Application of solvent microextraction to the analysis of nitroaromatic explosives in water samples

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Abstract

The application of solvent microextraction to the analysis of nitroaromatic explosives is presented. Extraction of 11 nitroaromatics was achieved by suspending 1 µl of organic solvent to the tip of a microsyringe in a stirred aqueous solution. Parameters such as extraction solvent, stirring rate, salt concentration and sampling time were studied and optimized. The limits of detection using bench-top quadrupole mass spectrometry and short extraction times (15 min) were found to be between 0.08 and 1.3 µg/l and the relative standard deviations ranged between 4.3 and 9.8%. Although precision and accuracy of quantification of the method are still needed, solvent microextraction proved to be a fast, simple and inexpensive tool for preconcentration and matrix isolation of nitroaromatics on a microscale. © 2001 Elsevier Science B.V. All rights reserved.

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1. Introduction

Site investigations of military installations in the United States and Europe, revealed that the uncontrolled disposal of generated waste as well as the testing of explosive weaponry has led, in most cases, to contamination of soil and groundwater [1]. High levels of 2,4,6-trinitrotoluene (TNT) constitute a health hazard, as it is a suspected mutagen and was demonstrated to be toxic to aquatic and terrestrial life [2]. The biodegradation and photolytical by-products of TNT introduce highly reactive compounds of often greater polarity and water solubility, which

may constitute an even greater environmental concern than TNT itself [3]. In this context, the development of new, efficient, simple and inexpensive analytical methods is of great importance.

Liquid–liquid extraction (LLE) and solid-phase extraction (SPE) are the most commonly used methods of sample pretreatment for isolation and/or enrichment of explosives [4] but have many disadvantages as they are tedious, labor-intensive and time-consuming. LLE in particular requires the use of large amounts of high-purity solvents, which are often hazardous and result in the production of toxic laboratory waste. Prior to chromatographic analysis LLE and SPE require solvent evaporation, in order to preconcentrate the samples. During this evaporation step, loss and/or deterioration of the target analytes has been reported [5]. To circumvent this problem

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Belardi and Pawliszyn [6] developed a new solvent-free extraction technique, termed solid-phase microextraction (SPME), whereby a thin fused-silica fiber coated with a stationary phase was exposed to a contaminated aqueous sample [7]. During exposure analytes sorbed to the stationary phase until equilibrium was achieved. SPME is an increasingly popular method for the extraction of organic analytes as it is rapid, solvent-free, easily automated and field usable [8,9]. The main drawbacks with this extraction technique are that SPME fibers are expensive and their lifetime is limited as they degrade with increased usage [10]. The partial loss of the SPME fiber stationary phase results into peaks that may coelute with target analytes affecting thus accuracy and precision.

A newly developed protocol, which overcomes the problems of solvent evaporation (LLE, SPE) and fiber degradation (SPME), is solvent microextraction. It is based on the traditional LLE technique but involves only a few microliters of organic solvent as extractant [11]. Jeannot and Cantwell developed two extremely simple approaches on solvent microextraction and preconcentrated analytes from aqueous solutions. They suspended a single microdrop at the end out of a PTFE rod [12] or at the tip of a gas chromatography (GC) microsyringe needle [13], and transferred it to the injection port of the GC system. Subsequently, another approach termed as dynamic liquid-phase microextraction used the microsyringe as a separatory funnel [14,15]. Most of these reports described theoretical models on the mass transfer kinetics involved in the system. Recently solvent microextraction has been successfully applied for the quantification of chlorobenzenes [15] and drugs [16] as well as for the screening of pesticides in water samples [17]. This paper examines whether solvent microextraction is a feasible protocol for the quantitative analysis of explosives in water samples. For the purpose of the present studies a microdrop of organic solvent was left suspended on the tip of a microsyringe needle, immersed in an aqueous spiked-solution, for a prescribed period of time. The microdrop was then retracted to the microsyringe and transferred to the GC injection port for further analysis. The parameters, which were controlled and studied in order to evaluate the performance of the method, are given in detail in the following sections.

