Trace Analysis of Explosives in Soil: Pressurized Fluid Extraction and Gas and Liquid Chromatography–Mass Spectrometry

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Abstract

Soil samples are collected from the former Open Burn/Open Detonation Unit, Makua Military Reservation, on the island of Oahu, Hawaii. The soil is the Helemano series. The soil samples are fortified with eight explosives for development of the analytical method. These analytes are 2-amino-4,6-dinitrotoluene; 1,3-dinitrobenzene; 2,4-dinitrotoluene (DNT); hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX); nitrobenzene (NB); octogen; 1,3,5-trinitrobenzene; and 2,4,6-trinitrotoluene. The analytes are recovered with pressurized fluid extraction and measured with liquid chromatography (LC), LC–mass spectrometry (MS), and gas chromatography–MS. Average recoveries of the seven analytes, except for NB, range from 67% to 110% from freshly fortified samples. The procedure fails to extract NB in soil. The average recoveries decrease from 67–110% to 41–81% as the soil is aged for 1 day to 6 months after fortification of the soil with the seven explosives. The field samples are analyzed for the presence of explosives, of which DNT and RDX are indeed detected. The results obtained with this procedure agree well with those obtained by an independent laboratory following the standard U.S. Environmental Protection Agency (EPA) method SW-846 8330. Compared with the EPA method, this new method provides MS confirmation of the analytes, and the extraction requires approximately 15 min, rather than 18 h by the EPA method.

Introduction

Nitroaromatic compounds such as 2,4-dinitrotoluene (DNT), 1,3-dinitrobenzene (DNB), hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX), octogen (HMX), 1,3,5-trinitrobenzene (TNB), and 2,4,6-trinitrotoluene (TNT) have been widely used in bombs and ammunitions (Figure 1). As a result, concentrations of these compounds in many training grounds may exceed acceptable levels in soil and water (1), thus, studies were done to determine the fate of such compounds in soil (2). Incineration of explosive-contaminated soil is an effective remediation method, but laborious and costly. Bioremediation (3–6) and phytoremediation (7,8) of such contaminated soil are being studied as a viable alternative method, where bacterial degradation contributes significantly to remediation (9,10). An analytical method combining quick and accurate extraction, detection, identification, and quantitation of these compounds at µg/g levels in soil is thus needed to support those studies.

The work described in this paper is the first part of a phytoremediation project being conducted at the Open Burn/Open Detonation (OB/OD) unit in the Makua Military Reservation, on the island of Oahu, Hawaii. The soil samples for testing the analytical methods were obtained from the Makua OB/OD site. The conventional method for extraction of explosive residues from soil is tedious and time consuming, involving sonication for 18 h (11). Soxhlet extraction (SE) was used for recovery of RDX and TNT, but SE is also time consuming (often as long as 48 h) and requires the use of a significant amount of organic solvent, with 200 mL/10-g sample (12). Pressurized fluid extraction (PFE) was used to successfully extract HMX and RDX from sediment (13) at levels as low as 0.5 µg/g. It has the advantage of using a reduced amount of organic solvent (20–30 mL/sample) and being fast (12–20 min/sample). Solid-phase microextraction also proved to be an adequate extraction method in the cases of contaminated water (14). Liquid chromatography (LC) with UV detection and gas chromatography (GC)– mass spectrometry (MS) are the techniques of choice for detection and identification (12,15–17). Our work focused on optimization of PFE parameters in conjunction with LC, LC–MS, and GC–MS for the detection and identification of the analytes of interest.

Experimental

Chemicals

Extraction solvents were optima-grade acetonitrile and methanol (Fisher Scientific, Pittsburgh, PA). The standard ana-

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lyte solution was a stock mixture purchased from Accustandard (New Haven, CT), containing the eight analytes (Figure 1) each at a concentration of 1.0 mg/mL in methanol–acetonitrile (1:1). Subsequent dilutions were made in the same solvent. All standard solutions were held at 4ºC.

