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Determination of RDX, 2,4,6-trinitrotoluene and other nitroaromatic compounds by high-performance liquid chromatography with photodiode-array detection

Monika Emmrich*
Institut für Hygiene, Freie Universität Berlin, Hindenburgdamm 27, 12200 Berlin (Germany)

Manfred Kaiser
Bundesinstitut für Chemisch-Technische Untersuchungen, Grosses Cent, 53913 Swisttal (Germany)

Henning Rüden and Stefan Sollinger
Institut für Hygiene, Freie Universität Berlin, Hindenburgdamm 27, 12200 Berlin (Germany)

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ABSTRACT

An HPLC method with photodiode-array detection was developed for the determination of RDX, TNT and the most important aromatic nitro compounds. Three mobile phases, isocratic methanol–water (50:50), a methanol–water gradient and a methanol–water gradient containing 2% of tetrahydrofuran were tested. Under the isocratic conditions used diaminonitrotoluene and the tetranitroazoxytoluene isomers cannot be measured within one HPLC run whereas this could be achieved with the two gradient systems. Good separations are achieved with the gradient systems with different elution orders for the nitrated benzene, toluene and aminonitrotoluene isomers.

INTRODUCTION

Residues of 2,4,6-trinitrotoluene (TNT), one of the most widely used explosives, are often accompanied by co-contaminants resulting from manufacturing impurities of the explosive, from hydrolysis or from biotransformation. Manufacturing impurities include 2,4- and 2,6-dinitrotoluene and dinitro- and trinitobenzene [1]. Many bacteria and fungi are capable of reducing the nitro groups to amines, hence important biodegradation products are 2-amino-4-nitro- and 4-amino-2-nitrotoluene, the aminodinitrotoluenes 4-amino-2,6- and 2-amino-4,6-dinitrotoluene and the diaminonitrotoluenes 2,4-diamino-6-nitro- and 2,6-diamino-4-nitrotoluene [1,2,3]. In addition, 2,2′,6,6′-tetranitro-4,4′-azoxytoluene and in trace amounts 4,4′,6,6′-tetranitro-2,2′-azoxytoluene have been observed as transformation products [4,5].

As these substances can be found in the environment (*e.g.*, soil), efficient and rapid analytical procedures for these compounds are necessary. Gas chromatography with flame ionization detection (FID) or as a more sensitive method electron-capture detection (ECD) is often used to determine these substances [6,7]. However, for the separation of thermally unstable and non-volatile compounds, high-per-

* Corresponding author.

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formance liquid chromatography (HPLC) with ultraviolet detection is ideally suited [8,9] and offers adequate detection limits for nitroaromatic compounds [10]. Environmental samples are very complex and the extracts may contain substances that co-elute with the nitroaromatic compounds. Hence the use of HPLC in combination with photodiode-array detection is advantageous, because peak identification is more reliable with the additional information from the UV spectrum. The spectra may be helpful in searching for particular pollutants in samples and give a first identification of the substances present. Further, by comparison of the various UV spectra at the peak front edge, peak apex and peak end edge, one can obtain information about homogeneity. Therefore, an HPLC method in combination with diode-array detection for rapid and efficient analysis was developed for hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX), TNT and its most important derivatives.

EXPERIMENTAL

Apparatus

The HPLC measurements were carried out on a Waters (Milford, MA, USA) Model 600E multi-solvent delivery system equipped with a Waters Model 700 automatic sample injection module and a Waters Model 990 photodiode-array detector. For separation a Merck LiChrospher 100 RP-18 column (25 cm × 4 mm I.D.) with a particle diameter of 5 μm in combination with a precolumn filled with the same material was used. The flow-rate was 1 ml/min and the volume injected was 20 μl. The mobile phase was a methanol–water programmed gradient starting with 35% methanol increased to 70% in 35 min and to 100% in the following 10 min. The column was then flushed for a further 5 min with pure methanol and re-equilibrated for 10 min. For a different elution order of the compounds a secondary gradient from methanol–water containing 2% of tetrahydrofuran (THF) to methanol was used.

