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Derivatization of aromatic amines for analysis in ammunition wastewater

II: Derivatization of methyl anilines by iodination with a Sandmeyer-like reaction

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Abstract An analytical method for the determination of aromatic amines in water is introduced that uses iodination with a Sandmeyer-like reaction to replace the amino group by iodine in aqueous solution. The non-polar derivatives are extracted with pentane or toluene, separated with gas chromatography and sensitively detected with an ECD. Thirteen major metabolites of nitroaromatic explosives were investigated. The method was used to analyze these metabolites in water samples from the site of a former ammunition plant. The results are compared with the derivatization of aromatic amines via bromination of the aromatic ring.

Introduction

The problems of separation and detection of aromatic amines in environmental samples and general derivatization approaches are thoroughly discussed in [1].

The method used in routine analysis of polar aromatic amines in water samples is diazotization and coupling with N-(1-naphthyl)ethylene diamine (NEDA) to give coloured azo dyes. The quantitative analysis (sum of aromatic amines) is done by photometrical detection and comparison with the reference analyte p-nitraniline [2]. Reitermayer [3] separated the yielding azo dyes with HPLC to identify polar aromatic amines in drain water.

Instead of preparing the azo dye, replacement of the azo-group with halogens in a Sandmeyer or Sandmeyer-

like reaction is another possibility of derivatization. The use of iodine is advantageous because no copper salt is required as catalyst. Furthermore, halogenation with iodine should allow the most sensitive detection with GC/ECD in comparison with other halogens [4]. In environmental analysis this derivatization method was rarely used. By now, only Kußmaul et al. [5, 6] and Kofmann et al. [7] used a Sandmeyer-like reaction to replace the amino group by iodine to detect degradation products of phenyl-urea and phenylamide herbicides in water.

We modified the described derivatization method to introduce a method for routine analysis. Thirteen major metabolites of mononitrotoluenes, dinitrotoluenes and trinitrotoluene were investigated.

Experimental

Materials

2-Amino-4-nitrotoluene, 2-amino-6-nitrotoluene, 4-amino-2-nitrotoluene, 2,4-diaminotoluene and 2,6-diaminotoluene were purchased from Aldrich (Steinheim, Germany), 4-aminotoluene and 3-aminotoluene from Fluka (Neu-Ulm, Germany) and 2-aminotoluene from Merck (Darmstadt, Germany). 2-Amino-4,6-dinitrotoluene, 4-amino-2,6-dinitrotoluene and 2,4,6-triaminotoluene were supplied by Promochem (Wesel, Germany). 2,4-Diamino-6-nitrotoluene and 2,6-diamino-4-nitrotoluene were synthesized in our group. The compounds, their CAS-numbers and the abbreviations used throughout the text are given in Table 1.

Pentane, toluene, amidosulfonic acid and hydrochloric acid were purchased from Riedel-de-Haën (Seelze, Germany), sodium sulfite, sodium nitrite, sodium hydroxide, potassium iodide and iodine from Merck (Darmstadt, Germany).

Real water samples were taken from the drain of a waste disposal site and a well at the former ammunition plant Stadtallendorf, Hessen, Germany. The samples were filled in brown 2.5 L glass bottles and stored at 6°C. They were analyzed within four weeks.

Apparatus

The gas chromatographic system consisted of a gas chromatograph HP 5890 II+ and autosampler unit HP 7673 (both from Hewlett-Packard, Waldbronn, Germany), equipped with an ECD and a

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Table 1 Investigated compounds with corresponding abbreviations, CAS-nos. and peak-nos. in chromatograms

