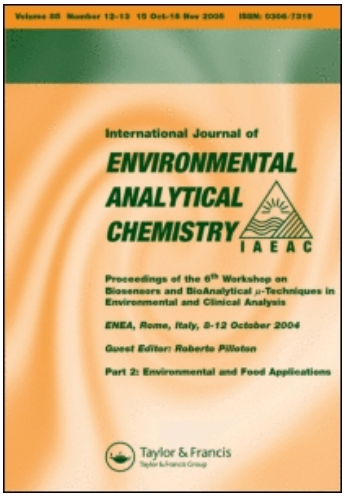

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Preconcentration and Analysis of Selected Pollutants in Industrial Effluents using LC Techniques

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The potential of using LC techniques for the analysis of industrial effluents has been explored. Twenty selected pollutants have been studied and investigated in standard solutions and actual waste water samples. The possibility of preconcentration and detection of most of these compounds at low levels (~1 ppb) in effluent samples has been explored. LC techniques are not suitable for fingerprinting purpose. Their major strength lies in the isolation and determination of specific compounds, or groups of compounds, as demonstrated for the case of methyldiaminobenzene. An LC-grade styrene-divinylbenzene polymer, marketed by Hamilton (PRP_1) has shown interesting characteristics to preconcentrate the majority of the pollutants studied. This approach coupled to chromatographic separation on the same materials and selective wavelength UV detection shows excellent potential for the analysis of priority pollutants in complex effluent samples.

KEY WORDS: Organic pollutants, XAD-type materials, preconcentration, water, samples, UV detection, HPLC, industrial effluents.

INTRODUCTION

It is well recognized that the determination of trace organic contaminants in water is fundamental to the solution of environmental protection problems. Since trace concentrations of organic pollutants are generally of interest, preconcentration prior to

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the analysis proper is often necessary. Beside extraction procedures, one of the most frequently employed methods of preconcentration of organic pollutants from water is direct extraction with solid sorbents.[1] Of all the sorbents, a low polarity styrene-divinylbenzene copolymer, Amberlite XAD-2, has been used most widely for the purpose.[1] Analytical procedures for the determination of organic pollutants in water employing Amberlite XAD-2 or chemically identical XAD-1 and XAD-4 presented in the literature include mainly off-line gas chromatographic techniques,[1] though a few on-line gas chromatographic (GC) methods have also been described.[2–4] Procedures employing high-performance liquid chromatography (HPLC) are also very attractive and have a wider range of application than GC methods. Volpe and Mallet,[5] e.g., employed off-line HPLC techniques for the determination of fenitrothion and its seven derivatives in water. However, especially convenient should be on-line techniques. The advantages of on-line techniques have been pointed out in many papers[6] and on-line HPLC techniques have become quite popular in the recent past. Hux *et al.*[7] used precolumns with Amberlite XAD-2 for direct injection HPLC determination of metaqualane in blood plasma while Cantwell and Mohammed[8] used such precolumns for sample clean-up of complex pharmaceutical samples. However, as Werkhoven-Goewie *et al.*[9] mentioned, for HPLC analysis of a multi-component sample, a precolumn should be packed with HPLC-quality sorbents, i.e., having 5–10 μm particle diameter. Since Amberlite XAD-2 is usually supplied as the 20–50 mesh beads, a chromatographer is required to crush and resize the irregular particles.[10,11]

Quite recently, a new spherical 10-μm styrene-divinylbenzene polymeric adsorbent, PRP_1, has been introduced by Hamilton Company. Comparative study of PRP_1 and XAD-2 done by Lee and Kindsvater[12] has shown that the quantitative chromatographic characteristics of both sorbents are identical; PRP_1 having a higher surface area retains organic compounds even better than Amberlite XAD-2. Such retention characteristics and the fact that PRP_1 is in the form of spherical microparticles make Hamilton PRP_1 reversed-phase resin very suitable for on-line HPLC determination of trace organic pollutants in water. This paper describes the use of PRP_1 sorbent for on-line preconcentration and HPLC determination of aromatics with chloro and/or amino substituents in industrial wastewater.

