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RECOVERY OF ORGANIC COMPOUNDS FROM LARGE-VOLUME AQUEOUS SAMPLES USING ON-LINE LIQUID CHROMATOGRAPHIC PRECONCENTRATION TECHNIQUES

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SUMMARY

It is demonstrated that in order to achieve maximal solute preconcentration on one or two given precolumns, it is necessary to use a sample volume larger than the breakthrough volume, even at the risk of obtaining recoveries lower than 100%. This is an important condition when traces of organic pollutants have to be determined in water samples with relatively low organic contamination.

A theoretical model is proposed for such conditions using a single precolumn or two precolumns in series and the experimental feasibility of such an approach (sample volume > breakthrough volume) is demonstrated. In addition, a critical comparison is made of different experimental methods for measuring amounts of preconcentrated solutes and the corresponding recoveries. A recommended procedure for the measurement of recoveries is proposed, involving the determination of variations of peak areas obtained during the determination of preconcentrated compounds in increasing volumes of water samples. In each sample, the concentration of the solutes is adjusted in order to have a constant amount. This method has the advantage that recoveries are measured under the same chromatographic conditions as those used for the real sample. At the same time breakthrough volumes can be estimated. Several solutes can be studied simultaneously and the efficiency of spiking can also be tested by this approach. A range of organic pollutants of medium to low polarity and two adsorbents, *viz.*, a bonded C_{18} silica and the polymer-based PRP-1, were chosen as a model system.

INTRODUCTION

The low concentrations of organics in relatively pure water (*e.g.*, natural compounds or pollutants in drinking water) necessitate the development of analytical techniques sensitive at the sub-ppb level. At this level, a concentration step is necessary before the analysis proper and the problem of component recoveries becomes paramount. In order to minimize sample loss and the risk of contamination and to

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increase the potential for automation, sample transfer steps, solvent evaporation and any manual manipulation should be eliminated or reduced to a minimum.

Sample concentration based on liquid–liquid sorption techniques has been shown to be a good alternative to liquid–liquid extraction, which requires several sample handling steps and evaporation of large solvent volumes[1]. When using off-line concentration techniques, trace components are concentrated on a convenient sorbent and then eluted by a small volume of suitable solvent; this volume has to be reduced and frequently only an aliquot is injected into the analytical system; in addition to the inherent disadvantage of an increased detection limit, there are still handling steps of the concentrated sample so that losses and contamination risks can still occur. For quantitative analysis one has to verify that there is no loss or degradation during the evaporation step; some studies have reported evaporation losses depending on the solute concentration and on the final volume[2], so that internal standards are often required.

Many of these drawbacks can be avoided by using on-line enrichment on precolumns. Trace compounds are trapped on a precolumn packed with a convenient sorbent; the precolumn is coupled to an analytical column via switching valves and the compounds adsorbed are then eluted directly from the precolumn to the analytical column with a suitable mobile phase. If adsorption and desorption are efficient, one can expect more accurate quantitative results as there is no sample manipulation between preconcentration and analysis.

Pertinent applications of on-line preconcentrations have been reported for the determination of specific compounds in complex aqueous mixtures[3–9]. Selectivity towards specific compounds can be provided with proper detection methods[10–12], but it can also be increased during the sample handling step by coupling different precolumns in series and by using more selective precolumns such as ion-exchange resins[4,13–15] or metal-loaded sorbents[16–19]. There are also examples of more general analyses, the compounds studied being very different in size and polarity; again, for greater selectivity in the sample handling steps the use of more than one precolumn is unavoidable. One example is the determination of organics in waste waters[14]; combination of three precolumns gives a satisfactory group separation prior to chromatographic analysis: a C_{18} bonded phase traps the non-polar compounds, a styrene–divinylbenzene copolymer the moderately polar compounds and a cation exchanger the more polar cations. In these procedures, each precolumn is eluted separately and the eluate analysed.

We applied this technique to drinking water by increasing the sample volume to 500 ml. When many compounds are to be quantified simultaneously, they are not concentrated on only one precolumn but often some solutes are recovered from two precolumns. Hence one has to take into account the recovery from each precolumn, because for some solutes breakthrough occurs so that the recovery is not complete whereas for others the recovery is 100%. When applying a comparison with pure water samples spiked with known amounts of solutes or a comparison with direct loop injection, solute amounts are quantified but the determination of the concentrations in the sample being analysed is linked to a knowledge of the recoveries. The aim of this study was to examine the recoveries in quantitative analyses when applying precolumn techniques to large sample volumes utilizing more than one precolumn. Although it is generally assumed that it is better to work with a 100% recovery, it will

be shown that for trace analysis the most important point is the amount available for detection, which has to be as high as possible for most compounds even if for some of them the recovery is not complete.

GENERAL CONSIDERATIONS FOR RECOVERY CALCULATION

Description of precolumn technique with two precolumns in series

Samples are percolated through two precolumns in series. In order to prevent band broadening in the analysis step, small sizes (about 1 cm × 2 mm I.D.) are chosen[20–23]. The affinity of solutes is stronger for the second than for the first precolumn[24]. After percolation of a known sample volume, the two precolumns are flushed with pure water in order to remove interferents. Each precolumn is then eluted separately on-line by the mobile phase to the analytical column. Extraction of solutes by sorbents is complete if breakthrough of analytes does not occur. Two criteria are responsible for breakthrough during the concentration step, *viz.*, the retention and the capacity.

Breakthrough volume

The most important parameter for trace analysis is the sensitivity of the method (or the minimal detectable concentration of the compound in water), determined by the sensitivity of the detector used, the compounds under study, the adsorption capacity of the sorbent in the precolumn, the sample volume, the desorption procedure and the chromatographic procedure. On percolating water containing organic compounds through a concentration column, a chromatographic process occurs which proceeds as frontal chromatography because the flow-rate of the water and the concentrations of compounds are constant. Compounds are extracted from the water by the sorbent according to their partition coefficients for the water–sorbent system. If sorption occurs, the water leaving the precolumn is free from solutes; after a certain percolated volume, breakthrough occurs and the effluent water contains the compounds again.

Fig. 1 shows frontal curves for two solutes having different affinities for sorbent A (solid line) and for sorbent B (broken line). These frontal curves were obtained by percolating water through only one precolumn at a time. Under ideal conditions, they have a bilogarithmic shape and the inflection point is the retention volume, V_r, of the solute eluted by pure water if the column is not overloaded. At 1% of the sample UV absorbance, we define the breakthrough volume, V_f, which corresponds to the sample volume that can be percolated without any elution of analyte; at 99% of the UV absorbance we define the maximal percolated volume, V_m. Theoretical studies of on-line preconcentration have been presented by Werkhoven-Goewie *et al.*[3,25] and Nondek and Chvalovsky[22,23]. We have the relationships

