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Journal of Chromatography B, 794 (2003) 137–148
JOURNAL OF CHROMATOGRAPHY B
www.elsevier.com/locate/chromb

New high-resolution mass spectrometric approach for the measurement of polychlorinated biphenyls and organochlorine pesticides in human serum

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Received 15 January 2003; received in revised form 19 May 2003; accepted 20 May 2003

Abstract

To increase our analytical throughput for measuring polychlorinated biphenyls (PCBs) and organochlorine (OC) pesticides without sacrificing data quality, we have developed and validated a combined PCB/OC pesticide gas chromatography–high-resolution mass spectrometry (GC–HRMS) analysis. In a single GC–HRMS analysis, both selected PCBs and OC pesticides are detected and quantified. Previously, this has been difficult, if not impossible, because of the major difference in masses of the most abundant electron-impact ions. However, we have identified slightly less abundant ions to monitor that allow us to successfully combine these analytes into a single analysis without sacrificing any analytical sensitivity or instrument reliability. Consequently, we have been able to double our analytical throughput by modification of mass spectrometric parameters alone. Our new methodology has been validated against our current GC–HRMS method, which entails using two separate injections, one for PCB analysis and one for OC pesticide analysis. The two methods differ by less than 4% overall, with no systematic bias. We used this method to analyze approximately 350 serum samples over a period of several months. We found that our new method was as reliable in automated, overnight runs as our current method.

Keywords: Polychlorinated biphenyls; Organochlorine pesticides

1. Introduction

Polychlorinated biphenyls (PCBs) and organochlorine (OC) pesticides are manmade chemicals that are pervasive in our environment. PCBs are chemicals that were produced commercially for a variety of applications, including dielectric fluid for capacitors and transformers; heat transfer liquids; lubricating fluids; and additives in a variety of products such as pesticides, adhesives and sealants [1]. PCBs are comprised of 209 congeners with various degrees of chlorination (1 to 10 chlorine atoms) on the biphenyl rings. These chemicals were marketed primarily as Aroclor, mixtures of various PCB congeners, by their major producer, Monsanto Corporation, from 1930 to 1977. The commercial utility of PCBs was largely dependent upon their chemical stability, low flammability and insulating

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1570-0232/03/$ – see front matter
doi:10.1016/S1570-0232(03)00451-3

properties [1]. PCBs have entered the environment both by use and careless disposal practices.

OC pesticides were used extensively in the USA as insecticides in the mid-20th century [2]. This class of pesticides includes cyclodienes, hexachlorocyclohexane isomers, and 1,1,1-trichloro-2-bis(*p*-chlorophenyl)ethane (DDT) and its analogues. Many public health benefits have been realized by the use of synthetic pesticides [3]. For instance, the food supply has become safer and more plentiful and vector borne diseases have been dramatically reduced. Despite the obvious benefits of OC pesticides in the mid-20th century, their impact on the environment and public health has been substantial.

Most OC pesticides and PCBs are considered persistent organic pollutants (POPs) in the environment. These POPs have long environmental half-lives and tend to bioaccumulate in humans and other animals, and thus biomagnify up to 70 000 times in the food chain [4,5]. Because migratory birds and other animals are at the top of the food chain, they carry these persistent compounds with them wherever they go and are then transferred to the very top of the food chain, humans [6]. Another manner in which these persistent compounds are transported transboundary is through a series of evaporation, deposit (condensation), evaporation, deposit steps; this is the so-called ''grasshopper effect'' [7]. By these two means, these persistent chemicals may be transported thousands of miles from their origin.

Nine of the OC pesticides, as well as PCBs and other chlorinated POPs, were the subject of the Stockholm Convention on Persistent Organic Pollutants in May 2001; this proposed treaty called for an immediate ban on the production, import, export, and use of most of these POPs as well as disposal guidelines [8]. The nine OC pesticides involved in this proposed treaty were aldrin, chlordane, DDT (note: DDT has a health-related exemption in some countries for the control of malaria-carrying mosquitoes), dieldrin, endrin, heptachlor, hexachlorobenzene, mirex, and toxaphene. Regardless, these compounds already exist in our environment and remain there for long periods of time during which exposures can occur. Even if the Stockholm Convention is ratified and becomes a Treaty that effectively eliminates the use of selected POPs, the persistent OC compounds will continue to be monitored in the ecosystems, including humans. The reasons for this are their toxicity (known animal toxicity, known and suspected human toxicity) and the possibilities of human exposure, primarily via food, especially dairy products, meat, and fish. In addition, the Stockholm Convention mandates continued human monitoring of these chemicals.

