Statistical method to evaluate clean-up procedures in polychlorinated biphenyl analysis

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The clean-up procedure involved in the trace analysis of polychlorinated biphenyls (PCBs) was evaluated by studying GC–MS chromatograms of PCB mixtures after the subsequent steps of the procedure. The complex chromatograms obtained were evaluated using two methods: the individual PCB congener method and a chemometric approach based on a study of the autocovariance function. The results obtained are statistically comparable, proving that the statistical approach is able to determine the total PCB content in the sample quantitatively. Moreover, since the autocovariance method is based on statistical evaluation of the whole complex chromatogram, it can overcome the problems that usually arise because of peak overlapping in PCB mixture chromatograms. It also provides accurate results when the chromatogram shows interfering peaks and low resolution. This is the case with Aroclor 1242, where some compounds released by the cartridges strongly interfere with the analysis, resulting in errors in the quantification of individual PCBs. Highly chlorinated PCBs (*i.e.*, Aroclor 1260) can be quantitatively recovered (mean recovery, 100 ± 1%). In contrast, lower proportions (less than 75%) of the less chlorinated compounds (*i.e.*, those containing two and three chlorine atoms) are recovered because they are selectively retained by silica and alumina columns.

Keywords: *Polychlorinated biphenyl analysis; clean-up procedure; chemometrics; multicomponent chromatograms; procedure evaluation*

Polychlorinated biphenyls (PCBs) are ubiquitous environmental pollutants world-wide: they can be found in soils, oils, sediments, waters, animal tissues and airborne particulates. The determination of PCBs in various complex matrices is mandatory for environmental monitoring, and for studying their toxic effects and biodegradation processes. The determination of trace levels of these organic compounds is a complicated procedure, consisting of many steps—sample homogenisation, extraction from the matrix, clean-up and concentration, gas chromatographic separation and detection—each of which can significantly contribute to the total error in the final determination.[1–9]

The clean-up procedure has proved fundamental in removing interferences and increasing the accuracy and precision of the final result.[1,9] Co-extracted compounds can interfere with the final determination of PCBs, since contamination can overload a high-resolution gas chromatographic (HRGC) column, create negative peaks or an erratic response when electron-capture detection (ECD) is used, or can lead to co-elution with PCBs, resulting in misidentification and incorrect determination.[1] Several methods have been proposed to remove interfering, co-extracted compounds. In particular, solid-phase extraction (SPE) has been widely employed with various chromatographic phases such as silica, Florisil, alumina and graphitic carbon. Moreover, the availability of inexpensive commercial cartridges for SPE has prompted widespread application to various complex matrices.[1,3,6]

Considerable attention has recently been paid to the quality of analytical results—in particular when they are derived from multi-step procedures—in order to ensure that the chemical measurements are comparable and accurate. A number of interlaboratory studies have been organised with the following objectives: to determine variations in PCB determinations; to identify what sources cause these variations; and to reduce them through a step-by-step learning process.[2–8] The sources of systematic, random errors produced by the various steps in the method can be evaluated by following the traceability chain of the analytical procedure in detail.[10–12] In particular, the different validation methods determine the loss of selected target compounds (single PCB congener method) and possible interferences introduced in the subsequent analysis steps. Current methods are based on area or height determination of selected target peaks present in the chromatogram. However, these quantities can be affected by several biasing factors: mutual overlapping of single component peaks, column efficiency degradation and sample overloading.

In this paper, an alternative approach is presented for evaluating the clean-up procedure, using SPE with silica and alumina cartridges. In essence the following properties of the chromatographic separation are estimated: (1) the number of single components (SCs), m, present in the mixture analysed; (2) their relative abundance compared with an internal standard; and (3) the peak capacity, n_c, *i.e.*, a check of column performance and any degradation. In this manner, each step of the clean-up procedure can be completely evaluated by controlling: the variation of m, the mean loss, or increase, in PCB concentration, and the efficiency of the separation system, affected either by any degradation in column efficiency or by sample overloading.

