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Determination of organic pollutants in small samples of groundwaters by liquid–liquid extraction and capillary gas chromatography

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Abstract

A method is presented for the determination of 22 organic compounds in polluted groundwaters. The method includes liquid–liquid extraction of the base/neutral organics from small, alkaline groundwater samples, followed by derivatisation and liquid–liquid extraction of phenolic compounds after neutralisation. The extracts were analysed by capillary gas chromatography. Dual detection by flame ionisation and electron capture was used to reduce analysis time.

1. Introduction

The determination of organic pollutants in aqueous environmental samples is nowadays commonplace and numerous chromatographic methodologies have been established for this purpose, e.g. the US Environmental Protection Agency methods for drinking water (500 series) and municipal and industrial waste water (600 series). Typically, large volumes of water (ca. 1 l) are pre-concentrated by either purge-and-trap [1] or liquid–liquid extraction followed by concentration on a Kuderna–Danish apparatus [2]. Our experiments, into the organic degradative and sorptive processes occurring in different redox zones of the aquifers surrounding two landfill sites, generated only small aqueous samples (10 ml) containing up to 150 ppb (w/w) of individual organic compounds. In these experiments, a cocktail of organic compounds (see Table 1) mixed with the groundwater from a given site was pumped through undisturbed sediment isolated in stainless-steel microcosms [3,4]. Once loaded with the spiked groundwater the microcosms were left in situ and samples were withdrawn from time to time for analysis. The sample volume was constrained to 10 ml so that a reasonable number of samples could be taken from the limited volumes in the microcosms. This in turn meant that, in order to achieve a reasonable degree of pre-concentration, the volume of pentane used for liquid–liquid extraction had to be very small (100 μl) and an ad hoc device was designed to facilitate loading the syringe with the extracted sample.

The technique of rapid liquid–liquid extraction with pentane has already been critically evaluated [5]. In our study the samples withdrawn

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Table 1
Boiling points, extraction efficiencies, water solubilities, log octanol–water partition coefficients, detection limits and relative response factors (R)

No.	Compound	B.p. (°C)	Extraction efficiency (%)	Solubility in water (ppm)	Log K_{ow}	Precision[a] (%)	Detection limit (ppb)	R
1	1,1,1-Trichloroethane	74.1	53	720	2.49	2.5 (ECD)	0.05	1.1[b]
2	Tetrachloromethane	76.5	56	800	2.83	4.5 (ECD)	0.01	4.2[b]
3	Benzene	80.1	30	1780	2.13	6.6 (FID)	1	0.99[c]
4	Trichloroethylene	87.0	58	1100	2.42	5.3 (ECD)	0.03	0.55[b]
–	BTCM (I.S.)	104.7	–	–	–	–	–	1.0[b]
5	Toluene	110.6	49	500	2.73	3.3 (FID)	1	1.0[c]
6	Tetrachloroethylene	121.0	65	150	3.40	1.8 (ECD)	0.015	1.9[b]
7	*o*-Xylene	144.4	53	175	3.12	2.2 (FID)	0.5	1.0[c]
8	1,1,2,2-Tetrachloroethane	146.2	–	2900	2.39	–	–	–
–	Cumene (I.S.)	159.2	–	–	–	–	–	1.0[c]
9	1,4-Dichlorobenzene	174.0	60	79	3.52	4.5 (FID)	1	0.47[c]
10	1,2-Dichlorobenzene	180.5	63	148	3.38	4.0 (FID)	1	0.49[c]
11	Phenol (Ester)	196.0	–	–	–	7.9 (FID)	1	–
12	Nitrobenzene	211.0	10	1900	1.85	4.0 (ECD)	2	0.26[b]
13	*o*-Cresol (ester)	–	–	–	–	4.9 (FID)	2	–
14	Naphthalene	218.0	73	30	3.30	6.1 (FID)	0.5	1.1[c]
–	2-Bromophenol (ester) (I.S.)	–	–	–	–	–	–	–
15	2,6-Dichlorophenol (ester)	–	–	–	–	6.9 (ECD)	1	–
16	2,4-Dichlorophenol (ester)	–	–	–	–	6.1 (ECD)	1	–
17	*o*-Nitrophenol (ester)	–	–	–	–	6.6 (ECD)	0.5	–
18	Biphenyl	255.9	75	7	3.90	2.2 (FID)	0.5	1.1[c]
19	*p*-Nitrophenol (ester)	–	–	–	–	5.3 (ECD)	0.5	–
	Hexadecane (I.S.)	287.0	–	–	–	–	–	–
20	Fluorenone	341.5	72	< 1	3.58	5.8 (ECD)	0.05	–
21	Dibenzothiophene	333.0	102	< 1	4.38	12.7 (FID)	0.5	0.95[c]
22	Pentachlorophenol (ester)	–	–	–	–	6.8 (ECD)	0.05	–
23	Phenanthrene	336.0	100	1	4.46	9.0 (FID)	0.5	1.1[c]