Many human epidemiologic and animal studies have been performed to help understand the health risks associated with exposure to POPs. These studies have associated POPs with a variety of adverse health outcomes [9]. However, links between POPs and many more adverse health outcomes remain to be studied.

Biological monitoring, such as measuring the internal dose, of POPs in human serum is the most common approach for exposure assessment of POPs in epidemiologic studies. The PCBs and OC pesticides often are included together in epidemiologic studies for a variety of reasons: (1) both persist in the environment and bioaccumulate in biological systems; (2) they were in heavy use during the same time frame; (3) they are typically found in the same environmental exposure media and their exposure pathways are similar; (4) both sequester in the lipid stores of animals; and (5) unlike dioxins, they are found at similar concentration levels in human serum. In addition, both PCBs and OC pesticides have similar extraction properties and can therefore be quantified in the same extract from serum, adipose tissue, breast milk, and even environmental samples.

PCBs and OC pesticides are most commonly measured as the intact chemical and/or its metabolite in whole blood, serum, plasma, and other lipid-rich matrices [10,11]. Aldrin is measured as its primary metabolite, dieldrin. Chlordane and heptachlor generally are used together and are monitored as their metabolites, oxychlordane and heptachlor epoxide, as well as their commercial by-product, *trans*-nonachlor. DDT is sometimes measured as DDT but more generally as its biodegraded product and metabolite, DDE (p,p'-dichlorodiphenyldichloroethylene).

Many methods exist in the literature in which PCBs and OC pesticides are measured either separately or concurrently. Typically, serum or plasma is extracted using liquid partitioning or solid-phase extraction (SPE), and the extract is analyzed using

dual-column capillary gas chromatography (GC) with electron-capture detection (ECD) [10,12,13]. These methods are sensitive and reliable and use affordable instrumentation. However, GC–ECD analyses have a higher potential for detecting interfering components than do more selective analysis techniques. Other methods for analysis of serum extracts include mass-selective detection [or single-stage mass spectrometry (MS)] [14–16], tandem mass spectrometry [17] and high-resolution mass spectrometry (HRMS) [11,18–20], some with isotope-dilution quantification [11,20]. Using isotope-dilution, each individual sample (i.e., unknown samples, calibration standards, quality controls and blanks) is enriched with stable isotope labeled analogues of the analytes of interest, usually ^{13}C-labeled for PCBs and pesticides. Chemically, the analytes and labeled analogues behave identically; however, they can be distinguished based upon their mass differences, allowing for a complete and automatic recovery correction for each analyte in each individual sample. These analyses are typically more accurate, selective, and sensitive than GC–ECD analyses; however, to obtain the needed sensitivity, the mass spectrometers must be operated in the selected ion monitoring (SIM) mode.

When SIM is performed on a magnetic sector mass spectrometer, each group of masses holds the field on the magnet at a constant setting; changing the acceleration voltage allows for the monitoring of different masses. The lowest mass in each group uses the highest acceleration voltage, and this voltage is decreased to analyze higher masses. The ratio of the highest to lowest mass in each group should typically be under 1.5 to prevent loss of sensitivity and stability at larger mass ratios. PCBs tend to elute from the GC column in the order of increasing mass. They also tend to yield strong molecular ions [M+] when analyzed by ionization with low electron energy. Conversely, the OC pesticides in the same extracts do not always elute with increasing mass and only produce lower molecular mass fragment ions with little apparent molecular ion. Because of the large mass differences between the PCB and OC pesticide ions and the problems these difference cause during analyses, PCBs and OC pesticides traditionally have been analyzed in two different GC–HRMS analytical runs [19]. Each of the runs usually employs unique GC and MS parameters. To increase the analytical throughput of our PCB and OC pesticides analyses, we have developed a method for the analysis of 38 PCB congeners relevant to human exposure and 11 OC pesticides or their metabolites in a single isotope-dilution (ID)–GC–HRMS analysis using one column in under 30 min. Using this method, we have been able to double our analytical throughput and reduce the analysis cost by reducing the personnel and instrumentation required for analysis.

2. Experimental

2.1. Human samples

All cross-method validation was completed on a human serum pool for which the concentrations of the OC pesticides and PCBs had been previously well characterized. To demonstrate the long-term reliability of the new method, we used it to analyze about 350 archived plasma samples that were collected in the 1960s. The study protocol complied with all national and institutional guidelines for the protection of human subjects.