Chemicals

RDX, TNT, 2-amino-4,6-dinitro- and 4-amino-2,6-dinitrotoluene (2A-4,6DNT, 4A-2,6DNT), 2,4-diamino-6-nitro- and 2,6-diamino-4-nitrotoluene (2,4DA-6NT, 2,6DA-4NT), 4,4',6,6'-tetranitro-2,2'-azoxytoluene (2,2'-Azoxy) and 2,2',6,6'-tetranitro-4,4'-azoxytoluene (4,4'-Azoxy) were prepared at the Bundesinstitut für Chemisch-Technische Untersuchungen (Swisttal, Germany). 1,4-Dinitrobenzene (1,4-DNB) and 2,6-dinitrotoluene (2,6-DNT) were obtained from Riedel-de Haën (Seelze, Germany) and 4-amino-2-nitrotoluene (4A-2NT) from Aldrich (Steinheim, Germany). The other reference compounds nitrobenzene (NB), 1,2- and 1,3-dinitrobenzene (1,2-DNB, 1,3-DNB), the nitrotoluene isomers (2-NT, 3-NT and 4-NT), the 2,3-, 2,4- and 3,4-dinitrotoluene iso-

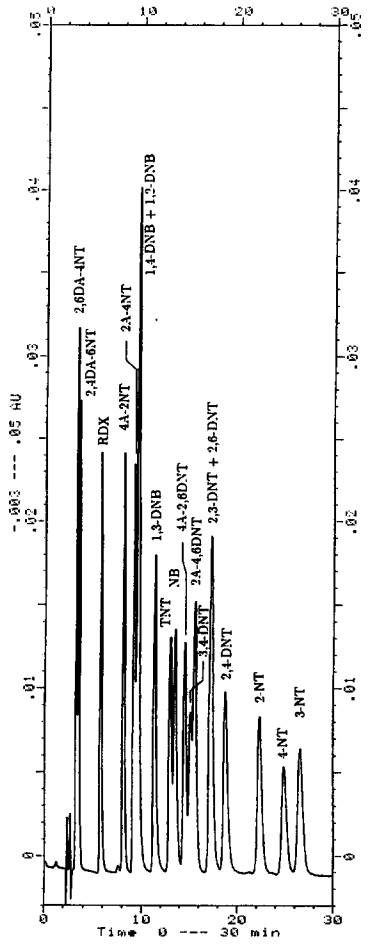

Fig. 1. Chromatogram of RDX, TNT and the nitroaromatic compounds with methanol–water (50:50) as mobile phase and detection at 254 nm.

mers (2,3-DNT, 2,4-DNT and 3,4-DNT) and 2-amino-4-nitrotoluene (2A-4NT) were purchased from Merck (Darmstadt, Germany). All chemicals were used as received. They were dissolved in methanol, with the exception of RDX, 2,2′-Azoxy and 4,4′-Azoxy, which were dissolved in acetonitrile and further diluted with methanol.

For mobile phase preparation, gradient-grade methanol, THF and water for chromatography were purchased from Merck.

RESULTS

Isocratic conditions

Common HPLC methods described in the literature [11,12] use reversed-phase columns with methanol–water under isocratic conditions. These methods have been optimized for the separation of explosives such as TNT, RDX, HMX, Tetryl and trinitrobenzene [9], but little is known about the elution order and separation of TNT and its degradation products. Therefore, RDX, TNT and the most important related nitroaromatic compounds were tested on a LiChrospher 100 RP-18 reversed phase column using methanol–water (50:50) as the mobile phase. A chromatogram recorded at 254 nm is shown in Fig. 1 and the retention times (t_R) are summarized in Table I. Under these conditions 2,6DA-4NT and 2,4DA-6NT are not retained and coincide with the solvent peak. In contrast, 2,2′-Azoxy and 4,4′-Azoxy do not elute within 70 min. To measure all these compounds one has to make several HPLC runs with different isocratic conditions.

