Determination of 2,4,6 – trinitrotoluene in the soil samples around the Cu Chi Tunnel area by gas chromatography – mass spectrometry

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Abstract

During Vietnam War, nitroaromatic compounds such as 2,4,6-trinitrotoluene (TNT) have been widely used many kinds of explosive which bombed the immense network of connecting underground tunnels in Cu Chi District, Ho Chi Minh City, Vietnam. This study aims to optimize the method of using gas chromatography − mass spectrometry (GC-MS) to analyze TNT and then apply it to determine TNT in soil samples which collected around the Cu Chi area tunnels. In this experiment, ultrasonic was used to extract TNT from the soil samples to Acetonitrile before injecting 1µL sample solution into GC-MS. This method had linear concentration range of TNT from (5.0 to 500) ng/g with limit of detection was 2.0 ng/g, the relative standard deviations of peak area were under 2.0 % and the recovery was from 80 % to 103 %. The results showed that TNT contamination in studied soil samples were not detected.

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Keywords
TNT, soil samples,
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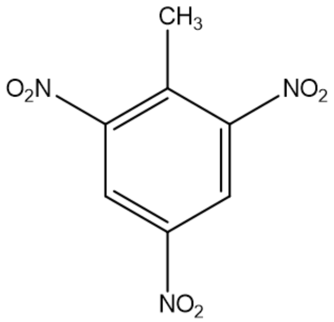
1 Introduction

During Vietnam War, nitroaromatic compounds such as 2,4,6-trinitrotoluene (TNT) have been widely used many kinds of explosive. Probably the most serious environmental problem facing the Vietnam Army today is soil contaminated with munitions residues at throughout the Vietnam. Soils have become contaminated over the last many years by (a) Vietnam War, (b) waste discharges from manufacturing of explosives and propellants, (c) military training. Among them Cu Chi tunnel, the immense network of connecting underground tunnels at Cu Chi District, Ho Chi Minh City, Vietnam, the number of American bombs and bullets dropped about 500,000 tons. On average, each resident suffered 1.5 tons of bombs from 1954 to 1975 [1]. TNT is highly toxic, carcinogenic to human and contaminate the environment. It can diffuse and quite mobile in water and soil sources. [2, 3]. When TNT is released into the environment, the processes of adsorption, biodegradation and photochemical decomposition of such contaminated soil are complex [4]. So, an analytical method combining quick and accurate extraction, identification, and quantitation of TNT at µg/g level in the soil is required. The method must be precise enough to allow quantification with a minimum number of replicate determinations. It must also be accurate over the range of concentrations measured to enable a decision when need to address the post-war consequences of bomb and mine [5]. Most previous methods for determining TNT in environmental matrices rely on chromatography. Liquid chromatography (LC) and gas chromatography (GC) – mass spectrometry (MS) are the techniques of choice for detection TNT in soil due to high sensitivity and reliability [6,7,8,9]. The known thermal instability of TNT makes LC a better choice for routine analysis than GC. The LC-MS limited to standard spectrum library database so the difficult to screen for TNT and its biological and photochemical degradation products. Meanwhile, the GC-MS has a big standard library database that can make the screening of TNT and degradation products easier [10]. TNT strongly adsorbs and forms many types of bonds with components in the soil sample [11, 12]. In the past, the Soxhlet extraction was commonly used for the extraction of TNT from the sample matrix in 48 h with MeOH solvent [13]. This method was time consuming and required an enormous amount of solvent in the extraction process. TNT can be extracted ultrasonically with acetonitrile for 18 h under cold conditions according to EPA 8330 B method [14]. This method gives the TNT recovery efficiency of nearly 100 %, but the continuous ultrasonic extraction for 18 h with high intensity of sound waves can affect humans and the working environment.

The objective of this research was to develop a method that could be used to determine the concentration of TNT in soil. The method should be suitable for commercial use by contractors analyzing large numbers of soil samples from Vietnam military installations which could be contaminated with TNT.