2.2. Sample preparation

The cross-method validation samples were prepared according to the procedure reported by DiPietro et al. [19]. Briefly, serum samples spiked with isotopically labeled standards for all PCBs and pesticides measured (Cambridge Isotope Labs., Andover, MA, USA) and were allowed to equilibrate. The serum samples were denatured with formic acid and extracted with cyclohexane. The organic extracts were purified on a Power Prep automated sample cleanup system (Fluid Management System, Waltham, MA, USA) with acidic silica gel, acid/base/neutral silica, and a carbon column in a sequential manner. PCB congeners and persistent pesticides were eluted from the carbon column in the forward direction using dichloromethane and cyclohexane. The eluates were concentrated using a nitrogen evaporator for analysis by GC–HRMS.

The instrument reliability samples were prepared using a newer SPE method [21]. The new prepara-

tion method has previously been shown to produce results indistinguishable from the method of DiPietro et al. [19]. Briefly, plasma samples were spiked with isotopically labeled standards of all PCBs and pesticides measured and were allowed to equilibrate. The plasma samples were denatured with acid and extracted using an Oasis SPE cartridge (Waters, Milford, MA, USA) and eluted with methanol–dichloromethane. The eluate was applied to a silica column, then eluted with hexane followed by dichloromethane–hexane. The extracts were further purified using gel permeation chromatography where a distinct fraction of eluate was collected. The eluates were concentrated for analysis by GC–HRMS.

2.3. Mass spectrometry

2.3.1. Persistent pesticide analysis

Chromatograms were recorded on an Autospec (Micromass, Manchester, UK; maximum acceleration voltage of 8 kV) or a MAT 95XL (ThermoFinnigan, Bremen, Germany; 5 kV) magnetic sector mass spectrometer. Both instruments were equipped with a Model 6890 GC system (Agilent Technologies, Palo Alto, CA, USA). A 30 m×0.25 mm DB-5MS (J&W Scientific, Folsom, CA, USA) column with a 0.25 μm film thickness was employed. The GC system was operated in the splitless injection mode with a constant flow of 1 ml/min of helium. The injector was set at 275 °C, and the transfer line was set at 270 °C. The initial column temperature was 100 °C and was held for 0.80 min. The oven was then heated to 220 °C at 18 °C/min and held for 6 min. The temperature was then increased to 320 °C at 25 °C/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, β-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).

2.3.2. PCB congener analysis

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

2.3.3. Combined PCB congener and persistent pesticide analysis

Spectra were obtained on the magnetic sector portion of a MAT 900 Trap hybrid mass spectrometer (ThermoFinnigan; maximum acceleration voltage 5 kV) equipped with a Model 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. The retention time windows, ions, and fragments are shown in Table 1. 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.

2.4. Cross-method validation

Pooled human serum samples were extracted as indicated above. Each extract was analyzed once using the older analysis method and then analyzed a second time using the new instrument method. Although this eliminated the introduction of error

Table 1
Retention time windows, ions, fragments, and additional specifications for the combined gas chromatography–high-resolution mass spectrometric analysis

Window/ cycle time	Time (min)	Group	Congener	t_R	Mass	Fragment	Ratio
1 0.6 s	6.15–8.55	Lock mass	NA	NA	242.9856	NA	Hi/Lo mass 1.35
1	6.15–8.55	Tri-PCBs	18 28 32	7.25 8.38 7.52	255.9613 257.9584 268.0016 269.9986	M M+2 L L+2	0.981
1	6.15–8.55	HCB	NA	6.40	283.8102 285.8072 289.8303 291.8273	M+2 M+4 L+2 L+4	0.815
1	6.15–8.55	HCCH	Beta Gamma	6.59 7.13	216.9145 218.9115 222.9347 224.9317	M-HCl_2 M+2-HCl_2 L-HCl_2 L+2-HCl_2	1.305
1	6.15–8.55	Calibration mass	NA	NA	292.9824	NA	NA
2 0.6 s	8.56–12.29	Lock mass	NA	NA	292.9824	NA	Hi/Lo mass 1.49
2	8.56–12.29	Tetra-PCBs	52 49 44 37 74 66	9.44 9.52 10.26 10.40 11.49 12.04	289.9224 291.9194 301.9626 303.9597	M M+2 L L+2	1.305
2	8.56–12.29	Heptachlor epoxide	NA	11.47	352.8442 354.8413 362.8777 364.8748	M+2-Cl M+4-Cl L+2-Cl L+4-Cl	0.815
2	8.56–12.29	Oxychlordane	NA	11.47	386.8052 388.8023 396.8388 398.8358	M+2-Cl M+4-Cl L+2-Cl L+4-Cl	0.977
2	8.56–12.29	Calibration mass	NA	NA	366.9792	NA	NA
3 0.5 s	12.30–13.30	Lock mass	NA	NA	316.9824	NA	Hi/Lo mass 1.293
3	12.30–13.30	*trans*-Nonachlor		13.20	406.7870 408.7840 416.8205 418.8176	M+2-Cl M+4-Cl L+2-Cl L+4-Cl	1.139
3	12.30–13.30	Penta-PCBs	101 99	12.59 13.10	323.8834 325.8804 335.9237 337.9207	M M+2 L L+2	1.629
3	12.30–13.30	Calibration mass	NA	NA	404.976	NA	NA

