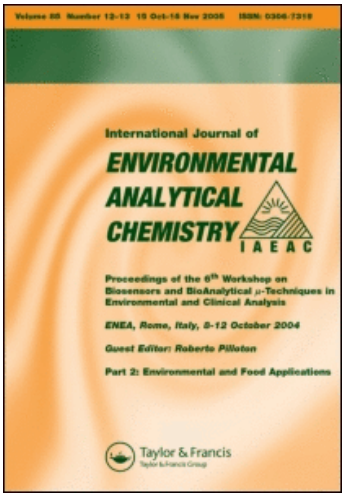

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Screening of Polychlorinated Biphenyls in Water Samples by Strategic Sample Composition-Solid Phase Extraction and Gas Chromatography Tandem Mass Spectrometry. Comparison of Different Strategies for Sample Composition

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SCREENING OF POLYCHLORINATED BIPHENYLS IN WATER SAMPLES BY STRATEGIC SAMPLE COMPOSITION-SOLID PHASE EXTRACTION AND GAS CHROMATOGRAPHY TANDEM MASS SPECTROMETRY. COMPARISON OF DIFFERENT STRATEGIES FOR SAMPLE COMPOSITION

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Strategic Sample Composition (SSC) is a new sample composition technique that allows the reduction of the number of analytical determinations to be carried out in screening campaigns down to the very number of the original sample specimens while providing information particularised to the original sample specimens instead of average information. The application of this technique in environmental screening studies is shown. Technical mixtures of polychlorinated biphenyls (Aroclors) have been chosen as model contaminants in water samples to show the usefulness and potential of the SSC technique in this field. EPA 1668/1668a protocols were used for sample treatment. Gas chromatography hyphenated to tandem mass spectrometry was used for the analysis of the samples. Two types of sample composition design matrices (a conventional Plackett–Burman screening matrix and a supersaturated matrix) were used and compared in the study. A total of 22 sample specimens were considered. Four of these sample specimens were contaminated at levels between 200 and 750 ng/L (total PCB concentration). A total of 24 experiments are needed to process these 22 sample specimens when applying the conventional Plackett–Burman matrix. Comparatively, only 13 analytical determinations are needed when using the supersaturated matrix. Both types of matrices allow clear identification of the contaminated sample specimens and produced satisfactory estimations of their concentration levels. Special emphasis has been put in investigating and demonstrating the robustness of the SSC technique.

Keywords: Sample composition; Experimental design; Supersaturated matrices; PCB analysis; SPE, GC-MS-MS

INTRODUCTION

Polychlorinated biphenyls (PCBs) are a well-known group of compounds of environmental concern, which were included in the lists of priority pollutants by the environmental agencies of USA and Europe several years ago. In spite of the fact that their use

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was banned in the 1970s, the analytical interest of PCBs still remains [1,2] because of their continued presence in the environment due to their extremely high resistance to degradation. In fact, the US EPA released its *Method 1668* [3] in 1997 and its revision *1668a* [4] in 1999 for the determination of these compounds in water, soil, sediment and tissues at the pg/L level (in the case of water). In order to obtain such low quantification limits, the procedure involves several steps including extraction, clean-up and concentration as well as special precautions to proper glassware decontamination. As a logical consequence and depending on the type of sample matrix, the whole analytical process per sample becomes very tedious and may take up to several days. Furthermore, the cost per analysis is quite high, not only because of the need of gas chromatography high resolution mass spectrometry (GC-HRMS) but also the use of ^{13}C labelled surrogate standards, which are also very expensive.

To alleviate some of these inconveniences, several alternative procedures have been developed, including enzyme immunoassay (EIA) [5] and phosphorimetry [6]. These techniques exhibit good selectivity and sensitivity while providing very fast results. However, only total PCB concentrations can be measured so it must be applied for gross screening campaigns. On the other hand, alternative procedures to liquid–liquid extraction or solid-phase extraction for sample preparation (e.g. solid-phase microextraction, [7]) have been proposed although Method 1668/1668a [3,4] continues to be the reference method for PCBs in environmental matrices. Laboratories lacking GC-HRMS demanded by the method approach the necessary selectivity and sensitivity by means of low-cost gas chromatography hyphenated to tandem mass spectrometry (GC-MS-MS) [8–11].

Another possibility in environmental screening studies is to resort to sample composition. Conventional sample composition is a well-known procedure to deal with a large number of sample specimens (throughout the text, the concept of *specimen* is defined as the individual samples where the analyst wants to detect and quantify the analytes of interest), aimed to increase the probability of detecting the analytes of interest while reducing the analytical costs [12]. For several decades, sample composition has been used when only the average or integrated properties of a population are of interest. Otherwise, if the interest was in the variability of the distribution of the sought-for components it is commonly accepted that any sample specimen should be treated on an individual basis [13,14]. Recently a new method for sample composition named Strategic Sample Composition (SSC) has been proposed that allows significant reductions in analytical costs while providing information directly on the original sample specimens [15–17]. SSC is clearly advantageous in cases such as the analysis of PCBs, as will be shown in this article.

In environmental screening studies, frequently the goal is to detect outliers in the huge amount of sample specimens taken from the studied area and not the attainment of an average value. Usually, it is expected that most sample specimens will exhibit the ''normal'' (unpolluted) state while a few (or none) of them will surpass permissible limits. Then the named effect sparsity (also denominated Pareto principle) applies in many cases. In these studies the interest is to detect and quantify the outliers although the time and cost of analyses is exactly the same for unpolluted sample specimens. So, the ideal situation will be to get a good estimate of the concentration level of all the samples without the need of analysing any original sample specimen on an individual basis. SSC uses special experimental design matrices to guide the sample composition process and uses the properties of these matrices to allow the evaluation of the original

sample specimens using only the analytical results of composite samples. SSC can use conventional screening matrices [18,19] or supersaturated matrices [20–26] to design composite samples. In the present article a comparison of sample composition carried out using both types of matrices is presented and applied to evaluate PCB concentrations in water samples.

