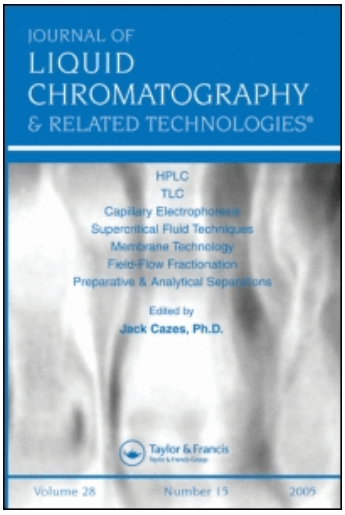


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# DETERMINATION OF NITROAROMATIC COMPOUNDS IN SOIL SAMPLES BY HPLC, USING ON-LINE PRECONCENTRATION

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## ABSTRACT

The aim of this paper is to develop a simple HPLC method for trace analysis of 17 nitroaromatic compounds (nitrophenols, nitroanilines, nitrotoluenes, and others) in soil samples. To improve the limit of determination, on-line preconcentration of water extracts has been used. The separation was achieved by using the column packed with 10 μm LiChrosorb RP-18 and mixture methanol–water as mobile phase. The SPE cartridge (on-line coupled to an analytical column) containing a C-18

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packing material was used to preconcentrate nitroaromatic compounds in water extracts of soil. The limits of the determination, at a wavelength of 254 nm (S/N = 5) 0.5–10 ppb, for studied analytes with recoveries of more than 85% were obtained. The developed method can be used for the study of nitroaromatic compounds during the degradation.

*Key Words*: Nitroaromatic compounds; HPLC; Soil preparation; On-line preconcentration



## INTRODUCTION

Nitroaromatic compounds are widely used in chemical industries and most are toxic and persistent pollutants. They are readily adsorbed by skin upon contact, lead to methemoglobinemia and liver damage, and they can uncouple the oxidative phosphorylation process. Nitrated organic compounds are the most widely used munitions components. Some nitrotoluenes are very important industrial and energetic compounds. 2,4,6-Trinitrotoluene is one of the most current military explosives, 2,4- and 2,6-dinitrotoluene are used both in the production of explosives and polyurethanes. Both dinitrotoluenes may be present as contaminants of trinitrotoluene in explosives, too. Many cases of serious soil contamination are known at former ammunition plants areas and other sites, where explosives were manipulated with, and used. These compounds have been, and continue to be, produced in large quantities and are, therefore, subject to regulation by environmental agencies.

The need to separate and quantitative the nitroaromatic compounds in complex samples has sustained continuing investigations of different separation methods including capillary electrophoresis,[1] supercritical fluid chromatography,[2] liquid chromatography,[3] and gas chromatography.[4] Generally, gas chromatography with either electron capture or mass spectrometry has been a more sensitive technique, but reproducibility suffers, especially for thermally labile compounds, such as RDX and HMX. HPLC with photodiode array detection, using either solvent extraction or solid phase extraction as a concentration step, is the standard method for munitions analysis.[5] Based on redox behavior of nitroaromatics, amperometric detection using pendant mercury electrodes, gold-mercury thin film electrodes or amalgamated gold electrodes, and glassy carbon electrode has been used together with LC, for the separation and the determination of several nitroaromatic compounds with various degrees of success.[6–8]

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Several new approaches for extracting organic analytes from environmental matrices have been developed, including pressurized liquid extraction (PLE, also known under the trade name ASE, accelerated solvent extraction), supercritical fluid extraction (SFE), supercritical water extraction, and others.[9,10]

The aim of this paper is to develop a simple HPLC method for trace analysis of nitroaromatic compounds in soil samples. To improve the limit of determination, on-line preconcentration of water extracts has been used.

## EXPERIMENTAL

### Apparatus

The measurements were carried out with a Hewlett-Packard HPLC system (series 1100) including a quaternary pump, a Rheodyne injection valve, and a photodiode array detector. The detection wavelength was 254 nm.

### Chromatographic Conditions

Separations were performed on columns (250 × 4 mm I.D.) packed with 10 μm LiChrosorb RP-18, (250 × 4 mm I.D.) packed with 10 μm LiChrosorb RP-8, and (250 × 4 mm I.D.) packed with 10 μm Separon SGX C-18.

