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Optimization of supercritical fluid extraction combined with dispersive liquid–liquid microextraction as an efficient sample preparation method for determination of 4-nitrotoluene and 3-nitrotoluene in a complex matrix

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ABSTRACT

Supercritical fluid extraction (SFE) combined with dispersive liquid–liquid microextraction (DLLME) followed by gas chromatography with flame ionization detector (GC-FID) was developed for the extraction and determination of 4-nitrotoluene and 3-nitrotoluene in soil sample. The effects of different SFE experimental parameters on the extraction recovery were studied simultaneously using a central composite design (CCD) after a 2^{n-1} fractional factorial experimental design. The variables of interest in SFE were pressure, temperature, modifier volume, and dynamic extraction time. From this statistical evolution the pressure, dynamic extraction time, and modifier volume were found to have significant effects on the results achieved from SFE-DLLME-GC-FID, while temperature was not statistically significant at a 95% confidence level. The optimal SFE conditions for 4-nitrotoluene and 3-nitrotoluene were a pressure of 350 atm, temperature of 35 °C, dynamic extraction time of 30 min and modifier volume of 150 μL. Under the optimal conditions, the extraction calibration plots were linear in the range of 0.25–25 mg kg^{-1} and the limits of detection (LODs) were 0.12 mg kg^{-1} for both of the analytes. Performance of the present method was evaluated for extraction and determination of 4-nitrotoluene and 3-nitrotoluene in soil samples, and satisfactory results were obtained (RSDs < 6.5%).

1. Introduction

Sample preparation is a critical step in chemical analysis, especially for the determination of trace analytes in complex sample matrices. An ideal extraction method should be simple, rapid, and quantitative. It should provide a sample that is immediately ready for analysis without additional concentration or class fractionation steps. Furthermore, it should generate no additional laboratory wastes. Traditional liquid solvent extraction techniques often require several hours to perform; may not lead to a quantitative recovery of analytes; and leave hazardous solvents [1,2]. Supercritical fluid extraction (SFE) has been utilized to overcome the difficulties involved in solid sample extraction for the past three decades. SFE requires a lower quantity of organic solvents, has a short extraction time, and is capable of extracting thermally labile compounds under mild conditions [3,4,1].

Dispersive liquid–liquid microextraction (DLLME) is a new mode of liquid-phase microextraction. In this method a cloudy solution is formed after rapid injection of an appropriate mixture of extraction and dispersion solvents into an aqueous sample. Due to the extremely large contact area between extraction solvent and sample solution, extraction equilibrium is reached rapidly [5–8]. Despite the fact that DLLME has several advantages, such as simplicity of operation, rapidity, low cost, and high preconcentration factor, this method is not suitable for compound extraction from complex matrices. Extra sample preparation steps are required prior to use DLLME [6,9].

SFE combined with DLLME is an efficient sample preparation method for compound determination in complex matrices. This combination prevents solvent vaporization after extraction and increases the preconcentration factor for organic compound determination in solid samples. In addition, it enables the easy application of DLLME toward complex matrices such as soils and sediments [10].

Isomers of mononitrotoluenes (MNTs) are used in the synthesis of intermediates for the production of dyes, rubber chemicals, pesticides, drugs, resin modifiers, optical brighteners, suntan lotions, and photographic developing agents, for example. Due to their significant number of uses, MNT substances are widely present in the biosphere and persist in environment as hazardous substances [11–14].

In this study, SFE-DLLME was applied as a sample preparation method for GC-FID to determine 4-nitrololuene and 3-nitrololuene in soil sample. The experimental parameters of SFE such as

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pressure, temperature, modifier volume, and dynamic extraction time, were optimized using a central composite design (CCD) based on a 2^{n-1} half fractional factorial experimental design.

2. Experimental

2.1. Chemicals and reagents

Nitrobenzene, 3-nitrotoluene, 4-nitrotoluene, methanol, carbon tetrachloride, and sodium chloride were purchased from Merck (Darmstadt, Germany). Carbon dioxide (99.99% purity), in a cylinder with an eductor tube, was obtained from Sabalan Co. (Tehran, Iran).

