ELSEVIER

Journal of Chromatography A, 810 (1998) 161–172

JOURNAL OF CHROMATOGRAPHY A

Gas chromatographic determination of aromatic amines in water samples after solid-phase extraction and derivatization with iodine I. Derivatization

Torsten C. Schmidt[a,*], Melanie Less[b], Rainer Haas[c], Eberhard von Löw[b], Klaus Steinbach[a], Gottfried Stork[a]

[a]*Department of Chemistry, Philipps-University, Hans-Meerwein-Strasse, D-35032 Marburg, Germany*

[b]*Environmental Hygiene and Immunology, Medical Centre of Hygiene, Philipps-University, Pilgrimstein 2, D-35037 Marburg, Germany*

[c]*Bureau for Exploration of Hazardous Waste Sites and Pollution Research, Stadtwaldstrasse 45a, D-35037 Marburg, Germany*

Received 13 January 1998; received in revised form 17 March 1998; accepted 17 March 1998

Abstract

A new method for the selective determination of aromatic amines is presented, which is based on the solid-phase extraction at pH 9 and subsequent derivatization of the analytes to the corresponding iodobenzenes. These can selectively and sensitively be determined with gas chromatography and electron-capture detection. Separation of at least 30 compounds in a single chromatographic run in 30 min is possible. With this method, 56 aromatic amines were investigated, and only in six cases no derivatives were obtained. Limits of quantitation were between 0.5 and 8 $\mu g\ l^{-1}$, but may still be lowered with higher sample volumes or different injection techniques. The application to water samples revealed the suitability for the investigation of ground, leachate and wastewater. © 1998 Elsevier Science B.V. All rights reserved.

Keywords: Water analysis; Derivatization, GC; Environmental analysis; Amines, aromatic; Iodine

1. Introduction

Aromatic amines are used in the chemical industry in high amounts for the production of dye stuffs, pesticides, plastics and pharmaceuticals [1]. The high consumption of aromatic amines in industrial processes leads to a release into the aquatic environment. An additional source for aromatic amines in the environment is the abiotic and biotic degradation of nitroaromatic compounds [2,3], azo dyes [4,5], several classes of pesticides [6–8] and possibly polyurethanes [9]. Aromatic amines are in general more polar than their precursors and thus have a higher solubility in water, which is responsible for the high mobility of aromatic amines in aquifers. Because of their widespread distribution and their mobility it does not surprise that a wide range of aromatic amines has been found in environmental matrices by several investigators, for example in river water [10–16] and ammunition wastewater [17–28].

An additional property of aromatic amines is their high acute and, maybe more important, chronic toxicity [29,30]. Many aromatic amines that were studied for their long-term toxicity in bioassays were found to be carcinogenic. For some of these com-

*Corresponding author.

0021-9673/98/$19.00 © 1998 Elsevier Science B.V. All rights reserved.
PII: S0021-9673(98)00233-7

pounds this is even well known for human beings. The widespread use and the harmfulness of aromatic amines make their analysis in environmental matrices important.

Because of their polarity, high-performance liquid chromatography (HPLC) is mostly used for the direct analysis of aromatic amines. The problem of HPLC is the rather low peak capacity. Therefore it is rather difficult to analyze a large number of analytes in one chromatographic run. Besides, the universal UV detector often lacks sensitivity, especially when analyzing real samples for which wavelengths below 230 nm often cannot be used for quantitation due to matrix interferences. Gas chromatography (GC) usually requires derivatization of the analytes [31]. Although it is possible to minimize the problems of peak tailing due to interactions with active sites in the injector or the column, it is rather tedious to maintain good peak shapes. Some aromatic amines cannot be separated at all by GC. Derivatization is usually done by perfluoracylation of the amines [8,32–36], and the derivatives can be detected by electron-capture detection (ECD) or mass spectrometry (MS). However, many of the reagents in use for perfluoracylation need a strictly anhydrous medium.

The objective of our work was to develop a generally applicable analytical method for the sensitive determination of aromatic amines in water. Therefore, we wanted to develop an alternative derivatization method for aromatic amines which is not restricted to anhydrous media and comparable or even superior in terms of ECD sensitivity. One way to increase the sensitivity of ECD is to introduce heavier halogens instead of fluorine into the molecules because the detector response increases in the order F<Cl<Br<I [37]. In former studies in our group we already investigated the usefulness of halogenation for the analysis of methyl anilines in ammunition wastewater [26,27]. Since the iodination via a Sandmeyer-like reaction appeared especially promising, we decided to study this derivatization method thoroughly for a wide range of aromatic amines. In this part of our contribution, the derivatization reaction, calibration and analysis of real samples is described. In a Part II [38], the developed solid-phase extraction (SPE) method is described in detail.

2. Experimental

2.1. Samples

Real water samples were taken from the drain of a waste disposal site and wells at the former ammunition plant Stadtallendorf, Hessen, Germany. The samples were stored at 4°C in brown glass bottles and analyzed within four weeks. No differences in results were observed when determining the contents of aromatic amines after this period of time.

2.2. Chemicals and reagents

Reference substances were obtained from various suppliers [Aldrich (Steinheim, Germany), Fluka (Neu-Ulm, Germany), Merck (Darmstadt, Germany), Promochem (Wesel, Germany), Mallinckrodt–Baker (Griesheim, Germany) and Riedel-de Haën (Seelze, Germany)] in the highest purity available. The compounds, their CAS numbers and the abbreviations used throughout the text are given in Table 1.

Pentane and sodium nitrite were purchased from Riedel-de Haën, hydriodic acid (ACS reagent, unstabilized, 55%), hydrobromic acid (ACS reagent, 45%) and amidosulfonic acid from Aldrich, hydrochloric acid, sodium sulphite, sodium hydroxide, phosphoric acid, potassium iodide and iodine from Merck, all in the highest purity available.