Semiquantitative relationships between chromatographic retention and composition of the water/methanol mobile phase for the above mentioned compounds have also been established. They permit the prediction of breakthrough capacities (volumes) of precolumns and hence the attainable enrichment factors for a particular precolumn and pollutant at 100% recovery conditions. Breakthrough volumes of selected organic compounds on the PRP_1 precolumns predicted on the basis of the relationships derived have been compared with experimentally determined values.

THEORETICAL ASPECTS

The relationship between the capacity factor, k', and the volume fraction of organic modifier, ϕ, in reversed-phase HPLC on C_{18}-bonded silica was studied by Schoenmakers *et al.*[13] In general a non-linear relationship was found for aqueous systems containing 10–100% of methanol which can be expressed as

$$\ln k' = A\phi^2 + B\phi + C \tag{1}$$

However, when using modifier in a ϕ range of at least 0.4–0.6 and with $k' > 1$ a linear approximation can be used for most solutes as follows:

$$\ln k' = \ln k'_{H_2O} - S\phi$$

where S can be expressed as

$$S = p + q \ln k'_{H_2O} \tag{3}$$

k'_{H_2O} is the capacity factor under pure aqueous conditions; p and q are empirically determined constants which depend on the stationary phase, type of modifier and column porosity.

$\ln k'_{H_2O}$ can also be determined for many compounds of interest such as, for example, chlorophenols[8] by measuring $\ln k'$ at two to three different ϕ values between 0 and 0.6 and then extrapolating the curve to $\phi = 0$. The retention volume, V_R, which permits computation of the trace enrichment factor, can be calculated from these

extrapolated k'_{H_2O} values as

$$V_R = V_O(1 + k'_{H_2O}) \tag{4}$$

V_O, the dead volume, can be determined experimentally or calculated from the porosity factor, ε_0, and the geometry (r = radius, l = length) of the precolumn as

$$V_O = \varepsilon_0 \pi r^2 l \tag{5}$$

The relationship between V_R and the breakthrough volume, V_B, is given by Eq. 6:

$$V_B = V_R - 2\sigma_v \tag{6}$$

and σ_v (band broadening) can be determined from a breakthrough curve as described earlier.[9]

A typical schematic for on-line operation is shown in Figure 1. Pump A is used for sample loading or trace enrichment and pump B for the desorption and on-line transfer to the separation column. In order to keep band broadening at a minimum it is desirable[6] that the same type of material (surface property and particle size) be used in precolumns and separation column. The length of the precolumn is usually very short (1–5 mm) and the diameter equal or smaller (4.6 to 1 mm I.D.) than that of the separation column.

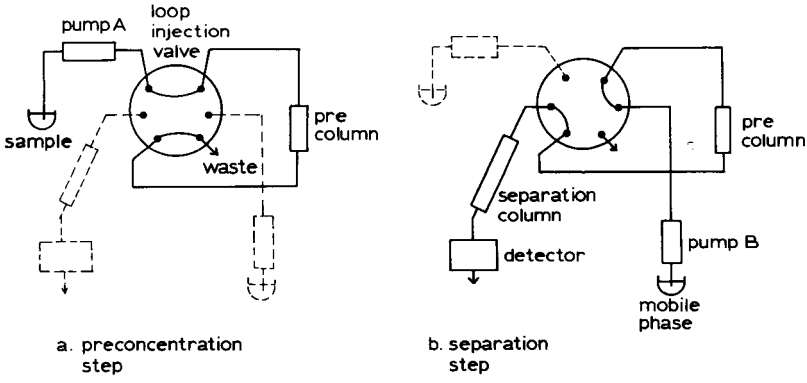

FIGURE 1 Scheme of on-line preconcentration procedure.

EXPERIMENTAL

Reagents

The organic chemicals of interest and used to prepare standard solutions and spiked water samples are listed in Table I. The water used to study breakthrough curves and the analyses of artificial mixtures, and also for the mobile phase, was demineralized water filtered over a Millipore filter (Millipore, Bedford, MA, USA). Other solvents used were absolute methanol and acetonitrile, both Analysed Reagents from Baker (Deventer, The Netherlands) and methanol p.a. from Merck (Darmstadt, GFR).

