NEW MASS SPECTROMETRIC APPROACHES FOR THE MEASUREMENT OF PCBS AND PERSISTENT PESTICIDES IN HUMAN SERUM

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Introduction

High quality and scientifically advanced human exposure assessment is essential for the effective diagnosis, treatment, and prevention of environmental disease. Biological monitoring is a capable exposure assessment tool that has provided unique information to support public health decisions. Data on the internal dose of a toxicant is required to strengthen and provide meaning to many epidemiological studies.

Polychlorinated biphenyls (PCBs) and persistent organochlorine pesticides are examples of persistent organic pollutants (POPs) that are found in the environment. These compounds can be found all over the world and due to their stable lipophilic nature they tend to bioaccumulate in humans and animals. Generally, human exposure to these compounds has been monitored with adipose tissue or blood serum measurements. Serum has become the primary matrix for measuring human body burden of PCBs and persistent pesticides because it can be collected in a more noninvasive manner than adipose tissue. However, serum concentrations tend to be comparatively low because serum contains a smaller fraction of lipids into which the POPs can partition.

To better assess health effects from exposure to POPs, it is useful to have measurements on many different compounds and classes of compounds from a single individual. It is also more cost-effective to obtain the greatest amount of information on exposure to POPs from a single sample, especially when conducting reference range studies. We have recently developed serum cleanup methods for sample preparation of dioxins, furans, coplanar PCBs, persistent pesticides and PCB congeners in a single sample. These POPs are eluted in two different fractions. One fraction contains the dioxins, furans and coplanar PCBs while a separate fraction includes the PCB congeners (38 compounds) and the persistent pesticides (13 compounds). Efficient analysis of the PCB-persistent pesticide fraction offers a great analytical challenge.

Many researchers have analyzed these POPs using GC with electron capture detection (ECD)[1-4]. However, many advantages are realized by using high resolution mass spectrometry (HRMS) for detection and measurement. HRMS offers an excellent degree of sensitivity and specificity for trace organic analysis, and unlike ECD detection, HRMS is ideally suited for isotope-dilution quantification, which automatically corrects for variable recoveries for each analyte in every sample. Therefore, we have developed isotope dilution GC/HRMS methods for the quantification of PCBs and persistent pesticides from extracts of human serum. Initially, the PCB/persistent pesticide fraction was analyzed using two separate GC/HRMS runs. We now report the analysis of all 38 PCBs and 13 persistent pesticides in a single, less than 30-minute GC/HRMS run using one column.

ANALYSIS II

Experimental

Sample Preparation

The samples were prepared according to the procedure reported by DiPietro *et al.*[5] Briefly, a serum sample is extracted with cyclohexane. The organic extract is eluted through acidic silica and acid/base/neutral silica and onto a carbon column. PCB congeners and persistent pesticides are eluted from the carbon column in the forward direction using dichloromethane/cyclohexane. Dioxin, furans, and coplanar PCBs are eluted from the carbon column in the reverse direction with toluene.

Mass Spectrometry

Persistent Pesticide Analysis. Chromatograms were recorded on a Micromass Autospec (maximum acceleration voltage of 8kV) or a MAT 95XL (5kV) magnetic sector mass spectrometer. Both instruments were equipped with an HP 6890 GC. A 30 m x 0.25 mm DB-5MS column with a 0.25 µm film thickness was employed. The GC was operated in the splitless injection mode with a constant flow of 1 mL/min of helium. The injector was set at 275 ºC and the transfer line was set to 270 ºC. The initial column temperature was set at 100 ºC; held for 0.80 min; heated to 220 ºC at 18 ºC/min; held for 6 min; temperature was increased to 320 ºC at 25 ºC/min; and held for 5 min. Molecular ions for aldrin and hexachlorobenzene were monitored. Fragment ions were monitored for o,p'-DDT, p,p'-DDT, DDE, ß-HCCH, γ-HCCH heptachlor epoxide, oxychlordane, *trans*-nonachlor, dieldrin and mirex. All spectra were recorded with low energy (30 or 40 eV) electron impact ionization with a resolution of 10,000 (10 % valley definition).

