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Sample cleanup and reversed-phase high-performance liquid chromatographic analysis of polar aromatic compounds in groundwater samples from a former gas plant

M.B. Müller, C. Zwiener, F.H. Frimmel*

Division of Water Chemistry, Engler-Bunte-Institut, Universität Karlsruhe, Engler-Bunte-Ring 1, 76131 Karlsruhe, Germany

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Abstract

A method for the analysis of the polar aromatic compounds 1*H*-quinolin-4-one (Q), 10*H*-acridin-9-one (A), 5*H*-phenanthridin-6-one (P) and 9*H*-fluoren-9-one (F) in aqueous solutions has been developed. The method comprises steps for sample preparation (solid-phase extraction, cleanup) and analytical determination by means of reversed-phase high-performance liquid chromatography (RP-HPLC). For the cleanup step the suitability of two different sorbents (alternative A: silica gel, alternative B: LiChrolut EN) was investigated. Alternative B depicted several advantages, in particular higher sorbent capacity, faster and less complicated handling, higher recovery and better reproducibility. For Q, A and P, reproducibility of all method steps is better than 13%, with recovery rates ranging from 76% to 105% ($n=3$). Alternative B was applied to groundwater samples from a former gas plant. The analytes A and P could be detected at concentrations in the μg/l range.

Keywords: Water analysis; Environmental analysis; Sample preparation; Quinolinone; Acridinone; Phenanthridinone; Fluorenone

1. Introduction

Various aromatic and heterocyclic compounds and their metabolites are typical contaminants in groundwaters of former gas plant sites [1–4]. In this work the three heterocyclic substances 1*H*-quinolin-4-one (Q), 10*H*-acridin-9-one (A), 5*H*-phenanthridin-6-one (P) and the aromatic compound 9*H*-fluoren-9-one (F) were of particular interest because of their relatively polar character compared to polycyclic aromatic compounds, and also because of their toxicity. Acridinone and phenanthridinone are reported as mutagenic and fluorenone as mutagenic and tumorigenic substances in a toxicological data base [5].

The aim of this work was to develop a method for the analysis of Q, A, P and F in aqueous samples with a very high degree of organic contamination [polycyclic aromatic hydrocarbons (PAHs) and other aromatic compounds]. A combination of steps for sample enrichment using solid-phase extraction

*Corresponding author. Tel.: +49-721-608-2580; fax: +49-721-699-154.

E-mail address: fritz.frimmel@ciw.uni-karlsruhe.de (F.H. Frimmel)

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(SPE) on the relatively nonpolar sorbent LiChrolut EN, cleanup and RP-HPLC–diode array detection (DAD) analysis was to be used. Two alternative cleanup sorbents, silica gel and LiChrolut EN were compared. Silica gel has been used as sorbent for a range of nonpolar organic contaminants like PAHs, polychlorinated biphenyls (PCBs), and organochlorine pesticides [6–10]. Several standardized methods with silica gel cleanup for the analysis of PAHs, PCBs and organochlorine insecticides exist [11–13]. Opposed to this, references for silica-gel cleanup of more polar compounds are scarce. Cleanup of triazines and PCBs has been reported in [14].

In this work recovery rates and RSDs for model solutions are reported for the individual method steps. The cleanup procedure was introduced to be able to make use of the widespread UV detection. The method can thus be applied using the standard equipment present in many laboratories and does not require specialized and sophisticated detection systems. The applicability of alternative B was tested using groundwater samples from a former gas plant which have a highly-concentrated organic matrix and are therefore not amenable to RP-HPLC analysis without sample enrichment and cleanup.

2. Experimental

2.1. Chemicals

All chemicals used for this work were of analytical or HPLC grade. The 1*H*-quinolin-4-one standard was purchased from Fluka (Neu-Ulm, Germany), all other analyte standards were obtained from Aldrich (Steinheim, Germany). The solvents methanol, acetonitrile and dichloromethane were from J.T. Baker (Griesheim, Germany); *n*-hexane and ethyl acetate from Merck (Darmstadt, Germany). For SPE, commercially available LiChrolut EN cartridges from Merck were used. Silica gel 40 for column chromatography (particle size 0.063 mm to 0.2 mm) was purchased from Fluka. Sodium sulfate was obtained from Merck. Ultrapure water (Milli-Q water) was produced in a Millipore system (Eschborn, Germany).