The home-packed columns were packed with PRP_1 spherical chromatographic resin, manufactured by Hamilton Company (Reno, NE, USA). The PRP_1 analytical columns (150×4.1 mm) were manufactured by Hamilton Company and purchased from Kontron (Zürich, Switzerland). All other chemicals were of analytical grade.

TABLE I
List of compounds studied

Name	Compound letter	Supplier	Purity (%)
p-Aminoazobenzene	A	Fluka	~99
1-Amino-2-methylbenzene	B	Fluka	>99.5
o-Anisidine (Methoxyaniline)	C	Fluka	>98
Benzylchloride	D	Fluka	>99
Chloranil (Tetrachloro-1,4-benzodinon)	E	Fluka	>99
p-Chlorophenol	F	Fluka	>99
Dianisidine (dimethoxybenzidine)	G	Fluka	>99
1,2-Dichlorobenzene	H	Merck	98
1,4-Dichlorobenzene	I	Merck	99
3,3'-Dichlorobenzidine	J	*	99.3
9-Ethyl-3-aminocarbazole	K	*	98
1-Methyl-2,4-diaminobenzene	L	Fluka	>98
Nitrobenzene	M	Fluka	99.5
2-Phenylaminonaphthalene	N	Fluka	~95
p-Phenylenediamine	O	Fluka	>99
1,2,3-Trichlorobenzene	P	Merck	99
1,2,4-Trichlorobenzene	Q	Fluka	~99
1,2,5-Trichlorobenzene	R	Fluka	>99
2-Aminoanthraquinone	S	Fluka	99
1-Aminoanthraquinone	T	Fluka	99

*Source unknown.

Chromatographic apparatus

A Spectra Physics 8000 (Santa Clara, CA, USA) liquid chromatograph was used for the measurements of the $\ln k'$ vs. ϕ curves and for a study of the influence of pH on k' values. It was programmed to give a constant flow of 1 ml/min while the recorder was 10 mV, and the UV detector range was set on 0.16 AUFS ($\lambda = 254$ nm).

The mobile phase solvents used with this apparatus were continuously degassed with helium to prevent the dissolving of air and the formation of air bubbles when mixing the two mobile phase components in the mixing chambers. At the end of the day the column was washed for about 20 minutes with pure methanol. For measuring the breakthrough curves a Kontron (Zürich, Switzerland) Analytical LC pump 410, a Kontron Uvikon 720 LC Spectrophotometer, a Kipp BD-8 multirange recorder (Emmen, The Netherlands) and a Rheodyne 990-3557 six-port valve (Berkeley, CA, USA) were used. The precolumn was a hand-packed 5×4.6 mm PRP_1 column.

For the separation of artificial mixtures and actual samples a Pye Unicam LC3 UV detector (Cambridge, England), a Kipp BD-8 multirange recorder, an Orlita TW 1515 double-head pump (Giessen, GFR) and a Rheodyne 089-0932 six-port valve were used. The flow was set to 1 ml/min and in all three setups the wavelength used was $\lambda = 254$ nm.

Analytical procedures

1. Sample preparation

— All stock solutions were prepared by weighing the compounds and dissolving in 10 ml methanol. From these solutions, a milliliter or less was taken and diluted further with methanol if necessary, to make measurements concerning retention time, etc.

— For preconcentration and breakthrough measurements, 10–50 μl from the methanol solutions were taken and diluted in water up to 100–1000 ml.

— Mixtures were made by dissolving several compounds in the same methanol solution, then diluted in water as above.

— For preconcentration of the real samples, the water was first filtered over a paper filter.

— To spike the real samples, a few μl of an artificial mixture were taken and added to the real sample solution.

2. Concentration on the precolumn Preconcentration of waste water and spiked waste water was carried out on a 5 mm × 4.6 mm I.D. precolumn.

Different amounts of waste water were preconcentrated, and then on-line desorbed with methanol.

When using another mobile phase for desorption and separation, it was necessary to flush the precolumn with pure methanol after the desorption step and then briefly with water (1–2 ml), before a new run was started.