2,4,6-Trinitrotoluen (TNT)

Fig. 1 Structure of TNT

Table 1 Physical and Chemical Properties of TNT [2]

Physical Description (physical state at room temperature)	**Yellow, odorless solid**
Molecular weight (g/mol)	227
Water solubility (mg/L at 25 °C)	130
Octanol-water partition coefficient (KOW)	1.6
Soil organic carbon-water coefficient (KOC)	300
Boiling point (°C)	240 (Explodes)
Melting point (°C)	80.1

2 Materials and methods

2.1 Chemicals

Extraction solvents were optima-grade acetonitrile (ACN), acetone, dichloromethane (DCM) and methanol (MeOH) (Schalaur, Spain). Standard 2,4,6 Trinitrotoluene (TNT) was obtained from the ministry of defense (Vietnam) − the general department of engineering − 14 mechanic and chemicals one member limited liability company. The standard analyte solution was prepared from TNT at a concentration of 1.0 mg/mL in ACN. Subsequent dilutions were made in the same solvent. All standard solutions were held at 4 ºC. The internal standard (IS), triphenyl phosphate (TPP) was purchased from Merck (Germany). The IS solution was 100 ng/mL TPP in ACN.

2.2 Instruments

All the extracts were analyzed with an Agilent 7890 A GC (USA) interfaced with an Agilent 5975 C MS (USA). A DB-5Ht capillary column was used, with dimensions of 30 m length, 0.25 m i.d., and 0.25 µm film thickness. The injection port liner was held at 250 ºC. The GC-MS transfer line and ion-source temperatures were 250 °C and 230 ºC, respectively. The GC oven temperature started at 90 ºC for 1 min, was ramped at 20 ºC/min to 140 ºC, then at 6 ºC/min to 200 ºC. The final temperature (200 ºC) was held for 6 min. Helium flow was 1.0 mL/min. The MS was operated in electron ionization (EI) and SIM modes. The total analysis time to complete GC-MS run was approximately 15 min/sample.

2.3 Analytical procedures

2.3.1 Soil samples

The soil samples were collected at sites around Cu Chi tunnel area in Cu Chi District, Ho Chi Minh City, Vietnam. They were stored in glass bottle on ice until arrival at the laboratory, where they were stored at –20 ºC until preparation and analysis. Reference samples, uncontaminated soil from the same series, were collected in the same manner in an area outside the Cu Chi tunnel. All soil samples were air-dried for 24 h, sieved through a #10 metal sieve (opening size 2.00 mm), and stored in clean glass bottle prior to extraction. A 2 g portion of each sample was reserved for moisture content determination.

2.3.2 Fortification procedure

The blank sample was analyzed to determine the background level of the TNT. It showed no presence of the TNT (below level of LOD was 16 ng/g). The soil samples (200 g) were fortified with a stock of standard chemicals in 150 mL of ACN and were homogenization with ah food processor. After the solvent was evaporated with a rotary evaporator, the soil could sit in a fume hood until dried at room temperature. The fortified soil was then stored and used for analysis. The spiking level was 50 ng/g for analyte. All the fortification samples were in triplicates.

2.3.3 Extraction procedure

Accurately weigh 2 g was extracted with 20 mL ACN in a 50 mL centrifuge tube. After immersing stand overnight, sonicated for 3 h at 20 °C. The sample was added powder $CaCl_2$ 0.2 % w/v and separated from suspension by centrifuging at 5,000 rpm for 10 min. The extraction was repeated one times in the same procedure. ACN was removed from the extract by a rotary evaporator and the dried residue was dissolved in 1 mL of the IS solution for GC-MS analysis.

3 Results and discussion

3.1 GC-MS Conditions

TNT has a high boiling point and can thermal decomposition at high temperatures then need controlled during the analysis [15]. The TNT standard solution is injected into the GC-MS system and the signal is recorded in scan mode. The EI mass spectra got are searchable in commercial MS libraries that are a precious tool for identifying unknowns. On the chromatogram showed that TNT eluted at 7.9 min (Figure 2) and no other peaks appeared in the chromatogram. The quantitative ion was m/z 210 and the qualitative ions were m/z 164, 180 and 193.