The method consists of a chemometric approach to evaluate multicomponent chromatograms, whenever there is a high degree of overlapping.[13–16] Distinct features of the method, based on a study of the autocovariance function,[17–25] are its ability to determine the mean number of single components (m) which is usually greater than the number of observed peaks, because of the severe peak overlapping present in multicomponent chromatograms.[13] The method was tested by simulation[20] and by application to test mixtures:[21,22] it was found to give unbiased estimation of both the peak capacity of the separation and the single component number of the analysed mixture. In the present paper, the simplified graphical version is applied,[23–25] which does not require complex mathematics: it gives estimates of comparable precision and accuracy and it was also shown to be able to detect column overloading effects.[23]

The aim of the present study was to test how the individual steps of a multi-step procedure affect the results of the final determination; this goal may be achieved by defining a method for a rapid and accurate estimation of multicomponent chromatograms. The final purpose of the proposed method was its application to real environmental samples; here, a preliminary study is reported referring to a simple synthetic sample.

Experimental

Reagents and apparatus

Aroclor 1242 and 1260 standard mixtures were obtained from Alltech Italia (Milan, Italy). Commercially available chromatographic cartridges of silica and neutral alumina were also supplied by Alltech Italia.

The chromatograph used was a Mega Series 5160 fitted with a 30 m × 0.25 mm id column coated with a 0.25 μm film of DB-5 (J & W Scientific, Rancho Cordova, CA, USA); it was equipped with a QMD1000 quadrupole mass spectrometer (Fisons Instruments, Milan, Italy).

Analytical methodology

Aroclor 1242 and 1260 standard mixtures (2 ml of 50 ppm solutions, samples 1242St and 1260St) were first eluted in silica cartridges with 4 ml of hexane (samples 1242Si and 1260Si, respectively), the solution was reduced to a volume of 2 ml in a Kuderna–Danish apparatus and then eluted in alumina cartridges with 4 ml of hexane–dichloromethane (98 + 2, v/v) (samples 1242Al and 1260Al, respectively). The same procedure was carried out on cartridges pre-washed with 3 ml of hexane before use (samples 1242Si pw, 1242Al pw, 1260Si pw, 1260Al pw). Solutions of Aroclor 1242 (50 ppm) in hexane and hexane–dichloromethane (98 + 2, v/v) were concentrated in a Kuderna–Danish apparatus (from 6 to 2 ml) and the samples 1242Hex and 1242CH_2Cl_2, respectively, were obtained. A standard solution of octachloronaphthalene at 5 ppm was added to each sample prior to chromatographic analysis as an internal standard in order to normalise the total area of all chromatograms. Each mixture was submitted to a procedure three times so that three chromatograms were obtained for each sample: the reported values are mean values calculated on the replicate determinations.

All the samples were analysed in the GC–MS system under programmed capillary GC conditions:[22] after 1 min at 90 °C, the column temperature was increased from 90 to 160 °C at 25 °C min^{-1}, and then from 160 to 300 °C at 3 °C min^{-1}; the final temperature of 300 °C was maintained for 30 min. Sample volumes (2 μl) were injected with splitting: the split ratio was 20 : 1, and the splitless time was 1 min.

The electron impact (EI) mass spectra were recorded, and the total ion current (TIC) chromatograms were automatically converted by an ADC (with Δt = 1 s) and stored in the GC–MS system. Obviously, a GC–MS analysis under selected ion monitoring (SIM) conditions may be much more selective than TIC detection:[22] in this paper, TIC detection was exploited in order to investigate general effects of sample contamination and sample loss during the steps of the clean-up procedure.

Procedure for chromatogram evaluation

Study of the complex chromatogram

The final step of PCB analysis always involves evaluation of a complex chromatogram since, in environmental matrices, PCB congeners usually occur as multicomponent mixtures of 40–50 congeners.[1] It has been demonstrated[13] that when a large number of single components (SCs) are present in a mixture, complex retention patterns, determined by peak overlapping, are always obtained even when HRGC systems are employed for separation. Quantitative analysis of PCB compounds in these complex chromatograms requires identification and quantification of some resolved peaks corresponding to single congeners (individual congener method): this determination is difficult, as well as time consuming, and can cause errors since it is not possible to achieve complete PCB separation.[1,2,6]