2.2. Method development

2.2.1. Sample preparation: solid-phase extraction and cleanup

For SPE, a J.T. Baker SPE system with Merck LiChrolut EN cartridges (sorbent mass 200 mg) was used. Aqueous model solutions were prepared by dilution of the appropriate standard solution of the analytes in methanol–Milli-Q water (50:50, v/v) (β=0.02 mg/l to 20 mg/l) with drinking water from the city of Karlsruhe (pH 7.30, $\beta(Ca^{2+})$=120 mg/l, $\beta(Na^{+})$=10.8 mg/l, $\beta(Mg^{2+})$=10.2 mg/l, $\beta(SO_4^{2-})$=66 mg/l, $\beta(Cl^{-})$=24 mg/l, chemical analysis in [15]). Preconditioning of the sorbent was done with one column volume (3 ml) methanol, followed by subsequent washing with approximately two column volumes Milli-Q water. 500 ml of the model solution were sucked through the preconditioned cartridge at a flow rate of 5 ml/min to 10 ml/min, then the sorbent was dried for 30 min in a nitrogen stream. Further treatment depended on the type of cleanup following the SPE step. In case of cleanup alternative A (silica gel column) the adsorbed compounds were eluted with five 1 ml portions of methanol–acetonitrile (60:40, v/v). The extract was evaporated to dryness under a nitrogen stream, then redissolved in 0.5 ml hexane–ethyl acetate (80:20, v/v) and transferred to the silica gel column. For alternative B (LiChrolut EN) sequential elution or cleanup of the analytes was done directly from the SPE column according to the elution scheme given in Table 2.

Silica gel columns for cleanup alternative A were packed directly before each fractionation experiment. A Pasteur pipette (length 150 mm×6 mm I.D.) was closed with a plug of glass wool and then filled with approximately 1 g of silica gel (deactivated with 3% water) suspended in *n*-hexane. The sorbent was covered by a 4 mm layer of dry Na_2SO_4. After settling, the column material was washed with about 15 ml hexane and then the sample extract was applied onto the top of the sodium sulfate layer without letting the sorbent get dry. Sequential elution was done according to Table 2.

The fractions obtained after sequential elution of the silica gel and LiChrolut EN column, respectively, were evaporated to dryness and redissolved in 0.5 ml

methanol+0.5 ml Milli-Q water. Thus the final sample volume was 1 ml. The concentrated and cleaned sample extracts were analyzed by RP-HPLC.

2.2.2. RP-HPLC analysis

A Hewlett-Packard 1100 system with HP Chem Station, a binary system gradient and a diode array detector were used for the HPLC analysis. The reversed-phase column was a Merck LiChroCart Purospher C_{18}-column (250 mm×4 mm diameter, 5 μm particle size). The linear gradient of acetonitrile (MeCN) and HPLC water (Milli-Q water) was as follows: at 0 min 20% MeCN, at 4 min 20% MeCN, at 30 min 50% MeCN, at 40 min 90% MeCN, at 45 min 100% MeCN. The flow rate was 1 ml/min; the injection volume was 100 μl by autosampler. Detection wavelengths were 230 nm for Q and P, and 250 nm for A and F, respectively. The compounds were identified according to peak retention times and UV spectra. Retention times for Q, A, P and F were 3.67 min, 17.84 min, 23.40 min and 35.91 min. The UV spectra of the analytes are shown in Fig. 1. All four compounds have very characteristic UV spectra and can be clearly distinguished from other aromatic substances if no coelution occurs.

Peak quantification was done by external calibration with standard solutions. For each compound, two calibration curves ranging from 0.02 mg/l to 1 mg/l, and 1 mg/l to 20 mg/l, were used. Correlation coefficients (R^2) were better than 0.998 for all analytes. Detection limits were calculated according to the method in [16]. For all four compounds the detection limit was 0.01 mg/l. Reproducibility of the HPLC measurements was better than 1.6% ($n=3$).

