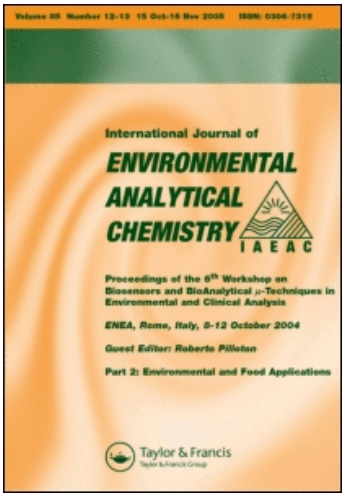

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Extraction and determination of trinitrotoluenes and products of their biotransformation in soil samples

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The separation of a mixture of trinitrotoluenes (TNTs) and their metabolites by reverse phase liquid chromatography using different columns with octadecyl stationary phase is presented. The retention behaviour of a mixture of TNTs and their metabolites was studied under different chromatographic conditions. The mixture of methanol/water was used as a mobile phase in isocratic and gradient modes. The effect of content of organic modifier and temperature of mobile phase has been investigated. Very good RP-HPLC separation of 14 nitroaromatic compounds was obtained using stepped gradient elution. Different properties of extraction procedures for isolation of trinitrotoluenes and their metabolites from soil were tested. Experimental conditions of Soxhlet warm extraction (extractant type, extraction time) suitable for efficient extraction of individual analytes were studied. Recoveries over 80% for all studied compounds and over 90% for almost all (11 compounds) were obtained in short extraction time (1 hour). The formation of precipitate during the Soxhlet extraction was observed and the further treatment of extract was necessary. The amount of precipitate formed from an artificial soil was higher than that from a natural one. THF was found as the best solvent suitable for total dissolution of precipitate.

Keywords: explosives; 2,4,6-TNT; metabolites; Soxhlet warm extraction; soil; artificial soil

1. Introduction

The most used worldwide highly energetic compounds like 2,4,6-trinitrotoluene (TNT) and other polynitro-organic compounds have been discharged into the environment since the WWI [1]. Considerable contamination of soil and water by these compounds is caused by various military activities (manufacturing, testing, training, demilitarisation, open burning/open detonation) [2].

In addition to 2,4,6-trinitrotoluene, its constitutional isomers (2,3,5-trinitrotoluene and 2,3,4-trinitrotoluene) were also distributed into the environment by wastewaters as a result of 2,4,6-TNT production (commonly known as the 'red water') [3]. Asymmetric trinitrotoluenes and other nitroaromatic compounds are relatively widespread.

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Asymmetric trinitrotoluenes have almost similar properties as 2,4,6-TNT. Higher hygroscopicity and lower thermal stability were determined [4]. However, only limited data about their toxicity and mutagenicity are available. TNTs and products of their degradation, aminodinitrotoluenes (Am-DNTs), diaminonitrotoluenes (DAm-NTs) and triaminotoluenes (TAmTs) have been found to be cytotoxic presumably due to induced oxidative stress and demonstrated mutagenic capability. 2,4,6-TNT is classified as possible human carcinogen. The evidence for human carcinogenicity is inadequate, and the animal carcinogenicity data are limited [5]. 2,4,6-TNT and several of its reduced metabolites isolated from human and rat urine showed mutagenic activity without metabolic activation in Salmonella mutagenicity assay [6,7].

Major processes affecting the fate and transport of 2,4,6-TNT in the environment can be classified as biotic and abiotic transformations. Especially adsorption and irreversible (covalent) bonding to soil organic matter play the significant role [8]. 2,4,6-trinitrotoluene can be biotransformed with soil indigenous microorganisms, photodegradated by sunlight or migrate through subsurface soil to cause groundwater contamination [2]. Because of low solubility in water (0.013 g/100 g water at 20°C) and high affinity of 2,4,6-TNT to soil (partition coefficient soil organic carbon/water = 6.38 L kg^{-1}), high contamination of soil caused by 2,4,6-TNT can occur. Consequently the most common process in soil is reversible sorption of 2,4,6-TNT. The detection of 2,4,6-TNT and its amine products in subsurface soil and groundwater clearly indicates the potential migration of these chemicals through subsurface soil to reach the water table [9]. Therefore both the knowledge of ecotoxicity of explosive compounds and their products of biodegradation, as well as concentration of these compounds in environment is essential.

To be able to provide insight into the environmental fate of explosives and the risk associated with their presence, analytical tools capable to analyse such chemicals and their transformation products in various environmental media has to be available. Although gas chromatography [10], capillary electrophoresis [11] and thin layer chromatography [12] have been used in special cases, high performance liquid chromatography (HPLC) has remained to be the major analytical tool for the detection and quantification of nitroaromatic compounds [13–17].

The most commonly used method for the analysis of explosives recommended by US Environmental Protection Agency (EPA) is HPLC with UV detection [18] due to its widespread availability, while HPLC combined with MS and electrochemical detection (ED) are also viable but less frequently available methods of detection and determination of explosives [19]. Previously published methods of explosives determination were focused on the use of C18 RP-HPLC and acetonitrile or methanol as the organic modifier of mobile phase [13]. Acetonitrile is of significantly greater health and environmental concern than methanol and therefore methods avoiding the use of acetonitrile are desirable [20]. In contrast to EPA recommended mixture we have used in our study a mixture of isomers of TNT and their amino-metabolites. These compounds are presumably present in contaminated sites, because of significant amount of asymmetric trinitrotoluenes in industrial production wastewater.

