

## FORENSIC ANALYSIS OF HIGH EXPLOSIVES RESIDUES IN POST-BLAST WATER SAMPLES EMPLOYING SOLID PHASE EXTRACTION FOR ANALYTE PRO-CONCENTRATION

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### Abstract

Nitroaromatic, nitramine and nitrate ester compounds are a major group of high order explosive or better known as military explosives. Octrahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX), 1,3,5-hexahydro -1,3,5-trinitrotriazine (RDX), 2,4,6-trinitro-toluene (TNT), pentaerythritol tetranitrate (PETN) and 2,4-dinitrotoluene (2,4-DNT) are secondary high explosives classified as most commonly used explosives components. There is an increasing demand for pre-concentration of these compounds in water samples as the sensitivity achieved by instrumental analytical methods for these high explosives residues are the main drawback in the application at trace levels for forensic analysis. Hence, a simple cartridge solid phase extraction (SPE) procedure was optimized as the off-line extraction and pre-concentration method to enhance the detection limit of high explosive residues using micellar electrokinetic chromatography (MEKC) and gas chromatography with electron-capture detection (GC-ECD) methods. The SPE cartridges utilized LiChrolut EN as the SPE adsorbent. By employing pre-concentration using SPE, the detection limits of the target analytes in water sample were lowered by more than 1000 times with good percentage recovery (> 87%) for MEKC method and lowered by 120 times with more than 2 % percentage recovery for GC-ECD methods. In order to test the feasibility of the developed method to real cases, post-blast water samples were analyzed. The post-blast water samples which were collected from Baling Bom training range, Ulu Kinta, Perak contained RDX and PETN in the range of 0.05 – 0.17 ppm and 0.0124 – 0.0390 ppm respectively.

**Keywords:** Solid phase extraction, high explosives, post-blast water samples, MEKC, GC-ECD, SPME.

### Introduction

Nitroaromatic, nitramine and nitrate ester compounds are a major group of high order explosive or better known as military explosives. Octrahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX), 1,3,5-hexahydro -1,3,5-trinitrotriazine (RDX), 2,4,6-trinitro-toluene (TNT), pentaerythritol tetranitrate (PETN) and 2,4-dinitrotoluene (2,4-DNT) are secondary high explosives classified as most commonly used explosives components [5].

Development of chemical analytical procedures for these high explosive residues is crucial for forensic analysis following increasing terrorist or other criminal activity [6]. Gas chromatography (GC) analysis of explosives was reported to be superior when it is coupled with powerful solid phase micro extraction (SPME) technique [3]. This method could not completely resolve thermally labile explosives, especially certain nitrate esters and nitramine such as HMX and RDX [3] and [2]. Capillary electrophoresis has been found to be a powerful alternative to chromatographic techniques, as shown by its rapidly expanding and widespread use in environmental, biological, clinical and forensic fields [13].

However, there is an increasing demand for pre-concentration of these compounds in water samples as the sensitivity achieved by instrumental analytical methods for these high explosives residues are the main drawback in the application at trace levels for forensic analysis. In order to reach the detection limits (LODs) demanded for high explosive residues in post blast water sample for forensic analysis, offline preconcentration steps (e.g., solid phase extraction (SPE)) are usually employed [10]. Most applications are based on chromatography such as HPLC [7] and [5] using various detection systems.

Hundreds of papers have appeared in the scientific journals describing various developments and applications of SPE in water analysis and many articles reporting the use of SPE for trace enrichment of explosives compounds from water can be found in literature, as well [11]. Apart from the main goal of extracting traces of the compounds of interest, SPE is also used to remove the interfering components of matrix [8].

In the present study, the analytical method involved off-line preconcentration of post-blast water samples employing LiChrolut EN SPE cartridges followed by instrumental analysis utilizing MEKC with UV detection, and GC-ECD methods. Thus, the aim of the study is to assess the possibilities of applying the developed methods coupled with SPE preconcentration technique to the determination of real samples.