EXPERIMENTAL

Standards and Reagents

PCB congeners: 2,4,4′-trichlorobiphenyl (CB-28), 2,2′,5,5′-tetrachlorobiphenyl (CB-52), 2,2′,3,3,4′,5′-hexachlorobiphenyl (CB-138) and 2,2′,4,4′,5,5′ hexachlorobiphenyl (CB-152) were obtained from Ultra Scientific (North Kingstown, RI, USA). 2,2′,4,5,5′-pentachlorobiphenyl (CB-101) and 2,2′,3,4,4′,5,5′-heptachlorobiphenyl (CB-180) were obtained from Dr Ehrenstofer GmbH (Augsburg, Germany).

A standard in *n*-nonane containing 5 μg/mL of each of the $^{13}C_{12}$-surrogate standards ($^{13}C_{12}$-CB-28, $^{13}C_{12}$-CB-52,$^{13}C_{12}$-CB-101, $^{13}C_{12}$-CB-138, $^{13}C_{12}$-CB-152, $^{13}C_{12}$-CB-180 and $^{13}C_{12}$-CB-209) was supplied by Cambridge Isotopic Labs (Andover, MA, USA). Standards of Aroclor-1254 and Aroclor-1260 with a concentration of 1000 μg/mL in isooctane were purchased from Supelco (Bellefonte, PA, USA). Working standards containing 10 ng/mL of $^{13}C_{12}$-surrogate standards and 1 μg/mL of Aroclor-1254 and Aroclor-1260 in acetone were prepared directly by diluting the commercial ones and used for spiking the appropriate specimens.

Methanol (HPLC grade), dichloromethane (pesticide grade) and anhydrous sulphate were from Scharlau (Barcelona, Spain); acetone (organic trace analysis grade) was from Merck (Darmstadt, Germany) and *n*-nonane (99%+) was supplied by Acros Organics (Geel, Belgium).

Apparatus

Solid-phase extraction manifold for 47 mm diameter extraction disks, C-18 Envi Disks, Kuderna–Danish apparatus, nitrogen blowdown concentrator and conical glass inserts for 2 mL vials were all obtained from Supelco (Bellefonte, PA, USA). Glass fibre filters (47 mm of diameter, 1 micron pore size) were supplied by Whatman (Kent, ME, UK).

Analyses were performed on a Varian (Wallnut Creek, CA, USA) CP-3800 gas chromatograph equipped with a 1079 split/splitless injector and an ion trap mass spectrometer (Satum 2000) with a waveboard generator for MS^n analysis. Beside common MS parameters (filament current: 90 μA, AGC target: 2000, interface temperature: 280°C, manifold temperature: 50°C, and trap temperature: 250°C), analysis was carried out in MS-MS mode with multiple reaction monitoring (MRM), by dividing the chromatographic run into six segments, optimising the MS-MS parameters for optimal sensitivity (Table I). The GC-MS-MS was controlled by a Satum GC-MS workstation version 5.4. A low bleed/MS CPSil-8 CB (30 m × 0.25 mm × 0.25 microns film thickness) analytical column was used in all described experiments. The GC oven temperature program was set as follows: initial temperature 90°C (held for 2 min), then ramped at 30°C/min to 170°C (held for 10 min) and finally ramped at 3°C/min to 300°C (held for 10 min). Helium was used as carrier gas at a constant flow of 1 mL/min. Injection

TABLE I GC-MS-MS optimal parameters in the analysis of PCBs

Segment number	*Group of compounds*	*Time window* (min)	*Parent ion* (m/z)	*Daughter ions* (m/z)	*Scan range* (m/z)	*Excitation storage level* (m/z)	*Excitation amptitude* (V)	*CID frequency offset* (Hz)
1	Solvent delay	0–12						
2	TriCB	12–17	258.0	186 + 188	180–280	133.0	1.14	600
	^{13}C–TriCB		270.0	196 + 198	180–280	141.0	1.13	600
3	TetraCB	17–22	292.0	220 + 222	210–325	157.0	1.10	600
	^{13}C–TetraCB		304.0	232 + 234	210–325	166.0	1.10	600
4	PentaCB	22–27	325.9	254 + 256	245–350	181.0	1.29	700
	^{13}C–PentaCB		337.9	266 + 268	245–350	190.0	1.35	700
5	HexaCB	27–34	359.8	288 + 290	280–380	206.0	1.59	700
	^{13}C–HexaCB		371.9	300 + 302	280–380	214.0	1.60	700
6	HeptaCB	34–38	395.8	324 + 326	315–415	231.0	1.71	800
	^{13}C–HeptaCB		407.8	336 + 338	315–415	240.0	1.70	800

TABLE II Plackett–Burman matrix used in sample composition experiments

	S_1	S_2	S_3	S_4	S_5	S_6	S_7	S_8	S_9	S_{10}	S_{11}
CS_1	0	1	0	1	1	1	0	0	0	1	0
CS_2	0	0	1	0	1	1	1	0	0	0	1
CS_3	1	0	0	1	0	1	1	1	0	0	0
CS_4	0	1	0	0	1	0	1	1	1	0	0
CS_5	0	0	1	0	0	1	0	1	1	1	0
CS_6	0	0	0	1	0	0	1	0	1	1	1
CS_7	1	0	0	0	1	0	0	1	0	1	1
CS_8	1	1	0	0	0	1	0	0	1	0	1
CS_9	1	1	1	0	0	0	1	0	0	1	0
CS_{10}	0	1	1	1	0	0	0	1	0	0	1
CS_{11}	1	0	1	1	1	0	0	0	1	0	0
CS_{12}	1	1	1	1	1	1	1	1	1	1	1

(2 μL) of samples and standards was carried out manually. Injector temperature was 250°C and a splitless time of 2 min.

Handling of Individual Sample Specimens and Sample Composition

In the experiments described in this article a series of 22 identical 2.5 L tap water sample specimens was taken. Some of them were randomly selected and spiked with variable amounts of Aroclor standards to obtain final concentration levels ranging between 200 and 750 ng/L. In this way, contaminated samples were below and above the 500 ng/L limit allowed for drinking water in USA [27]. Spiked samples were shaken vigorously for a while after the spike. Then, sample pH was adjusted to 2–3 with sulphuric acid and let to equilibrate for at least 24 h before sample processing and analysis. Composite samples were prepared by mixing manually equal volume aliquots of the original sample specimens and making up to a final volume of 1 L. Tables II and III were used to define sample composition strategies. Table II corresponds to a Plackett–Burman matrix [18,19] reversed to have in the last row all factors at high level. In SSC (Table III), one composite sample (for convenience, the last in the matrix), is designed to include all the original sample specimens. In both cases, this composite sample will be the first to be prepared and analysed.