Gradient programmed conditions

These compounds can be measured altogether in one chromatographic run with the use of a gradient programmed HPLC method. Fig. 2 shows a chromatogram obtained at 254 nm with

TABLE I

RETENTION TIMES, t_R, FOR METHANOL–WATER (50:50), A METHANOL–WATER GRADIENT AND A METHANOL–WATER GRADIENT CONTAINING 2% OF THF

Compound	t_R (min)		
	Methanol–water (50:50)	Methanol–water gradient	Methanol–water gradient +2% THF
2,6DA-4NT	3.3	5.2	6.2
2,4DA-6NT	3.6	6.4	8.4
RDX	5.8	11.1	16.3
4A-2NT	8.3	17.6	18.3
1,4-DNB	9.7	18.0	17.1
2A-4NT	9.3	19.0	19.2
1,2-DNB	9.7	19.5	20.6
1,3-DNB	11.5	20.3	20.2
TNT	13.1	23.0	24.1
NB	13.7	23.4	21.2
4A-2,6DNT	14.7	25.4	28.5
2A-4,6DNT	15.8	26.1	29.3
3,4-DNT	15.3	26.3	26.2
2,6-DNT	17.4	27.2	27.2
2,3-DNT	17.4	27.9	28.0
2,4-DNT	19.0	28.2	27.7
2-NT	22.6	30.7	28.8
4-NT	25.2	32.0	29.7
3-NT	26.9	32.8	30.7
4,4′-Azoxy	–	46.7	47.1
2,2′-Azoxy	–	48.8	48.9

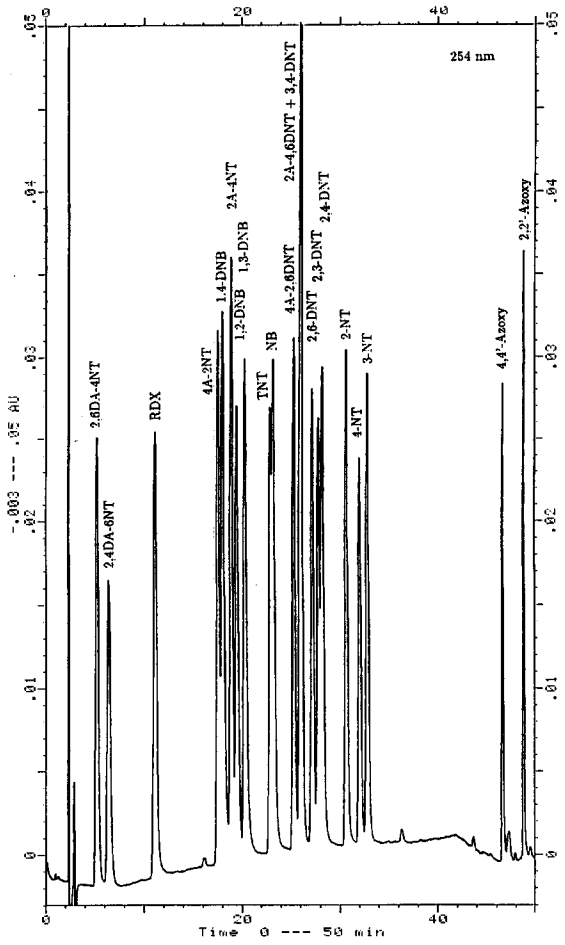

Fig. 2. Chromatogram of RDX, TNT and the nitroaromatic compounds with a gradient programmed mobile phase of methanol–water with detection at 254 nm.

a standard solution containing 6–12 ng/μl of each of the 21 substances. Retention times are summarized for the RP-18 column in Table I. Under the experimental conditions, the compounds are nearly completely separated. The two diaminonitrotoluene isomers are eluted first, followed by the nitramine RDX. The two aminonitrotoluene and the three dinitrobenzene isomers elute close together, but they are well separated. TNT and NB have nearly the same retention times and coincide. Then the aminodinitrotoluenes are eluted, but 2A-4,6DNT appears at the same time as 3,4-DNT, followed by the other DNT isomers. Of the mononitrotoluenes, 2-NT is eluted first, followed by 4-NT and 3-NT. 4,4'-Azoxy and 2,2'-Azoxy elute relatively late at 46.7 and 48.8 min, respectively. If it is desired to shorten the analysis time, 4,4'-Azoxy and 2,2'-Azoxy will elute earlier without changing the elution sequence and separation of the other compounds if the methanol concentration is increased rapidly to 100% after 35 min.

