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Development and validation of a solid phase extraction sample cleanup procedure for the recovery of trace levels of nitro-organic explosives in soil

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ABSTRACT

An improved cleanup method has been developed for the recovery of trace levels of 12 nitro-organic explosives in soil, which is important not only for the forensic community, but also has environmental implications. A wide variety of explosives or explosive-related compounds were evaluated, including nitramines, nitrate esters, nitroaromatics, and a nitroalkane. Fortified soil samples were extracted with acetone, processed via solid phase extraction (SPE), and then analyzed by gas chromatography with electron capture detection. The following three SPE sorbents in cartridge format were compared: EmporeTM SDB-XC, Oasis[®] HLB, and Bond Elut NEXUS cartridges. The NEXUS cartridges provided the best overall recoveries for the 12 explosives in potting soil (average 48%) and the fastest processing times (<30 min). It also rejected matrix components from spent motor oil on potting soil. The SPE method was validated by assessing limit of detection (LOD), processed sample stability, and interferences. All 12 compounds were detectable at 0.02 µg explosive/gram of soil or lower in the three matrices tested (potting soil, sand, and loam) over three days. Seven explosives were stable up to seven days at 2 µg/g and three were stable at 0.2 µg/g, both in processed loam, which was the most challenging matrix. In the interference study, five interferences above the determined LOD for soil were detected in matrices collected across the United States and in purchased all-purpose sand, potting soil, and loam. This represented a 3.2% false positive rate for the 13 matrices processed by the screening method for interferences. The reported SPE cleanup method provides a fast and simple extraction process for separating organic explosives from matrix components, facilitating sample throughput and reducing instrument maintenance. In addition, a comparison study of the validated SPE method versus conventional syringe filtration was completed and highlighted the benefits of sample cleanup for removing matrix interferences, while also providing lower supply cost, order of magnitude lower LODs for most explosives, higher percent recoveries for complex matrices, and fewer instrument maintenance issues.

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1. Introduction

The sampling and characterization of explosives in soil is vital for law enforcement purposes and environmental assessments.

Explosives are commonly used by terrorists to target people, buildings, institutions, and vessels in an attempt to destroy property and/or inflict mass casualties. As a result, there is a consistent need for a quick, efficient, and reliable way of recovering and detecting explosives residues in soil. Identification of the explosive(s) used in a bombing can be extremely useful for linking evidence back to its source (such as a person or organization) or to other events and for domestic or international prosecution. Firing

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ranges, manufacturing sites, and military activities have also contributed to significant levels of explosives contamination at different locations across the United States and around the world [1]. An additional major concern is that the explosives and their degradation products may persist in soil or groundwater and pose a threat to the health and safety of humans and the environment. Identification of the explosives present in soils can assist in site characterization and promote effective cleanup and/or treatment of the contaminated soil [1]. However, the task of identifying trace explosives can be very difficult, especially in complex matrices like soil.

Soil is a non-uniform and highly complex matrix comprised of minerals, organic matter, air, water, and organisms that varies greatly from one region to another with respect to its physico-chemical properties. Soil texture, defined by the relative proportion of sand (0.05–2 mm), silt (0.002–0.05 mm), and clay-sized (<0.002 mm) particles in the <2 mm size fraction [2], affects the retention capacity for water and chemicals. The clay-sized soil fraction contains organic material and minerals with higher specific surface area and more active surface charge, making clay-rich soils more challenging matrices for extraction and characterization of explosives residue. Nitroaromatic explosives like trinitrotoluene (TNT) can bind strongly to the electron-rich active sites in soil particles especially if the exchangeable cations at the surface of the soil particles are weakly hydrated (e.g. K^+ , NH_4^+), whereas non-aromatic explosives like cyclotrimethylene trinitramine (RDX) generally exhibit lower adsorption in soil particles [3,4]. Sand, on the other hand, is a simpler matrix with low surface area and surface charge. It also has the lowest water retention capacity. As a result of these properties, explosives are likely to permeate through sand more easily and to exhibit lower adsorption capacities. Other physical and chemical properties of soil that may affect the sorption/desorption and fate of the explosives present include, but are not limited to, pH, microbial content, and percentage of organic matter [5,6]. The percentage of organic matter will influence mobility and sorption of the explosives, with higher partitioning often seen for soils having high organic carbon content and explosives with aromatic moieties [7]. A higher organic matter or clay content may also translate into reduced extraction efficiencies for the organic explosives due to partitioning between the solvent and organic matter in the soil [8]. All of these properties add to the complexity of the soil, in addition to any contaminants that may be present, such as agricultural and industrial chemicals, small debris, and motor oil. As a result, the analysis of explosives in soil can be a challenging and time-consuming process.