The present paper is focused on the development of a simple extraction procedure and final analytical HPLC method for a group of 14 nitroaromatics (2,4,6-TNT, 2,3,5-TNT, 2,3,4-TNT, Am-DNTs and DAm-NTs) presented in Figure 1. The composition of the nitroaromatics group differs to a great extent from classical

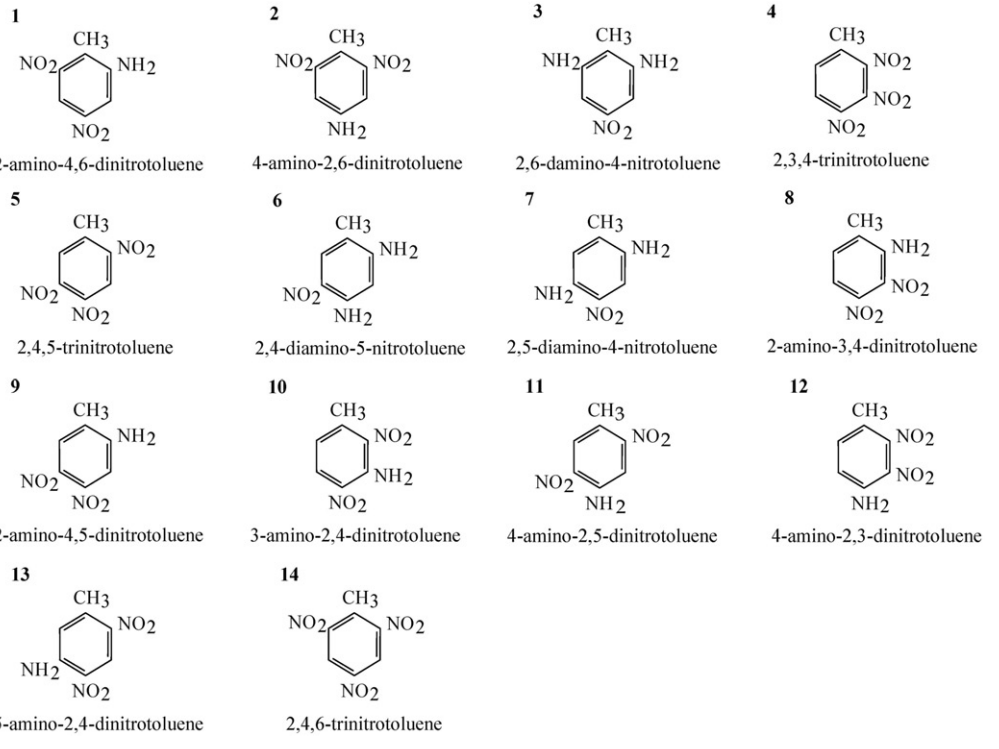

Figure 1. Nitroaromatic compounds used in the study. 2,4,6-TNT, 2-Am-4,6-DNT and 4-Am-2,6-DNT are identical for EPA method 8330.

EPA group. Only 3 identical substances (2,4,6-TNT, 2-Am-4,6-DNT and 4-Am-2, 6-DNT) are present in both groups.

For extraction of EPA explosives group ultrasonic extraction is recommended by US EPA method No. 8330 [18]. The most widespread extraction solvent is acetonitrile (MeCN). Extraction conditions for EPA explosives group were optimised in several studies focused on determination of explosives in contaminated soils [14,21,22]. The best recoveries (nearly 100%) were obtained after 18 hours in a cooled (6°C) sonic bath [21]. Also plant tissue extracts from plants growing on contaminated soils were prepared using the ultrasonic extraction method [14]. Soxhlet extraction was also used for extraction of explosives from soil samples. After one-stage 6 hour extraction period, more than 90% recovery of 2,4,6-TNT extraction was reached [23]. Microwave extraction was also used for determination of explosives in soil samples originated of different origin [24].

Soxhlet warm extraction used in this study for extraction of nitroaromatics from spiked natural and artificial soils enables to increase the efficiency and speed of extraction procedure in contrast to the standard Soxhlet extraction. The basic principles of Soxhlet warm extraction are the same as for standard Soxhlet extraction except that the extraction chamber of the apparatus is heated. The solubility of the analytes is increased by heating the content of extraction chamber. This reduces the

duration of the entire extraction process. The influence of different soil type on quality of extraction procedure and quantification of nitroaromatics content in spiked artificial and real soil samples is presented.

