Potential application of gas chromatography—tandem mass spectrometry in the measurement of coeluting isomers

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ABSTRACT - Despite the unprecedented popularity of separation chromatography, the measurement of coeluting isomeric chemicals remains an extremely difficult task. An analytical scheme capable of measuring two coeluting isomers was developed using a single chromatographic column and a gas chromatography-tandem mass spectrometry (GC/MS/MS) system. The protocol utilized two product (secondary) ion fragments, generated from a common parent molecular ion (primary) associated with the isomers, for quantitation. The utility of the analytical scheme was demonstrated with the measurements of several pairs of coeluting polychlorinated biphenyl (PCB) isomers in standard solutions and fish liver samples. Best results were achieved if the abundance ratio of the two product ion fragments was greater than unity for one isomer and less than unity for the other isomer. Analyses of seven fish liver samples collected from nearshore San Diego indicated that the domain that had been previously reported to comprise PCB 153 and PCB 168 actually contained PCB 153 only. Although only a selected number of PCB congeners were examined, the results indicate that the analytical scheme has the potential to be used to determine the concentrations of all chromatographically coeluted isomers.

INTRODUCTION

Chromatography is probably the most widely used separation tool in the physical sciences. Chromatography allows the separation of a sample into a series of chromatographic peaks, each representing a component in the sample. The ability to separate two components in a sample is defined as chromatographic resolution that is generally dictated by two parameters, column selectivity and column efficiency (Harvey 2000). Typically, chromatographic separation is provided by a column packed or coated with a desirable stationary phase. When a sample is applied to the column, the components undergo a number of cycles of fractionation between the stationary phase and a mobile phase, either a liquid or a gas. It is apparent that chromatographic resolution may be associated with a number of factors and can be altered by adjusting these factors against the properties of the components under consideration.

It frequently occurs that two chemicals may not be chromatographically separable under any chromatographic conditions or a group of components may not be completely separated with a single chromatographic column. Since mass spectrometry (MS) is capable of differentiating non-isomeric coeluting chemicals by their distinct fragmented ion profiles, this type of coeluting chemical is separable if a MS detector is employed. It is the isomeric coeluting chemicals that have posed a significant technical challenge to analytical chemists, because the conventional MS approach usually is unable to discriminate fragmented ion profiles from isomeric molecules.

One example of this challenge is the measurement of polychlorinated biphenyls (PCBs) on a congener-specific basis. Despite the tremendous efforts that have been devoted to the development of PCB congener-specific analysis methods (Pellizzari *et al.* 1985), no single chromatographic column has been able to separate all 209 congeners in a single run. Although multi-column approaches have been developed to separate all coeluting PCB congeners (Schulz *et al.* 1989, Larsen *et al.* 1992, Frame 1997), the techniques are technically difficult to implement and apparently not feasible for many analytical laboratories. On the other hand, the large variability of individual PCB congeners in toxicity and biodegradability necessitates the congener-specific measurement of PCB concentrations in environmental samples.

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We present herein an analytical scheme that allows separate measurements of two coeluting isomers with a Saturn 2000 ion trap gas chromatography/mass spectrometry (GC/MS) system (Varian Inc., Walnut Creek, CA). The analytical scheme is based on the concept of a two-component model (Zeng and Yu 1996), which does not require the generation of different quantitation ions from the coeluting isomers. Instead, the method utilizes the ratio of two predominant product ions fragmented from a common parent ion associated with the coeluting isomers. Since any commercially available ion trap GC/MS system has the capability of generating product ions from the parent ion of interest, the analytical scheme can be easily implemented and is highly affordable. The laboratories at the Southern California Coastal Water Research Project (SCCWRP) and the Wastewater Chemistry Laboratory of the City of San Diego (designated as San Diego hereafter) conducted independent experiments to demonstrate the utility of the analytical scheme with a subset of coeluting PCB isomers. This article reports the results of method calibration and analyses of spiked solutions performed at SCCWRP, and analyses of fish liver samples conducted in San Diego.