Autocovariance method

Different statistical methods have been developed to evaluate the properties of multicomponent chromatograms.[13–25] Among them, Dondi and co-workers proposed a procedure, based on a study of the use of the autocovariance function to estimate the attributes describing the chromatographic separation.[17–25] The method was widely investigated:[18,21] it was tested by using computer-simulated chromatograms and the validity of the results obtained was confirmed;[22,23] also, the applicability to experimental chromatograms[21,22,25] was verified. From the chromatogram, acquired in digitised form, the experimental autocovariance function (EACVF) can be directly calculated, according to the expression:

$$C(\tau) = \frac{1}{M}\sum_{j=1}^{N-k}(Y_j - \hat{Y})(Y_{j+k} - \hat{Y}) \quad k = 0, 1, 2, 3, \ldots M-1 \quad (1)$$

where Y_j and Y_{j+k} are the heights in the digitised chromatogram at the j and $(j + k)$ retention time positions, respectively; k is the fixed interdistance used for correlation, N the number of points in the digitised chromatogram, M - 1 the maximum extension over which EACVF is calculated and $\hat{Y}$ the mean calculated from the chromatogram as:

$$\hat{Y} = \frac{\sum_{i}^{N} Y_i}{N} \quad (2)$$

The autocovariance function expresses the intercorrelation between peak heights within the chromatogram as a function of time span, τ, *i.e.*, the interdistance between subsequent chromatographic peaks, expressed either by k or by the equation:

$$\tau = k\Delta t \quad (3)$$

where Δt is the interdistance between subsequent sampled points. In this paper the value Δt = 1 s was used; the time range of interest for all the chromatographic separations was 1200 s; therefore, the number of points N on which $C(\tau)$ was calculated was 1200. It has been demonstrated that the most significant information is contained in the first part of the $C(\tau)$ plot; therefore, it was calculated in the interval $0 \leqslant \tau \leqslant 16\,\sigma$, *i.e.*, M = 32, since in the chromatograms studied σ is approximately 2 s (see below).

The autocovariance function can be plotted against the time span, τ. Fig. 1 shows the EACVF plot calculated from the chromatogram of a PCB mixture, Aroclor 1260, reported in

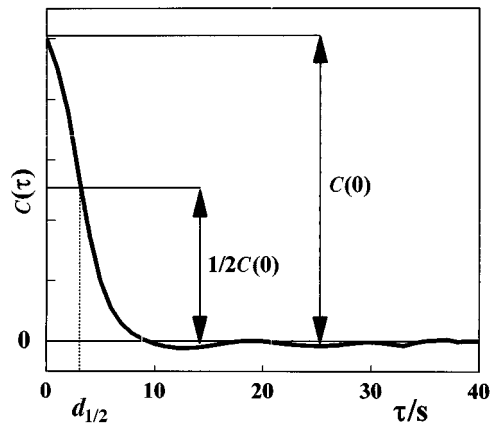

Fig. 1 Plot of the autocovariance function $C(\tau)$ calculated for a PCB mixture [sample 1260St, Fig. 2(*a*)]: $C(0)$ and $d_{1/2}$ are evaluated from graphical inspection and parameters m and $w_{1/2}$ are calculated [eqns. (4) and (5)].

Fig. 2(*a*). Such a plot appears as half of an approximately Gaussian peak, the properties of which proved to be related to two basic chromatographic separation parameters:[23] *m*, the number of components present in the mixture and σ, the mean standard deviation of the chromatographic peak of a single component. In particular:

The value of EACVF at the origin, *C*(0) in Fig. 1, estimates *m*:

$$m = \frac{A_T^2\left(\frac{\sigma_m^2}{a_m^2}+1\right)}{C(0)d_{1/2}2.129X} \quad (4)$$

where: σ_m^2/a_m^2 is the relative dispersion of heights of peak maxima, a_m the mean value of heights of peak maxima and σ_m^2 the standard deviation of peak maximum distribution; A_T is the total area of the acquired chromatogram (see below); X is the total time span in which the chromatographic separation is performed.