Compound	Abbreviation	CAS-no.	Peak-no.
2-Aminotoluene	2AT	95-53-4	1
3-Aminotoluene	3AT	108-44-1	1
4-Aminotoluene	4AT	106-49-0	1
2,4-Diaminotoluene	2,4-DAT	95-80-7	2
2,6-Diaminotoluene	2,6-DAT	823-40-5	7
2-Amino-4-nitrotoluene	2A4NT	99-55-8	5
2-Amino-6-nitrotoluene	2A6NT	603-83-8	3
4-Amino-2-nitrotoluene	4A2NT	119-32-4	4
2-Amino-4,6-dinitrotoluene	2A4,6DNT	35572-78-2	10
4-Amino-2,6-dinitrotoluene	4A2,6DNT	19406-51-0	9
2,4-Diamino-6-nitrotoluene	2,4-DA6NT	6629-29-4	6
2,6-Diamino-4-nitrotoluene	2,6-DA4NT	59229-75-3	8
2,4,6-Triaminotoluene	TAT	88-02-8	-

split/splitless injector. Control of the equipment and data acquisition was done with the PC program Gynksoft 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.

For the extractions the mechanical shaker SM (Bühler) was used.

The UV spectra of the iodinated products were recorded with a diode array detector UVD 340-S and the following HPLC equipment: M 480 pump, on-line degasser GT-103 and auto sampler GINA 50 (all Gynkotek, Germering, Germany). To separate the products from impurities and byproducts, a RP-18 column was used (25 cm × 3 mm i.d.). The analytes were eluted with methanol/water 60/40 (v/v).

The GC/MS investigations were carried out with a VG Trio 2 in the EI mode (70 eV). The separation column was a J&W DB5-column (30 m, 0.25 mm i.d., 0.25 mm d_f ; Fisons, Mainz, Germany). Carrier gas was helium with a column pressure of 50 kPa. For GC/MS the following temperature program was used: 40°C (1 min), 10°C/min to 250°C. Injection was on column with 1 μ L.

Enrichment and derivatization procedure

Depending on the expected contents of aromatic amines in the investigated water samples, 100 mL to 1 L water were acidified with 1 mL hydrochloric acid (conc. = 250 g/L). The acidic solution was reduced in volume to approximately 5 mL in a rotary evaporator at a water bath temperature of 70°C and in vacuum. Another 1 mL HCl was added and the solution transferred to a 24 mL vial. The solution was mixed with 0.5 mL sodium nitrite in water (conc. = 10 g/L) and shaken. After a reaction time of 20 min, 1 mL amidosulfonic acid in water (conc. = 50 g/L) was added to destroy the surplus of nitrite. The mixture was vigorously shaken for 60 min. Then 0.5 mL iodination solution, containing 50 g iodine and 100 g potassium iodide in 1 L water, was added. The mixture was heated for 5 min in a water bath with a temperature of 100°C and afterwards cooled down in water to room temperature. The surplus of iodine was destroyed with 100 μ L of a saturated aqueous solution of sodium sulphite. The solution was basified with 1.5 mL of a sodium hydroxide solution (conc. = 10 mol/L) and extracted for 15 min with 2 mL of pentane or toluene. During the extraction the vials were mechanically shaken. GC/ECD analysis was carried out on aliquots of the extracts which had been filled in autosampler vials. For HPLC investigations the solvent was blown off in a gentle stream of nitrogen and the dry residue dissolved in 250 μ L of methanol/water (1/1, v/v).

Gas chromatographic conditions

The temperatures of the injection block and the detector were 250 and 300°C, respectively. The injection volume was 1 or 5 μ L. For

the separation of the analytes a J&W DB5-column, 30 m, 0.25 mm i.d., 0.25 mm d_f (Fisons, Mainz, Germany) was used. The separation was started at an oven temperature of 150°C. After 11 min the temperature was raised using a rate of 10°C/min to 230°C and then held for another 6 min.

Results and discussion

Derivatization products

The iodination procedure is quantitative. No original analytes were detected after derivatization. Iodination gives only one product for the aminotoluenes and aminonitrotoluenes. As would be predicted from the present knowledge of the Sandmeyer and similar reactions, the corresponding iodotoluenes and idonitrotoluenes were generated.