There are several published methods in the literature describing the recovery of organic explosives in different matrices; however, these methods are often lengthy [9,10], complicated [9,11], or are validated for only a select number of explosives on simple matrices such as cotton or polyester [12] swabs. Lengthy and complicated methods (e.g. numerous steps and solvents required) can reduce sample throughput, be error prone or irreproducible, and delay obtaining results in a timely manner. For example, the EPA's Method 8330B recommends drying the soil at room temperature, which can take hours, followed by an 18-h sonication-based extraction process with acetonitrile for samples containing organic explosives [9]. This method is often used for bulk soil sampling and not practical for time-sensitive forensic cases involving trace detection of explosives. In addition, extraction of alkaline soil matrices with acetonitrile for such a long period of time can be problematic, as explosives tend to break down through alkaline hydrolysis when the soil has not been dried completely prior to extraction and residual water is present in the sample [13]. One fast and simple option often used for the preparation of explosives samples from soil is syringe filtration followed by evaporation of the organic solvent to concentrate the

extract. Filtration removes large debris from the soil extracts, but smaller (sub-2 micron) particles and soluble contaminants remain in the sample and may lead to instrumental issues, including signal suppression/enhancement, increased background noise, and extra/missing peaks. In addition, instrument performance loss may occur, leading to decreased sample throughput and increased maintenance costs. Therefore, removal of these unwanted contaminants and/or matrix components prior to instrumental analysis via a sample cleanup procedure is desirable, as it may provide more reliable results in successive analyses. The concentration step after syringe filtration may also cause analyte loss due to volatilization and degradation. This loss has been demonstrated by DeTata et al. for explosives (e.g. tetryl and TNT) in acetone after reduction of the solvent volume [14].

An alternate cleanup method to simple filtration that can be applied to soil extracts is solid phase extraction (SPE). With SPE, extraneous material in the soil extract is washed away with various solvents or retained on the sorbent and the analytes of interest are collected using a small volume of solvent, which promotes enrichment of trace levels of explosives present in large volume extracts. Final extracts can be eluted into autosampler vials with a solvent that is compatible with direct analysis. Avoiding an evaporation step reduces the chances of losing the more volatile explosives and saves time during sample preparation. It has been demonstrated that SPE provides high analyte recoveries for organic explosives [11,12,14], making it an attractive cleanup procedure. However, there is limited data on the application of SPE to the cleanup of soils containing organic explosives, as previous studies have focused on processing less complex samples such as spiked swabs/wipes [12] and standard solutions [14] or focused only on a few explosives. Because of the complexity of soil, it is important to have a method that targets all of the explosives of interest while leaving behind contaminants and other inherent components that may interfere with detection of the explosives and/or lead to instrumental issues.

The goal of this research was to evaluate new and different approaches to the cleanup of soil samples for explosives residue analysis with the aim of improving analyte detection and reducing instrument performance problems by focusing on an alternate sample processing procedure to the conventional syringe filtration technique currently in use. It was important to have an overall method that was quick and simple to facilitate sample throughput and that also targeted explosives that may be encountered in forensic casework, including nitramines, nitrate esters, nitroaromatics, and a nitroalkane. This study compares the performance of three copolymeric SPE cartridges that have been demonstrated for use with explosives on the basis of the following five factors: supply cost, method complexity, explosives recovery, processing time, and matrix rejection. The same criteria were used in a comparison with a simple syringe filtration method, and also included a limit of detection (LOD) study with potting soil. Gas chromatography with electron capture detection (GC/ECD) was selected for screening samples due to its sensitivity to a wide range of explosives and relatively rapid analysis time. As such, all performance evaluations of the cleanup process are focused on the analyte screening results. The organic explosives or explosives-related compounds studied were ethylene glycol dinitrate (EGDN), dimethyl dinitrobutane (DMDNB), 4-nitrotoluene (4-NT), nitroglycerin (NG), 2,4-dinitrotoluene (DNT), TNT, pentaerythritol tetranitrate (PETN), RDX, 2,4,6-trinitrophenylmethyl nitramine (tetryl), cyclotetramethylene tetranitramine (HMX), erythritol tetranitrate (ETN), and cyclotrimethylene trinitrosoamine (R-salt). These compounds will be collectively referred to as "explosives" for the remainder of the paper. Explosives were chosen based on threat potential, historical and emerging usage, and screening method being used. For example, PETN is the explosive fill in most

commercial detonation cords and this product may be used with other explosives like RDX and TNT in the main charge. Novel explosives like ETN and R-salt are gaining more notoriety [15] and

were therefore included in this research. The ability to recover these 12 explosives via a single, quick cleanup method will be valuable to the forensic and law enforcement communities.

