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Potential Application of Gas Chromatography/Tandem Mass Spectrometry in the Measurement of Coeluting Isomers

Eddy Y. Zeng,*,† C. C. Chou,‡ and Charlie Yu§

Southern California Coastal Water Research Project, 7171 Fenwick Lane, Westminster, California 92683, Wastewater Chemistry Laboratory, Environmental Monitoring and Technical Services, City of San Diego, 5530 Kiowa Drive, La Mesa, California 91942, Environmental Monitoring Division, City of Los Angeles, 12000 Vista del Mar, Playa del Rey, California 90893

Despite the unprecedented popularity of separation chromatography, the measurement of coeluting isomeric chemicals remains an extremely difficult task. We developed an analytical scheme capable of measuring two coeluting isomers using a single chromatographic column and a gas chromatography/tandem mass spectrometry system. The protocol utilized two product ion fragments generated from a common parent ion 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 given when a set of stringent constraints for the abundance ratio of the two product ion fragments was satisfied. Analyses of seven fish liver samples collected from nearshore San Diego, CA, 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 presented indicate that the analytical scheme has the potential to be used to determine the concentrations of all chromatographically coeluted isomers.

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.[1] 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 using a single chromatographic column. Since mass spectrometry (MS) is capable of differentiating nonisomeric coeluting chemicals by their distinct fragmented ion profiles, this type of coeluting chemicals 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,[2] no single chromatographic column has been able to separate all 209 congeners in a single run. Although multidimensional gas chromatography (GC) approaches have been developed to separate all coeluting PCB congeners,[3−5] 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.

We present herein an analytical scheme that allows separate measurements of two coeluting isomers with a Saturn 2000 ion trap GC/MS system (Varian Inc., Walnut Creek, CA). The analytical scheme is based on the concept of a two-component model,[6] 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

* Corresponding author: Fax: (714) 894-9699. E-mail: eddyz@sccwrp.org.
† Southern California Coastal Water Research Project.
‡ City of San Diego.
§ City of Los Angeles.

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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. Although the analytical scheme is demonstrated with PCB congeners as target analytes, it can potentially be applied to any other isomer pairs. The laboratories at the Southern California Coastal Water Research Project (SCCWRP) and Wastewater Chemistry Laboratory of the City of San Diego (designated as San Diego thereafter) conducted independent experiments to demonstrate the utility of the analytical scheme with a subset of coeluting PCB isomers. This paper 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 thereafter) that have a common parent ion. The parent ion can be fragmented into two product 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

$$A_1 = A_{11} + A_{21} \tag{1}$$

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

$$A_2 = A_{12} + A_{22} \tag{2}$$

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}, respectively; 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

$$C_1 = \frac{A_{11}C_{is}}{RRF_{11}A_{is}} \tag{3}$$

or

$$C_1 = \frac{A_{12}C_{is}}{RRF_{12}A_{is}} \tag{4}$$

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

$$C_2 = \frac{A_{21}C_{is}}{RRF_{21}A_{is}} \tag{5}$$

or

$$C_2 = \frac{A_{22}C_{is}}{RRF_{22}A_{is}} \tag{6}$$

Equations 3−6 can be converted into

$$A_{11} = \frac{C_1RRF_{11}A_{is}}{C_{is}} \tag{7}$$

$$A_{12} = \frac{C_1RRF_{12}A_{is}}{C_{is}} \tag{8}$$

$$A_{21} = \frac{C_2RRF_{21}A_{is}}{C_{is}} \tag{9}$$

$$A_{22} = \frac{C_2RRF_{22}A_{is}}{C_{is}} \tag{10}$$

Combining eqs 1−2 with eqs 7−10 leads to

$$A_1 = \frac{C_1RRF_{11}A_{is}}{C_{is}} + \frac{C_2RRF_{21}A_{is}}{C_{is}} \tag{11}$$

$$A_2 = \frac{C_1RRF_{12}A_{is}}{C_{is}} + \frac{C_2RRF_{22}A_{is}}{C_{is}} \tag{12}$$

Combining eqs 11 and 12 to solve for C_1 and C_2 yields

$$C_1 = \frac{C_{is}}{A_{is}} \frac{A_1RRF_{22} - A_2RRF_{21}}{RRF_{11}RRF_{22} - RRF_{12}RRF_{21}} \tag{13}$$

and

$$C_2 = \frac{C_{is}}{A_{is}} \frac{A_1RRF_{12} - A_2RRF_{11}}{RRF_{12}RRF_{21} - RRF_{11}RRF_{22}} \tag{14}$$

In both eqs 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 PCB1 and PCB 2 are determined correctly using eqs 13 and 14, the following constraint must be satisfied.

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

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 set of more stringent constraints, as described previously (Zeng and Yu 1996), is as follows: or

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

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

As demonstrated below, the variability in measurements tend to be large when the constraints in eq 16 are not satisfied. It should be noted that eqs 13 and 14 can be used to determine concentra-

tions of any two coeluting isomers, although they were originally derived for PCB isomers.