2. Experimental

2.1 *Chemicals and materials*

Standard substances of tested TNTs, Am-DNTs and Dam-NTs (Figure 1) were synthesised according to procedures optimised for preparation of clean substances (purity > 97%) used in toxicological [25] and phytotoxicological [26] studies. Most of them are commercially not available at the present time. Standard stock solutions (20–100 $\mu g\,mL^{-1}$) used for HPLC and extraction experiments were prepared by dissolution of each compound in methanol. Acetonitrile, methanol (Sigma Aldrich, St. Louis, MO), cyclohexane, *n*-hexane, tetrahydrofurane (Riedel de Haën, Germany) and dichloromethane (Sigma Aldrich, St. Louis, MO) used as solvents or mobile phase components were of HPLC-grade quality. The water used for HPLC analysis was prepared using Simplicity 185 equipment (Milipore, Molsheim, France).

2.2 *Sample preparation*

Soil bulk samples containing non-detectable amount of studied compounds (uncontaminated soil) collected in suburb of Brno (South Moravia, Czech Republic) were taken from a depth of 20–30 cm and air-dried at laboratory temperature. After removing of coarse materials (sticks, grits) the samples were passed through a 2 mm sieve and stored in a cold dark storage room. All soil samples were fortified with prepared solution of studied compounds dissolved in methanol, in concentration 0.2 $mg\,kg^{-1}$. Freshly spiked soil was prepared. Artificial soil used for study of extraction recovery of TNTs, Am-DNTs and DAm-NTs was prepared by mixing of 20% of kaolin clay, 70% of sand, 10% of peat and 0.8% of $CaCO_3$. The content of individual components was selected according to OECD recommendations [27]. The real soil samples were collected and treated by the same way as in the case of soil used for extraction experiments.

2.3 *Extraction procedures*

The B-811 extraction system (Büchi, Labortechnik AG, Switzerland) with 150 mL solvent vessel was used for Soxhlet warm extraction. The mode of Soxhlet warm extraction was evaluated based on a comparison of percentage extraction recovery of individual TNTs, Am-DNTs and DAm-NTs from spiked soil samples. Each extraction experiment was carried out in triplicate.

Five grams of soil samples were placed in each extraction tube and extracted with 140 mL of extraction solvent. At first, the soil samples were extracted for various extraction (40, 50 and 60 minutes) and stripping (20 and 30 minutes) times and using various extraction solvents. Temperature of hotplates was set in dependence on using solvent and its boiling point according to instrument set up recommended by the producer. Pure methanol, acetonitrile and their different ratio were used as extraction solvents.

The volume of extract concentrated in the extractor to 20 mL was subsequently reduced to 1 mL by solvent evaporation under a gentle stream of nitrogen. Evaporation of solvent was carried out in warm-up equipment at 30°C.

2.4 *Chromatographic separation and detection*

An Agilent 1100 series liquid chromatograph (Agilent Technologies, Inc., Palo Alto, CA) with a model 1100 DAD detector was used for chromatographic analyses. Chromatographic columns with octadecyl silica stationary phase, Zorbax Extend C18 (250 mm; 5 μm; 4.6 mm I.D., Agilent Technologies, US); Polaris C18-A (150 mm; 3 μm; 2 mm I.D, Meta-Chem, Technologies Inc.), ACE C18 (250 mm; 3 μm; 2.1 mm I.D.) and ACE C18 (150 mm; 3 μm; 2.1 mm I.D., Advanced Chromatography Technologies, Aberdeen, Scotland, UK) available in the laboratory were tested for analytical separation. The column dead time was determined using the retention time of uracile. The injection volume was 1 μL. The temperature was thermostatically equilibrated (20°C) to obtain a satisfactory reproducibility and peak broadening. After a series of measurements (20 analyses), the column was treated with a low flow (0.1 mL min^{-1}) of pure methanol. To obtain reproducible results, appropriate column conditioning with selected initial phase (up to 30 hold-time volumes) after column cleaning was performed. Chromatograms were recorded at wavelength 230 and 254 nm. Peaks were scanned from 200 to 600 nm to obtain spectrochromatograms for better compound identification. Quantitative measurements were performed on the basis of absolute calibration curve method within the range of 0.2 ng–0.2 μg per injection. Limits of detection (LOD) and limits of quantification (LOQ) for UV (230 nm) detection were calculated from calibration curves according to the Graham method [28] for optimised separation conditions. The flow rate for optimised separation method 0.2 mL min^{-1} and gradient of mobile phase: 0–30 min: 20–40% v/v methanol; 30–50 min: 40% v/v methanol; 50–70 min: 40–100% v/v methanol were used.

3. Results and discussion

3.1 *HPLC analysis*

Separation of TNTs and their metabolites is difficult due to common coelution of the structural isomers. The development of a method for determination of these compounds includes mainly selection of suitable chromatographic column possessing high separation efficiency, composition of mobile phase and temperature as well.

3.1.1 *Selection of chromatographic column*

Stationary phase of chromatographic columns tested for analytical separation of studied mixture (Figure 1) was octadecyl silica based. This type of stationary phase was selected according to physico-chemical properties of selected nitrocompounds and recommendation of EPA method used for determination of standard mixture of explosives. In the case of Zorbax Extend C18 (250 mm; 5 μm; 4.6 mm I.D.) column, having chromatographic parameters recommended by EPA 8330 method, total overlap of peaks of compounds 2,6-DAm-4-NT and 2,5-DAm-4-NT and also overlap of peaks of compounds 4-Am-2,6-DNT; 2-Am-4,6-DNT; 5-Am-2,4-DNT and 3-Am-2,4-DNT was observed.

