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Identification and quantification of polar naphthalene derivatives in contaminated groundwater of a former gas plant site by liquid chromatography–electrospray ionization tandem mass spectrometry

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Abstract

A liquid chromatography (LC) method followed by electrospray ionization (ESI) and tandem mass spectrometry (MS–MS) was developed for the quantification of acidic naphthalene derivatives in the concentration range 0.1 to 100 μg/l without excessive sample preparation. For optimal sensitivity the LC–MS–MS measurements were performed recording mass fragmentation by collision induced dissociation in the multiple reaction mode. The collision energy was optimized for every analyte. The matrix effects of the sample were investigated by spiking standards of 1-naphthoic acid with humic acid (HA) and with calcium chloride. While HA decreased the signal intensity an increase was observed in the presence of calcium chloride. For the investigated groundwater samples of a tar oil contaminated site a complete separation of the analytes from the sample matrix by reversed-phase separation could be obtained. The absence of matrix effects on quantification results was confirmed by comparison of results based on external calibration with those based on standard addition of the analytes to a groundwater sample. In four groundwater samples of the contaminated site naphthalene derivatives like 1-naphthoic acid, 2-naphthoic acid, 1-naphthylacetic acid, 2-naphthylacetic acid, 1-hydroxy-2-naphthoic acid, 2-hydroxy-3-naphthoic acid, and naphthyl-2-methylenesuccinic acid have been detected.

Keywords: Water analysis; Matrix effects; Naphthalenes; Polynuclear aromatic hydrocarbons; Humic acids; Naphthoic acids

1. Introduction

Gas production facilities running in Germany until 1970 often caused tar oil contamination of the underground. These contaminated sites have nowadays a high risk potential for ground water contamination. Tar oil is a complex mixture of compounds that can be divided into the groups of polycyclic aromatic compounds (PAHs, 85%, w/w), hetero-PAHs containing nitrogen, sulfur, and oxygen (5–13%, w/w), phenolic compounds (1–10%, w/w), and monoaromatic hydrocarbons (<1%, w/w) [1,2]. For groundwater contamination especially the polar compounds and metabolites of PAHs are of high relevance. Due to their good water solubility a high hydrogeological mobility is observed [3]. Common metabolites formed during the biochemical degradation of PAHs are naphthalene derivatives con-

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taining a carboxylic group. For aerobic degradation of phenanthrene and anthracene 1-hydroxy-2-naphthoic acid and 2-hydroxy-3-naphthoic acid were identified as metabolites [4–6]. 2-Naphthoic acid is formed as the main metabolite in the initial step of anaerobic naphthalene degradation by incorporation of bicarbonate into the carboxylic group [7,8]. In addition, 2-naphthoic acid was reported as metabolite of 2-methylnaphthalene in the anaerobic degradation pathway of sulfate-reducing bacteria [9]. Further metabolites in the degradation of 2-naphthoic acid are tetrahydro-2-naphthoic acid, octahydro-2-naphthoic acid and decahydro-2-naphthoic acid. In the upper degradation pathway of 2-methylnaphthalene to 2-naphthoic acid the major metabolites naphthyl-2-methylsuccinic acid and naphthyl-2-methylenesuccinic acid could be identified [9].

The analysis of PAH metabolites is often performed by GC–MS. However, an extensive sample preparation including extraction, cleanup, and derivatization steps have to be applied [10]. Alternative HPLC methods with UV detection work without a derivatization step but have the drawback of interferences of the UV detection due to coeluting substances and low selectivity and sensitivity [1,11]. For a continuous monitoring of the groundwater at contaminated sites, especially for the assessment of natural attenuation processes, an analytical method is needed that allows a rapid analysis of metabolites at low concentrations without complex sample preparation steps.

In this work a method for the direct analysis of polar naphthalene derivatives in ground water samples was developed on the basis of reversed-phase liquid chromatography, electrospray ionization (ESI) and tandem mass spectrometry (MS–MS). Since the ESI process is influenced by the sample matrix [12,13] the effects of humic substances and calcium chloride on the signal intensity were investigated. The absence of sample matrix effects for the investigated samples was confirmed by standard addition of the analytes to a groundwater sample.