3. Mobile phases The mobile phases used were water-methanol mixtures of different composition and prepared by premixing ultrasonicated solvents followed by degassing under stirring and vacuum. The bottle was then covered with parafilm to prevent the uptake of air.

For buffering the solutions to the various pH's, 0.1 M phosphate buffers were added so that the total buffer concentration in the solutions was 0.001 M.

RESULTS AND DISCUSSION

Measurement of ln k' vs. ϕ curves

Several of the more polar compounds of our list (Table I), viz. the compounds B, C, F, G, L, M, O, were investigated as to their retention at various ϕ values.

The above choice was made on the basis of preliminary experiments carried out on C-18 bonded silica with methanol as polarity modifier. These experiments showed that it was difficult to obtain a trace enrichment effect on C18-bonded silica supports since their retention was nil or very low and it was hoped to improve the situation on PRP_1 material. The curves, constructed from 4 to 8 data points, are shown in Figure 2. Since we are dealing with basic as well as acidic compounds, studies were carried out at three

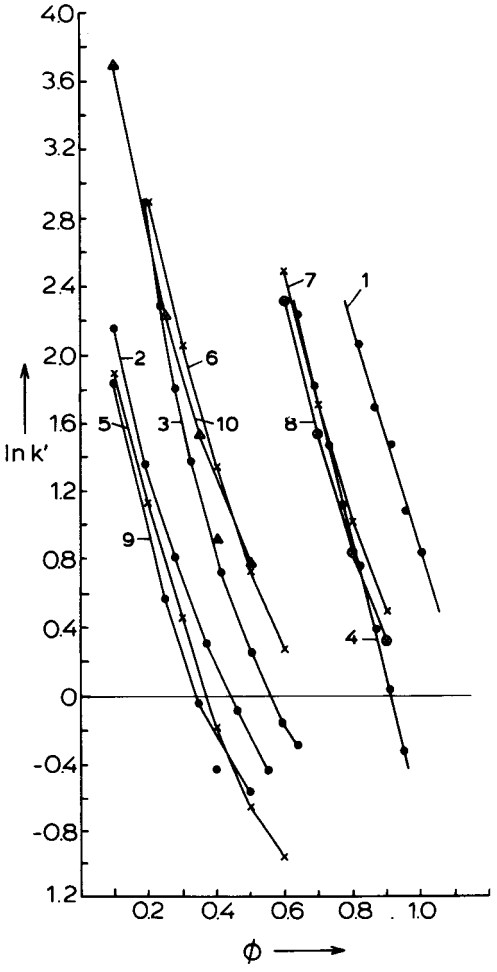

FIGURE 2 Ln k' vs. ϕ curves, flow 1 ml/min, UV detection at 254 nm, curve 1–4 pH = 3, curve 5–8 pH = 7, curve 9, 10 pH = 8.3. Mobile phase: water-methanol, ϕ values give methanol content; column 100 mm × 4.6 mm I.D. PRP_1 column. For identification of compounds see Table I. Curve (1) = M, (2) = C, (3) = G, (4) = F, (5) = O, (6) = L, (7) = D, (8) = B, (9) = Q, (10) = L.

different pH's: 3, 7 and 8.3. Home-packed PRP_1 columns with between 500 and 700 plates (for the compounds examined) were used for these experiments.

From Figure 2 it can be seen that, as expected, nitrobenzene and *p*-chlorophenol are retained resaonably strong on PRP_1 at a pH of 3. Retention for *o*-anisidine and dianisidine improved on going to pH 7 as shown in Figure 2 for the former. Very little retention has been found .at pH 7 for phenylenediamine and methyldiamino-benzene and going to pH 8.3 did not alter this situation. To buffer waste water samples to pH's much higher than this would hardly be feasible.

The influence of pH on the retention of some other compounds in Table I has been tested; the results are presented in Table II. Chromatographic conditions were the same as in the previous study. From Table II the general trend is an increase in k' values in going from lower to higher pH and, as expected, also an increase in going to a lower methanol content. However, the differences with pH are small and as a result one can recommend, also in view of Figure 2, pre-concentration to be carried out at $\sim$pH 7.

