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Journal of Chromatography A, 1065 (2005) 211–218
JOURNAL OF CHROMATOGRAPHY A
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Simultaneous determination of NSO-heterocycles, homocycles and their metabolites in groundwater of tar oil contaminated sites using LC with diode array UV and fluorescence detection

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Received 30 July 2004; received in revised form 17 December 2004; accepted 31 December 2004
Available online 26 January 2005

Abstract

For monitoring groundwater at tar oil contaminated sites a simple method of analysis was developed for the simultaneous detection of several NSO heterocyclic compounds, homocyclic compounds, mobile two- and three-cyclic PAHs and selected metabolites. The groundwater samples are enriched using SPE with polymer material at pH 4. Chromatographic separation and detection is performed by LC with diode array UV or fluorescence detection. The recoveries of 25 selected compounds were mostly between 80–110% and the detection limits were 0.4–2.4 μg/L for UV detection and for the fluorescence detectable compounds 0.4–140 ng/L. The method was successfully applied to groundwater samples from a wood preserving facility. Especially benzo(*b*)thiophene showed an increasing dominance downgradient of the source. Detection of metabolites, such as 1-hydroxyiso-, 2-hydroxyquinoline and 2-hydroxy-4-methylquinoline, 2-naphthoic acid, and 1-indanone, indicating in situ biodegradation, was confirmed by LC-ESI–MS analysis.

Keywords: NSO heterocyclic compounds; LC–DAD; LC–MS; Groundwater; Tar oil

1. Introduction

Tar oil and creosote are residuals from various chemical processes, mainly the gasification of coal for the production of coal gas (the so called “Leuchtgas”), or city gas. In Germany, more than 1000 former gasification plants are known [1]. Because of leakage and spillage, the subsurface and groundwater at these former gas plants, coke manufacturing and wood preserving facilities is often polluted with tar oil. This liquid consists of BTEX, PAHs and also up to 15% of more polar N-, S-, O-heterocyclic aromatic compounds, such as quinoline, benzo(*b*)thiophene and benzofuran, as well as the homocycles indane and indene (Fig. 1, [3–5]). Several NSO-heterocycles show significant ecotoxic effects. Furan, benzofuran, pyridine, quinoline, acridine and carbazole are genotoxic, mutagenic or carcinogenic [6–8]. Some biological transformation products, for instance benzothiophene-sulphoxide and hydroxy-quinoline, metabolites of benzothiophenes and quinoline, are also known to be mutagenic or at least genotoxic [9,10]. In addition, the di- and tricyclic heterocycles sometimes show high acute toxicity with LC_{50} values in the lower mg/L range [11,12].

At some sites in Denmark [3], Canada [13], the USA [14] and Germany [5,15], the occurrence of NSO-heterocycles was investigated, and mostly an increasing dominance downgradient of the source was observed. This can be explained by their high mobility caused by their high water solubility and low K_{ow} coefficients [15], and persistence against biological degradation. Under aerobic conditions, most compounds seem to be biodegradable [4,16]. However, most contaminated aquifers are anaerobic, and under these conditions varying results are described. Dyreborg et al. [17] observed an almost complete persistence of several compounds, such as thiophene, benzothiophene, benzofuran and dibenzofu-

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doi:10.1016/j.chroma.2004.12.098