## Experimental

### Chemicals

Analytical grade (AR) sodium dodecyl sulphate (SDS), boric acid, ortho-phosphoric acid (purity of 85 %), sodium chloride and sodium hydroxide pellets were purchased from Merck (Darmstadt, Germany). Acetonitrile and methanol (organic solvents) purchased from Merck (Darmstadt, Germany) were of HPLC grade. Analytical grade sodium tetraborate was obtained from Riedel-de-Haen (Seelze, Germany). Aqueous solutions were prepared in ultrapure, distilled, deionized water (DDDW) purified up to 18.2 M $\Omega$ . The DDDW was prepared in the laboratory, Department of Chemistry, UTM using a Simplicity<sup>TM</sup> water purification system obtained from Millipore (U.S.A). Liquid nitrogen, N<sub>2</sub> was industrial grade from Malaysian Oxygen Berhad (MOX), Petaling Jaya, Malaysia.

### Standard Solutions

Explosive standards of HMX and 2,4-DNT (1000 ppm, purity >99%) were purchased from Supelco (Bellefonte, USA). TNT, PETN and RDX analytical standards (1000 ppm) were provided by Science Technology Research Institute for Defense, Malaysia (STRIDE) Batu Arang, Selangor. Figure 1 provides the chemical structures of the five high explosives used in this study.

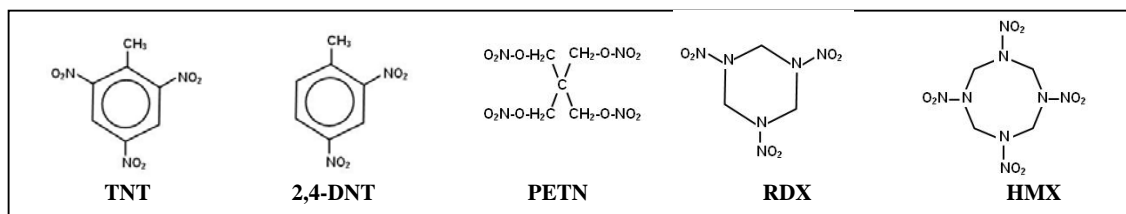


Figure 1: Chemical structures of the explosives analyzed

### Apparatus

All MEKC separations were performed using an HP<sup>3D</sup> capillary electrophoresis instrument (Agilent Technologies, Hanover, Germany) equipped with a built-in diode array detector and interfaced with a HP personal computer running HP<sup>3D</sup> Chemstation software to carry out system control and data acquisition. Standard bare fused silica capillary column (Agilent Technologies) with 112.5 cm total length, 104 cm effective length and 50  $\mu$ m i.d. was utilized for analyte separation. Injection offset was set at 4 mm. Polypropylene vials (Agilent Technologies) of 1 mL were used to place buffers and other solutions in the electrophoretic system. Standard samples were placed in the polypropylene glass lined 100  $\mu$ l vials. All solutions were filtered through a 0.45  $\mu$ m nylon filter disc (Whatman) prior to analysis.

All GC experiments were carried out on a Perkin Elmer Autosystem XL Gas Chromatograph equipped with an electron capture detector (ECD) was. Data acquisition and instrument control system was performed using Turbochrom Navigator Version 4.1. An Ultra 2 fused silica capillary column (25 m x 0.20 mm ID x 0.11  $\mu$ m film thickness) with helium as a carrier gas. Oven temperature was set at 100 $^{\circ}$ C (hold 3 min) that was increased at a rate of 15 $^{\circ}$ C/min up to 280 $^{\circ}$ C.

A Supelco SPME fiber manual holder, together with a 75  $\mu$ m film thickness Carboxen<sup>TM</sup>/polydimethylsiloxane (CAR/PDMS)-coated fiber obtained from Supelco (Bellefonte, USA) was employed in this study.

LiChrolut EN and LiChrolut RP-18 SPE cartridges (200 mg sorbent, 3 mL capacity) were purchased from Merck (Darmstadt, Germany). SPE cartridges were mounted on a 10-port vacuum manifold processing device (Agilent Technologies) coupled to a Fisherbrand vacuum pump (Fisher Scientific) for sample preparation. SPE eluates were concentrated off under a gentle stream of nitrogen gas.