Soil

The soil used in this study is referred to as Helemano series silty clay and classified as Rhodic eutrustox, very fine, kaolinitic isohyperthermic. A pH value of the soil, as measured in a 1:1 ratio (w/v) of soil–water, was 5.26. Its organic carbon content was 3.98%. All soil samples were air-dried for 24 h, sieved through a #10 metal sieve (Tyler equivalent 9 mesh, opening size 2.00 mm), and stored in clean glass jars prior to extraction. A 20-g portion of each sample was reserved for moisture content determination. Ottawa sand 20–30 mesh (Fisher Scientific) was used to initially assess the PFE performance and as an inert solid matrix for PFE extractions.

Sampling site

The soil samples were collected at a known detonation site in the OB/OD unit, nearby a road. They were stored in glass jars on ice until arrival at the laboratory, where they were stored at –20ºC until preparation and analysis. The sample holding time was less than two weeks. Reference samples, uncontaminated soil from the same series, were collected in the same manner in an area outside the OB/OD.

Fortification procedure

The soil samples were dried and sieved through a metal sieve (4-mm opening size) before the fortification. The blank sample was analyzed to determine the background level of the analytes. It showed no presence of the target chemicals. The soil samples (200 g) were fortified with a stock mixture of standard chemicals in 100–150 mL of methanol–acetonitrile (1:1, v/v) and were thoroughly mixed. After the solvent was evaporated with a rotary evaporator, the soil was allowed to sit in a fume hood for 24 h at room temperature. The fortified soil was then stored at 2–4ºC for later use. A portion of this spiked soil was set aside and aged for 60 and 180 days at room temperature (25ºC ± 2ºC) prior to extraction. The spiking level was 2.5 µg/g for each analyte. Also, the same level of the explosives was spiked directly on top of the Ottawa sand in the extraction cells as a control for the extraction processes. All the fortification samples were in triplicates.

Extraction procedure

A Dionex ASE 200 extractor (Salt Lake City, UT) was used for all extractions. The bottom of each 22-mL extraction cell was fitted with two cellulose filters covered with 2–3 cm^3 inert Ottawa sand. Each sample was mixed with 1–5 g of Ottawa sand as a dispersion agent and was loaded into the cell. The sand was used to fill up the cell. The cell was loaded onto the ASE 200 extractor. An extraction cycle began with the filling of the cell with a mixture of methanol–acetonitrile (1:1, v/v), then a 5-min preheating time, followed by a 5-min static extraction. The extract was flushed out of the system with a 60-s nitrogen purge into a glass collection vial. The sample extract (~ 30 mL) was concentrated with a rotary evaporator and brought to 4–5 mL for GC–MS or LC–MS determination.

GC–MS

All the extracts were analyzed with a Varian (Walnut Creek, CA) CP 3800 GC interfaced with a Varian Saturn 2000 MS and a Varian autosampler CP 8400. A DB-1 capillary column (J&W Scientific, Folsom, CA) was used, with dimensions of 10-m length, 0.18-mm i.d., and 0.4-µm film thickness. It is necessary to use a shorter column (10 m, rather than 30 m) with a thicker film (0.4 µm, compared with 0.25 µm) to minimize degradation of the very active analytes during separation processes. The injection port liner was the Silchrom type (Restek, Bellefonte, PA) and held at 210ºC. The GC–MS transfer line and ion-trap temperatures were

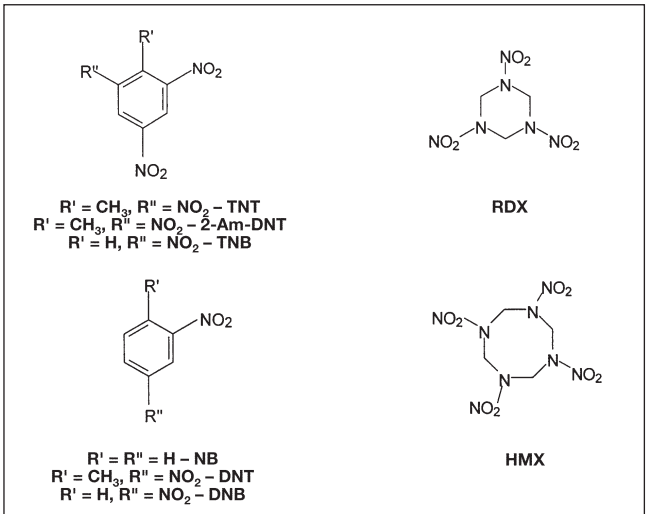

Figure 1. Structures of explosives.