Table 1. Continued

Window/ cycle time	Time (min)	Group	Congener	t_R	Mass	Fragment	Ratio
4 0.8 s	13.30–16.45	Lock mass	NA	NA	242.9856	NA	Hi/Lo mass 1.293
4	13.30–16.45	*o,p′*-DDT	NA	15.58	235.0081 237.0052 247.0484 249.0454	M-CCl_3 M+2-CCl_3 L-CCl_3 L+2-CCl_3	0.657
4	13.30–16.45	DDE	NA	14.10	246.0003 247.9973 258.0406 260.0376	M-Cl_2 M+2-Cl_2 L-Cl_2 L+2-Cl_2	0.657
4	13.30–16.45	Dieldrin	NA	14.19	260.8599 262.8570 267.8834 269.8805	M-C_5H_6ClO M+2-C_5H_6ClO L-C_5H_6ClO L+2-C_5H_6ClO	1.629
4	13.30–16.45	Penta-PCBs	87 110 118 105	14.02 14.23 15.30 16.31	323.8834 325.8804 335.9237 337.9207	M M+2 L L+2	1.629
4	13.30–16.45	Hexa-PCBs	151 149 146 153	14.50 15.20 16.06 16.22	289.9037 291.9008 301.9440 303.9411	M+2-Cl_2 M+4-Cl_2 L+2-Cl_2 L+4-Cl_2	0.492
4	13.30–16.45	Calibration mass	NA	NA	292.9824	NA	NA
5 0.8 s	16.46–18.50	Lock mass	NA	NA	242.9856	NA	Hi/Lo mass 1.438
5	16.46–18.50	*p,p′*-DDT	NA	17.28	235.0081 237.0052 247.0484 249.0454	M-CCl_3 M+2-CCl_3 L-CCl_3 L+2-CCl_3	0.657
5	16.46–18.50	Hexa-PCBs	138 158 128 167	17.28 17.28 18.30 18.38	289.9037 291.9008 301.9440 303.9411	M+2-Cl_2 M+4-Cl_2 L+2-Cl_2 L+4-Cl_2	0.492
5	16.46–18.50	Hepta-PCBs	178 187 183	17.41 18.03 18.17	323.8648 325.8618 335.9050 337.9021	M+2-Cl_2 M+4-Cl_2 L+2-Cl_2 L+4-Cl_2	0.653
5	16.46–18.50	Calibration mass	NA	NA	316.9824	NA	
6 0.5 s	18.51–20.30	Lock mass	NA	NA	292.9824	NA	Hi/Lo mass 1.166
6	18.51–20.30	Hexa-PCBs	156 157	19.30 19.41	289.9037 291.9008 301.9440 303.9411	M+2-Cl_2 M+4-Cl_2 L+2-Cl_2 L+4-Cl_2	0.492