2.2. SFE-DLLME procedure

For all extractions, a Suprex MPS/225 system (Pittsburgh, PA) in the SFE mode and equipped with a pump unit (Suprex) was used. Soil samples were air-dried at room temperature for 4 days and sieved for particle sizes in the range of 0.2–0.5 mm. A sample of 2.0 g was mixed with glass beads and placed in a SFE extraction vessel (3 mL). This sample was spiked with 50 μL of both the 4-nitrotoluene and 3-nitrotoluene standards (100 mg L^{-1}). After evaporation of the solution, 150 μL of methanol, as a modifier, was added directly into the sample. Extractions were carried out with supercritical carbon dioxide under the following conditions: pressure of 350 atm, oven temperature of 35 °C, dynamic extraction time of 30 min, and static extraction time of 10 min. A Duraflow manual variable restrictor (Suprex) was used in the SFE system to collect the extracted analytes. To prevent the sample from plugging the system, the restrictor point was warmed electrically. The extracted analytes were collected in a 2.0 mL volumetric flask at a flow rate of 0.4 ± 0.05 mL min^{-1}, using 1.0 mL methanol as the collector and dispersion solvent. To obtain a better collection efficiency, the collection vial was placed in an ice bath during the dynamic extraction time. Afterwards, 20 μL of carbon tetrachloride (extraction solvent) was added into the collector solvent. Finally, the resulting mixture was injected rapidly into a 5.0 mL water sample, which had an ionic strength of 3.0% (w/v) NaCl and was placed in a 14 mL screw-cap glass tube. A cloudy mixture was formed in the conical tube. Subsequently, the mixture was centrifuged at 3000 rpm for 5 min using a Hermle centrifuge (Germany). Upon centrifuging, the dispersed fine droplets of carbon tetrachloride were present as sediments at the bottom of the conical test tube (6.0 ± 0.4 μL), and 1.0 μL of the sediment phase was injected into the GC using a 10.0 μL SGE microsyringe.

2.3. GC analysis

Separation and detection of analytes were performed using an Agilent 7890A gas chromatograph (USA) equipped with a flame ionization detector. An HP-1 fused-silica capillary column (30 m × 0.25 mm i.d. × 0.01 μm film thickness) was applied for separation of the analytes. Nitrogen was used as a carrier gas at a constant flow rate of 1.5 mL min^{-1}. The oven temperature was initially held at 120 °C for 1 min and programmed to 160 °C at a rate of 4 °C min^{-1} and then to 250 °C at 20 °C min^{-1}.

2.4. Optimization strategy

As various parameters can potentially affect the extraction process, optimization of the experimental conditions represents a critical step in the development of a SFE method. In the present study, a 2^{n-1} fractional factorial design was applied for screening of the variables to choose the main factors. This was followed with central composite design (CCD) for their optimization. The experimental design matrix and data analysis were performed using the StatGraphics plus 5.1 package. The response measured was extraction recovery (ER), which was defined according to the following equation:

$$\text{ER\%} = \frac{n_2}{n_1} \times 100$$

where n_2 is the moles of analytes in the sedimented organic phase after extraction with the SFE-DLLME process. This parameter was calculated from a suitable calibration graph obtained by direct injection of standard analytes into the GC. The parameter n_1 is the total spiked moles of analytes in the sample.

Table 1
The experimental variables and levels of central composite design (CCD).

Variable	Level		
	Lower	Central	Upper
Pressure (atm)	100	225	350
Modifier volume (μL)	50	100	150
Dynamic time (min)	15	25	35

3. Results and discussion

In this study, SFE combined with DLLME and followed by GC-FID was developed for the analysis of 4-nitrotoluene and 3-nitrotoluene in soil sample. To reach a high extraction recovery of both analytes, the SFE conditions were optimized.