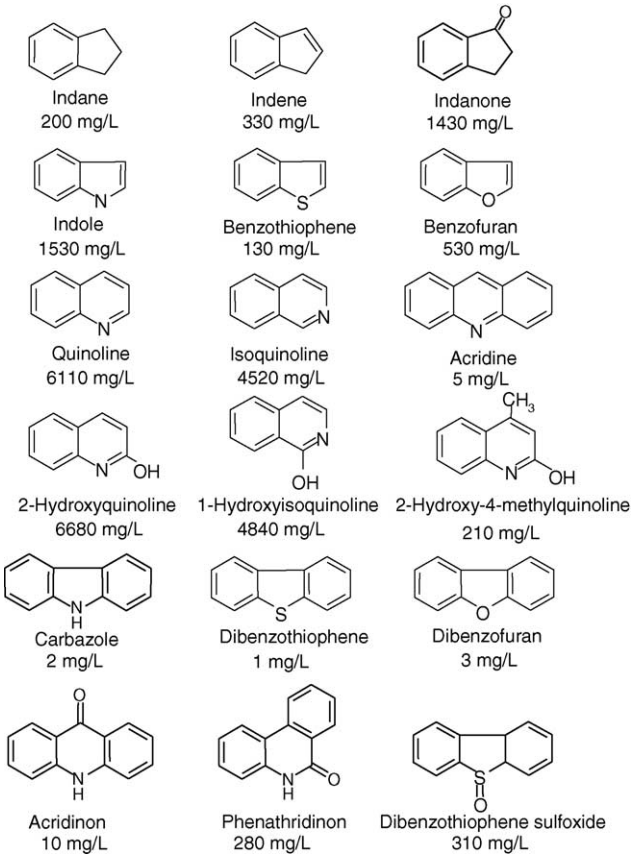

Fig. 1. Selected tar oil compounds with water solubility calculated by use of K_{ow} (EPIWIN [2]).

ran in denitrifying and sulphate-reducing laboratory studies, whereas other research groups described degradation of a few compounds, such as quinoline under methanogenic conditions [18], and indole and quinoline under nitrate- and sulphate-reducing, and methanogenic conditions [19–21]. For thiophene and benzo(*b*)thiophene only a cometabolic transformation has been reported under aerobic and anaerobic conditions [22–25]. In some laboratory experiments, and also at some sites, an enrichment of metabolites was reported. The quinoline and isoquinoline metabolites 2-hydroxyquinoline and 1-hydroxyisoquinoline were mostly observed in the plumes [3,14]. At other sites acridinone, phenanthridinone and carboxythiophenes were detected in groundwater samples [25–27].

In spite of their adverse effects, their mobility and persistence unlike PAHs and BTEX, NSO-heterocycles are not yet included in standard investigation programs. Several methods for groundwater monitoring of these NSO compounds have been previous described. The NSO-heterocycles were mostly analysed by GC–FID or GC–MS after liquid–liquid extraction [5,16] or solid phase micro-extraction [28,29]. By contrast, the metabolites were measured by LC with diode array or mass selective detection after solid phase extraction [26,27,30,31]. Meyer et al. [32] described a simultaneous determination of PAHs, hetero-PAHs and their degradation products in creosote-contaminated soils. Due to soil matrix and high contamination level of soil samples, they fractionated the different groups of contaminants using SPE with silica gel, basic anionic and acidic cation exchange material and quantified subsequently by GC–FID or LC–DAD UV. In contrast, in groundwater samples a lower contamination level is expected and hydrophobic compounds, such as higher molecular weight PAHs are not so relevant.

The aim of our study was to develop a simple method for the simultaneous determination of the parent NSO-heterocycles, the homocycles, as well as mobile two- and three-cyclic PAHs and known metabolites in groundwater samples. We selected approximately 25 typical tar oil compounds and some known metabolites, taking into account our previous screening of contaminated groundwater samples [5] and other investigations at gas plant sites [27]. After developing a suitable enrichment and analysis procedure, the method was applied to the investigation of groundwater samples from the contamination plume at a tar oil contaminated site.

2. Material and methods

2.1. Chemicals

1-Indanone, 2-naphthoic acid, 2-hydroxyquinoline, 1-hydroxyisoquinoline, 2-hydroxy-4-methylquinoline, 2-thiophenecarboxaldehyde, acenaphthene, acridinone, benzofuran, carbazole, dibenzothiophene, dibenzothiophene sulfoxide, fluorene, 9-hydroxyfluorene, indane, indene, trans-2-bromo-1-indanole, 5-bromo-1-indanone were purchased from Sigma-Aldrich (Taufkirchen, Germany). 2-Naphthalenol, acridine, benzo(*b*)thiophene, dibenzofuran, naphthalene, quinoline were purchased from Merck (Darmstadt, Germany). All commercially available substances were in 98% purity or higher. 1,1′-binaphthalene was self synthesized. 2-Carboxybenzothiophene was a gift from R. Meckenstock (GSF München). Acetonitrile (J. T. Baker, Deventer, The Netherlands) was used as ultra gradient grade. Water was obtained from a Milli-Q water purification system (Millipore, Eschborn, Germany).