Table 1. Continued

Window/ cycle time	Time (min)	Group	Congener	t_R	Mass	Fragment	Ratio
6	18.51–20.30	Hepta-PCBs	177	19.11	323.8648	M+2-Cl_2	0.653
			172	19.53	325.8618	M+4-Cl_2	
			180	20.09	335.905	L+2-Cl_2	
					337.9021	L+4-Cl_2	
6	18.51–20.30	Calibration mass	NA	NA	316.9824	NA	NA
7 0.6 s	20.31–22.35	Lock mass	NA	NA	292.9824	NA	Hi/Lo mass 1.49
7	20.31–22.35	Mirex	NA	21.25	271.8102	M+2-C_5Cl_6	0.815
					273.8072	M+4-C_5Cl_6	
					276.8269	L+2-C_5Cl_6	
					278.8240	L+4-C_5Cl_6	
7	20.31–22.35	Hepta-PCBs	170	21.09	323.8648	M+2-Cl_2	0.653
			189	21.51	325.8618	M+4-Cl_2	
					335.9050	L+2-Cl_2	
					337.9021	L+4-Cl_2	
7	20.31–22.35	Octa-PCBs	201	21.22	357.8258	M+2-Cl_2	0.815
			196	21.30	359.8229	M+4-Cl_2	
			203	21.30	369.8661	L+2-Cl_2	
			195	22.04	371.8631	L+4-Cl_2	
			194	22.25			
7	20.31–22.35	Nona-PCBs	208	22.02	403.8271	M+2-Cl_2	0.977
					405.8241	M+4-Cl_2	
7	20.31–22.35	Calibration mass	NA	NA	366.9792	NA	NA
8 0.5 s	22.36–24.00	Lock mass	NA	NA	454.9728	NA	Hi/Lo mass 1.125
8	22.36–24.00	Nona-PCBs	206	22.53	463.7216	M+4	0.759
					465.7187	M+6	
					475.7619	L+4	
					477.7589	L+6	
8	22.36–24.00	Deca-PCBs	209	23.16	497.6826	M+4	0.867
					499.6797	M+6	
					509.7229	L+4	
					511.7199	L+6	
8	22.36–24.00	Calibration mass	NA	NA	504.9697	NA	NA

NA=Not applicable; t_R=retention time; M=molecular ion of native analyte; L=molecular ion of labeled analyte; ratio=ratio of two chlorine isotope ions (except for lock mass rows); Hi/Lo mass=high mass to low mass ratio for the retention time window; HCCH=hexachlorocyclohexane; HCB=hexachlorobenzene; tri-PCB=trichlorinated biphenyl; tetra-PCB=tetrachlorinated biphenyl; penta-PCB=pentachlorinated biphenyl; hexa-PCB=hexachlorinated biphenyl; hepta-PCB=heptachlorinated biphenyl; octa-PCB=octachlorinated biphenyl; nona-PCB=nonachlorinated biphenyl; deca-PCB=decachlorinated biphenyl.

from sample preparation, it did not eliminate the interinstrument variability from the comparison. These data were analyzed using a paired *t*-test and a Pearson correlation analysis using SAS statistical software (SAS Institute, Cary, NC, USA).

2.5. Evaluation of method robustness and reliability

Archived serum samples were prepared and analyzed over a period of several months to ascertain the

robustness and reliability of the method under typical operating conditions. We subjectively evaluated these parameters by looking at various criteria over the study period, such as presence of interfering analytes in the chromatograms that prevented successful quantification of the analytes of interest, the number of injections that could be made during a single column's lifetime, repeat analyses required due to the shifting of peaks out of retention time windows, and the ability of the mass spectrometer to maintain lock and calibration during an automated overnight run of 25 samples.

3. Results and discussion

The analysis of human serum for PCBs and OC pesticides is an analytical challenge. One needs the specificity and sensitivity (in the SIM mode) offered by GC–HRMS; however, the simplicity of using other analytical techniques such as GC–ECD also is desirable. One traditional advantage of non-MS methods was that the analyst could easily include both PCBs and OC pesticides in a single analysis. However, oftentimes, an additional analysis on a different column creating orthogonal parameters was required to distinguish between coeluting species. Conversely, GC–HRMS analyses have the advantage of requiring either mass or chromatographic separation for analysis, so full chromatographic separation of coeluting species is not always required. However, in practice, HRMS has the disadvantage of requiring a relatively low mass spread of compounds analyzed in the same retention time window. For high-resolution mass spectrometers, such as the MAT or AutoSpec MS instruments, a maximum high mass to low mass ratio of 1.5 is recommended. In instances where higher ratios are used, the accelerating voltage is too low to allow reasonable sensitivity and the MS loses its ability to reliably focus on a single mass (i.e., loss of lock mass during run). This has been a particular problem with PCBs and OC