THEORY

We consider two coeluting PCB isomers (designated as PCB 1 and PCB 2 hereafter) that have a common parent molecular ion. The parent ion can be fragmented into two molecular ions, I_1 and I_2, with abundances of A_1 and A_2. If the contributions from PCB 1 and PCB 2 to A_1 are A_{11} and A_{21}, respectively, we have

$$A_1 = A_{11} + A_{21} \qquad (1)$$

Similarly, if the contributions to A_2 from PCB 1 and PCB 2 are A_{12} and A_{22}, respectively, we obtain

$$A_2 = A_{12} + A_{22} \qquad (2)$$

Apparently, A_1 and A_2 are measured from GC/MS experiments. The concentrations of PCB 1 and PCB 2 can be determined from abundances A_{11}, A_{12}, A_{21}, and A_{22} using an internal calibration method. If the relative response factors (RRFs) corresponding to A_{11}, A_{12}, A_{21}, and A_{22} are RRF_{11}, RRF_{12}, RRF_{21}, and RRF_{22}, A_{is} is the abundance of the internal standard, and C_{is} is the concentration of the internal standard, the concentration of PCB 1, C_1, in a sample can be determined by

$$C_1 = \frac{A_{11}C_{is}}{RRF_{11}A_{is}} \qquad (3)$$

or

$$C_1 = \frac{A_{12}C_{is}}{RRF_{12}A_{is}} \qquad (4)$$

Similarly, the concentration of PCB 2, C_2, can be obtained by

$$C_2 = \frac{A_{21}C_{is}}{RRF_{21}A_{is}} \qquad (5)$$

or

$$C_2 = \frac{A_{22}C_{is}}{RRF_{22}A_{is}} \qquad (6)$$

Equations (3-6) can be converted into:

$$A_{11} = \frac{C_1RRF_{11}A_{is}}{C_{is}} \qquad (7)$$

$$A_{12} = \frac{C_1RRF_{12}A_{is}}{C_{is}} \qquad (8)$$

$$A_{21} = \frac{C_2RRF_{21}A_{is}}{C_{is}} \qquad (9)$$

$$A_{22} = \frac{C_2RRF_{22}A_{is}}{C_{is}} \qquad (10)$$

Combining Equations (1-2) with Equations (7-10) leads to

$$A_1 = \frac{C_1RRF_{11}A_{is}}{C_{is}} + \frac{C_2RRF_{21}A_{is}}{C_{is}} \qquad (11)$$

$$A_2 = \frac{C_1RRF_{12}A_{is}}{C_{is}} + \frac{C_2RRF_{22}A_{is}}{C_{is}} \qquad (12)$$

Combining Equations (11) and (12) to solve for C_1 and C_2 yields

$$C_1 = \frac{A_1RRF_{22} - A_2RRF_{21}}{RRF_{11}RRF_{22} - RRF_{12}RRF_{21}} \qquad (13)$$

and

$$C_2 = \frac{A_1RRF_{12} - A_2RRF_{11}}{RRF_{12}RRF_{21} - RRF_{11}RRF_{22}} \qquad (14)$$

In both Equations (13) and (14), C_{is}, A_{is}, A_1, and A_2 are obtained from the analysis of the actual sample, and RF_{11}, RF_{12}, RF_{21}, and RF_{22} can be determined from the initial calibration experiments.

To ensure that the concentrations of PCB 1 and PCB 2 are determined correctly using Equations (13) and (14), the following constraint must be satisfied:

$$\frac{RRF_{12}}{RRF_{21}} \neq \frac{RRF_{21}}{RRF_{22}} \text{ or } \frac{A_{11}}{A_{12}} \neq \frac{A_{21}}{A_{22}} \quad (15)$$

Practically, A_{11}/A_{12} should be substantially different from A_{21}/A_{22} in order to obtain C_1 and C_2 without ambiguity. A more stringent constraint, as described previously (Zeng and Yu 1996), is as follows:

$$0 < \frac{A_{11}}{A_{12}} < 1 \text{ and } \frac{A_{21}}{A_{22}} > 1 \text{ or } \frac{A_{11}}{A_{12}} > 1 \text{ and } 0 < \frac{A_{21}}{A_{22}} < 1 \quad (16)$$

As demonstrated below, the variability in measurements tends to be large when the constraint in Equation (16) is not satisfied. It should be noted that Equations (13) and (14) can be used to determine concentrations of any two coeluting isomers, although they were originally derived for PCB isomers.

