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Optimization of dispersive liquid–liquid microextraction combined with gas chromatography for the analysis of nitroaromatic compounds in water

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ABSTRACT

Dispersive liquid–liquid microextraction (DLLME) coupled with gas chromatography-flame ionization detector (GC-FID) was developed for preconcentration and determination of some nitroaromatic compounds in wastewater samples. The effects of different variables on the extraction efficiency were studied simultaneously using experimental design. The variables of interest in the DLLME process were extraction and disperser solvent volumes, salt effect, sample volume, extraction temperature and extraction time. A Plackett–Burman design was performed for screening of variables in order to determine the significant variables affecting the extraction efficiency. Then, the significant factors were optimized by using a central composite design (CCD) and the response surface equations were derived. The optimum experimental conditions found from this statistical evaluation included: sample volume, 9 mL; extraction solvent (CCl_4) volume, 20 μL; disperser solvent (methanol) volume, 0.75 mL; sodium chloride concentration, 3% (w/v); extraction temperature, 20 °C and extraction time, 2 min. Under the optimum conditions, the preconcentration factors were between 202 and 314. Limit of detections (LODs) ranged from 0.09 $\mu g\,L^{-1}$ (for 2-nitrotoluene) to 0.5 $\mu g\,L^{-1}$ (for 2,4-dinitrotoluene). Linear dynamic ranges (LDRs) of 0.5–300 and 1–400 $\mu g\,L^{-1}$ were obtained for mononitrotoluenes (MNTs) and dinitrotoluenes (DNTs), respectively. Performance of the present method was evaluated for extraction and determination of nitroaromatic compounds in wastewater samples in the range of microgram per liter and satisfactory results were obtained (RSDs < 10.1%).

1. Introduction

Nitroaromatic compounds are released into the biosphere mainly from the chemical industry. They are highly toxic and can persist in environment [1]. Nitroaromatic compounds are used for production of pesticides, drugs, explosives, polymers, dyes, rubber chemicals, etc. [2,3]. Owing to severe toxicity and doubtful carcinogenicity, they should be abated from wastewater before disposal to the environment [4,5]. Determination of nitroaromatic compounds in water samples is generally performed by gas chromatography coupled to a sample pre-treatment such as the traditionally used liquid–liquid extraction (LLE) [6], solid-phase extraction (SPE) [7] or even the more recently introduced solid phase microextraction (SPME) [8], single drop microextraction (SDME) [9], head space solvent microextraction (HSME) [10], hollow fiber liquid phase microextraction [11] and homogeneous liquid–liquid extraction [12].

LLE is a time consuming and tedious procedure. Furthermore, it requires the use of large amounts of high-purity organic solvents, which are usually hazardous, resulting in the production of toxic laboratory wastes. The solvent microextraction technique effectively overcomes these problems by reducing the amount of organic solvent. Further, extraction, preconcentration and sample introduction are done in one step [13]. DLLME is one of the effective microextraction techniques that has been discussed in several papers [14–17]. In this method, a cloudy solution forms after the injection of appropriate mixture of extraction and disperser solvents rapidly into the aqueous sample. The simplicity of operation, rapidity, low time, high recovery and high enrichment factor are among the main advantages of DLLME [18].

In this study, DLLME followed by gas chromatography (GC) with FID detection was applied for determining the nitroaromatic compounds in wastewater samples. The experimental variables such as extraction solvent, disperser solvent, salt effect, sample volume, extraction temperature and extraction time were optimized by a multivariate strategy based on an experimental design using a Plackett–Burman design for screening and a central composite design for optimizing of the significant factors. The optimized

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Table 1
The experimental variables and levels of the Plackett–Burman design.

Variable	Level	
	Low	High
Extraction solvent volume (μL)	20	60
Disperser solvent volume (mL)	0.75	1.25
Sample volume (mL)	5	10
Extraction temperature (°C)	20	40
Ionic strength (NaCl concentration, w/v%)	0	3
Extraction time (min)	2	10

procedure was applied to determine nitroaromatic compounds in wastewater samples.