2. Experimental

2.1. Chemicals and sample preparation

All the target analytes were in the form of two separate 1-ml acetonitrile solutions (mix A and mix B) and were purchased from Supelco (Bellefonte, PA, USA). Mix A contained 2-amino-4,6-dinitrotoluene (2-ADNT), 1,3-dinitrobenzene (1,3-DNB), 2,4-dinitrotoluene (2,4-DNT), 1,3,5-trinitro-1,3,5-triazine (RDX), nitrobenzene (NB), 1,3,5,7-tetranitro-1,3,5,7-tetraazacyclooctane (HMX), 1,3,5-trinitrobenzene (1,3,5-TNB), 2,4,6-trinitrotoluene (TNT), each at 100 μg/ml. Mix B contained 4-amino-2,6-dinitrotoluene (4-ADNT), 2,6-dinitrotoluene (2,6-DNT), 2-nitrotoluene (2-NT), 3-nitrotoluene (3-NT), 4-nitrotoluene (4-NT) and tetryl, each at 100 μg/ml. A toluene solution (10 mg/l) of 2,3-dinitrotoluene (2,3-DNT) (Riedel-de Haën, Seelze, Germany) was prepared and used as an internal standard. All solvents were pesticide-grade (Merck, Darmstadt, Germany). Deionized water was prepared on a water purification system (EASYpure RF) supplied by Barnstead/Thermolyne (Dubuque, IA, USA). Working standards were prepared daily at the concentration levels of interest. Volumes (5-ml) of the spiked working standard solutions were transferred to 7-ml clear glass vials and sealed with black Viton septa and screw caps with hole, all purchased from Supelco.

2.2. Solvent microextraction

A 10-μl Hamilton gastight syringe (Hamilton Bonaduz, Bonaduz, Switzerland), Model 1701, with a bevel needle tip (length: 5.1 cm, I.D.: 0.013 cm, bevel 22°), containing 1 μl of the appropriate organic solvent was clamped above the vial containing the water sample. For all quantification experiments, 1 μl of a 10 mg/l toluene solution of the internal standard was used instead. The microsyringe was then lowered and its needle passed through the vial septum until the tip of the needle was 1 cm below the surface of the water sample. The plunger was depressed and the 1-μl drop of the organic phase was exposed to the sample. Magnetic stirring at 400 rpm (unless otherwise stated) was applied during extraction using a 0.5-in. (1 in.=2.54 cm) stir bar

(Supelco). After extracting for a prescribed period of time, the plunger was withdrawn and the microdrop was retracted into the microsyringe. The syringe was then transferred to the heated injection port of the GC–MS system for further analysis.

2.3. GC–MS analysis

All analyses were carried out on a Shimadzu GC-17A, Version 3, QP-5050A gas chromatograph–mass spectrometer system (Shimadzu, Kyoto, Japan). The ionization mode was electron impact (70 eV). Data was acquired in the full-scan detection mode from 45 to 300 u at rate of 0.5 scan/s. The interface temperature was set at 250°C and the detector voltage was at 1.40 kV. A solvent delay time of 3 min was used with the analysis starting at 4 min. Injections were performed in the splitless mode at 200°C. Separation was performed on a 10 m×0.25 mm, 0.25 μm HP-5MS capillary column (Hewlett-Packard, Palo Alto, CA, USA). Helium (>99.999% pure) was used as a carrier gas at a flow-rate of 2.0 ml/min. The head-pressure was set at 29 kPa. The column oven was initially held at 60°C for 4 min, programmed to 150°C at a rate of 10°C/min and then to 250°C at 20°C/min.

3. Results and discussion

3.1. Optimization of solvent microextraction

The initial objective was to develop and optimize solvent microextraction sampling conditions for the extraction of explosives from water samples. For solvent microextraction there are several parameters to control optimum performance such as extraction solvent, rate of sample agitation, organic drop volume and ionic strength of the solution.