The width, at half height, of the EACVF peak, $d_{1/2}$ in Fig. 1, gives an estimate of σ, the standard deviation of a pure single component peak, which is expressed in the chromatogram by the width at half the peak height:

$$w_{1/2} = d_{1/2}1.414 \quad (5)$$

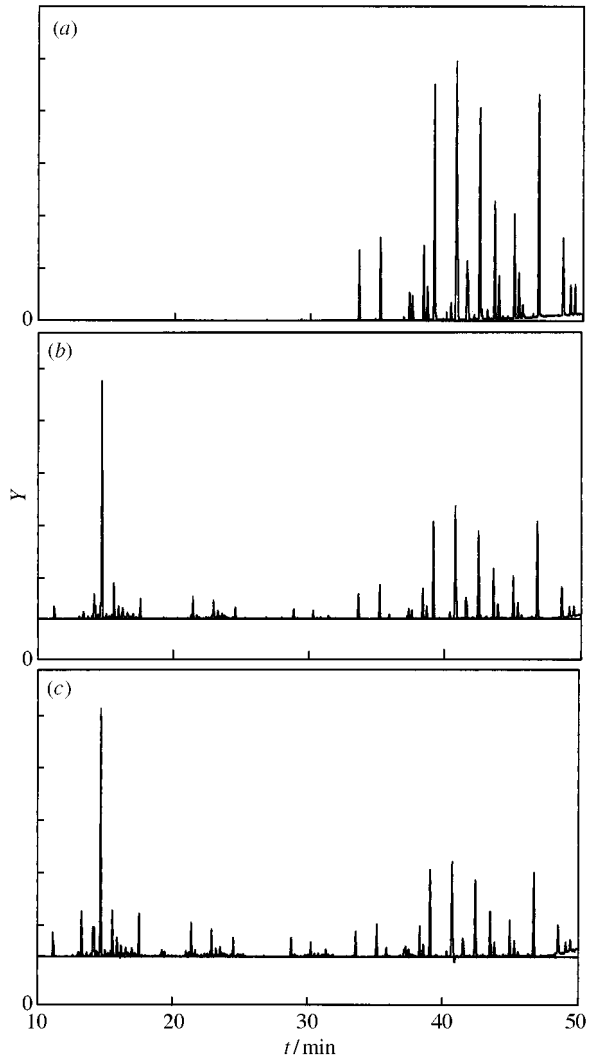

Fig. 2 Chromatograms obtained from 1260 mixture (50 ppm) after the different steps of the clean-up procedure: (*a*) original sample (sample 1260St); (*b*) after elution on silica cartridge (sample 1260Si); (*c*) after subsequent clean-up on silica and alumina (sample 1260Al).

A_T, the total area of the chromatogram, can be calculated from the digitised chromatogram according to the equation:

$$A_T = \sum_i^N Y_i \quad (6)$$

where N is the number of points of the acquired chromatogram (here, N = 1200).

From the two basic quantities, m and σ, all the other chromatographic parameters can be determined so that all PCB mixture properties can be evaluated.[21–24]

It must be emphasized that the proposed method can only be applied when the SC peak shape is constant, this is usually the case with programmed elution techniques. Moreover, since the method is based on determining the width of an EACVF peak at half height, it is sufficiently robust towards moderate SC peak shape asymmetry as is usually found in temperature programmed HRGC analysis of PCBs.[22–25] In addition, the method was able to detect column overloading effects.[23]

A basic parameter, describing the effectiveness of any chromatographic system, is the peak capacity, n_c, which, for constant peak width conditions, is:[19]

$$n_c = \frac{X}{4\sigma R_S} \quad (7)$$

n_c expresses how many SC peaks the chromatographic system can separate at a given resolution, R_s, when they are sequential, with a constant peak standard deviation, σ, over the chromatographic space, X. This parameter explains the performance of the separation system and can be determined and controlled during the procedure. In this paper an R_s value of 0.5—describing peak maximum separation—is assumed.

The extent of separation achieved is expressed by γ, the separation extent, *i.e.*, the ratio of SCs appearing in the chromatogram as peaks:

$$\gamma = \frac{p}{m} \quad (8)$$

Owing to peak overlapping, the number of peaks seen in the final chromatogram, p, is not the number m of components present in the mixture—*i.e.*, when each peak is a pure peak formed by a single component (SC)—but only a proportion of them since, in addition to the pure peaks, there are others formed by two or more SCs.

The mean area A_m of a single component can be calculated as:

$$A_m = \frac{A_T}{m} \quad (9)$$

The total area A_T expresses the total amount of PCBs present in the mixture, while A_m represents the mean amount of each congener present in the mixture. This amount is really an average value, also accounting for variations in detector sensitivity. To make different chromatograms comparable, the chromatographic response (peak heights or areas) must be normalised. In this work, this was achieved by referring the A_T values of all the chromatograms studied to the peak area of the internal standard (octachloronaphthalene, 5 ppm), which was added to all the samples immediately before the chromatographic analysis. This procedure increases the reproducibility of the measurements: the total area values of the normalised chromatograms exhibited a reproducibility of ±2%.