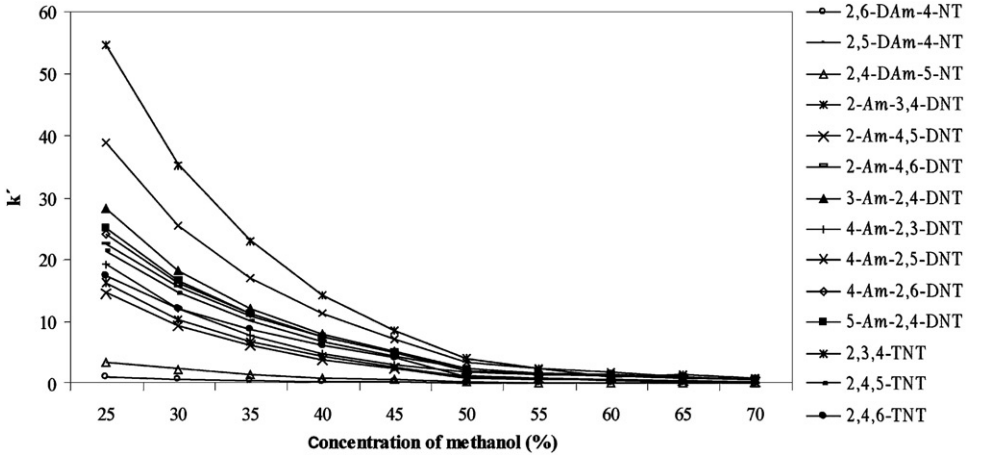

Figure 2. Effect of methanol content in mobile phase on retention (k′-capacity ratio) of nitroaromatic compounds. Column: ACE C18 (250 mm; 2.1 mm I.D.; 3 μm); mobile phase: methanol-water mixture, flow rate = 0.12 mL min^{-1}, mobile phase temperature 25°C.

The full separation of all compounds was obtained using ACE C18 (250 mm; 3 μm; 2.1 mm I.D.) column with smaller size of particles and column diameter. Because of high backpressure during HPLC separation decreasing of the flow rate of mobile phase was necessary. Consequently the separation of all compounds with sufficient resolutions was carried out in higher retention times. Because of high retention times of eluted compounds ACE C18 (150 mm; 3 μm; 2 mm I.D.) was used as a compromise solution with better resolution of critical peaks in comparison to Polaris C18-A (150 mm; 3 μm; 2 mm I.D.) column with similar geometrical parameters of stationary phase bed. The group of aminodinitrotoluenes coeluted in the same retention time on Polaris C18-A (150 mm; 3 μm; 2 mm I.D.) column, however.

3.1.2 *Effect of organic modifier content*

The effect of the methanol/water content ratio in mobile phase was investigated. Methanol content was changed in the range of 25–70% (v/v) for 4 different used columns. The stationary/mobile phase equilibrium was tested with the stability of column pressure, detector baseline and retention times of analytes, before each analysis. A volume of mobile phase equal to 30 column void volumes was sufficient to flush the chromatography column to equilibrate the initial separation conditions.

Generally, the decreasing of organic modifier content in the mobile phase increases retention time values for individual nitroaromatic compounds. The shape of curves expressing the effect of methanol content on capacity ratio differs for individual studied compounds (Figure 2). The change of retention order of some adjacent peaks is a result. Sufficient resolution of all studied compounds was achieved at nearly 25% methanol in the mobile phase. Because of high retention times of the latest eluted compounds gradient elution was developed.

Gradient elution using of gradient curves of different slopes was studied. Because of small overlaping of the first two peaks, 20% of methanol in mobile phase at start of

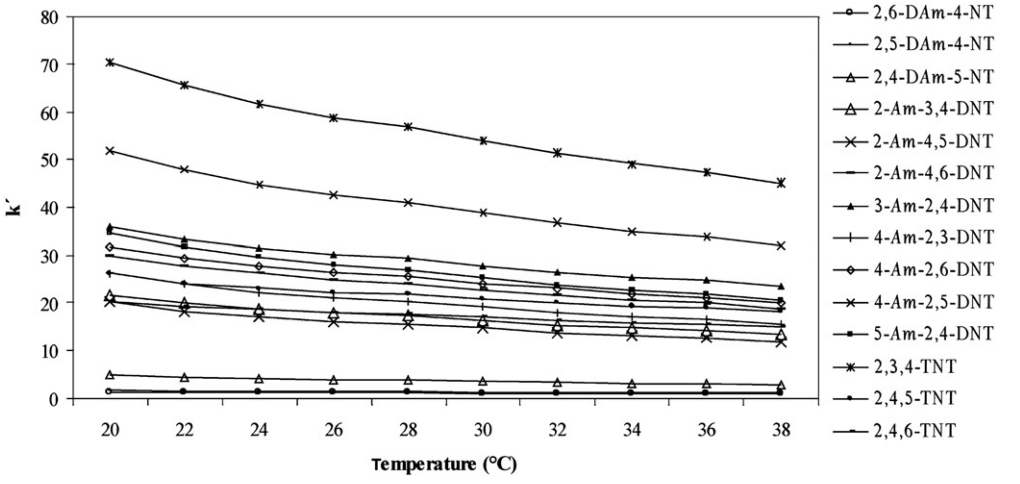

Figure 3. Effect of temperature of mobile phase on retention (k′-capacity ratio) of nitroaromatic compounds. Column: ACE C18 (250 mm; 2.1 mm I.D.; 3 μm); mobile phase: methanol : water 25 : 75 v/v, flow rate = 0.12 mL min^{-1}.