2. Experimental

2.1. Reagents and materials

Nitrobenzene (NB), 2-nitrotoluene (2-NT), 3-nitrotoluene (3-NT), 4-nitrotoluene (4-NT), 2,4-dinitrotoluene (2,4-DNT), 2,6-dinitrotoluene (2,6-DNT), methanol, carbon tetrachloride and sodium chloride were of the highest purity, all from the Merck (Darmstadt, Germany).

The wastewater samples were obtained from well and wastewater of the Research Center of Islamic Azad University (Tehran, Iran) and kept in polyethylene bottles at ambient temperature. Then the extraction was performed without the dilution of the samples.

2.2. Instrumentation

The Varian 3800 CP gas chromatography (3120 Hansen Way, Palo Alto, CA 94304-1030, USA) equipped with a flam ionization detector was employed for determination of the analytes. A CP-Sil-5 fused silica capillary column (25 m × 0.32 mm i.d. and 0.52 μm film thickness) was applied for separation of the analytes. The GC split valve was opened and nitrogen was used as a carrier gas at the constant flow rate of 2.0 mL min^{-1}.

The column oven was initially held at 60 °C for 4 min and programmed to 200 °C at a rate of 10 °C min^{-1} and then to 250 °C at 20 °C min^{-1}. Hettich centrifuge model EBA 20 (Oxford, England) was employed for phase separation.

2.3. Procedure

A 9 mL aqueous sample solution with the ionic strength of 3% (w/v) NaCl, containing 100 μg L^{-1} of each nitroaromatic compound and nitrobenzene (internal standard), was placed in a 12 mL screw cap glass test tube with conical bottom.

A 0.75 mL methanol (disperser solvent), containing 20 μL carbon tetrachloride (extraction solvent), was injected rapidly into the sample solution by a 1 mL Hamilton syringe (USA) and the mixture was gently shaken. A cloudy mixture was formed in the test tube. The mixture was centrifuged for 2 min at 3000 rpm. The fine droplets of carbon tetrachloride were sedimented at the bottom of the test tube. Volume of the sedimented phase was determined as about 19 μL using a 25 μL microsyringe.

One microliter of the sedimented phase was withdrawn using a 2 μL Hamilton plunger into the needle microsyringe, model 7002 (Bonaduz, Switzerland), and injected into the GC.

2.4. Optimization strategy

There are several factors like the volume of extraction and disperser solvents, salt effect, sample volume, extraction temperature and extraction time that affect the extraction process.

In order to obtain the optimum conditions of DLLME for extraction of nitroaromatic compounds from the wastewater samples, Plackett–Burman design was used for screening of the variables. After choosing the significant variables, in order to investigate the interaction between them, a central composite design (CCD) was performed and a response surface equation was derived.

The experimental design matrix and data analysis were performed using StatGraphics plus 5.1 package.

3. Results and discussion

In this study, DLLME combined with GC-FID was developed for the extraction and determination of nitroaromatic compounds in wastewater samples. Carbon tetrachloride was used as the extracting solvent because it is very slightly soluble in water, its density is higher than aqueous solutions and it is readily sedimented during the centrifugation in the bottom of the conic tube.

Disperser solvent was selected on the basis of its miscibility in both the extraction solvent and the aqueous phase. In the present work, the use of either acetonitrile or methanol as disperser solvents, gave similar results. Therefore, methanol was used as the disperser solvent because of its availability and low cost.

3.1. Screening design

Screening designs are used for testing of the factors for main effects in order to reduce their numbers. A particular type of these designs is Plackett–Burman design [19,20], which assumes that the interactions can be completely ignored, so the main effects are calculated with a reduced number of experiments.

