Fast Gas Chromatography/Isotope Dilution-High Resolution Mass Spectrometry For The Analysis Of PCDDs, PCDFs, and Coplanar PCBs In Human Serum

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Introduction

Polychlorinated dibenzo-*p*-dioxins (PCDD), polychlorinated dibenzofurans (PCDF) and coplanar PCBs (cPCB) are persistent organic pollutants found throughout the world. The human toxicity for PCDDs, PCDFs and cPCBs has been controversial and highly publicized. Considerable animal data on the toxicities of dioxins, furans and cPCBs has been gathered but there are concerns over interspecies differences, high-dose to low-dose extrapolations, and animal to human extrapolations. These uncertainties have indicated the need for more human studies to understand and perform human risk assessment.

To increase knowledge about the threat of PCDDs, PCDFs and cPCBs to human health, large human studies are needed. These studies require both a laboratory and epidemiology component. In order to increase the statistical power of epidemiologic studies by increasing the number of participants, faster and less costly analytical methods must be developed for the measurement of PCDDs, PCDFs, and cPCBs in human samples. The current HRGC-HRMS analytical method for dioxins, furans ,and coplanar PCBs is very time-consuming. We recently have developed methods which greatly increase the sample throughput by using thin film, short narrow-bore columns. This technique is known as fast gas chromatography. We also have recently been successful in coupling fast GC to high resolution mass spectrometry (HRMS). This has yielded a fast, sensitive and highly specific analysis for persistent organic pollutants (in this case PCDDS, PCDFs, and cPCBs). We have also coupled fast GC/HRMS to isotope dilution (ID) for the quantification of PCDDs, PCDFs and cPCBs in human serum. This fast GC/ID-HRMS method has been validated on serum QC materials against previously validated GC/ID-HRMS methods in our laboratory.

Experimental

60 meter GC/HRMS Analysis. The data was collected on a Micromass Autospec Ultima equipped with a HP 5890 GC. The instrument resolution was 10,000 (10% valley). The source temperature was 250°C. Ionization was performed by electron impact with an electron energy of 30 eV. The injector and transfer line were both 250°C. Helium was used as a carrier gas with a linear velocity of 30 cm/sec. The column was a 60 m DB-5MS with 0.25 mm ID and 0.25 μm film. The initial column temperature was 100°C which was held for 2 min. The column temperature was then increased at 25°C/min to 220° and held for 2 min. The oven temperature was then increased to 250°C at 15°C/min. and held for 32.2 min. The temperature was finally ramped to 300°C and held for 11 min.

30 meter GC/HRMS Analysis. The data was collected on a Micromass Autospec equipped with a HP 5890 GC. The mass spectrometer instrument resolution was 10,000 (10% valley). The source temperature was 250°C. Ionization was performed by electron impact with an electron energy of 30 eV. The injector and transfer line were both 250°C. A linear velocity of 30 cm/sec. with helium carrier gas was used. The column was a 30 m DB-5MS with 0.25 mm ID and 0.25 μm film. The initial column temperature was 100°C which was held for 2 min. The column temperature was then increased at 25°C/min to 220° and held for 2 min. The oven temperature was then increased to 250°C at 15°C/min. and held for 11.4 min. The temperature was finally ramped to 300°C and held for 4.2 min.

Fast GC/HRMS. The data was collected on a Micromass 70-4SE equipped with a HP 6890 GC. The instrument resolution was 10,000 (10% valley). The source temperature was 280°C. Ionization was performed by electron impact with an electron energy of 30 eV. The injector and transfer line were both 280°C. The injector port temperature was 260°C. The GC was operated in the constant flow mode with a linear velocity of 23 cm/sec. A 20 m DB-5 column was employed with a 0.1 mm ID and .1μm film. The initial column temperature was 100°C. The column temperature was then increased at 75°C/min to 185°. The temperature was finally ramped to 320°C at 50°C/min and held for 5 min.

