Comprehensive Two-Dimensional and Fast Single-Dimensional GC-HRMS Analysis of Human Serum for PCDDs, PCDFs, cPCBs, Congener PCBs, Persistent and Nonpersistent Pesticides, and PAHs

Donald G. Patterson, Jr., John R. Barr, Emily S. DiPietro, James Granger, Vaughn E. Green, C. R. Lapeza, Jr., Vincent L. Maggio, P. C. McClure, Sarath Sirimanne, and Wayman E. Turner

Division of Environmental Health Laboratory Sciences, National Center for Environmental Health, Centers for Disease Control and Prevention, 4770 Buford Highway, NE, Atlanta, Ga, USA 30341.

In order to increase the statistical power of epidemiologic studies by increasing the number of participants, faster and less costly (currently $1250 per sample) analytical methods must be developed for PCDDs, PCDFs, cPCBs, and pesticides. The current HRGC-HRMS analytical method (Patterson et al. 1991 and 1992; Turner et al.1991 and 1994) is very time consuming requiring a full 8 hours to analyze a five sample analytical run (3 of which are unknown study samples; one blank, and one QC sample). We have recently reported a new analytical approach (Liu, et al., 1994) which greatly increases the sample throughput by using a comprehensive two-dimensional gas chromatography system (C2DGC). This new approach is much faster than a one-dimensional GC system, more specific (two retention indices) and more sensitive (narrower peaks in the second dimension). Comprehensive two-dimensional separations differ from conventional multidimensional separations in that all substances are subjected to both dimensions of the separation processes. The theoretical peak capacity obtained from an orthogonal two-dimensional separation process is the product of the peak capacities obtained on each dimension. Unlike conventional heart-cutting techniques, comprehensive two-dimensional separations do not sacrifice any analytical range covered by a given sample.

The use of the C2DGC system to generate a fast, sensitive, and specific separation of a mixture of 14 pesticides and two internal standards within 3.5 minutes is shown in Figure 1A. The peak volume ratios between the pesticide analytes and the internal standards were used to generate calibration curves over a concentration range of 13 pg to 1.02 ng on column (Table 1). The limits of detection for several of the pesticides are given in Table 1 and the reproducibility of the method (6%-9%) was estimated by using the absolute peak volume of the analytes per picogram over the calibration range. The 14 pesticides in Figure 1A were baseline separated with 3.5 minutes using the C2DGC system while several of the pesticides could not be baseline resolved even after one hour with a one-dimensional GC system.