2.3. Groundwater samples

Sample collection has been described in detail elsewhere [17,18]. The samples were first filtered through 0.45 μm cellulose nitrate filters from Sartorius (Goettingen, Germany) before applying the appropriate volume (100 ml, 200 ml, or 500 ml of the original sample) onto the SPE column. All groundwater samples were treated according to cleanup alternative B and analyzed by RP-HPLC with gradient 2. General parameters (pH value, electrical conductivity and oxidation-reduction potential), as well as naphthalene and dissolved organic carbon (DOC) concentrations, were determined in routine laboratory analysis. The DOC measurements were performed with 10 ml of the filtered sample which were acidified with 0.1 ml concentrated phosphoric acid and purged with nitrogen prior to analysis with a Dohrmann Carbon Analyzer DC 80 (Schmidlin, Mutlangen, Germany).

3. Results and discussion

3.1. Solid-phase extraction (SPE)

In preliminary experiments several organic solvents were tested for completeness and reproducibility of elution from the LiChrolut EN cartridge. A defined mixture of methanol–acetonitrile (60:40, v/v) proved to be most efficient. The elution volume was 5 ml. Table 1 gives the average recovery rates (RRs) and relative standard deviations (RSDs) of analyte model solutions of four different concentration levels.

Recovery rates ranged from 91% to 103%, and RSDs were in all but one cases (A at concentration level 0.5 μg/l) better than 4%, which indicates a very good reproducibility of the extraction step. Average RRs and RSDs over all four levels were better than 93% and 4.3%, respectively. From the very high recoveries and the low RSDs, it can be concluded that with the LiChrolut EN sorbent the polar aromatic analytes can be extracted quantitatively and in a reproducible way from aqueous solutions.

3.2. Cleanup

For elution of the analytes from the cleanup columns a number of organic solvents was tested in preliminary experiments. With *n*-hexane and dichloromethane only up to 25% of the mass adsorbed onto the silica gel column could be eluted. Mixtures of the non-chlorinated solvents hexane, ethyl acetate and methanol of defined volume ratios, and hence defined polarity, were finally used in both cleanup alternatives. Table 2 contains the different solvents used for sequential elution of the analytes from the two cleanup columns.