Four water-immiscible solvents differing in polarity and water solubility were tested. Solvent selectivity was evaluated for the extraction of a 5-ml sample containing 100 μg/l of each analyte in deionized water. The stirred solution (400 rpm) was sampled for 10 min using 1 μl of the appropriate organic solvent. As the solvents examined here varied in terms of water solubility, longer sampling times and faster stirring rates were avoided. The results are

given in Fig. 1. The extraction efficiency was based on the average peak area counts of each analyte for three replicate analyses. The results show that the non-polar hexane microdrop extracted relatively well the NTs but its extraction capacity decreased with increasing polarity of the target compounds. A similar behavior was encountered for the diisopropyl ether drop which suffered problems of dilution as it possess the highest value of solubility in water compared to the others. Significant solvent loss was also encountered in the case of chloroform. Overall, toluene gave the best results by combining good selectivity and showing no significant solvent loss during extraction. In addition, the toluene drop was found easy to manipulate with the lowest incident of drop loss even when faster stirring rates were applied. The stability of the toluene drop, allowed the use of a 15-min sampling time for the rest of the experiments on optimization of the proposed method, in order to increase the analytical response of the instrument.

Toluene has been successfully used in the past for the LLE of nitroaromatics in water samples [18]. As expected from these studies, extraction of nitramines with this solvent is either limited (for RDX) or even not possible (for HMX). Problems with HMX elution were also attributed to the thermal instability of the above analyte as well as the value of carrier gas linear velocity necessary for the chromatographic separation of the rest of the analytes [1,19]. Thus, HMX and RDX were not included in the present investigation. In all cases, investigation of nitrobenzene was not possible, as it co-eluted with the organic solvent during GC–MS analysis.

Sample agitation enhances extraction and reduces extraction time, especially for higher-molecular-mass analytes [12]. For the purpose of the present study three replicate analyses were taken at three different stirring rates: 0 (static case), 400 and 700 rpm. Faster stirring rates were avoided as they resulted in dislodgment of the organic drop from the needle tip. In all cases, the 1 μl toluene drop was exposed for 15 min to a 5-ml water sample spiked with 100 μg/l of each analyte. The results show clearly that stirring produces a dramatic increase in the analytical signal when compared to the stagnant case (Fig. 2). This is consistent with the expected behavior of solvent microextraction based on the film theory of convec-
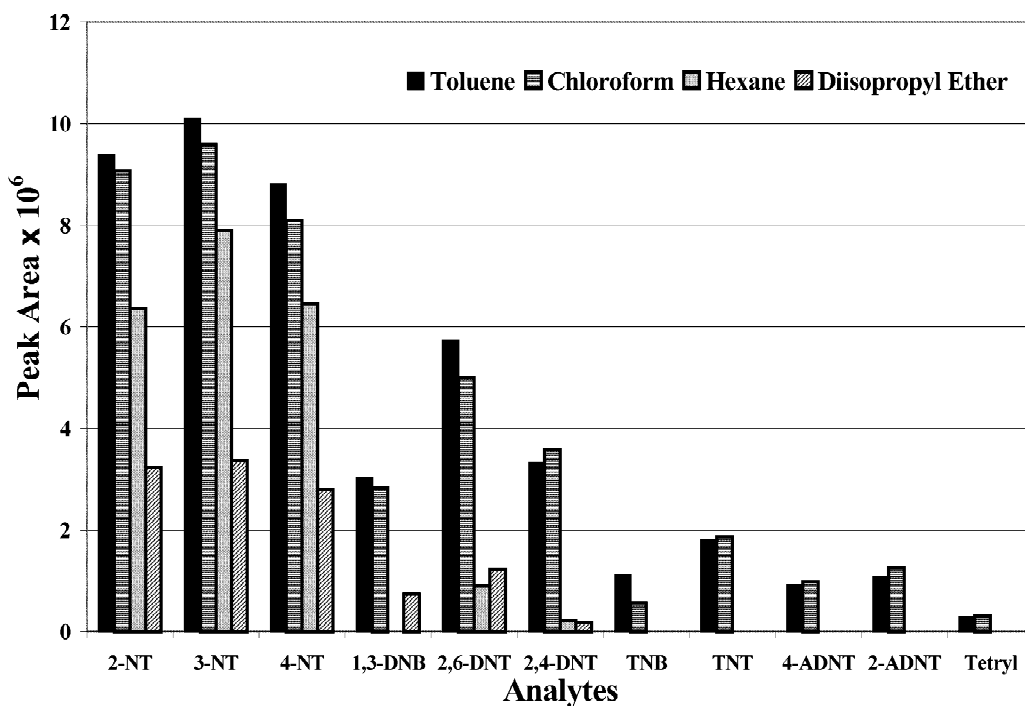
Fig. 1. Relative extraction efficiency after extracting for 10 min 5-ml spiked water samples (100 μg/l) of each analyte, using 1-μl drops of toluene, chloroform, hexane and diisopropyl ether, respectively.