From Figure 2 and some rough extrapolation work, one can also draw the preliminary conclusion that compounds with $\ln k'$ vs. ϕ

TABLE II

k' values for selected pollutants at different pH and methanol concentration

	$k'(\phi = 1.0)$	$k'(\phi = 0.9)$	
Compound	pH 6.8	pH 2.7	pH 6.8
B	0.7	1.3	1.4
D	1.8	5.0	5.6
E	1.5	5.5	5.5
F	—	—	1.1
I	2.5	7.3	8.1
J	3.0	10.7	11.75
K	2.5	0.1	7.5
M	1.6	3.7	3.7
N	8.6	—	—
Q	4.4	—	—
S	2.8	6.7	6.8

plots to the right of curve 6 including all the other, even more retained compounds (Tables II and IV) show a good preconcentration behaviour well above 10 ml sample volumes preconcentrated on a precolumn of 4.6 mm I.D. and 5 mm length. Of the others the methyldiaminobenzene will probably show breakthrough at a sample volume <5 ml and phenylenediamine even at <1 ml (Table III); hence alternative preconcentration techniques would have to be thought for those if low ppb-values are required. As to the shape of the curves (Figure 2), one can also say that they are roughly linear, some perhaps tending slightly to a quadratic relationship, but at any rate linear extrapolation of such curves would provide a more conservative and hence safer figure and is recommended (see also later data on breakthrough curves; Table III). The only somewhat odd data points in Figure 2 were observed for phenylenediamine and methyldiaminobenzene at pH 8.3 for $\phi=0.4$ and 0.45; for these, no explanation can be given.

TABLE III

Comparison of experimental V_R values with values calculated from extrapolation (Figure 2 and Eq. 4)

Compound	Extrapolation		Experimentally found results V_R(ml)
	Quadratic curves V_R(ml)	Linear curves V_R(ml)	
B	477	14.7	14.4
C	606	17.4	24.5
F	—	43.3	45.5
L	3.6	1.6	3.0
O	0.5	0.3	—[a]

[a]Too small to be accurately determined by breakthrough curves.

Breakthrough curves and retention volumes

From the above data it is possible to calculate breakthrough volumes and hence preconcentration factors without resorting to the tedious business of actually measuring breakthrough curves (see also theoretical introduction in this paper). This principle and the aspect and technique for determining breakthrough curves has been

discussed in detail earlier for C18[9] and for graphitized[14] surfaces and recently for PRP_1 supports.[15]

A few breakthrough curves have also been measured in this study and compared to data obtained by quadratic and linear extrapolation to check the validity of our postulations. The results are presented in Table III. One can observe a generallly good correlation between experimental data and the linear extrapolation approach. The precolumn used in these tests had a length of 2.5 mm and 4.6 mm I.D. The dead volume of the precolumn V_O was determined according to Eq. 5 with ε_0 taken as 0.65 from the literature[13] and was calculated to be $\sim 27\,\mu$l.

Separation of standard mixtures

The separation for a mixture of 15 compounds selected from Table I is shown in Figure 3. Two compounds (N and Q) do definitely not show in the chromatogram since their retention on PRP_1 is too high under the prevalent mobile phase condition. Without attempting to identify the various peaks we can already say that for a good resolution of such a complex standard mixture a gradient would be necessary. It is also noticeable that strong tailing occurs, which is an inherent disadvantage of PRP_1 materials and results from poor mass transfer characteristics. An attempt was made to use a gradient from 70% to 100% methanol. Unfortunately the PRP material responded very sluggishly to changes in mobile phase composition and the pressure drop of the column increased by about 100 bar along with a rapid rise of the baseline. Such phenomena have been reported earlier for gradient work with PRP material and must be attributed to swelling of the polymer beads.

It was therefore decided to work with three different mobile phases (8:1, 9:1 and 10:0 methanol/water, pH 7) and to separate the compounds into 3 groups according to their suitability to be separated isocratically from the other members in the group. The three groups and the relevant k' values are shown in Table IV.

