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Simultaneous Determination of Carbazoles in Water Samples by Cloud Point Extraction Coupled to HPLC

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Abstract

Cloud point extraction (CPE) as a rapid, simple and efficient method coupled with high performance liquid chromatography (HPLC) was used for sample preparation and subsequent determination of carbazole, trinitrocarbazole (TrNC) and tetra nitro carbazole (TNC) in water samples. Some effective parameters on extraction, such as volume of Triton X-100, extraction time, extraction temperature, ionic strength and centrifuging time were studied and optimized. Under the optimum conditions, recoveries of analytes were in the range of 98.0–102.0%. The calibration curve was linear in the range of 0.08 to 15.0 μg mL^{-1} for TNC, 0.06 to 15.2 μg mL^{-1} for TrNC, and 0.08–21.0 μg mL^{-1} for carbazole. Limit of detection (LOD) and limit of quantification (LOQ) were in the range of 0.009 -0.01 and 0.06-0.08μg mL^{-1}, respectively. The relative standard deviations (RSDs) were in the range of 4.0- 6.2 %. The obtained results show that CPE with a high performance liquid chromatography (HPLC) is a sensitive and simple method for the determination of carbazole, TrNC and TNC in water samples.

***Keywords*:** *Cloud point extraction, High performance liquid chromatography, Carbazole, Tri nitro carbazole, Tetra nitro carbazole, Water samples.*

Introduction

Nitroaromatic explosives are important compounds in both environmental and forensic science [1]. 1,3,6,8 Tetra nitrocarbazole (TNC) is a second type nitroaromatic explosive which was reported for the first time in 1904 by Graebe [2]. Industrial production of TNC was initiated with carbazolewhile other nitro carbazoles, such as TrNC, were produced as impurity. This material is known as nitrosane and as such is used as an insecticide [3, 4]. TNC is used in the octahydro-1,3,5,7-tetranitro-

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1,3,5,7-tetrazine(RDX), and hexahydro-1,3,5-trinitro-1,3,5-triazine (HMX) composites to reduce sensitivity and increase the stability of these types of explosive's composition [5]. Environmental waters including ponds, lakes, rivers, estuaries and coastal ocean area near the ammunition plants, military munitions sites, wartime activities area, sinking of warships, etc., containing residues of explosive compounds may be hazardous for natural life especially plants, animals and finally humans [6–8]. Releasing the unexploded ordinances, with various military exercises, in the ground or oceans/seas may cause contamination of surface, underground, and seawaters. Thus, rapid, sensitive and selective techniques for environmental monitoring of the explosives in aqueous samples are necessary [9–12]. Chemical structures of carbazole, TNC and TrNC were shown in Figure 1.

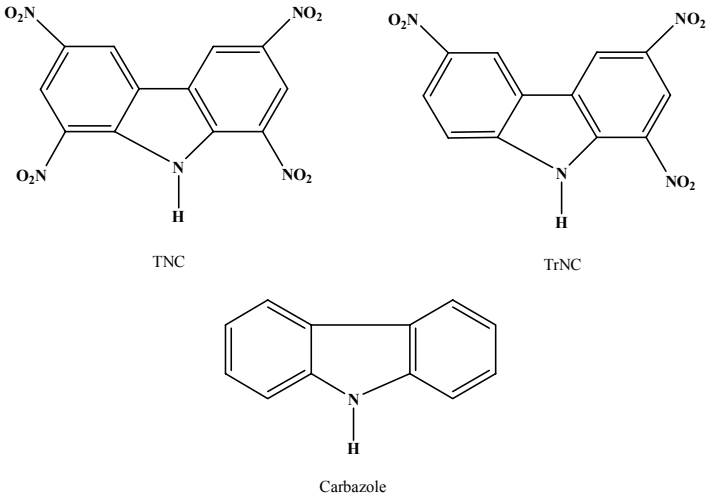

Figure 1. Chemical structures of carbazole, TNC and TrNC.

