# Full Paper

# Sensitive Colorimetric Detection of Explosive 2,6-Bis(picrylamino)pyridine after Preconcentration by Dispersive Liquid-Liquid Microextraction

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**Abstract**: An experimental investigation for sensitive spectrophotometric detection of explosive, 2,6-bis(picrylamino)pyridine (BPAP) using dispersive liquid-liquid microextraction was carried out. Based on this procedure, which is a dispersive-solvent-free technique, the extractant is dispersed in the aqueous sample solution using Aliquat 336 (acted as disperser agent and carrier to extraction solvent) and monitored with microvolume UV/Vis spectrophotometer. The effect of different variables such as pH, concentration of sodium hydroxide, type and volume of extraction solvent, concentration of Aliquat 336 solution and coexisting substances were systematically investigated and optimized. Interference tests showed that the developed method has a good selectivity and could be used conveniently for determination of explosive analyte. The proposed method is capable of determining BPAP over a range of 2.0–150.0 $ng\,mL^{-1}$ with a limit of detection 1.0 $ng\,mL^{-1}$. Relative standard deviations (RSD) for 20.0 and 80.0 $ng\,mL^{-1}$ of BPAP were 3.3 and 1.2% ($n = 10$), respectively. This colorimetric method was applied to determine BPAP in different water and soil samples.

**Keywords**: 2,6-Bis(picrylamino)pyridine · Aliquat 336 · Dispersive liquid-liquid microextraction

## 1 Introduction

Explosives mainly are used in military purposes, industries, mining, and agricultural activities. Production and disposal of these substances often result in anthropogenic contamination of soils and waters. Most of these materials are toxic, mutagenic, and are partly transformed to even more toxic compounds under environmental conditions [1, 2]. According to their chemical nature, explosives can be separated into nitroaromatics, nitrate esters, nitramines, and peroxides. Among them, nitroaromatic (NAC) explosive have been extensively used in military operations and criminal acts. The NAC materials are organic compounds that contain one or more nitro functional groups ($NO_2^-$), which they are often highly explosive when the compounds contain more than one nitro group [3].

Due to the increased attention on homeland security, human health, and environment issues, the investigation and detection of NAC explosives residues and explosive-like compounds is an issue of major importance in countering terrorist threats and contamination of soil and groundwater [4]. Hence, nowadays, several detection techniques have been developed and proposed for NAC explosive materials, such as liquid chromatography-mass spectrometry (LC-MS) [5] and gas chromatography-mass spectrometry (GC-MS) [6], surface-enhanced Raman spectroscopy (SERS) [7], ion-mobility spectrometry [8], Fourier-transform infrared (FTIR) [9], immunochemical method [10], and electrochemistry [11]. These methods require sophisticated instruments and are time-consuming with a certain cost, and they are not easily applied to in-field and real-time detection of explosives. Therefore, establishment of reliable methods for the rapid and sensitive determination of nitroaromatic explosives are in great demand.

In recent years, miniaturized preconcentration methodologies, in keeping with the principles of green analytical chemistry, have revolutionized the sample treatment steps

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in several aspects. Among them, liquid-phase microextraction (LPME) is one of the most reliable and serviceable separation tools, which can effectively improve the detection limit and eliminate the matrix interferences [12]. A new mode of liquid-phase microextraction, namely dispersive liquid-liquid microextraction (DLLME), has been developed by Assadi and co-workers in 2006 [13]. DLLME is based on ternary component solvent systems, which an appropriate mixture of extraction solvent and dispersive solvent is injected rapidly into an aqueous solution via a syringe. This results a cloudy solution consisting small droplets of the extraction solvent, which are dispersed into the aqueous sample, which markedly increased the contact surface between phases and reduce extraction time with the increasing enrichment factors. Some of its remarkable advantages are simplicity of operation, rapidity, high recovery and high enrichment factor, and low consumption of solvents and sample. DLLME has been used for separation and preconcentration of organic and inorganic compounds from different matrices [14–18].