The mobile phase was a mixture of methanol–water. The flow rate of mobile phase was 1 mL/min. All experiments were done at room temperature. The HPLC-grade methanol was purchased from Merck.

### Sample Preparation

The solutions of the standard mixtures were prepared by dissolving reference compounds in high-purity methanol or methanol/water 50/50 v/v, respectively.

For sample preparation 5 g of homogenized soil was extracted with 20 mL of water by sonication during 30 min. The extract was filtered and 10 mL of extract was injected into a precolumn Separon SGX C-18 (30 × 3.2 mm I.D., 5 μm) on-line coupled to an analytical column. The flow-rate of injection was 1 mL/min. Before injection the precolumn was washed with 5 mL of methanol and conditioned with 10 mL deionised water.

## RESULTS AND DISCUSSION

The separation of nitroaromatic compounds can be involved by type of stationary phase, nature and concentration of organic modifier in mobile phase, pH, and ionic strength of mobile phase. The pH and ionic strength have the smallest effect on the separation because none of the studied analytes are charged in the pH range of the analysis. Only very slight changes in the chromatographic separation when varying pH between 4–7 and the ionic strength from 0 to 80 mM, were observed. Initial experiments were conducted to determine the optimum stationary phase and mobile phase composition.

The analyses were carried out under isocratic and gradient conditions. When the isocratic elution was used, the two chromatographic systems must be used for the separation. The first system for the separation of TNX, 4-nitroaniline, RDX, 4-nitrophenol, 3-nitrophenol, 2-nitroaniline, 2,4-dinitroaniline, 2-nitrophenol, 2,4,6-trinitrotoluene, nitrobenzene, includes the mixture of methanol in water 40/60 (v/v) as the mobile phase. The second system has used the mixture 50% methanol in water (v/v) as the mobile phase for the separation of 2,6-dinitrotoluene, 2,4-dinitrotoluene, 2-nitrotoluene, 4-nitrotoluene, and 3-nitrotoluene. The stationary phase used for both chromatographic systems was C-18. The two C-18 (Lichrosorb RP-18 and Separon SGX C-18) columns used showed similar separation efficiency but the separation on Lichrosorb RP-18 column was faster. Only partial separation was obtained using C-8 type stationary phase (LiChrosorb RP-8). The compounds 4-nitrophenol, 3-nitrophenol, 2-nitroaniline, and 2,4-dinitroaniline were not separated. The best separation (the values of $R_{ij}$ higher than 1.2) of all compounds was obtained using stationary phase C-18 (LiChrosorb RP-18) and gradient elution with mobile phase methanol/water 40/60 (v/v) during 20 min, then 50/50 (v/v) during 15 min. The limits of the determination of nitroaromatic compounds under study are summarized in Table 1.

To improve the limit of the determination, on-line preconcentration was used. A reversed phase C18 packing material for the enrichment column was chosen. A high capacity is required for the precolumn, whereas selectivity and efficiency are important for the analytical column. The type and amount of packing material in the precolumn determine the maximum sample volume that can be passed through the column without sample components breaking through. In on-line experiments 3.2 mm diameter and 30 mm long precolumn was used. The breakthrough volumes of the compounds under study were determined in redistilled water at a spiking level of 100–250 ppb of nitroaromatic compounds, by using the method described in literature.[11] The results are shown in Table 2. Figure 1 demonstrated the typical break through curve for the concentration level of 200 ppb of 2,4-dinitrotoluene in redistilled water, with break through after