$$V_f = V_r - 2\sigma_v \quad (1)$$

and

$$V_m = V_r + 2\sigma_v \quad (2)$$

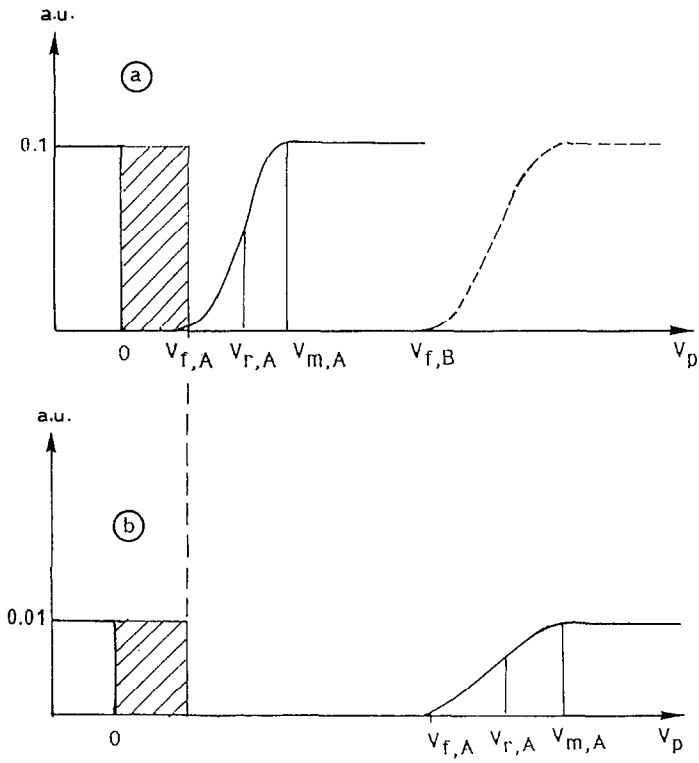

Fig. 1. Determination of breakthrough volumes from the recording of frontal chromatograms for two water samples spiked with (a) solute 1 and (b) solute 2. The sample UV response (total absorbance) is measured by coupling directly the spiked sample with the detector. At $V_p = 0$, sample is percolated through the precolumn; V_f is measured at 1%, V_r at 50% and V_m at 100% of the total absorbance. The solid line and broken line represent frontal chromatograms recorded with precolumn packed with sorbent A alone and with precolumn packed with sorbent B alone, respectively.

where σ_v is the standard deviation which is dependent on the axial dispersion of analyte along the bed of particles in the precolumn and can be expressed in terms of precolumn length (L), particle diameter (d_p) and reduced plate height (h) according to

$$\sigma_v = V_r (hd_p/L)^{\frac{1}{2}} \tag{3}$$

For trace analysis, the enrichment factor (f) is of prime importance; this term, defined by the ratio between the final concentration and the sample concentration can be expressed in terms of sample volume (V_p) desorption volume (V_d) and recovery (R) by

$$f = (V_p/V_d)R \tag{4}$$

Increasing f means increasing both the sample volume and recovery and reducing the desorption volume, so that preconcentrated analytes have to be eluted from the precolumn by the smallest volume equal at least to the void volume of the precol-

umn. Increasing f is at first an optimization of the precolumn geometry, which has been widely studied[21–25]; another method has been considered by Nondek and Chvalovsky[23], which consists in percolating a higher volume than V_m through the precolumn in order to extract a maximal amount of solute. This is particularly advantageous for analytes which have low V_r values. The maximal extracted amount is $V_r C_p$ and is obtained for a percolated volume V_p equal or higher than V_m owing to the symmetrical form of the frontal curve.

Recovery, defined by the ratio (amount extracted)/(amount percolated), is 100% only for $V_p < V_f$; the maximal amount extracted does not correspond to a 100% recovery but is reached by percolating a volume higher than V_m, in spite of a lower recovery. From a practical point of view, quantitative analysis is easier when the recovery is 100%. However, in addition to the case of poorly retained solutes as pointed out by Nondek and Chvalovsky[23], when several solutes are to be quantified simultaneously, Fig. 1 shows that it can be necessary to carry out analyses with partial recoveries; if solutes 1 and 2 are together, a 100% recovery for both solutes is reached for a maximal percolated volume equal to $V_{f,A}$ and the amount of solute 1 and 2 extracted by precolumn A correspond to the hatched areas in Fig. 1. If solute 2 has poor UV absorbance properties, the amount extracted is not enough to be detected, so the percolated volume has to be increased; the amount of solute 1 adsorbed remains constant but its recovery is then below 100%. When solutes are numerous it is impossible to adjust the sample volume in order to have 100% recovery for each one and the above situation will be encountered.

Let us now consider the second precolumn B coupled after A. For solute 1, breakthrough occurs for $V_{f,A}$ for precolumn A and for $V_{f,B}$ if precolumn B is alone; when A and B are coupled, breakthrough occurs for $V_{f,A} + V_{f,B}$; in fact, it is a first approximation because, for the second precolumn, conditions for frontal analysis are not required as the concentration of compound entering the second precolumn is not constant and increases depending on the shape of the breakthrough front from the first precolumn. Solute 1 will be extracted by both A and B for a percolated volume higher than $V_{f,A}$ and a knowledge of $V_{f,A}$ and $V_{f,B}$ is necessary in order to determine the respective recoveries.

Retention data are important because V_f can be estimated to a first approximation from V_r values. These retention values are often time consuming to measure in pure water. In reversed-phase chromatography, retention data can be extrapolated to pure water conditions from data known at two percentages of organic modifier because of the logarithmic relationship between capacity factor and methanol content[3,5]. There is also a very useful logarithmic relationship between capacity factors in water and the water–octanol partition coefficients of solutes[26,27].

Capacity

Breakthrough can occur if the precolumn capacity is exceeded. In overloading conditions, the volume denoted V_r does not correspond to the retention volume of solutes. The capacity of the sorbent depends on the type of stationary phase, on the bed volume of the precolumn and on the nature of the solute. One has to verify that the concentrations of analytes and interferences are low and that breakthrough due to overloading of the precolumn does not occur, otherwise the proposed theory is not valid.

TABLE I

EQUATIONS FOR EXTRACTED AMOUNTS, Q, AND RECOVERIES, R, ON C18 AND PRP1 DEPENDING ON PERCOLATED SAMPLE VOLUMES, V_p

See Fig. 1 for definition of characteristic volumes measured on breakthrough curves.

V_p	Q_{C18}	Q_{PRP1}	R_{C18}	R_{PRP1}
$< V_{f,C18}$	$C_p V_p$	0	1	0
$> V_{f,C18}$ $< V_{m,C18}$	$C_p V_{f,C18} +$ $+ \int[C_p - y(V_p)]dV_p$	$\int y(V_p)dV_p$	$1 - \int[y(V_p)/C_p V_p]dV_p$	$\int[y(V_p)/C_p V_p]dV_p$
$> V_{m,C18}$ $< V_{f,PRP1}$	$C_p V_{r,C18}$	$C_p(V_p - V_{r,C18})$	$V_{r,18}/V_p$	$1 - V_{r,C18}/V_p$
$> V_{f,PRP1}$ $< V_{m,PRP1}$	$C_p V_{r,C18}$	$C_p(V_{f,PRP1} - V_{r,C18}) +$ $+ \int[C_p - Z(V_p)]dV_p$	$V_{r,C18}/V_p$	$1 - (V_{r,C18}/V_p)$ $- \int[z(V_p)/C_p V_p]dV_p$
$> V_{m,PRP1}$	$C_p V_{r,C18}$	$C_p(V_{r,PRP1} - V_{r,C18})$	$V_{r,C18}/V_p$	$(V_{r,PRP1} - V_{r,C18})/V_p$