2.2. Sampling and sample preparation

On the site, groundwater samples were collected in 1-L glass bottles with gas tight Teflon-lined caps, adjusted to pH 1–2 by addition of 3 mL 32% HCl and transported to the laboratory. The samples were stored in the dark at 4 °C until analysis.

Prior to sample preparation the groundwater samples were adjusted to pH 4 by addition of 10 M NaOH solution. As internal standards 1,1′-binaphthalene, trans-2-bromo-1-indanole or 5-bromo-1-indanone were used. Hundred microliters of methanol stock solutions was added to 1 L sample, resulting in a final concentration of 20 μg/L. The samples were applied to 6 mL-SPE-cartridges (Isolute ENV+, SEPARTIS,

Grenzach-Wyhlen, Germany) with 200 mg polymer material (hydroxylated polystyrene-divinylbenzene copolymer, surface 1000 m^2/g, average particle diameter 90 μm) without prior conditioning. PTFE-tubes (25 cm × 3 mm I.D.) were fixed on the SPE-cartridge by PTFE-cartridge-tube adapters and used for loading the SPE-material with groundwater samples sucked directly from the flasks by a flow of approximately 10–15 mL/min generated by water-pump vacuum. The cartridges were eluted with 10 mL acetone–methanol (50/50, v/v). A total volume of 10 mL was collected in graduated 10-mL tubes and 500 μL of the extracts were transferred into microvials (500 μL). The remaining 9.5 mL were evaporated under a light nitrogen stream to 500 μL and also transferred in microvials. The extracts were stored in vials with a PTFE-lined cap at +4 °C until analysis.

For development, optimization and validation of the sample preparation, typical tar oil compounds and some known metabolites (Table 1) were added in different concentrations to tap water samples or non-contaminated groundwater.

2.3. LC–DAD UV/–FLD/–MS

The liquid chromatography was carried out with an LC system HP1100 (Agilent®, Waldbronn, Germany) with UV-diode array and fluorescence detectors in series. Separation was achieved on a RP-column Synergi™ Hydro RP C_{18} (250 mm × 2 mm I.D., 4 μm particle size, Phenomenex®, Aschaffenburg, Germany) by gradient elution; acetonitrile–phosphate buffer pH 7 5/95% (v/v) for 2 min, up to 50/50% (v/v) in 3 min, in 10 min up to 60/40% (v/v) in 12 min up to 75/25% (v/v) and in 7 min up to 100/0% (v/v) and then back to 5/95% in 15 min and for equilibration for 6 min. A flow rate of 0.2 mL/min and a column temperature of 25 °C was adjusted. The injection volume was usually 10 μL, but for highly contaminated samples 0.5 μL was used. The diode array detector was set up to detection wavelength λ = 210 nm and spectra acquisition was performed from λ = 190 nm up to λ = 400 nm. The determination of the two- and three-cyclic PAHs (naphthalene, 1-methylnaphthalene, 2-methylnaphthalene, fluorene, acenaphthene, phenanthrene, anthracene) was performed by fluorescence detection (λ_{ex} = 275 nm λ_{em} = 350 nm).

The ESI–MS analysis was performed with a Finnigan MAT SSQ 7000 after LC separation and diode array detection using 0.1% formic acid instead of phosphate buffer. The heated capillary was set to 200 °C, sheath gas to 4.137 bar (60 psi), spray voltage was adjusted to 3.3 kV in the positive mode, the CID (collision-induced dissociation) to 10 V.