Recovery evaluation

To evaluate the clean-up procedure, the PCB content in the original and cleaned-up mixtures was quantitatively calculated and compared. Two methods were used:

Individual PCB congener method.[1] In the complex chromatograms obtained, some resolved peaks are selected as corresponding to single PCB congeners. This procedure is fairly complex and time consuming: in chromatograms of Aroclor 1242, where peak overlapping is severe, only ten congeners could be identified with absolute certainty, while for the 1260 mixture, 16 PCBs were found. The recovery of each PCB is calculated by comparing areas of the corresponding peaks in the original and cleaned-up samples: the recovery of each mixture is calculated as the mean of the values for the selected congeners.

Autocovariance function method.[17–25] From the digitised chromatogram, A_T values are calculated and normalised relative to the area of the internal standard, octachloronaphthalene. Using the autocovariance method, the number of components present in the mixture, m, can be estimated according to eqn. (4) and from it the mean area, A_m, is calculated [eqn. (9)]. Both A_T and A_m can be used to calculate the PCB content in the mixture by comparing them to corresponding areas (total and mean, respectively) in the standard mixtures (samples 1242St and 1260St): the values obtained were determined with a precision of ±2%. The percentage variation of A_T and A_m among the original sample and those eluted on cartridges indicates the percentage recovery during the clean-up step.

Results and discussion

PCB mixtures were analysed in a system acquiring a TIC chromatogram. The GC–MS chromatograms obtained from the original solutions (samples 1242St and 1260St) were compared with those obtained after silica clean-up (samples 1242Si and 1260Si) and after subsequent elution on alumina (samples 1242Al and 1260Al). Chromatograms obtained using this procedure for Aroclor 1260 are reported in Fig. 2: original sample (*a*), mixture after elution on silica cartridge (*b*) and after subsequent elution on alumina (*c*). These steps were performed on non-pre-washed cartridges.

From the reported chromatograms it is evident that, when the mixture is eluted on a silica cartridge, nearly 15 peaks appear in the first part of the chromatogram (10–30 min) [Fig. 2(*b*)]. The same peaks are present at a higher concentration in the sample subsequently eluted on alumina [Fig. 2(*c*)]. They are also present in procedural blanks and chromatograms obtained from the 1242 mixture after clean-up. Moreover, these compounds severely interfere with the analysis of such a mixture, since they elute in the same chromatographic space as do the low-chlorinated PCBs contained in Aroclor 1242. In order to reduce this interference, cartridges were pre-washed with the solvent (3 ml of hexane) prior to use, as recommended by the supplier.[1] The chromatograms obtained (1242Si pw, 1242Al pw, 1260Si pw, 1260Al pw) showed that pre-washing moderately reduces the number of interfering peaks. MS analysis identified the interfering compounds as alkenes and alkyl phthalates: it has been reported that these compounds are used in the poly(propylene) housing and polyethylene frit,[26] or are impurities in the stationary phase,[27] and are thus released by the cartridges themselves.

Chromatogram evaluation

The characteristics of the chromatograms studied are reported in Table 1. Some values are directly calculated from the experimental chromatogram, *i.e.*, the number of observed peaks, p, and the total area, A_T. Other parameters can be estimated by the EACVF method, *i.e.*, the number of components, m, the mean chromatogram area, A_m, the mean width at half the chromatographic peak height, $w_{1/2}$, the capacity factor, n_c, and the separation extent γ. All the reported results refer to the chromatographic space actually used for the separation (10–30 and 30–50 min for 1242 and 1260 mixtures, respectively; hence, $X = 1200$ s for all the chromatograms).

With the $w_{1/2}$ values it is possible to evaluate separation system performance and follow it through the various analysis steps. The data obtained are small (4.5 ± 0.1 s) and constant in all the chromatograms studied: consequently, the n_c values [derived according to eqn. (7)] are also constant ($n_c = 316 \pm 8$). These results prove that the chromatographic system used performed well in all the separations reported. This control is important, as mentioned above, since it may single out a possible decline in column efficiency, a poor column choice, injection problems or uncontrolled sample overloading.[23] Among these problems, the last-named is particularly significant since it can substantially change the conditions under which single components can be quantified by evaluating area or height and can significantly affect the final analytical result.