$N,N'$-Bis(2,4,6-trinitrophenyl)-2,6-pyridinediamine (2,6-bis(picrylamino)pyridine, BPAP) is an energetic nitroaromatic compound (Figure 1), which is used for the production of particularly heatproof explosives for the oil exploration. Moreover, it has been widely utilized in areas such as aviation, space flight, drilling and applied as an intermediate for synthesis of explosive component, containing 2,6-bis(picrylamino)-3-nitropyridine (BPNP) and 2,6-bis(picrylamino)-3,5-dinitropyridine (PYX) [19, 20]. According to the attainable literature and to the best of our knowledge, no report is available so far for the determination of BPAP.

Therefore, the main aim of this work was to present a new, simple, rapid, and inexpensive dispersive liquid-liquid microextraction (DLLME) method for extraction/preconcentration of BPAP and determination by microvolume UV/Vis spectrophotometry. The proposed methodology is based on the extraction of the ion pair formed between the anion of BPAP, obtained in basic medium, and cationic ion of trioctylmethylammonium chloride (Aliquat 336) into a microvolume of organic solvent followed by spectrophotometric measurement.
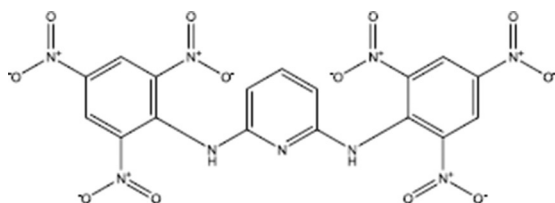


**Figure 1.** Chemical structure of BPAP.

## 2. Experimental Section

### 2.1 Apparatus

Recording the spectra and the absorbance measurements were made with a Jenway UV/Vis spectrophotometer model 6320 using 350 µL quartz microcells. A Jenway 3520 pH-meter with a combined glass electrode was used to adjust the pH of the solutions. A centrifuge model JENUS (model, TDL 80–2B) was used for the phase separation.

### 2.2 Reagents

All chemicals were of analytical grade and double distilled water was used throughout the work. A stock solution of BPAP (500 µg mL$^{-1}$) was prepared by dissolving 2,6-bis(picrylamino)pyridine (0.05 g, BPAP, Iranian Chemical Industry, Iran) in acetone (10 mL, Merck, Darmstadt, Germany) and diluting to 100 mL in a volumetric flask with water. Working solutions were prepared by an appropriate dilution of the stock solutions before use. A 1.0 % (w/v) solution of Aliquat 336 (Acros organics) was prepared by dissolving of Aliquat 336 (0.10 g, trioctylmethylammonium chloride) in carbon tetrachloride and diluting to 10 mL in a volumetric flask. pH adjustments were performed with 0.001–0.1 mol L$^{-1}$ HCl (Merck) and NaOH (Merck) solutions.

### 2.3. General DLLME Procedure

In a 10 mL volumetric flask, an aliquot of BPAP solution and sodium hydroxide (0.4 mL, 0.1 mol L$^{-1}$) were added and diluted to the mark with water. This solution was transferred to a conical glass test tube and the Aliquat 336 solution (1.0 % w/v, 200 µL) was rapidly injected into the produced solution using a microsyringe. Following gentle shaking (5 s), a cloudy solution, which consisted of very fine droplets of CCl$_4$ dispersed into the aqueous sample was formed. The mixture was centrifuged for 5 min at 5000 rpm, causing the dispersed fine droplets of the extraction phase to settle down in the bottom of the tube. The supernatant aqueous phase was discarded and the orange sediment phase (180–190 µL) was removed using a microsyringe, placed into the quartz microcell and its absorbance was measured at 460 nm against the blank. A blank solution was also run under the same procedure without adding any BPAP. The illustration of this procedure is shown in Figure 2.

### 2.4 Collection and Preparation of Samples

#### 2.4.1 Water Samples

The fresh water samples, including the water (Ahvaz, Iran), river water (Karun, Khuzestan Province, Iran), and effluent waste water (Ahvaz, Iran) were collected, filtered through a filter paper (Whatman No. 41) and stored at 5 °C and analyzed within 24 h by the recommended procedure.
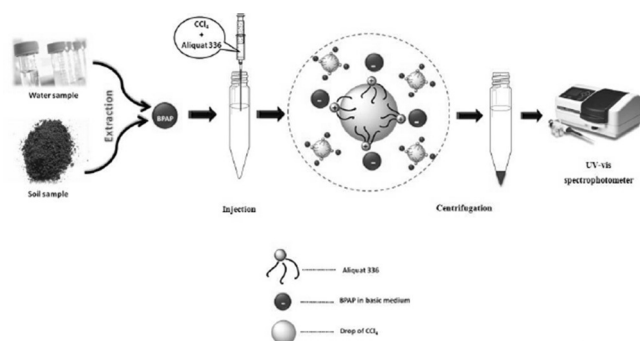
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**Figure 2.** Schematic procedure of proposed DLLME method.