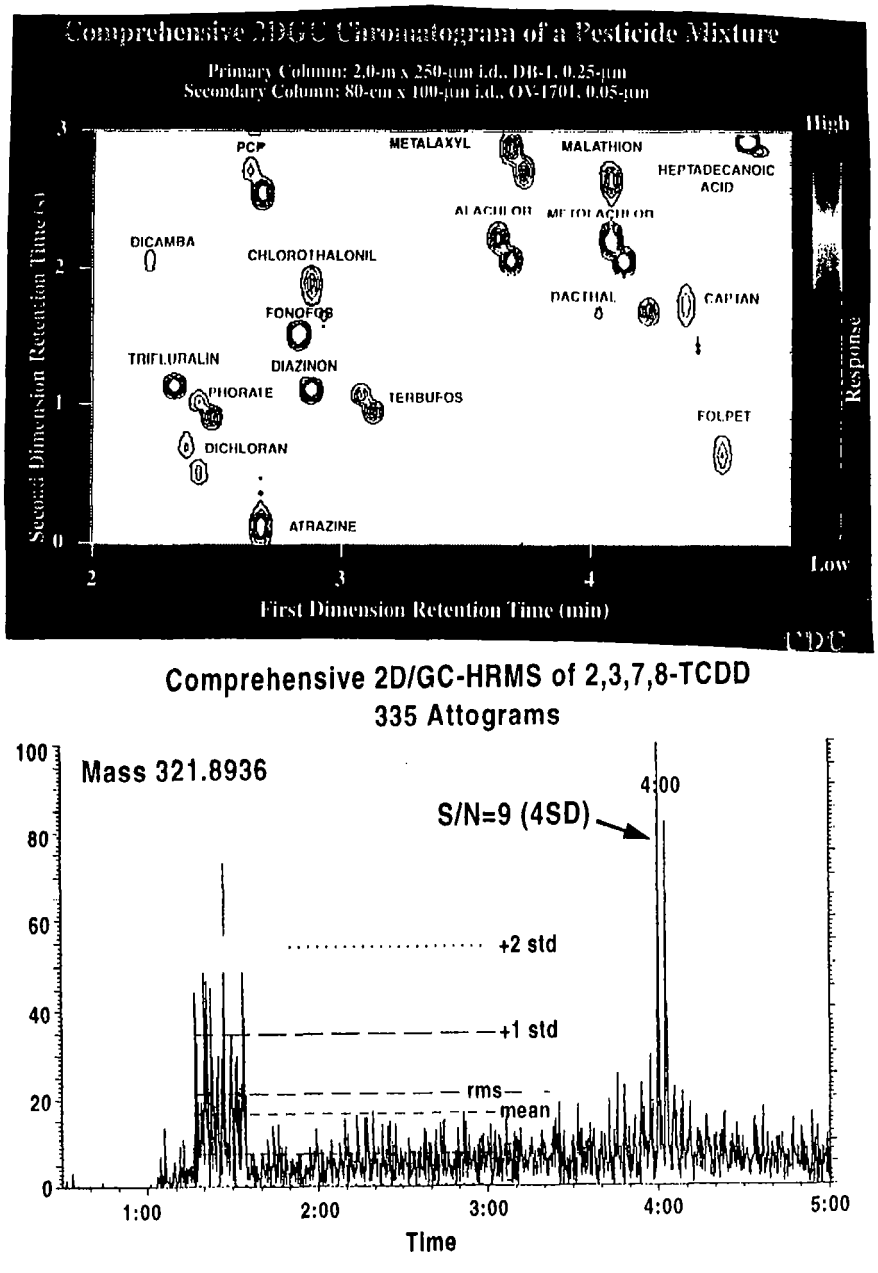

Figure 1. Using high-speed comprehensive 2D gas chromatography, peak volumes (instead of only peak areas or heights) can be obtained (1A). By measuring peak volumes, attogram detection is achievable (1B).

Table 1. Calibration results for fast analysis of pesticides[a]

Compound	Intercept	Slope	r[b]	LOD[c] (pg)
Phorate	0.14410	0.005240	0.99943	2.8
Fonofos	0.03766	0.006689	0.99942	1.8
Terbufos	0.13170	0.004511	0.99802	3.3
Atrazine	0.11340	0.005177	0.99845	2.7
Diazinon	0.07456	0.004977	0.99923	1.8
Dacthal	0.14560	0.002335	0.99915	3.8
Alachlor	0.12560	0.007348	0.99773	2.3
Metolachlor	0.01107	0.011073	0.99933	1.9

[a] The GC oven was kept at 118°C for 0.5 min and then programmed to 200°C at 15°C/min. The modulator chamber was kept at 150°C isothermal.
[b] Regression coefficient
[c] Limits of detection were measured as the analyte quantity on column using an FID detector.

We are currently developing methods to analyze PCDDs, PCDFs, cPCBs by C2DGC/HRMS. Preliminary results indicate that we can separate and analyze these toxicants an order of magnitude faster with attogram on-column sensitivity (Figure 1B). This new system should allow us to dramatically speed-up the analysis in human samples and thereby reduce the cost of these analyses. The normal 1 hour chromatogram for the HRGC/HRMS analysis of PCDDs/PCDFs/cPCBs on a 60 meter DB-5 capillary column is shown in Figure 2A. The analysis time can be dramatically reduced by using a 2 meter column with a 0.1 mm ID and 0.1μm film thickness. The same 24 compounds can be analyzed in just over 7 minutes(see Figure 2B) with the shorter column. The PCBs shown in Figure 3A can be analyzed in 10 minutes by employing the shorter narrower columns. The increase in sensitivity for 2378-TCDD analysis over time is shown in Figure 3B. This increase in sensitivity has been mainly achieved by ever more sensitive high resolution mass spectrometers. However, the new C2DGC technique has allowed us to have a 0.01 part-per-quadrillion detection limit for 2378-TCDD in 200 grams of human serum. This sensitivity enhancement could, however, also be used to allow the analysis of less serum. The use of smaller serum samples allows us to miniaturize and increase the speed of the sample cleanup procedures. The higher throughput and reduced cost will allow larger epidemiologic studies to be conducted with higher statistical power to find any potential association of body burden with health effects.