TABLE III Supersaturated matrix used in sample composition of water samples for PCBs determination

	S_1	S_2	S_3	S_4	S_5	S_6	S_7	S_8	S_9	S_{10}	S_{11}	S_{12}	S_{13}	S_{14}	S_{15}	S_{16}	S_{17}	S_{18}	S_{19}	S_{20}	S_{21}	S_{22}
CS_1	0	0	1	1	0	0	1	1	0	0	1	0	0	0	0	0	0	1	1	1	1	1
CS_2	0	0	0	1	0	0	1	0	1	1	0	1	0	0	1	1	1	0	1	1	0	0
CS_3	1	1	0	1	0	0	0	0	0	1	0	0	1	1	0	0	0	1	1	1	1	0
CS_4	0	0	1	0	1	0	0	0	1	1	0	1	1	0	0	1	0	1	0	0	1	1
CS_5	1	0	0	0	0	1	0	1	0	1	1	0	0	0	1	1	1	0	0	0	1	1
CS_6	0	0	1	0	0	1	0	1	1	0	0	0	1	1	1	0	1	0	1	0	0	1
CS_7	0	1	0	0	1	1	1	0	0	0	0	0	0	1	1	1	0	1	0	1	0	1
CS_8	0	1	0	0	1	0	0	1	1	0	1	1	0	1	0	0	1	0	0	1	1	0
CS_9	1	0	0	0	1	1	1	0	0	0	1	1	1	0	0	0	1	1	1	0	0	0
CS_{10}	1	1	1	1	0	0	0	0	0	0	1	1	1	1	1	1	0	0	0	0	0	0
CS_{11}	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0
CS_{12}	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1

Sample Analysis

All samples (composite and individuals) were extracted following the protocol of the US-EPA Methods 1668/1668a [3,4] and then analysed by isotope dilution GC-MS-MS in the above given conditions.

200 μL of a standard in acetone containing the ^{13}C-PCBs (10 ng/mL) were spiked to 1 L of sample and shaken for 15 min. Then 5 mL of methanol was added and the sample was further shaken. Samples were extracted by passing through the C-18 SPE membrane (previously conditioned with 15 mL of dichloromethane, 15 mL of methanol and 15 mL of ultrapure water), with a glass fibre filter on top, rinsing the sample container with ultrapure water.

The extraction membrane and filter were vacuum dried for 30 min and then eluted once with 5 mL of acetone and twice with 20 mL of dichloromethane. The obtained extract was dried with anhydrous sodium sulphate and concentrated to ca. 0.5 mL in a Kuderna–Danish apparatus and finally, changing the solvent to *n*-nonane, by nitrogen stream to a final volume of 20 μL. If not immediately injected into the GC, the extracts were kept capped in amber vials fitted with 250 μL conical inserts at −20°C until analysed by GC-MS-MS.

Software for SSC

The matrix in Table III is a supersaturated matrix developed by means of the Superga® software [28]. Once the composite samples have been analysed, the obtained results were treated by means of dedicated software (Gamich®) that consisted of a regression program driven by evolutionary algorithms. Fundamentals and details of these programs have been described elsewhere [29]. All software was developed and implemented in the laboratory using CA-Realizer V 3.0a programming language. Additional statistical calculations were carried out using Statgraphics Plus V 3.3 (Manugistics Inc.) [30].

RESULTS AND DISCUSSION

Sample Composition using SSC

Although the fundamentals of SSC have been recently presented elsewhere [16,17], they are new enough to justify a brief description here. SSC uses sample composition

matrices adapted to the problem in hand to conduct the sample composition process. These matrices have as many rows as experiments (the composite samples, in fact) to be carried out. In Tables II and III, composite samples have been denoted as $CS_{1,2,3...n}$. The number of columns in the SSC matrix correspond to the number of original sample specimens to be studied (named as $S_{1,2...m}$ in Tables II and III). Although these matrices can adopt any of the common coding types in experimental design, the simplest and the more fitted for the purposes of sample composition processes is the 0–1 coding. Using this coding, each column in the matrix indicates when a particular specimen S_j must be present (level = 1) or absent (Level = 0) in the composite sample CS_i. Thus, for example, in the matrix of Table II the first composite sample CS_1 will be composed by the original sample specimens S_2,S_4,S_5,S_6 and S_{10}.

In the matrices of Tables II and III it can be seen that the last row has all the sample specimens at level 1. This means that this (CS_n) composite sample is fully equivalent to a conventional composite sample. The basic principle of SSC is that the work and cost of analysis of a set of sample specimens should not be higher than the corresponding analysis of this set of samples by conventional sample composition or the analysis of the individual sample specimens. Accordingly, the CS_n composite sample must be the first to be prepared and analysed. If the results in the analyses of the CS_n sample indicated that one or some of the sample specimens considered are above limits, further analyses must be carried out. In conventional sample composition, all the individual sample specimens entering the composite sample must be analysed. Then, the total number of analytical determinations should be at least $n+1$, where n is the number of sample specimens. Of course, added reliability should advise carrying out replicate analyses, so the number of analytical measurements easily amounts to $2(n+1)$. In SSC, if the result for the CS_n composite sample is positive, all the remaining composite samples dictated by the composition matrix should be prepared and analysed. However, as we will see later, there is no need of replicate measurements because of the inherent properties of the applied design matrices. This means that the number of analytical measurements will be $(n+2)$ in the case of the matrix shown in Table II and $(n/2+2)$ for the supersaturated matrix in Table III. Here, it is assumed that the analysis of the CS_n composite sample is carried out in duplicate to control the risk of false negatives at reasonable levels. Of course, if the n original sample specimens were analysed on an individual basis a total of $2n$ analytical measurements should be made.