PCB Congener Analysis. Spectra were obtained on a MAT 95 XL magnetic sector mass spectrometer (maximum acceleration voltage 5 kV) equipped with a 6890 GC. A 30 m x 0.25 mm DB-5MS column with a 0.25 µm film thickness was employed. The GC was operated in the splitless injection mode with a constant flow of 1 mL/min of helium. The injector was set at 275 ºC and transfer line was set at 270 ºC. The initial column temperature was set at 100 ºC; held for 0.6 minute; heated to 200 ºC at 25 ºC/min; held at 200 ºC for 5 min; heated to 250 ºC at 4 ºC/min; and then heated to 320 ºC at 35 ºC/min and held at 320 ºC for 3 min. For each PCB congener (trichloro to decachlor), the two most intense chlorine isotope peaks in the PCB molecular ion clusters were monitored. All spectra were recorded with low energy (40 eV) electron impact ionization with a resolution of 10,000 (10 % valley definition).

Combined PCB Congener and Persistent Pesticide Analysis. Spectra were obtained on the magnetic sector portion of a MAT 900 Trap hybrid mass spectrometer (maximum acceleration voltage 5 kV) equipped with a 6890 GC. All GC operating parameters were identical to the PCB congener analysis described above. All spectra were recorded with low energy (40 eV) electron impact ionization with a resolution of 10,000 (10 % valley definition). Mass ions were optimized for each individual pesticide and PCB. Eight different retention time windows were used to collect data on all the PCB congeners and persistent pesticides. Window 1 included the trichloro PCBs 18 and 28 (M and M+2 ions were monitored), hexachlorobenzene (M, M+2), beta and gamma hexachlorocyclohexane [M-HCl_2, (M+2) -HCl_2]. The 13C labeled internal standards for each analyte were also monitored by SIM employing the accurate mass of the corresponding ions (e.g., PCB $^{13}C_{12}$ PCB 28 was monitored with the M and M+2 ions and $^{13}C_6$ hexachlorocyclohexane isomers were monitored with the masses for the corresponding M-HCl_2, (M+2) -HCl_2 ion). Window 2 included tetrachloro PCBs 44, 49, 52, 66, 74 (M, M+2), heptachlor epoxide [(M+2)-Cl,(M+4)-Cl], and oxychlordane [(M+2)-Cl, (M+4)-Cl]. Window 3 included pentachloro PCB 99, 101 (M, M+2), *trans*-nonachlor [(M+2)-Cl, (M+4)-Cl]. Window 4 monitored pentachloro PCB 87, 105, 110, 118 (M, M+2), hexachloro PCB 146, 149, 151, 153 [(M+2)-Cl_2, (M+4)-Cl_2], *o,p*-DDT [M-CCl_3, (M+2)-CCl_3], DDE [M-Cl_2, (M+2)-Cl_2], and dieldrin [M-C_5H_6OCl, (M+2)-C_5H_6OCl]. Window 5 included hexachloro PCB 128, 138/158, 167 [(M+2)-Cl_2,

(M+4)-Cl_2], heptachloro PCB 178, 183, 187 [(M+2)-Cl_2, (M+4)-Cl_2], and *p,p*-DDT [M-CCl_3, (M+2)-CCl_3]. Window 6 included hexachloro PCB 156, 157 [(M+2)-Cl_2, (M+4)-Cl_2], heptachloro PCB 172, 177, 180 [(M+2)-Cl_2, (M+4)-Cl_2]. Window 7 monitored ions for heptachloro PCB 170, 189 [(M+2)-Cl_2, (M+4)-Cl_2], octachloro PCB 194,195, 196/203, 201 [(M+2)-Cl_2, (M+4)-Cl_2], nonachloro PCB 208 [(M+2)-Cl_2, (M+4)-Cl_2], and Mirex [(M+2)-C_5Cl_6, (M+4)-C_5Cl_6]. Window 8 included nonachloro PCB 206 (M+2, M+4), and decachloro PCB 209 (M+2, M+4). The ions for each of the corresponding ^{13}C labeled compounds were monitored in the same windows as the native compounds and employed the accurate mass for the corresponding molecular or fragment ions for the labeled pesticide or PCB congener.

