New Fast Single and Multidimensional Gas Chromatographic Separations Coupled with High Resolution Mass Spectrometry and Time-of-Flight Mass Spectrometry for Assessing Human Exposure to Environmental Toxicants

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Introduction

Humans are exposed daily to an environment containing a number of potentially harmful substances of natural or anthropogenic origin. These environmental toxicants tend to share three insidious characteristics: environmental persistence, bio-accumulation, and high toxicity. Biomonitoring of environmental toxicants requires high throughput, sensitive, rugged and cost-effective methods of analysis. The current standard analytical methods for the analysis of the major environmental toxicants (such as dioxin, PCBs, PAHs, toxaphene, pesticides and their major metabolites) are based on gas chromatographic (GC) separation followed by high resolution mass spectrometric detection (HRMS). Recent efforts at the CDC have focused on decreasing analysis time (time-compression) through the use of fast one-dimensional and two-dimensional GC separations.

Fast one-dimensional GC uses shorter columns, faster temperature programming and higher carrier gas flow rates to reduce analysis time. Time-of-flight mass spectrometry (TOFMS) is ideally suited for this type of operation because of its fast scanning capabilities (1). The compressed separation time may be achieved at the expense of chromatographic resolution. However, the integrity of the chromatographic separation (component resolution) is recoverable in the mass spectral domain (2).

Comprehensive two-dimensional GC (GCxGC) differs fundamentally from one-dimensional GC. A GCxGC chromatogram has two dimensions of retention – substances are distributed over a retention plane rather than along a retention line. The key features of GCxGC are its large peak capacity, its higher speed, and its ability to separate substances in classes (3). The instrumental design (4) involves a thermal modulator interface that couples two serially connected GC columns. The thermal modulator accumulates, focuses, and re-injects fractions of the primary column effluent with high-speed onto the secondary column at regular intervals. GCxGC separations can usually be done in a time comparable to one-dimensional gas chromatographic separations that have less than one-tenth the total peak capacity.

In this work we assess the potential use of these technologies for fast, sensitive and reliable determinations of human exposure to environmental toxicants. A substantial reduction of the analysis time would result in reducing the overall cost per analysis of these determinations, which would allow for the analysis of larger numbers of samples and ultimately increase the statistical significance of epidemiological studies.

Materials and Methods

All standards and samples used were obtained from other CDC laboratories already working on existing methods for PCBs, pesticides, dioxins, toxaphene, PAHs, hydroxy-PAHs and hydroxy-PCBs, and their provenance and/or sample preparation procedures have been described previously (5-7).

Fast GC/TOF MS: A HP 6890+ (Hewlett Packard, Wilmington, DE) was interfaced with a LECO Pegasus II time-of-flight mass spectrometer (LECO, St. Joseph, MI). Samples were injected either manually or through the use of an autosampler. The injection volume was 1 μL. Inlet temperature was 250°C. A DB-5 MS column (15m, 250 μm i.d., 0.25 μm film) was used for the separation of the analytes. Temperature program regimes varied based on the samples that were analyzed. The GC/TOF MS transfer line temperature was 280°C. The electron impact ion source temperature was 200°C, and an ionization energy of 70 eV was applied. The bulk of the mass spectral data was acquired at a rate of 50 spectra/sec over a range of 40 to 550 amu. A solvent delay of 120 seconds was used. LECO deconvolution software was used for the analysis of the data.

GCxGC: A HP 6890 GC (Hewlett Packard, Wilmington, DE) equipped with a rotating thermal desorption modulator system (Zoex Corp., Lincoln, NE) was employed. A description of the operation of this type of system has been described elsewhere (4) and essentially is an automated on-column injection device between the first and second dimension columns. A flame ionization detector (FID) and a μ-electron capture detector (μ-ECD) were used. Injector temperature was 250°C. Injection volume was 1 μL (manual injection only); split injection was used. The first dimension column used dimethylpolysiloxane (1 m, 100 μm i.d., 3.5 μm film) as the stationary phase. The second dimension stationary phase was a 14% cyanopropyl methylpolysiloxane column (2 m, 100 μm, 0.05 μm film). Both columns were purchased from Quadrex (New Haven, CT). First and second dimension temperature programs varied according to the sample analyzed, and were also different from each other. The second dimension temperature was typically 20-25°C above the temperature of the first dimension. This decoupling of the temperature regimes allowed for optimization of chromatographic conditions in both dimensions. The data acquisition frequency was 100 Hz. The operating software for the modulator and data acquisition was written in LABVIEW 4.0 (National Instruments, Austin, TX).

