Development of a Comprehensive Multi-Dimensional GC Time-of-Flight Mass Spectrometric Method for the Simultaneous Determination of Substituted Polycyclic Aromatic Compounds, PCBs, Organochlorine Pesticides, PCDDs and PCDFs in Environmental Matrices

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Introduction

A new comprehensive multi-dimensional gas chromatographic time-of-flight mass spectrometric method (GCxGC-TOFMS) for the simultaneous determination of substituted polycyclic aromatic compounds (PAC), polychlorinated biphenyls (PCBs) and organochlorine pesticides (OC-pesticides) is presented. Methods were evaluated using different types of GC column combinations, temperature programs, peak modulation parameters and detection parameters. Overall, the new method has shown improved chromatographic resolving power, a remarkable increase in peak capacity and has significantly reduced the overall time required for sample analysis of all target compounds.

The Ontario Ministry of the Environment (MOE) methods currently used by our section include those for: (1) polycyclic aromatic hydrocarbons (PAH; methods PSA-PAH E3350 and E3425), which involve 40 minute-long analyses using GC-LRMS; (2) PCB congeners (method E3412), which involve 2 separate analyses using a dual column GC method with ECD taking approx 20 minutes; and (3) OC-pesticides (methods E3270, E3136 and E3155) which involve 2 separate analyses using a dual column GC method with ECD taking approx 12 minutes each.[1-3] In order to identify and quantify all of our target compounds, we must perform five separate analyses including the evaluation of all quality control measures on three different instruments. One of our preliminary objectives was to evaluate the use of GCxGC-TOFMS for the simultaneous analysis of all of these target analytes (PAH, PCBs and OC pesticides) in one single analysis. Another objective included the addition of several substituted polcyclic aromatic compounds including Nitro- and sulphur-substituted PAH (thia-arenes) and chlorinated dioxins and furans into our target analyte list.

The use of GCxGC-TOFMS technology allows us to maximize our chromatographic space in order to simultaneously determine hundreds of target analytes covering various compound classes. This is achieved by increasing peak capacity and resolving power while taking advantage of mass spectral peak deconvolution capabilities. GCxGC methods utilize the resolving power of two chromatographic columns in series, interfaced via a peak modulator to dramatically improve the separation of complex environmental mixtures in which matrix effects and target analytecoelutions are abundant. Peak modulation allows the refocusing of peaks, thereby enhancing sensitivity. Time-of-flight (TOF) mass spectrometers have the advantage of much faster scanning rates compared to conventional MS methods; scanning speeds of up to 500 spectra per second over the full mass range are obtainable by TOF-MS which ensures accurate characterization of narrow 2D GC peaks (peak widths on the order of 50 -250ms). Other advantages of TOFMS technology include mass spectral continuity and the potential for peak deconvolution during data analysis.[4,5]

Materials and Methods

The instrument used for this research is a Pegasus 4D GCxGC-TOFMS (Leco Corp., St. Joseph, MI). This system is based on a stationary quadruple jet dual-stage modulator; this consists of two cold nitrogen jets and two pulsed hot-air jets responsible for trapping and refocusing analyte peaks eluting from the ^{1}D column. The modulator is mounted in an Agilent 6890 gas chromatographic (GC) oven, and liquid nitrogen was used to cool the gas nitrogen cold jets. The GC is equipped with a split-splitless injection system. The source temperature used was 250ºC. Data processing and display of the GCxGC chromatograms were achieved using the LECOChromaTOF™ software (v2.2).

