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Derivatization of aromatic amines for analysis in ammunition wastewater

I. Derivatization via bromination of the aromatic ring

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Abstract A new derivatization method for aromatic amines is presented based on the bromination of the aromatic ring system in an acetic acid medium to yield bromo derivatives. The derivatization method is very simple to perform and overcomes several problems common in the analysis of polar aromatic amines. The derivatives are easy to extract with pentane and can be separated with gas chromatography. Due to the introduction of the electron withdrawing bromo substituents sensitive detection using an electron capture detector is possible. The method was used to investigate the contents of amino- and aminonitrotoluenes in water samples from the site of a former ammunition plant.

Introduction

Amino- and aminonitroaromatics are used as oxidation hair dyes [1] and herbicides [2]. They are even more important as precursors or metabolites of several classes of compounds, e.g. pesticides like phenylureas, anilides, carbamates and nitro phenols [3], toluene diisocyanates [4], azo dyes [5] and nitroaromatic explosives [6, 7].

Nitro and amino compounds are found in the leachate water from the area of former ammunition plants [8]. In recent studies of the bioremediation of 2,4,6-trinitrotoluene the 2,4-diamino and the 2,6-diamino metabolite were found [9].

For nitroaromatic explosives extraction of water samples with toluene, separation with gas chromatography and detection with electron capture detector (GC/ECD) is a method used in routine analysis in many laboratories. But there are several problems in measuring the contents of aromatic amines with the same method:

1. Amino- and diaminotoluenes are not detectable
2. Aminodinitro- and diaminonitrotoluenes are difficult to separate
3. All aminonitrotoluenes show strong tailing and decreased sensitivity in comparison with nitrotoluenes
4. The polarity of the analytes is responsible for the low extraction rates
5. In real water and soil samples from former ammunition plants nitroaromatic compounds are usually present in much higher contents than their aminometabolites, therefore removal of nitrotoluenes prior to the analysis is desirable.

Some, but not all, of these problems can be avoided by choosing another detection method (e.g. GC/MS) or chromatographic technique (HPLC). To overcome the problems and to develop a method for the determination of amino- and aminonitrotoluenes with GC/ECD, derivatization of aromatic amines seemed to be most promising.

Derivatization of amines (including aromatic amines) is already thoroughly discussed elsewhere [10, 11]. The two most common approaches for the subsequent GC analysis are perfluoracylation and silylation. Applications of these methods in environmental analysis of aromatic amines are found in [7, 12] and [7, 13], respectively.

For a sensitive detection of aromatic amines with GC/ECD it is essential to introduce electron withdrawing groups into the analyte. As mentioned above this can be done by perfluoroacylation. Since the detector sensitivity decreases in the order $I > Br > Cl > F$ it is

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necessary to introduce several fluoro groups. Introduction of other halogens for improved gas chromatographic analysis of aromatic amines was described for iodine [14–16] and bromine [16]. Since a simple and rugged method suitable for routine analysis was needed, the best known bromination method for aromatic amines in organic syntheses [17–19] was chosen, where bromine in glacial acetic acid is the reagent. The aim of this study was to investigate the usefulness of this easy derivatization method for 13 major metabolites of nitroaromatic explosives (see Table 1). In a forthcoming paper we will present the results of a derivatization method with iodine and compare the two methods.

Table 1 Investigated compounds with corresponding abbreviations and CAS number

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

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 and glacial acetic acid were purchased from Riedel-de-Haën (Seelze, Germany), sodium sulfite, sodium hydroxide and bromine from Merck.

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. In general 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 split/splitless injector. Control of the equipment and data acquisition was done with the PC program Gynkosoft 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 100 kPa.

For the extractions the mechanical shaker SM from Bühler was used.

The UV spectra of the brominated 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 by-products 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.

Enrichment and derivatization procedure. As no suitable extraction method for the enrichment of the investigated aromatic amines from water samples exists, the enrichment method used for the determination of the sum of diazotizable aromatic amines was applied [20]. Depending on the expected content of aromatic amines, 100 mL to 1 L of the water sample were acidified with 1 mL of 25% HCl and evaporated nearly to dryness at 70 °C in vacuum. The residue was dissolved in 3 mL glacial acetic acid. To reduce water and energy consumption and loss of volatile and labile amines, now a solid-phase extraction method with an ion-pair reagent at low pH is under investigation.