separation was necessary. At first continuous type of gradient was tested. However, no slopes of gradient gave good resolution and acceptable separation time at the same run. Therefore the stepped type of gradient with different profile of methanol content was studied. Satisfying resolution and shortest separation times was obtained with following gradient: 0–30 min: 20–40% v/v methanol; 30–50 min: 40% v/v methanol; 50–70 min: 40–100% v/v methanol.

3.1.3 *Effect of mobile phase temperature*

The effect of mobile phase temperature was investigated in the range of 20–38°C in mobile phases containing from 25% to 70% of methanol. The effect of temperature in mobile phase containing 25% methanol is presented in Figure 3 as an example. The increasing temperature of mobile phase reduces retention time of all analytes with significant influence on retention order, in the case of 2-Am-3,4-DNT and 2-Am-4,6-DNT. The best results were obtained in the range of 30–35°C, in 25% of methanol. The mobile phase temperature 33°C finally used enables the highest resolution of analytes mixture.

3.1.4 *Separation of TNTs, Am-DNTs and DAm-NTs mixture*

Satisfying resolution and chromatographic separation run time were achieved under the following conditions: Column: ACE C18, 150 mm × 2.1 mm I.D. 3 μm, methanol-water mobile phase gradient (0–30 min: 20–40% methanol; 30–50 min: 40% methanol; 50–70 min: 40–100% methanol), flow rate = 0.2 mL min^{-1} and temperature = 33°C. The rate of flow is affected by maximum value of backpressure recommended for stationary phase used. An example of separation with satisfactory resolution of all analytes in tested mixture can be seen in Figure 4. Twenty minutes of dwell time were included to re-establish the initial conditions before the next injection.

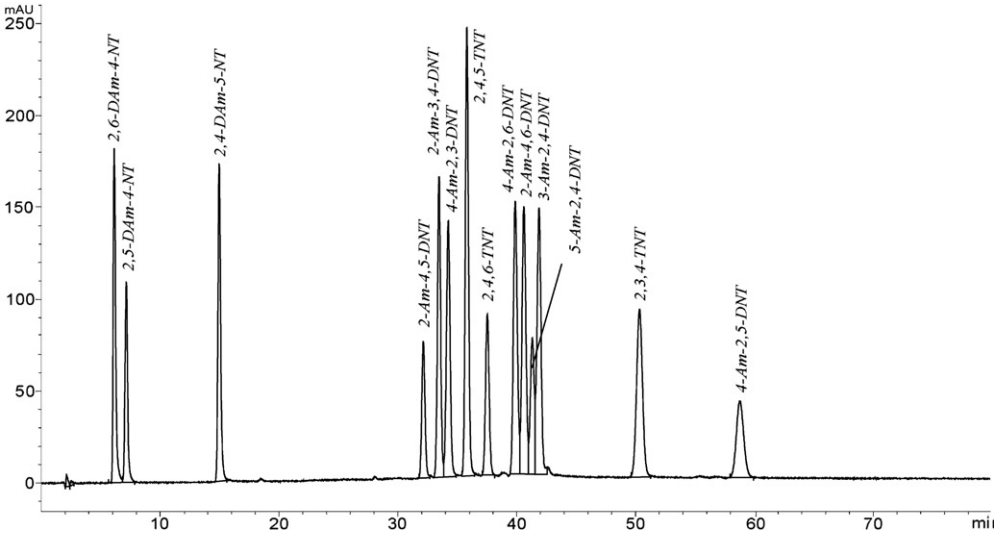

Figure 4. Separation of tested compounds. Column: ACE C18 (150 mm; 2.1 mm I.D. 3 μm); mobile phase: methanol/water (gradient = 0–30 min: 20–40% v/v methanol; 30–50 min: 40% v/v methanol; 50–70 min: 40–100% v/v methanol), flow rate = 0.2 mL min^{-1} and mobile phase temperature 33°C.

Table 1. Limits of detection of nitroaromatic compounds.