tive–diffusive mass transfer [13]. According to this theory, uniform, instantaneous and complete convective mixing exists at some distance δ_{aq} (Nernst diffusion film) away from the liquid–liquid interface. At steady state the aqueous phase mass transfer coefficient is given by $k_{aq} = D_{aq}/\delta_{aq}$ where D_{aq} is the diffusion coefficient in the aqueous phase, k_{aq} is the mass transfer coefficient. At faster stirring rates the thickness of the diffusion film decreases causing an increase in the mass transfer coefficient and hence increased the extraction rate of target analytes. There is a limit however as faster stirring rates (700 rpm) enhance extraction of target analytes as well as dissolution of the toluene drop into the spiked aqueous solution (especially when prolonged sampling times are applied). Thus, for all subsequent experiments a stirring rate of 400 rpm was used.

Addition of salt to the sample may have several effects on extraction. More commonly, the presence of salt increases the ionic strength of the solution and affects the solubility of organic analytes such as explosives [20]. Extraction is usually enhanced with

increasing salt concentration and increased polarity of the compound (salting-out effect). This effect is dominant in SPME, where sodium chloride concentrations greater than 1% were reported to increase adsorption onto the fiber [21] of pollutants such as explosives [22]. For solvent microextraction, the effect of salt was previously studied for the extraction of 10 chlorobenzenes [15]. These reports concerned dynamic liquid-phase microextraction, where a conventional microsyringe was used as a separatory funnel. The results revealed that the presence of salt significantly decreased the extraction efficiency. To our knowledge there are no reports concerning the effect of salt when the drop of organic solvent is directly exposed to the water sample. Thus, the effect of NaCl concentration (ranging from 0 to 30%) was investigated and the extraction efficiency was monitored. The results, based on triplicate analyses, reveal an unexpected decrease in extraction efficiency with increased ionic strength for the majority of target analytes, which is more pronounced for the less polar ones (Fig. 3). A
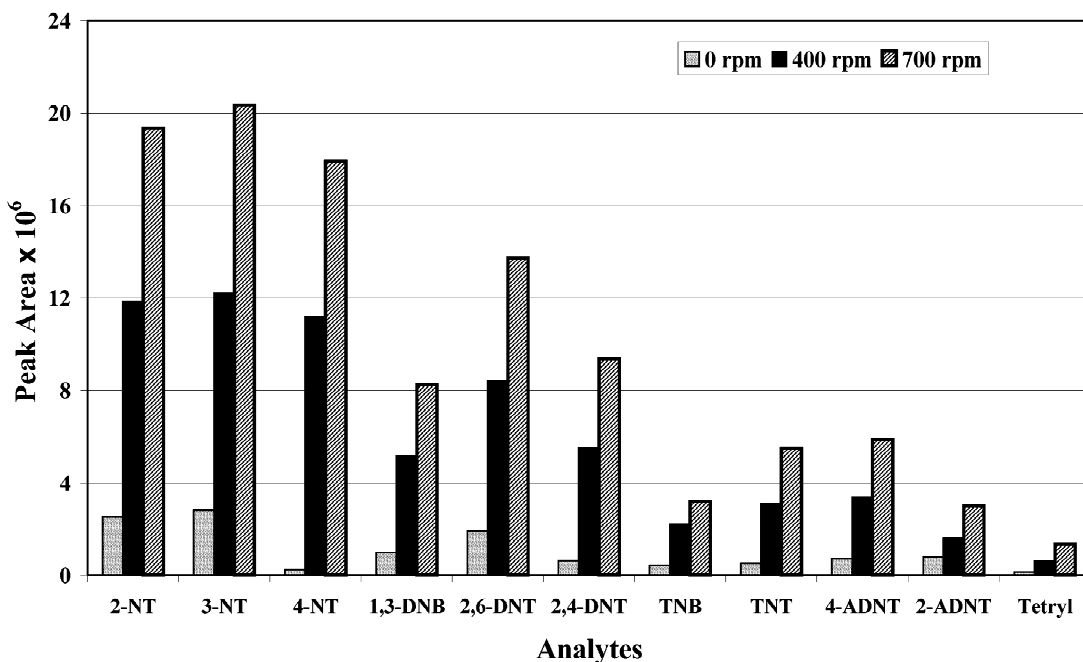
Fig. 2. Relative extraction efficiencies of target analytes with different stirring rates (0, 400 and 700 rpm): concentration 100 μg/l; sampling time 15 min; 1 μl organic drop.
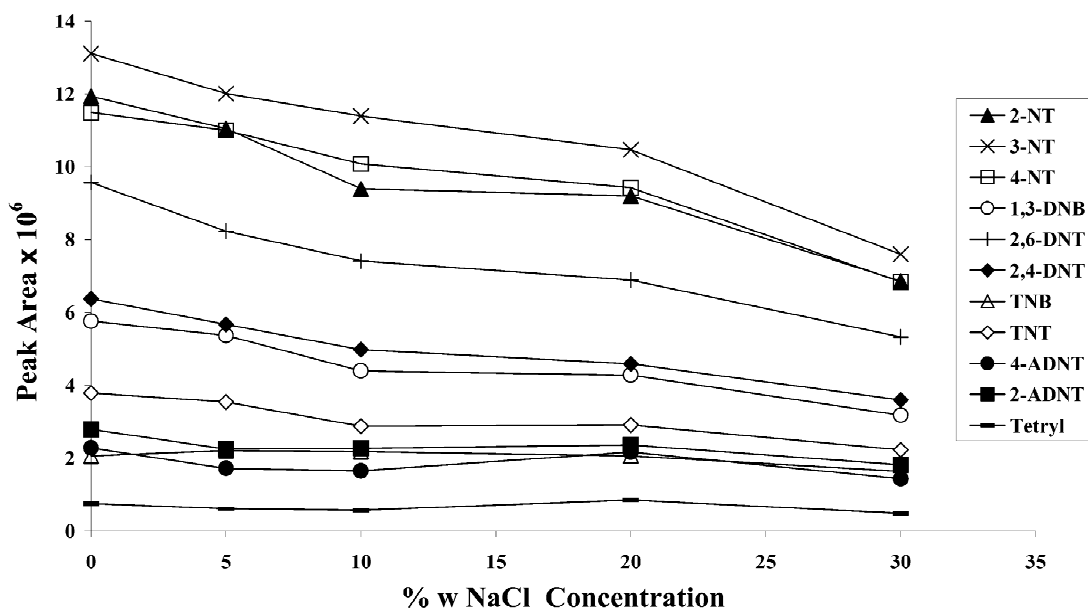