Results and Discussion

The current methods for GC/ID-HRMS are specific, accurate, and sensitive[1,2]. These methods have generated a large amount of data on PCDDs, PCDFs and cPCBs in human serum. Figure 1A shows the 60 meter GC/ID-MS run for standard of PCDDs, PCDFs and cPCBs and Figure 1B shows results from a serum QC sample. These are reliable methods but are slow and expensive. To improve the sample throughput and reduce the cost of analysis, new sample preparation procedures have been developed[3]. However, new mass spectrometric methods were also needed to increase the speed of analysis. First, a method which employed a 30 m x 0.25 mm DB-5MS was developed. A chromatogram with standards is shown in Figure 2A and a chromatogram of a serum QC extract is in Figure 2B. This method has been validated and is currently in use for dioxin studies.

We recently developed methods that utilize thin film narrow-bore GC columns. These fast GC methods were found to be accurate and reliable. The chromatograms for standards and serum QC extracts of PCDD, PCDF and cPCBs. are shown in Figures 3A and 3B, respectively. This method proved comparable to the previous method. Initially, there was some overlap of peaks that required a small change in the ions in which were monitored. These two changes were both in isotopically labeled channels of tetrachloro furan and pentchloro PCB. We previously monitored M and M+2 for both these labeled compounds but, now, monitor M+2 and M+4 ions by fast GC/ID-HRMS. Figure 4 shows the QC chart for the results of analyses on the 70-4SE by fast GC/ID-HRMS and on the Autospec on a 30 m x 0.25 mm column. These analyses were obtained on the same serum extracts; therefore, the comparisons can be considered purely instrumental. The Autospec would analyze 2 μL of a 5 μL extract on the 30 m column, then the 4-sector would analyze the next 2 μL by fast GC/ID-MS. For selected compounds, Tables 1 and 2 show the mean of the analyses for concentration and chlorine isotope ratios, respectively. These results were almost identical even though two different GC methods on two different instruments were used. We plan to implement the fast GC/ID-HRMS analysis for human studies in the near future.

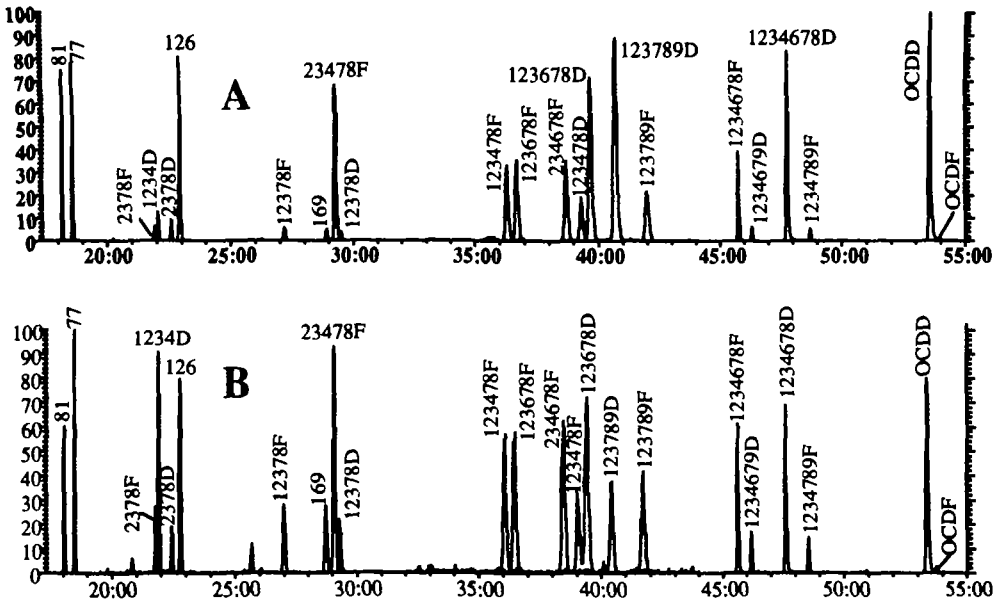

Figure 1: 60m x 0.25 mm DB-5MS GC/ID-HRMS of A) PCDD, PCDF, cPCB Standard and B) Serum Extract.

