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Determination of nitroaromatic explosives in water samples by direct ultrasound-assisted dispersive liquid–liquid microextraction followed by gas chromatography–mass spectrometry

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ABSTRACT

A fast, simple, inexpensive, sensitive, efficient and environmental friendly direct ultrasound-assisted dispersive liquid–liquid microextraction (DUSA-DLLME) procedure has been developed to concentrate five nitroaromatic explosives from water samples prior to quantification by gas chromatography–mass spectrometry (GC–MS). An efficient ultrasonic probe has been used to radiate directly the samples producing very fine emulsions from immiscible liquids. A D-optimal design was used for optimizing the factors and to evaluate their influential upon extraction. The optimum experimental conditions were: sample volume, 10 mL; extraction time, 60 s; cycles, 0.6 s(s^{-1}); power of ultrasound energy, 40% (70 W); and, extractant solvent (chlorobenzene) volume, 20 μL. Under the optimized experimental conditions the method presents good level of repeatability with coefficients of variation under 6% (n = 8; spiking level 10 μg L^{-1}). Calculated calibration curves gave high level of linearity with correlation coefficient values between 0.9949 and 0.9992. Limits of detection were ranged between 0.03 and 0.91 μg L^{-1}. Finally, the proposed method was applied to the analysis of two types of water samples, reservoir and effluent wastewater. The samples were previously analysed and confirmed free of target analytes. At 5 μg L^{-1} spiking level recovery values ranged between 75 and 96% for reservoir water sample showing that the matrix had a negligible effect upon extraction. However, a noticeable matrix effect (around 50% recovery) was observed for effluent wastewater sample. In order to alleviate this matrix effect, the standard addition calibration method was used for quantitative determination. This calibration method supplied recovery values ranged between 71 and 79%. The same conclusions have been obtained from an uncertainty budget evaluation study.

1. Introduction

Over the past few years the interest in nitroaromatic explosives (NE) analysis has increased due to military activities, but especially for the continuous upsurge in terrorist activity. This has generated tremendous demand for innovative analytical tools capable of detecting these compounds. Real samples are usually complicated matrices and these compounds are at low concentration, therefore, extraction is often recommended before detection. Conventional liquid–liquid extraction (LLE) and solid phase extraction (SPE) are the most commonly used methods of sample pretreatment for isolation and/or enrichment of nitroaromatic explosives [1]. However, they present many disadvantages as they are tedious, labor-intensive and time-consuming. In addition, LLE requires large amounts of organic solvents that are potentially toxic, and is difficult to automate. SPE uses much less solvent than LLE but can be relatively expensive. Besides, prior to chromatographic analysis LLE and SPE often require solvent evaporation in order to preconcentrate the samples. During this evaporation step, loss and/or deterioration of target analytes have been reported [2].

Efforts have been placed on the miniaturization of the SPE and LLE procedures to remove these disadvantages. Solid-phase microextraction (SPME) is a solvent-free extraction technique that incorporates sample pretreatment, concentration and sample introduction into a single procedure. However, the extraction fiber is expensive, fragile, requires conditioning and it has a limited lifetime, and in addition, sample carry-over between runs can be a problem [3]. Liquid–liquid microextraction (LLME) is a single-step extraction with a very high sample-to-solvent ratio which leads to high enrichment factor of analytes. In comparison to the traditional LLE and SPE, LLME has many advantages including wide selection of available solvents, low cost, simplicity and ease of use,

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short preconcentration time, virtually solventless and possibility of automation. In addition, it is characterized by its affordability, due to its independence of a commercial source. One of the LLME approach is based on analyte partitioning between a droplet of organic solvent (extractant phase) and the aqueous sample matrix, named single-drop microextraction (SDME) [4]. However, in SDME fast stirring speed and air bubbles cause drop instability and tend to break down the organic droplet, and relative low precision were often reported. Other configurations of LLME have been recently developed [5,6] with the aim of solving these problems. One of this novel liquid–liquid microextraction techniques is the dispersive liquid–liquid microextraction (DLLME), which was introduced by Assadi et al. [7,8]. DLLME is based on the formation of tiny droplets of extractant in the sample solution using water-immiscible organic solvent (extractant) dissolved in a water-miscible organic dispersive solvent. This novel technique has the advantages of simplicity, rapidity, low sample volume, low cost, high recovery, and a high enrichment factor. Both microextraction techniques (i.e., SPME and LLME) have been used for the determination of NE in soil and water samples [9–13].