[a]Relative standard deviation ($n = 5$).
[b]Area relative to the area of BTCM (i.e. ECD).
[c]Area relative to the area of cumene (i.e. FID).

from the microcosms were preserved by being immediately made alkaline with sodium hydroxide [2]. In some groundwaters addition of NaOH produced turbid precipitates of metal hydroxides. These were found to interfere with the phase separation and were removed before proceeding. Centrifugation, in preference to filtration, was used to avoid loss of organics by sorption to filter media. Liquid–liquid extraction with pentane was performed on the clarified sample and the extract was analysed by capillary gas chromatography (GC) on a 5% phenylmethylpolysiloxane (DB-5) column. The phenolic constituents of the spiked groundwater were not extracted into the pentane because in aqueous alkaline medium they exist as ionic species (phenolates). Therefore, after GC analysis of the pentane extract, the alkaline aqueous phase was neutralised in order to liberate the phenols which were then selectively derivatised to their acetate esters with acetic anhydride. The esters were extracted into pentane–diethyl ether and determined using the same DB-5 column.

Our aim was to develop a simple and robust methodology that would allow the analysis of a large number of small samples, containing a wide range of organic compounds, in a short time. The method was found to produce reliable

results in terms of precision and detection limits —the technique and the instrumental set up were both simple and total analysis time was relatively short.

2. Experimental

The cocktail was produced by mixing together accurately weighed amounts (ca. 1 g each) of all the compounds in Table 1 except for phenanthrene, fluorenone and dibenzothiophene which, because of their low water solubilities, had their contribution to the mixture reduced to about 0.2 g each. For the liquid–liquid extraction of base/neutrals *n*-pentane, containing 4 ppm of each of the following internal standards; cumene (isopropylbenzene), hexadecane and bromotrichloromethane (BTCM), was used. The derivatisation of the phenols was performed using orthophosphoric acid, borax and acetic anhydride. For the extraction of the esterified phenols *n*-pentane, containing 1 ppm of 2-bromophenol as internal standard, and diethyl ether were used. All chemicals were pure (>99%) and obtained from Aldrich UK except for the pentane and the ether, which were glass-distilled GC grade, and obtained from Rathburns UK.

The determinations were made with a Pye Unicam PU 4400 gas chromatograph equipped with flame ionisation detection (FID) and ^{63}Ni electron-capture detection (ECD) systems, both maintained at 310°C. The volume of extract injected was 3 μl. The injector (250°C) was used in the splitless mode with a vent flow of 55 ml min^{-1} and it was held closed initially for 0.5 min.