Theoretical recovery calculations from breakthrough curves

In this study the two coupled precolumns were packed with C_{18} bonded silica and with the styrene–divinylbenzene copolymer PRP-1 and will be denoted C18 and PRP1, respectively. The characteristic volumes in Fig. 1 were measured separately on each precolumn. When the water sample is percolated through the two precolumns, $V_{f,C18}$, $V_{r,C18}$ and $V_{m,C18}$ correspond to the volumes obtained when C18 is need alone, whereas $V_{f,PRP1}$, $V_{r,PRP1}$ and $V_{m,PRP1}$ represent the volumes obtained with PRP1 alone plus the values obtained for C18 alone. For most of the neutral solutes, these volumes increase in the following order:

$$V_{f,C18} < V_{r,C18} < V_{m,C18} < V_{f,PRP1} < V_{r,PRP1} < V_{m,PRP1} \quad (5)$$

The curves representing the front will not be mathematically expressed and are denoted $y(V_p)$ for C18 and $z(V_p)$ for PRP1. The amounts extracted on C18, PRP1 and on both (total) are Q_{C18}, Q_{PRP1} and Q_T, respectively, and the corresponding recoveries are R_{C18}, R_{PRP1} and R_T. The equations and corresponding data are reported in Table I and Fig. 2 shows the variations of (a) extracted amounts and (b) recoveries with the percolated sample volume. Variations of the extracted amounts are simple: for a sample volume smaller than the breakthrough volume of the first precolumn, $V_{f,C18}$, the extracted amount and percolated amount are equal and the recovery is 100%. For a percolated volume greater than $V_{f,C18}$, the amount extracted by C18 increases to a maximal value but is different from the percolated amount; the fraction eluted from C18 by the water sample is then extracted by the second precolumn PRP1 until $V_{f,PRP1}$ is reached. The recovery from C18 decreases whereas that from PRP1 increases, but the total recovery is always 100%. The maximal extracted amount is then obtained for a sample volume $V_{m,PRP1}$ but corresponds to a total recovery below

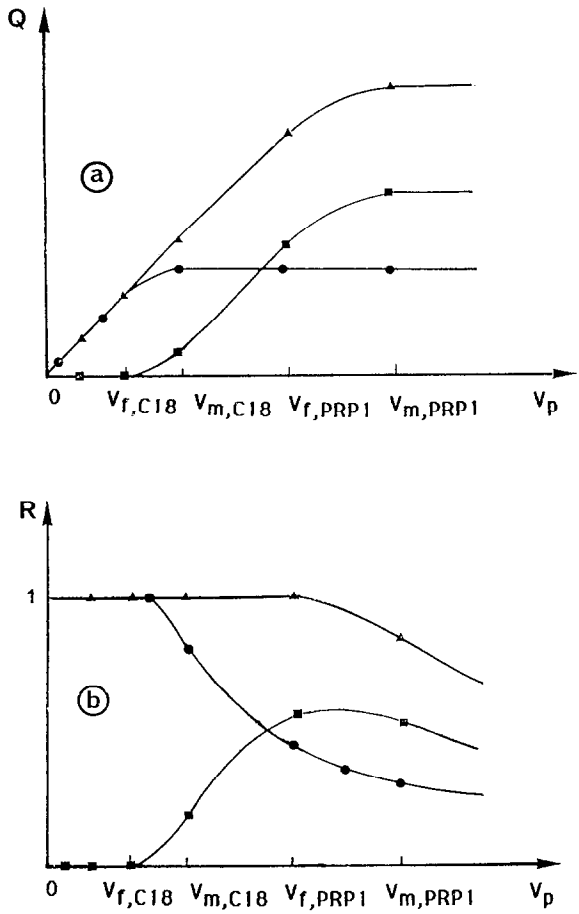

Fig. 2. Variations of (a) extracted amounts and (b) recoveries with percolated volume from C18 (●), from PRP1 (■) and total (▲) when the two precolumns are coupled together.

100%. We also note that when V_p is between V_f and V_m, the recovery and extracted amounts cannot be exactly calculated as the front curve has to be known.

Variations of extracted amounts and recoveries with percolated volumes are useful. In trace analysis, detection limits are the main interest. From Fig. 2a and b, the best condition for detection is to work with a sample volume higher than $V_{m,PRP1}$ because the amount extracted by PRP1 is higher than that extracted by C18 even if the corresponding recovery is then only 50%. This point is particularly important for solutes having poor detection properties.

Two procedures are carried out to determine solute concentrations in samples. The first consists in determining solute amounts by injecting directly a known amount of solute into the analytical column via a conventional loop injection; hence the amount in the percolated sample is determined by calibration graphs for the detector and of course recovery data are necessary in order to calculate the concentration. A second method, more widely used, consists in spiking water samples with known amounts of solutes and then measuring the corresponding extracted amount. This

method does not necessitate knowing recoveries if spiked samples have exactly the same volume as unknown samples and if the solubilization of the solutes on spiking is complete. If the sample volumes are changed, other spikings have to be performed; there are many examples where the sample volume has to be adapted for the same analysis (*e.g.*, determination of pollutants in waste waters and drinking waters).

It is therefore of interest to be able to optimize and adapt the sample volume to the analytical problem at hand by considering possible the initial concentrations and the detection mode. Recovery determinations are therefore necessary.

Theoretical calculations can be effectively made from breakthrough curves but we shall also show that from spiked samples it is possible, under certain conditions, to determine both recoveries and extracted amounts. In this first part we have shown a theoretical determination of recovery from the elution front, but we can also measure these recoveries directly with the two precolumns in series.

Experimental recovery calculation from peak areas

Percolation of several sample volumes is carried out with constant percolated amounts of solutes; hence, when the percolated sample volume increases, the concentrations (C_p) are adjusted in order to have a constant amount (C_pV_p). Depending on V_p and the breakthrough values, the solutes being determined are extracted only on the first precolumn or on both. On elution to the analytical column and analysis, peak areas can be measured and are proportional to the amount extracted. Fig. 3 shows two chromatograms obtained for 10 ml of water spiked with three herbicides at 120 μg/l and for 150 ml of water spiked with the same compounds at 8 μg/l. With the 10-ml sample, the three herbicides are extracted only by C18 whereas with the 150-ml sample they are recovered on both C18 and PRP1.