A sample preparation and preconcentration method is necessary prior to analysis of trace amount of explosives in water samples. There are many reports on the extraction and preconcentration prior to analysis of explosives in water samples by various techniques such as solvent extraction [13, 14], salting out liquid-liquid extraction (LLE) [15] or thermal desorption [16]. These methods suffer from some disadvantages such as time consuming and requiring large volumes of expensive organic solvents with high-purity [15]. These limitations led to the development of other methods such as solid-phase extraction (SPE) [17], solid-phase micro extraction (SPME) [18–20], micellar extraction [21], supercritical fluid extraction (SFE)[22] solvent micro extraction [23], single-drop micro extraction (SDME)[24], hollow fiber-based liquid-phase micro extraction (HF-LPME) [25], and cloud point extraction (CPE) [21]. These methods usually serve as sample preparation techniques which preconcentrate the analyte in the samples for analysis by

one of the following techniques: high-performance liquid chromatography (HPLC) [26], gas chromatography (GC) [27], ion mobility spectrometry [28], capillary electro chromatography (CE) [29],supercritical fluid chromatography (SFC) [30, 31] or electro kinetic capillary electrophoresis (EKCE) [32]. Simple, effective, and environment-friendly extraction procedures are still in demand. CPE or micelle-mediated extraction (MME), has been recognized as an alternative to the conventional extraction because of its performance, low cost, and less toxic. In CPE, a non-ionic surfactant is used as an extract analyte. The procedure is simple and the extraction of the analytes can be accomplished by optimizing the experimental conditions such as temperature, the addition of salts, etc. The extraction occurs at cloud-point temperature where the surfactant becomes cloudy, usually at a higher temperature than its critical temperature, resulting in a two phase separation involving the surfactant-rich phase (SRP) and the aqueous phase (AQ).Generally, analytes are present in the SRP which has a very small volume compared to the larger AQ volume. Thus, analytes are concentrated with a high preconcentration factor [33-37]. The SRP is then diluted with aminimum volume of organic solvent or mobile phase before being directly injected into the chromatographic system.

In this investigation, cloud point extraction coupled with high performance liquid chromatography has been used for simultaneous determination of TNC, TrNC and carbazole explosives in water samples. To the best of our knowledge, there is no literature on simultaneous determination of TNC explosives by CPE in aqueous samples.

Experimental

Materials

Acetone, methanol and acetonitrile were supplied from Acros (Geel, Belgium). The non-ionic surfactant Triton X-100 (Merck, Darmstadt, Germany) was used without further purification. Sodium acetate, acetic acid, ortho phosphoric acid, potassium dihydrogen phosphate, sodium carbonate, sodium sulfate and sodium chloride were purchased from Merck Chemical Company (Darmstadt, Germany). All solutions were prepared with deionized water from a Milli-Q system (Millipore, USA). Carbazole was purchased from Fluka Company (Buchs, Switzerland). TNC and TrNC were synthesized and recrystallized three times from acetone(purity of 99.7%) as reported previously [4]. To prepare the1000 mg L−1 standard solution of explosives, first, appropriate amount of each analyte was dissolved in small amount of the acetonitrile and then using deionized water the solution was diluted to the mark of a 100 mL volumetric flask. A stock Triton X-100 solution (25%, w/v)was prepared by dissolving it in an

appropriate amount in deionized water.

River, underground and drinking water samples, used for development of the method, were collected from Khorramabad (Iran) in amber glass bottles and stored at 4 ^{O}C and analyzed within 48 h of collection without any previous treatment.

Chromatographic system and operation conditions

The HPLC used system was from Knauer (Germany), consisted of a EA4300F smart line pump, fitted with a Rheodyne 7725i injector valve including of 20 µL injection loop, a S-2600 UV detector and a 250×4.6 mm Eurospher 100-5 C18column. Chromatographic data handling was performed using Chrom Gate V3.1.7 software. Separations of explosive compounds were carried out by following chromatographic conditions: The column was operated with a mobile phase of acetonitrile: water (75:25) at a flow rate of 1 mL min−1. The absorption wavelengths of TNC, TrNC and carbazole using three detecting channels UV–Vis detector were adjusted at287, 309, 291 nm, respectively. A centrifuge (HeruesLabofuge300) was used to accelerate the phase separation in the process of extraction. For pH measurements, a Metrohm digital pH-meter (model 692, Herisau, Switzerland) equipped with a glass combination electrode was used.