For the highly retained compounds one could also use C18 in place of PRP_1 material since these can likely also be pre-concentrated on C18-bonded silica, with the advantage of being separable by gradient elution on the same material. Earlier work[9,16] and some preliminary investigation in the course of this work

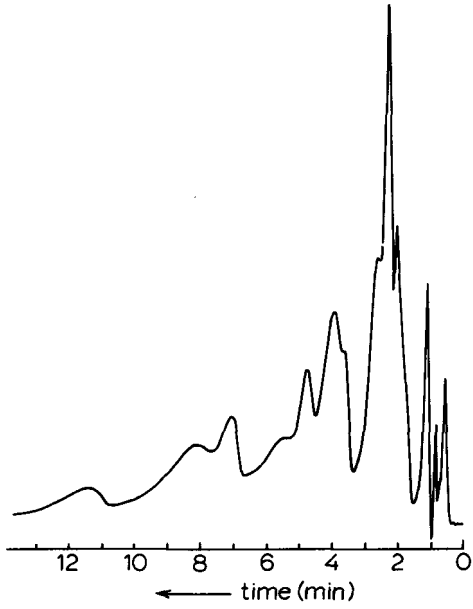

FIGURE 3 Separation of artificial mixture containing: B, C, D, E, F, G, I, J, K, L, M, N, O, Q and S. A 20 μl loop injection was done on a 100 × 4.6 mm I.D. 10 μm PRP_1 packed column; mobile phase, methanol-water (90:10), buffered with 0.01 M phosphate buffer to pH = 7, at flow 1 ml/min; UV detection at 254 nm, attenuation 0.08 AUFS.

TABLE IV

Mixtures suitable for isocratic separation for three different conditions

Mobile phase:	8:2 MeOH: H_2O[a]	9: MeOH: H_2O	100% MeOH
Compounds	O	B k' = 1.4	M k' = 1.6
	L	E k' = 5.5	I k' = 2.5
	C	K k' = 7.5	T k' = 3.4
	F	H k' = 6.6	S k' = 2.8
	G	A k' = 8.8	Q k' = 4.4
	D	J k' = 11.7	R k' = 7.4
		P k' = 12	N k' = 8.6

[a]Order of appearance: (k' 1–10): O < L < C < F < G < D.

support the feasibility of such an approach. Detection limits for the compounds tested were also determined at the chromatographic conditions suggested in Table IV. With absolute detection limits for most compounds being in the order of 1–20 ng (using UV detection) and 10-ml sample volumes being feasible for most compounds for on-line preconcentration with short pre-columns, with 100% recovery it should be possible to detect these pollutants at about the 1 ppb concentration range. The only borderline case of the compounds in Table I would be methyldiaminobenzene whereas phenylenediamine is hardly retained at all.

Analysis of real samples

Figure 4 shows a chromatogram for waste water taken after the biological cleaning step and preconcentrated on the usual precolumn

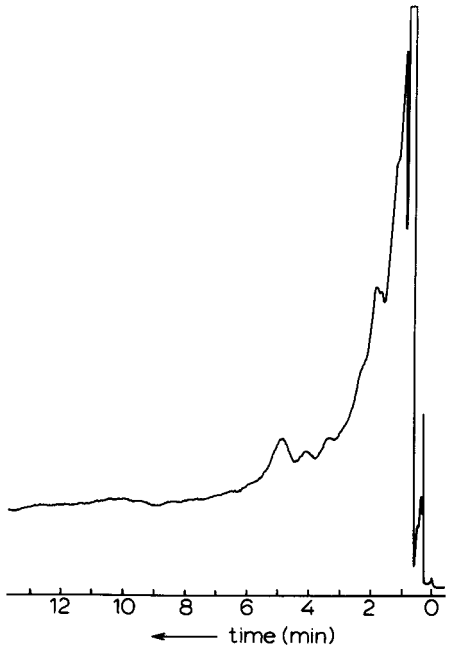

FIGURE 4 Chromatogram of a waste water sample. 100 ml sample preconcentrated on a 5 mm × 4.6 mm I.D. column packed with 10 μm PRP_1 resin; sampling rate 5 ml/min. HPLC on 150 mm × 4.6 mm I.D. column packed with 10 μm PRP_1; eluent 100% methanol at 1 ml/min; UV detection at 220 nm, attenuation 0.04 AUFS.