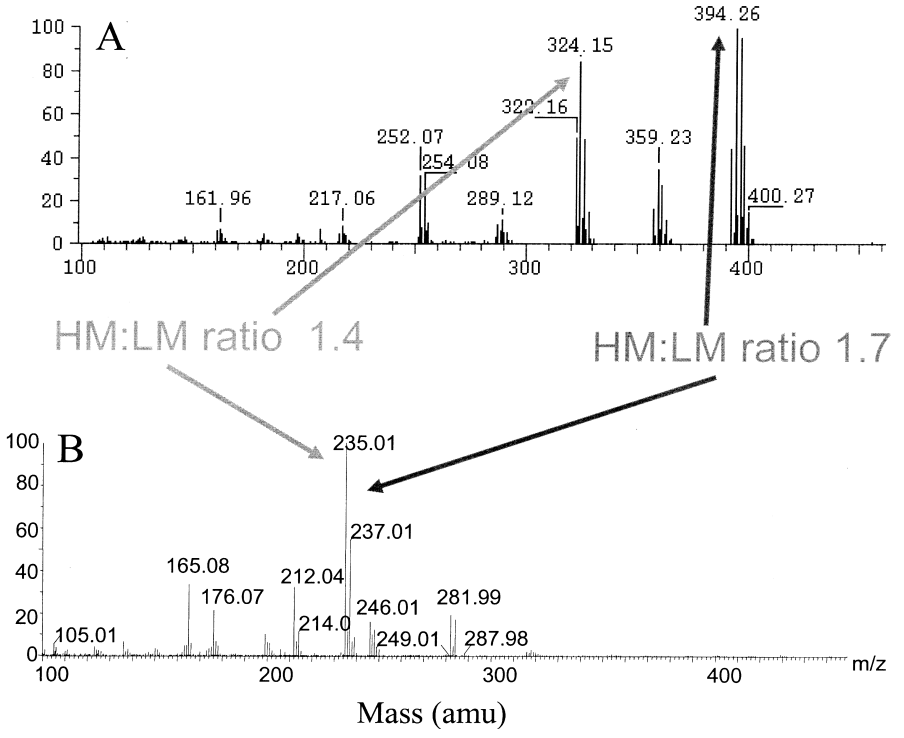

Fig. 1. Full scan mass spectra of PCB 178 (A) and *p,p′*-DDT (B). The high to low mass ratios are shown when two different PCB 178 ions are chosen for analysis. HM:LM=high mass to low mass ratio.

pesticides because the predominant ions in their mass spectra are widely spaced. For example, *p,p′*-DDT and the heptachloro PCB 178 are separated only by 14 s between which the hexachloro PCBs 138 and 151 eluted. Since virtually no molecular ion is seen for *p,p′*-DDT (Fig. 1), the mass separation between it and PCB 178 is large. The highest mass major fragment ion for *p,p′*-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 mass of 393.8025 (Fig. 1). The $^{13}C_{12}$ labeled PCB 178 has ions that are 405.8428 and 407.8389. This gives ratios between these two ions of almost 1.7 for the native and about 1.74 for the labeled PCBs. These differences are greater than the practical operating ratio limit of 1.5. Indeed, when we used these ions to analyze serum extracts, we routinely lost ''lock'' of the instrument; that is, we lost the ability for the mass spectrometer to recognize the magnet position and accurately calibrate the accelerating voltage required to analyze a specific mass. In addition, the timing of the lock loss was not predictable, sometimes occurring after only one injection or sometimes after several injections.

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 a MAT magnetic sector mass spectrometer. Decreasing the accelerating voltage below 3 or 3.5 kV leads to a huge loss in sensitivity and a considerable loss of resolution. Therefore, the mass spectrometer will typically not be able to retain lock throughout the analysis. To use the ions that have typically been monitored for PCB 178 and *p,p′*-DDT, the magnetic field must be changed between these peaks.

Changing the magnetic field is now a fast process on modern magnetic sector mass spectrometers; however, it is best to have a gap between peaks

Table 2
Limits of detection (LODs) and measured values obtained from serum sample analyses for selected PCB congeners and organochlorine pesticides