In the present work, based on the preliminary experiments, at least six factors might affect the experimental response. Therefore, six factors (extraction and disperser solvent volumes, salt effect, sample volume, extraction temperature and extraction time) at two levels were selected. The low and high values were selected from the results of previous experiments (Table 1). A Plackett–Burman design was used to determine the main effects. The overall design matrix showed 12 runs to be carried out randomly in order to eliminate the effects of extraneous or nuisance variables. The ANOVA results were evaluated for determining the main effects.

The normalized results of the experimental design were evaluated at a 5% of significance and analyzed by Standardized Pareto chart (Fig. 1). In fact, charts (A) and (B) were chosen as the representative examples of MNTs and DNTs, respectively, because three isomers of the studied MNTs and two isomers of the studied DNTs showed similar results. The standard effect is estimated for computing a *t*-statistic for each effect. The vertical line on the plot judges the effects that are statistically significant. The bars, extending beyond the line, correspond to the effects that are statistically significant at the 95% confidence level [21]. Furthermore, the positive or negative sign (corresponding to a black or white) response can be enhanced or reduced, respectively, when passing from the lowest to the highest level set for the specific factor.

According to Fig. 1, in this study, the extraction solvent volume was the most significant variable having a negative effect on the extraction efficiency of all analytes. Extraction temperature was the next most significant variable for three MNTs (the more volatile nitroaromatic compounds). Also sample volume was a significant variable for two DNTs (the less volatile nitroaromatic compounds). Fig. 1 also reveals that sodium chloride appeared to have a positive effect on the extraction efficiency. In fact, increasing the ionic strength (from 0 to 3% (w/v)) promoted the transport of the analyte into the organic phase.

Extraction time had no significant effect on the extraction efficiency. This is the most important advantage of DLLME, showing

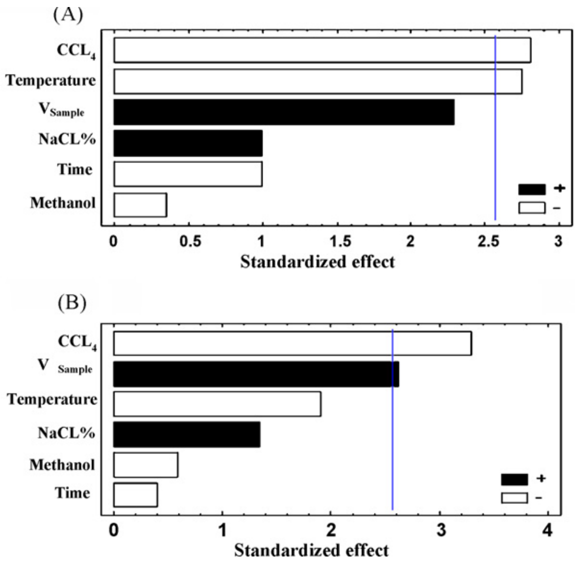

Fig. 1. Pareto charts of the main effects obtained from the Plackett–Burman design for MNTs (A) and DNTs (B).

that it is time-independent, because of an infinitely large surface area between the extraction solvent and the aqueous phase [22].

Disperser solvent volume (methanol) showed a non-significant negative effect, which is in agreement with the previously published results [17,18] and is attributed to the fact that at higher volumes disperser solvent, the solubility of nitroaromatic compounds in water increases, therefore, the extraction efficiency decreases [23].

Based on the results of the first screening study, to continue the optimization, three variables were fixed at suitable amounts (NaCl concentration: 3% (w/v), extraction time: 2 min and disperser solvent volume: 0.75 mL).

3.2. *Optimization design*

In the next step, a central composite design was applied to optimize the three factors (extraction solvent volume, sample volume and extraction temperature) that were chosen from the first screening design. The examined levels of the factors are given in Table 2.