Let us consider first that only one precolumn is used (*e.g.*, C18). If V_p is smaller than $V_{f,C18}$, Fig. 2 shows that extracted and percolated amounts are equal: as the percolated amount is constant from one percolation to another, the peak area is constant and for one given solute we have the condition

$$A(V_p) = kC_pV_p \tag{6}$$

When V_p is higher than $V_{m,C18}$, the extracted amount becomes equal to $C_pV_{r,C18}$, so that

$$A(V_p) = kC_pV_{r,C18} \tag{7}$$

Because the concentrations of the solutes in the sample are adjusted in order to have a constant percolated amount, C_p can be expressed in terms of C_{p1} and V_{p1}, for the first concentration and the first sample volume, respectively, and we then have

$$C_p = (C_{p1}V_{p1})/V_p \tag{8}$$

Denoting by A_1 the first percolation ($A_1 = kC_{p1}V_{p1}$) for which it is verified that V_{p1} is smaller than $V_{f,C18}$, eqn. 7 is expressed by

$$A(V_p) = A_1(V_{r,C18}/V_p) \tag{9}$$

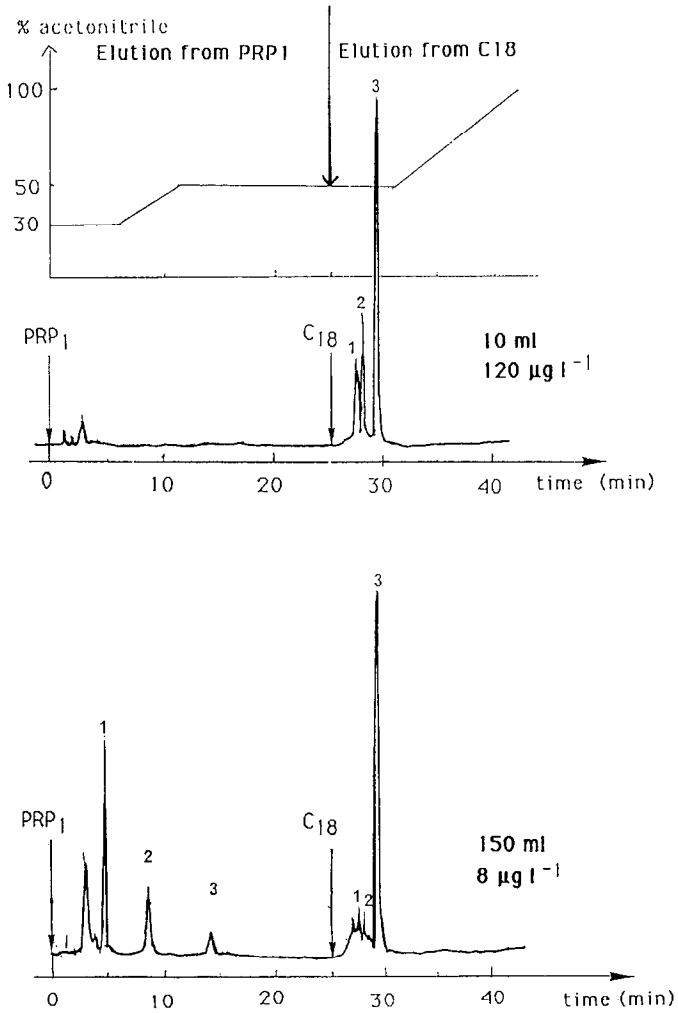

Fig. 3. Analysis of two water samples spiked with the same amounts of herbicides: (a) preconcentration of 10 ml of water spiked at 120 µg/l for each solute; (b) preconcentration of 150 ml of water spiked at 8 µg/l for each solute. Solutes: 1 = simazine; 2 = atrazine; 3 = linuron. Preconcentration through two precolumns (1 × 0.21 cm I.D.) in series packed with RP-18 silica and PRP-1 copolymer at a flow-rate of 3 ml/min; elution to analytical column (ODS-2; 15 × 0.46 cm I.D.) of PRP-1 first by a acetonitrile gradient from 30 to 50% and then RP-18 from 50 to 100% at a flow-rate of 1.5 ml/min.

If peak areas are normalized by areas obtained for a first percolation under A_1 conditions, we have the following relationships:

$$V_p < V_{f,C18}: \qquad A(V_p)/A_1 = 1 \tag{10}$$

and

$$V_p > V_{m,C18}: \qquad A(V_p)/A_1 = V_{r,C18}/V_p \tag{11}$$

Comparison of these with those in Table I indicates that they represent recoveries obtained when C18 is used alone.

When the two precolumns are used in series, there is a decrease of peak areas with V_p obtained on C18 elution which is accompanied by an increase of peak areas from PRP1. With the same notations for the characteristic volumes as those defined for breakthrough studies, the values of the normalized areas obtained on C18 and PRP1 on elution and analysis of extracted compounds are as follows:

$V_p < V_{f,C18}$:

$$A(V_{p,C18})/A_1 = 1 \quad (12)$$

$$A(V_{p,PRP1})/A_1 = 0 \quad (13)$$

$V_{m,C18} < V_p < V_{f,PRP1}$:

$$A(V_{p,C18})/A_1 = V_{r,C18}/V_p \quad (14)$$

$$A(V_{p,PRP1})/A_1 = 1 - (V_{r,C18}/V_p) \quad (15)$$

$V_p > V_{m,PRP1}$:

$$A(V_{p,C18})/A_1 = V_{r,C18}/V_p \quad (14)$$

$$A(V_{p,PRP1})/A_1 = (V_{r,PRP1} - V_{r,C18})/V_p \quad (16)$$

Comparing eqns. 12–16 with the calculations in Table I, one can see that the normalized peak areas represent exact recoveries; measurement of peak areas when preconcentrating several sample volumes spiked with a constant amount of solute allows one to determine recoveries and extracted amounts.

EXPERIMENTAL

Apparatus

Percolation of water was performed with a Milton Roy pump (LDC, Riviera Beach, FL, U.S.A.) and precolumn elutions and analyses were carried out with a Model 5060 liquid chromatograph (Varian, Palo Alto, CA, U.S.A.) equipped with a UV 200 variable-wavelength UV spectrophotometer, a Polychrom 9060 diode-array detector (Varian) and a fluorimetric detector (Kratos, Ramsey, NJ, U.S.A.). Precolumn and analytical column switching was performed with two valves (Rheodyne, Berkeley, CA, U.S.A.). Quantitative measurements of peak areas were made with a CR3A integrator–computer (Shimadzu, Kyoto, Japan).

Stationary phases and columns

Water samples were preconcentrated on 1 cm × 2.1 mm I.D. stainless-steel precolumns available from Chrompack (Middelburg, The Netherlands), which were packed manually with a thick slurry using a microspatula or with a thin slurry using a syringe. The stationary phases were 10-μm octadecylsilica RP-18 (Merck, Darmstadt, F.R.G.) and spherical 10-μm styrene–divinylbenzene copolymer PRP-1 (Hamilton, Reno, NV, U.S.A.). The analytical column was a 15 cm × 4.6 mm I.D. stainless-steel column prepacked with 5-μm octadecylsilica Spherisorb ODS 2 (Whatman, Clifton, NJ, U.S.A.).

Chemicals

HPLC-grade acetonitrile was obtained from Rathburn (Walkerburn, U.K.)