Cloud point extraction procedure

Under the finally optimized conditions, 10.0 mL of aqueous sample solution containing mixture of TNC, TrNC and carbazole was placed in a 15 mL conical bottom glass tube. A 0.4 mL of Triton X-100 stock solution and 1.0 g of NaCl were consequently added, and the contents were mixed well with a Vortex (CAY-1, Beijing Changan Instrumental Factory, PR China) for 1 min, and then incubated in a thermo stated water bath at 50 °C for 10 min. After centrifuging for 10 min at 4000 rpm, the aqueous phase was then carefully removed, using a syringe with a long needle that passed through the surfactant-rich phase. A surfactant-rich phase was diluted with methanol (1 mL) to decrease viscosity before injection. Finally, 20 µL of this solution was injected into the HPLC system for analysis.

Results and discussion

Spectrum of TNC, TrNC and carbazole

The correct selection of absorption wavelengths in HPLC coupled with UV–Vis analysis system has an important role in the selectivity of the method. Thus, the UV–Vis spectrum of 1,3,6,8 tetra nitro carbazole (TNC), three nitro carbazole (TrNC) and carbazole were investigated and spectrums of them are shown in Figure 2. There are two main absorption peaks; the first is observed in the UV region and the second is near the visible region. The UV peak corresponds to the benzene rings

and the visible peak is due to the resonance of benzoic rings in carbazole structure. Although, the UV peaks are more sharp and selection of this peak as determination wavelengths lead to sharper HPLC peaks; but, in order to reduction of foreign interface and obtaining higher selectivity by using three detecting channels UV–Visdetector, the wavelengths of the explosives were adjusted at287, 309, 291 nm for TNC, TrNC and carbazole, respectively. Differences between three spectrums as illustrated correspond to the nitro groups and their effect on the resonance of carbazole backbone.

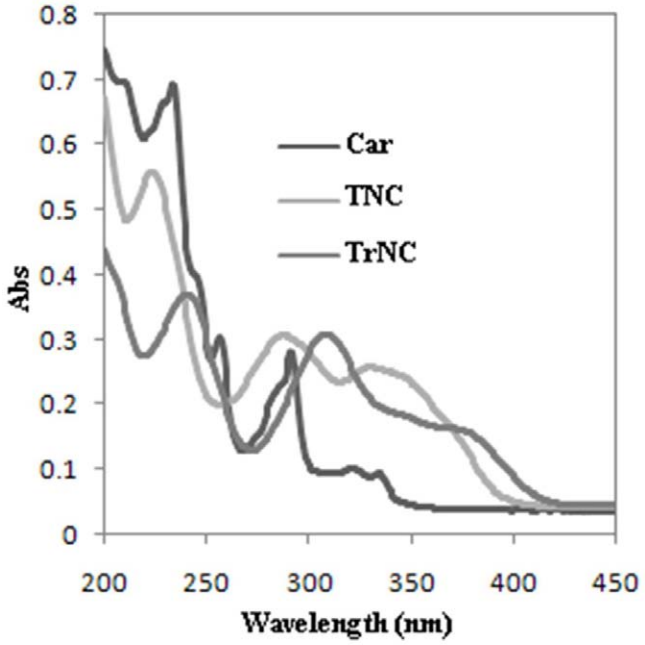

Figure 2. Absorbance spectra of the investigated explosives (concentration of TNC, TrNC and carbazole was about 10^{-5} M in acetonitrile).

Effect of Triton X-100 volume

During CPE, it is necessary to optimize the surfactant concentration for sufficient extraction of target analytes. The effect of the surfactant concentration on the extraction was determined by measuring a series of aqueous solutions containing analytes and different concentrations of Triton X-100.Figure3 shows the effect of surfactant concentration on the extraction efficiencies of analytes. The extraction efficiencies of these compounds increase sharply when Triton X-100volume increases up to 0.4 ml, from which point extraction efficiency of analytes remain constant. In the light of these results, 0.4 mL of stock Triton X-100 solution is established to extract analytes.