A hotplate stirrer model HS 0707V2 from Favorit (United Kingdom) and Teflon magnetic stir bar were used to agitate the buffer. A pH meter Model 510 Cyberscan (Eutech Instruments, Singapore) was employed for pH adjustment of buffer used for MEKC method.

#### MEKC Separation Procedures

The capillary was rinsed with methanol for 10 minutes followed by deionised water for 5 minutes. It was then rinsed with 1 M NaOH to activate the silanol groups of the capillary. This protocol was implemented as a daily start-up procedure. The separation buffer was composed of 2.5 mM sodium tetraborate and 12.5 mM boric acid containing 50 mM SDS [4] and 5% organic modifier (ACN:MeOH). The final pH of the buffer after combining all components was adjusted to pH 8.15. Preconcentrated samples were introduced into the capillary hydrodynamically for 5s at 50 mbar. Unless otherwise indicated, the run time was 30 min with preconditioning flushes of the capillary after each run in the following sequence: 0.1 M NaOH (3 min) and running buffer (5 min). The total analysis time was therefore 38 min. Separation runs were carried out at positive end (anodic injection) at 30 kV with a constant temperature of 25°C. The operating current under the above conditions were typically in the range of 12-15  $\mu$ Ampere. All the solutions were degassed using a sonicator prior to analysis in order to obtain good baseline.

#### SPE Procedure

The LiChrolut EN cartridges were placed on the SPE vacuum manifold and conditioned with 10 mL of MeOH at gravity flow, followed by 20 mL of distilled water (~ 1 mL/min). Sample passage (200 mL) was carried out at a flow-rate of ~ 3 mL/min as controlled by the vacuum pump. Once all the samples have been applied to the SPE cartridge, the cartridge was dried under vacuum for 5 min. The elution of retained components was done with 3 portions of 3 mL acetonitrile and eluates directly collected in a 10 mL vial. The organic phase thus obtained was brought to near dryness under a stream of nitrogen, after which the residue was reconstituted with acetonitrile to required volume prior to analysis.

#### SPME procedure

In DI-SPME procedure, the fiber was exposed to 5 mL aqueous samples in a 10 mL vial sealed with a rubber septum prior to thermal desorption in GC injection port.

#### Preparation of Spiked Sample

200 mL of water was spiked with 200  $\mu$ L of standard mixture solution (200 ppm each). The spiked sample was then subjected to SPE. Blank samples of unspiked water samples were prepared in a similar way.

#### Sampling Activities

The sampling area was a gazetted area for explosion situated next to Pasukan Gerakan Am PDRM (PGA), Ulu Kinta, approximately 15 km from Ipoh. The impact area where the detonation took place was an open hilly area covered with coppice and known to be free from the types of explosives analyzed. The location of sampling point is given in Figure 2 and the types of explosives detonated at each sampling point are given in Table 1. The weather was damp with intermittent drizzling on the day of sampling. The sampling points were at least 50 m apart from each other so that cross-contamination due to each explosion would not occur.

Table 2: Information of Explosives Exploded at Different Sampling Points

Sampling Point	Nature of trace amount of explosive	Weight of explosive (g)
R	RDX	100
P	PETN	150

### Sampling at Point R

The RDX explosive (100 g) was placed at point R at a slope near the stream. After explosion, water samples were collected from the stream at various times within 5 min to 45 min.