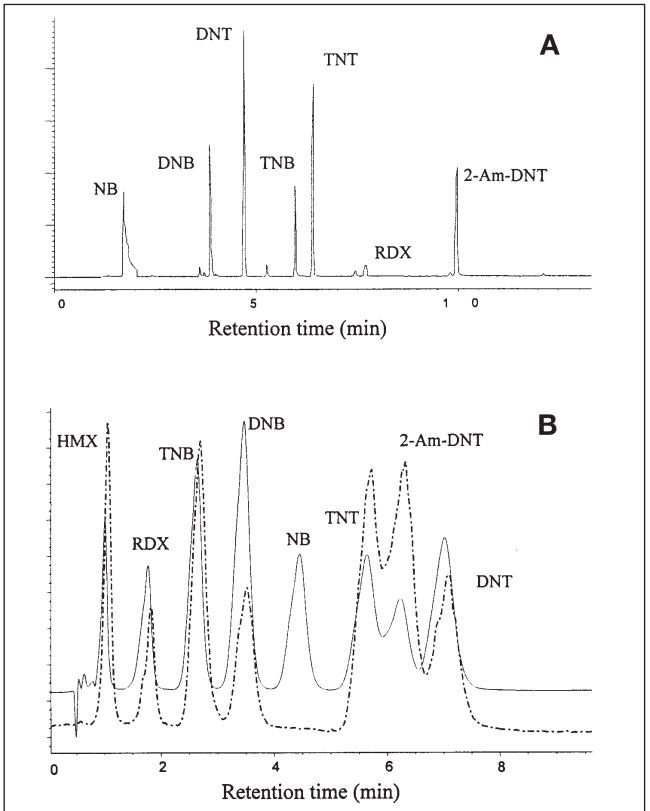

Figure 2. GC–MS full scan chromatogram of explosives (A) and HPLC–DAD (—) (254 nm) and MS (--) full scan chromatogram of explosives (B).

200°C and 210ºC, respectively. The GC oven temperature started at 80ºC for 1 min, was ramped at 20ºC/min to 140ºC, then at 6ºC/min to 200ºC. The final temperature (200ºC) was held for 16 min. The helium flow was 1.4 mL/min. The MS was operated in electron ionization (EI) and full scan modes to monitor a 100–650-amu range. The total analysis time from extraction to complete GC–MS run was approximately 40 min/sample.

LC–MS

An Agilent 1100 series LC (Wilmington, DE) equipped with an autosampler, diode array detector (DAD), and MSD SL model MS was used for the analysis. The column was a Hewlett-Packard Zorbax SB-C_{18} (narrow bore 2.1 cm × 150 mm × 5 µm) held at 44ºC (Palo Alto, CA). The injection volume was 10 µL set with a methanol needle wash. The flow rate was 0.7 mL/min. The mobile phase was gradually changed from 75:25 to 65:35 of water–methanol in 15 min, then back to 75% water–25% methanol at 20 min, which was held for 5 min. The elution was monitored at 254 nm, as well as with a full UV spectrum. The MS was operated with the atmospheric pressure chemical ionization (APCI) source in negative mode for a mass range of 100–400 amu. The fragmentor was set at 70 V, the nitrogen (5 L/min) temperature at 350ºC, the vaporizer at 350ºC, and the nebulizer pressure was 60 psi. The capillary voltage was 4000 V and the corona current 25 µA.