Sonochemistry is a growing topic in science and technology [14]. The effects of ultrasound on chemical and physical transformations are through the phenomenon of cavitation. Cavitation is the production of microbubbles in a liquid when a large negative pressure is applied to it [14]. Sonication can be used to produce very fine emulsions from immiscible liquids, which result in very large interfacial contact areas between the liquids and a corresponding dramatic increase in the mass transfer between two immiscible phases. This leads to an increment in the extraction efficiency of the procedure in a minimum time [15,16]. For this reason, sonochemistry has been combined with dispersive liquid–liquid microextraction and the result has been a new technique called ultrasound-assisted emulsification–microextraction (USAEME) or ultrasound-assisted dispersive liquid–liquid microextraction (USA-DLLME). The use of ultrasound energy to disrupt the extractant phase reduces the consumption of organic solvent, because the necessity of using a third component (disperser solvent) is not needed. Moreover, the use of disperser solvent usually decreases the partition coefficient of analytes into the extractant solvent. Therefore, these drawbacks are eliminated when sonochemistry is introduced. This technique was for the first time applied to the determination of synthetic musk fragrances, phthalate esters and lindane in water samples [17]. Fontana et al. [18] reported the extraction and preconcentration of polybrominated diphenyl ethers from environmental waters. Ozcan et al. [19,20] extracted some selected polychlorinated biphenyls and organochlorine pesticides from water samples, and Cortada et al. [21] extracted geosmin and 2-methylisoborneol in water and wine samples. Simultaneous USAEME and derivatization have been combined to extract parabens, triclosan and related phenols in water samples [22]. More recently Li et al. [23] have introduced an ionic liquid-based ultrasound-assisted dispersive liquid–liquid microextraction method (IL-based USA-DLLME) for cadmium determination in water samples.

In most of the published papers cited above [17–23] an ultrasonic bath has been used to irradiate the liquids. Nevertheless, it is well known that ultrasonic bath is less efficient in energy transmission than ultrasonic probe [14], and hence, the former produces a lower rate of emulsification [24].

Therefore, the main aim of this work was to develop a new, simple, rapid, inexpensive, sensitive, and efficient direct ultrasound-assisted dispersive liquid–liquid microextraction (DUSA-DLLME) method for extraction of NEs from aqueous samples. In this method the ultrasonic probe (i.e., sonotrode) was directly introduced into the sample increasing the efficiency of energy transmission. DUSA-DLLME extraction parameters (i.e., sample volume, extraction time, cycles, power of ultrasound energy, extractant solvent type and volume) were optimized and the procedure was then applied to the determination of five nitroaromatic explosives in real reservoir water and effluent wastewater samples. Determinations were carried out by gas chromatography–mass spectrometry (GC–MS). The optimization of the microextraction conditions has been done using experimental design [25], and uncertainty budget [26] has been used for establishing confidence as a more realistic approach to the regulatory environment.

2. Experimental

2.1. Chemicals, "real-world" water samples and apparatus

Nitrobenzene, 2-nitrotoluene, 2,6-dinitrotoluene, 2,4,6-trinitrotoluene and 2-amino-4,6-dinitrotoluene (2-ADNT) were obtained from Sigma-Aldrich (St Louis, MO, USA). Chlorobenzene, carbon tetrachloride, tetrachloroethylene and methanol pesticide-grade were also obtained from Sigma-Aldrich. De-ionised water was prepared on a water purification system (Gradient A10) supplied by Millipore (Billerica, MA, USA). Stock standard solution of 2 mg L^{-1} of target compounds was prepared in methanol. Working solutions were prepared by proper dilution of stock standard solution in water. All solutions were stored in the dark at 4 °C.

The recovery studies were carried out using reservoir water (Seville, Spain) and effluent wastewater (Ourense, Spain) samples, which were also stored in the dark at 4 °C. Initial analysis confirmed that they were free of all target analytes.