2. Experimental

2.1. Chemicals

1-Naphthoic acid (98%), 1-naphthylacetic acid (95%), 2-naphthylacetic acid (99%), 1-hydroxy-2-naphthoic acid and 2-hydroxy-3-naphthoic acid were purchased from Acros (Geel, Belgium). 2-Naphthoic acid was from Fluka (Neu-Ulm, Germany). Naphthyl-2-methylsuccinic acid was formed by catalytic reduction of naphthyl-2-methylenesuccinic acid with palladium on activated carbon at a hydrogen pressure of 100 kPa as described elsewhere [9]. Acetic acid (100%) and formic acid (98–100%) were of analytical grade and purchased from Merck (Darmstadt, Germany). Acetonitrile (ACN; HPLC grade) was purchased from J.T. Baker (Griesheim, Germany).

For all analytes, stock solutions in acetonitrile–water (50:50, v/v) were prepared in 10 ml flasks with a concentration of approximately 250 mg/l and diluted as required.

For the measurement of matrix effects a stock solution of humic acid (HA, Aldrich, Germany) was prepared by dissolving 8 g of HA in 1000 ml demineralized water. The solution was sonificated for 30 min, shaken for 24 h, and finally centrifuged for 60 min at 5000 rev./min and filtered with a 0.45 μm cellulose acetate membrane filter (Sartorius, Germany). The final concentration was 1460 mg/l dissolved organic carbon (DOC). An aliquot was neutralized by sodium hydroxide solution to pH 7 and filtered again through a 0.22 μm filter (Millex-GS, Millipore, Molsheim, France). After dilution with demineralized water in a ratio of 1:2 the concentration of 480 mg/l DOC was obtained.

All DOC measurements were performed with a total organic carbon analyzer TOC-5000 (Shimadzu). A calcium chloride stock solution with a final calcium chloride concentration of 3030.8 mg/l was prepared by calcium chloride dihydrate (analytical grade, Merck Darmstadt, Germany).

2.2. Groundwater samples

Groundwater samples were taken from the contaminated site ‘‘Testfeld Süd’’ of a former gas plant facility in southern Germany. Pollution on this site originated from gasification and gas purification processes due to leakage from storage facilities and water leaching of coal and tar dumping grounds. More details on the site are given elsewhere [14–17]. Groundwater samples were collected from four different wells with a submersible pump. Then, 20

ml of the water samples were diluted with 20 ml of acetonitrile to avoid bacterial degradation and collected in 42 ml vials sealed with PTFE septa. The samples were cooled immediately and stored at 4 °C. Before analysis the samples were filtered with a prewashed 0.22 μm filter (Millex-GS).

The wells 22, 49 and 14 were located near the contamination source and showed high contamination levels whereas well 85 was located downstream in the contamination plume with a low contamination level [18].

2.3. LC–ESI–MS–MS analysis

The analyses were performed with a HPLC system Agilent 1100 equipped with binary pump, degasser, column oven, and autosampler which was coupled with an electrospray ionization source (TurboIon Spray, Applied Biosystems Sciex) to the triple quadrupole mass spectrometer API 3000 (Applied Biosystems Sciex). The separation column was a Purospher RP-18e, 250×4 mm, 5 μm particles from Merck. The column oven was set to 35±2 °C. The eluents consist of (A) 100% water with 0.1% acetic acid; and (B) 100% acetonitrile. Eluent B raised from 20 to 90% within 35 min. The flow-rate of the eluent was 0.5 ml/min and the injection volume was 50 μl. The ion spray voltage of the ESI system was set to −5500 V, the nebulizer gas flow to 1.5 l/min, the dry gas flow to 6 ml/min, the dry gas temperature to 450 °C and the curtain gas flow to 1.4 l/min. MS measurements were performed as negative product ion scan (PIS) or in the negative multiple reaction mode (MRM). In the MRM the most abundant fragment ion was recorded after collision induced dissociation (CID). The collision gas thickness was set to $2.19 \cdot 10^{17}$ molecules N_2/ml and the scan time was 500 ms. The optimized MS parameters for the analytes are summarized in Table 1.

The collision energy was optimized by injection of a standard solution with a concentration of 5 mg/l into the eluent stream that was flowing without chromatographic separation into the ESI source of the MS. The eluent was acetonitrile–water+0.1% acetic acid (65:35). This eluent composition was equivalent to the composition of the eluent at the retention time of the analytes.