Fig. 3. Effect of salt concentration on the extraction efficiency: concentration 100 μg/l; stirring rate 400 rpm; sampling time 15 min; 1 μl organic drop.

possible explanation for this observation may be that apart from the salting-out effect, the NaCl dissolved in the aqueous solution may have changed the physical properties [23] of the Nernst diffusion film and reduced the rate of diffusion of the target analytes into the drop. This means that with increased salt concentration the diffusion of analytes towards the organic drop becomes more and more difficult limiting thus extraction. It is noteworthy, that for the more polar compounds (TNB to tetryl) the two effects seem to compensate each other, as the presence of salt causes insignificant changes on extraction efficiency.

3.2. Extraction time profiles

A series of spiked-water samples (100 μg/l) were prepared and the variation of the analytical signal for each analyte was studied as a function of exposure time (Fig. 4). In general, the more the toluene microdrop is exposed to the stirred contaminated sample, the more analytes are transferred in the organic drop [14]. However, solvent microextraction is not an exhaustive extraction method and analytes are partitioned between the bulk aqueous phase and the organic microdrop [12–15]. The total amount of analytes transferred in the drop reaches its maximum

when equilibrium between the two immiscible phases is established. Longer equilibration times were avoided as they resulted in solvent loss and drop displacement. On the basis of the curves obtained most analytes reached equilibrium after 45 min.

For quantitative analysis, it is not necessary for the analytes to have reached equilibrium, only to allow sufficient mass transfer into the drop and exact reproducible extraction time [14,22]. To avoid incidents of drop loss or dissolution, a 15-min extraction time was adopted, even though analytes had not reached equilibrium at this time point. The chosen sampling time was similar to the chromatography run time allowing thus maximum sample throughput.