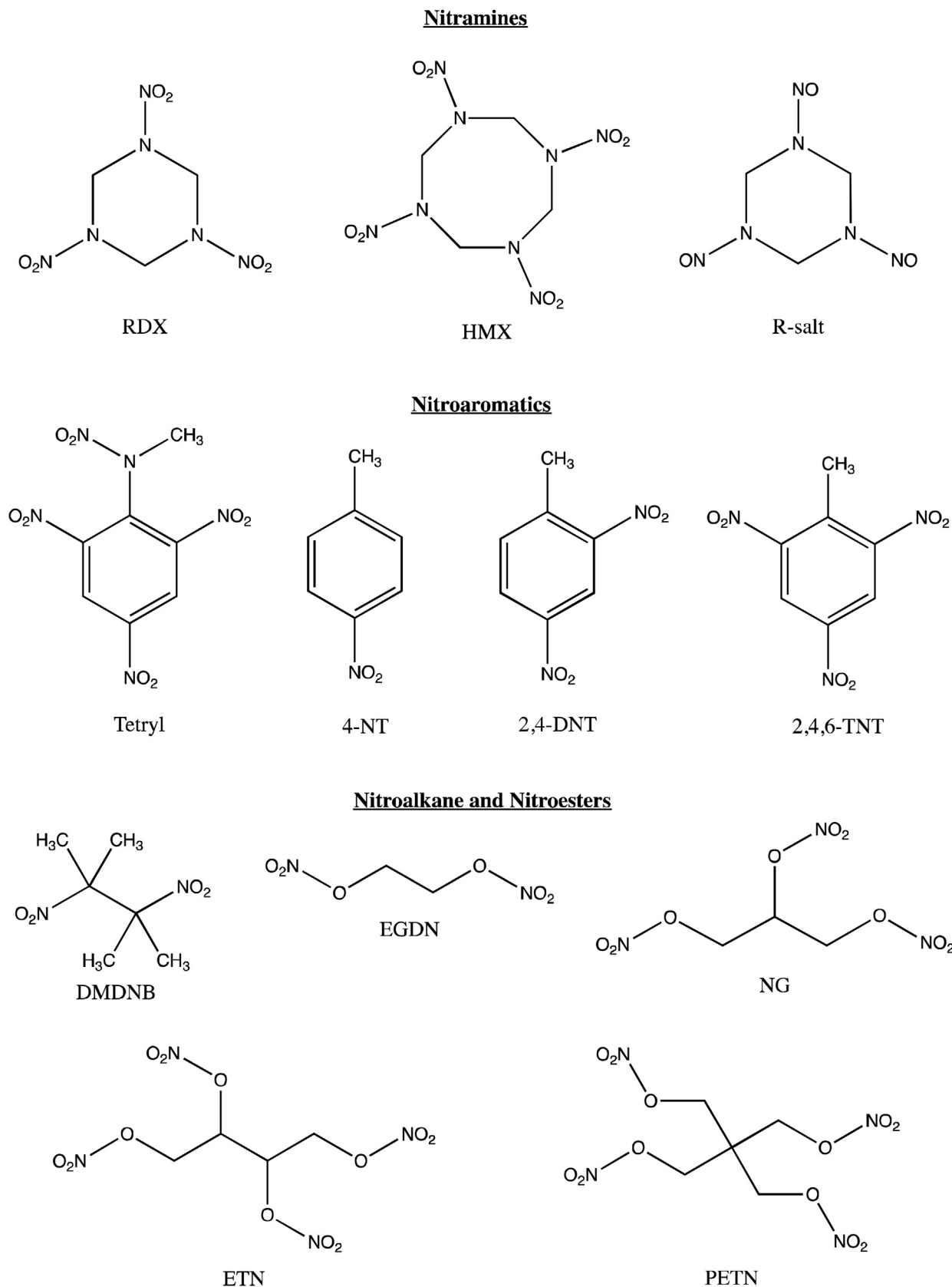


Fig. 1. Chemical structures of the 12 organic explosives or explosives-related compounds of interest.