METHODS

Experimental Design

Several sets of experiments were conducted to demonstrate the utility of the analytical scheme. First, a screening test was run to identify potentially coeluting PCB isomers. A series of standard solutions containing 209 PCB congeners was prepared with each containing distinctly separable congeners (based on prior findings). Second, based on the results of the screening test, a selected group of PCB congeners was subject to further investigation. A list of PCB isomer pairs deemed inseparable under the chromatographic conditions used in the present study was identified from this set of experiments. Third, the selected PCB isomer pairs were prepared in two groups at five concentration levels. The stability of the ratio of two quantifying ions was examined over the mass scanning range of each chromatographic peak at each of the concentration levels. The RRF of each congener was also obtained. Fourth, the analytical scheme was validated with analyses of a series of spiked solutions containing the selected PCB congeners at varying concentrations. Measured concentrations of the PCB congeners were obtained with the newly acquired RRFs and Equations (13) and (14) and compared with the spiking concentrations. Finally, the analytical scheme was applied to the measurement of PCB 153 and PCB 168 in fish liver samples.

Chemicals

Custom-made solutions of PCBs with 20 mg/mL for each congener in hexane:isooctane (98:2) were acquired from AccuStandard (New Heaven, CT). Individual PCB standards in isooctane were also obtained from AccuStandard with various concentrations. Hexane and methylene chloride of pesticide grade or equivalent were purchased from VWR International (West Chester, PA) and used as supplied. Anhydrous sodium sulfate was purchased from Fisher Scientific (Pittsburgh, PA) and purified by heating at 550°C for at least 1 h in a shallow tray or at 100°C overnight in an oven before use. Concentrated sulfuric acid (certified A.C.S.) was also obtained from Fisher Scientific and used as supplied. Mercury was recycled from mercury-containing devices such as thermometers, filtered, and washed with hexane before use.

Preparation of Standard Solutions

Standard solutions with various combinations of PCB congeners at various concentrations were prepared in hexane with appropriate glassware. Each solution also contained internal standards (PCB 30 and 205) at 200 ng/mL each.

Collection and Extraction of Fish Samples

Several species of flat and rock fish were collected in April 2000 from the coastal areas off San Diego using a standard protocol (City of San Diego 2001a) and cooled with ice on board and during transportation to the San Diego laboratory, where individual fish species were dissected immediately. Fish liver samples were kept at –20°C from the time of dissection until extraction.

Fish liver was homogenized using a tissue homogenizer and approximately 0.3 g of each liver sample was weighed into a 50 mL beaker and mixed with sodium sulfate. The mixture was transferred to a stainless steel extraction cell (33 mL in size) containing 3 g of alumina powders. The sample was extracted using an automated accelerated solvent extractor system (ASE 200; Dionex, Sunnyvale, CA) at 100°C and 1500 psi with methylene chloride:hexane

(1:1) mixture as a solvent. The extract was dried with sodium sulfate and concentrated to 1 mL using a TurboVap 500 concentrator (Zymark, Hopkinton, MA). One to three drops of mercury were added to the extract cell and the cell was subsequently agitated on a vortex mixer for at least 2 min. The extract cell was centrifuged for at least 15 min to separate the sample from the mercury layer. About 0.5 to 1 mL of concentrated sulfuric acid was added to the extract cell and the cell was manually shaken for 5 min. The extract was centrifuged for 30 min and the organic layer was removed with a Pasteur pipette into a clear 2-mL vial for instrumental analysis.