2.3. Sample preparation and enrichment procedure

Optimization of the solid-phase extraction method is described in detail in Part II [38] of this communication. Immediately before measurements, the samples were adjusted to about pH 9 with a concentrated sodium hydroxide solution (10 mol l^{-1} in water). If necessary, the sample was filtered through 0.45-μm cellulose nitrate membrane filters (Sartorius, Göttingen, Germany) prior to the enrichment with a polystyrene–divinylbenzene [HR-P-phase from Macherey–Nagel (Düren, Germany)].

Prefilled 3-ml polypropylene cartridges with 200 mg of the solid phase, which was kept between two polyethylene frits, were used for all extractions. The solid phase was conditioned two times with 1 ml of methanol, followed by two times 1 ml of acetonitrile, and washed two times with 1 ml of distilled water,

Table 1
Aromatic amines used in this study with abbreviations, CAS Nos. and suppliers

Compound	Abbreviation	CAS No.	Supplier
2-Aminotoluene	2AT	95-53-4	Merck
3-Aminotoluene	3AT	108-44-1	Fluka
4-Aminotoluene	4AT	106-49-0	Fluka
2,3-Diaminotoluene	2,3DAT	2687-25-4	Aldrich
2,4-Diaminotoluene	2,4DAT	95-80-7	Aldrich
2,6-Diaminotoluene	2,6DAT	823-40-5	Aldrich
3,4-Diaminotoluene	3,4DAT	496-72-0	Promochem
2-Amino-3-nitrotoluene	2A3NT	570-24-1	Riedel-de Haën
2-Amino-4-nitrotoluene	2A4NT	99-55-8	Promochem
2-Amino-5-nitrotoluene	2A5NT	99-52-5	Riedel-de Haën
2-Amino-6-nitrotoluene	2A6NT	603-83-8	Aldrich
4-Amino-2-nitrotoluene	4A2NT	119-32-4	Aldrich
2-Amino-4,6-dinitrotoluene	2A4,6DNT	35572-78-2	Promochem
4-Amino-2,6-dinitrotoluene	4A2,6DNT	19406-51-0	Promochem
2,4-Diamino-6-nitrotoluene	2,4DA6NT	6629-29-4	Promochem
2,6-Diamino-4-nitrotoluene	2,6DA4NT	59229-75-3	Synthesized in our group
Aniline	–	62-53-3	Aldrich
1,2-Phenylendiamine	1,2PDA	95-54-5	Merck
1,3-Phenylendiamine	1,3PDA	108-45-2	Aldrich
1,4-Phenylendiamine	1,4PDA	106-50-3	Aldrich
2-Nitroaniline	2NA	88-74-4	Baker
3-Nitroaniline	3NA	99-09-2	Baker
4-Nitroaniline	4NA	100-01-6	Baker
2,4-Dinitroaniline	2,4DNA	97-02-9	Aldrich
2,5-Dinitroaniline	2,5DNA	619-18-1	Aldrich
2,6-Dinitroaniline	2,6DNA	606-22-4	Fluka
3,5-Dinitroaniline	3,5DNA	618-87-1	Riedel-de Haën
o-Anisidine	2MOA	90-04-0	Aldrich
m-Anisidine	3MOA	536-90-3	Aldrich
p-Anisidine	4MOA	104-94-9	Aldrich
2,3-Dimethylaniline	2,3DMA	87-59-2	Aldrich
2,4-Dimethylaniline	2,4DMA	95-68-1	Aldrich
2,5-Dimethylaniline	2,5DMA	95-78-3	Aldrich
2,6-Dimethylaniline	2,6DMA	87-62-7	Aldrich
3,4-Dimethylaniline	3,4DMA	95-64-7	Aldrich
3,5-Dimethylaniline	3,5DMA	108-69-0	Aldrich
N,*N*-Dimethylaniline	N,NDMA	121-69-7	Aldrich
Diphenylamine	DPA	122-39-4	Aldrich
1-Naphthylamine	1NPA	134-32-7	Merck
2-Naphthylamine	2NPA	91-59-8	Aldrich
Benzidine	–	92-87-5	Fluka
2-Aminobiphenyl	2ABP	90-41-5	Aldrich
4-Aminobiphenyl	4ABP	92-67-1	Aldrich
4-Isopropylaniline	4IPA	99-88-7	Aldrich
2,6-Diethylaniline	2,6DEA	579-66-8	Aldrich
2-Ethyl-6-methylaniline	2E6MA	24549-06-2	Aldrich
4-Chloro-*N*-methylaniline	4CNMA	932-96-7	Aldrich
2-Chloroaniline	2CA	95-51-2	Riedel-de Haën
3-Chloroaniline	3CA	108-42-9	Riedel-de Haën
4-Chloroaniline	4CA	106-47-8	Riedel-de Haën
3,4-Dichloroaniline	3,4DCA	95-76-1	Riedel-de Haën
4-Chloro-2-methylaniline	4C2MA	95-69-2	Riedel-de Haën
3-Chloro-4-methylaniline	3C4MA	95-74-9	Riedel-de Haën
3-Chloro-4-methoxyaniline	3C4MOA	5345-54-0	Riedel-de Haën
3-Chloro-4-fluoraniline	3C4FA	367-21-5	Riedel-de Haën
4-Bromoaniline	4BrA	106-40-1	Riedel-de Haën

adjusted to pH 9. The sample was passed through the cartridge with a peristaltic pump, set to a flow-rate of 10 ± 0.2 ml min^{-1}.

After washing with distilled water the cartridges were dried under vacuum for 1 min, and eluted three times with 1 ml methanol–acetonitrile (1:1, v/v). For the subsequent derivatization the eluates were transferred to 24-ml borosilicate glass vials and reduced in volume under a gentle stream of nitrogen at 40°C to less than 0.5 ml (exact measurement of the volume was not necessary).