For isotope-dilution PAH analysis, solutions of deuterated analogues of target analytes were used as internal standards (crystalline solids from Cambridge Isotope Laboratories Inc., Andover, MA, USA). The isotope-dilution quantification standard mixture including 14 deuterium-labeled PAHs was used to fortify samples prior to

extraction.[1,3] Additional surrogates are added just prior to sample solvent evaporation (naphthalene-d_8) in order to track recovery from the sample preparation procedures. The internal standard, which was added to the extract just prior to analysis, was composed of ^{13}C-phenanthrene and perylene-d_{12}. Standard solutions containing substituted PAC including various nitro-PAH, thia-arenes (sulphur-substituted PAH), alkylated-PAH and quinones were used (described elsewhere).[6] Standard solutions utilized for PCB, PCDD, PCDF and organochlorine pesticide analyses are described in Ontario Ministry of the Environment analytical methods.[1-3]

Environmental samples and reference materials including river sediments were extracted using accelerated solvent extraction (Dionex ASE 200 - Accelerated Solvent Extractor, Dionex Canada Ltd., Oakville, ON, Canada) in dichloromethane. All solvents used were of glass-distilled analytical grade. AZymarkTurbovap LV evaporating system (Zymark Corp., Hopkinton, MA, USA) was used to evaporate solvents from samples. Details regarding the sample preparation methods have been reported elsewhere.[3]

Chromatographic column combinations here presented include the following two sets of analytical conditions: (A) a 30m DB17-HT, 0.25mm i.d., 0.15µm film thickness in the first dimension (J and W Scientific, Folsom, CA), a 2m RTX-PCB, 0.18mm i.d., 0.18µm film thickness (Restek Corp., Bellefonte, PA) connected using a deactivated universal pressfit glass union (Restek Corp.); (B) a 10m DB-5, 0.18mm i.d., 0.18µm film thickness (first dimension), a 2m DB17, 0.1mm i.d., 0.1µm film thickness (J and W Scientific, Folsom, CA) also connected using a universal pressfit glass union. Analyses were performed as follows: (A) 1 µL splitless injection in a Siltek Drilled Uniliner (Restek Corp.) 4mm i.d., injector 275ºC, modulation period 4 s., modulator temp. offset 20ºC, Helium flow 1.0mL/min, hot pulse 1.2sec, primary oven 90ºC (1min) to 300ºC (2.5ºC /min, hold 7min), secondary oven 30ºC temperature offset (except 315ºC final temperature), (B) 0.2 µL splitless injection in a 1.5mm double tapered liner (Chromatographic Specialties, Brockville, ON), injector 280ºC, Helium flow 0.8mL/min, modulation period 4 s, modulator temp. offset 60ºC, hot pulse 0.7sec, primary oven 90ºC (1min) to 150ºC (10ºC /min), to 250ºC (3ºC /min), to 300ºC (5ºC /min), secondary oven 40ºC temperature offset (except 320ºC final temperature). Transfer line temperature was 250ºC. The modulation period of 3-4 seconds generally resulted in the production of 4 to 5 slices across each first dimension peak.

Results and Discussion

Analyses revealed that most PCBs, OC pesticides and PACs may be selectively determined using GCxGC-TOFMS. The development of this method yields the simultaneous detection and quantification of over 150 different target analytes spanning five compound classes in one analysis. A determination of this scale is normally performed using five or more different analytical runs on different instruments; each analytical run taking from 23 - 100 minutes. Several orthogonal column combinations were evaluated. The best overall separation was achieved using a combination of a DB17-HT (first dimension) coupled with an RTX-PCB column (second dimension).

The top chromatogram in Figure 1illustrates the GCxGC-TOFMS total ion chromatogram (TIC) contour plot for the analysis of a contaminated river sediment (analytical conditions: A). This 2D chromatogram depicts the simultaneous separation and deconvolution of a mixture of polycyclic aromatic compounds (PAC), polychlorinated biphenyls (PCBs) and organochlorine pesticides (OC pesticides). In this case, proper orthogonal conditions have placed chemically-related compounds in ordered structures (or diagonal bands). These structures greatly facilitate group-type analysis and the provisional classification of unknowns. Various target compound classifications are illustrated on the chromatogram using circled classification regions. These classification regions have been identified through the analysis of standard solutions.