### 2.4.2 Soil Samples

Three different soil samples (collected from different fields in Khuzestan, Iran) were sieved through a 40-mesh sieves to remove larger aggregates. For removing the humidity content, the finely ground soils were dried in an oven at 110 °C for 1 h, cooled to room temperature and spiked with BPAP (100 µg g$^{-1}$). An amount of these spiked soils (0.1 g) were accurately weighted and introduced into a 30 mL centrifuge tube. Next, acetone (5 mL) was added and the mixture was shaken for 30 min. After this period, the contents of tube were centrifuged at 4000 rpm for 10 min and the liquid was filtered through a filter paper (Whatman). The filtrate solution was diluted to 100 mL with distilled water in a volumetric flask [14]. An aliquot of these solutions was analyzed for determination of BPAP using the proposed DLLME procedure.

## 3 Results and Discussion

Our preliminary investigation showed that no extraction of BPAB was observed in the organic phase, but in the presence of Aliquat 336, which acts as an anionic carrier, its tendency towards extraction to the organic phase was increased. BPAP is converted to an anionic form in basic medium and created an ion-pair with trioctylmethylammonium ion (cation of Aliquat 336) (Figure 3). In order to determine BPAP spectrophotometrically, its absorption spectrum after DLLME procedure was recorded and it showed
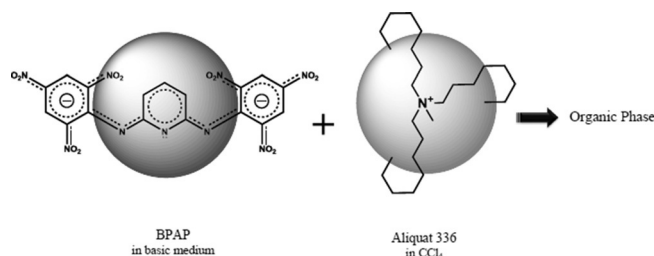


**Figure 3.** Reaction mechanism for the extraction of BPAP in organic phase.

that the maximum absorbance of the sediment phase containing BPAP and Aliquat 336 is at 460 nm. Thus 460 nm was chosen for measuring the absorbance throughout this study. For achieving the highest efficiency and sensitivity in DLLME of BPAP the influence of effective variables was investigated and optimum conditions were obtained.

### 3.1 Effect of Sodium Hydroxide Concentration

The initial experiments indicated that the maximum absorbance intensity of BPAP in sediment phase was observed in basic media, and decreasing the pH of the solution decreased the absorbance. To identify a suiTable basic medium, the effect of different concentrations of sodium hydroxide at the range of 0.001–0.010 M in aqueous solution was investigated. As Figure 4 shows, it was found that the absorbance increased with sodium hydroxide concentration up to 0.004 M, and it was almost constant after this value. Therefore, a concentration of 0.005 M of NaOH in the final solution was chosen for further experiments.