2.4. Site description

Groundwater from several wells in the plume of the tar oil contaminated sites “Wülknitz” was investigated by the method developed. The site at Wülknitz, Saxony, Germany, was used over many decades for timber preservation. So far, reclamation of the non-saturated soil zone has taken place. A secondary source of contaminated material with a semi-residual coal tar phase, consisting of PAH, BTEX and phenols, remained in the horizon below the water table. A contamination plume of approximately 250 m is expected. Due to sulphate concentrations in the groundwater in the range of 150–500 mg/L and nitrate concentrations up to 20 mg/L, the redox conditions are suggested to be mainly sulphate reducing. For our investigation, two wells near the source and four wells in the plume were available.

3. Results and discussion

3.1. Sample preparation

In preliminary experiments, solid phase extraction cartridges with the polymer material ISOLUTE ENV+ were compared to cartridges with the polymer in combination with RP18 material in regard to their extraction efficiency of approximately 20 compounds added to tap water samples. Since the RP18 material did not improve the recovery, ISOLUTE ENV+ cartridges without the RP18 material were used in all further experiments. In order to enrich acids, such as naphthoic acid, as well as basic compounds, such as quinoline, in one extraction step, recoveries were measured at two pHs. In weakly basic conditions (pH 8), the naphthoic acids were poorly sorbed to the SPE material, resulting in recovery rates lower than 30%. In contrast, in acidic conditions (pH 4) all compounds, including the slightly basic heterocycles quinoline and acridine, showed recoveries above 60%. In the next step, the elution solvent was optimized. In order to allow also GC–MS measurements, a mixture of acetone–methanol was chosen. The mixture was varied from 50:50 (v/v) acetone–methanol up to 70:30 (v/v) with a higher elution force for non-polar compounds. The higher acetone concentration did not lead to higher extraction yields, so that in all further experiments elution was performed with acetone–methanol 50:50 (v/v). In Table 1, the average recovery rates and relative standard deviations for tap water samples spiked with selected tar oil compounds and their metabolites at two different concentration levels are shown. Most compounds showed recovery rates in the range from 80% to 110% with acceptable relative standard deviations. The basic and acidic compounds quinoline, acridine and naphthoic acids showed the highest standard deviations and some compounds slight over estimation. This is probably caused by broad peaks in the LC chromatogram as shown for instance in Fig. 2 for the quinoline and acridine peaks (peaks numbers 10 and 17).

Recovery in SPE was further checked with spiked non-contaminated groundwater samples (Table 1). Some compounds, such as 1-hydroxyisoquinoline, 1- and 2-methylnapthalene were investigated additionally, because they were detected in the meantime often in groundwater samples. As expected the recovery rates were very similar for groundwater samples compared to tap water samples. Only

Table 1
Limit of detection, intra day deviation, inter day deviation, recovery rates (RR) and relative standard deviations (RSD) of selected NSO-heterocycles, homocycles and their metabolites after SPE of tap water with two concentration levels or spiked non-contaminated groundwater