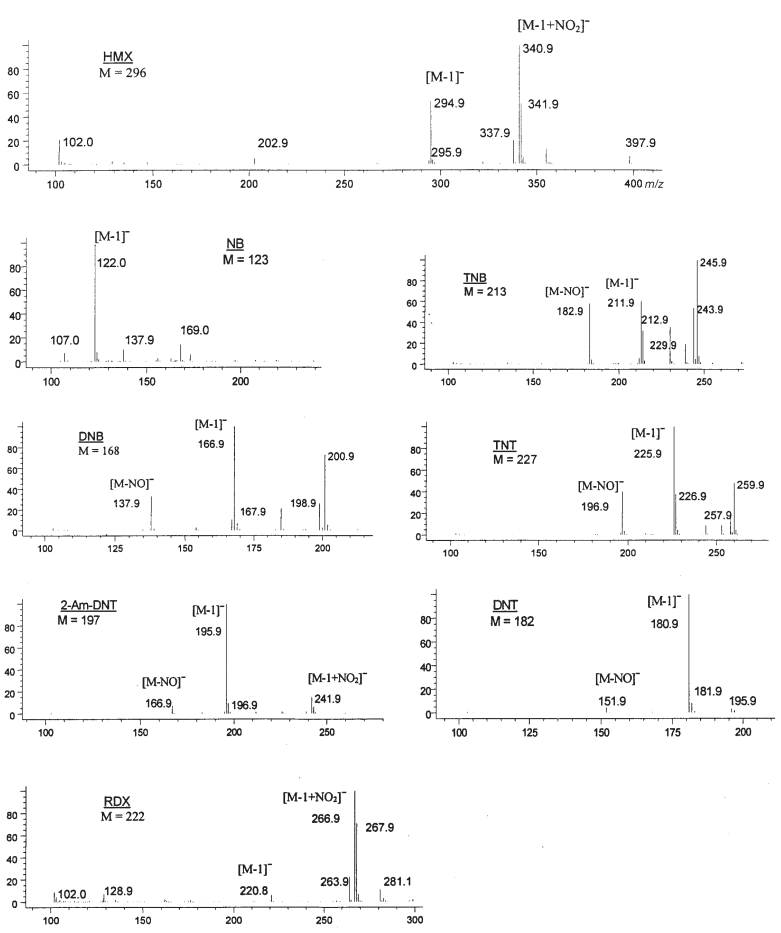

Figure 3. LC–MS spectra of explosives (APCI, negative mode, full scan).

Safety precautions

Care should be taken when standard stock solutions of the explosives are handled. Also, soils suspected to have high levels of munitions residues (> 100 ppm) should not be ground.

Results and Discussion

Comparison of GC–MS, HPLC–DAD, and LC–MS for the detection of explosives standard solution

GC–MS was compared with LC–MS for the analysis of explosives. The thermal lability and high reactivity of explosives make high-performance liquid chromatography (HPLC)–DAD a conventional method of analysis for explosives (11). This technique does not, however, allow for identification of analytes other than by absorbance spectral match and comparison of retention times with those of standard compounds. GC–MS procedures were developed, which enable solid confirmation of identity by MS analysis (16–17). Figure 2A shows a GC–MS chromatogram of a mixed-standard solution of eight explosives each at 10 µg/mL. The EI mass spectra obtained are searchable in commercial MS libraries that are a very valuable tool for identifying unknowns. It is noted that HMX was always absent from the GC chromatogram, with decomposition in the GC injection liner or in the ion trap as a very probable cause.

LC–MS offers the advantage of HPLC giving minimal thermodegradation of the explosives during chromatographic separation and of MS, producing mass spectra for identification. Figure 2B shows the superimposed chromatograms of UV at 254 nm and APCI–MS for the same 10-µg/mL standard solution. Nitrobenzene (NB), at this level, was detected by UV, but not by MS. When more concentrated NB solutions were injected into LC–MS, it was detected only above 1000 µg/mL. It is suggested that NB vaporizes quickly in the hot ion source and spreads as a gas in the source. Combined with a low ionization efficiency of the compound, this may explain the nondetection of NB by the MS. Figure 3 shows the normalized spectra of all eight compounds. HMX, RDX, and 2-amino-4,6-dinitrotoluene (2-am-DNT) showed an ion of $[M-1]^-$, as well as an adduct $[M-1+NO2]^-$ formed in the ion source during fragmentation. TNB, DNB, TNT, DNT, and 2-am-DNT showed an ion of $[M-1]^-$, as well as a fragment $[M-1-NO]^-$.

EI mass spectra have the advantage of being searchable for standard mass spectra in commercial databases, whereas APCI mass spectra do not. A user library was created that contained the spectra of each analyte obtained by LC–MS, along with information such as mobile phase, flow, and MS conditions. Once established, the user library can be searched for unknown samples for the detection of the explosives. The LC–MS–DAD system was used to quantitate all of the extracts.