Compound	R^2	LOD (ng mL^{-1})	LOQ (ng mL^{-1})	X_D^{α} (ng mL^{-1})	X_D^{β} (ng mL^{-1})
2,3,4-TNT	0.9981	9.94	33.13	12.56	40.51
2,4,5-TNT	0.9944	12.32	41.07	7.59	35.20
2,4,6-TNT	0.9977	6.05	20.15	13.13	39.45
2,4-DAm-5-NT	0.9974	11.13	37.11	16.30	43.65
2,5-DAm-4-NT	0.9984	7.81	26.04	22.10	49.91
2,6-DAm-4-NT	0.9969	6.97	23.24	14.71	40.32
2-Am-3,4-DNT	0.9967	8.33	27.76	13.94	41.74
2-Am-4,5-DNT	0.9958	14.81	49.36	6.95	59.14
2-Am-4,6-DNT	0.9975	5.59	18.64	15.25	42.09
3-Am-2,4-DNT	0.9972	5.84	19.48	10.52	31.48
4-Am-2,3-DNT	0.9971	4.01	13.35	13.35	37.71
4-Am-2,5-DNT	0.9975	7.27	24.23	15.02	41.83
4-Am-2,6-DNT	0.9974	6.14	20.48	13.21	38.64
5-Am-2,4-DNT	0.9989	13.39	44.64	9.84	30.00

3.1.5 *Limits of detection*

Calibration curves based on seven equidistantly prepared calibration samples were analysed by the linear regression analysis (Table 1). The instrumental limits of detection X_D^{α} and X_D^{β} were calculated according to the Graham method [28] from the calibration plots. Their values obtained from confidence intervals are compared with limits of detection (LOD) and limits of quantification (LOQ) calculated from the baseline noise of calibration samples according to ISO 11843-1 and ISO 11843-2 [29,30]. Values of detection limits X_D^{α} and X_D^{β} were obtained using the *Solver* function of the *Microsoft® Excel* program.

3.2 *Soxhlet extraction*

3.2.1 *Artificial soil*

Artificial soil, as a common indifferent medium often used in various comparison studies was tested. Methanol was used as a Soxhlet extraction solvent and programme including 40 minutes of extraction and 20 minutes of stripping (to remove residue of the extract) was applied. During extraction process of spiked artificial soil significant amount of precipitate was observed. After filtration of formed precipitate significant loss of the analysed compounds and therefore low recovery (30–40%) were observed. Sorption of nitroaromatic compounds on the surface of precipitate would be the reason. Therefore the way to resolve the precipitate was studied. Sonication and Vortex mixing were used. No evident changes in precipitate form were observed, however. Therefore 1 mL of hexane, cyclohexane, dichloromethane (DCM) or tetrahydrofurane (THF) was added to 1 mL of soil extracts containing the precipitate. Only THF demonstrated the ability to resolve the precipitate completely. However, adding of more than 2 mL was able to dissolve all of the precipitate. Extraction solvent was therefore to last drop evaporated from soil extract and 1 mL of THF was added finally. Procedures together with the results of precipitate dissolution are presented in Figures 5 and 6.

3.2.2 *Natural soil*

The same procedures as in the case of artificial soil were applied to spiked natural soil samples. During extraction procedures of natural soil less amount of precipitate was observed. Twenty different natural soil samples were extracted for comparison. In dependence of soil type and sampling place various look and amount of precipitate was observed. Comparison of amount of precipitate in the extract from different natural soils and artificial soil is demonstrated in Figure 7. Significant and different loss of analysed compounds can be reached if filtration is used for precipitate elimination.

3.2.3 *Extraction recovery*

A methanol-acetonitrile or acetone-hexane mixtures are recommended as an extractant for polar and non-polar compounds. Pure methanol was tested as an extractant since it was a component of mobile phase in HPLC analysis. Two commonly used types of extraction tube filling were compared: zig-zag fold filter-paper tube and cotton-wool layer. The extraction recovery of individual analytes from fresh spiked uncontaminated natural soils was studied (Figure 8). All soil samples were fortified with prepared solution of studied compounds dissolved in methanol, in concentration 1 and 10 $\mu g\,mL^{-1}$. The average recovery of most compounds was over 90% if cotton-wool layer was used. This type of filling of extraction tube was used for consequent experiments.

The effect of acetonitrile content (25, 50, 75 and 100%, v/v) in extraction mixture with methanol was studied. Increasing recovery for most of tested compounds was observed in the case of 25 or 50% (v/v) of acetonitrile but with considerable decrease in the case of 2,4,5-TNT, 5-Am-2,4-DNT and 2,5-Dam-4-NT (see Table 2).

Finally, the effect of time of individual steps of Soxhlet warm extraction was studied in order to improve the recoveries of selected analytes. The changes in extraction time (40, 50 and 60 minutes) and stripping time (20 and 30 minutes) give no increase of recovery.