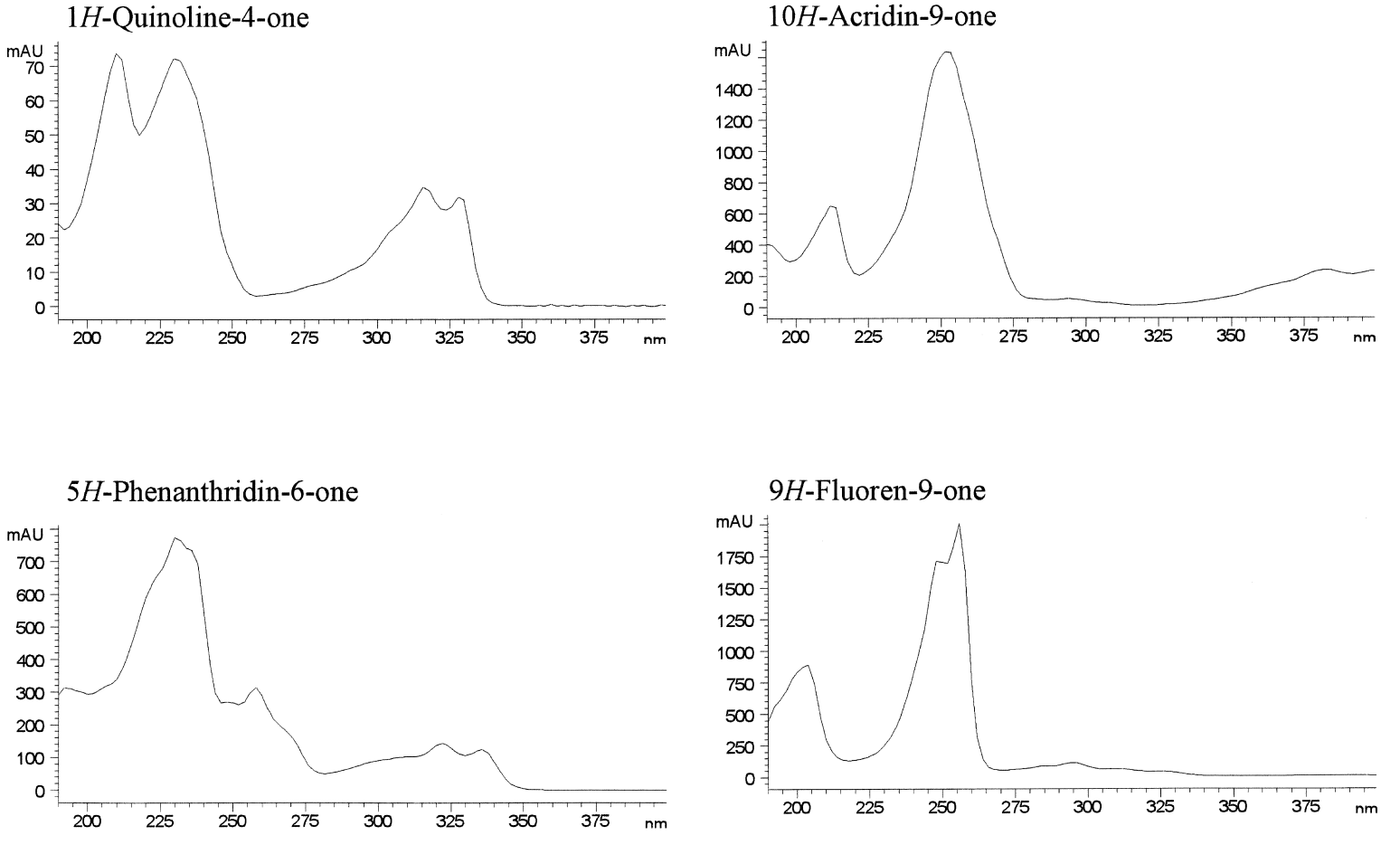

Fig. 1. UV spectra of 1*H*-quinolin-4-one (Q), 10*H*-acridin-9-one (A), 5*H*-phenanthridin-6-one (P) and 9*H*-fluoren-9-one (F).

Table 1
Recovery rates RRs and RSDs for SPE on LiChrolut EN (sorbent mass 200 mg) of four concentration levels (0.5 μg/l to 10 μg/l per compound, pH 7, concentration factor=500, 3 replicates per level)

Compound	β=0.5 μg/l		β=1 μg/l		β=5 μg/l		β=10 μg/l		Average	
	RR (%)	RSD (%)	RR (%)	RSD (%)	RR (%)	RSD (%)	RR (%)	RSD (%)	RR (%)	RSD (%)
Q	94	1.6	94	0.7	97	0.4	97	1.2	96	1.0
A	103	11.8	95	0.4	100	2.7	98	2.1	99	4.3
P	92	3.8	91	0.6	96	2.1	94	1.1	93	1.9
F	96	2.2	97	1.0	96	0.8	94	1.0	96	1.3

Table 2
Solvents used for sequential elution of Q, A, P and F from the cleanup columns A (silica gel) and B (LiChrolut EN); elution volume 5 ml per fraction

Fraction	Solvents
1	*n*-Hexane–ethyl acetate (A: 50:50, v/v; B: 80:20, v/v)
2	*n*-Hexane–ethyl acetate (50:50, v/v)
3	*n*-Hexane–ethyl acetate (50:50, v/v)
4	Ethyl acetate
5	Ethyl acetate–methanol (80:20, v/v)
6	Ethyl acetate–methanol (50:50, v/v)

3.2.1. Alternative A: silica gel column

Fig. 2 shows the fractionation of 0.5 ml of a model solution (analyte concentration β=1 mg/l, pH 7) after sequential elution from the silica gel column (sorbent mass 1 g).

Total RRs (sum of recovery rates of all six fractions) were>91% for all substances, with RSDs ranging from 2.6% (Q) to 39.6% (F). The very high RSD for fluorenone shows that reproducible elution of this compound is critical. Generally, it can be stated that fractionation using silica gel is feasible, however, the need to transfer the sample extract from the SPE onto the cleanup column results in an unwanted loss of the target compounds due to solubility problems, thereby reducing the efficiency of this alternative.