The acidic solution was filled in a 24 mL vial. 250 µL of the bromination solution (consisting of 50 mL bromine in 200 mL glacial acetic acid) were added and shaken vigorously. After 15 min time the surplus of bromine was destroyed with 750 µL of a saturated solution of sodium sulfite. After addition of 5 mL of water the solution was basified with 7 mL of a sodium hydroxide solution (c = 10 mol/L), cooled to room temperature in a water bath and extracted two times for 15 min with 2 mL of pentane. During the extraction the vials were mechanically shaken. The extracts were combined and the solvent blown off with a gentle stream of nitrogen. When analysed with GC/ECD the residue was dissolved in 400 µL pentane, for HPLC investigations 250 µL of methanol/water (50/50, v/v) were added.

Gas chromatographic conditions. The temperatures of the injection block and the detector were 250 and 300 °C, respectively. The injection volume was 1 µL. 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 170 °C, after 18 min the temperature was raised with a rate of 20 °C/min to 240 °C and then held for another 18.5 min.

Results and discussion

Derivatization

In general, substitution by bromine takes place in all available ortho-and para-positions to the amino-groups. Because of the substitution pattern of the compounds under investigation all analytes give dibromo derivatives except 3AT, which gives the tribromo product (see Table 2).

Bromination gives only one product except for the following three cases. For 4A2NT one side product is obtained with a corresponding peak area of about 7% compared to that of the main product, which may be caused by a contamination of the reference substance. For 2,6-DA4NT three main peaks can be seen in the

Table 2 Brominated derivatives with chromatographic data (measured with GC/ECD)

Mother compound	Derivative	t_R/min	Peak no.	Detection limit/(ng/μL)	Extraction rate/%
2AT	3,5-Dibromo-2-aminotoluene [17]	8.58	2	0.1	99
3AT	2,4,6-Tribromo-3-aminotoluene [17]	17.91	3	0.02	100
4AT	3,5-Dibromo-4-aminotoluene [17]	6.74	1	0.05	100
2,4-DAT	3,5-Dibromo-2,4-diaminotoluene[c]	18.33	4	0.3	97
2,6-DAT	3,5-Dibromo-2,6-diaminotoluene[c]	20.66	6	0.5	99
2A4NT	3,5-Dibromo-2-amino-4-nitrotoluene [21]	21.62	7	0.08	99
2A6NT	3,5-Dibromo-2-amino-6-nitrotoluene [22]	19.54	5	0.06	100
4A2NT[a]	3,5-Dibromo-4-amino-2-nitrotoluene [21]	22.96	9	0.1	98
4A2NT[b]	–[d]	23.49	–	–[d]	–[d]
2A4,6DNT	3,5-Dibromo-2-amino-4,6-dinitrotoluene[c]	28.85	12	0.2	95
4A2,6DNT	3,5-Dibromo-4-amino-2,6-dinitrotoluene[c]	24.79	10	0.1	99
2,4-DA6NT	3,5-Dibromo-2,4-diamino-6-nitrotoluene[c]	26.98	11	0.1	87
2,6-DA4NT[a]	3,5-Dibromo-2,6-diamino-4-nitrotoluene[c]	22.09	8	0.3	88
2,6,-DA4NT[b]	–[d]	24.01		–[d]	–[d]
2,6-DA4NT[b]	–[d]	24.20		–[d]	–[d]

[a] Main peak, used for determination of detection limit; [b] Most important side peaks; [c] Bromination degree proved by mass spectrometry; [d] Not determined

chromatograms, the main peak and two peaks, which are classified as side peaks in Table 2 (about 70% of the peak area of the main peak). TAT is the only compound under investigation, which does not give any detectable brominated product. The electronic effect of three amino groups should favour an electrophilic substitution by bromine and steric effects should not be more important than in the aminodinitro or diaminonitro compounds, which do react, but no peaks at all could be detected.