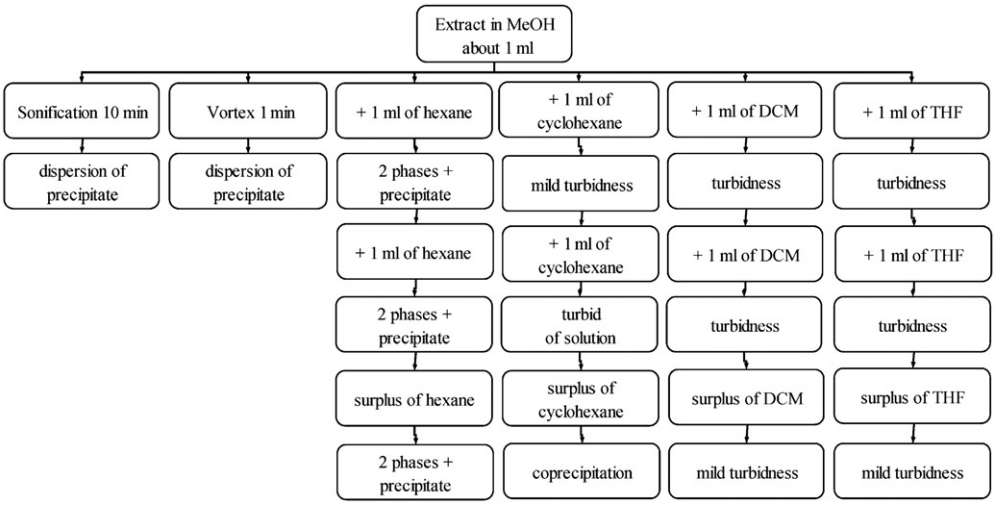

Figure 5. Dissolution of precipitate in methanol's extract.

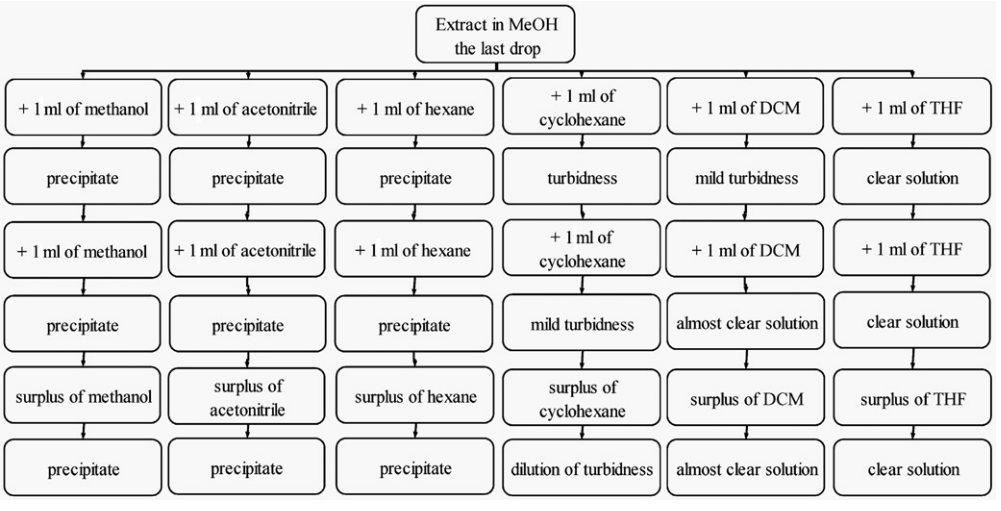

Figure 6. Dissolution of precipitate in evaporated methanol's extract.

3.3 *Determination of TNTs, Am-DNTs and DAm-NTs in real soil samples*

The HPLC method developed was used for determination of TNTs and products of their biotransformation in real soil samples collected in the vicinity of factory producing energetic materials near the Slavičín town in the industrial part of Moravia (Czech Republic). Sampling point was chosen as a part of a started pilot study focused on monitoring of explosives and their biotransformation in the environment. Significant content of TNTs isomers was found in analysed samples, up to $1\,mg\,kg^{-1}$ 2,4,6-TNT and between 0.18 and $1.17\,\mu g\,kg^{-1}$ for asymmetric TNTs. The content of aminoderivatives reached 2.1 and $2.3\,\mu g\,kg^{-1}$ in the case of 2-Am-4,6-DNT and 4-Am-2,6-DNT, respectively.

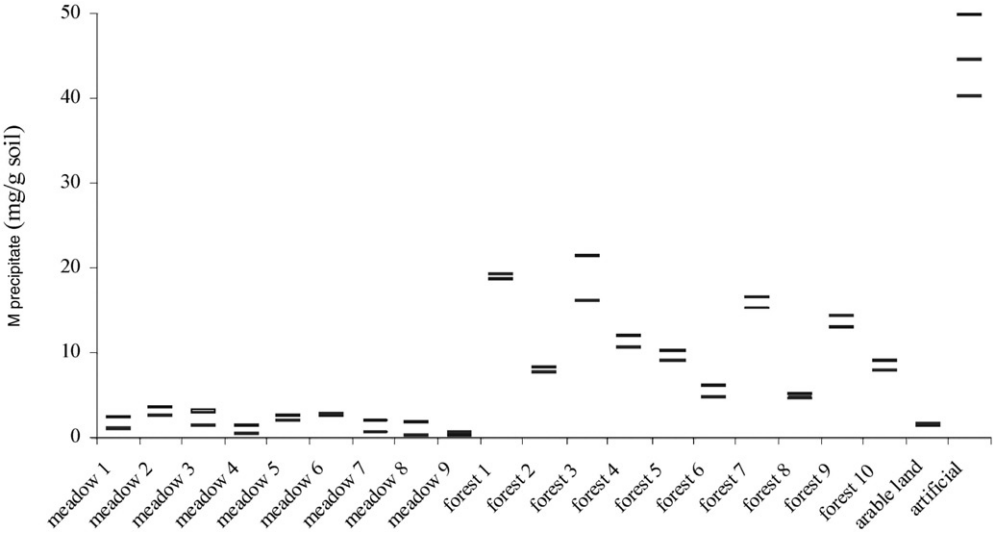

Figure 7. Comparison of precipitate formation in extract from different soil type.