3.2.2. Alternative B: LiChrolut EN column

In alternative B, SPE and cleanup were performed with the same column. Sequential elution from the LiChrolut EN cartridge (sorbent mass 200 mg) was done according to Table 2. Since the groundwater samples have a very high degree of matrix contami-

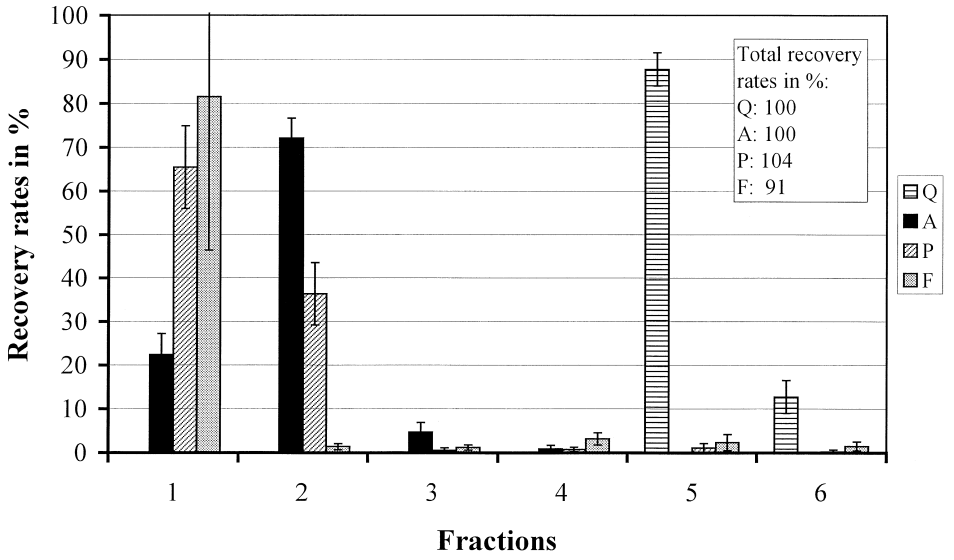

Fig. 2. Fractionation of analytes on silica gel column (analyte concentration β=1 mg/l, sorbent mass 1 g, pH 7). Error bars correspond to the 95% confidence interval (*n*=3).

Table 3
Total recovery rates (RRs) and RSDs for three different concentration levels for cleanup on LiChrolut EN column (pH 7, sorbent mass 200 mg, concentration factor=500, $n=3$)

Compound	β=4 μg/l		β=20 μg/l		β=40 μg/l		Average	
	RR (%)	RSD (%)	RR (%)	RSD (%)	RR (%)	RSD (%)	RR (%)	RSD (%)
Q	76	4.7	93	1.9	97	2.0	89	2.9
A	93	1.1	102	2.3	105	1.2	100	1.5
P	99	2.4	104	1.0	101	4.9	101	2.8
F	105	6.6	67	24.9	96	3.9	89	11.8

nation (various aromatic and heterocyclic substances), six fractions were required to fractionate not only the compounds of interest, but also the matrix components. This did not reduce the sensitivity of the method as the analytes were distributed over two fractions at the most. Three different concentration levels were investigated using this cleanup alternative. Table 3 gives the total RRs and RSDs for 4 μg/l, 20 μg/l and 40 μg/l (concentration of each analyte in an aqueous model solution, pH 7).

Except for one case, RRs were greater than 76% and RSDs better than 7%. Average recovery rates over all three concentration levels are also given in Table 3. Average RRs are better than 89%, with average RSDs below 2.9% except for fluorenone. Cleanup with the LiChrolut EN column hence was more reproducible than the silica gel cleanup (lower RSDs). The fractionation of fluorenone, however, also showed considerable variations (average RSD= 11.8%).

The fractionation of a model solution on the LiChrolut EN column at the concentration level β= 40 μg/l is shown in Fig. 3. It can be seen that the elution order of the analytes is similar to that from the silica gel column even though the sorbents differ in composition and polarity.