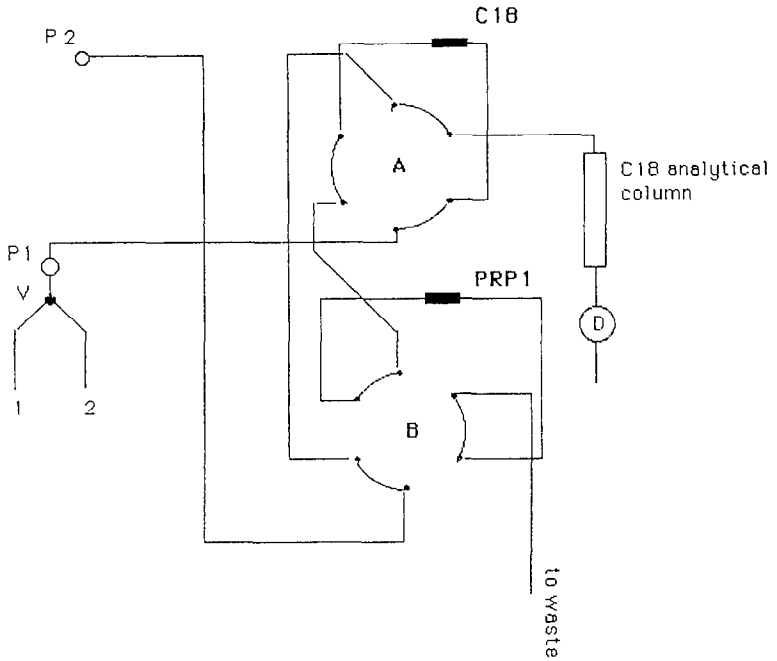

Fig. 4. Experimental set up for the on-line trace enrichment and analysis of water samples. P1 = pre-concentration pump; P2 = high-pressure pump; V = low-pressure valve for sample (1) and flushing with 0.001 *M* perchloric acid (2); A and B = high-pressure six-port switching valve; C18 = precolumn (1 x 0.21 cm I.D.) packed with RP-18 silica; PRP1 = precolumn (1 × 0.21 cm I.D.) packed with PRP-1 copolymer; analytical column, 15 × 0.46 cm I.D. packed with ODS-2 silica.

and methanol from Prolabo (Paris, France). Water was deionized and evaporated in a quartz apparatus from Quartex (Paris, France). The various solutes were supplied by Prolabo, Merck or Fluka (Buchs, Switzerland).

Procedure

Stock solutions of selected solutes were prepared by weighing and dissolving them in methanol. LC-grade water samples were spiked with these solutions at the ppb level and adjusted to pH 3 with perchloric acid. The final standard solutions did not contain more than 0.5% of methanol.

Breakthrough curves of selected analytes were recorded as follows: the standard solution was first directly coupled to the detector to measure the absorbance of the studied solute and then coupled to the precolumn (which has been previously conditioned) at a flow-rate of 2.5 ml min^{-1}.

The experimental set up is shown in Fig. 4 according to ref. 14. A water sample was introduced via pump P1 onto the two precolumns in series; the precolumns were flushed with 4 ml of 10^{-3} *M* perchloric acid. Each precolumn was then separately coupled to the analytical column by switching valve A or B and backflush-eluted by an acetonitrile gradient via pump P2. Precolumns in series were cleaned with pure acetonitrile and regenerated with 25 ml of 10^{-3} *M* perchloric acid.

RESULTS AND DISCUSSION

Breakthrough curves

Fig. 5 shows breakthrough curves recorded for a few solutes such as phthalates, herbicides and phenolic compounds on (a) C18 or (b) PRP1. Neither of these frontal analysis chromatograms has the ideal shape of a bilogarithmic front which would correspond to a gaussian peak in elution chromatography and the fronts are different from one solute to another. One reason is that the geometric volumes of these precolumns are small (35 μl) and, as they are filled with a microspatula, their plate number is not very high. Another reason is that solutes have different exchange kinetics between the sorbent and the aqueous mobile phase. Breakthrough volumes and maximal volumes were measured on the curves at 1% and 99% of the sample absorbance and retention volumes at 50%. With non-symmetrical curves, this determination of retention volumes is an approximation and one should probably use momentum theory to obtain more accurate retention values from breakthrough curves. These values are reported in Table II. The breakthrough volumes are higher with PRP1 than with C18 for all solutes except for trinitrophenol, which is in fact ionized at pH 3 because of the influence of the three nitro groups ($pK_a = 0.3$); it can be easily verified that apolar compounds are extracted by C18; for instance, when percolating 100 ml with solutes such as linuron and diethyl phthalate of course all other phthalates and less polar solutes (hydrocarbons, etc.) will also be adsorbed. Moderately polar compounds such as phenolics are better extracted by PRP1 than by C18. When percolating 100 ml of 2,4,5-trimethylphenol (solute No. 5) it is impossible to neglect the amount extracted by C18, whereas for 2-nitrophenol (solute No. 2) one can neglect it although these two solutes have similar breakthrough volumes on PRP1. It is also noticeable that there is a great difference beween V_r and V_f; *e.g.*, simazine has a

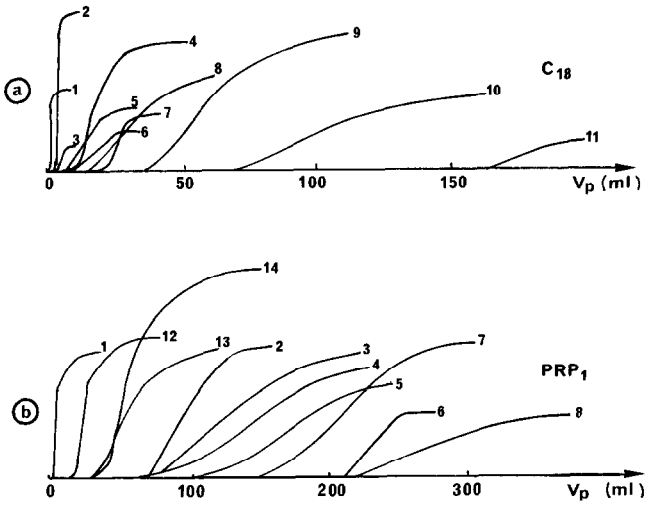

Fig. 5. Breakthrough curves for solutes with (a) C18 packed with RP-18 and (b) PRP1 packed with PRP-1 copolymer. Samples: dilute solutions in water at pH 3 (10^{-3} *M* perchloric acid); flow-rate, 2.5 ml/min; UV detection at variable wavelength. See Table II for solute numbers.

TABLE II

CHARACTERISTIC VOLUMES (ml) FROM BREAKTHROUGH CURVES RECORDED SEPARATELY ON C18 AND PRP1

V_f = Breakthrough volume corresponding to 1% of total front height; V_r = retention volume corresponding to 50% of total height; V_m = maximal volume corresponding to 99% of total height.

No.	*Solute*	*C18*			*PRP1*		
		V_f	V_r	V_m	V_f	V_r	V_m
1	2,4,6-Trinitrophenol	0.1	0.7	5	2	5	30
2	2-Nitrophenol	1.5	2.5	7	70	95	130
3	Toluene	2.5	5	9	65	140	230
4	*m*-Xylene	6	14	27	65	150	250
5	2,4,5-Trimethylphenol	5	15	34	60	145	220
6	Dimethyl phthalate	7	15	28	212	230	270
7	2,4,6-Trichlorophenol	14	31	58	215	283	360
8	Simazine	19	26	36	130	207	280
9	Atrazine	37	60	110	–	–	–
10	Linuron	70	105	165	–	–	–
11	Diethyl phthalate	165	180	195	–	–	–
12	2-Methylphenol	–	–	–	15	23	61
13	2,4-Dinitrophenol	–	–	–	27	50	103
14	2,4-Dimethylphenol	–	–	–	26	52	105

breakthrough volume of 130 ml on PRP1 and a retention volume of 207 ml; therefore, calculation of V_f from V_r, widely used in the literature, is to be considered only as a first approximation.