3.3. Evaluation of method performance

Calibration curves were calculated using five spiking levels in the concentration range 20–1000 μg/l. For each spiking level three replicate analyses were performed. All procedures were carried out in triplicate to evaluate the inter-day reproducibility. The toluene microdrop contained this time a known amount of internal standard in order to quantify target analytes. The correlation coefficient (r^2) ranged from 0.9498 to 0.9857 as shown in Table 1.
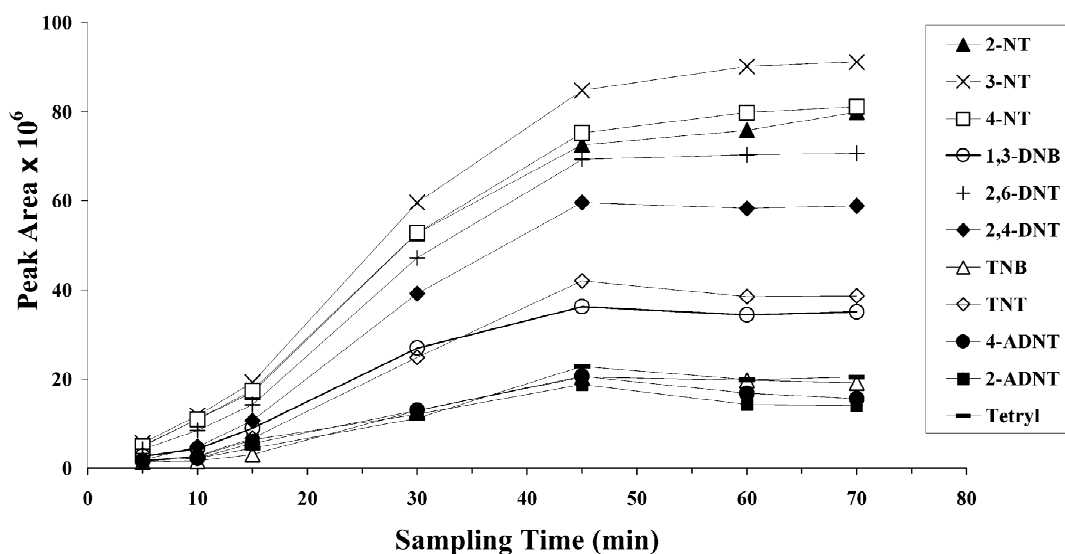

Fig. 4. Extraction time profiles for determination of optimum sampling times: concentration 100 μg/l; stirring rate 400 rpm; 1 μl organic drop.

Table 1
Main method parameters for solvent microextraction of 5-ml spiked water samples after exposing 1-μl toluene drop for 15 min

Analyte	Correlation coefficient (r^2)[a]	LOD $(\mu g/l)$[b]	RSD (%) $(n=5)$[c]
2-NT	0.9857	0.11	9.3
3-NT	0.9849	0.08	8.2
4-NT	0.9850	0.09	8.1
1,3-DNB	0.9608	0.47	4.5
2,6-DNT	0.9777	0.41	4.3
2,4-DNT	0.9652	0.53	8.2
TNB	0.9584	0.71	8.4
TNT	0.9711	0.40	6.8
4-ADNT	0.9656	1.3	8.9
2-ADNT	0.9784	0.80	8.7
Tetryl	0.9498	1.2	9.8

[a] Average of three experiments performed on different days.

[b] Lowest detectable concentration for a S/N ratio of approximately 3.

[c] Spiking level 100 μg/l.