An ultrasonic processor (175 W, 24 kHz) with a titanium cylindrical sonotrode (7 mm o.d.; 110 mm long, reference S7) from Dr. Hielscher (Teltow, Germany) was used as the sonic probe. The instrument can be used in pulsed mode to enable rhythmic processing of media. With a pulse setting of "1" the reaction mixture is sonicated without interruption whereas with a pulse setting, for example, of "0.5" the reaction mixture is sonicated for 0.5 s and then sonication stops for 0.5 s. Hence, in pulse mode the ratio of sound-emission time to cyclic pause time can be adjusted continuously from 0 to 100% per second. Centrifuge table GS-6R model of Beckman (Fullerton, CA, USA) was used for centrifugation of the samples.

2.2. Direct ultrasound-assisted dispersive liquid–liquid microextraction (DUSA-DLLME)

10 mL of sample were placed into a 20 mL glass test tube with conical bottom. 20 μL of chlorobenzene as extractant solvent (Caution: be aware with the hazardous for the environment. Especially precaution should be taken for waste management and residues after extraction) were dropped into the sample solution and then the mixture was homogenized to avoid that the solvent remains in the cone inhibiting dispersion. 75 mm of the titanium cylindrical sonotrode was directly introduced into the reaction mixture for 60 s. Once the extraction was finished the mixture was centrifugated for 4 min at 2300 rpm in the centrifuge table. Finally, 2 μL of the extractant phase located at the bottom of the tube were manually injected into the GC–MS system for analysis.

2.3. GC–MS determination

All analyses were carried-out on a Varian 3900-Saturn 2100T Gas Chromatography/Mass Spectrometer system (Walnut Creek, CA, USA) equipped with a Meta.X5 Tecknokroma column (30 m × 0.25 mm, 1.0 μm) (Barcelona, Spain). The mass spectrometer employed was an ion trap (20 μA) with 0.82 s of scan time. The injector was maintained at 220 °C and operated in the splitless mode with the split closed for 0.75 min. Helium (>99.999% pure)

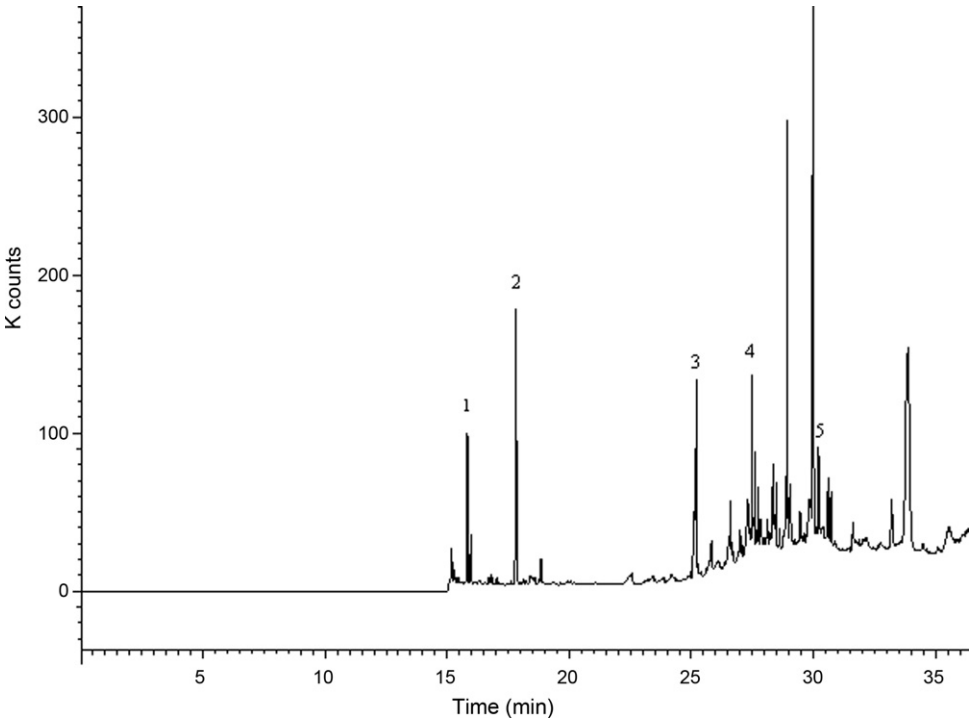

Fig. 1. Typical chromatogram of a de-ionized water sample (10 μg L^{-1}) extracted by the DUSA-DLLME-GC-MS method. (1) Nitrobenzene; (2) 2-nitrotoluene; (3) 2,6-dinitrotoluene; (4) 2,4,6-trinitrotoluene; (5) 2-ADNT. Conditions: extractant (chlorobenzene) volume, 20 μL; sample volume, 10 mL; extraction time, 60 s; cycles, 0.6 s(s^{-1}); and power, 40% (70 W).