For aminodinitrotoluenes, diaminonitrotoluenes and diaminotoluenes several side reactions occurred. Deamination was observed for all compounds of the three classes. The following products were identified by GC/MS:

- 2,4-Dinitrotoluene from 2A4,6DNT
- 2,6-Dinitrotoluene from 4A2,6DNT
- 2A6NT and 4A2NT from 2,4-DA6NT
- 2A4NT from 2,6-DA4NT.

The concentrations of the byproducts are about 1–2% of the concentration of the original analytes.

For all diamino compounds, substitution of one amino-group with chlorine was observed as a side reaction, when hydrochloric acid was used to acidify the samples. For 2,4-DA6NT the corresponding iodo-chloro-6-nitrotoluene was the main peak in GC/MS-studies, whereas for 2,6-DA4NT the diiodo derivative was found to be the most prominent peak. Substitution patterns for the iodo-chloro-compounds could not be identified. For the diaminotoluenes several products were obtained, which could not be identified so far. At least the MS-spectra show that no amino group remained in the derivatives of all diamino compounds.

To eliminate chlorination as side reaction, the diaminotoluenes were acidified with 100 mL concentrated phosphoric acid instead of hydrochloric acid. Under these con-

ditions 2,4-DAT and 2,6-DAT yield only one product each, 6-iodo-indazole and 4-iodo-indazole, respectively. Formation of indazoles from ortho-substituted anilines is a well known reaction in preparative organic chemistry, but the iodo derivatives were not described in literature so far. The indazole formation will be further investigated.

2,4,6-Triaminotoluene is the only compound under investigation that does not give any detectable iodinated product, although diazotation takes place, as can be seen in the following coupling reaction with NEDA, which gives an azo dye.

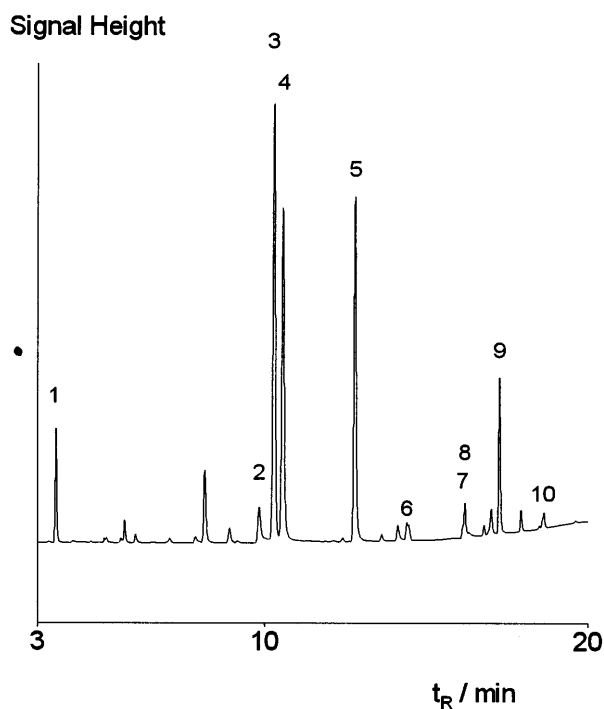


Fig. 1 Chromatogram of a standard mixture of iodinated amino- and aminonitrotoluenes. Derivatives of: 1: 2AT (25), 3AT (25) and 4AT (5), 2: 2,4-DAT (25), 3: 2A6NT (5), 4: 4A2NT (5), 5: 2A4NT (5), 6: 2,4-DA6NT (25), 7: 2,6-DAT (100), 8: 2,6-DA4NT (25), 9: 4A2,6DNT (25), 10: 2A4,6DNT (25). Concentration of analytes in ng/ μ L given in brackets

Table 2 Iodinated derivatives (only greatest peak) with retention times and detection limits (measured with GC/ECD)