A retention gap of deactivated silica tubing (5 m × 0.32 mm I.D.) was fitted before the DB-5.625 (J&W Scientific) capillary column (30 m × 0.32 mm I.D., 0.5 μm film thickness). A deactivated glass effluent splitter divided the column effluent into two streams. One stream was routed through a short length of PTE 5 (Supelco UK) capillary column (10 cm × 0.25 mm I.D., 0.25 μm film thickness) to the FID system and the other was directed through a longer length of deactivated silica tubing (2.5 m × 0.25 mm I.D.) to the ECD system. The helium carrier gas flow-rate was 2.6 ml min^{-1} (oven at 33°C). The make-up gas flow-rate to the FID system was 28 ml min^{-1} (helium) and that to the ECD system was 100 ml min^{-1} (nitrogen).

After an injection the oven was held at 33°C for 0.5 min, then raised at 0.2°C/min to 34°C and then at 15°C/min to 250°C. The chromatographic peaks were integrated using a Nelson 900 A/D interface and Nelson 2600 software (Perkin-Elmer UK). The same chromatographic conditions were used with either type of sample (base/neutral or phenolic esters) and a run was accomplished in 20 min.

Aqueous samples, taken in the field, were dispensed into 11-ml glass-stoppered test tubes (Quickfit MF24/1) each containing 50 μl of 3 *M* NaOH. The tubes were filled completely so that insertion of the stopper eliminated headspace. The samples, stored in ice were dispatched to the laboratory as quickly as possible. There the tubes were centrifuged and 10 ml of each supernatant transferred to a 10-ml graduated flask.

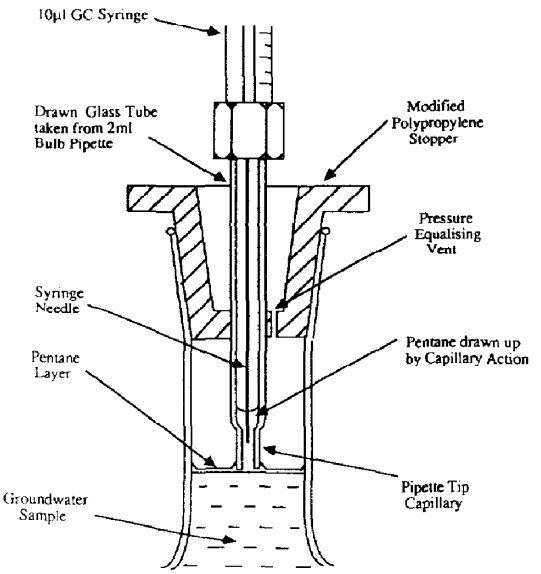

Fig. 1. GC syringe loading device. The glass tube of the device, which is a snug fit in the stopper, is gently lowered until the tip just contacts the thin layer of pentane extract which then rises by capillary action into the tip. The device is so constructed that when the GC syringe is fully inserted the needle's tip is completely immersed in the column of pentane extract.