Precolumn capacity

Fig. 6 shows breakthrough curves recorded for increasing concentrations of dimethyl phthalate in water on C18. For water spiked with 0.3 and 0.9 ppm, breakthrough occurs at the same percolated volumes (and V_r is the same), but for higher concentrations the breakthrough volumes decrease. Assuming a Langmuir adsorption isotherm, overloading occurs when 20 μg of dimethyl phthalate are adsorbed on C18. The capacity depends on the size of the solute and on its steric configuration. Under the same conditions, it was estimated as 50 μg for xylene. Our results correspond to an adsorption of up to 4 mg/g of bonded silica for xylene. The results in the literature vary considerably and of course depend on the solutes and on the types of bonded silicas. Capacity values for bonded silicas up to 15–60 mg/g of packing material have been reported[24]. Although we did not measure the capacity of PRP1, it is considered to be higher than that of C18. A value reported earlier[8] is 186 mg/g for 2,6-dichlorophenol, which is much higher than the capacity of C18. Another study[27] estimated that the capacities of both C18 and PRP1 are greater than 1 mg/g of sorbent.

One has to make sure that no breakthrough occurs due to overloading of sorbent in the precolumns. Although we should consider the total concentration of both solutes and interferences, concentrations in surface water samples typically are at the ng/l to the μg/l level, so that overloading is rather unlikely to occur. The concentrations of standard solutions were adjusted so that the sum of the adsorbed species was always below 15 μg in all our investigations.

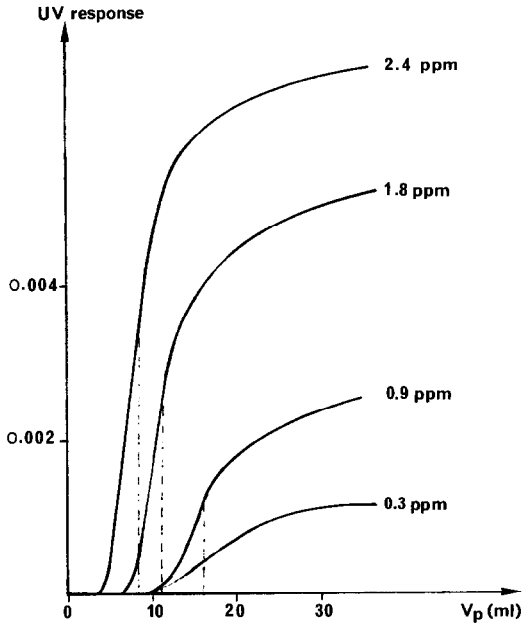

Fig. 6. Estimation of the capacity of C18 by recording breakthrough curves for water samples with increasing concentrations of dimethyl phthalate. Precolumn (1 × 0.21 cm I.D.) packed with RP-18 silica; flow-rate, 2.5 ml/min; UV detection at 260 nm; sensitivity, 0.1 a.u.f.s.

Recoveries from breakthrough volumes

Recoveries are calculated from data reported in Tables I and II. Variations with sample volume are shown in Fig. 7 for six solutes with increasing breakthrough volumes. For a solute such as nitrophenol, having a small $V_{f,C18}$, the recoveries from C18 and from PRP1 are 10% and 90%, respectively, for a 25-ml sample volume, whereas the same values are obtained for dimethyl phthalate with a 150-ml sample volume.

Frontal analysis is therefore a powerful method for rigorously calculating recoveries but requires the measurement of the characteristic volumes from breakthrough curves. The curves in Fig. 7 also show that a knowledge of retention data is not sufficient for an accurate determination of recovery.

Before measuring experimental recoveries by percolations with constant amount of solutes, one must also test the desorption efficiency of the coupled system, as the detected amount is generally determined via the whole system by measuring peak area of peak height.

Comparison of results obtained by direct injection and on-line preconcentration analysis

Comparison of quantitative results obtained by direct injection into the analytical column and by preconcentration of a water sample containing the same amount of solutes is made in order to test the efficiency of coupling a precolumn to an analytical column, that is, the desorption and transfer of solutes from the precolumn to the analytical column. In order to eliminate the problems inherent in spiking samples with hydrophobic compounds, the moderately polar 2-chlorophenol was chosen and

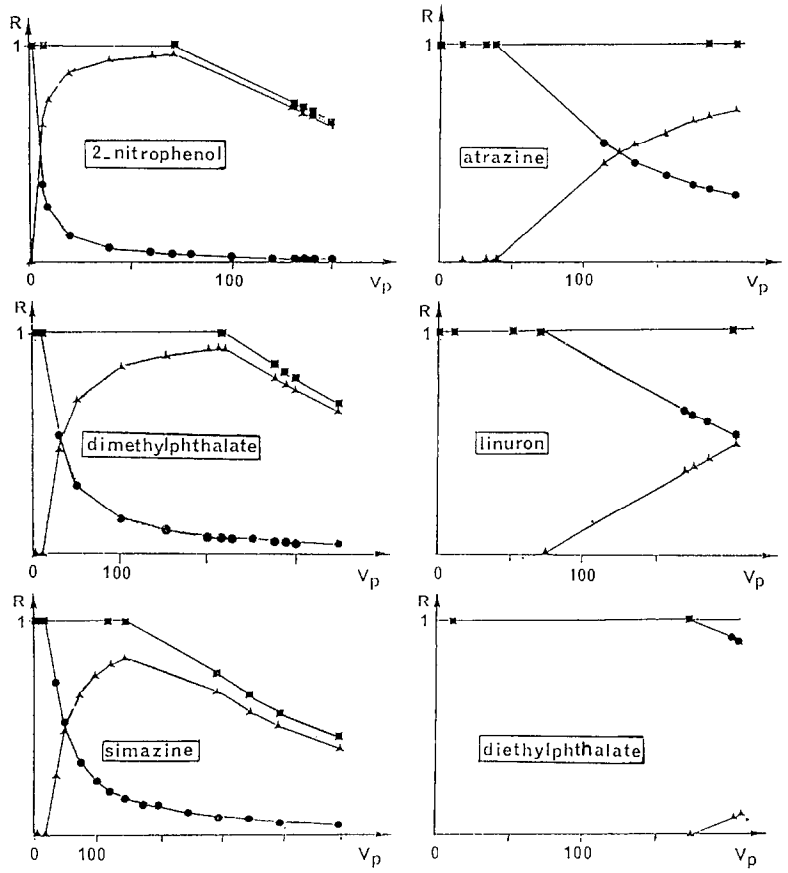

Fig. 7. Variation of recoveries calculated from characteristic volumes determined from experimental breakthrough curves (Table II) with percolated volume of samples. (●) Recovery from C18; (▲) recovery from PRP1; (■) recovery from C18 and PRP1 together (total).

the study was carried out with PRP1 alone (V_f = 32 ml). In order to test also the reproducibility of precolumn packings, a series of four precolumns were packed with a thin or thick slurry. Table III reports the retention time, plate number, peak area and peak height for three replicate analyses of 2-chlorophenol directly injected or preconcentrated from 10-ml water samples on the four different PRP1 columns.