An important advantage of alternative B is the fact that extraction and cleanup can be performed with the same column. Transfer of sample extracts and solvent exchange consequently are not necessary, and losses of the analytes due to insufficient solu-

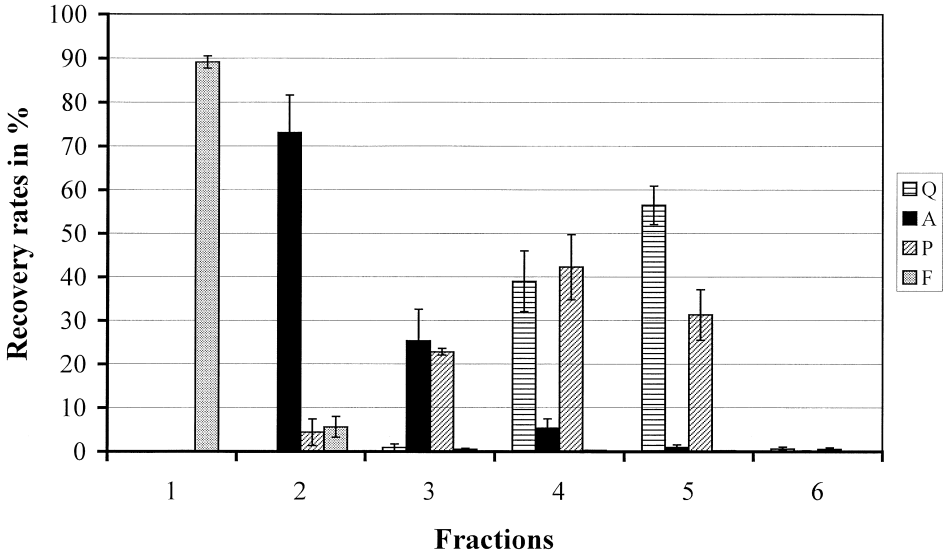

Fig. 3. Fractionation of analytes on LiChrolut EN column (analyte concentration β=40 μg/l, sorbent mass 200 mg, pH 7). Error bars correspond to the 95% confidence interval ($n=3$).

bility can be avoided. The achievable enrichment factor is only limited by the sorption capacity of the sorbent. For LiChrolut EN it is very high and amounts to 500 mg caffeine or diisodecylphthalate per 200 mg sorbent [19]. The single-step extraction and cleanup also allows a faster and more reproducible pretreatment procedure since pre-packed cartridges with a defined sorbent mass and activity can be used. Therefore, all groundwater samples were treated according to alternative B. Fig. 4 shows the HPLC chromatograms of a groundwater sample (B51/6) without cleanup, and three fractions obtained after cleanup of the same sample with LiChrolut EN (concentration factor was 100 in all cases, only chromatograms detected at λ=230 nm are shown).

In fraction 3, acridinone and phenanthridinone could be detected at retention times of 17.22 min and 22.88 min. The peak of A was better detectable at λ=250 nm (chromatogram not shown). Peak height was about 4-fold higher at 250 nm than at 230 nm. Identification of fluorenone was not unequivocally possible for the groundwater samples. UV spectra indicated coelution with another nonpolar aromatic substance. The particular 4-hydroxy isomer of Q could not be found in the sample.

3.3. Groundwater samples

The general parameters temperature, pH value, electrical conductivity and oxidation-reduction potential (ORP) were measured directly on the sampling site. The ORP was used as an indicator for the degree of contamination of the individual samples. ORP values ranged from −330 mV (B44/2) to +150 mV (B41). Groundwater samples with negative ORP values were assumed to be highly contaminated, and therefore a smaller sample volume (100 ml or 200 ml) was pre-concentrated and extracted. A sample volume of 500 ml for samples with a low level of contamination (greater ORP values) was used.

Alternative B was well applicable for the compounds A and P. Fig. 5 illustrates the concentration profiles for the two substances, naphthalene, and for the DOC values in the groundwater samples from the contaminated site.