Substance	CAS	R_t	Limit of detection		Intra day (50 μg/L)	Inter day[a] (50 μg/L)	5 μg/L (tap water)[a]		50 μg/L (tap water)[a]		50 μg/L (groundwater)[b]	
			UV (μg/L)	FLD (ng/L)			RR	±RSD	RR	±RSD	RR	±RSD
2-Hydroxyquinoline	59-31-4	13.8	0.4	140.0	1	3	111	5	107	3	107	3
1-Hydroxyisoquinoline	491-30-5	14.2	1.2	4.0	n.d.	n.d.	–[c]	–[c]	–[c]	–[c]	92	1
2-Thiophenecarboxaldehyde[d]	98-03-3	15.3	0.7	n.f.	1	14	107	9	92	7	–[c]	–[c]
Acridinone[d]	578-95-0	16.2	2.4	4.0	3	3	96	5	106	13	136	4
1-Indanone	83-33-0	16.9	0.4	n.f.	7	10	118	2	105	12	110	3
2-Naphthoic acid	93-09-4	18.2	1.2	12.0	1	4	80	12	132	28	112	3
trans-2-Bromo-1-indanole (ISTD)	10368-44-2	18.5	n.d.	n.f.	1	6	87	9	107	5	98	2
2-Naphthalenol	135-19-3	19.0	0.4	0.4	2	5	104	4	106	6	118	3
9-Hydroxyfluorene	1689-64-1	19.4	0.4	n.f.	1	4	110	2	103	2	108	2
Dibenzothiophene sulfoxide	1016-05-3	19.6	0.6	n.f.	2	2	110	2	110	1	118	3
Quinoline	91-22-5	20.3	1.0	n.f.	2	2	109	23	131	7	96	4
5-Bromo-1-indanone (ISTD)	34598-49-7	21.7	n.d.	n.f.	2	3	91	5	110	3	98	1
Benzofuran	271-89-6	23.5	0.4	n.f.	8	6	105	10	98	4	88	3
Carbazole	86-74-8	25.8	0.4	n.f.	2	3	–[c]	–[c]	104	3	97	2
Benzo(*b*)thiophene	95-15-8	26.8	0.8	n.f.	7	5	109	10	99	3	87	3
Indene	95-13-6	27.0	0.4	n.f.	8	7	–[c]	–[c]	95	3	83	2
Naphthalene	91-20-3	28.7	0.2	2.0	11	5	80	8	99	12	91	1
Acridine[d]	260-94-6	28.9	0.0	n.f.	10	9	110	16	98	19	106	2
Indane	496-11-7	31.2	1.6	n.f.	9	8	98	3	92	2	62	3
1-Methylnaphthalene	90-12-0	32.2	0.4	1.4	n.d.	n.d.	–[c]	–[c]	–[c]	–[c]	87	1
Dibenzofuran	132-64-9	32.6	0.4	1.2	7	5	102	6	93	3	104	2
2-Methylnaphthalene	91-57-6	32.9	0.4	1.0	n.d.	n.d.	–[c]	–[c]	–[c]	–[c]	89	1
Fluorene	86-73-7	34.1	0.6	1.0	6	4	98	7	93	3	82	3
Acenaphthene	83-32-9	34.3	1.2	1.0	6	5	106	9	96	3	–[c]	–[c]
Dibenzothiophene	132-65-0	35.5	0.8	0.8	5	5	93	16	89	2	97	2
1,3-Dimethylnaphthalene	575-417	36.4	0.4	0.4	n.d.	n.d.	–[c]	–[c]	–[c]	–[c]	82	1
1,1′-Binaphthalene (ISTD)	604-53-5	38.4	n.d.	n.d.	7	7	96	4	92	6	101	2

n.d.: Not determined, n.f. not fluorescence active.
[a] $n = 9$, At three different days.
[b] $n = 2$.
[c] Not added to the sample.
[d] Detection wavelength 254 nm.

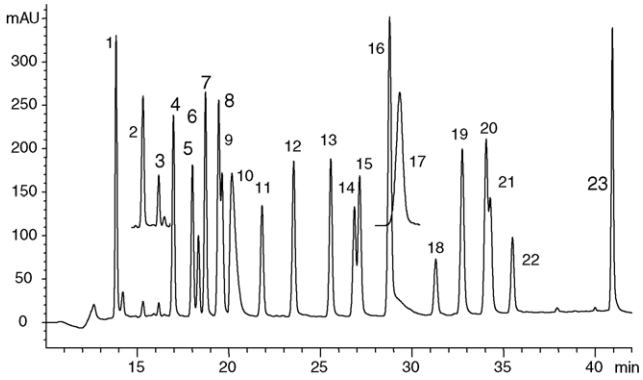

Fig. 2. LC–DAD UV-chromatogram of a standard with heterocycles, homocycles and their metabolites (5 mg/L) corresponding to 50 μg/L in groundwater samples: (1) 2-hydroxyquinoline; (2) 2-thiophenecarboxaldehyde*; (3) acridinone*; (4) 1-indanone; (5) 2-naphthoic acid; (6) trans-2-bromo-1-indanol (ISTD); (7) 2-naphthalenol; (8) 9-hydroxyfluorene; (9) dibenzothiophene sulfoxide; (10) quinoline; (11) 5-bromo-1-indanone (ISTD); (12) benzofuran; (13) carbazole; (14) benzo(*b*)thiophene; (15) indene; (16) naphthalene; (17) acridine*; (18) indane; (19) dibenzofuran; (20) fluorene; (21) acenaphthene; (22) dibenzothiophene; (23) 1,1′-binaphthalene (ISTD). Compounds with (*), denotes quantified at λ = 254 nm; all others quantified at λ = 210 nm.

indane showed low recoveries, what cannot be explained at the moment.