Extraction efficiency and solvent optimization

Ottawa sand fortified with the explosives was first used to estimate possible losses of each analytical step and chemical stability of the analytes at the extraction conditions. All analytes, except for NB (55%), have had average recoveries of 90–120% (Table I) with standard deviations in a range of 3–11%. The results showed that these analytes, except for NB, are stable at the PFE condition and not lost in this step. Rotary evaporation of pure NB dissolved in methanol–acetonitrile (1:1, v/v) showed no loss of NB from rotary evaporation processes.

The PFE extraction conditions were then optimized for Helemano soil spiked at a 2.5-µg/g level. Because of the thermally unstable nature of the explosives, the extraction temperature was held at 100ºC to minimize thermal decomposition but retain the extraction efficiency. The static extraction time was set at 5 min. Previous studies showed that pressure does not greatly influence extraction efficiencies (18,19), so it was held at 1500 psi. The extraction efficiency was investigated with the following solvent: acetonitrile–methanol (1:1, v/v) premixed in one bottle ("premix") and acetonitrile and methanol in two separate bottles pumped and mixed with the PFE ("flowmix"). Premixed acetonitrile–methanol gave, overall, the best results with average recoveries ranging from 67% to 110% and the lowest standard deviations varying from 0% to 14% (Figure 4). However, NB was not recovered. Also, there was a nonnegligible difference between extraction with one bottle (premix) and two bottles (flowmix) for that solvent mixture. When the solvents were not premixed, the standard deviations increased from a range of 0.3–13% (average 7%) for the premix, to 4–28% (average 12%) for the flowmix. NB was recovered only from the Helemano soil in the instance of methanol extraction for a recovery of 18 ± 2% compared with 55 ± 11% from the freshly spiked sand. To summarize, the optimal extraction conditions were chosen to be 100ºC, 1500 psi, a solvent mixture premixed of methanol–acetonitrile in equal volumes, and one extraction cycle (30 mL solvent) for 10 min.

Hawaiian soils, particularly from the Wahiawa series, tend to strongly bind with organic analytes. It is often quite challenging to obtain good recoveries, like in the case of NB. Several studies (20,21) showed that premixing the soil sample before extraction with Na_4EDTA can dramatically improve the recoveries of carboxylic acids and phenol classes of chemicals. This possibility was investigated here by mixing the soil with 5% (w/w) Na_4EDTA and 15% (w/w) deionized water, but the presence of Na_4EDTA did not improve the recoveries for this type of analyte.

Table I. Recoveries of Eight Explosives from Ottawa Sand and Helemano Soil

	%Recovery*			
Analyte	Ottawa sand	Soil, 1 day†	Soil, 2 months†	Soil, 6 months†
2-am-DNT	120 ± 7	110 ± 13	127 ± 3	78 ± 2
DNB	90 ± 7	67 ± 14	63 ± 6	41 ± 2
DNT	91 ± 7	69 ± 13	63 ± 4	42 ± 1
HMX	111 ± 3	107 ± 5	105 ± 2	81 ± 2
NB	55 ± 1	0 ± 0	13 ± 2	0 ± 0
RDX	112 ± 4	96 ± 0	82 ± 3	76 ± 4
TNB	107 ± 5	88 ± 3	76 ± 2	68 ± 1
TNT	99 ± 7	74 ± 5	47 ± 2	54 ± 1

* Extraction conditions were: 100°C, 1500 psi, 5 min static extraction, premixed acetonitrile–methanol (1:1, v/v).
† Helemano soil was spiked at 2.5 µg/g of each of the eight analytes and kept at room temperature in the dark.