***Table 1.*** The Limits of the Determination of Nitroaromatic Compounds Without and Using On-Line Preconcentration

| | Limit of Determination (ppb) | |
|---|---|---|
| Analyte | Without Preconcentration | With Preconcentration |
| 2,4-Dinitrophenol | 100 | 1 |
| RDX | 100 | 1 |
| 4-Nitroaniline | 100 | 1 |
| Hexahydro-1,3,5-trinitrozo-1,3,5-triazine | 80 | 0.5 |
| 3-Nitroaniline | 50 | 0.5 |
| 4-Nitrophenol | 100 | 1 |
| 3-Nitrophenol | 100 | 1 |
| 2-Nitroaniline | 100 | 5 |
| 2,4-Dinitroaniline | 100 | 5 |
| 2-Nitrophenol | 100 | 1 |
| 2,4,6-Trinitrotoluene | 100 | 1 |
| Nitrobenzene | 150 | 5 |
| 2,6-Dinitrotoluene | 50 | 5 |
| 2,4-Dinitrotoluene | 50 | 5 |
| 2-Nitrotoluene | 100 | 10 |
| 4-Nitrotoluene | 200 | 10 |
| 3-Nitrotoluene | 200 | 10 |



11 mL. A volume of only 10 mL was used for the analysis of the water extract of soil (spiking $\leq$ 10 ppb).

The limits of the determination of nitroaromatic compounds obtained with on-line preconcentration are in ppb range (Table 1). On the other hand, the limits of the determination without on-line preconcentration, at the same chromatographic conditions, are about 100-times higher. The recoveries of nitroaromatic compounds under study at the 5 and 10 ppb concentration levels were 93–96 $\pm$ 3.0–2.7% ($n = 4$) and 91–88 $\pm$ 3.4–4.0% ($n = 4$), respectively. These results indicate that breakthrough did not occur during preconcentration. These studies also confirm complete elution of the analyte from the precolumn to the analytical column. Next, standard curves for the studied analytes were constructed. The dependences of peak area on concentration were linear (correlation coefficients were about 0.98) in the range of the concentration, from the limits of the determination (Table 1) to 10 ppb for all compounds.

The example of the real sample analysis spiked (10 μg) extract of the soil after on-line preconcentration, is shown in Fig. 2.

***Table 2.*** The Breakthrough Volumes of Nitroaromatic Compounds. Precolumn: Separon SGX C-18 ($30 \times 3.2$ mm I.D., 5 μm), flow rate: 1 mL/min

| Analyte | Concentration (ppb) | Breakthrough Volume (mL) |
|---|---|---|
| RDX | 100 | 1.5 |
| 4-Nitroaniline | 100 | 1.5 |
| Hexahydro-1,3,5-trinitrozo-1,3,5-triazine | 100 | 1.4 |
| 3-Nitroaniline | 100 | 1.4 |
| 4-Nitrophenol | 100 | 2.2 |
| 3-Nitrophenol | 100 | 2.4 |
| 2-Nitroaniline | 100 | 2.6 |
| 2,4-Dinitroaniline | 100 | 7.0 |
| 2-Nitrophenol | 100 | 8.6 |
| 2,4,6-Trinitrotoluene | 100 | 8.5 |
| Nitrobenzene | 100 | 9.4 |
| 2,6-Dinitrotoluene | 100 | 11.0 |
| 2,4-Dinitrotoluene | 200 | 11.2 |
| 2-Nitrotoluene | 250 | 17.0 |
| 4-Nitrotoluene | 250 | 16.1 |
| 3-Nitrotoluene | 200 | 17.6 |



The stability of hexahydro-1,3,5-trinitrozo-1,3,5-triazine, RDX, 4-nitrophenol, 2-nitrophenol, 2,4,6-trinitrotoluene, and nitrobenzene in water extract at room conditions (the concentration level 1 ppm) was studied using the developed HPLC method. It was found, that the hexahydro-1,3,5-trinitrozo-1,3,5-triazine was degraded more effectively than other studied compounds (Table 3). The degradation of 4-nitrophenol and 2-nitrophenol in water solution was also studied under irradiation ($\lambda = 254$ nm for 4-nitrophenol and 2-nitrophenol). These experiments showed that both nitrophenols were thermally and photochemically very stable (Table 3).