Mother compound	Derivative (derived from mass spectrometric results)	tR/min	Detection limit (ng/ μ L)	Extraction rate/%
2AT	2-Iodotoluene	3.58	1.0	95
3AT	3-Iodotoluene	3.58	1.5	95
4AT	4-Iodotoluene	3.58	0.1	95
2,4-DAT	until now not identified ^b	9.86	1.0	90
2,6-DAT	until now not identified ^b	16.13	10	28
2A4NT	2-Iodo-4-nitrotoluene	12.64	0.02	95
2A6NT	2-Iodo-6-nitrotoluene	10.38	0.02	95
4A2NT	4-Iodo-2-nitrotoluene	10.65	0.03	95
2A4,6DNT	2-Iodo-4,6-dinitrotoluene	18.63	0.4	91
4A2,6DNT	4-Iodo-2,6-dinitrotoluene	17.28	0.05	91
2,4-DA6NT	Iodo-chloro-6-nitrotoluene ^a	14.45	2.5	100
2,6-DA4NT	2,6-Diiodo-4-nitrotoluene	16.20	1	100

^aThe position of the chloro- and iodo-substituent could not be elucidated from the MS data
^bSee text for details

The iodination procedure is inert against methanol content of the solution. Standard mixtures were compared that had either been dissolved in 2 mL methanol or of which methanol had been blown off with nitrogen prior to the iodination reaction. No significant differences were observed in the obtained chromatograms.

The extraction was usually done with pentane. Extraction rates exceeded 90% in a single extraction step for all analytes except the derivative of 2,6-DAT (extraction rate 28%).

Toluene shows a similar extraction efficiency, but it is harder to remove in the blow-off step.

To test possible interferences by nitroaromatic compounds we treated a reference mixture of nitroaromatics (2,4,6-trinitrotoluene, 2,4-dinitrotoluene, 2,6-dinitrotoluene, 3,4-dinitrotoluene, 2-nitrotoluene, 3-nitrotoluene, 4-nitrotoluene) as described in the experimental section. Only the dinitrotoluenes were found under these conditions, for the other nitro compounds extraction rates between 0 and 10 % were obtained.

Gas chromatography with electron-capture detection

Separation of most of the iodinated compounds was achieved with the temperature program described above. In Fig. 1 a chromatogram of a reference mixture is shown. The critical analyte pair consists of the derivatives of 2,6-DAT and 2,6-DA4NT (resolution: 0.6). As can be seen in the mentioned chromatogram, they are only partially resolved. The derivatives of 2-aminotoluene, 3-aminotoluene and 4-aminotoluene are not separated and give only one peak. A partial separation was achieved when an initial temperature of 90°C had been applied. To obtain complete separation of the iodotoluene isomers, the following GC columns were tested: DB5, DB17, DB1701, DB624 and Supelcowax. The column dimensions were 30 m, 0.25 mm i.d., 0.25 mm d_f for all columns except the DB624, which was 60 m long. With all of these columns a complete separation of the three compounds was not possible.

Table 2 shows retention times, the applied temperature program and the respective detection limits for the main

Table 3 UV-maxima and GC/MS-data of the derivatives listed in Table 2

Derivative of	UV-max. 1/nm	UV-max. 2/nm	UV-max. 3/nm	m/z (100 %)	m/z (M+)
2AT	n.d.	n.d.	n.d.	91	218
3AT	n.d.	n.d.	n.d.	91	218
4AT	n.d.	n.d.	n.d.	91	218
2,4-DAT	235.4	255.8	277.1 (s)	— ^a	— ^a
2,6-DAT	246.3	289.2	381.5	— ^a	— ^a
2A4NT	229.8	277.5	—	90	263
2A6NT	229.0	—	—	90	263
4A2NT	228.1	—	—	90	263
2A4,6DNT	221.6	254.4	—	89	308
4A2,6DNT	220.7	325.0	—	89	308
2,4-DA6NT	229.1 (s)	339	—	89	297
2,6-DA4NT	256.2	285.0 (s)	347.0	389	389

(s): shoulder

n.d.: not determined

^a until now, derivatives are not identified

products detected with an ECD. The detection limits are estimated from the peak height with the 3σ -method and were obtained with dilute mixtures of the reference substances. For real samples, the detection limits can be further improved by the extraction step (theoretical enrichment factor: 250 to 2500) and an injection volume of 5 μ L (factor: 5).