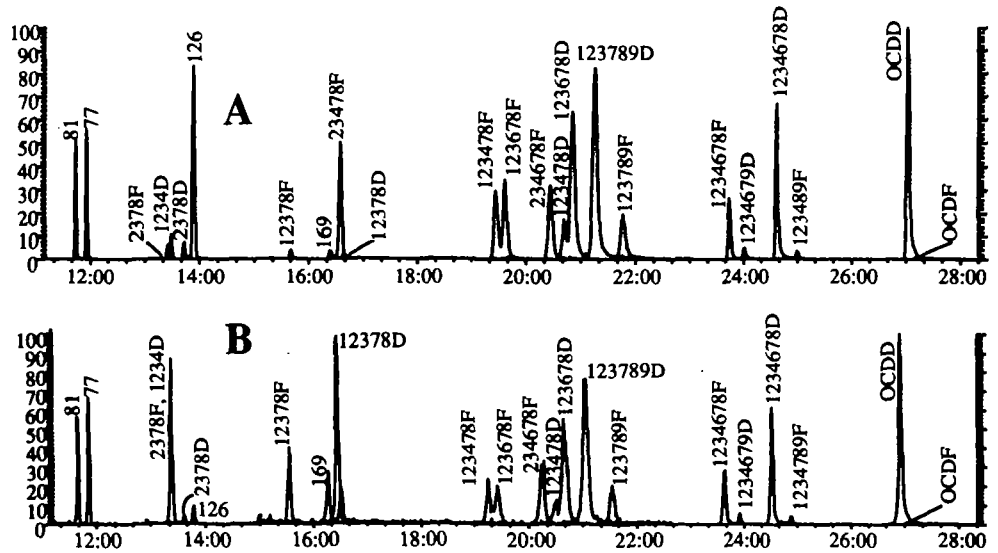

Figure 2: 30m x 0.25 mm DB-5MS GC/ID-HRMS of A) PCDD, PCDF, cPCB Standard and B) Serum Extract.

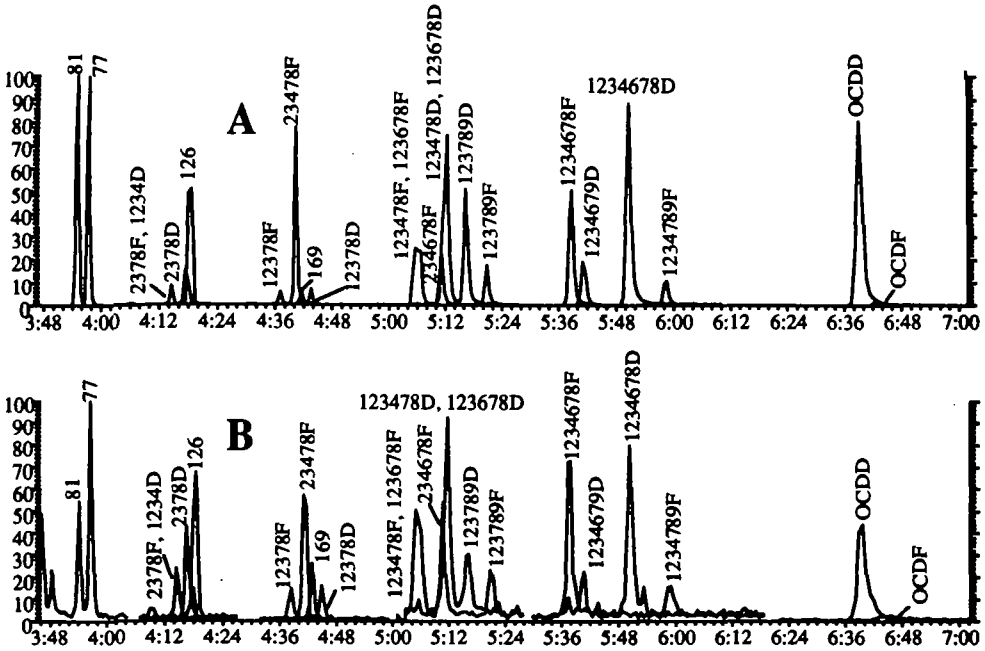

Figure 3: 20m x 0.1 mm DB-5 Fast GC/ID-HRMS of A) PCDD, PCDF, cPCB Standard and B) Serum Extract.