was used as the carrier gas at a flow rate of 1.0 mL min^{-1}. The column oven was initially set at 60 °C for 2 min, then programmed at 5 °C min^{-1} to 150 °C where it was held for 2 min, and finally to 250 °C at 20 °C min^{-1} rate, where it was held for 10 min. The interface temperature was set at 200 °C and the detector voltage at 4 V. A 15 min solvent cut time was allowed for all analyses. The base peak ion and two other significant ions of each analyte were isolated during the whole chromatogram and were chosen as the quantifying ions. The base peaks ion (*m*/*z*) for the target analytes were: nitrobenzene: 77; 2-nitrotoluene: 65; 2,6-dinitrotoluene: 165; 2,4,6-trinitrotoluene: 210 and 2-amino-4,6-dinitrotoluene: 180. Prior to quantification, the identification of all target compounds was based on their mass spectra and GC retention times. Fig. 1 shows a typical chromatogram obtained after extraction of a spiked de-ionised sample containing 10 μg L^{-1} of each target analyte.

2.4. Data handling and processing

According to a previous work, the response of the instrument used in the design of experiments study was based on each area of the peaks individually obtained during GC–MS analysis [27].

Experimental design matrices were constructed and the results were evaluated using the Statgraphics Statistical Computer Package "Statgraphics Plus 5.1" (Warrenton, VA, USA).

3. Results and discussion

3.1. Study of experimental factors involved in DUSA-DLLME

3.1.1. Solvent study

The first step in the optimization procedure was to select an appropriate extraction solvent. The solvents were selected on the basis of higher density than water, extraction capability of target compounds and good gas chromatography behaviour. Carbon tetrachloride, chlorobenzene and tetrachloroethylene were tested as potential acceptor phases. Solvent selectivity was evaluated with 20 μL of each extractant solvent into a 10 mL of de-ionized water sample spiked at 100 μg L^{-1} with all target analytes. From the three tested solvents chlorobenzene gave the best results, as can be seen in Fig. 2. Polarity of the solvents is similar, where values in terms of log *P* are 2.91, 2.84 and 3.07 for carbon tetrachloride, chlorobenzene and tetrachloroethylene, respectively. However, chlorobenzene has an aromatic ring which allows π–π interaction with the NEs, and therefore, higher extraction.

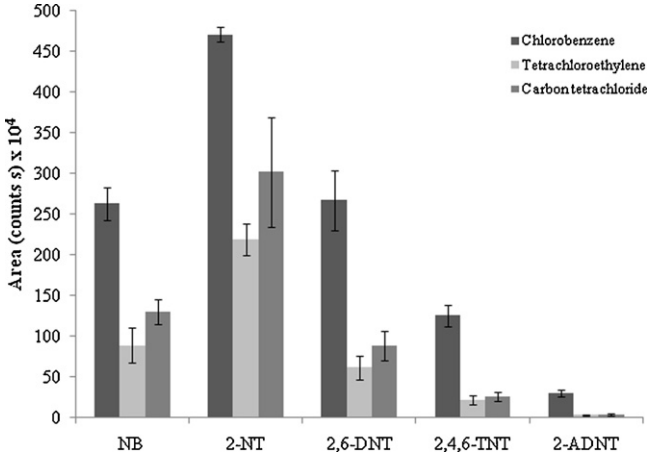

Fig. 2. Response of the three organic solvents tested. De-ionised water samples spiked at concentration of 100 μg L^{-1}. Conditions: extractant volume, 20 μL; sample volume, 10 mL; extraction time, 5 min; cycles, 0.5 s(s^{-1}); and power, 55% (96 W).

3.1.2. Study of others experimental factors

Different factors can affect the extraction yield in the direct ultrasound-assisted dispersive liquid–liquid microextraction procedure and in most cases they are correlated. Therefore, their

Table 1
Experimental factors and levels studied on the D-optimal design.