Instrumental Analysis

Tandem mass spectra are generated by the ion trap GC/MS system via four steps: (1) ion production and matrix ion ejection; (2) parent ion isolation; (3) product ion formation by collision induced dissociation (CID); and (4) product ion mass scanning (Varian 1995). The selective storage and dissociation of the ions of interest may eliminate a large portion of background interferences. Two CID methods are available for use to generate product ions: resonant and non-resonant excitation. The resonant excitation method applies a high-frequency supplemental dipole field to the end cap of the ion trap. The frequency is chosen so that a specific chemical bond or group is dissociated. Therefore, the resultant mass fragment profile produced by resonant excitation is very sensitive to the structure of the molecule under consideration. Parent ions from closely eluting isomers with a small difference in molecular structure can be fragmented into identical product ions, but the mass spectral profiles could be vastly different. Leonards *et al.* (1996) demonstrated that the product ions of PCB 77 and PCB 126 could be generated sequentially with varying CID excitation voltages using the resonant excitation mode. With all of these considerations, the resonant excitation mode was chosen in the present study.

Both laboratories used the Varian Saturn 2000 GC/ion trap-MS system equipped with a 60 m × 0.32 mm i.d. (0.25 µm film thickness) DB-XLB column (J&W Scientific, Folsom, CA). The oven temperature program used by researchers at SCCWRP was as follows: initial temperature was set at 60°C (held for 1 min), programmed to 180°C at a rate of 15°C/min, ramped to 280°C at 2°C/min, and further increased to 310°C (no holding time) at 5°C/min. The injector temperature was initially set at 60°C (held for 0.3 min), increased to 310°C at a rate of 200°C/min, and held for 25 min. The temperature program used by the San Diego laboratory was slightly different but achieved similar chromatographic separation efficacy.

RESULTS AND DISCUSSION

Identification of Coeluting PCB Isomers

Several sets of retention time data for 209 PCB congeners are available in the literature. Mullin *et al.* (1984) used a 50 m×0.2 mm i.d. SE-54 column to acquire retention times for 209 PCB congeners in a 140-minute run. J&W Scientific (1996) published retention times of 209 PCB congeners on three DB-XLB columns. Cochran and Reese (1997) obtained relative retention times of 209 PCB congeners on one DB-XLB column and one experimental XLB column. The criteria for resolved peaks might depend on the column type, temperature program, instrument conditions, and desired accuracy of the peak assignment. We considered two peaks as resolvable if the retention time difference was greater than 0.2 minutes under the chromatographic conditions employed in the present study. Since non-isomeric congeners can be adequately separated by the conventional GC/MS method, they are regarded as separable even if they coelute chromatographically.

Although we did not set out to measure all coeluting PCB isomers, initial efforts were made to identify all possible coeluting isomers and select the appropriate ones for further investigation. Thirty-two domains (each containing at least one pair of coeluting PCB isomers) were identified based on the retention times of 209 PCB congeners published by J&W Scientific (1996), comprising 80 PCB congeners in total. The retention times of these PCB congeners were obtained initially using the full scan mode. A close analysis of the chromatograms for these congeners revealed that only 18 domains containing a total of 41 PCB congeners were deemed inseparable.

These congeners were prepared in two solutions (each containing one of the coeluting isomers) and analyzed again using the resonant CID method based on the automated method development mode after fine-tuning the GC/MS system. It was found that three pairs of PCB isomers (Table 1) remained coeluted and satisfied the constraint contained in Equation (16). They were used for further investigations. An additional three pairs of coeluting PCB isomers (Table 1) not complying with Equation (16) but complying with Equation (15) were also selected for comparison.

Table 1. Retention times and characteristic ion fragments for 6 pairs of PCB isomers selected for investigations. [a]

	Group 1	Structure	RT (min)	Group 2	Structure	RT (min)	Parent ion	Product Ion	
(A)									
	42	2, 2', 3, 4'	30.60	59	2, 3, 3', 6	30.56	292	257	222
	153	2, 2', 4, 4', 5, 5'	42.25	168	2, 3', 4, 4', 5', 6	42.25	360	325	290
	182	2, 2', 3, 4, 4', 5, 6'	45.25	175	2, 2', 3, 3', 4, 5', 6	45.23	396	361	326
(B)									
	10	2, 6	19.52	4	2, 2'	19.48	222	187	152
	143	2, 2', 3, 4, 5, 6'	40.13	139	2, 2', 3, 4, 4', 6	40.10	360	325	290
	203	2, 2', 3, 4, 4', 5, 5', 6	53.30	196	2, 2', 3, 3', 4, 4', 5, 6'	53.15	430	395	360

[a]Coeluting isomers were prepared in separate solutions to obtain retention times.