This design permitted the response to be modeled by fitting a second-order polynomial, which can be expressed as the following equation:

$$y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \beta_{12} x_1 x_2 + \beta_{13} x_1 x_3 + \beta_{23} x_2 x_3 + \beta_{11} x_1^2 + \beta_{22} x_2^2 + \beta_{33} x_3^2$$

where x_1, x_2 and x_3 are the independent variables, β_0 is an intercept, β_1–β_{33} are the regression coefficients and y is the response function (relative area). The number of experiments is defined by the expression: $(2^f + 2f + C)$, where f is the number of factors and C is the number of center points.

This design consists of a factorial design (2^f) augmented with ($2f$) star points and central points (C) [24].

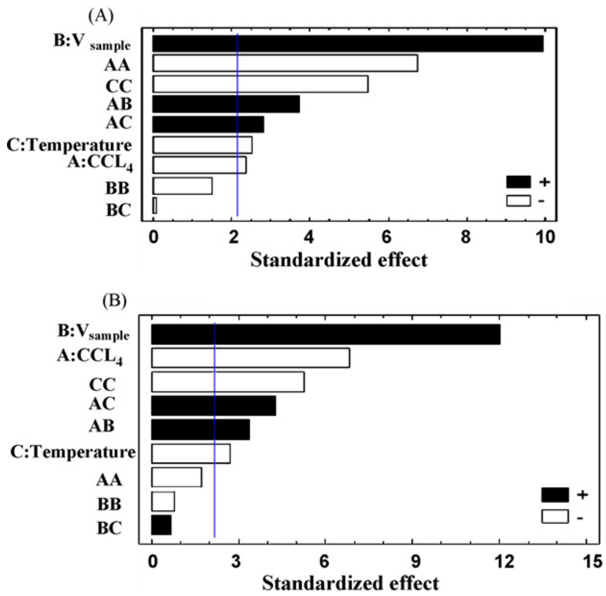

Fig. 2. Pareto charts of the main effects in the central composite design for (A) MNTs and (B) DNTs. AA, BB and CC are the quadratic effects of the extraction solvent volume, sample volume and extraction temperature, respectively. AB, AC and BC are the interaction effects between the extraction solvent volume and the sample volume, between the extraction solvent volume and the extraction temperature, and between the sample volume and the extraction temperature, respectively.

The star points are located at $+\alpha$ and $-\alpha$ from the center of the experimental domain. An axial distance, α, was selected with a value of 1.682 in order to establish the rotatability condition of the central composite design.

In this study, f and C were set at 3 and 9, respectively, which meant that 23 experiments had to be done.

The experimental data showed a good accordance with the second-order poly nominal equations. The coefficients of determination, R^2, were more than 0.95 for the relative areas, which were statistically acceptable at $p < 0.05$ levels.

The data obtained were evaluated by ANOVA test and the effects are shown by using Pareto chart in Fig. 2. Based on the central composite design, each of the three factors (extraction solvent volume, sample volume and extraction temperature) was the most important variable for all of the analytes.

As Fig. 2 shows, the sample volume has the largest influence on the relative area and a positive effect upon the extraction. In fact, by increasing of the sample volume at the fixed concentration of the analytes, the amount of analytes will increase. On the other hand, at higher sample volumes, a better cloudy state will form compared with lower sample volumes. Therefore, the response begins to increase at higher sample volumes.

Fig. 2 shows that the extraction solvent volume has a significant negative effect upon the extraction efficiency. By increasing of the extraction solvent volume (carbon tetrachloride), due to increasing of the sedimented phase and dilution of the analytes, the enrichment factor will decrease. Further, temperature has a significant negative effect on the extraction recovery, due to the decrease of distribution coefficient and also further dissolution

Table 2
The experimental variables, levels and star points of the central composite design (CCD).