### 3.2 Selection of Extraction and Disperser Solvent

The selection of extraction solvent is very important for DLLME processing. The primary requirements for an efficient extraction solvent include low solubility in water, larger density than water, and high extraction efficiency for the analytes of interest. Three solvents, including carbon tetrachloride, trichloromethane, and dichloromethane, which satisfy these requirements, were evaluated in this work. The results in previously research were revealed that carbon tetrachloride gave the highest absorbance for the target analytes among the three solvents investigated, and therefore, $CCl_4$ was selected as the extraction solvent for further experiments [14].
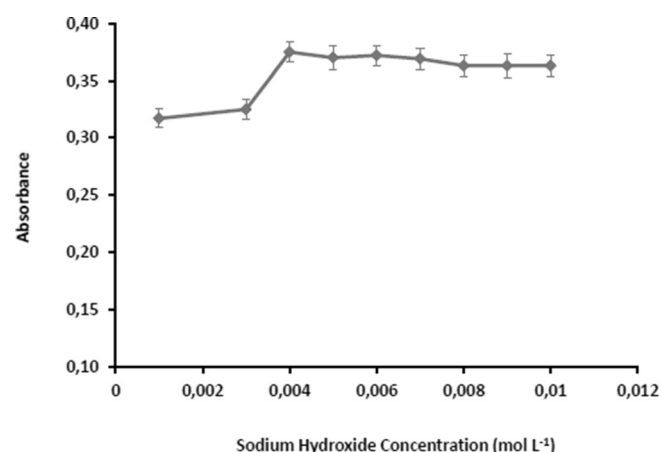


**Figure 4.** Effect of sodium hydroxide concentration in determination of BPAP. Conditions: aqueous sample volume: 10 mL; BPAP concentration: 50 ng mL$^{-1}$; volume of Aliquat 336 solution: 200 µL; shacking time: 5 s; centrifugation time: 5 min.

In this study, the Aliquat 336 was added to the water immiscible extraction solvent to enhance the dispersion of extraction solvent in the aqueous sample. Following the addition of the extraction solvent (containing Aliquat 336) to the aqueous phase and after gentle shaking, the solvent was dispersed in the solution, and a sTable emulsion system was formed. In comparison with conventional DLLME methods, in this research there is no need for an additional of dispersive solvents, due to injection of routine dispersers (such as methanol, ethanol, acetonitrile, and acetone) together with carbon tetrachloride caused the dissolving a portion of CCl$_4$ into aqueous phase and so, decreased the volume of sediment phase and extraction efficiency. Finally, the proposed method was applied only by injecting the extraction solvent containing appropriate amount of Aliquat 336 (without additional disperser solvent) to the sample solutions of BPAP.

### 3.3 Effect of Aliquat 336 Concentration in CCl$_4$

In this method, Aliquat-336 was used as the carrier and the disperser agent simultaneously and variations in its concentration cause changes in the extraction efficiency and absorbance of sediment phase, therefore it was investigated. For this purpose, the solutions of carbon tetrachloride containing various concentrations of Aliquat-336 in the range of 0.0 to 2.0 % (w/v) were prepared and 200 μL of these solutions injected to the same sample solutions of BPAP. As can be seen in the Figure 5, up to 0.8 % (w/v) Aliquat-336 solution the absorbance of sediment phase was increased and remained constant above this concentration. So, the 1.0 % (w/v) was selected as the optimum concentration of Aliquat 336 solution.
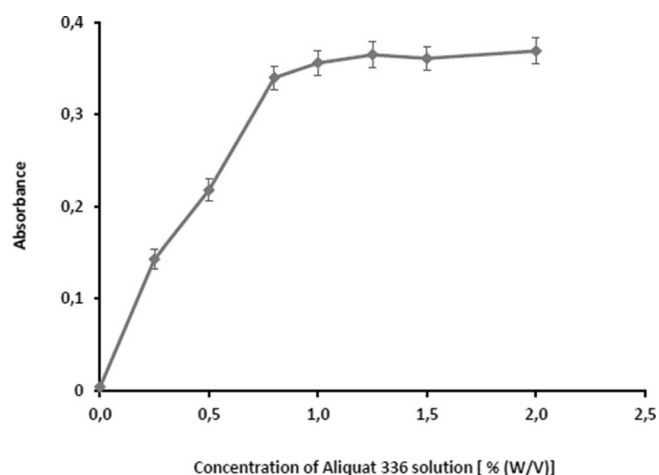