Analyte	Separate analyses		Combined analysis	
	LOD	Concentration (pg/g)	LOD	Concentration (pg/g)
PCB 28	0.50	531±32	0.36	525±19
PCB 74	0.50	358±21	0.52	355±13
PCB 99	0.50	462±29	0.66	472±31
PCB 105	0.50	493±23	0.74	506±19
PCB 118	0.50	549±27	0.92	560±32
PCB 138/158	0.50	1046±59	0.88	1023±76
PCB 146	0.50	502±33	1.31	487±70
PCB 153	0.50	618±56	1.20	594±83
PCB 156	0.50	470±31	0.81	462±39
PCB 170	0.50	435±50	0.94	428±43
PCB 180	0.50	503±36	0.99	486±43
PCB 201	0.50	354±55	0.71	368±53
B-HCCH	5	955±87	0.75	940±92
G-HCCH	5	637±55	0.65	620±53
Dieldrin	5	705±74	2.56	682±95
HCB	5	696±73	3.3	691±93
Heptachlor epoxide	5	624±53	0.57	635±31
o,p′-DDT	5	725±69	2.44	742±52
p,p′-DDT	5	716±55	0.69	692±54
p,p′-DDE	5	3212±256	0.80	3257±248
Oxychlor	5	562±47	0.98	556±83
trans-Nonachlor	5	767±56	0.88	756±44
Mirex	5	415±96	2.15	436±96

B-HCCH=β-HCCH; G-HCCH=γ-HCCH.

because changes in retention times among samples (often seen with serum extracts) can cause peaks to be missed. Therefore, this approach is not a practical one for this analysis. Instead, we reinvestigated the full scan spectra of each analyte to determine whether alternative ions could be monitored by the mass spectrometer without reducing the sensitivity, precision, or accuracy of the analysis. Indeed, for the problematic retention time windows with large high to low mass ratios, we were able to select less abundant fragment ions of the PCBs without sacrificing the method sensitivity (Table 2). We did this by carefully optimizing the electron energy for the chosen fragment ions for several of the PCBs that elute near the lower molecular mass pesticides. Specifically, we selected fragment ions that represented the loss of two chlorine atoms for the hexa-, hepta-, octachloro PCBs and also for the nonachloro PCB 208. A total ion chromatogram of the PCBs and OC pesticides is shown in Fig. 2. All compounds were separated by time or mass except for the hexachloro PCBs 138 and 158 and the octachloro PCBs 196 and 203; these isomer pairs are 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 11 persistent pesticides in a single mass spectral run were nearly identical to those of the PCBs and OC pesticides in separate mass spectral runs (Table 2). A paired *t*-test analysis confirmed that the two data sets were statistically indistinguishable. The means of the extracts analyzed were within 4% of each other and there was no systematic bias between methods.

The precision and limits of detection of the two mass spectrometric methods were comparable. The limits of detection were nearly identical in the low pg/ml range. The relative standard deviation (RSD)

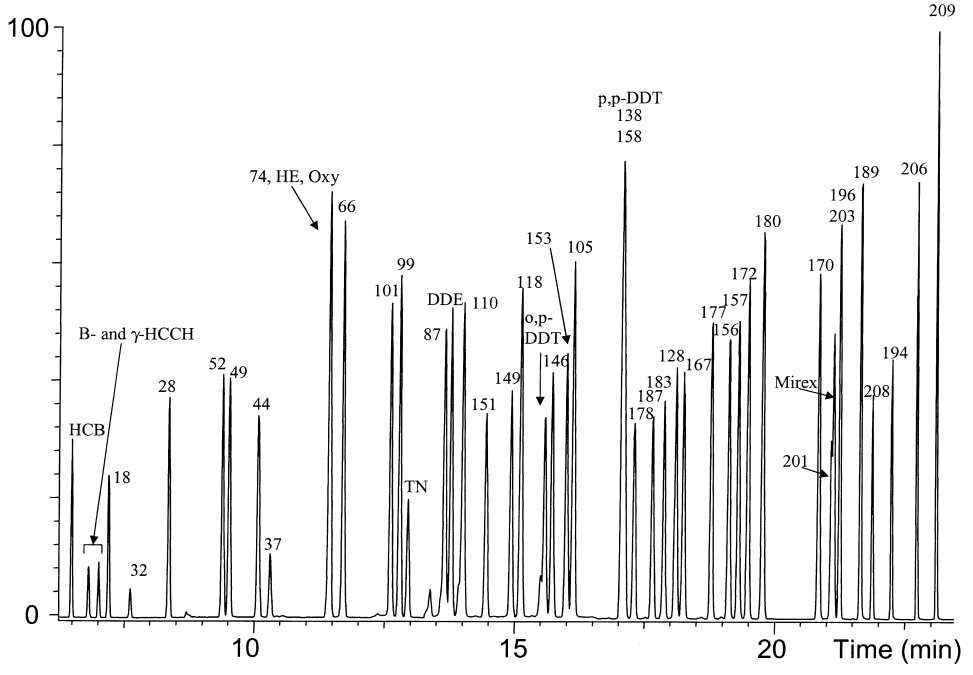

Fig. 2. Total ion chromatogram of polychlorinated biphenyls and organochlorine pesticides in a pooled serum extract. HE=heptachlor epoxide; TN=*trans*-nonachlor; Oxy=oxychlordane; HCB=hexachlorobenzene; β- and γ-HCCH=the beta and gamma isomers of hexachlorocyclohexane.