The limits of detection (LODs) were determined according to published guidelines by comparing the signal-to-noise (S/N) ratio of the lowest detectable concentration to a S/N ratio of 3 [23]. Although a bench-top quadrupole mass spectrometer was used, the resulting LODs were found in the sub-ppb level (Table 1), well below the drinking water standards and health advisory numbers of the US Environmental Protection Agency for increased cancer risk of 10^{-4} (100 μg/l for TNT; 5 μg/l for DNB, 2,4-DNT and 2,6-DNT) [24]. Lower LODs are to be expected when using a larger sample and organic drop volume combined with the single ion monitoring (SIM) method in the mass spectrometer instead of the full-scan method used here. When SPME is used as a preconcentration tool, the resulting LODs vary depending on the polarity of the SPME fiber and the type of detector connected to the GC instrument. For example, detection of nitrobenzene and dinitrotoluenes using the non-polar polydimethylsiloxane (PDMS)-coated fiber and GC–flame ionization detection (FID), yielded detection limits from 9 to 15 μg/l [19]. However, the use of more polar SPME fiber coatings greatly improves sensitivities [19,22]. For example, when the Carbowax–divinylbenzene (CW–DVB) SPME fiber was coupled to GC–ion-trap (IT) MS, the detection limits were found in the low range of 0.005–0.010 μg/l for TNT and its amino metabolites [22]. Other preconcentration techniques such as LLE coupled to GC–electron-capture detection (ECD) yielded LODs as low as 0.003 for 2,6-DNT, 0.040 for 2,4-DNT and 0.060 for TNT μg/l [18]. For the SPE–GC–ECD combination, when 500-ml water samples were preconcentrated to 5 ml acetonitrile extracts, the LODs ranged from 0.04 to 0.20 μg/l [1].

The precision of the method was determined by conducting five replicate analyses of water samples spiked with 100 μg/l of each target analyte, under identical operating conditions. The results were based on the ratio of the analyte peak area to the internal standard peak area (Table 1). The precision of the proposed method was good, with the relative standard deviations (RSDs) ranging from 4.3 to 9.8%. Since no real-world samples were available, tap water samples from a chemistry laboratory and groundwater samples from a well in Pelekapina–Chania, were spiked with 100 μg/l of each target analyte and analyzed under the selected conditions in order to check the matrix effect on determination. Because solvent microextraction is a non-exhaustive extraction procedure, the relative recovery (determined as the ratio of the concentrations found in environmental and deionized water samples, spiked with the same amount of analytes), instead of the absolute recovery (used in exhaustive extraction procedures), was employed. As seen in Table 2, acceptable relative recoveries and RSD values were obtained for both environmental water samples, revealing that in the present context the matrix has little effect on the analysis of samples.

The r^2 and RSD values are not completely satisfactory, however they are comparable to the ones obtained in other SPME [22] and solvent microextraction procedures [14,17]. There are several possible reasons, which may explain these values. Firstly, at 15 min all target analytes are in the rising portion of the equilibration time profile, making precise timing essential for good precision [22]. During the experiments there was an unavoidable tolerance of few seconds, which may have increased the error for each measurement [14]. It is also possible that, during extraction, the internal standard present in the organic drop is partitioned between the two liquid phases, affecting thus precision [17]. Finally, the stir plate may have slightly increased the temperature of the samples, causing evaporation of

Table 2
Average relative recoveries and relative standard deviations (RSDs) for each target analyte after exposing 1-μl toluene drop for 15 min in stirred 5-ml tap water and groundwater samples spiked with 100 μg/l of each analyte ($n=5$)

Analyte	Tap water		Groundwater	
	Relative recovery[a] (%)	RSD (%)	Relative recovery[a] (%)	RSD (%)
2-NT	83	11.0	93	9.3
3-NT	84	10.4	94	9.0
4-NT	82	11.3	92	9.7
1,3-DNB	88	7.5	92	10.5
2,6-DNT	85	9.6	91	10.9
2,4-DNT	84	8.8	89	10.9
TNB	86	6.0	100	12.2
TNT	85	8.5	98	11.3
4-ADNT	97	8.7	92	12.0
2-ADNT	102	7.1	89	13.0
Tetryl	92	13.1	93	10.4

[a] Mean values for five determinations.

the more volatile contaminants into the headspace [17]. It is possible that lower RSD values will be obtained by automating the technique and controlling the temperature of the samples.

4. Conclusions

In this study, solvent microextraction has been demonstrated as a viable means of quantifying trace levels of nitroaromatics in water samples. The overall sample preparation time as well as the consumption of toxic organic solvents is minimized. For solvent microextraction there is no need of dedicated and expensive apparatus Although precision and accuracy are still in need, the extreme simplicity and cost-effectiveness of the proposed method makes solvent microextraction quite attractive when compared to SPME and other more labor-intensive methods such as LLE or SPE.

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