In Table II the different UV absorbance maxima are summarized together with the molar absorptivities (ϵ) for all substances.

The reproducibility of the method was tested by making repeated injections of a standard solution of the compounds. The relative standard deviations of the mean retention times for each compound determined for ten injections are summarized in Table III. The relative standard deviations of the peak retention times are very low and the method has good reproducibility.

For checking the linearity of the detector six different concentrations were tested, each with a minimum of four injections. The calibration graphs with intercept a and slope b are given in Table III for a wavelength of 254 nm. They were calculated as regression lines based on a least-

TABLE II

ABSORBANCE MAXIMA, λ_{max} (nm), AND MOLAR ABSORPTIVITIES, ϵ_{max} (10^3 cm^2/mol), IN THE MEASURED RANGE 210–350 nm

Compound	λ_{max}	ϵ_{max}	λ_{max}	ϵ_{max}	λ_{max}	ϵ_{max}
2,6DA-4NT	210	29.8	240	13.4	338	3.6
2,4DA-6NT	213	30.8	239	11.9	350	1.7
RDX	210	11.0				
4A-2NT	234	15.0				
1,4-DNB	259	13.5				
2A-4NT	226	12.3	249	11.8	287	4.6
1,2-DNB	210	11.3				
1,3-DNB	233	15.6				
TNT	227	17.4				
NB	258	7.4				
4A-2,6DNT	232	20.6	367	1.9		
2A-4,6DNT	226	17.4				
3,4-DNT	215	11.8	260	5.8		
2,6-DNT	210	9.0	233	8.5		
2,3-DNT	210	10.0	254	5.0		
2,4-DNT	241	12.7				
2-NT	210	6.2	256	5.1		
4-NT	210	6.6	273	8.8		
3-NT	210	7.8	263	6.9		
4,4'-Azoxy	239	34.0				
2,2'-Azoxy	227	23.5	313	18.7		

TABLE III

CALIBRATION GRAPHS AND REPRODUCIBILITY (WAVELENGTH 254 nm) FOR RDX, TNT AND THE NITROAROMATIC COMPOUNDS

Compound	Concentration (ng/μl)		Calibration graph[a]		r^2	R.S.D.[b] ($n = 36$) (%)
	Min.	Max.	a	b		
RDX	0.41	78	−9	596	1.000	1.3
4A-2NT	0.30	58	−128	836	1.000	1.4
1,4-DNB	0.20	38	−812	1565	0.999	1.5
2A-4NT	0.22	43	−366	1218	0.999	1.4
1,2-DNB	0.31	60	−680	749	0.999	1.6
1,3-DNB	0.17	33	−671	1703	0.998	1.5
TNT	0.22	42				
			−216	1030	1.000	0.9
NB	0.22	43				
4A-2,6DNT	0.28	54	−32	764	1.000	1.3
2A-4,6DNT	0.24	47				
			−232	942	1.000	1.4
3,4-DNT	0.24	46				
2,6-DNT	0.26	50	−99	782	1.000	1.0
2,3-DNT	0.32	62	−4	520	0.999	1.1
2,4-DNT	0.16	30	−304	1645	0.999	1.1
2-NT	0.33	64	−139	676	1.000	0.9
4-NT	0.31	59	−207	565	1.000	0.9
3-NT	0.31	60	−235	704	1.000	0.9
4,4′-Azoxy	0.08	16	−16	1489	1.000	0.2
2,2′-Azoxy	0.11	22	−10	1161	1.000	0.1

[a] $y = a + bx$.
[b] Relative standard deviation of retention time.

squares fit. With values between 0.998 to 1.000 for the squared regression coefficients (r^2) all compounds show good linearity. The slope b calculated for 254 nm is a measure of the sensitivity and will have other values for different wavelengths, depending on the UV spectrum.

The detection limit based on the amount required to give a response three times the standard deviation of the noise is in the range 40–80 pg/μl. With an injection volume of 20 μl a minimum amount of 0.8–1.6 ng of each compound is needed.