3.1. Optimization of SFE experimental conditions

3.1.1. Fractional factorial design

There are several factors that affect the extraction process in SFE. In the present work, a 2^{n-1} fractional factorial design method was used to test all possible variables for main effects in order to optimize extraction conditions with fewer experiments. Based on these preliminary experiments, four factors were found to potentially affect the experimental response. Therefore, the four factors of pressure (A), dynamic extraction time (B), modifier volume (C), and temperature (D) at two levels with three center points were studied. The low and high values selected from the results of previous experiments included: pressures of 100 and 350 atm; temperatures of 35 and 85 °C; modifier volumes of 0 and 100 μL; and dynamic extraction times of 15 and 35 min. According to initial experiments, the static extraction time was fixed at 10 min. The overall design matrix showed 11 runs to be carried out randomly. Analysis of the results was visualized at a 95% confidence level using standardized main effect Pareto charts (Fig. 1). The positive or negative sign (corresponding to pink or red) response could 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, pressure was the most significant variable, having a positive effect on the extraction of 4-nitrotoluene and 3-nitrotoluene. The modifier volume and dynamic extraction time were the next most important parameters, respectively. Based on the results obtained from the screening step to continue the optimization, the temperature was fixed at 35 °C.

3.2. Central composite design (CCD)

In the next step, a CCD was applied to optimize the three selected factors (i.e., pressure, modifier volume, and dynamic extraction time) at three levels. The examined levels of the factors are given in Table 1. The number of experiments is defined by the expression: $(2f + 2f + C)$, where f is the number of factors and C is the number

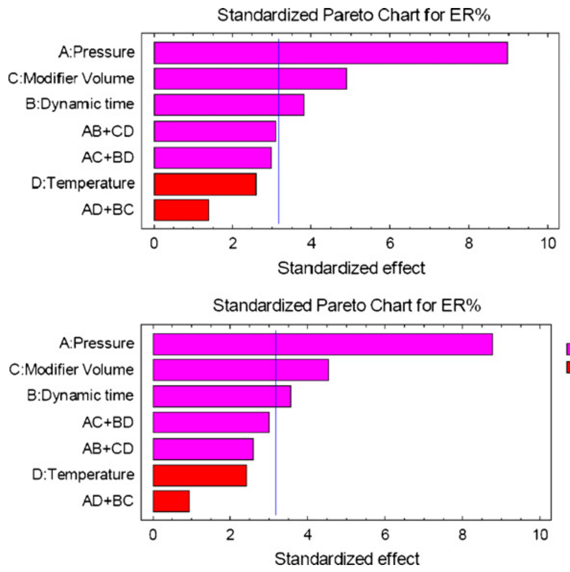

Fig. 1. Pareto charts of the main effects obtained from the 2^{n-1} fractional factorial design: (a) 4-nitrotoluene and (b) 3-nitrotoluene. AB, AC, and AD are the interaction effects of pressure (atm) with dynamic extraction time (min), modifier volume (μL), and temperature (°C), respectively. BC and BD are the interaction effects of dynamic extraction time with modifier volume and temperature, respectively. CD is the interaction effect of modifier volume with temperature.

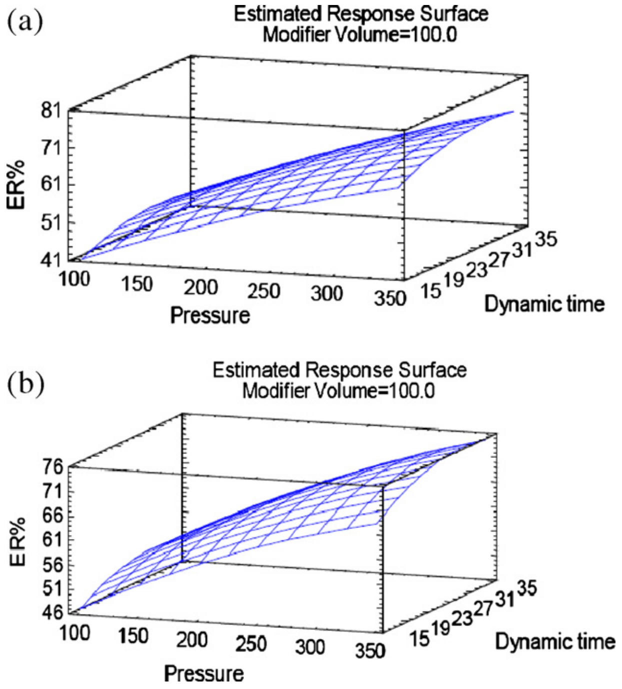

Fig. 2. Response surfaces using the central composite design obtained by plotting pressure (atm) vs. dynamic extraction time (min): (a) 4-nitrotoluene and (b) 3-nitrotoluene.