The limits of detection and limits of quantification were determined according to the calibration method of DIN 32645 (DIN) [19]. Therefore, 10 standard samples in the range 0.1 to 1.0 μg/l were prepared and analyzed. The limits were calculated by Eqs. (1) and (2):

Limit of detection (*LOD*):

$$\mathrm{LOD} = s_{x0} t_{n-2;1-\alpha} \sqrt{\frac{1}{n_1} + \frac{1}{n_2} + \frac{\bar{x}^2}{\sum (x_i - \bar{x})^2}} \qquad (1)$$

where s_{x0} is the standard deviation; t the student factor; n_1 the number of sample measurements ($n_1 = 1$); n_2 the number of calibration points ($n_2 = 10$); $\bar{x}$ the arithmetic mean of the x-values; x_i the x-value; and α the level of significance ($\alpha = 0.01$).

Limit of quantification (*LOQ*):

$$\mathrm{LOQ} = k s_{x0} t_{n-2;1-\frac{\alpha}{2}} \sqrt{\frac{1}{n_1} + \frac{1}{n_2} + \frac{(k\mathrm{NG} - \bar{x})^2}{\sum (x_i - \bar{x})^2}} \qquad (2)$$

where k is the element of uncertainty ($k = 3$).

For the investigation of matrix effects a standard solution of 2-naphthoic acid with a concentration of

Table 1
Operating parameters for the MS–MS measurements

Compound	Molecular mass	Precursor ion $[M-H]^-$	Fragment ion	Declustering potential (DP) (V)	Focus potential (FP) (V)	Entrance potential (EP) (V)	Collision energy (CE) (eV)	Collision cell exit potential (CXP) (V)
1-Naphthoic acid	172.19	171.1	127.1	−30	−130	8	−19	−4
2-Naphthoic acid	172.19	171.1	127.1	−30	−130	8	−19	−4
1-Naphthylacetic acid	186.21	185.1	141.1	−11	−100	7	−10	−5
2-Naphthylacetic acid	186.21	185.1	141.1	−11	−100	7	−10	−5
1-Hydroxy-2-naphthoic acid	188.18	187.1	143.1	−30	−140	8	−26	−5
2-Hydroxy-3-naphthoic acid	188.18	187.1	143.1	−30	−140	8	−26	−5
Naphthyl-2-methylsuccinic acid	258.27	257.2	169.2	−35	−160	8	−25	−5

20 μg/l was prepared. The solution was diluted in a ratio of 1:1 with aqueous solutions of HA with different DOC concentrations, resulting in a final 2-naphthoic acid concentration of 10 μg/l and DOC concentrations in the range 1.25 to 50 mg/l. In the same way a 2-naphthoic acid solution was diluted with aqueous calcium chloride solutions resulting in a 2-naphthoic acid concentration of 10 μg/l and calcium chloride concentrations in the range 7.5 to 1500 mg/l. Then, 20 μl of the Aldrich HA and calcium chloride samples were injected in an eluent of acetonitrile–water (65:35) or acetonitrile–water + 0.1% acetic acid (65:35).

For the standard addition experiments a standard solution in acetonitrile–water (50:50) was prepared which contained a concentration of the analytes eleven times higher then the groundwater sample of well 49. From this standard solution 0–50 μl were added to 450 μl of the groundwater sample and the volume was filled up with acetonitrile–water (50:50) to set 500 μl. The highest concentrations of the analytes in these standard addition samples were twice as high as the concentrations in the original sample.

3. Results and discussion

For quantitative analysis of polar compounds at concentrations of 1 μg/l an excellent sensitivity of the analytical method is needed. With ESI-MS–MS the best sensitivity was obtained in the multiple reaction mode. A variation of the collision energy was performed to identify the most intensive fragment for the quantification of the analyte. In Fig. 1 the intensities of CID-MS fragments of naphthyl-2-methylsuccinic acid were shown at varied collision energies. For quantitative analysis the collision energy at the maximum of the most intensive fragment was used. The optimum collision energies for all analytes are listed in Table 1. Under these conditions limits of quantification between 0.16 and 0.29 μg/l and limits of detection between 0.05 and 0.09 μg/l were obtained.