Variable	Level			Star points (α = 1.682)	
	Lower	Central	Upper	$-\alpha$	$+\alpha$
Extraction solvent volume (mL)	20	40	60	6.36	73.64
Sample volume (mL)	5	7.5	10	3.3	11.7
Extraction temperature (°C)	20	30	40	13.2	46.8

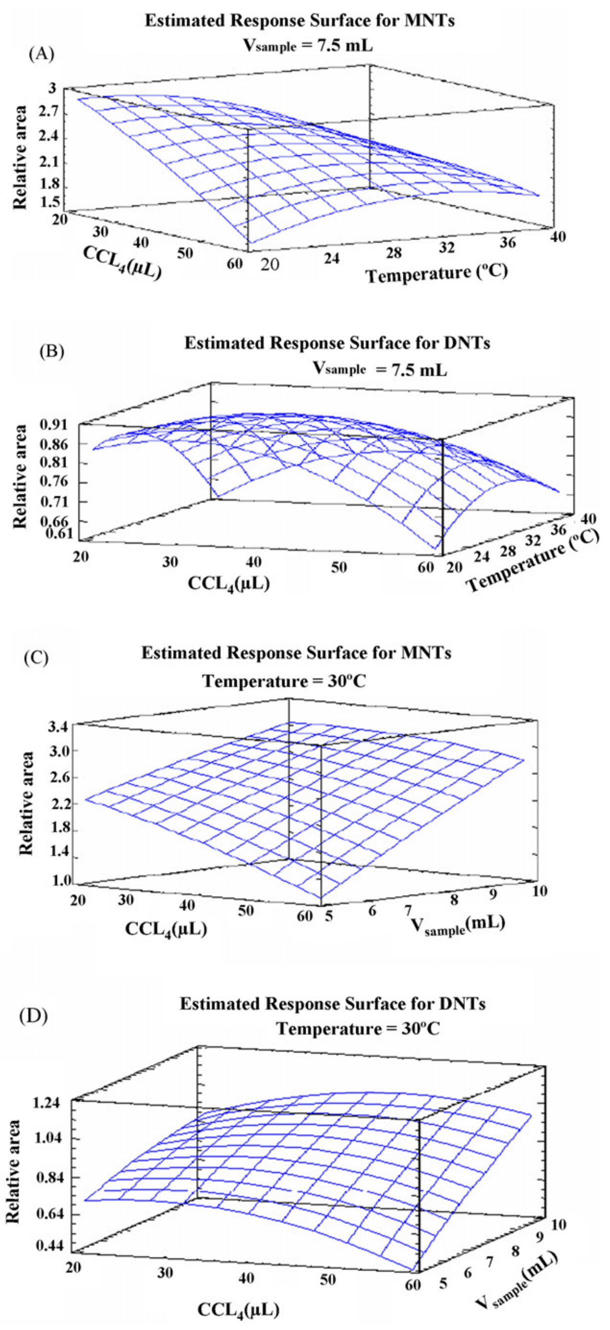

Fig. 3. Response surfaces for MNTs and DNTs using the central composite design obtained by plotting of: (A) the extraction solvent volume *vs* the extraction temperature for MNTs, (B) the extraction solvent volume *vs* the extraction temperature for DNTs, (C) the sample volume *vs* the extraction temperature for MNTs and (D) the sample volume *vs* the extraction temperature for DNTs.

of the extraction solvent at higher temperatures. These observations can be explained by the following extraction recovery (R) equation:

$$R\% = \frac{K_D}{K_D + V_{aq}/V_{sed}} \times 100$$

where k_D is distribution coefficient and V_{sed} and V_{aq} are the volumes of the sedimented phase and sample solutions, respectively.

As Fig. 2A and B shows, the quadratic term of temperature (CC) and interactions between the extraction solvent volume/temperature (AC) and the extraction solvent volume/sample volume (AB) showed a significant effect on the extraction recovery.