2.4. Derivatization

A 5-ml volume of water was added to the extract and the solution acidified with 0.2 ml of hydriodic acid. The solution was mixed with 0.5 ml sodium nitrite in water (10 g l^{-1}) and shaken. After a reaction time of 20 min, 1 ml amidosulfonic acid in water (50 g l^{-1}) was added to destroy the surplus of nitrite and the mixture was vigorously shaken for 45 min. The solution was heated for 5 min in a water bath, temperature 100°C, and afterwards cooled down in water to room temperature. The surplus of iodine was destroyed with 0.25 ml of a saturated aqueous solution of sodium sulphite. The solution was basified with 0.5 ml of a 10 mol l^{-1} sodium hydroxide solution and extracted for 15 min with 2 ml of pentane. During the extraction the vials were mechanically shaken. GC–ECD or GC–MS analysis was carried out on aliquots of the extracts which had been filled in autosampler vials.

2.5. Gas chromatography

The GC system consisted of a gas chromatograph HP 5890 II+ and an autosampler unit HP 7673 (both from Hewlett-Packard, Waldbronn, Germany), equipped with an ECD system and a split/splitless injector. Control of the equipment and data acquisition was done with the personal computer program Gynkosoft V 5.32 (Gynkotek, Germering, Germany). Carrier gas was nitrogen, which was further purified using a Megasorb reactor by Messer-Griesheim (Frankfurt, Germany). The column pressure was set to 100 kPa, the make-up-gas flow to approx. 20 ml min^{-1} and the split ratio to 1:120. The temperatures of the injection block and the detector were 250 and 300°C, respectively. The injection volume was 1 or 5 μl. For the separation of the analytes a (5%-phenyl)-methylpolysiloxane column, 30 m×0.25 mm I.D., 0.25 μm d_f (DB5 from J&W, Köln, Germany) was used. The separation was started at an oven temperature of 135°C. After 20.5 min the temperature was raised using a rate of 12.5°C/min to 235°C and then held for another 8.5 min.

The GC–MS investigations were carried out with a VG Trio 2 in the electron impact ionization (EI) mode (70 eV). The same type of capillary column as mentioned above was used. Carrier gas was helium with a column pressure of 50 kPa. The temperatures of the injection block and the transfer line were 250°C and 230°C, respectively. For GC–MS the following temperature program was used: 40°C (1 min), 15°C/min to 270°C. Injection was on-column with 1 μl.

3. Results and discussion

3.1. Derivatization

The derivatization takes place in two steps according to the reaction scheme given in Fig. 1.

The amino group is first diazotized at room temperature with nitrite in an acidic medium. In the second step, the diazo group is substituted by iodine at elevated temperatures.

Fifty-six aromatic amines were investigated with the method using hydriodic acid. The derivatives are given in Table 2. Only six of them were not derivatized at all. For the three *ortho*-diaminobenzene compounds it was expected that they would give no iodinated derivatives since they yield triazonium compounds under the conditions during diazotisation [39]. Two of the electron-poor dinit-

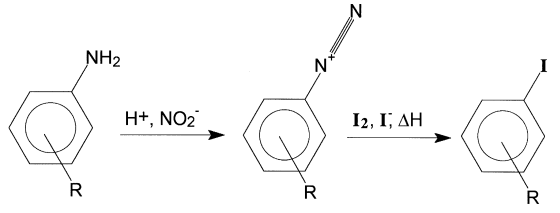

Fig. 1. Reaction scheme of the iodination of aromatic amines.