Concentrations of A and P decrease from south to north and indicate the presence of a contamination

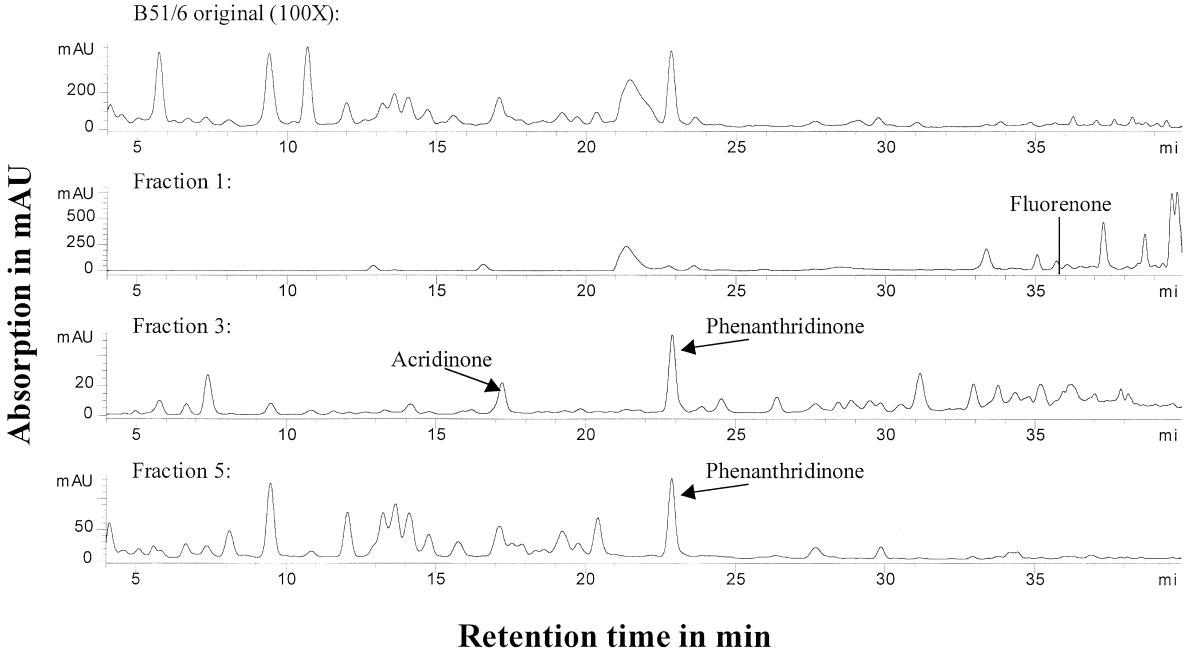

Fig. 4. RP-HPLC chromatogram of original B51/6 sample and fractions 1, 3 and 5 after LiChrolut EN cleanup (concentration factor was 100, only λ=230 nm detection shown).