Fragmentation Patterns of Coeluting PCB Isomers

To generate different fragment patterns from coeluting isomers, the MS/MS parameters were optimized within the specified mass scanning windows. Besides CID amplitude, CID time and ionization storage level were also adjusted manually. Other CID-related parameters, including (but not limited to) emission current, target total ion current (TIC), prescan ionization time, ejection amplitude, isolation window, low edge offset, high edge offset, high edge amplitude, isolation time, waveform type, modulation range, modulation rate, number of frequencies, excitation time, CID frequency adjustment, and CID bandwidth, were adjusted automatically by the software package supplied with the Saturn 2000 GC/MS system (Varian 1995). The automated method development mode was used to vary the values of individual parameters to obtain the fragmentation pattern of interest. More than one ion fragment associated with the optimized parameters were simultaneously scanned using the multiple reaction monitoring (MRM) approach. A series of MRM methods, covering all the mass scanning windows, were integrated into a specific GC/MS/MS method.

It was found that the CID amplitude was the most important factor that dictated the abundance ratios of product ions from coeluting PCB isomers. In the case of PCB 153 and PCB 168, as the CID excitation amplitude increased, the abundance ratio of *m*/*z* 290 to *m*/*z* 325 for PCB 168 increased faster than that for PCB 153. When the excitation amplitude was greater than 1.5 V, the abundance ratio for PCB 168 became greater than unity while that for PCB 153 remained smaller than unity (Figure 1) (Chou 2000). This example illustrates that careful adjustment of the CID excitation amplitude could generate two product ions from PCB isomers in compliance with the constraint in Equation (16).

Stability of Abundance Ratios

Abundance ratio is a critical variable in the current approach to separation of coeluting PCB isomers. Since peak areas are normally used for quantitation, the accuracy of the measured concentrations of two coeluting isomers is highly dependent upon the stability of abundance ratios over a chromatographic peak. A set of experiments was conducted to assess the stability of abundance ratios at

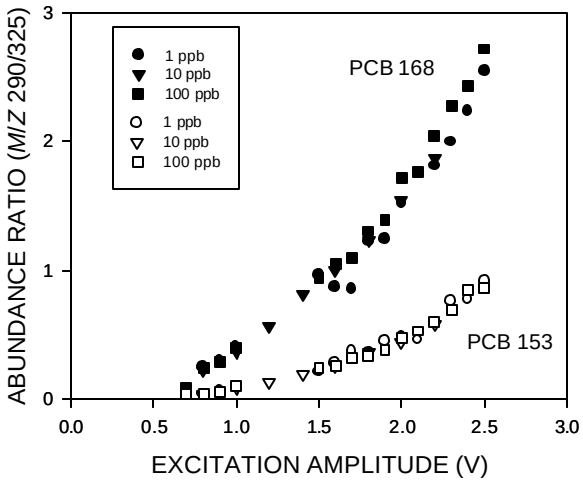
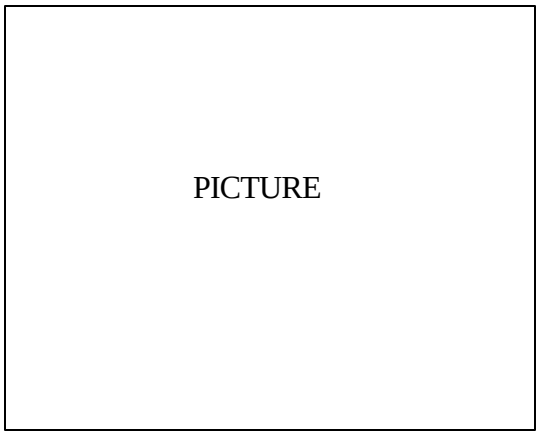


Figure 1. Variation of abundance ratios of *m*/*z* 290 to *m*/*z* 325 for PCB 168 and PCB 153 with CID amplitude.

each of the peak scans, and the results are described below.