EXPERIMENTAL SECTION

Chemicals. Custom-made solutions of PCBs with 20 μg/mL for each congener in hexane/isooctane (98:2) were acquired from AccuStandard (New Heaven, CT). Individual PCB standards in isooctane with various concentrations were also obtained from AccuStandard. 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 using appropriate glassware. Each solution also contained internal standards (PCB 30 and 205) and surrogate standards (PCB 65 and 209) at 200 ng/mL each.

Collection and Extraction of Fish Samples. Several species of flat and rock fish were collected from the coastal areas off San Diego using a standard protocol[7] 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 ~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 33-mL extraction cell containing 3 g of alumina powders. Surrogate standards in solution were added to the cell at 533 ng/g. About 15 mL of methylene chloride/hexane (1:1) mixture was added to the cell, and the sample was extracted using an accelerated solvent extractor system (ASE 200; Dionex, Sunnyvale, CA). The extract was 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 pipet into a clear 2-mL vial. Internal standards were added to the extract prior to instrumental analysis.

Instrumental Analysis. Both laboratories conducted chromatographic analyses using the Varian Saturn 2000 GC/ion trap-MS system. Chromatographic separation was provided by 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 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.

With the resonant excitation mode, we found that collision-induced dissociation (CID) amplitude was the single most critical parameter associated with the formation of product ion fragments. Hence, efforts were mainly directed toward the adjustment of CID amplitude to obtain the desirable product ion fragment patterns.

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.[8] used a 50 m × 0.2 mm-i.d. SE-54 column to acquire retention times for 209 PCB congeners in a 140-min run. J&W Scientific[9] published retention times of 209 PCB congeners on three DB-XLB columns. Cochran and Reese[10] obtained relative retention times of 209 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 >0.2 min under the chromatographic conditions employed in the present study. Nonisomeric congeners can be unambiguously identified by the conventional GC/MS method, because different characteristic ion fragments may be generated and used for quantitation. Therefore, 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 investigations. A subset of the 209 PCB congeners suspected of being coeluting on the basis of their retention times determined by J&W Scientific[9] were analyzed chromatographically with 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 on the basis of the retention time criteria.

These congeners were prepared in two solutions (each containing one of the coeluting isomers) and analyzed again using the resonant CID method on the basis of the automated method development mode after fine-tuning the GC/MS system. It was found that only three pairs of PCB isomers (Table 1) clearly satisfied the constraint expressed in eq 16. They were used for further investigations. An additional three pairs of coeluting PCB isomers (Table 1) not complying with eq 16 but complying with eq 15 were also selected for comparison. For the purpose of demonstration, we decided to choose only six isomer pairs for detailed examination. This allowed us to sufficiently demonstrate the efficacy of the analytical scheme without considering all possible scenarios.

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Table 1. Retention Times and Characteristic Ion Fragments for Six Pairs of PCB Isomers Selected for Investigations[a]

group 1	structure	R_T (min)	group 2	structure	R_T (min)	parent ion	product ion	
			(A) Isomer Pairs Complying with Eq 16					
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	362	327	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) Isomer Pairs not Complying with Eq 16					
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 (groups 1 and 2, respectively) to obtain retention times.

Fragmentation Patterns of Coeluting PCB Isomers. The molecular ion of a PCB congener was generated via electron impact reaction. Two daughter ions corresponding to the dissociation of one chlorine (^{35}Cl or ^{37}Cl) and two chlorines, respectively, from the molecular ion, were produced using the CID mode. To generate different fragment patterns from coeluting isomers, the MS/MS parameters were optimized within the specified mass scanning windows. In addition to CID amplitude, CID time and ionization storage level were also adjusted manually. Other CID-related parameters were adjusted automatically by the software package supplied with the Saturn 2000 GC/MS system.[11] 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 168, as the CID excitation amplitude increased, the abundance ratio of *m*/*z* 290−325 for PCB 168 increased faster than that for PCB 153. When the excitation amplitude was >1.5 V, the abundance ratio for PCB 168 became greater than unity, but that for PCB 153 remained smaller than unity (Figure 1).[12] 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 eq 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 each of the peak scans, and the results are described below.

The three pairs of PCB isomers (Table 1) in compliance with eq 16 were prepared in two solutions (each containing one of the coeluting isomers) at 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.

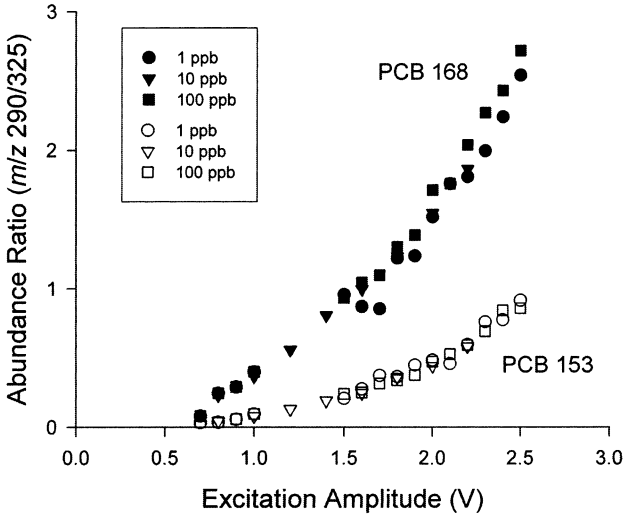

Figure 1. Variation of abundance ratios of *m*/*z* 290−325 for PCB 168 and 153 with CID amplitude.