Results and Discussion

The analysis of human serum for PCBs and persistent pesticides is an analytical challenge. GC/HRMS provides a high degree of specificity and selected ion monitoring (SIM) yields high sensitivity. We are able to successfully separate and analyze all 38 PCBs and 13 persistent pesticides in a single analytical run. One major challenge in this HRMS method is the mass difference between closely eluting peaks. Peaks in the same retention time window can have mass differences too great to be successfully monitored in the same window by the mass spectrometer. One example of this was DDT and the heptachloro PCB-178, which were only separated by 14 seconds and between which the hexacloro PCB-s 138 and 151 eluted. This creates a problem since virtually no molecular ion is seen for DDT; the highest weight major fragment ion for DDT is m/z 235.0081. The M+2 and M+4 ions of the molecular ion cluster for PCB-178 are the major ions recorded and the M+2 ion has a molecular weight of 393.8025. The molecular mass of the $^{13}C_{12}$ labeled PCB-178 have ions that are 405.8428 and 407.8389. This gives a ratio between these two ions of almost 1.7 for the native and higher than 1.7 for the labeled PCB. These differences are greater than the practical operating ratio limit of 1.5. In SIM, the magnet is held constant for every ion in a single group and the accelerating voltage is varied to record different masses. The accelerating voltage for the lowest mass ion in a group is generally 5 KV on this type of magnetic sector mass spectrometer. Decreasing the accelerating voltage below 3 or 3.5 KV leads to a huge loss in sensitivity and considerable loss of resolution. The magnetic field must therefore be changed between these peaks. Changing the magnetic field is now a fast process on modern magnetic sector mass spectrometers but it is best to have a gap between peaks since changes in retention times (often seen with serum extracts) can cause peaks to be missed.

We have optimized the electron energy and have begun monitoring fragment ions for several of the PCBs, which elute near the lower molecular weight pesticides so that both classes of compounds can be analyzed in a single isotope dilution GC/HRMS run. A chromatogram of the GC/HRMS run of the 38 PCBs and 13 persistent pesticides can be seen in Figure 1. All compounds are separated by time or mass except for the hexachloro PCBs 138/158 and the octachloro PCBs 196/203; each of the two isomer pairs is typically quantified together. We validated the mass spectrometric analysis by consecutively analyzing the same serum extracts using the old analysis method (i.e., two separate analyses for PCBs and pesticides) and the new analysis (i.e, a single analytical run). The quantified concentrations for the analysis of the 38 PCB congeners and 13 persistent pesticides in a single mass spectral run are nearly identical to those of the PCBs and pesticides in separate mass spectral runs. The means of several runs of QC materials are within 2% of each other and there was no systematic bias between methods. The coefficients of variation (CV) of both methods are nearly identical.

Conclusion

The careful use of fragment ions and the careful selection of mass spectral SIM groups have allowed us to analyze all 38 PCBs and 13 or the persistent pesticides in a single serum sample with a

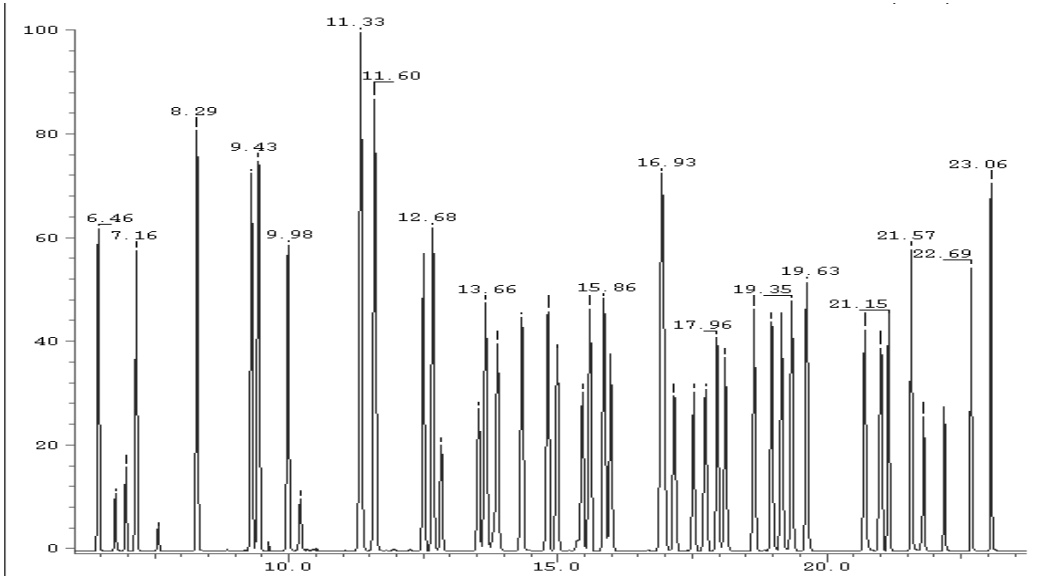

Figure 1. Chromatogram of 38 PCB congeners and 13 persistent pesticides

single mass spectral run. This is a sensitive chromatographic method that has detection limits in the very low parts-per-trillion (ppt) range and is selective. We have been able to analyze unattended almost 50 samples per day of both classes of compounds.

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