Factor	Level		
	Low (−1)	Central (0)	High (+1)
Extractant volume (μL)	20	–	40
Sample volume (mL)	5	–	10
Extraction time (s)	30	–	60
Cycles (s(s^{-1}))	0.4	0.7	1.0
Power (%)[a]	40 (70)	70 (123)	100 (175)

[a] Numbers in parentheses are absolute ultrasound power values in W.

Table 2
Parameters of the evaluation of significance of the proposed model.

Analyte	*R*-square (%)	*p*-value[a]
Nitrobenzene	99.9	0.0915
2-Nitrotoluene	99.6	0.1151
2,6-Dinitrotoluene	97.2	0.3140
2,4,6-Trinitrotoluene	98.3	0.2690
2-ADNT	98.8	0.4016

[a] Significance level: 0.05.

optimization through a multivariate approach is recommended. The methodology based on the design of experiments (DOEs) is a useful tool that can be used to find the best experimental conditions. Various types of designs can be applied as factorial designs [20] that are appropriate for assessing main effects and interactions between factors; fractional designs [28] that only concerned with the main effects, and central composite, Doehlert designs and those designs based on the simplex method that are used for optimizing the experimental conditions when dealing with continuous factors [29,30]. In many cases, it is not possible to apply these classical experimental designs due to constraints either on the experimental domain (cost of certain reagents, safety or incompatibility in the experimental conditions, etc.) or on the number of experiments (time-consuming analysis, cost, material, etc.). These limitations obliged to reduce experimentation by selecting those experiments that, complying with the constraints enforced, keep the highest quality of the design, the reliability of the estimations and therefore the conclusions derived from it. In the present work, in order to reduce resources (time, reagents and sample) invested, and that two out of the five factors under study were not expected to have a linear behaviour, the experiments to be performed were selected according to the D-optimality criterion [31]. D-optimal designs have the property that the estimations of the coefficients of the model are the most precise possible [31]. More detailed information of D-optimal design can be found in Ref. [31].

The first step of the experimental design methodology was the selection of the factors, experimental domain and the response function. Three of these factors named extractant volume, sample volume and extraction time were chosen at two levels, low (−1) and high (+1), and the other two factors, namely cycles and power of ultrasound energy were chosen at three, low (−1), central (0) and high (+1). The selected values chosen for each factor are given in Table 1. Three levels must be chosen for cycles and power of ultrasound energy in order to avoid the problem associated to lack of linearity. It should be emphasized that the number of experiments needed using a full factorial design is 72. However, using D-optimal design the number of experiments selected by the program is only 21 (see Supplementary material for more information). As it has been described above, the peak area of each analyte has been used as a response of the proposed model.

The second step was the selection of the mathematical model which represents the phenomenon studied, and a second-order

Table 3
Estimated effects, *p*-values and levels of the coefficients of factors of the model.

Coefficient	Nitrobenzene			2-Nitrotoluene			2,6-Dinitrotoluene			2,4,6-Trinitrotoluene			2-ADNT		
	Estimation	*p*-value[a]	Level	Estimation	*p*-value[a]	Level	Estimation	*p*-value[a]	Level	Estimation	*p*-value[a]	Level	Estimation	*p*-value[a]	Level
b_1	−106,691.0	0.0023	−	−357,101.0	0.0053	−	−388,579.0	0.0382	−	−482,200.0	0.0186	−	−68,693.0	0.0178	−
b_2	26,942.9	0.0333	+	114,989.0	0.0468	+	200,871.0	0.1220	+	41,371.0	0.5944	+	9727.5	0.4008	+
b_3	24,860.6	0.0313	+	101,961.0	0.0475	+	84,568.5	0.3463	+	204,764.0	0.0738	+	29,785.8	0.0683	+
b_4	−24,265.4	0.0489	−	−44,926.1	0.2559	−	53,330.1	0.5969	+	−43,426.6	0.6117	−	−23,740.9	0.1445	−
b_5	−31,846.6	0.0184	−	−75,051.9	0.0787	−	−16,694.4	0.8271	−	7696.4	0.9054	+	−13,769.1	0.2266	−

[a] Significance level: 0.05.

Table 4
Optimum extraction conditions for each analyte.