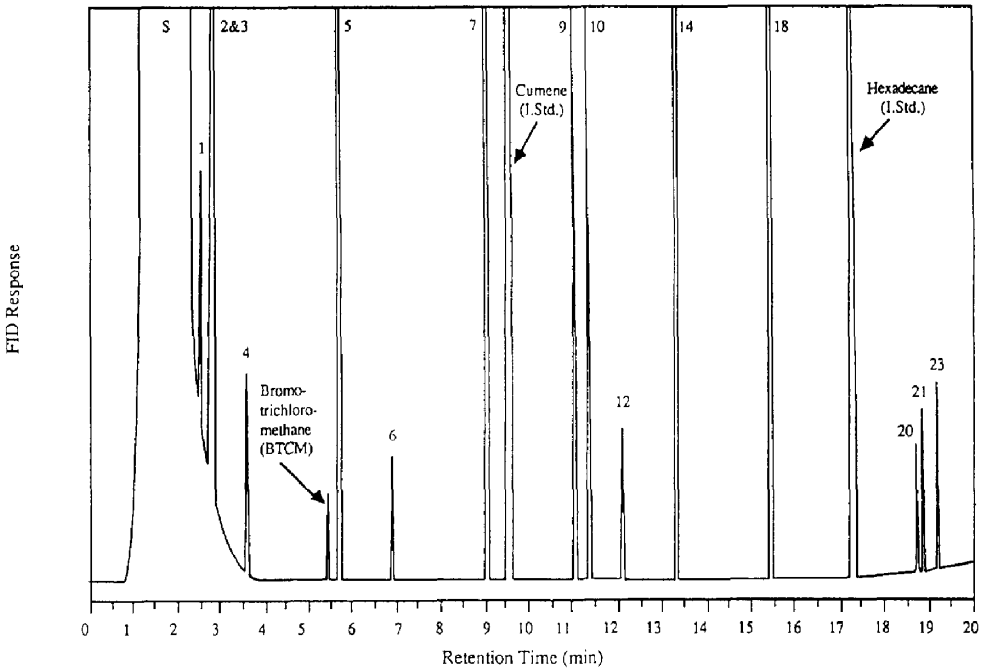

Fig. 2. Chromatogram (FID) of the pentane extract of spiked groundwater [1,1,2,2-tetrachloroethane (8) excluded]. Each component 37.5 ppb except for fluorenone (20), dibenzothiophene (21) and phenanthrene (23); these three each 7.5 ppb. I.Std. = Internal standard.

Using a 250-μl gas tight syringe, 100 μl of pentane containing internal standards was added to each flask. Glass stoppers, with PTFE sleeves, were inserted in the flasks and they were shaken vigorously for 1 min. This time had been found by experiment to be more than sufficient to establish extraction equilibrium. They were then stored bottom up in a refrigerator. To analyse, each sample was placed the right way up and allowed to stand for a few minutes to allow the phases to separate. The glass stopper was removed and replaced by an ad hoc device (Fig. 1) to facilitate loading of the GC syringe (Hamilton 701, 10 μl). A 3-μl volume of pentane extract was taken by syringe and injected into the gas chromatograph.

The remnants of the pentane layer, after the base/neutrals GC sample had been taken, were pipetted from the surface of the aqueous sample and discarded. Then 9 g of the aqueous sample were weighed out into a glass vial, provided with a PTFE-lined screw closure, ready for derivatisation and liquid–liquid extraction of the phenols as their acetate esters [6] which was carried out as follows: the pH was adjusted to 7.0 ± 0.2 with orthophosphoric acid and then 0.2 g borax were added and allowed to dissolve. From gas-tight GC syringes, 400 μl of 1 ppm 2-bromophenol in pentane and 40 μl of acetic anhydride were added. The vial was shaken on a mechanical shaker for 3 min before adding 400 μl of ether and shaking for a further 2 min. The organic layer was allowed to separate and 3 μl, taken by syringe, were injected onto the gas chromatograph.

3. Results and discussion

3.1. Separation

A slow injection technique was necessary with the system because of the small volume of the injection liner (76 mm × 2 mm I.D.). Fast injec-

tion of a 3-μl sample caused problems of solvent flooding and backflashing. Optimum performance was achieved when samples were injected at the rate of 3 μl in 10 s. Typical chromatograms of the pentane extract detected by FID and ECD are shown in Figs. 2 and 3, respectively. Those of the pentane–ether extract which contained the phenolic compounds are shown in Figs. 4 and 5. The peaks are numbered as in Table 1 and are referred to in the text by their numbers in parentheses.

The organic components of the spiked groundwaters eluted roughly in order of their boiling points [7,8] as expected on a relatively non-polar stationary phase like DB-5. Some low-boiling components appeared on the solvent tail (FID) viz. 1,1,1-trichloroethane (1), tetrachloromethane (2) and benzene (3). This did not matter for the two chlorinated aliphatics because they gave clear ECD peaks. For benzene the precision did not seem greatly affected even though its quantitation was complicated by the coelution of tetrachloromethane (a coelution compensation factor was derived by comparing the FID and ECD responses of tetrachloromethane). Cumene and hexadecane were used as internal standards for FID and since peaks for neither appeared in any of the groundwater blanks, variations in their area ratio could be used to warn of possible interference. One of the compounds, 1,1,2,2-tetrachloroethane (8), used initially in the cocktail was later excluded, because it was found to undergo rapid dehydrohalogenation to trichloroethylene (4) in alkaline aqueous medium. Its instability in neutral aqueous media has been noted by other workers [9,10].