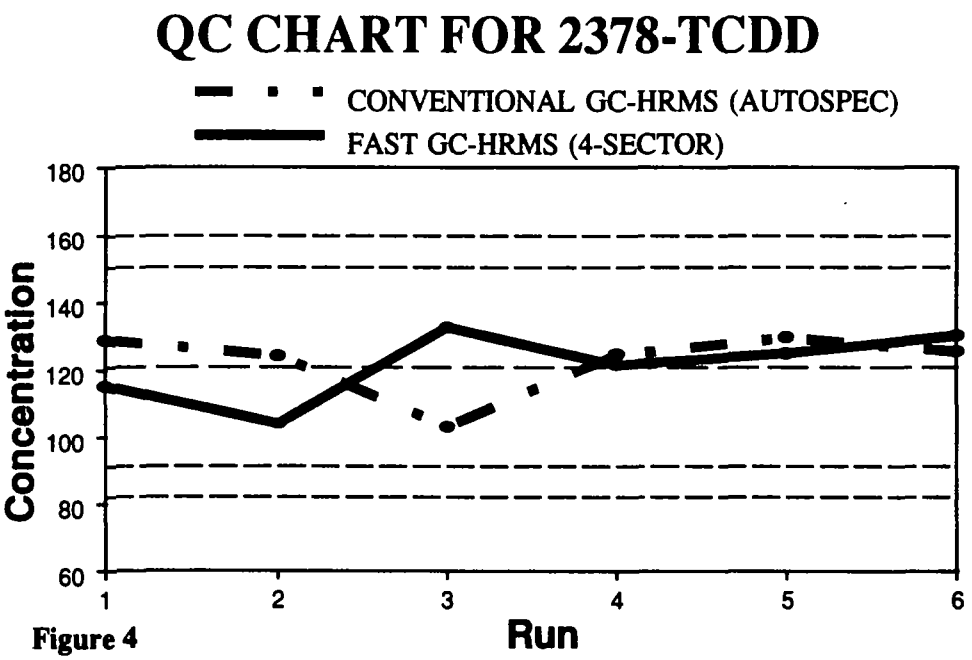

Figure 4

Table 1 : Comparison of Concentrations (ppq) in Serum QC Pool Samples by Conventional and Fast GC/HRMS

	CONVENTIONAL GC/HRMS AUTOSPEC, n=6	FAST GC/HRMS 4-SECTOR n=6
2378D	123 ±9.0 cv = 7.3%	122 ± 9.7 cv = 8.0%
12378D	119 ±11.4 cv = 9.6%	102 ±10.6 cv = 10.3%
23478F	135 ±12.1 cv = 8.9%	133 ±12.1 cv = 9.1%

Table 2 : Comparison of Isotope Ratios in Serum QC Pool Samples by Conventional and Fast GC/HRMS

	CONVENTIONAL GC/HRMS AUTOSPEC, n=6	FAST GC/HRMS 4-SECTOR n=6
2378D	0.75 ± 0.025 cv = 3.4%	0.79 ± 0.092 cv = 11.6%
12378D	0.62 ± 0.021 cv = 3.4%	0.63 ±0.082 cv = 13.0%
23478F	0.62 ±0.024 cv = 3.9%	0.62 ±0.073 cv = 11.8%

References

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3. Dipietro, E.S., Turner, W.E., Lapeza, C.R., Jr., Cash, T.P., Green, V.E., Gill, J.B., Patterson, D.P., Jr., *Organohalogen Compounds*, **1997**.