The ionization process of the ESI is very sensitive to sample matrix and therefore quantification may be a problem in matrix rich samples using external calibration. To investigate the influence of sample

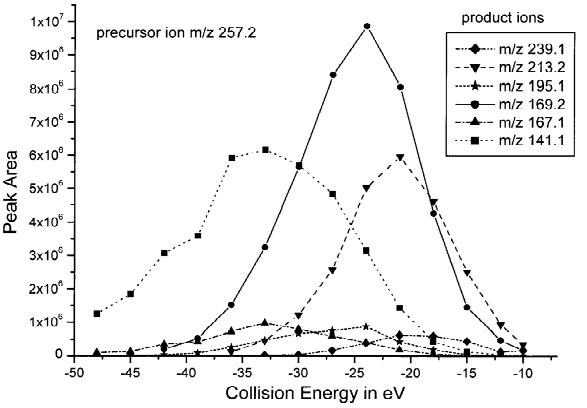

Fig. 1. Intensities of the main CID-MS fragment ions of naphthyl-2-methylsuccinic acid at different collision energies.

matrix effects on the ESI process, standards of 2-naphthoic acid were spiked with a humic acid and with calcium chloride. The spiked samples were injected in eluents of acetonitrile–water (65:35) or acetonitrile–water + 0.1% acetic acid (65:35) and analyzed without chromatographic separation. With increasing concentration of HA a decrease of the signal intensity was observed for both eluents (Fig. 2). Already at a DOC concentration of 1.2 mg/l the signal area decreased to 80%. When acetic acid was present in the eluent it acted as a modifier and generated a 25% higher signal in absence of HA compared to the eluent without acetic acid. With increasing DOC concentrations a higher decrease of the signal intensity was observed in the presence of

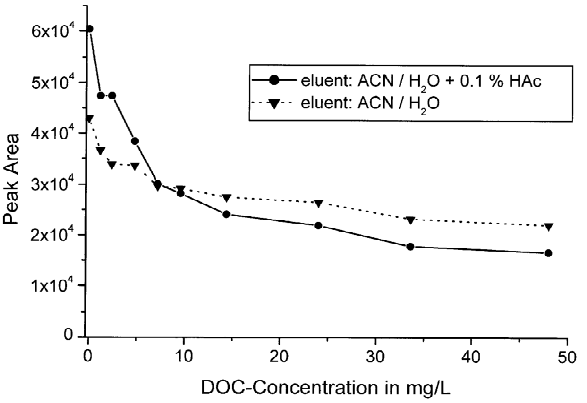

Fig. 2. Signal intensities of 10 μg/l 2-naphthoic acid at different concentrations of HA. CID-MS–MS (m/z 171.1→m/z 127.1).

acetic acid. At a DOC concentration of 48 mg/l the signal area decreased to 27% of its original signal area with acetic acid and only to 51% without.

The observed effects can be explained by changes of the spray process of the ESI. In general the yield of gas-phase ionization is dependent on the formation and evaporation of fine droplets and the electrolyte concentration of the eluent. The droplet size decreases with decreasing surface tension. Thus, substances with surfactant properties such as organic modifiers (e.g. acetic acid) increase the formation of fine droplets and therefore increase the formation of gas-phase ions producing higher signal intensities [20]. A decrease in droplet size is also induced by an increased conductivity of the solution caused by an increased inorganic salt content [21]. In addition a competition effect of analyte ions with electrolyte ions (e.g. from the matrix or modifier) in the conversion process to gas-phase ions has to be taken into account. This effect is due to the competition of ions for the surface of the droplet and due to differences in the rate constants of gas-phase ion formation. Humic acid molecules present in the sample are also ionized and therefore in competition with the analyte ions for the droplet surface. As a result, less of the analyte molecules have the possibility to leave into the gas-phase. This causes the observed decrease in signal intensity in the presence of humic acids. However, acetic acid as a modifier improved the spray process. More small droplets may be generated. They have a larger surface area compared to bigger droplets formed from the same liquid volume. At low acetic acid concentrations the effect of increased surface area is superimposing that effect of competition between analyte and acetic acid anions for occupying the droplet surfaces. That is the reason why an increased signal in the presence of acetic acid is observed. In the presence of humic acid the modifying effect of the acetic acid on the spray process is reduced because the humic acid has also a positive effect on the droplet size due to its surfactant properties. But this effect is superimposed by the negative effect of droplet surface occupation. This means, that at a certain humic acid concentration, the acetic acid has nearly no additional effect on the droplet size but the acetate ions compete for the surface of the droplets. This effect can be observed in Fig. 2 at the point where the two plots are crossing. At the corresponding humic acid concentration (DOC) of 8 mg/l the spray process is modified almost by the humic acid and the acetic acid has nearly no additional effect but is also competing for the droplet surface. As a result the signal in the presence of acetic acid at humic acid concentrations above 8 mg/l is smaller then in absence of acetic acid.