3.2. Extraction efficiency

The extraction combines preconcentration with the transfer of the base/neutrals to the pentane phase. The transfer of low concentrations of components to small volumes of solvent has been shown to produce effects not expected from the known partition coefficients [5] which

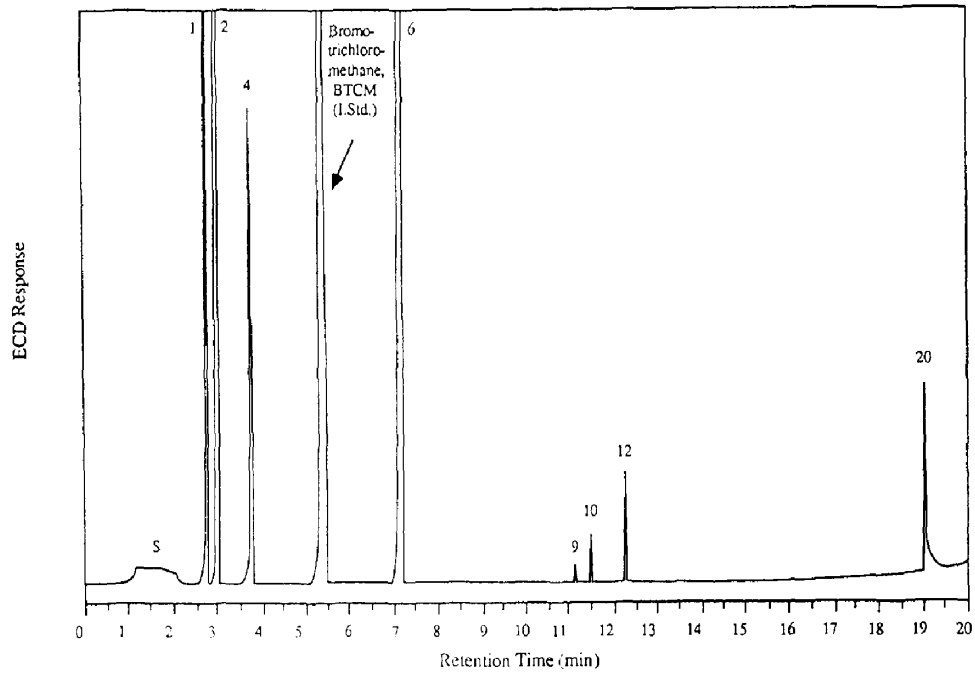

Fig. 3. Chromatogram (ECD) of the pentane extract of spiked groundwater [1,1,2,2-tetrachloroethane (8) excluded]. Each component 37.5 ppb except for fluorenone (20), dibenzothiophene (21) and phenanthrene (23); these three each 7.5 ppb.