The bromination procedure is inert against the methanol content of the solution. Standard mixtures were compared, which were either dissolved in 2 mL methanol or where methanol was blown off with nitrogen before the addition of the reagents. No significant difference in the chromatograms was obtained. On the contrary it is important that the acetic acid concentration is not decreased by the addition of water. Addition of 3 mL of water diminishes the reaction rates for some of the analytes to less than 10% in comparison to those obtained without water. The aminonitrotoluenes and aminodinitrotoluenes are hardly affected by the water content.

The extraction was usually done with pentane. Extraction rates exceeded 90% in a single extraction step for all analytes except the derivaties of 2,4-DAT, 2ADNT, 2,4-DANT and 2,6-DANT. For the former two 80 and 78% were obtained, respectively. For the dibromodiaminonitrotoluenes extraction rates were even less (60% for both analytes). In order to recover all analytes quantitatively, two consecutive extraction steps were performed. In Table 2 the extraction rates for the combined extracts are given. For the dibromodiaminonitrotoluenes 87% were achieved, for all the other compounds more than 95%. Toluene shows a similar extraction efficiency, but it is harder to remove in the blow-off step.

Interferences by nitroaromatic compounds

In order to test possible interferences by nitroaromatic compounds, a mixture of reference substances (2,4,6-trinitrotoluene, 2,4-dinitrotoluene, 2,6-dinitrotoluene, 3,4-dinitrotoluene, 2-nitrotoluene, 3-nitrotoluene, 4-nitrotoluene) were treated as described in the experimental section. As expected, no bromination of the compounds took place and the extraction rates were so low, that they did not interfere at all, even when present in high concentrations.

Gas chromatography

Separation of all brominated compounds was achieved with the temperature program described above. In Fig. 1 a chromatogram of a reference mixture is shown. The critical analyte pair are the derivatives of 3AT and 2,4-DAT (resolution: 1.4). Between 23 and 24.5 min three rather big peaks are obtained, which refer to side products of the bromination of 4A2NT and 2,6-DA4NT.

In Table 2 retention times and detection limits for the procedure are given. The detection limits are estimated from the peak height with the 3σ-method and were obtained with diluted 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 μL (factor: 5). The former could be done since

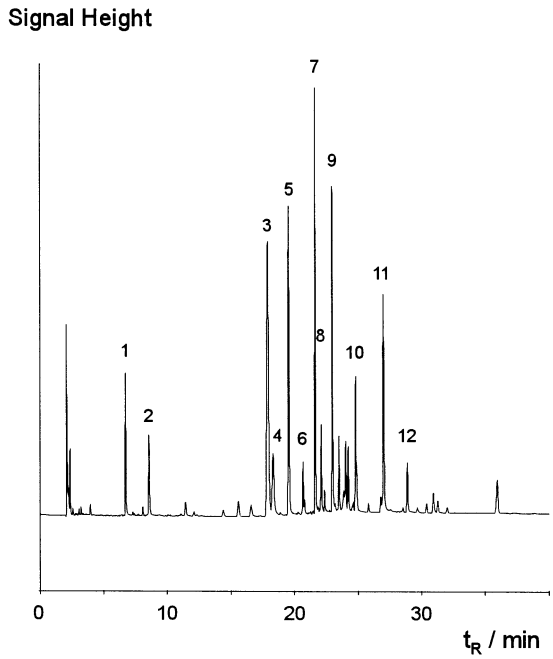

Fig. 1 Chromatogram of a reference mixture of brominated amino- and aminonitrotoluenes. Derivatives of: *1*: 4AT (25), *2*: 2AT (25), *3*: 3AT (5.0), *4*: 2,4-DAT (22), *5*: 2A6NT (6.3), *6*: 2,6-DAT (82), *7*: 2A4NT (5.0), *8*: 2,6-DANT (27), *9*: 4A2NT (6.1), *10*: 4A2,6DNT (26), *11*: 2,4-DANT (26), *12*: 2A4,6DNT (27). Concentration of analytes in ng/μL given in brackets