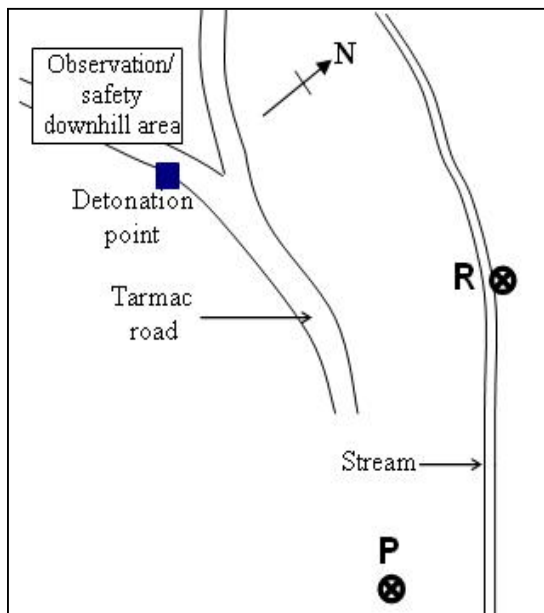


Figure 2: Position of point R and point P at sampling area

### Sampling at Point P

Plastic trays that were filled with 0.5 L of tap water were hooked to the ground using stainless steel hook. The trays were placed according to the position of labeled metal pegs placed at specified distances from the pit area (3-5 m). These trays and plates were hooked to avoid them being blown off during the blast. After the explosion took place, the post blast water samples from the trays were transferred into sample bottles using a plastic funnel. The sample bottles were labeled accordingly

### Sample Storage

All samples were placed in a cooler box immediately after collection. This was to keep the sample cool and to maintain the integrity of the samples. The sample bottles were sealed tightly using plastic cap and were arranged closely in the cooler box so that they do not fall or shake during transportation back to the laboratory. Freezer bags and ice cubes were used to keep the water samples cool ( $\sim 4^{\circ}\text{C}$ ). Once transported to the lab, all the samples were stored in the refrigerator prior to analyses.

## Results and discussion

### Separation of Analytes

A number of MEKC methods for the analysis of nitro compounds have been published [4] and [12]. The method of [4] proved to be better suited to this application and was utilized in the work presented. Figure 3 shows MEKC and GC-ECD separations of explosive constituents in a five-component standard mixture. Identification of individual components in the electropherogram was made by spiking individual components on subsequent runs.