and on-line transferred to the analysis column with a mobile phase containing 100% methanol. The detection wavelength was 220 nm. Chromatograms detected at 254 nm under otherwise identical conditions were even less conclusive. In general one can conclude that LC techniques are not very well suited for fingerprinting purposes and for a rapid assessment of the overall performance of a water treatment plant. The chromatographic resolution is much too low and GC techniques even with classical columns as described elsewhere[18] would have to be given preference.

However, LC techniques with on-line preconcentration show considerable promise for the isolation and detection of individual or small groups of pollutants as demonstrated with the following example. Figure 5 shows a chromatogram for a 4-ml waste water

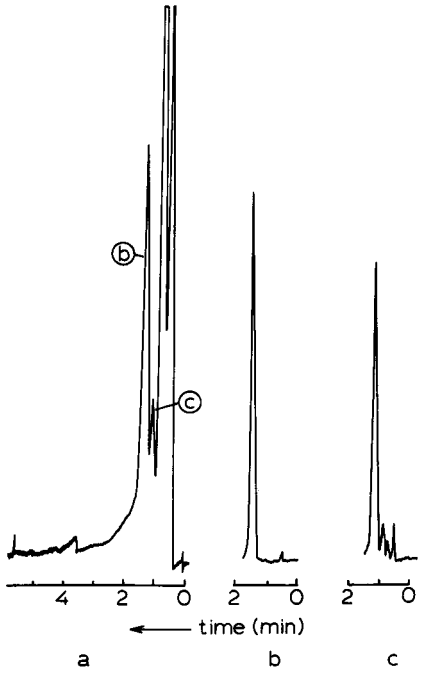

FIGURE 5 Identification of compounds in a waste water sample. 4 ml sample preconcentrated. All conditions as in Figure 4 except for HPLC eluent (9:1 methanol-water buffered to pH = 7 with 0.01 M phosphate buffer) and attenuation (0.4 AUFS). Figure 5a waste water. Figure 5b *p*-chlorophenol, 10 μl injection (0.1 mg/ml). Figure 5c 1-methyl-2, 4-diaminobenzene, 10 μl injection (0.25 mg/ml).

sample preconcentrated in the usual manner and separated with a 9:1 (v/v) methanol/water mixture on a PRP_1 column. The detection wavelength is again 220 nm. According to k' values peaks *b* and *c* can be assigned to *p*-chlorophenol and methyldiaminobenzene respectively. Further evidence for the presence of the latter compound was gathered. Detection at different wavelengths was also investigated as shown in Figure 6 were chromatograms for 4 ml samples are recorded at 200, 220, 254 and 280 nm, respectively using a variable wavelength UV detector. Good evidence for the actual presence of methyldiaminobenzene can be gathered from these chromatograms when comparing them to standard chromatograms of the pure compound detected at the same wavelength. Very clear and reasonably well separated peaks can be observed for 200 and 220 nm detection, whereas with 254 and 280 nm the compound is barely visible which is in accordance with the UV absorption spectrum for this compound.