The vector of analytical responses obtained in the processing of composite samples should be regressed on the design matrix, providing an estimation of the factor effects (namely the concentration level of the target analytes for the original sample specimens), according to the model:

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \cdots + \beta_{m-1} X_{m-1} + \beta_m X_m + \varepsilon.$$

The output of SSC is a decreased analytical effort and cost, more evident when supersaturated matices are applied. As mentioned before, to be able to use these types of matrices the effect of sparsity hypothesis needs to be assumed. This means that only a few of the original sample specimens are expected to be really above limits, so the analysis strategy becomes an outlier-identification problem. Simulation studies [15] have shown that from a practical point of view, SSC based on supersaturated matrices

runs smoothly if the ratio of expected real positive specimens *versus* the total number of sample specimens is not greater than 0.3. If this condition cannot be reasonably assumed it should be preferable to resort to conventional screening matrices such as Plackett–Burman designs as used in this article. Of course, in real cases the number of really polluted specimens cannot be known beforehand so this decision becomes difficult. Moreover, in SSC the original sample specimens are not analysed individually so there is not an apparent way of validating the results. Software developed for SSC [29] has some built in tools to help the analyst in evaluating the quality of the results produced. Some of them are related to the apparent difficulties in convergence or anomalous residuals in the regression process, although the more efficient one is the use of flagged samples in the composition process. This approach involves the substitution of two randomly selected original sample specimens by a known standard and a blank although the process of sample composition runs exactly in the described manner. However, finally we have means of checking the results at the price of processing two specimens less. If the number of predicted positive specimens is low (effect sparsity true) and the flagged specimens are predicted accurately there is no reason to be wary of the obtained results.

Another question is related to the size of the design matrix when using supersaturated matrices. Enormous supersaturated matrices can be found in the literature [22,24] and the analyst can fall into the temptation of using them for SSC. In our experience, although in simulation these big matrices can be managed successfully, in real applications since the real number of polluted specimens is unknown, it is not advisable to get over a column to rows ratio of 3 : 1. Ratios approaching 2 : 1 give robust reliable results while still providing clear cost and work reduction advantages.

In the experiments described here, four of the 22 water sample specimens were randomly chosen and spiked with different amounts of Aroclors. Aroclors 1254 and 1260 were selected for this study as they have been extensively used previously and are likely to be found in environmental samples. Samples S_2 (750 ng/L in Aroclor 1254), S_5 (200 ng/L in Aroclor 1260), S_9 (600 ng/L in Aroclor 1260) and S_{10} (250 ng/L in Aroclor 1254) were prepared and handled as described in the experimental section. All the sample specimens were managed by their associated numbers. In this experiments no flagged samples were used, so all the available sample specimens entered the composition when using the matrix in Table III and were divided into two sets of consecutive samples when using the matrix in Table II.

Performance of the Analytical Method

As described in the experimental section the analytical methods applied follow prescriptions of US-EPA Method 1668/1668a, although GC-MS-MS was used instead of GC-HRMS. The whole analytical procedure was tested for recovery, quantification limits (defined as 10 times the standard deviation of the intercept divided by the slope of the calibration curve) and chromatographic repeatability (Table IV). Acceptable values of absolute recovery (between 63 and 91%) and chromatographic repeatability (RSDs between 1.4 and 4.4%) were obtained. Furthermore, quantification limits are low enough (between 0.1 and 0.3 ng/L) to ensure that polluted sample specimens would be detected in spite of the dilution of the original samples due to the sample composition.

TABLE IV Performance of the analytical method for PCBs in water samples

PCB congener	*Retention time* (min)	*Correlation coefficient**	*Chromatographic repeatability/RSD* ($n=10$)	*Average % absolute recoveries* ($n=3$)**	*Limit of quantification* (ng/L)
CB-28	15.49	0.9998	3.7	67 ± 2	0.1
CB-52	18.07	0.9998	1.4	62 ± 1	0.2
CB-101	24.27	0.9986	4.0	71 ± 3	0.2
CB-153	29.88	0.9990	3.5	78 ± 3	0.3
CB-138	31.56	0.9997	4.4	85 ± 1	0.2
CB-180	35.68	0.9999	3.5	91 ± 6	0.3

*Calibration between 5 and 750 ng/mL; **Spike level = 20 ng/L.

TABLE V Results obtained in the analysis of the composite samples prepared according to the design matrix shown in Table III (all concentrations are expressed in ng/L, nd = non-detected)

	CB-28	*CB-52*	*CB-101*	*CB-153*	*CB-138*	*CB-180*
SC_1	nd	nd	nd	nd	nd	nd
SC_2	nd	1.0	1.6	3.2	3.4	2.8
SC_3	0.2	2.4	3.2	3.4	4.1	1.0
SC_4	nd	1.0	1.9	4.3	4.4	4.7
SC_5	nd	0.9	0.9	0.8	0.8	0.1
SC_6	nd	nd	0.6	1.5	2.3	1.9
SC_7	0.2	1.9	2.6	1.9	3.1	0.9
SC_8	0.2	1.9	3.3	3.3	5.9	3.0
SC_9	nd	nd	0.5	0.6	0.9	0.7
SC_{10}	0.2	1.7	2.4	1.2	2.7	0.4
SC_{11}	0.3	2.7	4.1	4.3	8.0	3.5
SC_{12}	0.3	3.4	4.9	4.5	5.8	2.7

Screening of PCBs in Water Samples. Comparison of Plackett–Burman and Supersaturated Matrices

All polluted samples (S_2, S_5, S_9, and S_{10}) were individually analysed prior to sample composition in order to have reference values of the actual concentration of PCBs that can be compared with the predicted values to be obtained through SSC. Then, the experiment using the supersaturated matrix shown in Table III was carried out. All the 22 sample specimens entered in the composition process. Finally, the experiment using the Plackett–Burman matrix shown in Table II was undertaken. In that case, the whole set of sample specimens was divided into two subsets of 11 samples that were composed and analysed successively. The results in the analyses of the composite samples have been summarised in Tables V and VI respectively for the first and second experiments. Obviously in the second experiment, because the four randomly spiked samples fall in the first subset, composite sample SC_{22} did not give any detectable amounts of PCB congeners, thus, the remaining composite samples in the second set were not prepared or analysed. As a consequence, since composite samples CS_{12} and CS_{22} were measured in duplicate this second experiment required only 15 analytical measurements of the theoretical 24 to be carried out.