2. Materials and methods

2.1. Materials

2.1.1. Standard solutions

Individual standards of DNT, EGDN, HMX, 4-NT, NG, PETN, RDX, tetryl, and TNT at 1000 µg/mL were purchased from Restek Corporation (Bellefonte, PA, USA). Solid DMDNB was purchased from Sigma–Aldrich® (St. Louis, MO, USA) and dissolved in acetonitrile to make a 1000 µg/mL standard. ETN and R-salt standards at 1000 µg/mL were purchased from AccuStandard® (New Haven, CT, USA). These standards were used for comparison in order to determine peak retention times of each explosive in the soil extracts. A certified quantitative standard mixture of nine of the explosives (DMDNB, DNT, HMX, 4-NT, NG, PETN, RDX, tetryl, and TNT) at 1000 µg/mL was also purchased from Restek®. In order to make the 12-component test mix for spiking the matrices, the four stock solutions (9-component explosives mixture, EGDN, ETN, and R-salt) were combined and diluted to 20 µg/mL in either acetone or acetonitrile. When necessary, the mixture was diluted to the appropriate concentration and then spiked onto the soil samples. Calibration curves were also generated from the standard mixture and used to estimate percent recoveries for the spiked soils. For replicate samples, the relative standard deviation (RSD) was calculated for each compound and then averaged for method comparison. The chemical structures of the organic explosives and related compounds are given in Fig. 1. The internal standard, 1,1-dinitrocyclopentane (DNCP), was used during validation to verify the efficacy of the extraction process. DNCP was purchased from Sigma–Aldrich® as a solid and made into a 1000 µg/mL solution with acetone.

2.1.2. Reagents

Reagent grade or higher acetone, acetonitrile, methanol, and calcium chloride dihydrate were purchased from Thermo-Fisher Scientific (Pittsburgh, PA, USA). A Barnstead NANOpure® Infinity ultrapure water system generated 18.2 megohm (deionized) water that was used to dilute the soil extracts prior to SPE. The addition of water to the organic extracts promoted adsorption of the analytes onto the SPE sorbent. Laboratory personnel at the Federal Bureau of Investigation (FBI) donated the spent automotive motor oil that was used to make the oil-contaminated soil samples for method development and validation.

2.1.3. Soils

The recovery of organic explosives was evaluated using clean clay loam, all-purpose sand, and potting soil. The sand and potting soil were purchased from a local retail store. According to the manufacturer, the potting soil is composed of materials derived from one or more of the following ingredients: rich blend of mineral soils, dolomitic limestone, composted bark fines, sand, and perlite. The loam was purchased from Sigma–Aldrich (product number CLNSOIL2) [16]. It was a certified reference material (CRM) containing 1.85% organic matter and is classified as a loam according to its textural properties (10% clay, 50% sand, 40% silt) [2]. These soil samples were spiked with the compounds of

interest, extracted with acetone, and processed using the validated SPE method in order to evaluate the method for analyzing post-blast soil samples containing residue and other components. In addition, two actual post-blast residue samples were used to test the finalized cleanup method; post-explosion soil from Africa and soil from the demolition range at Marine Corps Base Quantico (MCBQ) in Quantico, VA. Finally, 10 natural soils from the U.S. and its territories, representing a wide range of climate, vegetation, soil texture, organic content, and pH were assessed for potential interferences. In this paper, soil will refer to all types of soils used in experiments including natural soils as well as potting soil, all-purpose sand, and loam.

2.2. Sample preparation

2.2.1. Solid phase extraction

A 5-g sample of the matrix was added to a beaker, spiked with the internal standard and explosives test mix, allowed to sit for 5 min at room temperature, and extracted for 5 min with 5 mL of acetone. The soil, covered with acetone, was swirled initially by hand for a few seconds to ensure that the entire sample was saturated and then allowed to settle for the 5 min without further disturbance. The oil-contaminated soils were prepared by adding 0.1 g (2% wt/wt) of spent motor oil to the soil prior to the addition of any explosives or internal standard. The acetone extract was recovered (typically 3–4 mL) from the soil using a disposable transfer pipette and diluted to 50 mL with deionized water. This dilution brought the aqueous concentration of the loading portion to about 90% or above. A centrifugation step for 2.5 min at 3500 rpm was added prior to dilution only if acetone extracts formed a thick slurry, typical of the clay-loam soils.