From the data in Table 1 it is apparent how interfering, cartridge-released compounds cause errors in the analysis of Aroclor 1242 samples: the observable parameters, *i.e.*, the number of peaks, p, and the total area, A_T, increase significantly in the mixtures eluted on cartridges. It is possible to calculate the total PCB content in each mixture by comparing the total chromatogram area, A_T, with the area of a standard Aroclor chromatogram (sample 1242St). The results obtained (second column in Table 1**)** show that for cleaned-up 1242 samples (samples 1242Si, 1242Si pw, 1242Al, 1242Al pw), the PCB content is erroneously over-estimated (72–74 ppm of total PCBs); the A_T values are higher because there are interfering compounds in the chromatographic space.

The EACVF method was applied to evaluate m [eqn. (4)]: the results obtained (third column in Table 1) agree with the data describing Aroclor mixture composition.[1,22] It should be noted that, since it is a parameter statistically evaluated, it is affected by an error of $\sqrt{m}$.[17] Nearly 20 additional components are estimated in the 1242 mixture after elution on the silica and alumina cartridges: this is why the PCB content was over-estimated. The A_m values [calculated from A_T and m according to eqn. (9) and reported in the fifth column of Table 1] are not so severely affected by interference as are the A_T values since, in this case, higher A_T values are compensated for by higher m. From A_m, the total PCB content can be calculated (sixth column in Table 1) with reference to the A_m of standard mixtures (samples 1242St and 1260St): the results obtained are not affected by over-estimation and can be considered a correct estimation of the total PCB content of the samples analysed.

Table 1 Properties of chromatograms of Aroclor samples obtained after different clean-up steps: p, number of observed peaks; PCB content calculated from A_T values; m, number of components; $w_{1/2}$, mean value of peak width at half the chromatographic peak height; A_m, mean area [eqn. (9)]; PCB content calculated from A_m values; γ, separation extent [eqn. (8)

Sample	p	PCB content from A_T (ppm)	m	$w_{1/2}$/s	A_m	PCB content from A_m (ppm)	γ
1242St	35	50	45	4.5	64 676	50	0.78
1242Hex	36	50	46	4.5	63 663	50	0.78
1242CH_2Cl_2	36	51	45	4.5	64 532	50	0.80
1242Si	42	72	66	4.5	51 741	40	0.63
1242Si pw	40	73	62	4.5	59 735	42	0.64
1242Al	41	74	65	4.5	45 272	35	0.63
1242Al pw	39	72	61	4.5	47 859	37	0.64
1260St	38	50	47	4.5	42 242	50	0.81
1260Si	38	51	48	4.5	42 115	50	0.79
1260Si pw	36	50	46	4.5	42 199	50	0.78
1260Al	37	40	47	4.5	41 397	49	0.79
1260Al pw	37	51	46	4.5	41 927	50	0.80

The γ values (seventh column in Table 1) provide information on global peak overlapping in the chromatogram. In the chromatogram of cleaned-up 1242 mixtures, the extent of separation attained is significantly lower than in standard Aroclor (γ decreases from 0.8 to 0.6). This is due to the interference of compounds released by the cartridges. Prewashing the cartridges does not significantly reduce the number of interfering compounds (compare samples 1242Si and 124Al with samples 1242Si pw and 1242Al pw, respectively): therefore, in all the chromatograms obtained after clean-up procedures, the degree of overlapping is so severe that only 60% of the compounds present in Aroclor 1242 produce chromatographic peaks.

For Aroclor 1260, the chromatographic separation attained is not affected by interferences released during the clean-up procedure, as described by the constancy of the parameters *p*, *m*, γ and PCB contents reported in Table 1. This result is expected, since compounds derived from cartridge elution are not arranged in the chromatographic space where the PCBs of the sample are placed [see Fig. 2(*a*)–(*c*)]: for this mixture a separation extent of 0.8 is always achieved.

Recovery determination

PCB recovery after different clean-up steps was determined using individual PCB and autocovariance function methods. The results are illustrated in Table 2: mean recovery and reproducibility (% RSD) from triplicate measurements are reported and compared. It should be noted that by use of the individual PCB method the PCB content of each mixture is determined as the mean value of only ten PCBs for Aroclor 1242, the chromatograms of which exhibit the most severe peak overlapping (γ values nearly 0.6, Table 1).