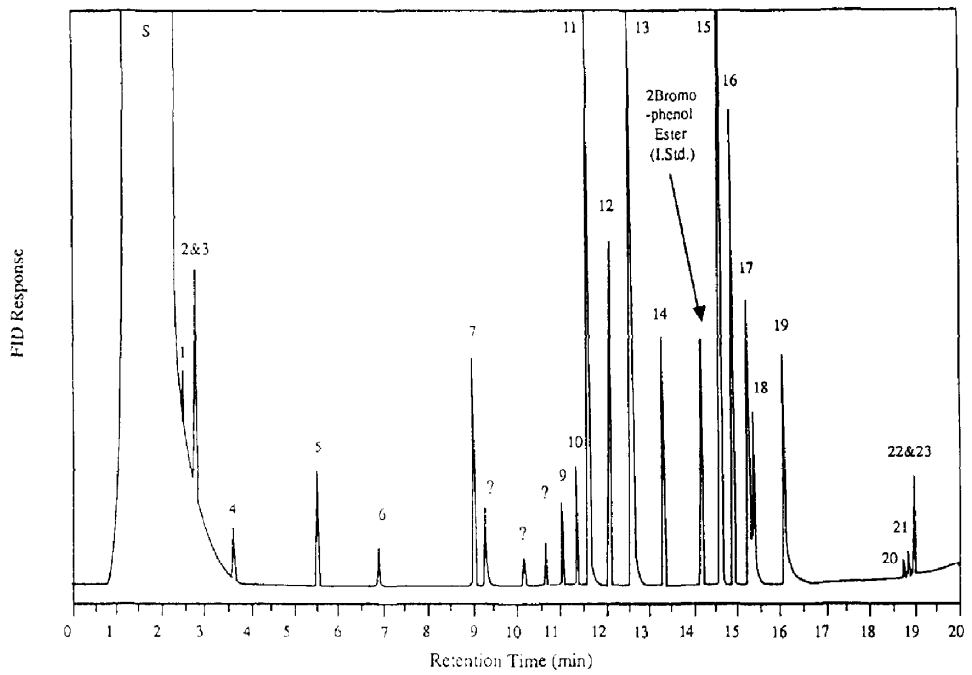

Fig. 4. Chromatogram (FID) of the pentane–ether extract of spiked groundwater [1,1,2,2-tetrachloroethane (8) excluded] following neutralisation and esterification of the aqueous phase remaining after initial pentane extraction. Each phenol 150 ppb. Peaks labelled "?" attributed to impurities and reaction products from the esterification. Note peaks due to re-extraction of base/neutrals remaining after pentane extraction.

influence the degree of preconcentration. Nevertheless, to some extent the observed extraction efficiencies correlate with the water solubility and K_{ow}, the octanol–water partition coefficient [11] (see Table 1). The calculation of extraction efficiency, which was based on comparing the response factors of the pure components in pentane with those obtained from extracted aqueous solutions, was not extended to the phenolic esters as only a few of the requisite acetates are available commercially. The remainder would require laboratory synthesis and purification [12] in order to obtain their extraction efficiencies.

3.3. Precision and detection limits

The precision (relative standard deviation, R.S.D.) was determined by analysing a spiked groundwater sample five times and was found to range from 1.8 to 12.7%. The values for each compound are shown in Table 1. Calibration curves, for compounds determined by FID, were all linear with correlation coefficients of 0.996 to 1.000. Calibration curves, for compounds determined by ECD were all non-linear.

The detection limits defined refer to the minimum concentrations of compounds in spiked groundwater that give clearly defined peaks (signal-to-noise ratio ca. 4). Lower levels could have been achieved by ECD by increasing the detector current level on the electron-capture amplifier. This, however, would have led to even greater calibration non-linearity, as a result of increased detector overload at high concentrations. The ECD sensitivity was, therefore, set at a compromise value appropriate to the range of concentrations of compounds encountered in the degradation experiments.