included both the error from the sample preparation and the instrumental methods. The RSDs of both methods were close for the PCBs (mean RSDs=10.6 and 11.2%, for the two injection and single injection methods, respectively) and the OC pesticides (mean RSDs=10.2 and 11.1%, for the two injection and single injection methods, respectively). Plots showing the correlation between the two methods for PCBs and OC pesticides are shown in Figs. 3 and 4. The slopes of linear regression analyses of the plots were within 4% of unity indicating good agreement among the methods. Pearson correlation analysis showed the two data sets were highly (r=0.9738 for PCBs and 0.9848 for pesticides) and significantly (P<0.0001) correlated.

We evaluated the reliability and robustness of the mass spectrometric method over the typical duration of a study using archived serum samples. We found that the instrument was able to maintain lock during overnight runs throughout the duration of the study. The routine maintenance required for successful daily operation was no different than that required for the older methods. No significant problems were observed in the chromatograms and no unusual interferences were observed during peak integration. Overall, the instrument performance and maintenance was similar to that of the older method indicating that the combined analysis is a reliable method.

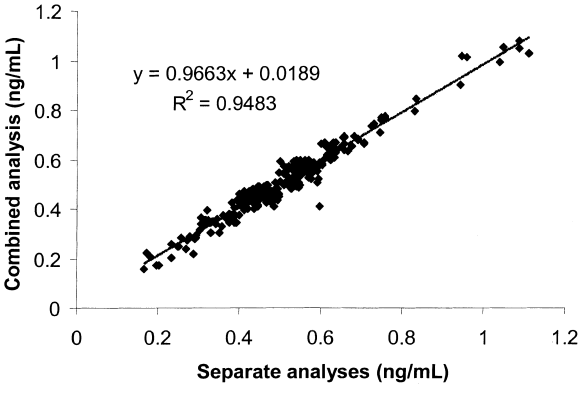

Fig. 3. Comparison of new and old polychlorinated biphenyl and organochlorine pesticides methods (N=284). For the two data sets, a Pearson correlation of 0.9738 and a P value <0.0001 were obtained.

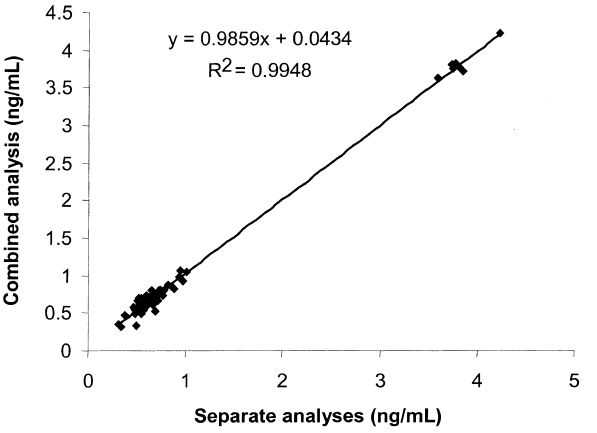

Fig. 4. Comparison of new and old organochlorine pesticide methods (N=70). No values were in the mid-range in our quality control material. If the upper data points are eliminated, a similar correlation still exists. For the two data sets, a Pearson correlation of 0.9848 and a P value <0.0001 were obtained.

4. Conclusions

The careful use of fragment ions, optimization of electron energy, and selection of mass spectral SIM groups allowed us to analyze 38 biologically relevant PCBs and 11 OC pesticides in a single serum sample with a single mass spectral analysis. This is a sensitive, selective and reliable method with detection limits in the low pg/ml or parts-per-trillion (ppt) range. This method has also been found to be amenable to automation. We have been able to successfully analyze almost 50 samples per day of both classes of compounds. Because of the timesavings due to both the combined analysis and automation, the overall cost of the analysis has decreased significantly.

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