Figures 1(a–f) compare the concentrations predicted by regression of the results in Tables V and VI with the theoretical concentrations (expected on the basis of the

TABLE VI Results obtained in the analysis of the composite samples prepared according to the design matrix shown in Table II (all concentrations are expressed in ng/L, nd = non-detected)

	CB-28	*CB-52*	*CB-101*	*CB-153*	*CB-138*	*CB-180*
SC_1	0.6	5.1	15.0	3.3	6.8	3.2
SC_2	nd	0.3	1.3	1.1	1.4	1.6
SC_3	nd	nd	nd	nd	nd	nd
SC_4	0.3	4.2	11.6	5.5	9.5	5.2
SC_5	0.2	1.7	6.7	2.6	4.7	2.8
SC_6	0.1	1.5	5.1	3.1	5.3	4.5
SC_7	0.5	1.9	3.3	2.3	3.5	1.5
SC_8	0.3	4.0	4.8	4.8	7.1	3.2
SC_9	0.5	5.6	6.1	3.7	5.2	0.7
SC_{10}	0.6	4.8	5.8	2.2	3.6	nd
SC_{11}	nd	0.3	2.7	3.3	4.6	3.6
SC_{12}	0.5	5.2	5.7	4.5	6.8	2.2
SC_{22}	nd	nd	nd	nd	nd	nd
SC_{13} to SC_{21}	Not prepared not analysed					

added amounts of Aroclors to the original sample specimens and the relative proportions of the chosen PCB congeners in those Aroclors) and the result in the individual checks for spiked sample specimens. From this figure, it can be concluded that SSC was able to identify which sample specimens were really contaminated and to provide a good estimation of the concentration levels for the considered PCB congeners. Small false positives appearing in Figure 1 were evaluated statistically and discarded at the confidence level of 95%, without additional experimental work.

Some differences between expected, found and predicted concentrations can be justified by the time needed to carry out all the analyses and thus the time to complete all experiments. In fact the composite samples using the Plackett–Burman matrix were carried out two weeks after the preparation of the original specimens. This can lead to higher sources of error due to the well-known tendency of PCBs to adsorb on glass walls of the container with time. In principle, it is not expected that conventional Plackett–Burman screening matrices would give worse results than supersaturated matrices since the number of degrees of freedom is higher. In any case, the differences fall between reasonable limits and the results point out that supersaturated matrices exhibit comparable efficiency to conventional screening matrices in sample composition processes provided effect sparsity. In this case, 13 analytical determinations allow evaluation of the concentration level of several PCBs in 22 water samples. This means a saving factor greater than two when compared to the individual analysis of these samples. It should be noticed that saving factors provided by SSC are direct because of the total number of full analytical processes which is reduced and not only the time and costs of some of the analytical stages. Logically, SSC can be combined with fast screening alternative analytical methods [5,6] thus providing additional savings in time and costs. The use of conventional screening matrices involves additional work as compared to SSC based on supersaturated matrices but provides enhanced reliability. Moreover, conventional matrices can be applied without the need of having any special regression tool because most commercial statistical packages provide excellent procedures to solve these conventional screening matrices. In any case, it should be taken into account that regression applied to SSC involves a non-negativity constraint for coefficients. Evolutionary algorithm regression [29] allows the easy handling of this constraint while most regression packages do not.

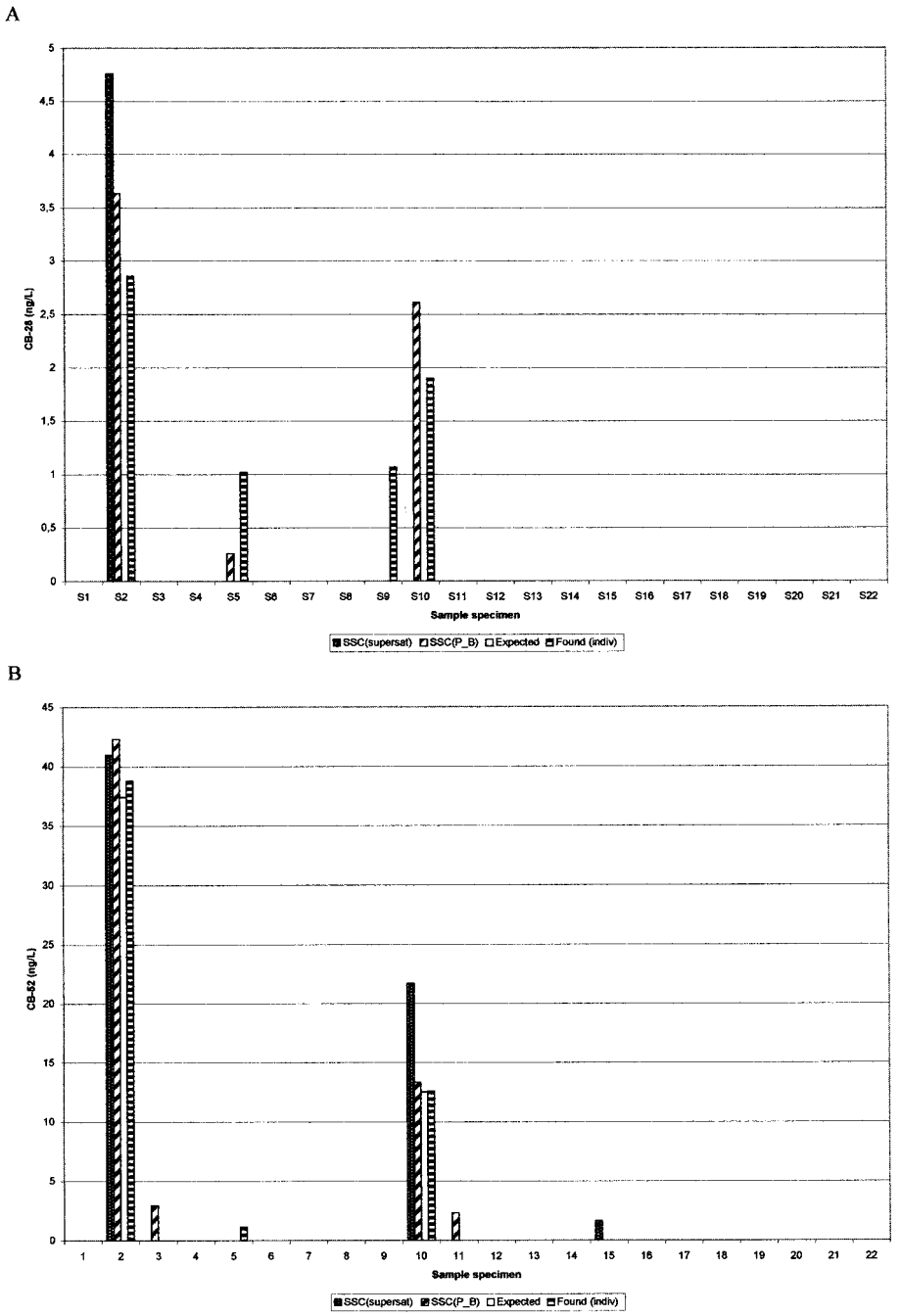
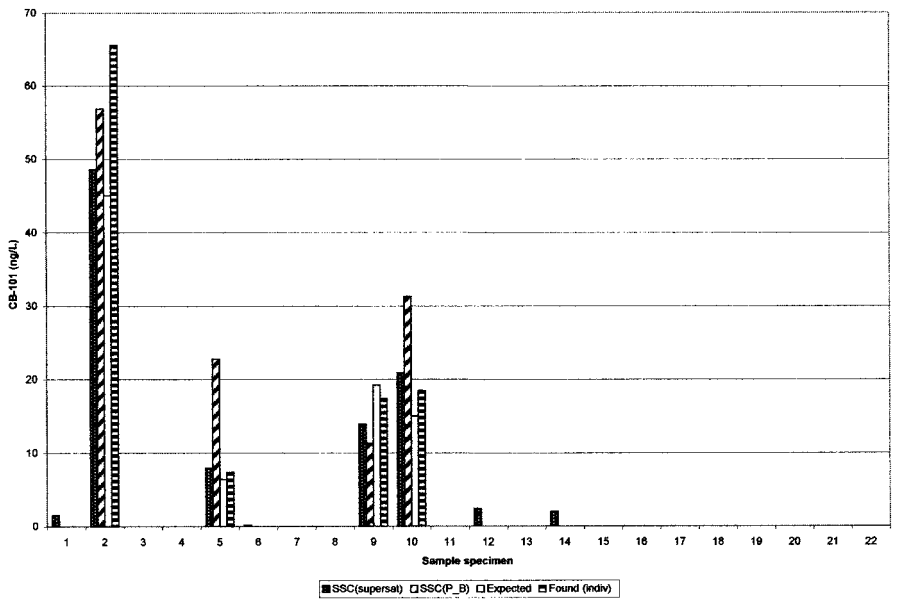