of center points. In this study, *f* and *C* were set at 3, meaning that 17 experiments were necessary. The experimental data for these three factors and the obtained results are elaborated 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:

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{11} {X_1}^2 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{22} {X_2}^2 + \beta_{23} X_2 X_3 + \beta_{33} X^2$$

where X_1, X_2 and X_3 are the independent variables, β_0 is an intercept, β_1, β_2, β_3, β_{12}, β_{13}, β_{23}, β_{11}, β_{22} and β_{33} are the regression coefficients and *Y* is the response function (ER%). Model terms were selected or rejected based on the *p* value with a 95% confidence level.

Fig. 2 shows the response surface obtained by plotting pressure vs. dynamic extraction time. The modifier volume was fixed at 100 μL. Fig. 2 shows the extraction of both analytes increased when the dynamic extraction time was increased from 15 min to 30 min. This subsequently decreased when increasing the dynamic extraction time from 30 min to 35 min due to the increased amount of CO_2 emitted from the restrictor, which led to a higher loss of analytes. Further, extraction recovery increases when increasing the pressure from 100 atm to 350 atm owing to the increase in solvation power of supercritical CO_2. Fig. 3 shows the response surface developed for pressure and modifier volume, while keeping the dynamic extraction time at 25 min. Fig. 3 confirms that the highest extraction recovery for both the analytes was obtained at a modifier volume of 150 μL. This result can be explained by the fact that increasing the modifier volume from 50 μL to 150 μL increases the ability of CO_2 to extract polar compounds.

According to the overall results of the optimization study, the following experimental conditions were chosen: pressure of 350 atm, dynamic extraction time of 30 min, and modifier volume of 150 μL.

Table 2
The central composite design (CCD) program and results for SFE-DLLME-GC-FID of 4-nitrotoluene and 3-nitrotoluene.

Run no.	Pressure (atm)	Dynamic time (min)	Modifier volume (μL)	ER%	
				3-Nitrotoluene	4-Nitrotoluene
1	350	35	50	61.76	58.74
2	225	25	150	72.32	69.30
3	225	15	100	55.87	52.55
4	350	15	150	71.68	68.66
5	350	35	150	73.51	70.50
6	100	15	150	45.80	42.78
7	225	25	50	50.32	47.31
8	225	25	100	55.72	52.69
9	100	25	100	44.52	41.50
10	225	25	50	53.12	50.11
11	100	15	50	38.44	35.42
12	350	15	50	52.84	49.81
13	225	35	100	62.59	59.57
14	225	25	100	62.54	59.53
15	350	25	100	70.76	67.74
16	100	35	150	49.87	46.76
17	225	25	50	51.88	48.86

Table 3
Figures of merit for the SFE-DLLME-GC-FID of 4-nitrotoluene and 3-nitrotoluene.

Analyte	LDR (mg kg^{-1})	R^2	LOD (mg kg^{-1})	(RSD%, $n=3$)	PF	ER%
4-Nitrotoluene	0.25–25/0	0.997	0.12	6.32	118.06	70.84
3-Nitrotoluene	0.25–25/0	0.998	0.12	7.12	122.85	73.71