Comparison of efficiencies. Band broadening comes from the transfer of the solute from the precolumn to the analytical column; from a geometric point of view, in order to avoid band broadening it is necessary to use precolumns of small dimensions compared with those of the analytical column, a condition which has been easily realized. The fact that the precolumn and analytical column are packed with different stationary phases can also be a cause of band broadening[24], especially when the retention of the solute by the precolumn sorbent is greater than that by the analytical column sorbent, as for 2-chlorophenol in this study. Band broadening can be suppressed by compressing the relatively broad profile in the precolumn at the top of the analytical column by the choice of a proper mobile phase and stationary phase com-

TABLE III

COMPARISON OF RESULTS OBTAINED BY DIRECT INJECTION OF 20 μl OF A SOLUTION OF 2-CHLOROPHENOL AND BY PRECONCENTRATION OF 10 ml OF WATER SAMPLE SPIKED WITH THE SAME AMOUNT OF 2-CHLOROPHENOL

Reported values are average values of three identical analyses; relative standard deviations (R.S.D.) are also reported; retention volumes are in ml; efficiency was calculated using $N = 5.54\,[t_R/w_{0.5}]^2$, where $w_{0.5}$ is the peak width at half-height; peak areas and heights are in arbitrary units; PRP-1 precolumns packed manually with (a) a thin slurry using a syringe or (b) with a thick slurry using a microspatula.

	Retention volume	*Efficiency (plate number, N)*	*Peak area*	*Peak height*
Direct injection	5.06	6060	2 047 323	9.35
R.S.D. (%)	0.2	3	0.3	2.6
Precolumn A (a)	5.08	6330	1 889 877	9.0
R.S.D. (%)	0.2	2.3	7.2	9
Precolumn B (a)	5.10	6160	1 847 978	8.7
R.S.D. (%)	1	5.6	1.4	4.6
Precolumn C (b)	4.65	6120	1 865 798	9.7
R.S.D. (%)	1.5	4.3	7	4.7
Precolumn D (b)	4.8	6100	1 834 194	9.35
R.S.D. (%)	1.5	1.8	5.5	5.2

bination[16]. In our study, backflush-desorption is used and Table III shows that no band broadening effect occurs; the efficiencies are identical with and without the precolumn. A relative standard deviation (R.S.D.) below 6% is observed when a precolumn is coupled to the analytical column and this is not very different from the R.S.D. of 3% measured with the analytical column alone; hence one can conclude that the introduction of the precolumn does not modify the apparent efficiency of the analytical column for the four precolumns.

Comparison of peak areas and heights. It can be seen (Table III) that the average peak area obtained with direct injection is slightly higher (about 10%) than the average peak areas with all the precolumns tested. This decrease cannot be explained by a possible breakthrough as the sample volume and the flushing volume (10 + 4 ml) before elution are far below the breakthrough volume of 2-chlorophenol (about 30 ml). This discrepancy can therefore be attributed to imprecisions in the percolated volume and in the calibration of the 20-μl injection loop, which has not been verified.

Reproducibility of precolumn packing. For the four packed precolumns, the reproducibility between peak areas and peak heights obtained for three consecutive preconcentrations is good, within an average R.S.D. of 3% for peak areas and 6% for peak heights (see Table III). This indicates that reproducible quantitative analyses are possible, provided that reproducible breakthrough volumes are obtained. Table IV reports V_f values for various solutes with the four precolumns. Up to V_f values of 20 ml the four precolumns are not very different; for 2-chlorophenol, 2-nitroaniline and 2-nitrophenol, A, B and D give similar V_f values but C gives lower values. These results show that differences in packing homogeneity are more critical when high volumes are percolated. This study does not show a clear influence of the packing

TABLE IV

TEST OF REPRODUCIBILITY OF PRECOLUMNS BY MEASURING BREAKTHROUGH VOLUMES OF VARIOUS SOLUTES WITH PRP1

See Fig. 5 for experimental conditions.

Solute	*A*	*B*	*C*	*D*
Phenol	3.5	4	2.5	3.5
4-Chloroaniline	4.5	6	6.5	8
2-Cresol	16.5	19	16	22
2-Naphthylamine	30	30	22	35
2-Chlorophenol	32	30	23	30
2-Nitroaniline	62	75	36	58
2-Nitrophenol	–	115	70	101

procedure (thin or thick slurry). We did not test commercially available precolumns but one can assume that they are packed under more reproducible conditions.

Recovery from peak areas with percolations at constant C_pV_p

Several 10-ml water samples spiked with different solutes are concentrated and peak areas are measured on elution. The recorded values are taken for A_1 values; several percolations with a constant amount of solute but a different volume are performed and, as described above, the recoveries correspond to the ratio of the peak areas measured for a volume V_p and of peak areas for a 10-ml volume. These values are reported in Table V for concentrations on C18 alone or on PRP1 alone. For most of the solutes the variations in recovery with sample volumes correspond to those

TABLE V

NORMALIZED PEAK AREAS WITH PERCOLATED SAMPLES HAVING THE SAME AMOUNT OF SOLUTES

A_1 measured with 10-ml samples; recoveries calculated with each precolumn.

Precolumn for concentration	*Solute*	*Sample volume (ml)*					
		10	*25*	*50*	*100*	*200*	*400*
C18	Dimethyl phthalate	1.00	0.84	0.51	0.25	0.13	0.11
	Diethyl phthalate	1.00	0.88	0.84	0.83	0.83	0.63
	Dibutyl phthalate	1.00	0.86	0.79	0.86	0.95	1.07
	Pyrene	1.00	0.92	0.82	1.16	1.51	1.90
PRP1	2-Methylphenol	1.00	0.90	0.62	0.35	–	–
	2,4-Dimethylphenol	1.00	0.93	0.92	0.88	–	–
	2,4,5-Trimethylphenol	1.00	0.92	0.91	0.91	–	–
	2-Chlorophenol	1.00	0.94	0.84	0.57	–	–
	2,4-Dichlorophenol	1.00	0.91	0.97	0.85	–	–
	2,4,6-Trichlorophenol	1.00	0.96	0.94	1.03	–	–
	2-Nitrophenol	1.00	0.96	0.94	0.90	–	–
	2,4-Dinitrophenol	1.00	0.98	0.94	0.89	–	–
	2,4,6-Trinitrophenol	1.00	0.63	0.44	0.33	–	–

calculated from breakthrough curves, *viz.*, a decrease in recovery for sample volumes above V_f.