Analyte	Extractant volume (μL)	Sample volume (mL)	Extraction time (s)	Cycles (s(s^{-1}))	Power (%)[a]
Nitrobenzene	20	10.0	58.3	0.65	40 (70)
2-Nitrotoluene	20	10.0	59.3	0.67	41 (72)
2,6-Dinitrotoluene	20	10.0	54.0	0.88	40 (70)
2,4,6-Trinitrotoluene	20	10.0	60.0	0.65	68 (119)
2-ADNT	20	9.2	60.0	0.61	40 (70)

[a] Numbers in parentheses are absolute ultrasound power values in W.

linear model was proposed:

$$y = b_0 + b_1x_1 + b_2x_2 + b_3x_3 + b_4x_4 + b_5x_5 + b_6x_1x_2 + b_7x_1x_3 + b_8x_1x_4 + b_9x_1x_5 + b_{10}x_2x_3 + b_{11}x_2x_4 + b_{12}x_2x_5 + b_{13}x_3x_4 + b_{14}x_3x_5 + b_{15}x_4x_5 + b_{16}x_4^2 + b_{17}x_5^2$$

where y is the response function (peak area), b_0 is the intercept, b_{1-17} are the regression coefficients that estimate the effect of each factor (b_{1-5}), interactions (b_{6-15}) and quadratic effects of two factors (b_{16-17}), and x_i are the independent factors, where x_1 is extractant volume, x_2 is sample volume, x_3 is extraction time, x_4 is cycles and x_5 is power of ultrasound energy.

From the analysis of the 21 experiments results, the *R*-square and *p*-values from Durbin–Watson statistical test [32] are shown in Table 2. As can be seen, values of *R*-square are between 97.2 and 99.9% and these values indicate that the model chosen explains the variability of the factors with the response (y). The Durbin–Watson statistic is a test to determine if there is any residual autocorrelation. The significance level was settled at 0.05. All *p*-values shown in Table 2 are greater than 0.05, therefore the null hypothesis is accepted, which considers that there is no autocorrelation in the residuals, and therefore the model proposed for this study was valid.

The data obtained were evaluated by ANOVA test, and the coefficients of the factors are listed in Table 3. Only coefficients of main factors are presented in Table 3, due to only few interactions for three out of the five analytes were significant, and the results were also according to those of the main factors. Therefore, to select the optimum conditions of the extraction procedure only the effects of main factors were considered. In order to determine if a coefficient is significant and consequently, if the corresponding factor affects the extraction procedure, the significance level was also settled at 0.05. Therefore, those coefficients, whose *p*-value is smaller than 0.05, will be considered statistically significant.

As can be seen, extractant volume shows a negative significant effect upon extraction for all target analytes. Indeed, decreasing extractant volume increases the concentration of analyte extracted and therefore the signal increased. Sample volume and extraction time show a positive effect upon extraction for all target analytes but only for nitrobenzene and 2-nitrotoluene have significant effect. Indeed, increasing the sample volume results in an increase of the total amount of analyte extracted. In case of extraction time, the amount of analytes extracted is also increased when time is increased. Nevertheless, extraction time is not significant for three of the five compounds, and this could be due to equilibrium is reached in few seconds once the solvent is fully dispersed.

Cycles show a negative non-significant effect for all targets except a negative significant effect for nitrobenzene and a positive non-significant effect for 2,6-dinitrotoluene. Decreasing the cycles the amount of analyte extracted increases. This effect could be explained because an increase in cycles results in a temperature increase at a microscale level that will raise the vapour pressure of a medium, and so lead to easier cavitation but less violent collapse. This large number of cavitation bubbles will act as a barrier to sound transmission and dampen the effective ultrasonic energy from the source which enters the liquid medium. Power shows a non-significant negative effect for all the compounds except for 2,4,6-trinitrotoluene where the effect is positive and for nitrobenzene that shows a negative significant effect. A minimum of ultrasound energy is required to reach the cavitation threshold, but when a large amount of ultrasonic power enters a system, a great number of cavitation bubbles are generated in the solution. These will certainly act as a barrier to sound transmission as in the cycles effect [14]. Optimum extraction conditions for each target compound are shown in Table 4. However, compromise values chosen as optimum for the simultaneous extraction of all NE compounds with this method were: extractant volume, 20 μL; sample volume, 10 mL; extraction time, 60 s; cycles, 0.6 s(s^{-1}); and power, 40% (70 W).