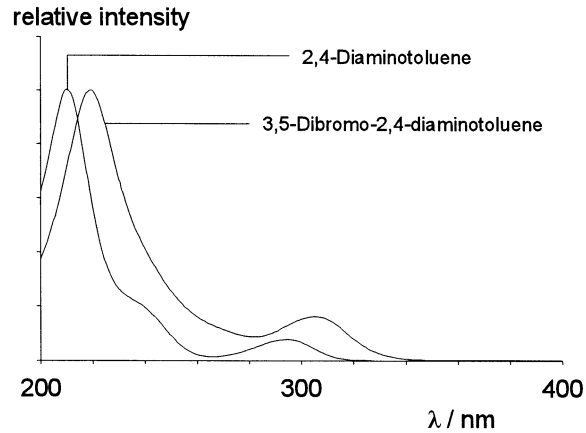

Fig. 2 Comparison of the UV spectra of 2,4-DAT and 3,5-dibromo-2,4-diaminotoluene

the signal/noise ratio was actually improved by a factor of 5 with the increased injection volume.

UV and MS data

UV data of the derivatives are given in Table 3. It can be seen, that the UV spectra do not differ very much. In general three maxima are present: the first and strongest absorption band between 210 and 220 nm, a second one, sometimes as a shoulder, between 230 and 250 nm and a third one between 300 and 315 nm. The introduction of one or more bromo groups does not have a strong influence on the UV absorption of most of the analytes, as can be seen in Fig. 2, which shows UV spectra for 2,4-DAT and its derivative in the wavelength range from 200 to 350 nm. Nevertheless, if the identification of analytes is critical, the retention behavior in HPLC and spectral data could be used to confirm identity.

MS data of the derivatives are presented in Table 3. For the analytes without additional nitro substituent the 100%-peak was in general equal to the M^+-peak, whereas for the other brominated compounds the most intense peak is obtained at a fragment far below the M^+-peak. The bromination degree of the compound is easily derived from the isotope pattern. Hence, GC/MS is a convenient means of identifying the derivatives. As an example for the obtained mass spectra, Fig. 3 shows the mass spectrum for 3,5-dibromo-2,6-diaminotoluene. The typical 1:2:1 isotope pattern for a dibromo compound at m/z = 278/280/282 and – after

Table 3 UV-maxima and GC/MS-data of the derivatives.

Derivative of	UV-max. 1 /nm	UV-max. 2 /nm	UV-max. 3 /nm	m/z (100%)	m/z (M^+)
2AT	208.8	243.7	301.0	265	265
3AT	215.0	245.9	308.9	343	343
4AT	210.7	232.0[a]	303.0	265	265
2,4-DAT	219.1	–	305.0	280	280
2,6-DAT	219.2	244.0[a]	306.2	280	280
2A4NT	209.4	248.0	302.6	104	310
2A6NT	210.0	247.2	302.5	104	310
4A2NT	215.9	250.4	315.8	104	310
2A4,6DNT	209.9	250.2	–	44	355
4A2,6DNT	214.1	239.0[a]	320.0	43	355
2,4-DA6NT	221.3	250.0[a]	305.5	119	325
2,6-DA4NT	222.6	250.0[a]	306.7	119	325