Breakthrough was tested by using two SPE cartridges in sequence. Extraction of a groundwater sample spiked with 25 compounds in a concentration of 300 μg/L led to no detection of any compound in the extract of the second cartridge. This indicates that also highly contaminated samples can be investigated by the procedure. Of course, it is also possible to extract only 100 mL of high-polluted samples.

In order to improve sensitivity for low contaminated groundwater samples, the SPE extracts were concentrated by evaporation under a light nitrogen stream, but the recovery of many compounds decreased significantly (data not shown). The recovery rates of the volatile compounds indane, indene and benzofuran were even below 25%. The selected internal standards did not show such a high decrease so that they could not be used for correction of volatilization loss. Because of these results, quantification was performed with non-concentrated extracts.

3.2. Chromatographic separation, detection limits and accuracy of the method

In Fig. 2, a typical LC–DAD UV-chromatogram of a standard mixture is shown. The chosen chromatographic conditions yielded baseline separation for many peaks. The compound pairs hydroxyfluorene–dibenzothiophene sulfoxide, benzo(*b*)thiophene–indene, naphthalene–acridine and fluorene–acenaphthene showed double peaks, but quantification is possible. The separation of the two PAHs fluorene and acenapthene is known to be difficult [33]. For acridine, 2-thiophene carboxyaldehyde and acridinone calibration and

Table 2
Concentration of NSO-heterocycles, homocycles and their metabolites in groundwater samples from the timber impregnation site Wülknitz

Substances[a]	Concentration in groundwater samples from different wells (μg/L)					
	IW1 near source	IW2 near source	GWM 1/97 ~60 m	IW5 ~100 m	13/01 ~120 m	14/01 ~250 m
Naphthalene	1070	720	740	50	10	10
1-Methylnaphthalene	–[b]	450	300	130	<[c]	Trace[d]
2-Methylnaphthalene	<	70	300	<	<	Trace[d]
Fluorene	160	60	20	20	40	<
Acenaphthene	480	220	190	240	290	<
Indene	650	220	210	100	n.d.	<
Quinoline	Trace[d]	<	<	<	<	<
Carbazole	180	20	20	30	30	<
Benzo(*b*)thiophene	920	330	280	210	140	40
Benzofuran	<	110	<	<	<	<
Dibenzofuran	n.d.	100	<	80	130	1
2-Naphthalenol	720	<	<	<	<	<
1-Naphthalenol	<	20	<	50	20	<
2-Naphthoic acid	<	<	40	100	Trace[d]	<
2-Hydroxyquinoline	720	900	100	70	50	<
1-Hydroxyisoquinoline	700	810	140	440	n.d.	<
Acridinone	Trace[d]	<	<	<	<	<
Phenanthridinone	Trace[d]	<	<	<	<	<
1-Indanone	<	60	<	<	<	<
4-Methylquinoline	<	<	<	<	<	<
1,3-Dimethylnaphthalene	<	<	<	<	<	<

[a] The following compounds were not detected in any samples: 2-thiophen-carboxyaldehyde, dibenzothiophene sulfoxide, indane, acridine, phenanthridine, hydroxyfluorene, 2-hydroxy-4-methylquinoline, 5-carboxybenzothiophene.
[b] Not determinable.
[c] <LOD.
[d] Detected but below limit of quantitation.

quantification was carried out at 254 nm because the spectra of these compounds showed higher UV-absorption at this wavelength. As indicated in Table 1, several compounds, especially PAHs, could be determined sensitively by fluorescence detection, too.