FIGURE 1 Predicted *versus* expected and actually found concentrations for several PCB congeners in the original sample specimens. (A) Results for CB-28, (B) results for CB-52, (C) results for CB-101, (D) results for CB-153, (E) results for CB-138 and (F) results for CB-180. (Legend key: ''SSC(supersat)'' results found by the SSC method using the supersaturated matrix in Table III; ''SSC(P_B)'', results found by the SSC method using the Plackett–Burman matrix in Table II; ''Expected'', PCB congeners concentration expected from the added Aroclor amounts; ''Found(indiv)'', actual concentrations of spiked sample specimens measured individually (no replicated)).

C



D

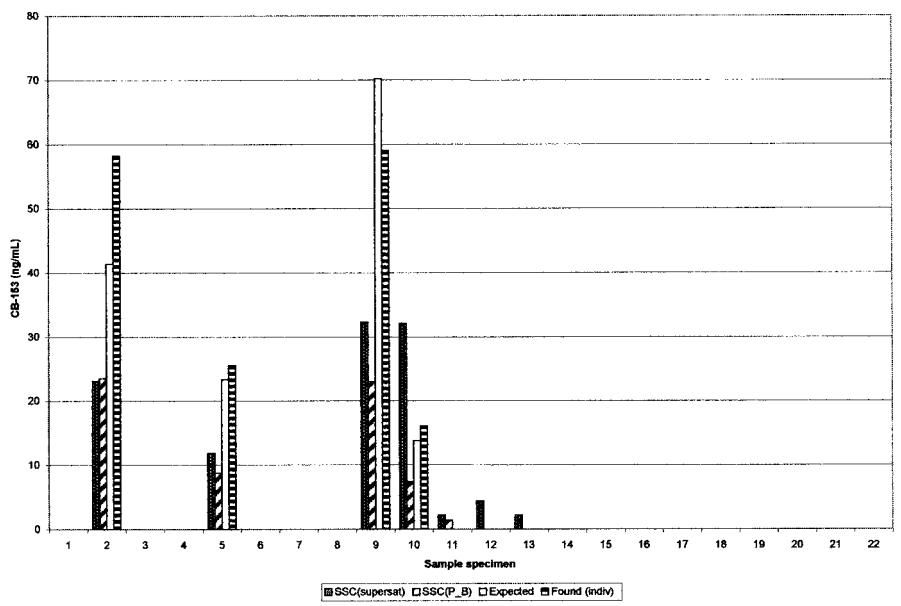


FIGURE 1 (Continued).

Robustness of the Proposed Strategy

An important advantage in SSC derives from the fact that no duplicate measurements need to be carried out to evaluate the composite samples. To justify this affirmation, SSC must be robust and support significant errors without losing prediction ability. Otherwise, composite samples formed should be analysed at least in duplicate. To