3.2. Analytical figures of merit

The optimum conditions of direct ultrasound-assisted dispersive liquid–liquid microextraction were used to assess the applicability of the proposed method for quantitative determination of target analytes by GC–MS. A calibration study was performed by spiking de-ionized water with analytes over the concentration range of 1–10 μg L^{-1}. The calculated calibration curves gave a high level of linearity for all target analytes with correlation coefficients (r) between 0.9949 and 0.9992 (Table 5). The repeatability of the proposed method, expressed as coefficient of variation (CV), was evaluated by extracting and analyzing eight consecutive aqueous samples spiked at 10 μg L^{-1} with each target analyte, and the CV values were ranged between 3.9 and 5.1% (Table 5). The limits of detection (LODs) for all target analytes were determined according to a signal-to-noise-ratio (S/N) of three and the limits

Table 5
Main method (DUSA-DLLME-GC-MS) parameters for NEs determination in water samples.

Analyte	Correlation coefficient (r)[a]	CV (%)[b]	LOD (μg L^{-1})[c]	LOQ (μg L^{-1})[d]
Nitrobenzene	0.9992	4.1	0.10	0.3
2-Nitrotoluene	0.9989	4.6	0.03	0.1
2,6-Dinitrotoluene	0.9949	4.3	0.06	0.2
2,4,6-Trinitrotoluene	0.9957	3.9	0.17	0.6
2-ADNT	0.9981	5.1	0.91	3.0

[a] Linear range: 1–10 μg L^{-1} (number of calibration points = 5, replicates = 3 for level).
[b] Coefficient of variation (CV): mean value for 8 replicate analyses; spiking level 10 μg L^{-1}.
[c] Limits of detection (LODs): calculated for a three signal to noise ratio (S/N = 3).
[d] Limits of quantification (LOQs): calculated for a ten signal to noise ratio (S/N = 10).

Table 6
Comparison of DUSA-DLLME-GC-MS method with other analytical methods for the determination of NEs.

Preconcentration method	Separation/detection technique	Linear range ($\mu g\,L^{-1}$)	LOD ($\mu g\,L^{-1}$)	Solvent (volume)	Extraction time (min)	Volume sample (mL)	References
SOE[a]	HPLC-UV	1–50	0.04–0.31	Acetonitrile (165 mL)	100	770	[1]
SPME[b]	GC–MS	20–1000	0.03–0.29	–	15	5	[9]
SDME[c]	GC–MS	20–1000	0.11–0.80	Toluene (1 μL)	15	5	[10]
HF-LPME[d]	GC–MS	10–500	0.3–0.64	Toluene (3 μL)	20	5	[11]
DLLME[e]	GC-FID	0.5–300	0.09–0.4	Carbon tetrachloride/methanol (20/750 μL)	2	9	[13]
DUSA-DLLME	GC–MS	1–10	0.03–0.91	Chlorobenzene (20 μL)	1	10	This work

[a] Salting-out extraction.
[b] Solid phase microextraction.
[c] Single drop microextraction.
[d] Hollow fiber liquid-phase microextraction.
[e] Dispersive liquid–liquid microextraction.

of quantification (LOQs) as ten times the above mentioned ratio. As can be seen in Table 5 the LODs and LOQs values were found to be in the low $\mu g\,L^{-1}$ level. Limit of detection, extraction time, extractant solvent, solvent and sample volume and linear range of different methods are shown in Table 6 [1,9–11,13]. The LODs of the proposed method are of the same order or lower for some of the analytes than those obtained in previous works. However, DLLME and DUSA-DLLME are faster (1–2 min) and easier-handle methods. On the other hand, LODs obtained in the present work for 2-nitrotoluene and 2,6-dinitrotoluene are three times lower and almost one order of magnitude lower, respectively, than LODs obtained with DLLME method [13].

3.3. Analysis of "real-world" samples

Two different types of water samples were extracted using the DUSA-DLLME method and the extracts were quantified by GC–MS. Five aliquots of the reservoir water and effluent wastewater samples were spiked at 5 $\mu g\,L^{-1}$ with all target contaminants and analysed under the optimized experimental conditions. Preliminary analysis showed that samples were free of all studied compounds.