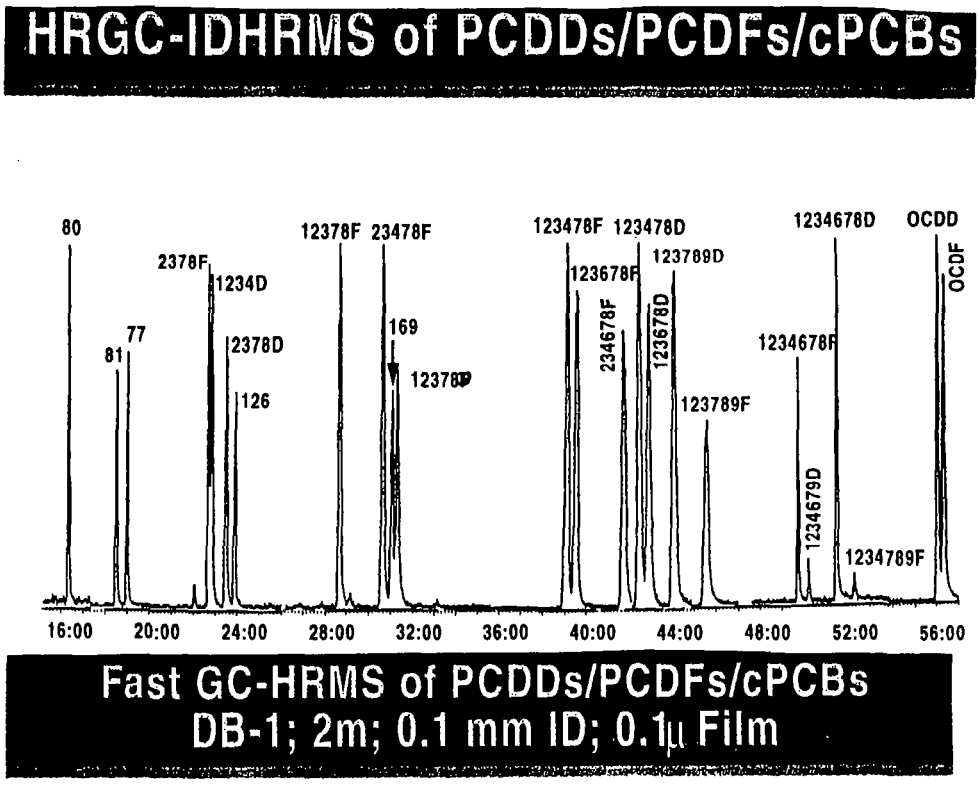

Figure 2. PCDDs, PCDFs, and cPCBs can be adequately separated and analyzed in about 1 hr using high-resolution gas chromatography/high-resolution mass spectrometry (HRMS) (2A). By interfacing fast chromatography with HRMS, the same compounds can be separated and analyzed in less than 8 min.