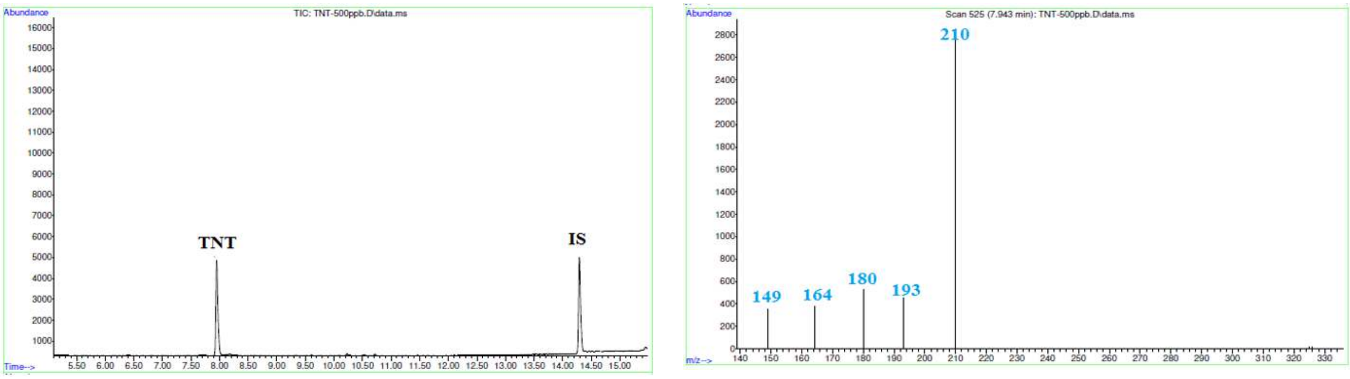


Fig. 2 GC-MS full scan chromatogram of TNT and spectrum

3.2 Extraction

3.2.1 Investigation of extraction solvents

The 15 samples collected around in the Cu Chi tunnel area were screened for TNT according to the optimized sonication method. Before extraction, all samples were manually cleaned of debris such as rocks, root balls, and metal pieces. Soil is a heterogeneous assembly of biotic and abiotic components. The mechanisms which bind not completely understood, but one can speculate that they may include contributions from Van der Waals interactions, hydrogen bonding, electrostatic attraction through exchangeable cations and a degree of covalent bonding through silanol esters.

Because of the inhomogeneity of soils and the variety of binding mechanisms that appear to be involved, an extracting solvent must perform several functions. First it must be capable of displacing analyte from high-energy binding sites. It should also maintain the soil organic matter in an open structure in which imbibed organics can diffuse. The extractant must provide the analyte with adequate solubility so that any equilibrium partitioning between the solvent and the soil organic matter will be strongly in favor of the extracting solvent.

For these functions, a relatively polar organic solvent is expected to be optimum for TNT. In this research, we evaluated acetone, CAN, DCM and MeOH for extraction of TNT from fortified soil and sonicated in 60 min prior to GC-MS determination. The results (Figure 3) show that polar solvents such as ACN, MeOH give higher extraction efficiency than less polar solvents, but the extraction efficiency is still low and needs further optimization.

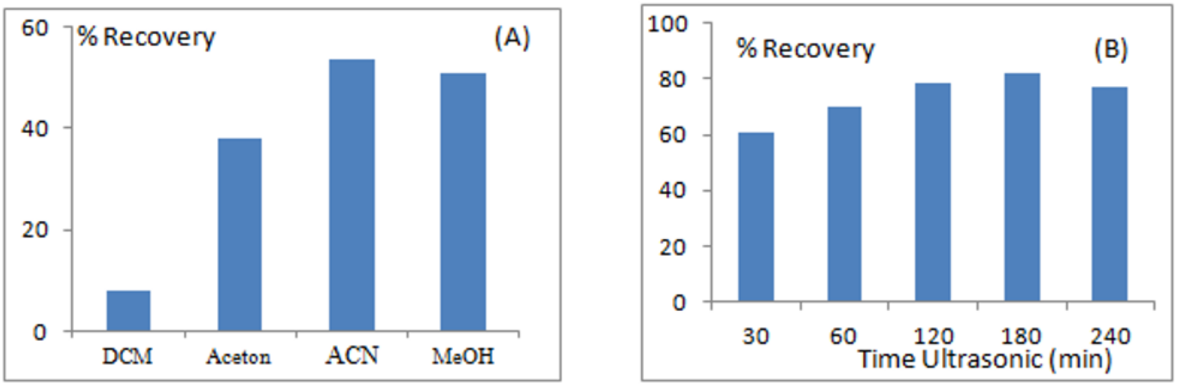


Fig. 3 Extraction solvents (A) and the ultrasonic extraction time (B)