**Figure 5.** Effect of concentration of the Aliquat 336 solution in determination of BPAP. Conditions: aqueous sample volume: 10 mL; BPAP concentration: 50 ng mL$^{-1}$; NaOH concentration: 0.005 mol L$^{-1}$; volume of injection solution: 180 μL; shacking time: 5 s; centrifugation time: 5 min.
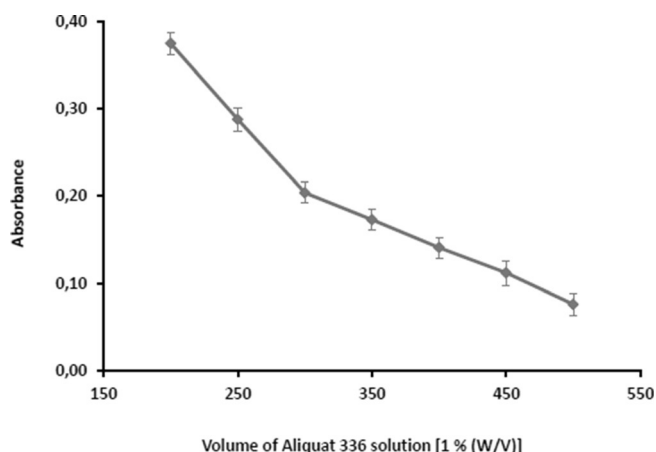


**Figure 6.** Effect of injection solution volume in determination of BPAP. Conditions: aqueous sample volume: 10 mL; BPAP concentration: 50 ng mL$^{-1}$; NaOH concentration: 0.005 mol L$^{-1}$; concentration of Aliquat 336 solution: 1 % (w/v); shacking time: 5 s; centrifugation time: 5 min.

### 3.4 Effect of Injection Solution Volume

The volume of extraction solvent is a crucial parameter and has an important effect on the extraction efficiency. The volume of collected extraction solvent is increased as the extraction solvent volume is increased, while the preconcentration factor and sensitivity of the method is decreased. To study the effect of extraction solvent volume, different volumes of organic solvent containing 1.0 % (w/v) of Aliquat 336 solution (in the range of 200–500 μL) were subjected to DLLME method keeping other parameters constant. It was observed that insufficient extraction solvent was collected when the volume less than 200 μL was used, and the absorbance signal could not be measured by spectrophotometer fitted with microcells. According to the obtained results in Figure 6, 200 μL was selected as the optimum volume of the 1.0 % (w/v) of Aliquat 336 solution.

### 3.5 Effect of Time

For breaking up the organic phase into fine droplets in aqueous solutions, it was necessary to shake the solution after injection of extraction solvent. Accordingly, the effect of shaking time on the DLLME of BPAP was investigated in the range of 0–20 s. The obtained results revealed that the highest absorbance was achieved about 5 s and above that shaking time had no significant effect on the absorbance of sediment phase. Hence, 5 s was selected for shacking time.

In addition, the effect of extraction time (interval time after the shaking of mixture of the Aliquat 336 and the extraction solvent in the aqueous sample and prior to centrifugation onset) on the DLLME method was investigated and evaluated. This parameter was studied over the range of 0–5 min under constant experimental conditions. The obtained results showed that extraction procedure was

time independent. It is verified that an infinite large surface is developed between extraction solvent and aqueous phase after the formation of cloudy solution, therefore the extraction time was not the remarkable effect on the extraction efficiency.

In this method, the most time-consuming step is the centrifugation used for phase separation, which is about 5 min at 5000 rpm.

## 3.6 Interference Studies

The effect of interferences on the determination of BPAP was investigated using the optimized preconcentration conditions. Various ions and molecules were added individually to a solutions containing 50 ng mL$^{-1}$ of BPAP and the proposed procedure was applied. The effect of each species was considered as interference when the analytical signal showed absorbance deviation of more than $\pm 5\%$. The results (Table 1) show that the presence of interfering at the ratios that usually occur in real samples are almost quantified by using developed method.

## 3.7 Analytical Figures of Merit

Under optimal conditions, analytical parameters (i.e., linear rage, limit of detection, repeatability, and preconcentration factor) were investigated. Linear calibration graph was obtained in the range of 2.0–150.0 ng mL$^{-1}$ for BPAP in the initial solution. The equations of the line is $A = 0.0115C + 0.0199$, where $C$ is the concentration of BPAP (ng mL$^{-1}$) in the initial aqueous solution. The regression coefficient for the line is 0.9996. The limit of detection based on $3S_b$ was 1.0 ng mL$^{-1}$ for BPAP. The relative standard deviation for ten replicate measurements of 20 and 80 ng mL$^{-1}$ was 3.3 and 1.2%, respectively.