The calibration was performed with at least six different concentrations in the range of 100–10,000 μg/L (DAD UV detection) and 0.1–100 μg/L (FLD detection) corresponding to groundwater concentrations of 1–100 μg/L and 0.001–1 μg/L, respectively. The calibration curves were linear with correlation coefficients > 0.997. The detection limits were determined using the signal-to-noise ratio >3. They were after enrichment 0.4–2.4 μg/L for the compounds determined by DAD UV detection and 0.4–140 ng/L for the fluorescence active compounds. These limits are sufficient for the investigation of groundwater samples in the plume. To determine the robustness of the method, the preparations of spiked samples and analysis of extracts were made in triplicates at three different days. Intra day deviation was mostly below 10%. The inter day deviation, shown in Table 1, for most compounds, is not greater than the intra day deviation.

3.3. Application to groundwater samples from a tar oil contaminated site

The method developed was applied to groundwater samples from the timber impregnation site Wülknitz described in the Section 2. In order to determine the occurrence and fate of tar oil compounds, we analysed groundwater samples near the source of contamination and downstream the plume. The identification of tar oil compounds was performed by comparison of retention time and UV spectra or in the case of PAHs fluorescence spectra with standard substances. In addition to UV spectra, identification of polar compounds was confirmed by LC–MS measurements. Naphthalene, acenaphthene, indene, benzofuran and benzo(*b*)thiophene were detected in high concentrations near the source (Table 2). Downgradient of the source the concentrations of naphthalene, indene and benzofuran decreased more than those of