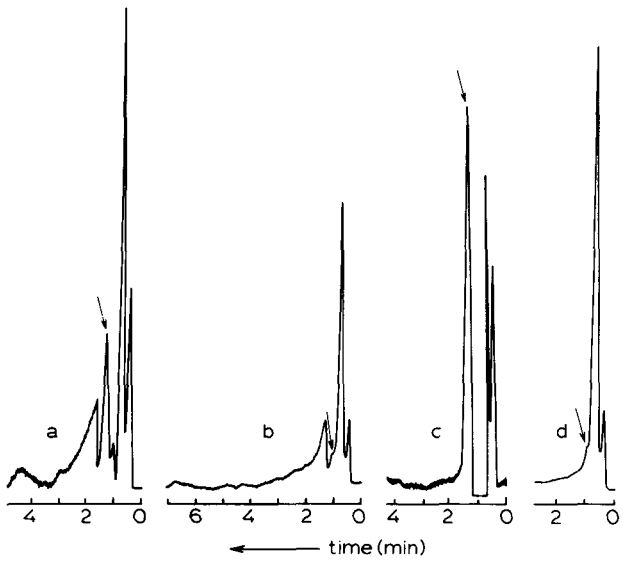

FIGURE 6 Analyses of 4 ml preconcentrated water samples at four different wavelengths. All conditions as in Figure 4 except for HPLC eluent (9:1 methanol-water) UV detection at (a) 220 nm attenuation 0.4 AUFS, (b) 254 nm attenuation 0.4 AUFS, (c) nm attenuation 0.4 AUFS, (d) 280 nm attenuation 0.2 AUFS. Arrow points to the peak of interest (1-Methyl-2,4-diaminobenzene).

Although the quantitation aspect of this technique has not been further explored, there is good evidence that standard addition techniques can be used to quantitate certain compounds, provided that they are reasonably well separated. The enhanced selectivity obtained through proper selection of detection wavelength in UV detection is quite promising.

CONCLUSIONS

On-line pre-concentration of the 20 pollutants investigated (Table I) is essentially possible on PRP_1 sorbents with the exception of phenylenediamine (<1 ml). For methyldiaminobenzene only below 5 ml sample sizes are possible. With on-line transfer of the enriched sample to the separation column no further losses or contamination can occur and the method can be fully automated if required. It should be realized that even with 10 ml samples an enrichment factor of 100 is obtained by this pre-concentration technique.

The use of C_{18} sorbents for the less polar compounds can be complementary to PRP_1 sorbents with the advantage of being compatible with gradient elution techniques. This is not the case for PRP_1 sorbents where swelling of the beads prevents the use of gradient elution; hence compounds have to be classified in groups for isocratic separation. The suitability of HPLC techniques for fingerprinting purpose is rather doubtful. The major strength of the HPLC approach lies in the possibility to selectively determine specific pollutants. This has been demonstrated with the detection of methyldiaminobenzene in effluent water using selective wavelength detection. Obviously definite proofs would have to be given by MS as an on-line or off-line detection device.

Nevertheless this points in the direction of use of diode array UV detectors, such as now commercially available from various companies, for isolation of single compounds or groups of compounds (selective mapping) in complex sample matrices. The use of other possibly more selective sorbents (other than PRP_1 and C_{18}) such as ion exchangers and metal-loaded supports for selective pre-concentration of ionic and (or) complexing pollutants is currently investigated and shows good promise (i.e. pre-concentration of chlorophenols on anion exchanger). Another approach with good possibilities is the coupling of pre-column technology with selective

detectors (see also refs. 14, 6). A recent example was the selective detection of chlorophenols by photolytic dechlorination and fluorescence detection of the non-substituted phenol produced.[14] Reaction detectors such as this show particularly interesting possibilities.[17]

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Appendix

COLUMNS AND COLUMN PACKING PROCEDURE

Several PRP_1 columns were used: home packed 15 cm ($N = 865$) and 10 cm ($N = 1290$) × 0.4 cm I.D. columns, and Hamilton 15 cm × 0.4 cm

I.D. columns ($N = 1120$). A packing procedure slightly modified from the one described by Lee and Kindsvater[12] was used. The columns were packed as follows: The resin (1 g) was slightly moistened with acetone and then some packing solvent was added. When the slurry was good, more packing solvent was added up to a total of 20 ml slurry. The packing solvent was 2.5% sodium chloride and 20% glycerine in water. The slurry was agitated under vacuum in an ultrasonic bath and the column was filled with the slurry. Then the tube was connected to the slurry vessel filled with the rest of the slurry and then with packing solvent. The tube was connected to a Haskel pneumatic pump and the column was packed downward at 5000 psi. After 3 strokes (± 30 min) the column was disconnected from the tube. The plate number of the columns were tested using a 9:1 MeOH:H_2O mobile phase and nitrobenzene as a test compound. With this mobile phase nitrobenzene has a k' value of 3.5 which is sufficiently high to give a reliable value of the plate number.