## CONCLUSION

The presented gradient HPLC method, with on-line preconcentration, is suitable for separation of 17 nitroaromatic compounds in less than 35 min. The method used a C-18 stationary phase with a methanol/water 40/60 (v/v) during 20 min, then 50/50 (v/v) during 15 min, as mobile phase. The SPE cartridge

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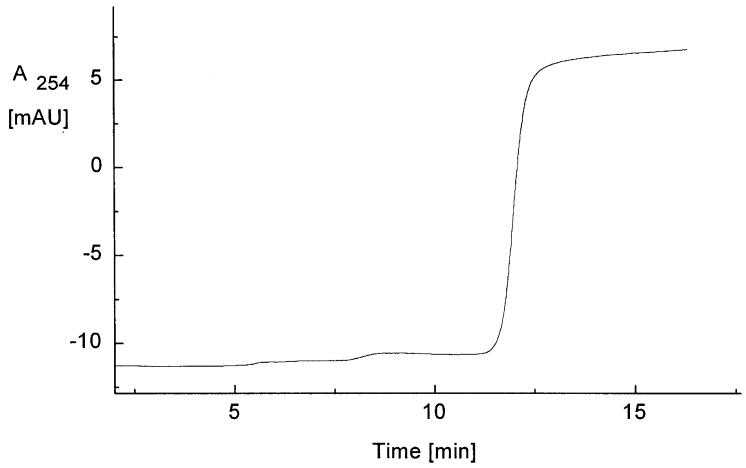


***Figure 1.*** The break through curve of 200 ppb of 2,4-dinitrotoluene. Precolumn: Separon C-18 (30 × 3.2 mm I.D.), flow rate 1 mL/min. Chromatographic conditions: see in the experimental.

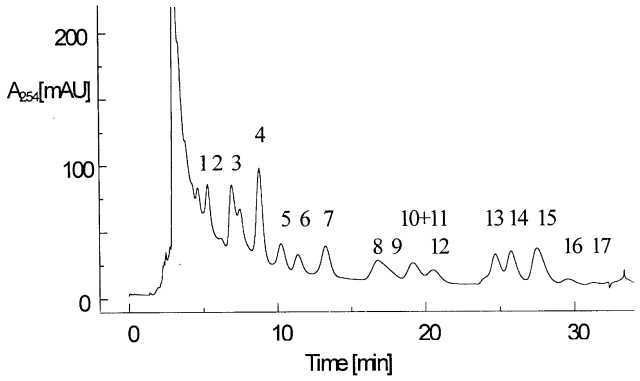


***Figure 2.*** Chromatogram of the spiked soil extract (10 μg of each compound) after on-line preconcentration. Chromatographic condition: see in the experimental. Peaks: 1—2, 4-dinitrophenol; 2—RDX; 3—4-nitroaniline; 4—hexahydro -1,3,5-trinitrozo -1,3,5-triazine; 5—3-nitroaniline; 6—4-nitrophenol; 7—3-nitrophenol; 8—2-nitroaniline; 9—2, 4-dinitroaniline; 10—2-nitrophenol; 11—2,4,6-trinitrotoluene; 12—nitrobenzene; 13—2, 6-dinitrotoluene; 14—2,4-dinitrotoluene; 15—2-nitrotoluene; 16—4-nitrotoluene; 17—3-nitrotoluene.

***Table 3.*** The Stability of Some Nitroaromatic Compounds in Water at Room Temperature and After Irradiation ($\lambda = 254$ nm)

| | Stability in Water at Room Temperature (%) | Stability in Water After Irradiation (%) |
|---|---|---|
| Nitrobenzene | | |
| 0 h | 100 | 100 |
| 1 h | 99.5 | 98.2 |
| 2 h | 99.4 | 97.4 |
| 4 h | 99.5 | 96.6 |
| 4-Nitrophenol | | |
| 0 min | 100 | 100 |
| 15 min | 99.7 | 99.2 |
| 30 min | 99.5 | 99.3 |
| 45 min | 99.3 | 99.5 |
| 2-Nitrophenol | | |
| 0 min | 100 | 100 |
| 15 min | 99.6 | 94.1 |
| 30 min | 99.3 | 92.2 |
| 45 min | 99.1 | 91.5 |



(on-line coupled to analytical column) containing a C-18 packing material was used to concentrate nitroaromatic compounds. The determination limit (S/N = 5) 0.5–10 ppb for studied analytes with recoveries of more than 85% were obtained. The developed method can be used for the study of nitroaromatic compounds during the degradation.

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