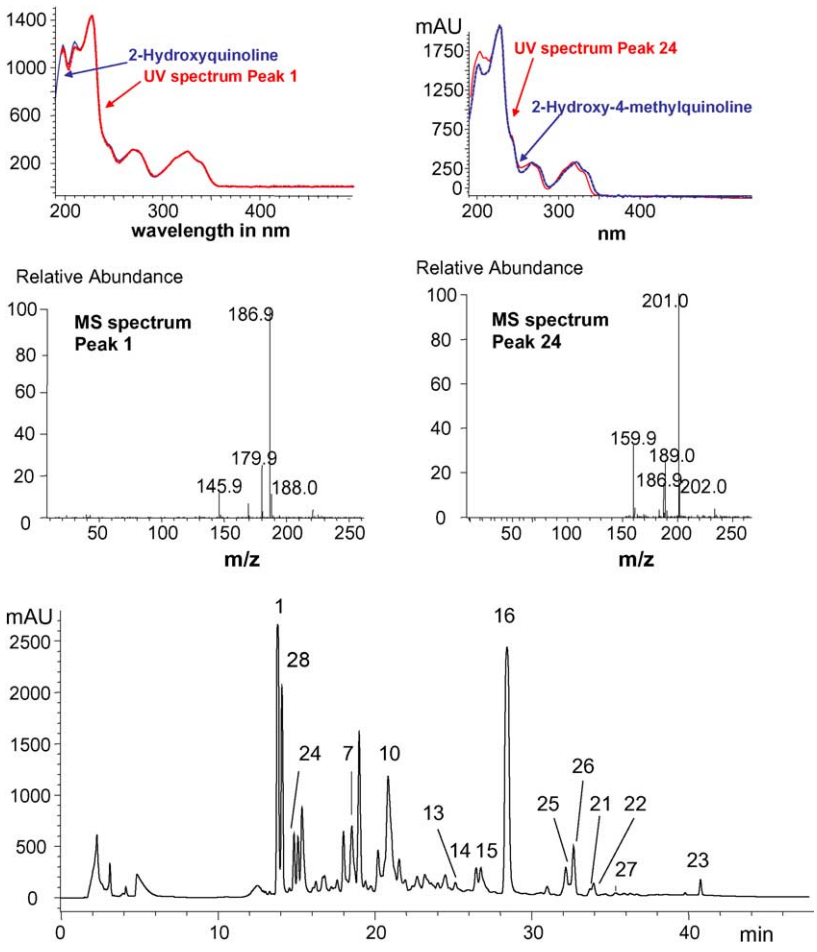

Fig. 3. LC–DAD UV-chromatogram of a highly contaminated groundwater sample from the timber impregnation site with ESI–MS and UV spectra of peak 1 (2-hydroxyquinoline) $m/z = 186.9$ $[M^+H^+MeCN]^+$ and peak 24 (2-hydroxy-4-methylquinoline) $m/z = 201.0$ $[M^+H^+MeCN]^+$. (24) 2-Hydroxy-4-methylquinoline; (25) 1-methylnapthalene; (26) 2-methylnapthalene; (27) phenanthrene; (28) 1-hydroxyisoquinoline other peak numbers explained in Fig. 2.

benzo(*b*)thiophene, carbazole and acenaphthene, leading to increasing dominance of these compounds. This greater persistence is in accordance with several investigations described in the literature [3,5].

Several hydroxylated metabolites were detected simultaneously in the chromatogram as shown for a high-contaminated groundwater sample from Wülknitz in Fig. 3. For instance, the mass and UV spectra of two hydroxyquinolines are shown in Fig. 3, too. Besides the protonated molecule ions (1-hydroxyiso- and 2-hydroxyquinoline, *m*/*z* 146, 2-hydroxy-4-methylquinoline, *m*/*z* 160), the acetonitrile adducts (*m*/*z* 187, 201) were detected under the performed electrospray conditions. We found high concentrations of 1-hydroxyiso- and 2-hydroxy-quinoline near the source and in the plume, indicating transformation of the likely parent compounds quinoline and isoquinoline [38,39]. Further transformation of these compounds in the aquifer seems to be difficult because they were still detected 120 m downgradient. Accordingly, Zamfirescu and Grathwohl [5] also found them at a distance of 185 m from the contamination source. The 1- and 2-naphthalenol found at Wülknitz may derive from tar oil [34] or can be intermediates in degradation of naphthalene under aerobic conditions [35]. In contrast, the presence of 2-naphthoic acid showed unequivocally the transformation of PAHs at this site. It may derive from degradation of naphthalene or 2-methylnaphthalene under sulphate-reducing conditions [36]. In addition, near the source 1-indanone was found in the groundwater from well IW2, which may originate from indane or indene degradation [16]. Acridinone and phenanthridinone were identified in concentrations below the limit of quantification only near the source, too. Probably, they derive from acridine and phenanthridine, which were not detected [37]. Müller et al. [27] found them in concentrations up to 175 μg/L near the source at another tar oil contaminated site.

In general, at the Wülknitz site, natural attenuation processes seem to occur in the plume because most compounds and their metabolites were no longer found in the groundwater from well 14/01. Benzo(*b*)thiophene showed the highest persistence at this site in that it is still found 250 m downstream of the source. So far, benzo(*b*)thiophene is known to be transformed only cometabolically [25]. The metabolite 5-carboxybenzothiophene, which was formed under sulphate-reducing conditions at another tar oil contaminated site [25,40], was detected in no groundwater sample.

Analysis of further groundwater samples as well as additional hydrological investigations has to be performed at the site to confirm the occurrence and fate of the heterocyclic compounds and their metabolites in the plume. So far, the results show that the simple method described here is suitable to investigate a broad spectrum of parent compounds and their metabolites in contaminated groundwater typically found at tar oil contaminated sites downgradient the source.

Acknowledgement

This study was supported in the priority program “Natural Attenuation: Retention and Degradation Processes to reduce Contamination in Groundwater and Soil” (KORA) funded by the German Federal Ministry of Education and Research. The authors appreciate the help and technical assistance of U. Kaufmann and M. Meindorf. We thanks N. Hüsers, H. Lorbeer, S. Schönekerl, P. Werner of TU Dresden for groundwater sampling at the site. We are grateful to R. Meckenstock for supply of 2-carboxybenzothiophene. Special thanks go to G. Rippen for helpful discussion and W. Dott for continuous support.

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