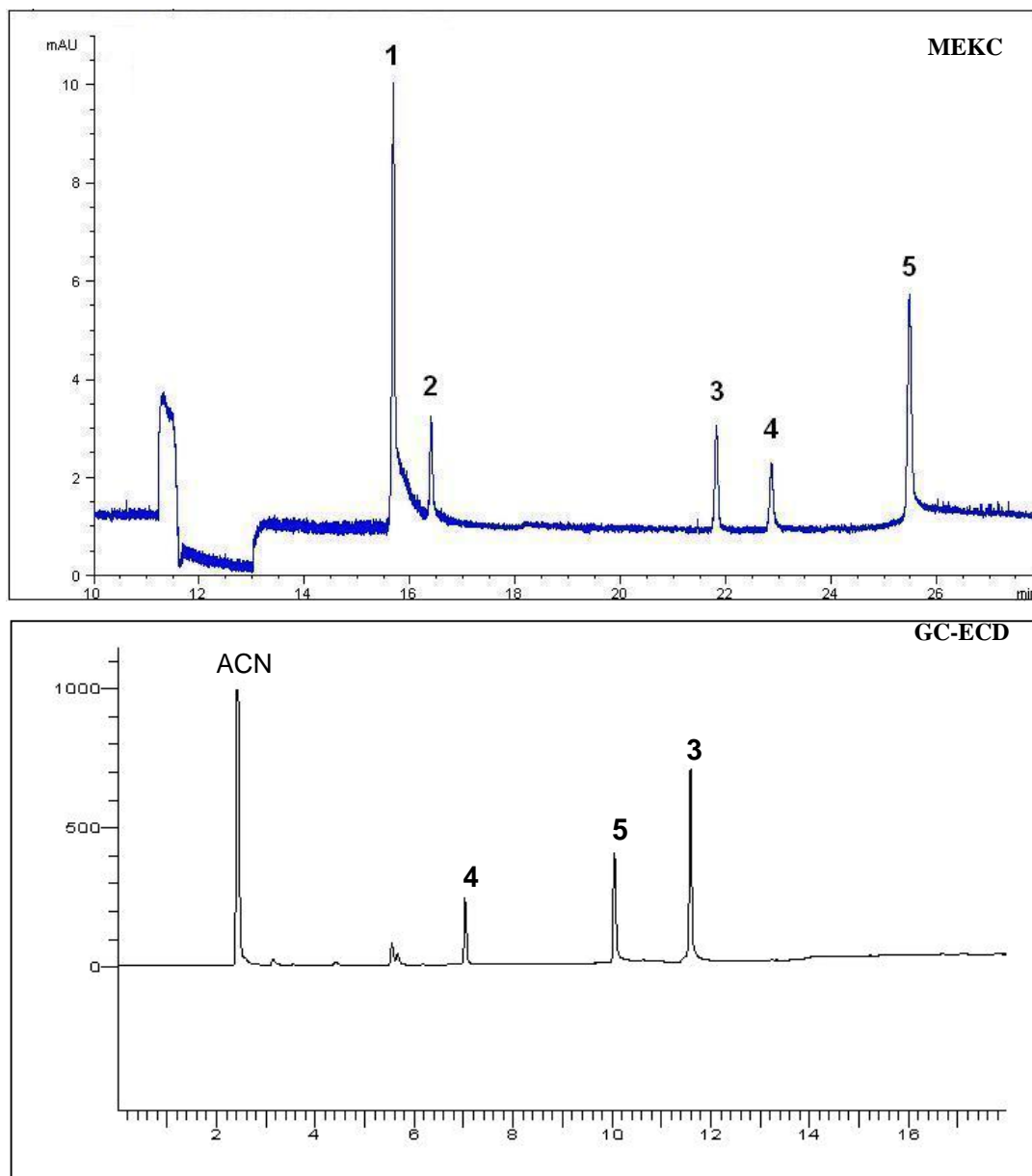


Figure 3: MEKC and GC-ECD separation of a mixture of explosives. For chromatographic conditions, see text. Peak identity: (1) HMX (2) RDX (3) TNT (4) PETN and (5) 2,4-DNT.

The separation order obtained with both methods is not the same, due to the dissimilar separation modes. The quality of the MEKC and GC-ECD separations are readily apparent, as all the compounds are baseline resolved in both methods. The non-aromatic HMX and RDX heterocyclic compounds should be solubilized to a lesser degree within the hydrophobic region created by the micellar aggregates than the nitroaromatic rings and nitrate ester. These theoretical considerations are consistent with the results presented here, which showed HMX eluted first, followed by RDX, then the other nitroaromatics (TNT, 2,4-DNT) and nitrate ester (PETN) which agree with the findings by Kleiböhmer *et al.*, 1993.

### Preconcentration Using SPE system

The analysis of explosives in real samples such as soil, groundwater or seawater, requires the incorporation of a sample preparation step in order to preconcentrate the explosives residue from sample. For explosives, this has been accomplished very efficiently utilizing solid phase extraction [11]. Optimization of SPE method has been studied in detail in previous study [1].

### Real Sample Analysis

#### Point P

Concentration of PETN residues in post blast water samples from collection trays using SPE-DI-SPME/GC-ECD is shown in Table 3.

Table 3: Concentration of PETN in water samples from collection trays using SPE-DI-SPME/GC-ECD.

Sample	Distance from Explosion Point (m)	Concentration of PETN (ppm)
PA1	3	0.020
PA2	4	ND
PA3	5	ND
PB1	3	0.039
PB2	4	0.023
PB3	5	ND
PB4	10	ND
PB5	13	ND
PC	0	ND

As can be interpreted from Table 3, the distance of the point of explosion to the sample collection tray did not show specific relationship with the concentration of the explosives. The concentration of water sample at point PB1 was the highest among the three samples. It was followed by PB2 and lastly, PA1, and undetectable amount of PETN in PC. This phenomenon may be attributed to the explosive distribution pattern in which the soil containing explosives residues would randomly spread from the explosion point during explosion. Most of the mass of the explosive compounds resided in big pieces, resulting in non-symmetrical and non-uniform distribution of explosive residue.