The results for each set of experiments are summarized in Table 7. The amount of the extracted analytes is presented as found concentration and recovery values. The latter, ranged between 75 and 96% for reservoir water, and therefore no noticeable matrix effects were observed. However, effluent wastewater shows recoveries between 49 and 88%, where 2,4,6-trinitrotoluene and 2-ADNT shows values around 50%. This implies that there is a significant matrix effect.The found concentration was accompanied by expanded uncertainty (Table 7), which values were calculated based on Refs. [21,26]. Uncertainty of measurement is a component of uncertainty in all individual steps of an analytical procedure. Hence, it is necessary to determinate the sources and types of uncertainty for all these steps. Estimation of uncertainty leads to better measurement reliability, renders data from inter-laboratory studies comparable, and helps to assess the statistical significance of the difference between the measurement and a relevant reference value [26]. As can be seen, for reservoir water no systematic error is concluded since reference value (spiked value) is included into the found concentration ± expanded uncertainty intervals. In the case of effluent wastewater sample a systematic error is concluded for nitrobenzene, 2,4,6-trinitrotoluene and 2-ADNT, which was confirmed with the low recovery values.

Table 7
Found concentration and mean recoveries of NEs in water samples.

Analyte	Found concentration ± U ($k=2$)[a] $\mu g\,L^{-1}$ (recovery ± CV, %)[b]	
	Reservoir water	Effluent wastewater
Nitrobenzene	4.8 ± 1.8 (96 ± 13)	3.6 ± 1.3 (72 ± 16)
2-Nitrotoluene	4.6 ± 2.0 (93 ± 13)	3.6 ± 1.5 (72 ± 15)
2,6-Dinitrotoluene	3.8 ± 1.7 (75 ± 14)	4.4 ± 1.6 (88 ± 12)
2,4,6-Trinitrotoluene	4.0 ± 1.6 (80 ± 14)	2.6 ± 1.6 (52 ± 13)
2-ADNT	4.1 ± 2.4 (81 ± 11)	2.5 ± 2.0 (49 ± 10)

[a] U = expanded uncertainty.
[b] Five replicate analyses at 5 $\mu g\,L^{-1}$ spiking level.

Table 8
Found concentration of NEs in effluent wastewater samples with standard addition calibration method.

Analyte	Correlation coefficient (r)[a]	Found concentration ± U ($k=2$)[b] $\mu g\,L^{-1}$ (recovery ± CV, %)[c]
Nitrobenzene	0.9940	3.1 ± 1.3 (77 ± 14)
2-Nitrotoluene	0.9967	2.9 ± 1.1 (71 ± 8)
2,6-Dinitrotoluene	0.9965	3.0 ± 1.2 (74 ± 9)
2,4,6-Trinitrotoluene	0.9924	3.2 ± 1.2 (79 ± 10)
2-ADNT	0.9963	3.1 ± 1.9 (78 ± 12)

[a] Linear range: 0–6 $\mu g\,L^{-1}$ (number of calibration points = 4).
[b] U = expanded uncertainty.
[c] Three replicate analyses at 4 $\mu g\,L^{-1}$ spiking level.

For this reason, the standard addition calibration method was used for quantitative determination of the NEs in effluent wastewater due to the matrix effects observed. The standard addition calibration curve was performed with effluent wastewater until 6 $\mu g\,L^{-1}$ and gave a high level of linearity for all target analytes with correlation coefficients (r) between 0.9924 and 0.9967. The three aliquots of effluent wastewater samples were spiked at 4 $\mu g\,L^{-1}$ with all target compounds. The results obtained are summarized in Table 8. Recovery values are ranged between 71 and 79% and it can be seen now that reference value is included into the found concentration ± expanded uncertainty intervals, and therefore no systematic error is presented.

4. Conclusions

A new, simple, rapid, inexpensive, sensitive and efficient DUSA-DLLME method has been introduced for extraction of five NEs from aqueous samples. Ultrasonic probe is directly introduced into the sample increasing the efficiency of energy transmission.

The new sample preparation method has been optimized with a non-common DOE approach (D-optimal design). This optimization approach significantly reduces the number of experiments and introduces experimental conditions that are not able to study with classical experimental designs.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.talanta.2011.08.011.

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