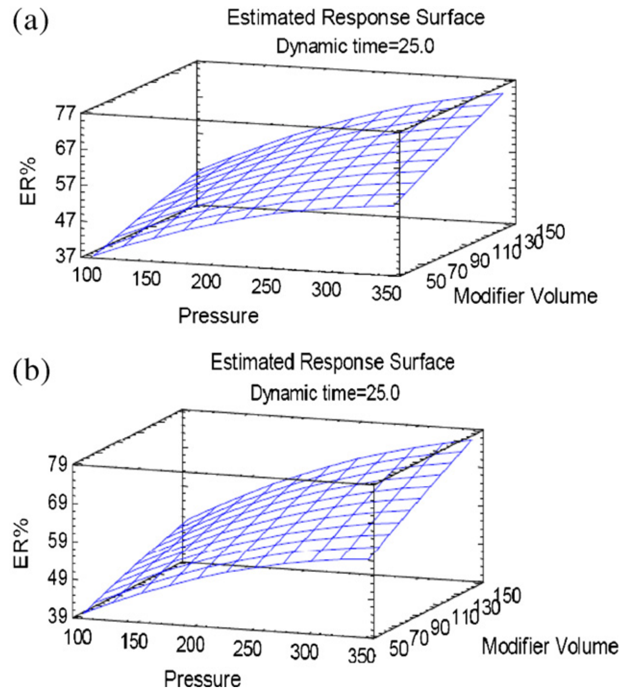

Fig. 3. Response surfaces using the central composite design obtained by plotting pressure (atm) vs. modifier volume (μL): (a) 4-nitrotoluene and (b) 3-nitrotoluene.

3.3. *Quantitative analysis*

Linear dynamic ranges (LDRs), coefficients of determinations (R^2), relative standard deviations (RSDs, %), and limits of detection (LODs) based on a signal to noise ratio (S/N) of 3 were calculated at optimal conditions. Preconcentration factors (PFs) were calculated based on the extraction of 2.5 mg kg^{-1} for each analyte. The results are summarized in Table 3.

3.4. *Real soil sample analysis*

The efficiency of the presented method was evaluated by determining 4-nitrotoluene and 3-nitrotoluene concentrations in soil sample. Contaminated soil sample was collected from the Pars Simin Company (Rasht, Guilan, Iran). Soil sample was air-dried at room temperature for 4 days and sieved for particle sizes in the range of 0.2–0.5 mm. Afterward, the sample was analyzed by GC-FID, following the developed SFE-DLLME procedure. The results are shown in Table 4.

Fig. 4 shows the chromatograms obtained for the soil and spiked soil samples at a concentration level of 0.25 mg kg^{-1} of each analyte. The relative analyte recoveries were 80% and 84% for 4-nitrotoluene and 3-nitrotoluene, respectively (Table 4).

Table 4
Results obtained from the analysis of a soil sample.

Sample		4-Nitrotoluene	3-Nitrotoluene
Soil	C_{initial} (mg kg^{-1})	–	0.55
	C_{added} (mg kg^{-1})	0.25	0.25
	C_{found} (mg kg^{-1})	0.20	0.76
	Relative recovery %	80	84
	RSD%	5.75	6.50

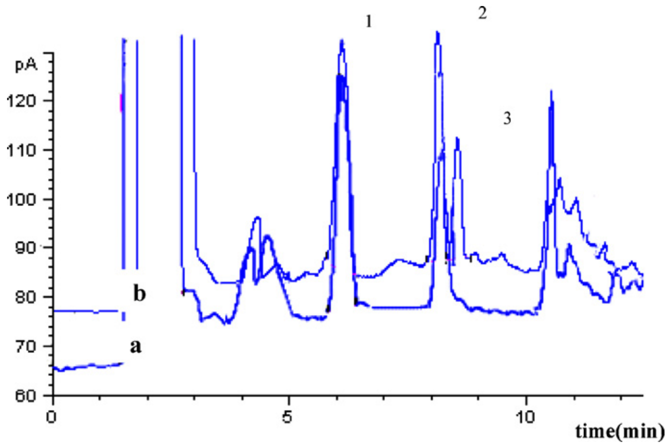

Fig. 4. GC-FID chromatograms of the (a) nonspiked and (b) 0.25 mg kg^{-1} of 4-nitrotoluene and 3-nitrotoluene spiked soil sample. 1: nitrobenzene, 2: 3-nitrotoluene, 3: 4-nitrotoluene.

4. Conclusion

In this study, SFE method was combined with a DLLME technique. This combination was successfully applied toward the extraction and preconcentration of 4-nitrotoluene and 3-nitrotoluene from soil sample prior to analysis by GC-FID. SFE-DLLME leads to a high preconcentration factor for determining organic compounds in solid samples and eliminates the collecting solvent evaporation step at the end of SFE. To optimize the SFE conditions, a central composite design (CCD) based on a 2^{n-1} fractional factorial design was employed. The optimal experimental conditions found from this statistical evaluation included: pressure of 350 atm, dynamic extraction time of 30 min, and modifier volume of 150 μL.

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