Table 2
GC and MS data for iodinated anilines

Compound	Iodinated derivative	t_R (min) the derivative	Peak No. in the chromatograms	m/z (M^+)	m/z (100%)
2AT	2-Iodotoluene	3.89	2	218	91
3AT	3-Iodotoluene	3.89	3	218	91
4AT	4-Iodtoluene	3.89	1	218	91
2,3DAT	n.d.	–	–	–	–
2,4DAT	2,4-Diiodotoluene	16.13	4	344	344
2,6DAT	2,6-Diiodotoluene	16.51	6	344	90
3,4DAT	n.d.	–	–	–	–
2A3NT	2-Iodo-3-nitrotoluene	16.98	24	263	263
2A4NT	2-Iodo-4-nitrotoluene	18.64	9	263	90
2A5NT	2-Iodo-5-nitrotoluene	17.71	25	263	263
2A6NT	2-Iodo-6-nitrotoluene	14.09	7	263	90
4A2NT	4-Iodo-2-nitrotoluene	14.59	5	263	90
2A4,6DNT	2-Iodo-4,6-dinitrotoluene	27.82	12	308	89
4A2,6DNT	4-Iodo-2,6-dinitrotoluene	26.48	10	308	89
2,4DA6NT	2,4-Diiodo-6-nitrotoluene	28.41	11	389	89
2,6DA4NT	2,6-Diiodo-4-nitrotoluene	29.62	8	389	389
Aniline	Iodobenzene	2.95	13	204	77
1,2PDA	n.d.	–	–	–	–
1,3PDA	1,3-Diiodobenzene	9.96	14	330	330
1,4-PDA	1,4-Diiodobenzene	9.85	15	330	330
2NA	1-Iodo-2-nitrobenzene	10.76	16	249	249
3NA	1-Iodo-3-nitrobenzene	10.67	17	249	249
4NA	1-Iodo-4-nitrobenzene	10.97	18	249	249
2,4DNA	n.d.	–	–	–	–
2,5DNA	n.d.	–	–	–	–
2,6DNA	1-Iodo-2,6-dinitrobenzene	22.61	50	294	294
3,5DNA	1-Iodo-3,5-dinitrobenzene	26.19	51	294	75
2MOA	1-Iodo-2-methoxybenzene	6.09	21	234	234
3MOA	1-Iodo-3-methoxybenzene	5.86	22	234	234
4MOA	1-Iodo-4-methoxybenzene	6.12	23	234	234
2,3DMA	1-Iodo-2,3-dimethylbenzene	5.94	26	232	232
2,4DMA	1-Iodo-2,4-dimethylbenzene	5.31	27	232	232
2,5DMA	1-Iodo-2,5-dimethylbenzene	5.28	28	232	232
2,6DMA	1-Iodo-2,6-dimethylbenzene	5.46	29	232	232
3,4DMA	1-Iodo-3,4-dimethylbenzene	5.88	30	232	232
3,5DMA	1-Iodo-3,5-dimethylbenzene	5.25	31	232	232
DPA	n.d.	–	–		
1NPA	1-Iodonaphthalin	19.81	33	254	127
2NPA	2-Iodonaphthalin	19.40	34	254	127
2ABP	2-Iodobiphenyl	23.83	35	280	280
4ABP	4-Iodobiphenyl	27.01	36	280	280
Benzidine	4.4′-Diiodobiphenyl	34.87	52	406	406
4IPA	4-Isopropyl-1-iodobenzene	6.36	37	246	104
2,6DEA	2,6-Diethyl-1-iodobenzene	9.60	38	260	260
2E6MA	2-Ethyl-1-iodo-6-methylbenzene	7.20	39	246	246
4CNMA	4-Chloro-1-iodobenzene	4.65	40	238	111
2CA	2-Chloro-1-iodobenzene	5.03	41	238	238
3CA	3-Chloro-1-iodobenzene	4.64	42	238	238
4CA	4-Chloro-1-iodobenzene	4.64	43	238	238
3,4DCA	3,4-Dichloro-1-iodobenzene	8.64	44	272	145
3C4MA	3-Chloro-1-iodo-4-methylbenzene	6.75	45	252	252
4C2MA	4-Chloro-1-iodo-2-methylbenzene	6.74	46	252	252
3C4MOA	3-Chloro-1-iodo-4-methoxybenzene	12.98	47	268	268
3C4FA	3-Chloro-4-fluoro-1-iodobenzene	4.54	48	256	256
4BrA	4-Bromo-1-iodobenzene	6.31	49	282	282

n.d.=No derivatives detectable with GC–ECD or GC–MS.

roanilines did not react. For polynitroanilines it is well known that they do not readily give diazonium salts [40]. Surprisingly, two other dinitroanilines yield the diazonium salt and are subsequently iodinated. One of the latter two is 2,6-dinitroaniline which should reduce the basicity of the amino group more efficiently than 2,5-dinitroaniline which does not react. The reason for this behavior is still unknown.

3.2. Choice of the acid

In a previously published method for the iodination of aminotoluenes [27] we used hydrochloric acid and sodium nitrite to generate nitrous acid as the diazotisation agent. A disadvantage of this method was the formation of chlorinated by-products because of the competition between chloride ions and iodide ions in the substitution reaction. With diamino aromatics the partly chlorinated derivatives were sometimes the main products. To study the effect of the acid used, we compared hydrochloric, hydrobromic, and hydriodic acid and as an acid with a non-nucleophilic corresponding base orthophosphoric acid in the derivatization of six diamino aromatic compounds. In Table 3 the main products with the use of each acid are given.

When the solutions were acidified with hydrobromic acid, derivatization yielded several products with such a low efficiency that identification with GC–MS was not possible except in the case of 1,4PDA which was derivatized to 1-iodo-4-bromobenzene. With orthophosphoric acid, iodination was not completed or formation of a iodoindazole occurred. Only hydriodic acid always gave the diiodo derivatives without formation of side products. Since the sensitivity of the method was enhanced in the presence of hydriodic acid compared to the other acids it was chosen for all further experiments. Another advantage was the formation of iodine in the unstabilized acid, which made subsequent addition of the iodination solution (iodine in potassium iodide solution) obsolete.

3.3. GC separation and GC–MS data

The retention times of the derivatives with GC–ECD and the temperature programme mentioned above together with MS data are given in Table 2.

The mass spectra of the derivatives are easy to interpret. For 32 of the 49 derivatives the M^+ peak is equivalent to the base peak. For 12 derivatives, mainly of amino and aminonitro compounds, the base peak is obtained for the toluene or benzene fragment. In the other cases the base peak represents the radical cation after cleavage of a iodo substituent. Examples for the mass spectra of a mono- and a diiodo derivative are given in Fig. 2.

The main fragments for 1-iodo-2,5-dimethylbenzene besides the M^+ peak (m/z =232) are obtained at m/z =105 (after cleavage of the iodo substituent) and m/z=75 (after subsequent cleavage of the two methyl groups). For 1,3-diiodobenzene the main fragments besides the M^+ peak (m/z=330) are obtained at m/z=203 (cleavage of the first iodo substituent) and m/z=76 (cleavage of the second iodo substituent).

Thirty-three of the derivatives could be separated

Table 3
Main products of iodination with the use of different acids

Compound	Main products with the use of			
	HCl	HBr	HI	H_3PO_4
1,3PDA	1,3-Diiodobenzene	–	1,3-Diiodobenzene	–
1,4PDA	Iodo-chlorobenzene	1-Iodo-4-bromobenzene	1,4-Diiodobenzene	–
2,4DAT	4-Iodo-1H-indazole[a]	–	2,4-Diiodotoluene	4-Iodo-1H-indazole
2,6DAT	6-Iodo-1H-indazole[a]	–	2,6-Diiodotoluene	6-Iodo-1H-indazole
2,4DA6NT	Iodo-chloro-6-nitrotoluene	–	2,4-Diiodo-6-nitrotoluene	n.i.
2,6DA4NT	2,6-Diiodo-4-nitrotoluene	–	2,6-Diiodo-4-nitrotoluene	2-Iodo-4-nitro-6-aminotoluene

[a] Major by-product, main product not identified.
n.i.=Not investigated.
– No derivatives observed with GC–ECD.