The obtained regression models were used to calculate the response surface for each variable separately. Fig. 3 shows the response surface plots for the relative areas. Accordingly, the plots given in Fig. 3 were used for interpreting graphically the variation of the relative areas as a function of each pair of the independent variables. Fig. 3A and B shows the response surfaces obtained by plotting the extraction solvent volume (CCl_4) *vs* the extraction temperature with the sample volume fixed at 7.5 mL. Fig. 3C and D shows the response surfaces developed for the extraction solvent volume (CCl_4) and the sample volume, whilst keeping the extraction temperature at 30 °C.

As can be seen, the presence of low extraction solvent volume enhances the extraction of all target analytes, reaching a maximum at 6.4 μL of carbon tetrachloride. The negative effect of the higher volumes of extraction solvent has been discussed by many authors [14,16,18].

In general, at higher extraction solvent volumes, the relative peak area (sensitivity) decreased due to increasing of the sedimented phase volume and dilution effect. Subsequently, at lower extraction solvent volumes, higher enrichment factors will be obtained.

Fig. 3A confirms the existence of a significant positive interaction between the extraction solvent volume and the extraction temperature, suggesting that room temperature is the optimum value upon extraction. The results also revealed that the relative pick areas of the target analytes increased when the temperature increased from 20 to 30 °C and then decreased by increasing of the temperature from 30 to 40 °C. Therefore, at higher temperatures, because of the decrease in distribution coefficient, lower extraction efficiencies were obtained. Accordingly, room temperature (∼20 °C) is suitable for doing the extraction procedure. The surface plot of the relative peak area response (Fig. 3C) showed a pronounced increase as the sample volume increased. It can be explained by the fact that by increasing of the sample volume, the amount of analytes will increase. According to the overall results of the optimization study, the following experimental conditions were chosen: carbon tetrachloride volume, 20 μL; methanol volume, 0.75; sample volume, 9 mL; sodium chloride concentration, 3% (w/v) and extraction time, 2 min.

3.3. *Evaluation of the method performance*

The corresponding regression equations, correlation of determinations (r^2), dynamic linear ranges (DLRs), the limit of

Table 3
The limit of detections, regression equations, correlation of determinations, dynamic linear ranges and preconcentration factors for DLLME.

Analyte	LOD ($\mu g\,L^{-1}$)	r^2	Regression equation	DLR ($\mu g\,L^{-1}$)	Preconcentration factor
2-Nitrotoluene	0.09	0.997	$Y = 0.021X + 0.694$	0.5–300	314
3-Nitrotoluene	0.106	0.995	$Y = 0.020X + 0.516$	0.5–300	314
4-Nitrotoluene	0.094	0.998	$Y = 0.022X + 0.348$	0.5–300	305
2,4-Dinitrotoluene	0.5	0.994	$Y = 0.009X + 0.347$	1–400	202
2,6-Dinitrotoluene	0.4	0.990	$Y = 0.009X + 0.517$	1–400	250