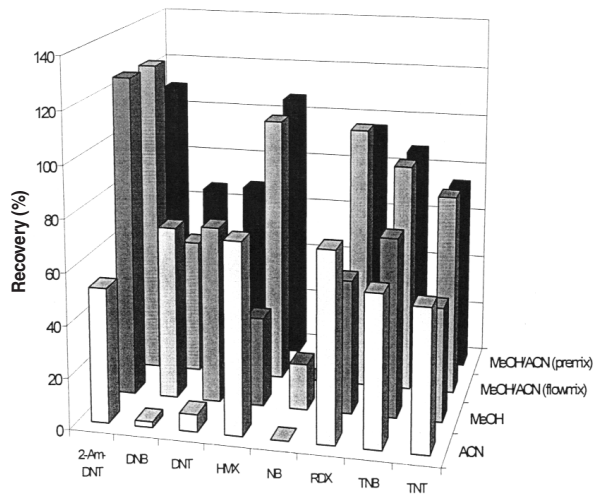

Figure 4. Solvent optimization for PFE of explosives fortified at 2.5 mg/g in Helemano soil. PFE conditions were 100°C and 1500 psi. MeOH = methanol, ACN = acetonitrile.

Aged spiked samples

In order to determine the influence of aging on the adsorption of the analytes to the Helemano soil, some soil was spiked at 2.5 µg/g of each analyte and incubated at room temperature in glass jars in the dark. Samples were analyzed after 1 day, 2 months, and 6 months. Aging tended to decrease the amounts recovered from the soil (Table I). With an exception of NB, average recoveries of the other explosives varied from 41% to 81% after 6 months, compared with 67% to 110% after 1 day. Analytes appear to be more tightly bound to the soil after a long period of time, or to have suffered degradation.

Table II. Comparison of Concentrations of RDX and DNT in the Soil Samples Determined by Two Independent Laboratories

Sample Location	RDX (µg/g) This laboratory	RDX (µg/g) SAS*	DNT (µg/g) This laboratory	DNT (µg/g) SAS*
1	0.1	0.1	0.2	0.1
2	0.4	0.3	0.3	0.1
3	0.8	0.5	0.2	0.1
4	ND	ND	0.3	0.1
5	ND	ND	ND	160
6	ND	2.1	0.1	ND
7	ND	ND	ND	ND
8	0.1	0.1	0.2	0.05
9	7.2	8.4	ND	ND

* SAS, Sound Analytical Services Laboratory, following EPA method SW-846 8330.
† ND, not detected.

Field samples

The nine samples collected in the OB/OD were screened for the presence of explosives according to the optimized PFE method described previously. The detection and identification were done with the HPLC–DAD–MS system. Before extraction, all samples were manually cleaned from debris such as rocks, root balls, and metal pieces. Table II shows the concentrations obtained for the samples. The limit of quantitation varied from 0.05 µg/g for 2-Am-DNT to 0.3 µg/g for TNT and HMX. It is noted that three of the samples contained HMX. Samples 3, 8, and 9 had 0.5, 0.3, and 1.5 µg/g of HMX, respectively. These samples were also analyzed by an independent commercial laboratory following EPA method SW-846 8330 (10). These two sets of results agreed well except for one sample, which showed 160 µg/g of DNT by the commercial analysis compared with a nondetectable level by our analysis. This discrepancy may be attributed to uneven distribution of munitions residues in the soil sample. All the explosive levels were found to be below the EPA limit values in soil.

Conclusion

The results showed that this is an improved extraction and identification method for trace analysis of explosive residues in soil. The soil samples were extracted with 1:1 volume of premixed methanol–acetonitrile at 1500 psi and 100ºC. The extracts were analyzed with HPLC–DAD, LC–MS, and GC–MS for detection and identification. The chemicals that showed 67–110% of average recoveries were 2-Am-DNT, DNB, DNT, RDX, HMX, TNB, and TNT. It should be noted that the procedure is not suitable for NB in soil. The advantages of the method include short analysis time, reduced amount of extraction solvents, and improved extraction efficiency for the above seven analytes. Some soil samples were collected from a training ground in the Makua military reservation, Oahu, Hawaii. These samples were analyzed by two laboratories for explosives. DNT and RDX were detected in these samples and showed comparable results, except one sample, from the two laboratories.

Acknowledgments

The work was supported in part by a contractual agreement with the State of Hawaii Department of Health (Office of Hazard Evaluation and Emergency Response), agreement with U.S. DOD (920150-002), U.S.-EPA award (X989512-01-1), and USDA-TSTAR award (0034135-9576). We thank Ron Honnold (Varian Inc.) and David Presser (Agilent Technologies) for their technical assistance.

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Manuscript accepted May 19, 2003.