The detection limits of the 4-iodo-derivatives (4-iodotoluene, 4-iodo-2-nitrotoluene and 4-iodo-2,6-dinitrotoluene) are 10 times lower than the detection limits of the corresponding 2-iodo-derivatives.

The peak-tailing of the iodinated derivatives is lower than the tailing of the original analytes (if they can be detected at all with GC/ECD). For example the peak base width of the aminodinitrotoluenes is, due to the strong tailing, about two times higher than for the corresponding iodo-derivatives.

UV and MS data

UV data of the derivatives are given in Table 3. Only the derivatives of the diamino compounds show remarkable distinctions in their UV spectra in comparison with the spectra for the corresponding amino compounds. Despite the good accordance of UV spectra for derivatized and underivatized monoamino compounds the iodinated products always lack the typical broad maximum at 360 nm. As expected, retardation of the iodotoluenes was in general much stronger than for the aminotoluenes. After derivatization two unknown compounds were detected in chromatographic runs for several reference substances. Compound 1 was obtained for 2,4-DA6NT and 2A4,6DNT, compound 2 for these two and in much smaller amounts for 2,6-DA4NT and 4A2,6DNT. In Fig. 2 the UV spectra of these compounds are presented.

MS data of the derivatives are presented in Table 3. Except for 2,6-diiodo-4-nitrotoluene the most prominent mass fragment (100%-peak) constitutes the toluene rest. Depending on the number of substituents this fragment is obtained between $m/z = 89$ and 91. The molecule peak (M^+) is present in all cases, allowing the easy derivation of the molecule mass. If a nitro group in ortho-position to the methyl group is present, fragmentation of OH^\bullet was

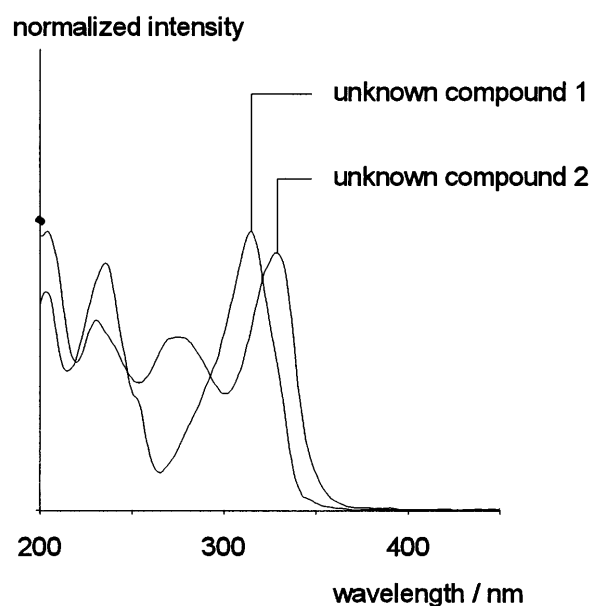


Fig. 2 UV spectra of two unknown compounds generated during the iodination procedure of several analytes

observed. For the two diaminotoluenes several peaks were obtained with GC/MS after derivatization, which could not be identified.