It is apparent that most of the results obtained by the two methods are statistically comparable, proving that the chemometric approach based on 'statistical evaluation' estimates nearly the same PCB content as does the classical individual PCB method. Some discrepancies exist in the data concerning 1242 samples: recovery values calculated by the individual PCB method are significantly higher than those estimated by autocovariance. This result is due to over-estimation of individual PCB content—the basis of the single PCB method—in the samples eluted on the cartridges, a consequence of interference and the low separation attained. It should be noted that the autocovariance method provides the more accurate results, since it is based on a statistical evaluation of the whole complex chromatogram and it is able to determine the properties hidden in the complex retention pattern due to peak overlapping.[24] Moreover, from Table 2 it is apparent that recovery values estimated with the autocovariance method show a better reproducibility, with relative standard deviations below 3%.

Table 2 PCB % recovery (% relative standard deviation of three repeated measurements) obtained with the individual PCB method and the proposed method

Sample	Individual PCB method	Proposed method
1242Hex	101* (5)	99 (3)
1242CH_2Cl_2	98* (4)	99 (2)
1242Si	89* (4)	80 (2)
1242Si pw	93* (3)	85 (3)
1242Al	80* (7)	70 (2)
1242Al pw	84* (5)	74 (3)
1260Si	99† (4)	100 (2)
1260Si pw	100† (5)	100 (2)
1260Al	101† (7)	98 (3)
1260Al pw	99† (5)	99 (2)

* Mean value calculated on ten PCBs. † Mean value calculated on 16 PCBs.

The results obtained for Aroclor 1260 show that the clean-up procedure is efficient, since all the PCBs are quantitatively recovered (mean recovery, 100 ± 2%).

For Aroclor 1242, mean recovery values near 80% were achieved for all samples. Less chlorinated compounds (*i.e.*, those containing two and three chlorine atoms) are recovered in lower proportions (lower than 75%) than the others.[28] A possible reason for this low recovery may be the selective evaporation of lighter PCB congeners during concentration in the Kuderna–Danish apparatus. To test this hypothesis, the concentration steps with hexane (sample 1242Hex) and with hexane–dichloromethane (sample 1242CH_2Cl_2) were examined separately. In this case, quantitative recoveries were obtained (see Table 2): this proves that evaporation is not the cause of PCB loss.[21] Therefore, the low recovery obtained must be ascribed to different causes, *i.e.*, the fact that PCBs with lower chlorination levels are selectively retained by the silica and alumina columns.[29]

Conclusions

The results obtained have demonstrated that the chemometric approach, based on a study of the autocovariance function, is a rapid, simple and precise method for estimating the properties of complex chromatograms. It overcomes the problems usually encountered in such evaluations due to peak overlapping present therein: the complexity of the mixture can be correctly estimated and the performance of the separation system can also be checked. Therefore, the approach can be proposed as a tool with which to evaluate an analytical procedure based on a chromatographic determination of multicomponent mixtures; it can be applied to monitor the traceability chain of any given method—by testing how the different steps involved in the procedure affect the final result—to eliminate bias and provide long-term stability and accuracy of measurements.

One drawback of the autocovariance method is that it is based on a 'statistical evaluation' of the number of components, *m*, realised on a large number of single components. In contrast, the 'individual PCB congener' method is based on the assumption that each peak is made up of a single component, whereas the peak might be formed by two or more components. Owing to peak overlapping,[13] this effect is severe in multicomponent chromatograms, such as those of PCB mixtures, and causes erroneous analytical results.

In this work, MS detection under TIC conditions was investigated, although different detection systems—such as MS under SIM conditions and ECD—with different linearity and sensitivity variations, may also be considered in a subsequent handling. The autocovariance method appears very promising for ECD—afflicted with limited linearity range—since it can immediately detect loss of system efficiency due to chromatographic column overloading or ageing.

Moreover, it has been verified that even a widely used procedure, such as clean-up with SPE cartridges, may introduce significant systematic errors in the final determination, because of the release of interfering compounds and low recovery. A strategy for ensuring the best quality of analytical measurements is always required in order to evaluate each individual step present in the methodology.

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