In addition to explosive distribution pattern, the direction of the wind during simulated blasting of PETN also played an important role in this study. There could be a change in direction of the wind during explosion which might blow the soil or debris to other places. Therefore, the residues collected in these sampling trays were very limited, even reached an undetectable level or less than the detection limit of PETN in this study. These two factors could be probable explanations for the failure in detecting traces of PETN residues in water samples at point PA2, PA3, PB3, PB4, PB5 and PC.

#### Point R

Samples at point R were taken at certain time after the explosion took place as listed in Table 4. Post blast water samples from point R were preconcentrated using SPE technique as described previously and was analyzed by MEKC, and the results are presented in Table 5.

Table 4: List of samples collected at Point R

Sample Code	Time collected (min), after explosion
R-1	5
R-2	10
R-3	15
R-4	30
R-5	45
R-6	75

Table 5: Concentrations of RDX found in post-blast water sample at point R, determined with MEKC.

Sample	MEKC		
	R <sup>2</sup>	Conc., (µg/L) of RDX	R.S.D, (%)
R-1	0.9862	156.75	12.76
R-2	0.9922	268.42	16.97
R-3	0.9917	70.80	0.06
R-4	0.9917	51.03	9.35
R-5	0.9847	ND	-
R-M	0.9847	ND	-

Based upon calibration curves, concentration of explosive (RDX) for MEKC analysis were found to range from 0.156 mg/L in the earliest sample collected to 0.051 mg/L in sample collected at 30 minutes after explosion. Sample R-M showed negative result. The concentrations of residues in samples at point R were found to decrease drastically after the sample was taken at 10 minutes. This phenomenon could be best explained using Figure 8 that shows the change in concentration observed with time at which the samples were taken. This might be due to the explosives residues that were carried away by the current flow of the stream where the sampling took place.

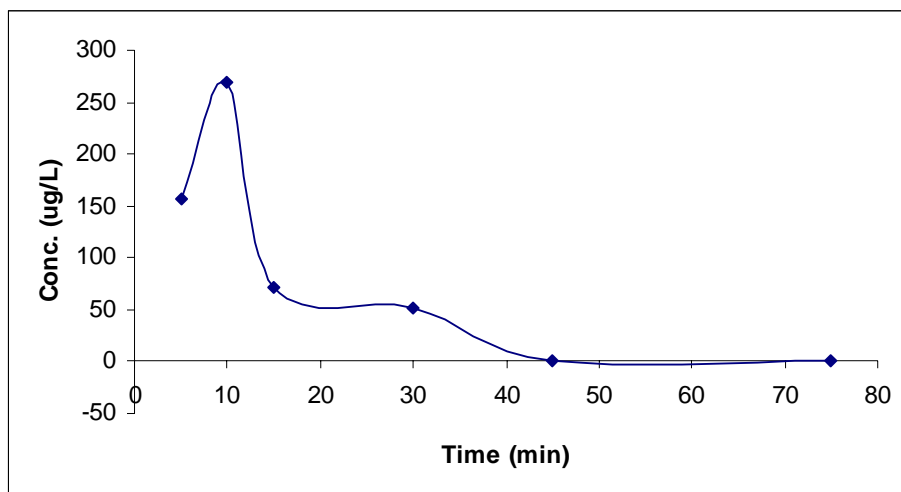


Figure 8: Concentration of RDX with time using MEKC-SPE.

#### Conclusion

The SPE sorbent LiChrolut EN provided a good percentage of recovery (>87%) for all the five analytes studied for MEKC. Concentration enhancement using SPE ranges from 878 times to 1000 times. The MEKC method coupled to SPE was successfully applied to analysis of post blast water sample containing explosives residues. RDX was detected in the water samples in the range of 51.03 – 156.75 µg/L using MEKC. However, better improved limit of detection is needed. Meanwhile, 0.0124 – 0.0390 ppm of PETN was detected in samples at point P using GC-ECD. Hence, SPE preconcentration is crucial for analysis of explosives in post-blast water samples.

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