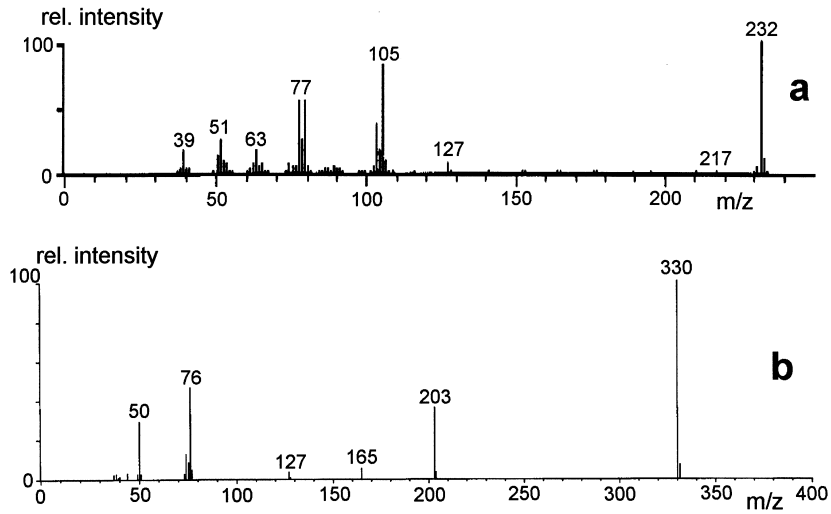

Fig. 2. Mass spectra for a mono and a diiodo derivative. (a) 1-Iodo-2,5-dimethylbenzene, (b) 1,3-diiodobenzene.

in a single chromatographic run in about 35 min on a standard DB5 column, which was not specially deactivated (Fig. 3). The major difficulty is the separation of positional isomers. Some of them could not be separated at all, neither with the use of other columns of different polarity and film thickness nor with lower temperatures (e.g., the three iodotoluenes). To our knowledge, no separation of iodotoluene isomers on capillary GC columns has been published yet, therefore this remains a problem still to be solved.

The effect of the iodination on the peak shape is shown in Fig. 4 with the example of 3,4-dichloroaniline. Although this is not a very polar

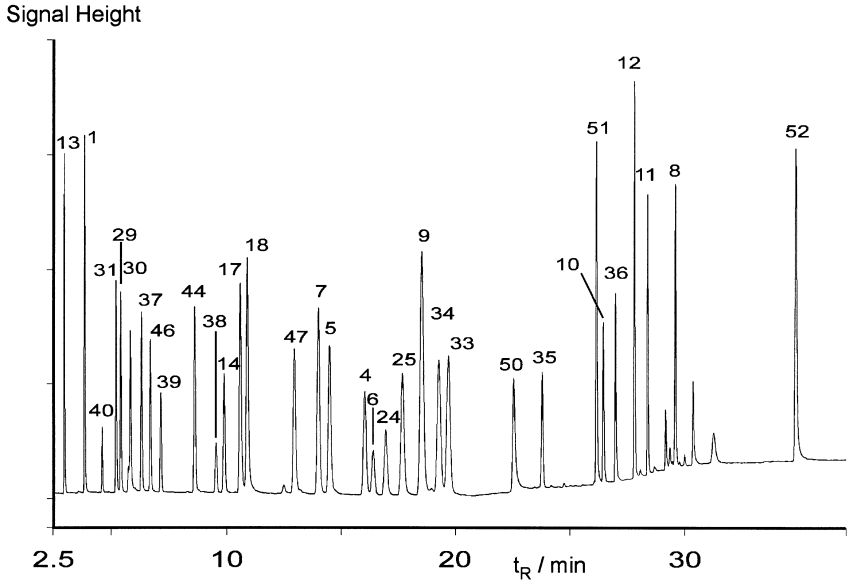

Fig. 3. GC separation of 33 iodinated derivatives of aromatic amines after enrichment on HR-P phase. Detection: ECD. Peak numbers refer to Table 2. Concentration of analytes between 10 and 50 μg l^{-1}, prior to the SPE.

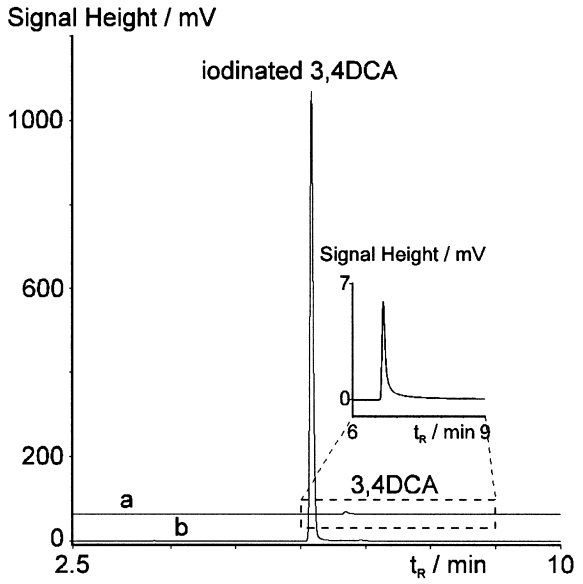

Fig. 4. Comparison of GC–ECD chromatograms of 3,4-dichloroaniline (a) and 3,4-dichloro-1-iodobenzene (b).

aromatic amine, it shows strong peak tailing on a standard column (skewness: 2.27). In contrast, the peak for the derivative is nearly symmetric (skewness: 1.24). In both experiments the same concentration of 3,4-DCA was used. The introduction of iodine increases the ECD response by a factor of about 150. This shows the much greater electron affinity of iodinated aromatic compounds compared to other haloaromatic compounds [37].