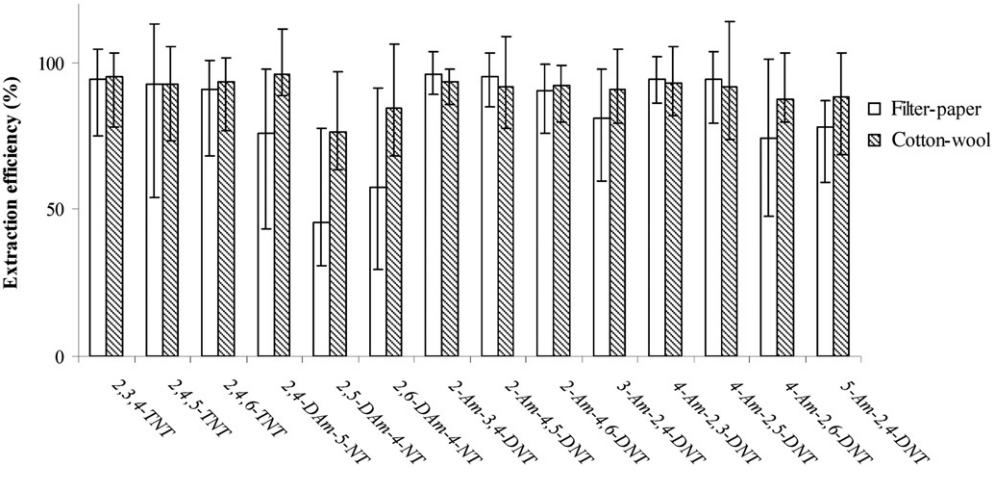

Figure 8. Methanol extraction recovery of nitroaromatic compounds. Soxhlet warm extraction with methanol as an extractant, 40 minutes of extraction and 20 minutes of stripping.

The average content of the 2-Am-4,5-DNT, 4-Am-2,5-DNT and 5-Am-2,4-DNT was on the limit of quantification. A content of the rest of aminoderivatives was under limit of quantification.

4. Conclusions

The HPLC retention behaviour of nitroaromatic explosives and their metabolites on C18 type stationary phase was investigated. Effect of selected chromatographic columns, organic modifier content and temperature of mobile phase was studied. Because of coelution of the group of aminodinitrotoluenes under US EPA recommended conditions,

Table 2. Comparison of extraction recovery of tested compounds in percentage using Soxhlet warm extraction.

	methanol/acetonitrile (v/v)				
	100/0	75/25	50/50	25/75	0/100
2,3,4-TNT	95	94	89	55	48
2,4,5-TNT	93	55	43	17	25
2,4,6-TNT	94	98	105	69	49
2,4-DAm-5-NT	96	100	100	72	52
2,5-DAm-4-NT	76	73	64	46	20
2,6-DAm-4-NT	84	91	90	71	46
2-Am-3,4-DNT	93	102	106	77	63
2-Am-4,5-DNT	92	102	106	77	61
2-Am-4,6-DNT	92	101	104	76	61
3-Am-2,4-DNT	91	97	104	75	54
4-Am-2,3-DNT	93	101	105	77	63
4-Am-2,5-DNT	92	100	103	75	60
4-Am-2,6-DNT	87	95	94	70	54
5-Am-2,4-DNT	88	82	80	63	30

the column with small-size (3 μm) particles and lower column diameter has to be used. Sufficient resolution of all studied compounds was only achieved in mobile phase with low organic modifier content (25% methanol). Because of high retention times of the latest eluted compounds gradient elution was developed (0–30 min: 20–40% v/v methanol; 30–50 min: 40% v/v methanol 50–70 min: 40–100% v/v methanol). Changes in temperature of mobile phase (20–38°C) allow precise shifting in retention of tested compounds. At the temperature 33°C the best resolution of nitroaromatic compounds and analysis time less than 60 minutes was achieved.

The Soxhlet warm extraction of nitroaromatic explosives and their metabolites was studied. Effect of extractant content (acetonitrile/methanol), time of extraction and type of sample placing was tested. Using methanol as an extractant, 40 minutes of extraction and 20 minutes of stripping, recoveries over 90% for most extracted compounds were obtained.

Formation of precipitate during Soxhlet warm extraction and extract treatment may underestimate the final analytical results, especially if artificial soil is used for experiments focused on the fate and toxicity effects of nitroaromatics in soil environment. The amount of precipitate originated from artificial soil was much higher than from natural one. THF was used as the best solvent for precipitate dissolution. Rigorous analysis of precipitate formed during sample preparation and its effect on extraction recovery of polar organic pollutants from soil samples of various sources will be a subject of following work. As a comparison the formation of precipitate using different techniques (ASE, ultrasonic extraction) and different organic solvents as extraction medium will be studied.

The method developed was used for determination of studied compounds in real soil samples. Results demonstrate necessity to use detection method with lower limits of detection. Therefore LC/MS/MS will be optimised for pilot study focused on TNTs and their metabolites at contaminated sites in future work.

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