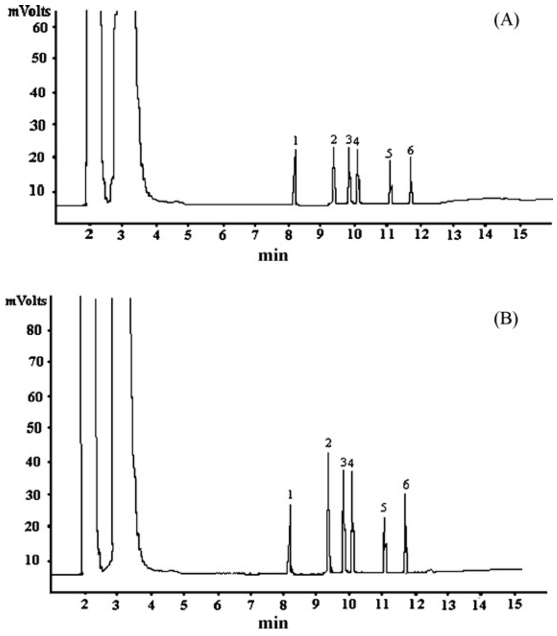

Fig. 4. (A) Chromatogram of the standard solution (50 μg L^{-1}) of nitroaromatic compounds after DLLME under optimum conditions; (1) nitrobenzene, (2) 2-nitrotoluene, (3) 3-nitrotoluene, (4) 4-nitrotoluene, (5) 2,6-dinitrotoluene and (6) 2,4-dinitrotoluene. (B) Chromatogram of the wastewater sample of the Research Center of the Islamic Azad University after DLLME under optimum conditions: (1) nitrobenzene, (2) 2-nitrotoluene, (3) 3-nitrotoluene, (4) 4-nitrotoluene, (5) 2,6-dinitrotoluene and (6) 2,4-dinitrotoluene.

detections (LODs) and preconcentration factors (PFs) were calculated under optimized conditions and the results are summarized in Table 3.

The LODs were calculated as the concentration of the analytes equal to three times of the standard deviation of the blank signal divided by the slope of calibration curve. The LODs in the range from 0.09 μg L^{-1} (for 2-nitrotoluene) to 0.5 μg L^{-1} (for 2,4-dinitrotoluene) were obtained.

In order to calculate the preconcentration factor of each analyte, four replicate extractions were performed under optimal conditions from the aqueous solution containing 100 μg L^{-1} of the analytes. The preconcentration factor was calculated as the ratio of the final concentration of the analyte in the sedimented phase and its concentration in the original solution. The preconcentration factors were obtained in the range of 202–314.

Table 4
Determination of nitroaromatic compounds in the well and wastewater samples of the Research Center of Islamic Azad University (Tehran, Iran).

Sample	Analyte	C_{added} (μg L^{-1})	C_{found} (μg L^{-1})	RSD%	Error%
Wastewater (R.C.)	2-Nitrotoluene	–	110	8.3	–
		50.0	164	8.8	8
	3-Nitrotoluene	–	105	8.9	–
		50.0	160	9.1	10
	4-Nitrotoluene	–	105	9.8	–
		50.0	158	10.1	6
	2,4-Dinitrotoluene	–	94	8.6	–
		50.0	140	8.7	−8
	2,6-Dinitrotoluene	–	92	7.8	–
		50.0	139	8.2	−6
Well water	2-Nitrotoluene	–	–	–	
		100.0	105	5.6	5
	3-Nitrotoluene	–	–	–	
		100.0	99	4.8	−1
	4-Nitrotoluene	–	–	–	
		100.0	103	4.5	3
	2,4-Dinitrotoluene	–	–	–	
		100.0	98	3.9	−2
	2,6-Dinitrotoluene	–	–	–	
		100.0	93	3.8	−7

3.4. *Real water sample analysis*

Well and wastewater samples were collected from the Research Center of Islamic Azad University (Tehran, Iran) and analyzed by DLLME combined with GC-FID. The analysis of well water samples showed that they were free of nitroaromatic compounds. But in the wastewater samples, nitroaromatic compounds were detected and confirmed by spiking 50 and 100 μg L^{-1} of the nitroaromatic compounds into the samples.

Fig. 4A shows the chromatogram obtained for the standard solution containing 50 μg L^{-1} of the analytes after extraction by DLLME under optimum conditions. The chromatogram of the wastewater samples from the Research Center of the Islamic Azad University after DLLME procedure is shown in Fig. 4B.

Table 4 shows that the results of three replicate analysis of each sample obtained by the proposed DLLME method and that the amount of the added nitroaromatic compounds is in satisfactory agreement.

4. Conclusions

In this work, a multivariate optimization strategy was used to obtain the optimum conditions for the extraction of nitroaromatic compounds by DLLME.

Optimization of the DLLME variables was carried out using the response surface methodology and experimental design. The optimized DLLME coupled to GC-FID allowed quantification of trace levels of nitroaromatic compounds in the water.

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