3.4. Extraction with pentane

In Table 4 the average peak areas for the derivatives in three parallel extractions are given (measured by GC–ECD). The relative standard deviations (R.S.D.s) are $<10\%$ for all derivatives, for 37 of the 49 derivatives $<6\%$. These values were obtained although all transfer steps with pipettes were done manually, which is sometimes difficult to do reproducible when handling pentane. With automation it should be possible to improve the R.S.D.s further.

Extraction rates were determined with three consecutive extractions of the same solution, all done in triplicate. After the third extraction step the extraction rate was set to 100%. This procedure overestimates only extraction rates of derivatives with very low extraction efficiencies because in these cases the derivatives are not extracted completely after the third extraction.

For the derivatives studied here, extraction rates were always $>90\%$ except for 1-iodo-2,6-dinitroaniline (57%). If an additional halogen substituent was present in the aniline, extraction rates reached 99% after one extraction. After the second extraction step for all analytes $\geq 99\%$ were extracted, again with the exception of 1-iodo-2,6-dinitroaniline (85%). R.S.D.s for the extraction rates were generally $\leq 3\%$.

3.5. Calibration

Calibration was done for the whole analytical procedure, including enrichment with SPE, iodination, extraction of the derivatives with pentane and measurement with GC–ECD. For the calibration, three mixtures of reference substances were prepared because not all of the derivatives could be separated in one chromatographic run. The first mixture comprised 33 aromatic amines, 13 other substances were divided in two more mixtures. All analytes which previously had been detected in real samples were part of reference mixture 1. The concentration of analytes in the mixtures was between 0.4 and 5 μg ml^{-1}. One hundred ml samples of drinking water, which were spiked with different volumes (50, 250, 500, 750, 1000 μl) of the reference mixtures, were adjusted to pH 9 with 0.1 mol l^{-1} sodium hydroxide solution and extracted with the HR-P phase. All spiking experiments were done in duplicate. Table 5 gives the calibration data for 46 analytes. R.S.D.s were between 2.6 and 12%, for most compounds $\leq 8\%$. Considering that the whole analytical procedure is enclosed, these values are rather good. Limits of quantitation (LOQs) were calculated according to Ref. [41] and depend on the method standard deviation. The LOQs were between 0.5 and 7.8 μg l^{-1}, referring to the enrichment of 100 ml sample volume. The LOQs may still be lowered via the enrichment of larger sample volumes, higher injection volumes or different injection techniques (e.g., on-column or large-volume injection).

Table 4
Extraction rates of iodinated anilines in pentane

Derivative of	Average peak area (mV min) after first extraction (n=3)	R.S.D. (%)	Extraction rate (%) after first extraction (n=3)	R.S.D. (%)
2AT	14.3	3	97	0.9
3AT	4.99	7	92	3.1
4AT	10.6	4	96	1.1
2,4DAT	10.4	8	94	2.1
2,6DAT	8.16	7	96	0.5
2A3NT	50.6	4	98	0.8
2A4NT	64.7	3	96	1.6
2A5NT	97.2	3	98	0.2
2A6NT	82.4	5	98	0.3
4A2NT	64.9	2	96	1.4
2A4,6DNT	27.5	6	91	1.8
4A2,6DNT	37.0	6	95	0.4
2,4DA6NT	30.5	5	94	2.1
2,6DA4NT	32.3	7	94	0.8
Aniline	50.7	1	94	2.1
1,3PDA	6.67	7	95	1.1
1,4PDA	7.15	7	96	3.0
2NA	47.4	5	97	0.7
3NA	81.9	3	98	0.4
4NA	67.0	2	97	0.8
2,6DNA	5.00	1	57	0.6
3,5DNA	44.4	3	94	0.6
2MOA	12.2	4	96	0.5
3MOA	32.2	1	97	1.0
4MOA	20.3	8	97	0.3
2,3DMA	14.4	2	93	1.8
2,4DMA	19.0	2	91	1.1
2,5DMA	10.5	2	92	2.0
2,6DMA	12.9	5	96	1.9
3,4DMA	10.0	3	93	1.6
3,5DMA	8.10	4	94	1.6
1NPA	30.3	5	90	1.7
2NPA	34.8	5	91	1.7
2ABP	36.6	7	98	0.1
4ABP	65.4	5	97	2.2
Benzidin	72.1	5	90	1.5
4IPA	24.7	4	91	1.2
2,6DEA	24.3	4	91	1.1
2E6MA	24.4	5	92	0.5
4CNMA	6.78	3	96	0.6
2CA	65.4	5	99	0.3
3CA	72.0	7	99	<0.1
4CA	64.1	7	99	0.2
3,4DCA	44.8	4	99	0.2
3C4MA	34.8	3	99	0.2
4C2MA	71.1	2	99	0.1
3C4MOA	46.9	3	99	0.3
3C4FA	69.4	2	99	0.1
4BrA	55.1	3	99	1.0

Table 5
Calibration data and limits of quantitation (LOQs) for 46 aromatic amines