[a] shoulder

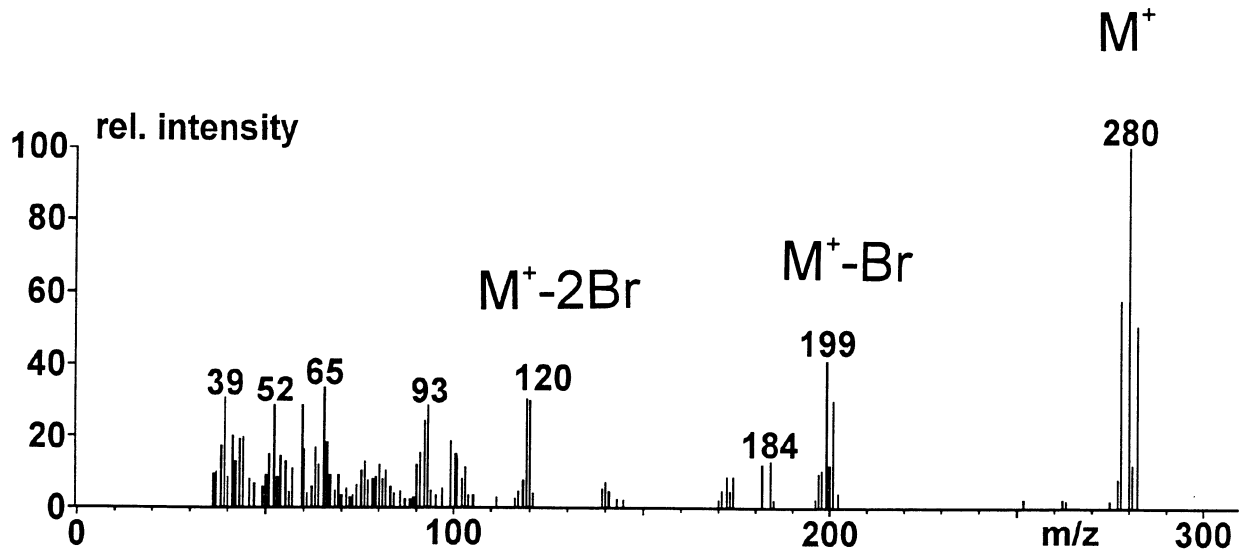

Fig. 3. Mass spectrum of 3,5-dibromo-2,6-diaminotoluene with the main fragments M^+, M^+-Br, M^+-2Br

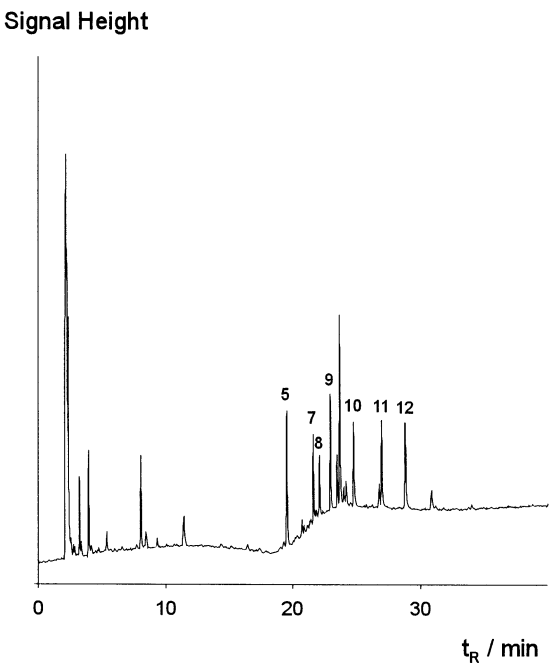

Fig. 4. Chromatogram of a water sample from a well near an ammunition waste landfill. Peak identification as in Fig 1

cleavage of one bromo substituent – the 1:1 pattern for a monobromo compound at m/z = 199/201 can be seen. The two fragments at m/z = 120 and 119 are explained by subtraction of Br and HBr, respectively.

Analysis of real samples

In Fig. 4 a chromatogram for a real sample (ASB II) is given. It is a water sample of a well situated near an ammunition waste landfill. It can be seen, that no amino- or diaminotoluenes without additional nitro groups are present in concentrations above the detection limit. In addition, no nitroaromatics without amino groups are detected although their concentration in the real sample is in the mg/L level. The following analytes were detected (concentration in µg/L in brackets): 2A4NT (1.0), 2A6NT (2.6), 4A2NT (2.4), 2ADNT (48), 4ADNT (27), 2,4-DANT (10), 2,6-DANT (18). If one uses the absorption of the corresponding azo dyes with N-(1-naphthyl)ethylenediamine referred to the absorption of p-nitroaniline [20] the sum of these aromatic amines is 21.5 µg/L. Photometrically a value of 22.7 µg/L was determined in routine analysis. This indicates that the content of aromatic amines in the investigated water should be almost entirely due to the seven analytes detected.

Conclusions

Bromination of aromatic amines is a simple means of derivatization, which is able to overcome the problems mentioned above. It is easy to perform and gave excellent results in the investigation of real samples, therefore it may be used in routine analysis for amino- and aminonitrotoluenes. In the moment we are testing the derivatization reaction for other aniline and xylidine derivatives to find out its possible use for monitoring of an even wider range of aromatic amines.

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