3.2.2 Optimization of Sonic Bath Extraction

According to the extraction process of TNT from the soil sample of U.S. EPA 8330 B, the sample was extracted by ultrasonic for 18 hours at a cold temperature of 6 °C and achieved a recovery efficiency of nearly 100 %. But the longtime of ultrasonic could affect to the laboratorial environment. In this research, soil samples were extracted with an immersed overnight in organic solvents and investigated the ultrasonic extraction time to evaluate the recovery efficiency in order to minimize the time ultrasonic. Seven sub-samples of soil were processed using ACN. An amount of 2 g sub-sample of each soil was weighed out to the nearest 0.01 g and transferred to a 50 mL centrifuge tube. Aliquots of 20 mL of each solvent were added to each test tube, and the soil was immersed in the solvent overnight. After that, one-milliliter aliquots were removed for analysis after 30 min, and (1, 2, 3 and 4) h in the sonic bath. Experimental results showed that 3 h was the best time for sonic bath extraction. To enhance the extraction efficiency, the extraction was repeated one time in the same procedure.

3.3 GC-MS Validation

EI mass spectra have the advantage of being searchable for standard mass spectra in a commercial database. So, the GC-MS method used for the quantitation TNT content and evaluates the ability to analyze and detect its biological and photochemical degradation products in the soil sample to serve the research team's next research directions. The GC oven temperature started at 90 °C and solvent delay time at 5.5 min to minimize the MS system's contamination.

Linear concentration range: Inject standard solutions of TNT in the concentration range from (5 to 500) ng/mL and record chromatograms under optimal conditions. The calibration curve equations and correlation coefficients are showed in (Table 2).

Accuracy (% RSD): Perform 6 replicates sub-sample of soil containing TNT at a concentration of 50 ng/g and record the chromatograms. The accuracy of the method was assessed through the RSD value (Table 2).

Limit of detection (LOD) and limit of quantitation (LOQ): Record 7 chromatograms of the fortified sample containing TNT concentration at 50 ng/mL, the LOD and LOQ values are calculated by the formula [15]:

$$\text{LOD} = t_{0.99,f=7} \times \text{SD}\ ,\ \text{LOQ} = 10 \times \text{SD}$$

Where SD is the standard deviation of the ratio of the analyte signal to the internal standard signal and $t_{0.99,f=7}$ is the Student's *t*-value appropriate for the single-tailed 99 percentile *t*-statistic and a standard deviation estimate with $n - 1$ degrees of freedom [16]. The calculated LOD and LOQ values are shown in the Table 2 below.

Field samples

The 15 samples collected around Cu Chi tunnels area were screened for TNT according to the optimized method described previously. The detection and identification were done with the GC-MS system. Table 3 shows the concentrations got for the samples. the recovery was from 80 % to 103 %. The results showed that TNT contamination in studied soil samples were not detected.

Table 2 GC-MS validation results

Rt (min)	Concentration range (ng/mL)	Calibration Curve	R^2	%RSD	Recovery efficiency (%)	SD	LOD (ng/g)	LOQ (ng/g)
7.943	5.0-500	$Y = 0.0008X - 0.0126$	0.9975	7.1	80-103	5.1	16	51

Table 3 Concentrations of TNT in the Soil Samples

Nº	Sample	Results (ng/g)	
		Cu Chi Tunnel	Recovery (%)
1	QTTB-CC-OB1-30	*ND	80.2
2	QTTB-CC-OB1-60	ND	83.0
3	QTTB-CC-OB1-90	ND	86.0
4	QTTB-CC-OB1-120	ND	82.1
5	QTTB-CC-OB1-150	ND	88.6
6	QTTB-CC-OB2-30	ND	103.1
7	QTTB-CC-OB2-60	ND	99.2
8	QTTB-CC-OB2-90	ND	89.3
9	QTTB-CC-OB2-120	ND	86.8
10	QTTB-CC-OB2-150	ND	101.2
11	QTTB-CC-OB3-30	ND	99.0
12	QTTB-CC-OB3-60	ND	91.6
13	QTTB-CC-OB3-90	ND	86.1
14	QTTB-CC-OB3-120	ND	90.2
15	QTTB-CC-OB3-150	ND	99.0