The three pairs of PCB isomers (Table 1) in compliance with Equation (16) were prepared in two solutions (each containing one of the coeluting isomers) with concentrations of 4, 10, 20, 50, 100, and 200 ng/mL and analyzed using the resonant CID method. Responses from the 4 and 10 ng/mL solutions were fairly weak and were not used for further evaluation. Abundance ratios of two product ions from each parent ion (Table 1) were calculated at each scan point. An average abundance ratio was calculated from all the scan points constituting a peak.



It is obvious that the abundance ratios for the three pairs of coeluting isomers were quite stable (Table 2). The average abundance ratio for each congener varied within a small range at each concentration level, as indicated by the small relative standard deviation (RSD) values ranging from 8.3 to 25.3% (calculated from Table 2). Only 2 out of 24 measured RSDs exceeded 20% (20.3% and 25.3%). Therefore, highly stable abundance ratios of two product ions could be obtained by the ion trap MS technique. In addition, the average abundance ratios obtained for various congener concentration levels essentially overlapped. The small variability in the abundance ratios with respect to the scan points and concentration range justified the use of the abundance ratios to determine the concentrations of two coeluting isomers using Equations (13) and (14).

We summarized all the peak abundances from the same experiments (Table 2) for each ion fragment and each concentration level and conducted linear regressions over the five concentration levels (including zero concentration). Most of the regressions carried a coefficient of variation of 0.99 or 1.00 (except for one regression with $r^2 = 0.97$ and another regression with $r^2 = 0.98$). This indicated that either product ion from each isomer could be used for quantitation purposes if a regular GC/MS method is employed.

Validation Experiments: Analyses of Spiked Solutions

To use Equations (13) and (14) to estimate the concentrations of two coeluting PCB isomers, four RRFs related to the two product ion fragments need to be determined. By definition, these RRFs are obtained from peak abundances of the product ion fragments from the coeluting isomers and the related internal standard using Equations (3), (4), (5), and (6). The analyses of the four solutions presented in Table 2 were used to obtain RRFs. As described above, the abundance ratios for the three pairs of coeluting PCB isomers satisfying Equation (16) were quite stable over the scans of an entire peak for any of the coeluting PCB isomers. This ensured the reliability of the peak areas used to calculate RRFs. These RRFs would be used to calculate the concentrations of the coeluting isomers in spiked and field samples.

Table 2. Average abundance ratios from the scans of an applicable peak at various calibration concentrations. [a]

		20 ppb		50 ppb		100 ppb		200 ppb	
Ion Ratio	PCB	AVE	SD	AVE	SD	AVE	SD	AVE	SD
222/257	42	0.65	0.10 (14)	0.67	0.06 (15)	0.65	0.07 (17)	0.62	0.07 (18)
	59	3.04	0.51 (15)	3.74	0.59 (17)	4.19	0.37 (20)	4.16	0.48 (21)
290/327	153	0.56	0.14 (13)	0.53	0.11 (17)	0.59	0.07 (19)	0.62	0.09 (20)
	168	1.29	0.23 (13)	1.50	0.25 (19)	1.82	0.34 (20)	2.08	0.38 (22)
292/327	182	1.27	0.24 (12)	1.47	0.16 (16)	1.50	0.17 (20)	1.41	0.16 (21)
	175	0.83	0.13 (15)	0.96	0.11 (17)	0.99	0.14 (19)	0.92	0.13 (23)

[a] AVE = average; SD = standard deviation. The number in parentheses is the number of scans for the chromatographic peaks.

Table 3. Measured recoveries (%) of coeluting PCB congeners from various spiked samples.