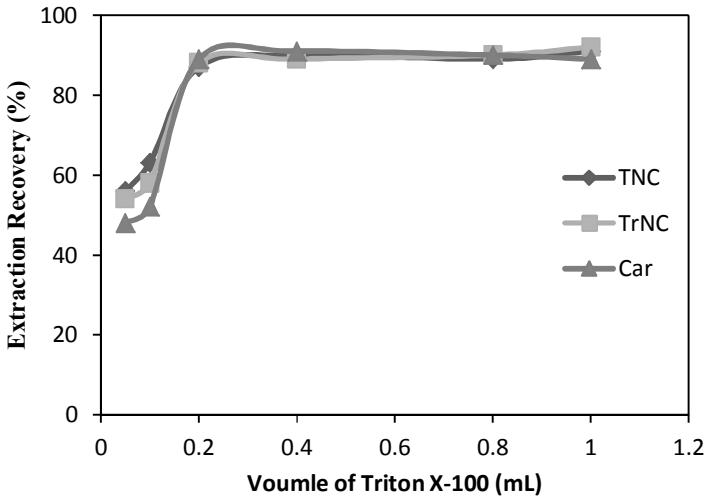

Figure 3.Effect of Triton X-100 volume on extraction efficiency. Extraction conditions: temperature; 50 ^{O}C, incubation time; 10 min, centrifuging time; 10 min, pH; 6.2, NaCl concentration; 0.4 M.

Effect of sample pH

The pH of the sample solution is a significant parameter, which has an important effect on the extraction of analytes from water samples. Figure 4 shows effect of pH on the extraction recoveries of analytes. As shown in this Figure, the extraction recoveries increased until pH≈7; while, in higher values of pH, the extraction recoveries decrease. Therefore, the pH of aqueous sample was adjusted to pH=6.2.

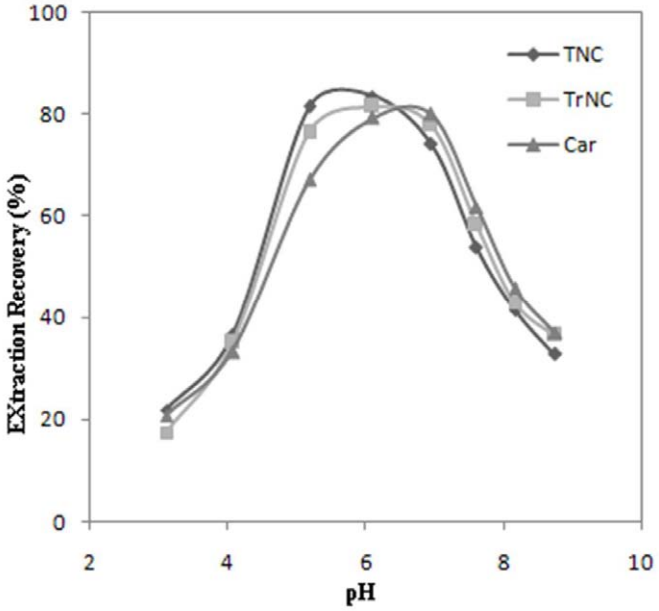

Figure 4. Effect of solution pH on extraction efficiency. Extraction conditions: temperature; 50 ^{O}C, incubation time; 10 min, centrifuging time; 10 min, Triton X-100 volume; 0.4 mL, NaCl concentration; 0.4 M.

Effect of incubation time

The influence of the extraction time on the ability of TritonX-100 to extract the analytes from water was examined in the

range between 5 min and 30 min (Figure 5). Extraction time of less than 10 min produced low signal responses. As shown in Figure 5, 10 min extraction was enough to give the highest signal response; therefore, extraction with duration of 10 min was recommended.