The lower chromatogram in Figure 1 depicts the same sediment sample run TIC visualised in three dimensions (a surface plot). Signal intensity is indicated by peak height and color variation. The hundreds of peaks seen in this surface plot suggest the presence of hundreds of analytes, many matrix-derived. The majority of these analytes would be masked if conventional analytical methods were used. Although many of the peaks partially or completely coelute in the first dimension, all of the target analytes are chromatographically separated in the second dimension and spectrally deconvoluted and can thereby be quantified individually.

Using conventional methods of gas chromatography with electron-capture detection or mass spectral detection, separation of PCB critical pairs often proves difficult. It is our goal to optimize chromatography in order to resolve the most highly toxic PCBs (those which exhibit dioxin-like toxicity) from other PCB congeners in complex mixtures. Using this GCxGC-TOFMS method, the majority of critical pairs were either chromatographically resolved or deconvoluted

(on the basis of mass spectral differences) enabling quantification of these PCBs up to deca-PCB 209. Critical coelutinganalyte pairs such as PCB-81/PCB-87 and PCB-77/PCB-110 (see Figure 2, top chromatograms depicting a standard solution) which are normally difficult to resolve (e.g., DB5 column, 1DGC) are easily separated in this analysis. PCBs 81 and 77 (marked with asterisks on the contour plot) are well known as having dioxin-like toxicity and as such, accurate quantification is desirable. In addition, dioxin-like PCB-126 was completely resolved from any coelutions (PCB-126/PCB-178 coelution is problematic on the DB-5 stationary phase). Other tentative PCB identifications will be confirmed using individual standards.

Coelutions of certain target PAH (e.g., phenanthrene/anthracene, benzofluoranthenes, benzo[*e*]pyrene B[*e*]P)/benzo[*a*] pyrene (B[*a*]P)) often prove difficult to resolve on conventional column stationary phases causing results to be biased high for those target analytes.[7] Accurate quantification of the individual benzofluoranthenes commonly provides a challenge (benzo[*b*]fluoranthene (B[*b*]F), benzo[*k*]fluoranthene (B[*k*]F) and benzo[*j*]fluoranthene (B[*j*]F)) two of which have been designated as priority PAH due to their carcinogenic and mutagenic properties (US EPA Method 610, World Health Organization, European Economic Community). This group of structural isomers cannot be resolved mass spectrometrically by conventional MS or by TOFMSdeconvolution software. Using this method, all of the benzofluoranthene target analytes were completely resolved to baseline (see Figure 2, middle chromatograms depicting a sediment sample). All substituted PAC from naphthalene (128 Da) to the dibenzopyrenes (302 Da) including various nitro-PAH, thia-arenes (sulphur-substituted PAH), alkylated-PAH and quinones were resolved and quantifiable. Calibration curves constructed using isotope-dilution PAH calibration standards for each priority PAH yielded correlation coefficients for linearity (R) exceeding 0.999 (linear range of 10 to 5000 pg injected).

The lower chromatograms in Figure 2 illustrate the separation of the seventeen 2,3,7,8-substituted PCDD and PCDF congeners. The contour plot depicts the separation achieved for the most critical hexa-substituted congeners. Further analyses of environmental samples for these congeners are being performed.

Overall TOF instrumental sensitivity was lower for high molecular weight planar compounds (above 250 Da) and peak-tailing was an issue for large planar aromatic compounds like PAH. It was determined that increasing the hot pulse time and transfer line temperature (300ºC) and utilization of the DB-17HT stationary phase provides an increase in sensitivity for high mass planar aromatics. These parameters also provide a reduction in chromatographic peak tailing and an increase in chromatographic resolution for critical compound pairs.

This “mega method” appears to have potential as a robust analytical method for the simultaneous analysis of substituted PACs, PCBs and OC pesticides and must be further evaluated with other samples from various environmental matrices. This method may potentially replace various laboratory analytical methods, or be shortened for use as a sample screening method for signature compounds, effectively reducing the sample preparation time, and the number of instruments and analytical runs required. Analytical conditions targeted to increase sensitivity for the determination of other compound classes, including dioxins, furans and dioxin-like compounds are currently under investigation.