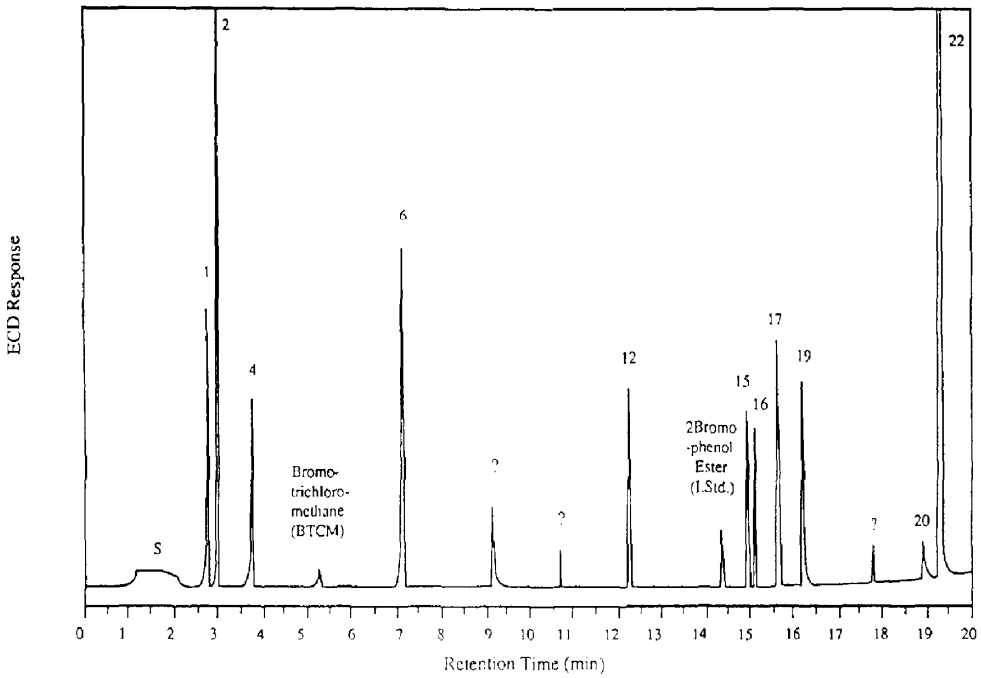

Fig. 5. Chromatogram (ECD) of the pentane–ether extract of spiked groundwater [1,1,2,2-tetrachloroethane (8) excluded] following neutralisation and esterification of the aqueous phase remaining after initial pentane extraction. Each phenol 150 ppb. Peaks labelled "?" attributed to impurities and reaction products from the esterification. Note peaks due to re-extraction of base/neutrals remaining after pentane extraction.

3.4. Sample stability studies and the effect of the precipitate

Experiments (using 50-ml samples and 500 μl pentane) showed that if the clarified samples were stored inverted and refrigerated after shaking with pentane they could be regarded as relatively stable. Thus plots of concentration against time over a 105-day period were smooth showing a slight downward trend corresponding to some loss of analytes (typically ca. 3%). There were, however, much greater variations in the response factors for the components of the pentane extract if the hydroxide precipitate was not removed. Possibly, random fluctuations in the amount of precipitate at the interface caused the poor reproducibility. The values obtained for the clarified sample were slightly lower than for samples containing precipitate, indicating some sorption to the precipitate, but the effect was reproducible and was included in the calculation of extraction efficiency.

Conversely, the esters of the phenols were found to be unstable in their aqueous ether–pentane matrix. Once derivatisation/extraction had been performed the ester sample needed to be analysed as soon as possible. Even when stored in a refrigerator there was a significant loss of analytes after only 12 h. However, storage of the alkaline groundwater (containing the unesterified phenols in their ionic form) for 14 days in a refrigerator resulted in no discernible loss of analytes. Accordingly, derivatisation, extraction and analysis were always carried out on the same day.

3.5. Application

The method described was formulated specifically for the analysis of organic compounds

containing a wide range of functional groups. It was designed to operate with small volumes of aqueous sample and the determination did not require sophisticated apparatus or instrumentation. A simple base/neutrals liquid–liquid extraction was first performed on spiked alkaline groundwater followed by derivatisation/extraction of the remaining aqueous phase to determine phenols. The method, successfully employed to analyse over 2000 samples, has proved to be reliable and should, in principle, be applicable to most situations where only small volumes of sample are available. Analysis time is short permitting a relatively high throughput of samples. Storage of the pentane extracts, inverted in a refrigerator, is permissible for short periods. The alkaline aqueous phase remaining, after discarding the pentane extract, may be stored similarly. Once derivatised however, the ester samples require prompt analysis.

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