The efficiency of spiking is clearly shown in Table V when only one precolumn was used; it is difficult to ensure that a recovery below 100% is due to breakthrough of the solute and not to inefficient solubilization of the test compounds or possible adsorption on the vessels and connection tubes. For a sample volume smaller than V_f, the recovery should be 100% and, taking account of the reproducibility of peak area measurements and of spiking, a value between 95% and 105% is acceptable. This is the case for methyl-, nitro- and substituted phenols as these compounds are sufficiently soluble in water; however, for butyl phthalate and pyrene, which are more hydrophobic compounds and difficult to solubilize in water, we see that for a volume higher than 100 ml the recovery increases to 190%; increasing the sample volume and decreasing the concentration (as C_pV_p remains constant) allows a more efficient solubilization so that the peak areas increase. For these solutes the lowest percolation volume to normalize peak areas is not 10 ml but at least 400 ml.

Fig. 8 shows the results for three herbicides concentrated on the two precolumns in series and can be compared with Fig. 7b, c and d. The sum of peak areas recovered from C18 and PRP1 is constant within a 4% deviation so that up to 200 ml there is no breakthrough from PRP1 for any of the three compounds. Thus, from a 100-ml sample, only 45% of simazine, about 95% of atrazine and 100% of linuron are recovered from C18, whereas simazine is concentrated to 55% and atrazine 5% on PRP1. One can say that PRP1 alone would, of course, concentrate the three compounds to 100%, but this is a typical example demonstrating the advantage of coupling precolumns. If only PRP1 is used, all other non-polar compounds are trapped together with the analytes and interfere during the analysis. However, with C18 coupled before PRP1 some non-polar interferents are trapped, hence rendering analysis of the PRP1 fraction more selective.

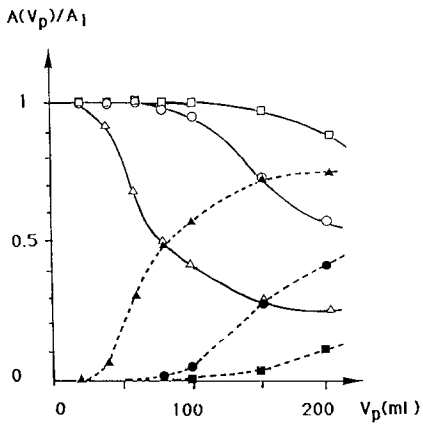

Fig. 8. Experimental variations of normalized peak areas with percolated water samples having a constant amount of herbicides. Preconcentration of samples at pH 3 (10^{-3} *M* perchloric acid) through the two precolumns in series; see Fig. 3 for analytical gradient and other experimental conditions. Solutes: (△) simazine; (○) atrazine; (□) linuron; peak areas measured during elution of C18; (▲) simazine; (●) atrazine; (■) linuron; peak areas measured during elution of PRP1.

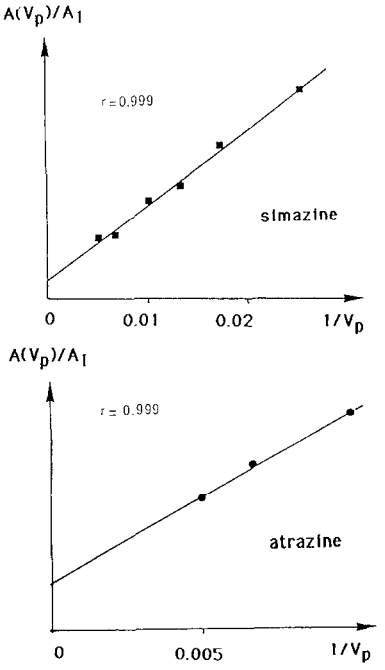

Fig. 9. Determination of breakthrough for (■) simazine and (●) atrazine by extrapolating the variations of normalized peak areas with the inverse of the percolated volume. Peak areas measured during elution of C18. Experimental conditions as in Fig. 8.

Another application of peak-area variations is an extrapolation to breakthrough values. $V_{f,C18}$ corresponds to the volume from which recovery from C18 begins to decrease and $V_{f,PRP1}$ to the volume from which the total recovery begins to decrease. From Fig. 8, the breakthrough volumes of simazine, atrazine and linuron can be roughly estimated to be 20, 50 and 75 ml on C18 whereas the real values determined from breakthrough curves are 19, 37 and 70 ml, respectively.

Fig. 9 shows the variations of $A(V_{p,C18})/A_1$ with the inverse of the percolated volume for simazine and atrazine when V_p is higher than $V_{f,C18}$. As seen from eqn. 14, these variations should be linear for V_p higher than $V_{m,C18}$, whereas for V_p between $V_{f,C18}$ and $V_{m,C18}$ there is no simple theoretical expression for the recovery as it is related to the shape of the front. For the two herbicides, excellent linear relationships are obtained (Fig. 9), hence showing the validity of eqn. 14 and the validity of measuring recoveries from peak areas. The last points of the straight line correspond to extrapolated V_m values and are about 40 ml for simazine and 100 ml for atrazine. Values taken from the breakthrough curves are 36 and 110 ml, respectively.

Table VI shows the differences between recoveries measured from variations in peak areas with various percolated volumes and those calculated from breakthrough curves. The latter are always slighty higher (about 15%). These differences are linked to the procedures in the two methods. The breakthrough method is based on calculations from experimental values carried out with only one precolumn and recovery determinations depend on the accuracy of the recorded breakthrough curves and

TABLE VI

COMPARISON OF RECOVERIES FROM C18 CALCULATED FROM BREAKTHROUGH CURVES AND FROM VARIATIONS OF PEAK AREAS WITH SAMPLE VOLUME (V_p, ml) WHEN PERCOLATING SAMPLES AT CONSTANT C_pV_p THROUGH THE TWO PRECOLUMNS IN SERIES

Solute	V_p	*Calculated recoveries from breakthrough curves*	*Calculated recoveries from peak areas*
Simazine	50	0.52	0.80
	100	0.26	0.43
	150	0.17	0.28
Atrazine	50	0.95	1.00
	100	0.60	0.96
	150	0.40	0.72
Linuron	50	1.00	1.00
	100	0.90	1.00
	150	0.70	0.96

especially on the reading of characteristic volumes. In order to not overload the precolumn, frontal analysis chromatograms have to be obtained with very dilute solutions and are recorded at a low UV range (0.005 to 0.01 a.u.), as shown in Fig. 5; hence for compounds having poor UV absorbance properties, measurement of V_f at 1% of the total front height is not always accurate, especially if the front is spread over a large volume range. In addition, uncertainties from spiking affect these calculations.

Recoveries determined from peak-area measurements, on the other hand, are certainly more accurate as it is always easier to measure peak areas than fronts and if the first two or three percolations with small volumes (assumed to be smaller than the breakthrough volume on the first precolumn) give the same areas. Determinations are rapid because several solutes can be studied simultaneously even if several percolations are necessary. However, the most important point is that these determinations are performed via the whole on-line system and the same operating conditions as used for actual analysis. Errors due to inefficient spiking can occur but, as shown above, they can easily be detected.

From a practical point of view, it seems interesting and more accurate to determine quantitative results by this latter method. As alreadey mentioned, quantitative analyses are carried out by studying spiked samples in order to calibrate detectors and to test the reproducibility of the system. We therefore recommend spiking the samples with a constant amount of solutes and different volumes. There are many advantages to such an approach: recoveries and extracted amounts can be calculated, the efficiency of spiking can be verified, sample volumes can be adjusted to the detection properties of the solutes and breakthrough volumes can be estimated.

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