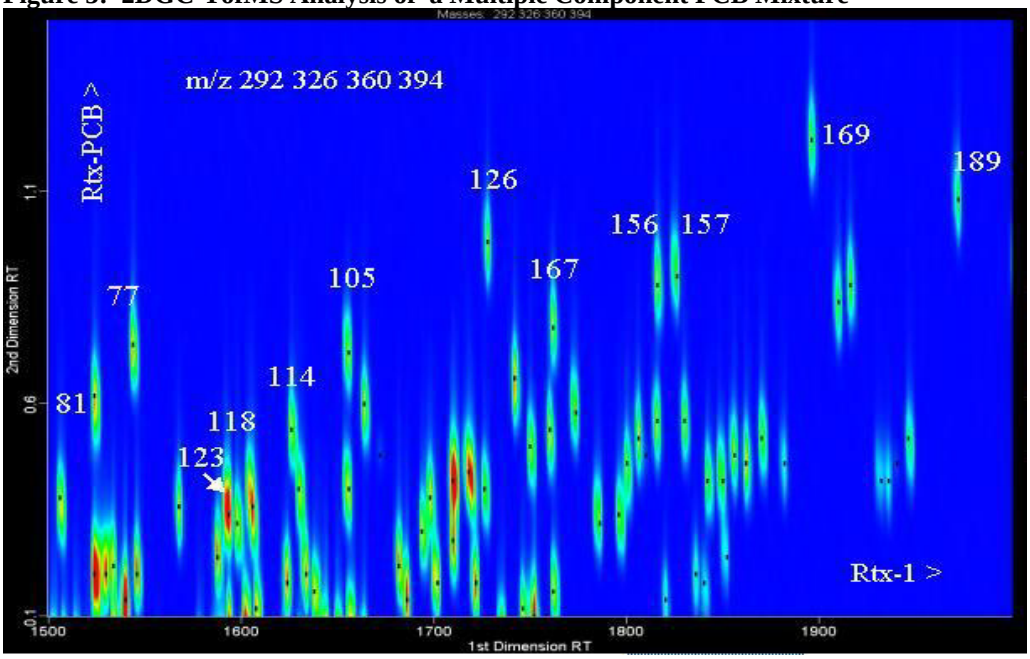
Figure 2 below shows the column performance mix for DB-225 analysed with a Rtx-Dioxin2 column. The Rtx-Dioxin2 column is clearly able to separate 2378-TCDF from any neighbouring congeners in the mix.
In flyash samples, an unidentified congener appears between 2378 and 1269, but does not effect the quantitative values of results. Currently, only 123789-HxCDF has been identified as having a coelution with a non 2378-substituted congener.

Figure 2: TCDF column performance mix analysed on a Rtx-Dioxin2 column



Due to the ability to use to stationary phases in series, comprehensive 2 dimensional gas chromatography can be used to separate many coelutions previously not possible in a single analytical run. Figure 3 shows the separation of the 12 WHO PCBs on an Rtx-1 / Rtx-PCB column set. These 12 PCB congeners appear as 2 bands with the coplanar PCB (77, 81, 126 and 169) band eluting later than the mono-ortho band (105, 114, 118, 123, 156, 157, 167, 189). Many of these congeners coelute with higher chlorinated congeners (e.g. 77 with 110 , 81 with 87) on a standard 5% phenyl phase, but can be chromatographically be isolated from coeluting congeners with comprehensive 2D GC.

Figure 3: 2DGC-TofMS Analysis of a Multiple Component PCB Mixture



Conclusions

A number of chromatographic enhancements can be made to improve resolution, reduce analysis times and increase peak capacity. Fast GC with TOFMS is able to deconvolute coeluting peaks with unique mass spectra separated by at least 150 ms. Analyte specific columns like Rtx-Dioxin2 can resolve all the 2,3,7,8 containing congeners except 1,2,3,7,8,9-HxCDF. This compound is typically present at the lowest concentration or not detected and makes up less than 1% of the TEQ. Comprehensive 2D GC can be used to separate a number of difficult coelutions for PCBs and other halogenated organics.

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