Influence of THF

TNT is one of the most often used explosives and frequently its measurement is of special interest. In unknown and complex systems such as environmental samples TNT might be accompanied by co-contaminants. NB interferes with TNT and the accurate determination of TNT can be affected. Therefore, another HPLC system that offers the detection of TNT without co-elution of NB was developed. A ternary mobile phase mixture was used, consisting of water and methanol mixed with 2% of THF with the same gradient programme as mentioned above.

A chromatogram with methanol–THF–water as mobile phase is shown in Fig. 3. The retention times are given in Table I. The elution order with methanol–THF–water as mobile phase is different from that with methanol–water. There is now an excellent separation of TNT and NB in about 2 min, with NB eluting first. Further, the addition of 2% of THF has a significant influence in RDX, which is retarded by about 5 min and now elutes before 1,4-DNB. The retention times of 3,4-DNT, 2,6-DNT, 2,4-DNT and 2,3-DNT

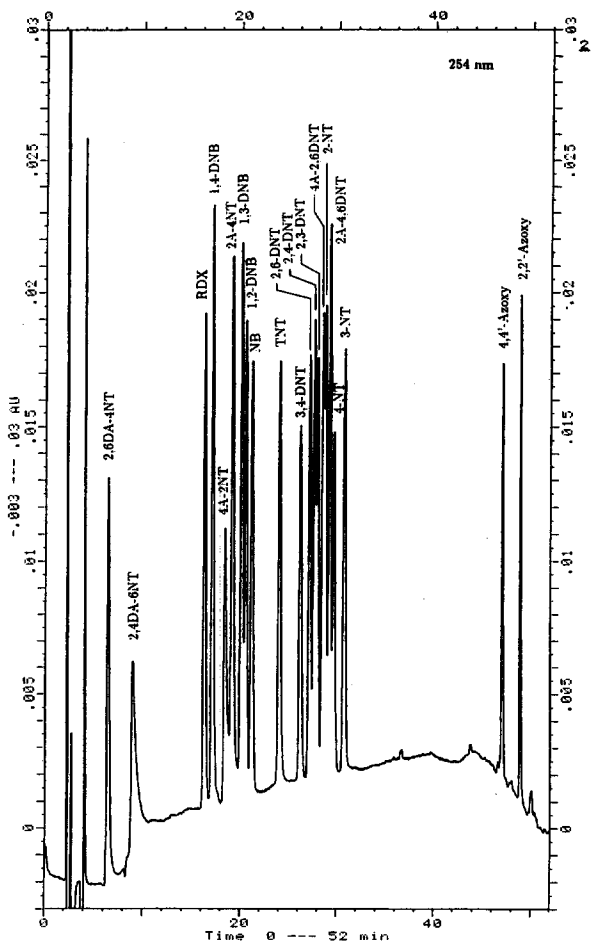

Fig. 3. Chromatogram of RDX, TNT and the nitroaromatic compounds with a gradient programmed mobile phase of methanol–water containing 2% of THF with detection at 254 nm.

are nearly the same. With the methanol–water gradient system 4A-2,6DNT elutes after TNT and NB whereas 2A-4,6DNT coincides with 3,4-DNT. When 2% of THF is added to the mobile phase both are retained about 3 min longer and elute close together with 2-NT. Likewise, the elution order of 4A-2NT and 1,4-DNB changes and 4A-2NT now elutes in front of 2A-4NT.

CONCLUSIONS

With the developed HPLC-method with a methanol–water gradient as the mobile phase, the determination of RDX, TNT and other relevant compounds within one HPLC run is possible. This cannot be achieved under isocratic conditions and one has to use different solvent strengths, *e.g.*, 20% methanol, to separate 2,4DA-6NT from 2,6DA-4NT, 50% methanol for TNT and its derivatives and 75% methanol for 2,2′-Azoxy and 4,4′-Azoxy [11].

The behaviour of the compounds on a reversed-phase RP-18 column with and without the addition of THF to methanol–water was investigated. The separation for TNT and NB is excellent when THF is added, but the performance for the dinitrotoluene isomers, the aminodinitrotoluenes and 2-NT is better when the methanol–water gradient system alone is used. Hence it might depend on the complexity of the sample matrix and the compounds of interest which of the two systems, methanol–water or methanol–THF–water, is to be preferred. In combination with photodiode-array detection one can obtain further information of peak identity.

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