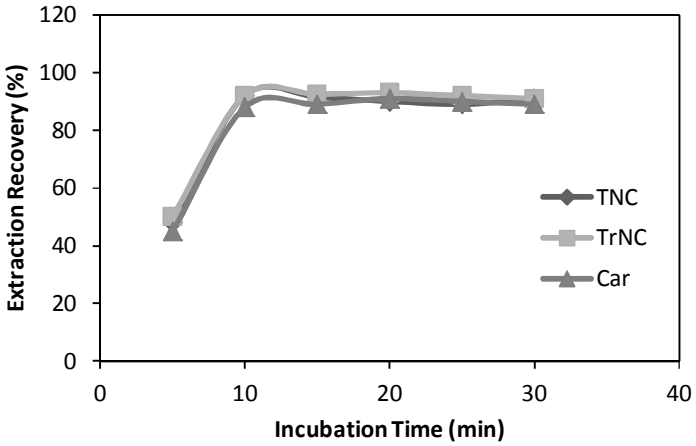

Figure 5. Effect of incubation time on extraction efficiency. Extraction conditions: temperature; 50 ^{O}C, Triton X-100 volume; 0.4 mL, centrifuging time; 10 min, pH; 6.2, NaCl concentration; 0.4 M.

Effect of ionic strength

In order to investigate the influence of ionic strength on the extraction recoveries various concentrations of sodium chloride solutions were added to aqueous samples. As shown in Figure 6, the extraction recoveries were increased with increasing the ionic strength up to a concentrating of 0.04 M; while, in higher ionic strengths, the resulted signals were constant. This behavior arises from salting out effect on extraction. Therefore, a salt concentration of 0.04 M sodium chloride was selected as the optimal concentration of salt in the CPE procedure.

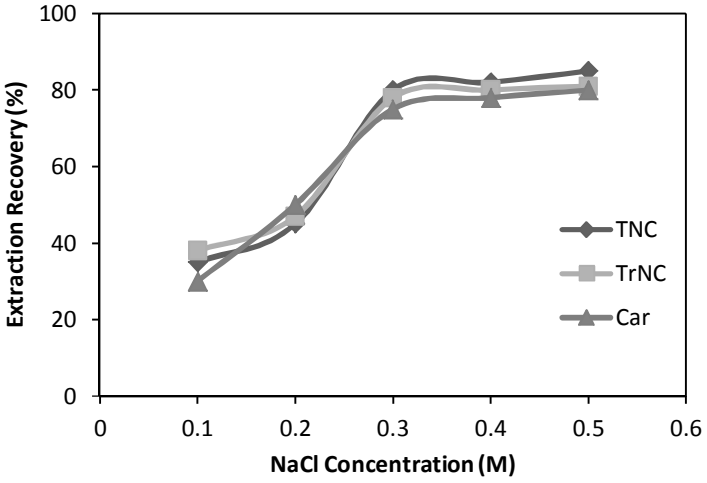

Figure 6. Effect of ionic strength on extraction efficiency. Extraction conditions: temperature; 50 ^{O}C, incubation time; 10 min, centrifuging time; 10 min, pH; 6.2, Triton X-100 volume; 0.4 ml**.**

Effect of centrifuging time

Centrifuging time has an important effect on extraction efficiency. In this study, the effect of centrifuging time was tested at the constant centrifuge speed of 4000 rpm. The dependence of extraction recovery of sample species on centrifuge time (2-20min) was presented in Figure 7. As shown in this Figure, the variations of extraction recovery versus centrifuge time are remarkable, which indicate that the centrifuging time has considerable effect on extraction efficiency. Therefore, effect of this parameter on the efficiency of the procedure was investigated and the time of 10 min was chosen as the optimum centrifuging time.