Analysis of real samples

In Fig. 3 a chromatogram for a real sample (ASB I) is given. It is a water sample of a well situated near an ammunition waste landfill. As can be seen, no nitrotoluenes are present in concentrations above the detection limit. In the original extract without derivatization they are the main analytes. The following analytes were detected (concentration in brackets): 2A4NT (6.6 μ g/L), 2A6NT (2.2 μ g/L), 4A2NT (13 μ g/L), 2A4,6DNT (196 μ g/L) and 4A2,6DNT (56 μ g/L). If one uses the absorption of the corresponding azo dyes with N-(1-naphthyl)ethylenediamine (NEDA) referred to the absorption of p-nitraniline [3], the sum of these aromatic amines is 56.6 μ g/L. Photometrically a value of 20.6 μ g/L was determined in rou-

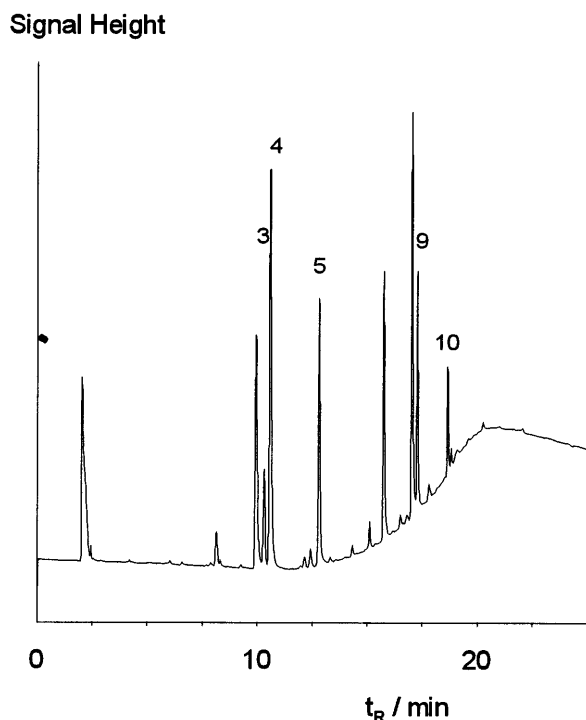


Fig. 3 Chromatogram of a water sample from a well near an ammunition waste landfill. Peak identification as in Fig. 1

tine analysis. This means that either some of the found amines were destroyed during the enrichment for the photometrical method or that the aminodinitrotoluenes content was overestimated with the iodination method. At present we undertake comparative studies with the bromination method described in [1] to find out if there is a systematic error in one of the derivatization methods or the photometrical routine method.

Comparison of iodination and bromination of aromatic amines

Iodination yielded more side products than bromination. Only for the monoamino and monoaminonitro compounds single products were obtained with iodination, whereas bromination always gave single products except for 2,6-DA4NT and 4A2NT. The brominated derivatives were easy to identify by mass spectrometry due to the bromine isotope pattern and to the sole substitution of hy-

drogen. For the iodinated derivatives sometimes no identification was possible so far. The chosen iodination procedure is selective for aromatic amines, whereas other analytes (e.g. BTXE aromatics) are brominated as well. The derivatization procedure (without enrichment and extraction) for bromination needs about 25 min to complete, for iodination 90 min are needed, although most of the time the samples are shaken with a mechanical shaker. The extraction with pentane is more effective in the case of the iodo compounds (except for 2,6-DAT), hence only one extraction step seemed to be sufficient. Separation needed 30 min for brominated products and 20 min for iodinated ones, but in the latter case separation of the derivatives of the toluidines was not possible and the resolution for the critical peak pair was much worse. The detection limits (LOD) were comparable for 2A4,6DNT and 4A2,6DNT, for the aminonitrotoluenes iodination gave 3 to 4 times lower LOD, for the other compounds bromination was more sensitive (LOD 2 to 10 times lower). With both methods it was not possible to detect TAT.

Conclusions

The comparison of the derivatization of aromatic amines with iodination and bromination shows that they supplement each other in terms of sensitivity and selectivity. Both methods were suitable to analyze contents of methyl anilines in well and leachate water from the site of a former ammunition plant. However, the use of these methods is not restricted to ammunition waste water. First results were obtained for the investigation of leachate water from a former garbage dump [8].

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