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Figure 1: GCxGC-TOFMS total ion chromatogram of a contaminated river sediment: 2D contour plot showing target compound classifications (top), 3D surface plot (bottom).

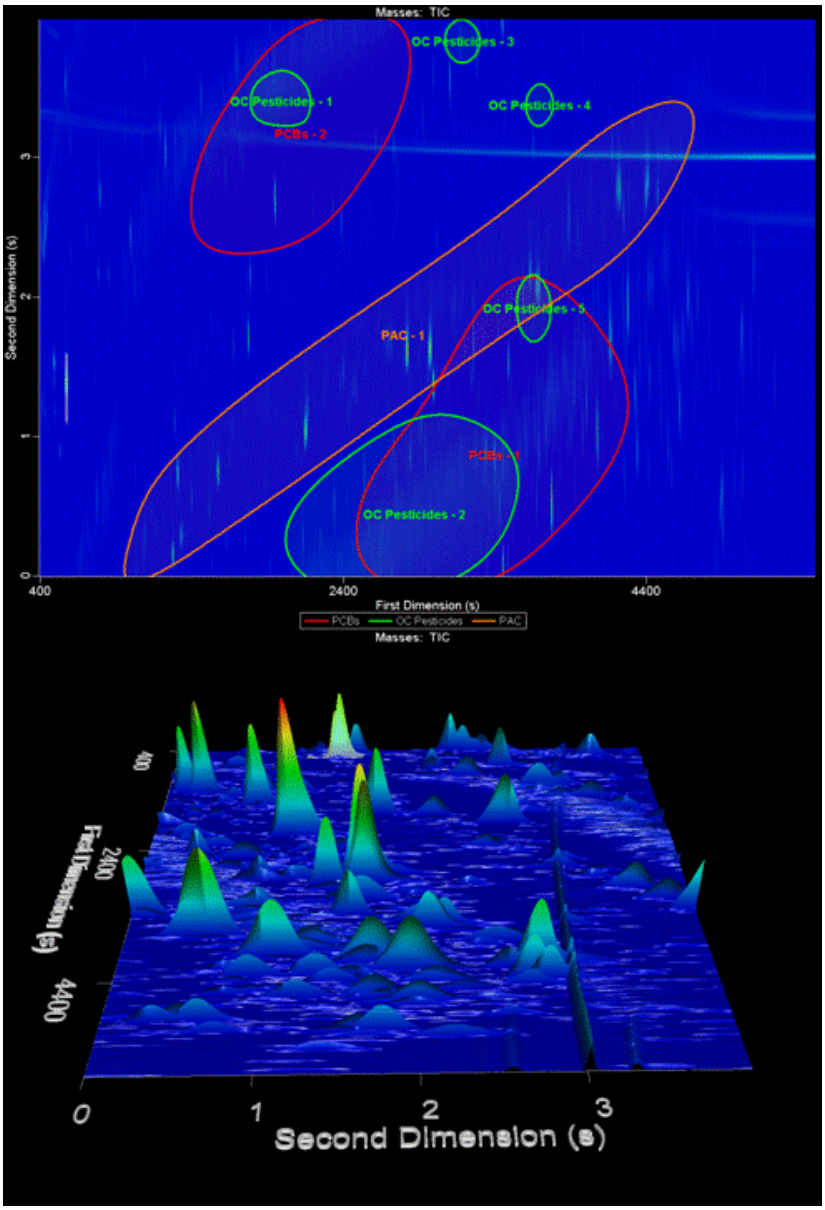

Figure 2: GCxGC-TOFMS extracted ion chromatograms: 2D contour plot showing target PCBs (left-top), PACs (left-middle) and PCDFs/PCDDs (left-bottom), 3D surface plot of all target PCBs (right-top), PACs (right-middle) and PCDFs/PCDDs (right-bottom).