Derivative of	Intercept (mV min)	Slope [(mV min)/(μg l^{-1})]	s_x (μg l^{-1})	R.S.D. (%)	LOQ (μg l^{-1})
2AT	0.010	0.031	0.843	8.6	2.8
3AT	−0.035	0.033	0.937	11	3.3
4AT	−0.019	0.032	0.744	7.5	2.4
2,4DAT	−0.022	0.019	0.661	7.0	2.2
2,6DAT	−0.007	0.018	0.975	9.5	3.2
2A3NT	0.009	0.011	0.616	12	2.0
2A4NT	0.011	0.061	0.326	3.2	1.1
2A5NT	0.017	0.060	0.230	4.5	0.75
2A6NT	0.006	0.068	0.194	3.6	0.63
4A2NT	0.011	0.047	0.254	5.1	0.83
2A4,6DNT	−0.007	0.034	0.182	3.6	0.59
4A2,6DNT	−0.001	0.027	0.123	2.4	0.40
2,4DA6NT	0.001	0.030	0.423	8.3	1.4
2,6DA4NT	0.012	0.039	0.500	9.1	1.6
Aniline	−0.011	0.052	0.420	8.4	1.4
1,3PDA	−0.015	0.004	2.38	9.3	7.8
2NA	0.010	0.067	0.362	7.1	1.2
3NA	0.008	0.057	0.208	4.1	0.68
4NA	0.013	0.088	0.142	2.8	0.46
2,6DNA	−0.013	0.009	1.52	6.0	5.0
3,5DNA	−0.016	0.053	0.131	2.7	0.43
2MOA	−0.029	0.052	0.491	6.3	1.7
3MOA	−0.015	0.046	0.250	6.5	0.87
4MOA	−0.023	0.037	1.09	11	3.6
2,3DMA	0.021	0.035	1.09	11	3.6
2,4DMA	−0.055	0.052	0.863	11	3.0
2,5DMA	0.018	0.030	1.21	12	4.0
2,6DMA	0.007	0.029	0.946	9.5	3.1
3,4DMA	−0.004	0.011	0.955	9.3	3.1
3,5DMA	−0.005	0.015	0.750	7.5	2.5
1NPA	0.007	0.026	0.451	4.0	1.5
2NPA	−0.003	0.029	0.348	3.4	1.1
2ABP	−0.001	0.023	0.148	2.9	0.49
4ABP	−0.007	0.037	0.388	7.8	1.3
Benzidine	−0.002	0.031	0.211	3.8	0.69
4IPA	−0.017	0.031	0.653	6.9	2.1
2,6DEA	0.003	0.027	0.955	9.4	3.1
2E6MA	0.005	0.027	0.920	8.9	3.0
4CNMA	0.002	0.003	1.09	4.6	3.6
2CA	−0.050	0.082	0.504	12	1.8
3CA	0.003	0.064	0.152	6.4	0.50
3,4DCA	0.008	0.048	0.341	6.6	1.1
3C4MA	0.003	0.065	0.157	7.1	0.51
4C2MA	−0.004	0.060	0.364	7.2	1.2
3C4MOA	−0.008	0.070	0.230	4.6	0.75
4BrA	−0.022	0.074	0.338	8.7	1.2

s_x = Method standard deviation, as defined in Ref. [41].

3.6. Application to water samples

The method was first tested with water samples from the former ammunition plant in Stadtallendorf/Hessen, which were previously studied in our group [26,27]. In Fig. 5 a typical GC–ECD chromatogram

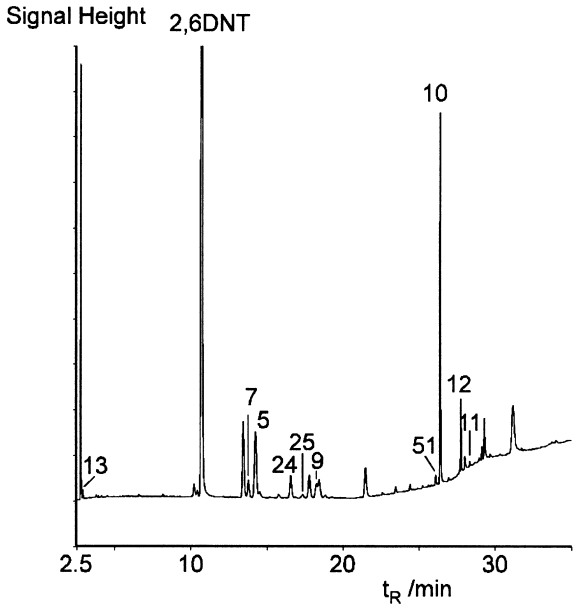

Fig. 5. GC–ECD chromatogram of a ground water sample from Stadtallendorf. Peak numbers refer to Table 2.

of a ground water sample is shown. The sample contained mainly aminonitrotoluenes from the microbial reduction of nitrotoluene explosives. Besides, aniline and 3,5-dinitroaniline were found in concentrations <1 μg l^{-1}. At $t_R=10.34$ min 2,6-dinitrotoluene is eluted. Table 6 summarizes the concentrations of aromatic amines in two representative ground water samples. In addition to ammunition wastewater, samples from an industrial sewage plant, from a former gas plant and leachate water from a former landfill were investigated. Although the matrix of the samples was rather complex the method gave reliable results. The investigation of these samples with two different derivatization methods will be described in detail in a later paper.

Table 6
Aromatic amines in ground water samples from Stadtallendorf

Compound	Concentration in sample (μg l^{-1})	
	ASB 1	ASB 2
Aniline	0.41	0.41
2A6NT	0.63	0.37
4A2NT	4.3	0.35
2A3NT	5.7	2.8
2A5NT	0.23	0.26
2A4NT	0.25	n.d.
3,5DNA	0.60	0.86
4A2,6DNT	18[a]	22[a]
2A4,6DNT	2.6	3.2
2,4DA6NT	0.22	0.29

[a] Concentration exceeding the calibration range (and extrapolated).
n.d.=Not detectable.

4. Conclusions

Iodination of aromatic amines is generally applicable for the derivatization of a wide range of aromatic amines. The derivatives can be separated with GC and detected sensitively by ECD. The investigation of real samples revealed the potential of the method in environmental analysis. Further investigations will concentrate on the use of atomic emission detection for a more selective detection and the application to an even wider range of water samples.