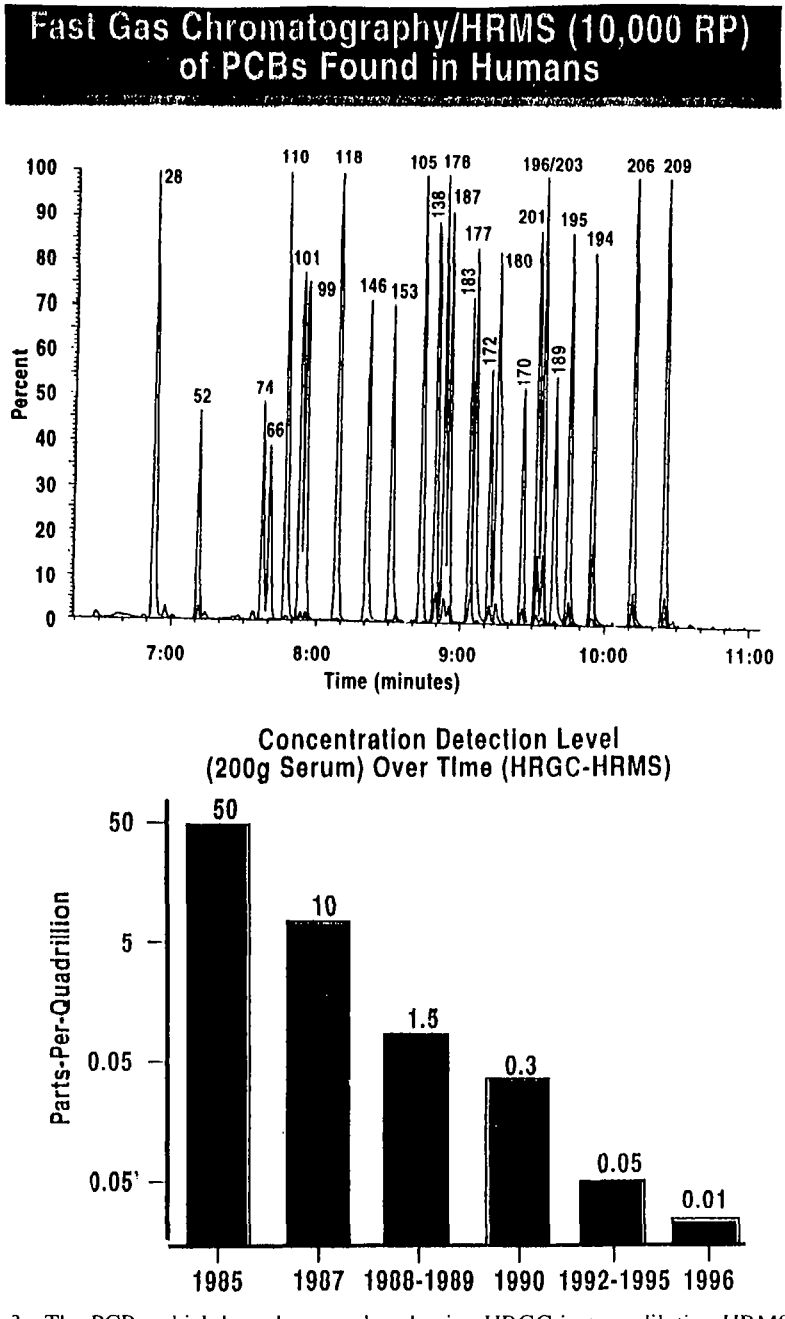

Figure 3. The PCBs which have been analyzed using HRGC-isotope dilution-HRMS in about 1hr can now be analyzed in about 10 mins (3A) using the shorter, narrower column. The decrease in detection limits of 2,3,7,8-TCDD over a decade (3B) is mainly attributable to more sensitive high-resolution mass spectrometers. However, the new C2DGC technique has allowed the detection of only 0.01 ppq 2,3,7,8-TCDD in a 200g serum sample

References

Liu, Z., Sirimanne, S.R., Patterson, D.G. Jr., Needham, L.L. (1994). "Comprehensive two-dimensional gas chromatography for the fast separation and determination of pesticides extracted from human serum." Anal. Chem. 66:3086-3092.

Patterson, D.G. Jr., Isaacs, S.G., Alexander, L. R., Turner, W.E., Hampton, L., Bernert, J.T., and Needham L.L. (1991). "Method 6: Determination of specific polychlorinated dibenzo-p-dioxins and dibenzofurans in blood and adipose tissue by isotope-dilution high-resolution mass spectrometry." In: Rappe C., Buser H.R., Dodet B., and O'Neill I.K., eds. Environmental Carcinogens Methods of Analysis and Exposure Measurement, Vol 11--Polychlorinated Dioxins and Dibenzofurans. IARC Scientific Publications No. 108, 299-342.

Patterson, D.G. Jr., Alexander, L.R., Turner, W.E., Isaacs, S.G., and Needham, L.L. (1992). "The development and application of a high resolution mass spectrometry method for measuring polychlorinated dibenzo-p-dioxins and dibenzofurans in serum." Chapter 6 In: Clement, R.E., Sui, K.W., and Hill, H.H., eds., Instrumentation for Trace Organic Monitoring, Lewis Publishers, Chelsea, MI, 119-154.

Turner, W.E., Isaacs, S.G., Patterson D.G. Jr., and Needham L.L. (1991): Method 7: Enrichment of Biologicals by the Semi-Automated Smith, Stalling, and Johnson Method. In: Rappe C., Buser H.R., Dodet B., and O'Neill I.K., eds. Environmental Carcinogens Methods of Analysis and Exposure Measurement, Vol 11--Polychlorinated Dioxins and Dibenzofurans. IARC Scientific Publications No. 108, 343-355.

Turner, W.E., DiPietro E.S., Cash, T.P., McClure, P.C., Patterson, D.G. Jr., and Shirkhan, H. (1994). "An improved SPE extraction and automated sample cleanup method for serum PCDDs, PCDFs, and coplanar PCBs." Organohalogen Compounds, Vol 19, Analytical Methods and Emission Control, 31-36.