Results and Discussion

Figure 1 is a GC-TOF chromatogram of 38 PCB standards by isotope dilution. This analysis was performed in under 5 minutes. The current HRGC/HRMS method has an analysis time of 40 minutes. The sample throughput gained by the GC/TOF method is significant. The same co-eluting pairs in the 40 minute run are, not surprisingly, co-eluting in the GC/TOF run. However, the deconvolution software and the careful selection of unique ions for the co-eluting peaks allowed complete spectral resolution of the two critical pairs.

Figure 2 is an isotope-dilution calibration curve for PCB206. The calibration curve shows good linearity over the selected range, with limits of detection in the low ppb range. Some quality control samples were run to check the accuracy of the curve and to compare it with results obtained in the previous method by HRGC/HRMS. Fast GC/TOF results were consistent with the HRGC/HRMS results within the 95% confidence interval limits. These results are very encouraging, and show that Fast GC/TOF MS allows for analysis cycles that are one order of magnitude faster than HRGC/HRMS methods without a loss in qualitative or quantitative power.

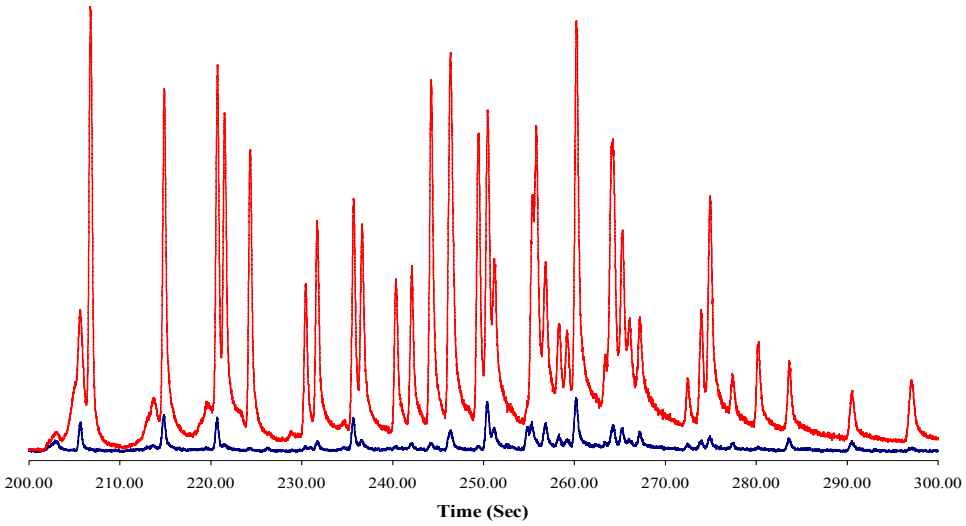

Figure 1: Chromatogram of the 38 PCBs most prevalent in human serum by isotope-dilution

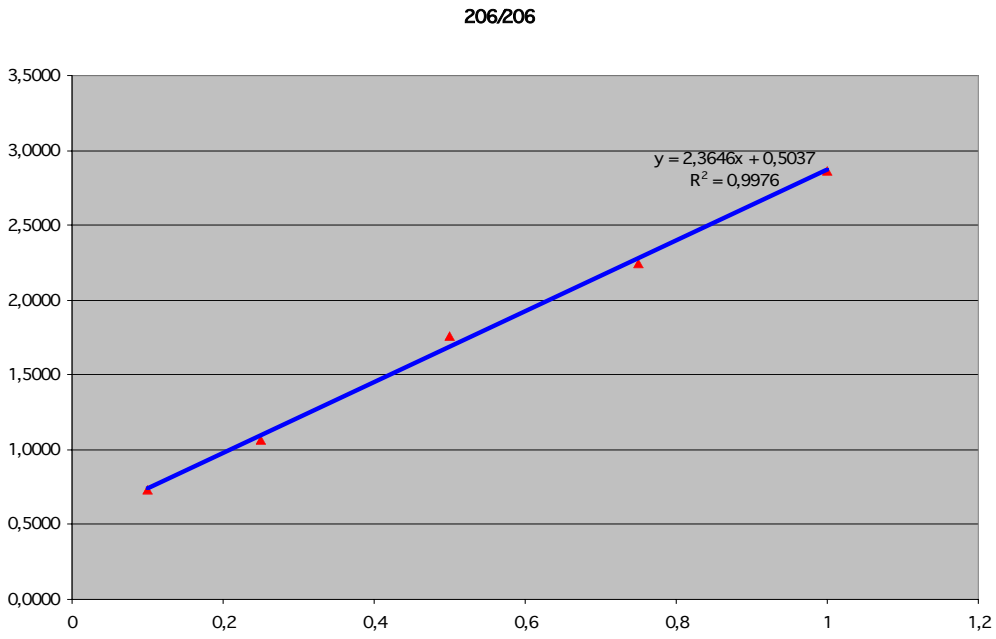

Figure 2: Calibration curve for PCB-206. Concentration axis is in parts-per-billion (ppb)

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