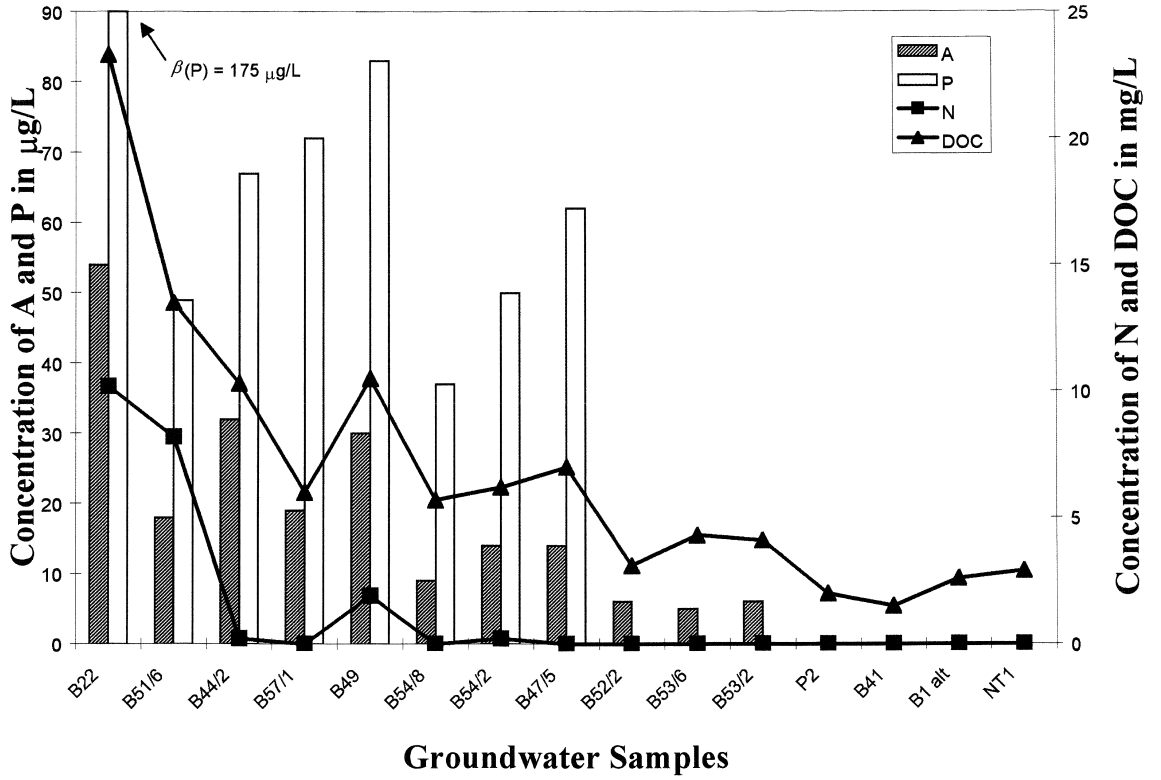

Fig. 5. Contaminant concentrations of acridinone, phenanthridinone, naphthalene and DOC in groundwater samples from a former gas plant (sample order corresponds to well location from south to north, 1 replicate per well).

plume. Acridinone could be detected at concentrations ranging from 5 µg/l to 54 µg/l, and P at concentrations ranging from 37 µg/l to 175 µg/l, respectively. The sampling site B22 seems to be situated next to a contamination source like the high concentrations of A and P, namely 54 µg/l and 175 µg/l reveal. Comparison with the concentrations of the typical PAH contaminant naphthalene (β=10.2 mg/l) and the general parameter DOC (β=23.3 mg/l) support this assumption. In earlier investigations the parameter DOC was found to be a good indicator of the degree of contamination of the investigated site. DOC values above a background level of 3 mg/l to 4 mg/l are mostly due to the presence of anthropogenic organic compounds [17]. In samples with a DOC below 3 mg/l, no analytes could be detected. The compounds quinolinone and fluorenone could not be found in any of the groundwater samples. The particular 4-hydroxy isomer of Q probably was not present in the samples, and other isomers could not be identified due to the lack of an appropriate standard. Fluorenone appears together with other nonpolar aromatic hydrocarbons in the nonpolar fraction 1 of the cleanup step. With HPLC, a baseline separation of all peaks could not be achieved for this fraction and therefore peak identification on the basis of the UV absorption spectra was not possible due to coelution.

4. Conclusions

Sample preparation according to alternative B including SPE and cleanup with the polymer sorbent LiChrolut EN (alternative B) and RP-HPLC analysis are well applicable for aqueous model solutions of the polar aromatic compounds 1*H*-quinolin-4-one, 10*H*-acridin-9-one, 5*H*-phenanthridin-6-one and 9*H*-fluoren-9-one. Both pre-concentration by SPE and fractionation (cleanup) can be performed in a quan-

titative and reproducible way. Advantages of cleanup alternative B are fast single-step extraction, preconcentration and cleanup of samples with only one column and the use of pre-packed cartridges with a high and well-defined sorption capacity. Opposed to this, cleanup alternative A with self-made silica gel columns is more time-consuming, and sample preconcentration is limited due to the need of a transfer step from the SPE to the cleanup column. Performance of the cleanup step before RP-HPLC separation allows detection of the compounds of interest using a UV diode array detector, that is, a common detection system available in most laboratories. Successful application of alternative B for the analytes acridinone and phenanthridinone in groundwater samples from the contaminated area of a former gas plant site was shown in this paper. The 4-hydroxy isomer of quinolinone could not be found in the samples, however, the presence of other isomers can not be excluded. Fluorenone as the least polar analyte could not be separated from other polycyclic aromatic contaminants in the groundwater samples even though fractionation of the analytes in model solutions was well feasible with cleanup alternative B. For this compound, a different analytical method is needed, and GC–MS analysis is currently being tested.

Acknowledgements

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