The influence of calcium chloride on the signal intensity is shown in Fig. 3. The signal increased at a calcium chloride concentration of 1500 mg/l by approximately 300% in the presence of the modifier acetic acid and approximately 700% in the absence of it.

The increase of the signal intensity can be explained by the modifying effect of the calcium chloride. Optimum calcium chloride concentrations were between 200 and 500 mg/l. With the eluent that contains already acetic acid as modifier this effect is lower.

The results show that for precise quantification a complete separation of the sample matrix from the analytes during the chromatographic process is important if external calibration is applied. With the described method the analytes were eluting with relatively long retention times (Fig. 4), resulting in capacity factors (k') between 4.63 and 5.80 ($t_0 = 3.38$ min).

Separation of the analytes from sample matrix—which effects the ionisation process—was obtained. This was proved by comparison of standard addition and external calibration of all analytes in a ground-

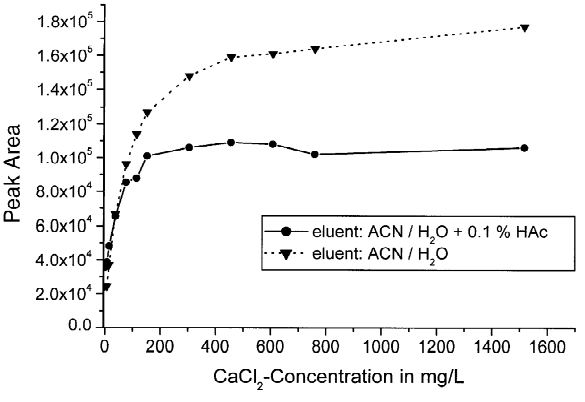

Fig. 3. Signal intensities of 10 μg/l 2-naphthoic acid at varied concentrations of $CaCl_2$. CID-MS–MS (m/z 171.1→m/z 127.1).

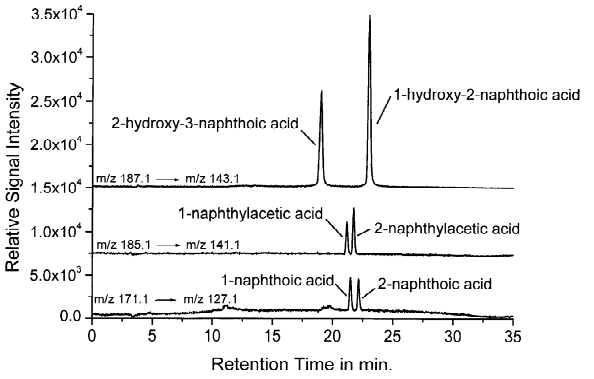

Fig. 4. MRM chromatogram of six different acidic naphthalene derivatives with a concentration of 1 μg/l each.

water sample of well 49. Nearly the same slopes of the straight lines of the external calibration and the standard addition were obtained, proving the absence of matrix effects.

For the analysis of metabolites of aerobic and anaerobic naphthalene degradation four groundwater wells were chosen that showed fairly high naphtalene concentrations of up to 5.8 mg/l [18,22]. The following analytes could be quantified by LC–ESI-MS–MS (Fig. 5): 1-naphthoic acid, 2-naphthoic acid, 1-naphthylacetic acid, 2-naphthylacetic acid, 1-hydroxy-2-naphthoic acid, 2-hydroxy-3-naphthoic acid, and naphthyl-2-methylenesuccinic acid. The hydroxynaphthoic acids occur only in relatively small amounts with concentrations between 0.2 and