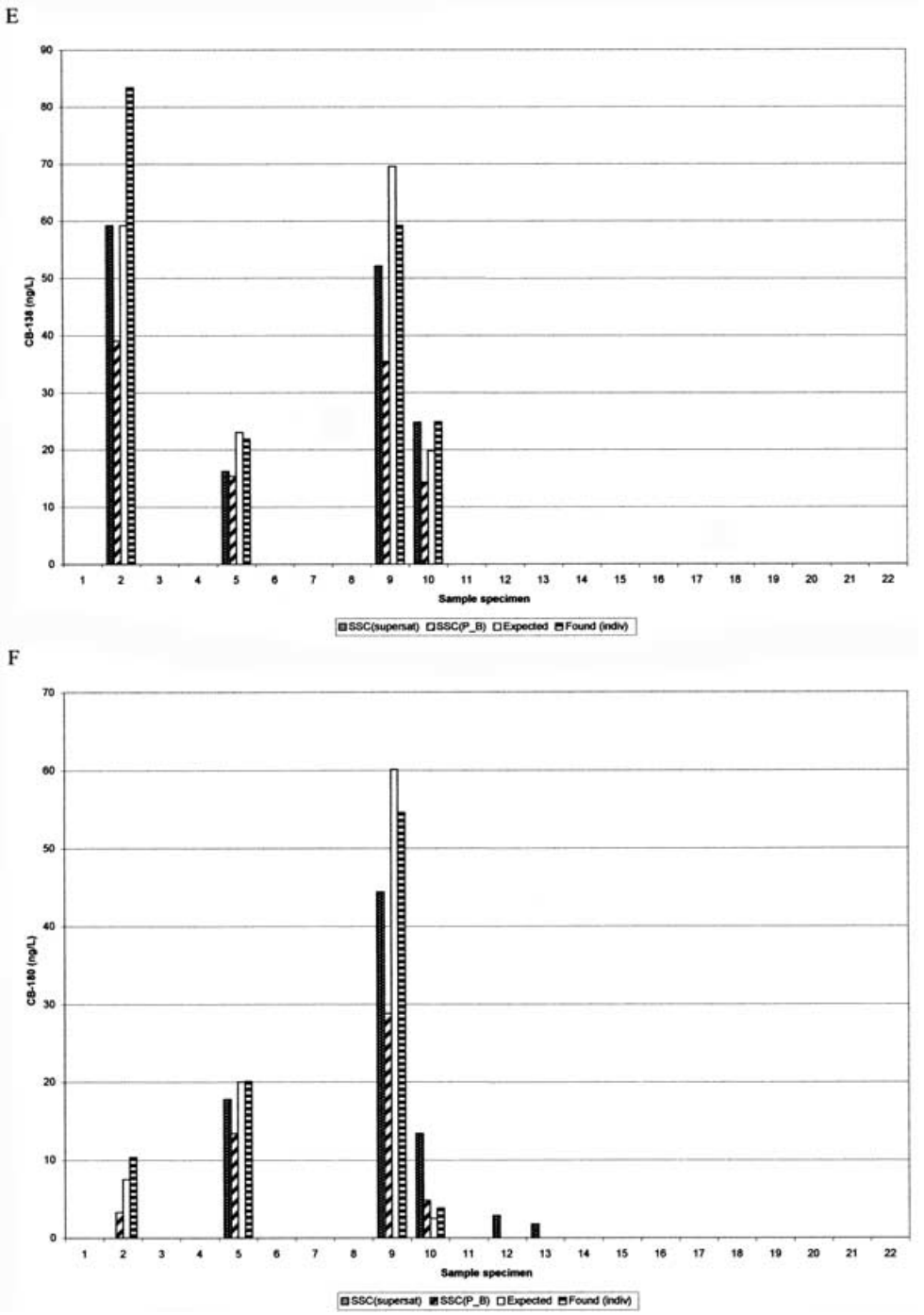


FIGURE 1 (Continued).

TABLE VII Real and manipulated results for CB-52 and CB-138 (runs affected by manipulations have been highlighted). In all cases, concentrations are expressed in ng/L

	SSC using supersaturated matrix in Table III				*SSC using Plackett–Burman matrix in Table II*			
Composite sample	*CB-52 (true)*	*CB-52 (manipulated)*	*CB-138 (true)*	*CB-138 (manipulated)*	*CB-52 (true)*	*CB-52 (manipulated)*	*CB-138 (true)*	*CB-138 (manipulated)*
SC1	nd	nd	nd	0.5	5.1	5.1	6.8	6.8
SC2	1.0	2.1	3.4	3.4	0.3	0.3	1.4	1.4
SC3	2.4	2.4	4.1	4.1	nd	nd	nd	nd
SC4	1.0	1.0	4.4	4.4	4.2	3.0	9.5	7.0
SC5	0.9	1.3	0.8	0.8	1.7	1.7	4.7	4.7
SC6	nd	nd	2.3	2.3	1.5	1.5	5.3	5.3
SC7	1.9	1.9	3.1	3.9	1.9	1.9	3.5	3.5
SC8	1.9	1.9	5.9	5.9	4.0	4.0	7.1	7.1
SC9	nd	nd	0.9	0.9	5.6	5.6	5.2	5.2
SC10	1.7	1.7	2.7	2.7	4.8	4.8	3.6	4.5
SC11	2.7	2.7	8.0	7.0	0.3	nd	4.6	4.6
SC12	3.4	3.4	5.8	5.8	5.2	5.2	6.8	6.8

show the robustness of the proposed strategy the data for CB 52 and CB 138 in Tables V and VI were chosen and manipulated. True and manipulated values have been summarised in Table VII. Manipulated data consist of the exchange of randomly selected true experimental results by numbers that simulate the occurrence of gross accidental errors in the measurements of some composite samples for the PCB congeners selected. Table VII shows that in some cases errors greater than 100% have been introduced. In one case three over twelve erroneous results have been posted. This is a rather unusual situation in practice, but a challenging task for SSC strategy since it represents a bad performance of the analytical method applied. Because in SSC only the CS_n composite sample is measured in duplicate it is clear that if these errors take place, they will go unnoticed by the analyst.

To carry out this robustness study, fully independent regression processes were run presenting to the program the real and the manipulated sets of results. In all cases, the process parameters [29] were maintained identical (population size = 100; maximum number of generations allowed = 500; initialisation = random; selection operator = roulette wheel; elitist mode = enabled; crossover type = single; crossover operators = simple + heuristic; crossover operators probability = 0.6 + 0.7 (equally balanced); mutation operators = uniform + non-uniform; mutation probability = 0.01 + 0.01 (equally balanced); fitness function = squared Euclidean distance (no scaling). These parameters, defaults on Gamich® software [29] are not necessarily the best ones to solve these particular cases but allow an objective comparison of the results. The obtained results in all these regression processes have been depicted in Figure 2(a) and (b) and compared with the expected amounts as well as with the results of the checking for the individual sample specimens.