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 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 were essentially constant. 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 eqs 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 0 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 $r^2 = 0.98$).

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Table 2. Average Abundance Ratios from the Scans of an Applicable Peak at Various Calibration Concentrations

		20 ppb		50 ppb		100 ppb		200 ppb	
ion ratio	PCB	av[a]	SD[b]	av	SD	av	SD	av	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)
326/361	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] Average. [b] Standard deviation. The numbers in a parentheses are the numbers 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	av[b]	STDEV[c]
(A)							
42	109.6	121.0	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. [b] Average value of five recoveries. [c] Standard deviation from five recoveries.

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 eqs 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 eqs 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 eq 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.

The recoveries of six pairs of coeluting PCB congeners in five spiked solutions were calculated from eqs 13 and 14 and the known spiking concentrations (Table 3). In general, the three pairs of coeluting isomers (42/59,153/168, and 182/175) in compliance with eq 16 exhibited better recoveries than the other three pairs (4/10, 143/139, and 196/203) not satisfying eq 16. This emphasizes the importance of the constraint contained in eq 16 in achieving a unique mathematical solution to the two-component model.[6] Among the three pairs in compliance with eq 16, the measured recoveries were fairly consistent indicated by small values of standard deviation (<15%). The recoveries were best with PCB 42 and 59 and appeared disproportional to the RRFs (not shown). For the three pairs not in compliance with eq 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 the analytical scheme to the measure coeluting isomers would gain the best results if the constraint in eq 16 were strictly satisfied.

Analyses of Fish Liver Samples. Upon validation, the analytical scheme was applied to analyses of the fish liver samples. The focus of the analysis was to determine the concentrations of PCB 153 and 168 that coeluted under the chromatographic conditions currently used. These congeners were part of the 41-PCB congener list for a regional coastal environmental survey on the Southern California Bight conducted in 1998.[13] Among the 41 PCB congeners, PCB 153 and 168 were the only coeluting isomers, based on a DB-XLB column, and 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 168 have been reported as one entity in the annual monitoring reports.[14]

Seven fish liver samples were processed and analyzed by the San Diego laboratory. The concentrations of PCB 153 and 168 were determined using peak areas from two product ions (m/z = 290 and 325) and eqs 13 and 14. Since the San Diego laboratory used the external calibration method, response factors instead of relative response factors were used in eqs 13 and 14. It can be shown that the concentrations of PCB 168 in the fish liver extracts were essentially 0 (Table 4). The chromatographic domain believed to comprise of PCB 153 and 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.[15]

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Table 4. Concentrations of PCB 153 and 168 in Fish Liver Extracts

	peak area		concn (ppb)[a]	
sample	*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 eqs 13 and 14. Relative response factors required by eqs 13 and 14 were obtained prior to the analyses of the fish liver extracts.

Final Remarks. Three issues remain to be addressed in order to fully utilize the potentials of the analytical scheme presented for the measurement of coeluting isomers. The first issue is how the accuracy of the concentrations calculated from eqs 13 and 14 is affected by the variability in other experimental parameters. The accuracy of the calculated concentrations could be determined by a second analysis employing a chromatographic column capable of separating the isomers. In reality, however, that separation capability is not always available. Another approach would be to estimate the uncertainty of the calculated concentrations from the measurement errors associated with the measured parameters via eqs 13 and 14.

The second issue is the relationship between the reliability of the concentrations calculated from eqs 13 and 14 and the abundance ratios of two product ions for the coeluting isomers under consideration. As indicated in eqs 13−15, the calculated concentrations would become ridiculous as the abundance ratios of two product ions from two coeluting isomers approach the same value. Since the abundance ratios are obtained experimentally, the measured values may become statistically indistinguishable, even though the apparent values are different. Therefore, it is desirable to determine the acceptable ranges for the abundance ratios with which the concentrations of the coeluting isomers can be calculated reliably. That is why the constraints in eq 16 can be used as a reliable threshold before specific acceptable ranges are developed.

The third issue involves the expansion of the analytical scheme into measurements of more than two coeluting isomers. The applicability of the analytical scheme would be limited if only two-isomer systems could be dealt with. To determine the concentrations of more than two coeluting isomers, the number of product ions generated from a common molecular ion should be the same as the number of coeluting isomers under consideration. The equations (equivalent to eqs 13 and 14 for two-isomer systems) for calculating isomer concentrations would have to be obtained from an $N \times N$ coefficient matrix, where N is the number of coeluting isomers.

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