	Spiking Concentration (ng/mL)[a]						
PCB	20/20	40/40	100/100	40/100	100/40	AVE[b]	STDEV[b]
(A)							
42	109.6	121	121.8	123.5	126.5	120.5	6.4
59	84.5	109.4	106.0	105.2	111.6	103.3	10.8
153	134.9	130.2	119.9	132.3	131.4	129.7	5.8
168	65.2	84.7	92.8	91.2	61.2	79.0	14.8
182	71.4	81.6	64.5	60.2	98.5	75.2	15.3
175	135.3	141.9	153.2	124.8	160.8	143.2	14.3
(B)							
10	98.6	114.2	130.9	110.9	132.8	117.5	14.4
4	97.1	122.6	121.5	114.6	135.0	118.2	13.9
143	238.9	276.6	272.1	553.6	196.8	307.6	141.2
139	-25.0	-42.5	-44.6	-58.1	-72.9	-48.6	18.0
203	6.0	4.6	33.9	44.3	60.1	29.8	24.2
196	226.1	233.0	193.8	136.2	270.6	211.9	50.4

[a]The spiking concentrations (n/m) are labeled as follows: n is the concentration of the first isomer and m the concentration of the second isomer.

The recoveries of six pairs of coeluting PCB congeners in five spiked solutions were calculated from Equations (13) and (14) and the known spiking concentrations (Table 3). In general, the three pairs of co-eluting isomers (42/59, 153/168, and 182/175) in compliance with Equation (16) exhibited better recoveries than the other three pairs (4/10, 143/139, and 196/203) not satisfying Equation (16). This emphasizes the importance of the constraint contained in Equation (16) in achieving a unique mathematical solution to the two-component model (Zeng and Yu 1996). Among the three pairs in compliance with Equation (16), the measured recoveries were fairly consistent, as indicated by the small values of standard deviation (<15%). The recoveries were best with PCB 42 and PCB 59 and appeared disproportional to the RRFs (not shown). For the three pairs not in compliance with Equation (16), only 10/4 showed reasonable recoveries and standard deviations. The recoveries of the other two pairs were highly unacceptable (the recoveries of PCB 139 were even negative at all the spiking concentrations). Therefore, the use of an analytical scheme to measure coeluting isomers would gain the best results if the constraint in Equation (16) were strictly satisfied.

Analyses of Fish Liver Samples

Upon validation, the analytical scheme was applied to the analysis of the fish liver samples. The focus of the analysis was to determine the concentrations of PCB 153 and PCB 168 that coeluted under the chromatographic conditions currently used. These congeners were part of the 41-PCB congener list for a regional environmental survey on the Southern California Bight coordinated by SCCWRP and participated in by more than 40 organizations in 1998 (Noblet *et al.* 2002). Among the 41 PCB congeners, PCB 153 and PCB 168 were the only coeluting isomers and were therefore critical for assessing the ecological implications of PCB contamination in the Southern California Bight. This analyte list has also been adopted by the City of San Diego in its ocean monitoring program for the measurement of PCB congeners in fish samples, but PCB 153 and PCB 168 have been reported as one entity in the annual monitoring report (City of San Diego 2001b).

Seven fish liver samples were processed and analyzed by the San Diego laboratory. The concentrations of PCB 153 and PCB 168 were determined using peak areas from two product ions (m/z = 290 and 325) and Equations (13) and (14). Since the San Diego laboratory used the external calibration method, response factors instead of relative response factors were used in Equations (13) and (14). It can be shown that the concentrations of PCB 168 in the fish liver extracts were essentially zero (Table 4). The chromatographic domain believed to comprise PCB 153 and PCB 168 actually contained PCB 153 only. This result might imply a different toxicity indication from the routine PCB analysis in general, since PCB 153 was believed to possess a slightly higher toxicity impact than PCB 168 (McFarland and Clarke 1989).

Table 4. Concentrations of PCB 153 and PCB 168 in fish liver extracts.

Sample	Peak Area		Concentration (ppb)[a]	
	m/z 325	*m/z* 290	PCB 153	PCB 168
1	118825	99566	80	-1.0
2	21394	18126	17	-0.2
3	17030	14855	13	0.1
4	29663	24129	23	-1.1
5	33396	29550	25	0.6
6	23656	20154	18	-0.2
7	27611	24058	21	0.2

[a]Calculated using the peak areas and Equations (13) and (14). Response factors required by Equations (13) and (14) were obtained prior to the analyses of the fish liver extracts.

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