In Figure 2 it can be clearly appreciated that in all cases the original sample specimens really containing CB-52 and/or CB-138 were detected as such. Interestingly, even the concentration levels are not very different to the expected ones and those predicted when using non-manipulated results. Of course, a number of small false positives appeared. These false positives can be judged as mentioned before and, where their concentration value appears statistically significant, checked experimentally. This demonstrates the robustness of the proposed strategy that supports really gross

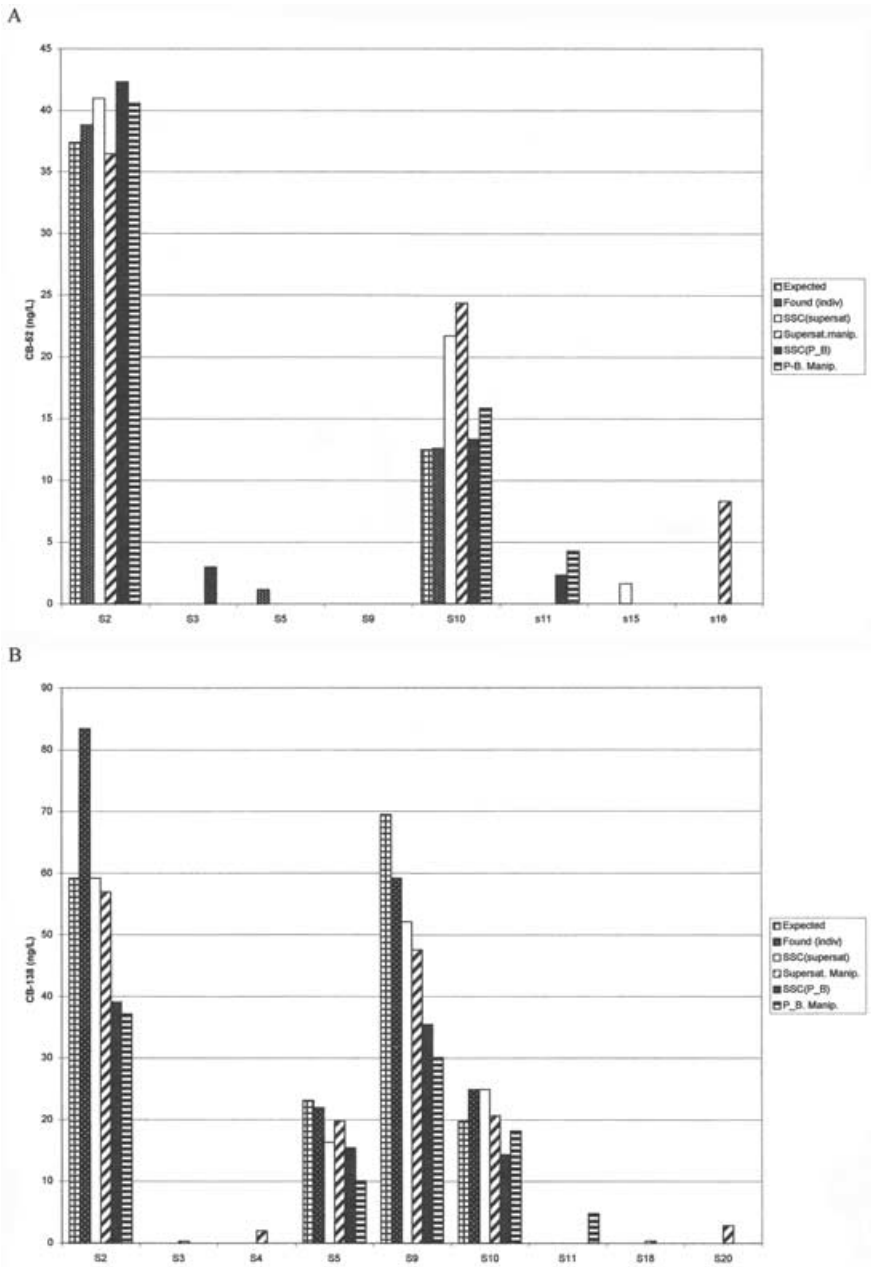


FIGURE 2 Comparison between true and manipulated data used in SSC. (A) Data for CB-52; (B) data for CB-138 (Legend key: ''Expected'', concentration expected for the added Aroclor amount to each sample specimen; ''Found (indiv)'', actual concentrations of spiked sample specimens measured individually (no replicated); ''SSC(supersat)'', results founded by the SSC method using the supersaturated matrix in Table III. Experimental data without any manipulation; ''Supersat.manip.'', results predicted by the SSC method using the supersaturated matrix in Table III. Experimental data manipulated as shown in Table VII (columns two and four); ''SSC(P_B)'', Results predicted by the SSC method using the Plackett–Burman matrix in Table II. Experimental data without manipulation; ''P_B.Manip'', results predicted by the SSC method using the Plackett–Burman matrix in Table II. Experimental data manipulated as shown in Table VII (columns seven and nine).

errors in the analytical determinations without losing its ability to detect and identify the real contaminated sample specimens. These errors, however, cannot be corrected because of the absence of replicated analyses; apparently they do not compromise the reliability of the detection and even the production of acceptable estimates for the concentration levels in the original sample specimens. Obviously, the robustness is a consequence of the mathematical characteristics of design matrices applied to prepare the composite samples, which means that the selection of this matrix becomes of utmost importance.

When supersaturated matrices and the more conventional Plackett–Burman matrices are compared in terms of robustness it appears that both perform similarly. This means that the main advantages of using Plackett–Burman matrices rely on the robustness (no need of replicated measurements in composite samples) and reduced dependence on the effect sparsity (ratio of contaminated sample specimens and the total sample specimens to be handled). Supersaturated matrices are equally robust although much more dependent on the effect sparsity. However, they allow a significant reduction (more than twice) of the total number of analyses to be carried out.

CONCLUSIONS

The described experiments show the features and real applicability of the Strategic Sample Composition technique. Taking advantage of SSC, the screening of water samples for PCBs can be made using a limited number of analytical determinations without the need of analysing the original sample specimens on an individual basis. It has been shown that supersaturated design matrices, as well as the more conventional Plackett–Burman screening matrices can reliably be used in SSC although the use of supersaturated matrices is clearly advisable. Moreover, the technique has shown an excellent robustness when gross accidental errors are present in the analytical results for composite samples. This characteristic avoids the need of replicate measurements, that decisively contributes to reduce the cost and analytical effort in screening campaigns, which are the main objective for the SSC technique. The analysis of PCB congeners in water samples is a challenging case study although many other pollutants can be equally good candidates for this type of strategic sample composition approach.

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