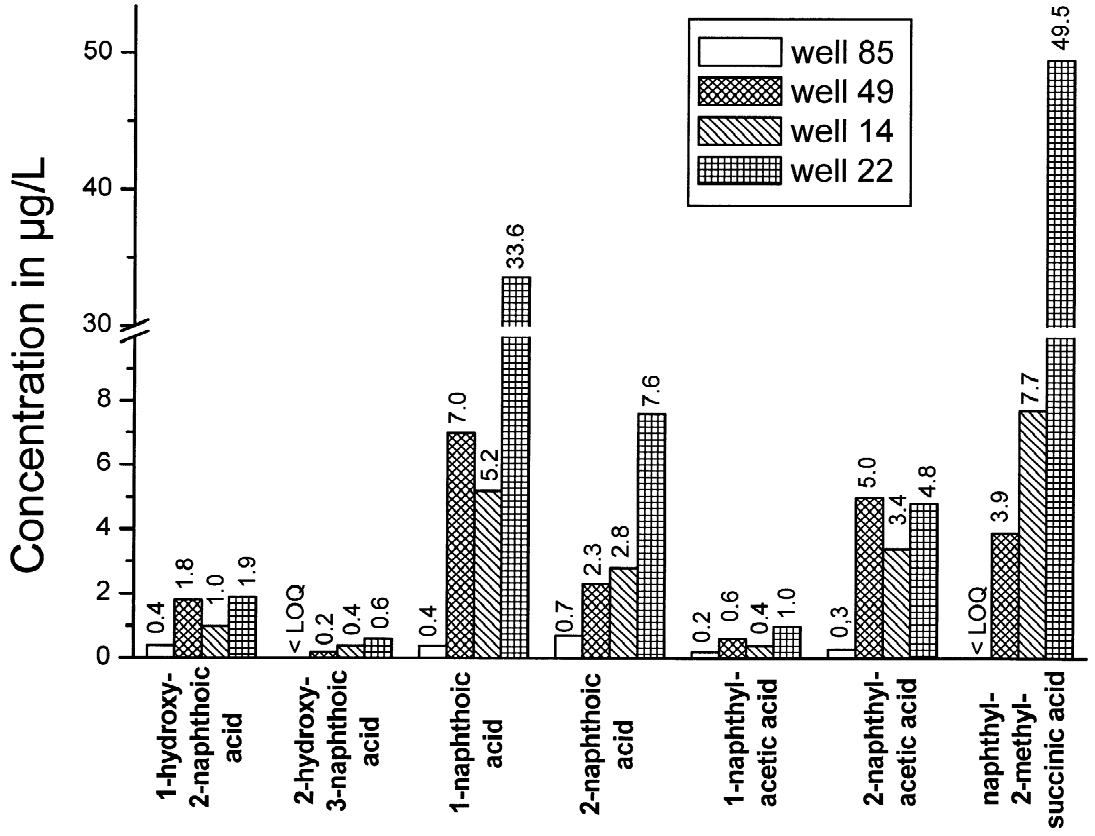

Fig. 5. Concentrations of the investigated acidic naphthalene derivatives in the groundwater samples of four wells on the contaminated site. (<LOQ: below limit of quantification).

1.9 μg/l which might be explained by the anoxic conditions in the groundwater. The hydroxynaphthoic acids are known as metabolites of aerobic PAH degradation and their formation should be only possible in aerobic environments at the fringes of the contamination plume.

Relatively high concentrations in the range of 0.4 to 33.6 μg/l were observed for the naphthoic acids which are metabolites of anaerobic degradation of methylnaphthalene and naphthalene. In addition, large amounts of naphthyl-2-methylsuccinic acid were found which is a metabolite of anaerobic 2-methylnaphthalene degradation. The source of the naphthylacetic acids which occur in concentrations of 0.2 to 5.0 μg/l is not obvious. Nevertheless, we assume that these compounds are also degradation products of PAHs.

4. Conclusions

Rapid and sensitive analysis methods are required to monitor natural attenuation processes of contaminants in groundwater. Above all, there is a lack of suitable methods for polar analytes such as metabolites of biochemical degradation. The developed method based on LC–ESI-MS–MS allows a quantification of acidic naphthalene derivatives in the concentration range 0.1–100 μg/l without prior sample preparation and therefore turned out to be a powerful tool for investigating biochemical degradation pathways of pollutants in former gas production sites and other dumping areas. For the samples investigated a complete chromatographic separation of the analytes from the sample matrix that was influencing the ionisation process was obtained. Therefore matrix effects did not influence quantification by ESI-MS–MS on the basis of external calibration.

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