* ND: Not detected

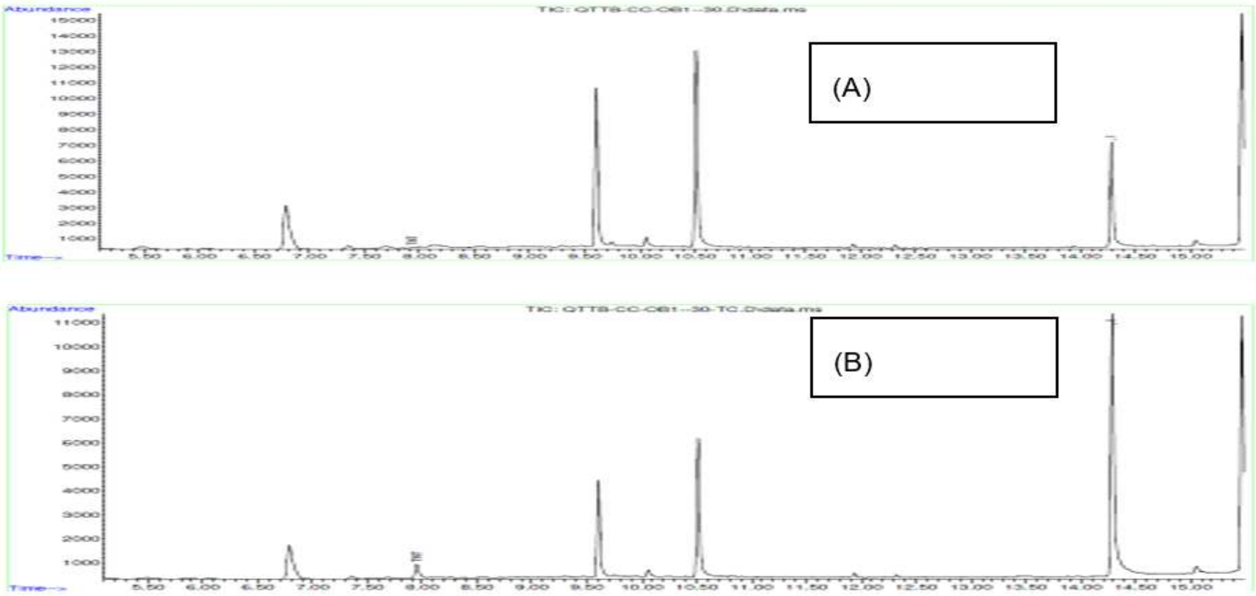


Fig. 4 The chromatograms of the soil sample (A) and the spiked soil samples (B)

4 Conclusion

In this study, the extraction method using the ultrasonic extraction for TNT in the soil sample showed high efficiency and stability. The GC-MS methods can determine TNT at trace levels in soil samples. Soil samples collected around the Cu Chi tunnels did not detect TNT, which is a positive signal for human health and the environment in this area. With this result, the research direction can be extended to the screening of TNT and its degradation products in groundwater samples or water sources, pond sediments in the areas around the Cu Chi tunnels, in order to overall assessment to prevent contamination of TNT to the environment and public health.

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Xác định 2,4,6–trinitrotoluen trong các mẫu đất xung quanh khu vực địa đạo Củ Chi bằng phương pháp sắc kí khí – khối phổ

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Tóm tắt Trong chiến tranh Việt Nam, các hợp chất nitroaromatic như 2,4,6–trinitrotoluene (TNT) đã được sử dụng rộng rãi trong nhiều loại chất nổ dùng để đánh bom mạng lưới rộng lớn kết nối địa đạo Củ Chi, Thành phố Hồ Chí Minh, Việt Nam. Nghiên cứu này nhằm tối ưu hóa phương pháp sử dụng phương pháp sắc kí khí − khối phổ (GC-MS) để phân tích TNT và sau đó ứng dụng để xác định TNT trong các mẫu đất lấy xung quanh khu vực địa đạo Củ Chi. Trong thí nghiệm này, sóng siêu âm được sử dụng để tách TNT từ mẫu đất sang Acetonitril trước khi bơm 1 µL dung dịch mẫu vào GC-MS. Phương pháp này có dải nồng độ tuyến tính của TNT từ (5-500) ng/g với giới hạn phát hiện là 2 ng/g, độ lệch chuẩn tương đối của diện tích peak nhỏ hơn 2 % và độ thu hồi từ (80-103) %. Kết quả cho thấy không phát hiện ô nhiễm TNT trong các mẫu đất nghiên cứu.

Từ khóa TNT, mẫu đất, địa đạo Củ Chi