## 3.8. Application to Real Samples

In order to validate the suitability of proposed DLLME procedure, it was verified by employing the method to determine the BPAP in different water and soil samples. The results showed that the real sample analyzed were either free

**Table 1.** Tolerance limits for some tested species for the determination of BPAP (50 ng mL$^{-1}$).

| Species | Tolerance ratio (w/w) |
|---|---|
| Na$^+$, Cl$^-$, NO$_3^-$, SO$_4^{2-}$, Al$^{3+}$, Ag$^+$ | 1000 |
| K$^+$, Mg$^{2+}$, HCO$_3^-$, CO$_3^{2-}$, NH$_4^+$ | 800 |
| PO$_4^{3-}$, Ca$^{2+}$, Cu$^{2+}$, CO$^{2+}$, I$^-$, F$^-$, Ni$^{2+}$ | 500 |
| Mn$^{2+}$, RDX,[a] HMX,[b] Picric acid | 200 |
| Fe$^{3+}$, Tetryl,[c] Tetranitrocarbazole | 100 |
| TNT[d] | 50 |

a) 1,3,5-Trinitroperhydro-1,3,5-triazine. b) Octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine. c) 2,4,6-Trinitrophenylmethylnitramine. d) Trinitrotoluene.

**Table 2.** Determination of BPAP in different water samples.

| Sample | Added [ng mL$^{-1}$] | Found[a] [ng mL$^{-1}$] | Recovery [%] |
|---|---|---|---|
| Tap water (Ahvaz) | 0 | N.D[b] | – |
| | 20 | 19.6 ± 0.6 | 98.0 |
| | 40 | 41.3 ± 1.4 | 103.2 |
| River water (Karun) | 0 | N.D | – |
| | 20 | 20.4 ± 0.7 | 102.0 |
| | 40 | 40.9 ± 1.3 | 102.2 |
| Effluent waste water (Ahvaz) | 0 | N.D | – |
| | 20 | 20.8 ± 0.7 | 104.0 |
| | 40 | 39.5 ± 1.3 | 98.7 |

a) Mean ± standard deviation ($n = 3$). b) N. D. = not detected.

**Table 3.** Determination of BPAP in soil samples.

| Soil sample | Added [ng mL$^{-1}$] | Found[a] [ng mL$^{-1}$] | Recovery [%] |
|---|---|---|---|
| 1[b] | 0 25.3 ± 0.8 | – | |
| | 20 | 46.0 ± 1.5 | 103.5 |
| | 40 | 66.4 ± 2.2 | 102.7 |
| 2[c] | 0 | 24.1 ± 0.8 | – |
| | 20 | 44.3 ± 1.5 | 101.0 |
| | 40 | 63.9 ± 2.1 | 99.5 |
| 3[d] | 0 | 26.0 ± 0.8 | – |
| | 20 | 45.1 ± 1.5 | 95.5 |
| | 40 | 64.7 ± 2.1 | 96.7 |

a) Mean ± standard deviation ($n = 3$). b) Amount of BPAP was 101.2 µg g$^{-1}$. c) Amount of BPAP was 96.4 µg g$^{-1}$. d) Amount of BPAP was 104.0 µg g$^{-1}$.

of BPAP or had concentrations below detection limits. The recovery tests were performed by the analysis of the samples spiked with known amounts of analyte (at to concentration levels, containing 20 and 40 ng mL$^{-1}$). The analytical data of this investigation are given in Table 2 and Table 3. As can be seen, the recoveries for the spiked samples are quantitative (95.5–104.0%). These results demonstrate that the matrix effect from water and soil samples on the DLLME for determination of BPAP was not significant.

## 4 Conclusions

The combination of DLLME method with microvolume UV/Vis spectrophotometry provided a simple route for preconcentration and determination of BPAP explosive. The main benefits of the DLLME method are: reduced environmental hazards and consumption of less extracting solvent, simplicity, low cost, and increased sensitivity. Compared with the conventional dispersive liquid-liquid microextraction, in this procedure no conventional disperser solvent has to be applied, because the Aliquat 336 was used as anion carrier and disperser agent. The good accuracy and precision offered by this method, makes it useful for the analysis of BPAP in water and soil samples.

## Acknowledgements

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