Acknowledgements

We gratefully acknowledge the financial support of the German Research Foundation (DFG), and we thank Jörg Bettelhäuser and Kathrin Spiegel for providing ground water samples, and Danny Stingel for the preparation of 2,6-diamino-4-nitrotoluene.

References

[1] P.F. Vogt, J.J. Geralis, in: Ullmann's Encyclopedia of Industrial Chemistry, Vol. A 2, 5th ed., VCH, Weinheim, 1985, p. 37.
[2] J.C. Spain (Ed.), Biodegradation of Nitroaromatic Compounds, Plenum Press, New York, 1995.
[3] R.A. Larson, E.J. Weber, Reaction Mechanisms in Environmental Organic Chemistry, Lewis, Boca Raton, FL, 1994.
[4] E.A. Clarke, R. Anliker, in: O. Hutzinger (Ed.), Handbook of Environmental Chemistry, Vol. 3A, Springer, Heidelberg, 1980, p. 181.
[5] G.L. Baughman, E.J. Weber, Environ. Sci. Technol. 28 (1994) 267.

[6] K.H. Domsch, Pestizide im Boden, VCH, Weinheim, 1992.
[7] E.M. Lores, D.W. Bristol, R.F. Moseman, J. Chromatogr. Sci. 16 (1978) 358.
[8] A. de Kok, Y.J. Vos, C. van Garderen, T. de Jong, M. van Opstal, R.W. Frei, R.B. Geerdink, U.A.Th. Brinkman, J. Chromatogr. 288 (1984) 71.
[9] G. Skarping, M. Dalene, P. Lind, J. Chromatogr. A 663 (1994) 199.
[10] R.C.C. Wegman, G.A.L. de Korte, Int. J. Environ. Anal. Chem. 9 (1981) 1.
[11] B. Scholz, N. Palauschek, Fresenius' Z. Anal. Chem. 331 (1988) 282.
[12] V. Coquart, M.-C. Hennion, Chromatographia 37 (1993) 392.
[13] D. Bethmann, F. Sacher, H.-J. Brauch, Vom Wasser 87 (1996) 171.
[14] H. Börnick, V. Hultsch, T. Grischek, D. Lienig, E. Worch, Vom Wasser 87 (1996) 305.
[15] S. Müller, J. Efer, W. Engewald, Fresenius' J. Anal. Chem. 357 (1997) 558.
[16] S. Paul, D. Lienig, E. Worch, Vom Wasser 88 (1997) 273.
[17] R. Haas, Konzepte zur Untersuchung von Rüstungsaltlasten, Erich Schmidt, Berlin, 1992.
[18] K. Levsen, P. Mussmann, E. Berger-Preiss, A. Preiss, D. Volmer, G. Wünsch, Acta Hydrochim. Hydrobiol. 21 (1993) 153.
[19] P. Mussmann, A. Preiss, K. Levsen, G. Wünsch, Vom Wasser 82 (1994) 79.
[20] P.M. Gates, E.T. Furlong, T.F. Dorsey, M.R. Burkhardt, Trends Anal. Chem. 15 (1996) 319.
[21] U. Lewin, J. Efer, W. Engewald, J. Chromatogr. A 730 (1996) 161.
[22] A. Preiss, K. Levsen, E. Humpfer, M. Spraul, Fresenius' J. Anal. Chem. 356 (1996) 445.
[23] L. Wennrich, U. Lewin, J. Efer, W. Engewald, Vom Wasser 86 (1996) 341.
[24] U. Lewin, L. Wennrich, J. Efer, W. Engewald, Chromatographia 45 (1997) 91.
[25] K. Spiegel, T. Welsch, Fresenius' J. Anal. Chem. 357 (1997) 333.
[26] T.C. Schmidt, R. Haas, K. Steinbach, E. von Löw, Fresenius' J. Anal. Chem. 357 (1997) 909.
[27] R. Haas, T.C. Schmidt, K. Steinbach, E. von Löw, Fresenius' J. Anal. Chem. 359 (1997) 497.
[28] T.C. Schmidt, D. Meinzer, L. Kaminski, E. von Löw, G. Stork, Chromatographia 46 (1997) 501.
[29] T.S. Scott, Carcinogenic and Chronic Toxic Hazards of Aromatic Amines, Elsevier, Amsterdam, 1962.
[30] L. Fishbein, Toxicol. Environ. Chem. Rev. 3 (1980) 145.
[31] H. Kataoka, J. Chromatogr. A 733 (1996) 19.
[32] D.E. Bradway, T. Shafik, J. Chromatogr. Sci. 15 (1977) 322.
[33] G. Skarping, L. Renman, B.E.F. Smith, J. Chromatogr. 267 (1983) 315.
[34] H.-B. Lee, J. Chromatogr. 457 (1988) 267.
[35] P. Mussmann, R. Eisert, K. Levsen, G. Wünsch, Acta Hydrochim. Hydrobiol. 23 (1995) 13.
[36] M. Longo, A. Cavallaro, J. Chromatogr. A 753 (1996) 91.
[37] K. Blau, J.M. Halket (Eds.), Handbook of Derivatives for Chromatography, Wiley, Chichester, 1993.
[38] M. Less, T.C. Schmidt, E. von Löw, G. Stork, J. Chromatogr. A 810 (1998) 173.
[39] E. Reitermayer, Ph.D. Thesis, Philipps University, Marburg, 1991.
[40] L.F. Fieser, M. Fieser, Organische Chemie, 2nd ed., VCH, Weinheim, 1968.
[41] DIN 38402, Part 51, in: Deutsche Einheitsverfahren zur Wasser-, Abwasser- und Schlammuntersuchung, Beuth, Berlin, 1986.