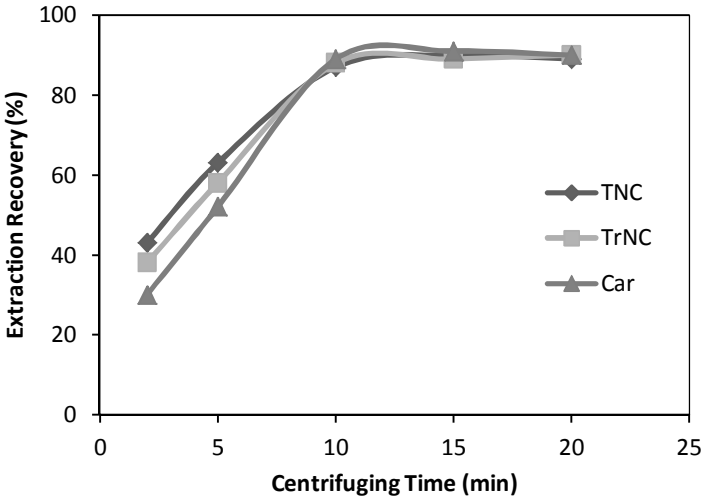

Figure 7. Effect of centrifuging time on extraction efficiency. Extraction conditions: temperature; 50 ^{O}C, incubation time; 10 min, Triton X-100 volume; 0.4 mL, pH; 6.2, NaCl concentration; 0.4 M.

Evaluation analytical performance of method

Calibration curves were obtained by analyzing standard solutions containing different concentrations of the explosives under the optimum conditions. Linearity ranges were: 0.08 to 15.0 μg mL−1 for TNC, 0.06 to 15.2 μg mL−1 for TrNC, and 0.08–21.0 μg mL−1 for carbazole. The relative standard deviations (RSDs, n=6) for the extraction of TNC, TrNC and carbazole varied between 4.0 and 6.2%. The limit of detection (LOD), based on signal-to noise ratio (S/N) of 3, ranged from 0.009 to 0.01ng mL-1.The statistical parameters of the CPE method were detailed in Table 1.

Table 1. Performance of the method for determination of analytes in aqueous samples.

Analyte	RSD[a] (%)	LR[b] (μg mL^{-1})	r^2	LOD[c] (μg mL^{-1})	LOQ[d] (μg mL^{-1})
TNC	6.2	0.08-15.0	0.9988	0.01	0.08
TrNC	4.0	0.06-15.2	0.9993	0.009	0.06
Carbazole	5.2	0.08-21.0	0.9989	0.01	0.08

[a] RSD for concentration of TNC (0.1 μg mL^{-1}),TrNC (0.08 μg mL^{-1}) and carbazole (0.1 μg mL^{-1})
[b] LR: Linear range.
[c] LOD: Limit of detection for S/N=3
[d] LOQ: Limit of quantification for S/N=10

Application of the procedure to real aqueous sample analysis

River, underground and drinking water samples were collected in 1 L amber glass bottles (without any further treatment) and cooled in refrigerator. Prior to performing CPE, each sample was filtered through a 0.45μmmembrane filter and then was used for extraction. The spiked samples were extracted using the optimized CPE procedure and then analyzed using HPLC.The real aqueous samples were spiking with standard at concentrations of 0.2 μg mL^{-1} for TNC,0.3μg mL^{-1} for TrNC and 0.4 μg mL^{-1} for carbazol. The spiked samples were extracted using the optimized CPE procedure and then analyzed using HPLC. Itshows the chromatograms obtained from real water samples spiked with TNC, TrNC and carbazole standard solutions. The results (Table2) indicated that the recoveries for spiked environmental aqueous samples were in the range of 98.0–102.0 %.These results demonstrate that CPE method can be used for the preconcentration of carbazole based explosives in aqueous samples.

Table 2. Results for analysis of environmental aqueous samples

Compound	Recovery (%)					
	Underground water		River water		Drinking water	
	Blank	Spiked	Blank	Spiked	Blank	Spiked
TNC	nd[a]	98.7	nd	100.2	nd	98.8
TrNC	nd	100.9	nd	98.6	nd	101.6
Carbazole	nd	102.0	nd	99.2	nd	101.5

[a]Not detected

Conclusions

The present study exhibited an efficient method for simultaneous determination of nitrocarbazole based explosives using cloud point extraction combined with HPLC. This method provides excellent linear range, limits of detection (LOD) and repeatability. The use of CPE method as alternatives to other techniques of separation and preconcentration offers several advantages including low cost, safety and high capacity to preconcentrate variety organic compounds, with high recoveries and good enrichment factors. This method utilizes a single step extraction of analytes. The developed CPE method has various advantages such as simplicity, facile operation and low cost. The analysis of real aqueous samples by this method proved that CPE has significant a proper potential for usage in the routine analysis of the investigated explosives.

Acknowledgments

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