For sample cleanup, the acetone extracts were processed via solid phase extraction using three different copolymer sorbents in cartridge format: Bond Elut NEXUS (Agilent Technologies, Palo Alto, CA, USA), Empore™ SDB-XC (3M, Eagan, MN, USA), and Oasis® HLB (Waters Corporation, Milford, MA, USA). Table 1 gives a summary of the characteristics of each SPE cartridge. Methods from the literature (Table 2) were initially used to evaluate the three cartridges and then modified to increase performance [11,12]. Samples were processed on a 24-port Visiprep™ SPE vacuum manifold with disposable liners (Sigma–Aldrich). Final extracts were collected in autosampler vials to facilitate direct analysis by GC/ECD.

2.2.2. Syringe filtration

Acetone extracts prepared identically to the SPE soil extracts were also processed by syringe filtration for comparison. EMD Millipore Millex™ (Fisher Scientific) non-sterile nylon syringe filters were first attached to a 5-mL plastic syringe and conditioned with 1–2 mL of acetone. The pore size and diameter of the filters were 0.2 µm and 33 mm, respectively. After conditioning the filters, the acetone extract was added to the syringe barrel and forced through the filter, which removed large particulates present in the sample [17]. The filtered extract was then collected in a test tube, reduced to 1 mL at 40 °C under nitrogen gas, transferred to an autosampler vial, and analyzed by GC/ECD.

Table 1

Comparison of the SPE cartridges based on composition, polarity, packing, particle size, pore size, and cartridge volume.

Cartridge	Composition	Polarity	Packing	Particle size	Pore size	Cartridge volume
Oasis HLB	<i>m</i> -Divinylbenzene	Polar and nonpolar	60 mg	30 µm	80 Å	3 cc
Bond Elut NEXUS	N-Vinylpyrrolidone	Nonpolar	60 mg	70 µm	100 Å	3 cc
Empore SDB-XC	Styrene divinyl-benzene Methyl methacrylate	Nonpolar	Disk in SPE format	12 µm	450 Å	6 cc
	Poly(styrene divinylbenzene)				80 Å	

Table 2
Solid phase extraction procedures for Oasis HLB [11], Empore SDB-XC [11], and Bond Elut NEXUS [12] cartridges from the literature.

Oasis HLB	
Conditioning	3 × 10 mL methanol @ 5 mL/min 10 mL water @ 5 mL/min
Load	Extract in acetone diluted to 50 mL with water @ <2 mL/min
Wash	3 mL 50% methanol:50% water @ 2 mL/min
Dry	5 min @ full vacuum
Elute	1 mL methanol @ <2 mL/min
Empore SDB-XC	
Conditioning	2 × 3 mL acetone @ 5 mL/min 2 × 3 mL acetonitrile @ 5 mL/min 3 mL methanol @ 4 mL/min 10 mL water @ 3 mL/min
Load	Extract in acetone diluted to 50 mL with water @ 3 mL/min
Wash	3 mL water @ 3 mL/min
Dry	10 min @ full vacuum
Elute	0.5 mL methanol ^a @ <2 mL/min
Bond Elut NEXUS^b	
Conditioning	3 mL methanol 3 mL water
Load	Extract in 60:40 methanol:water, ca. 8 mL
Wash	0.5 mL water
Dry	Do not dry
Elute	1 mL acetonitrile

^a Used 100% methanol instead of 90:10 methanol:water and diluted to 1 mL.

^b Gravity flow for all steps.

2.3. Instrumentation

Final extracts, standard solutions, and solvent blanks were analyzed on an Agilent 6890 gas chromatograph with an electron capture detector. The inlet was operated in split mode (5:1) at 225 °C and 1 µL of sample was injected onto the system using an Agilent 7683 autosampler. The GC inlet was operated at a nominal head pressure of ~9.5 psi. A J&W DB-5 ms (Agilent Technologies) column (6 m × 0.25 mm × 0.25 µm) was used with helium as the carrier gas. The temperature program for the GC/ECD started at 50 °C for 1.5 min and then increased to a final temperature of 250 °C at a ramp of 25 °C/min. The ECD was operated at 275 °C and nitrogen (99.99% purity) was used as the makeup gas with a combined flow of 25 mL/min (column plus makeup). The final analysis time was 10 min with an approximate average carrier velocity of 101 cm/s. The instrument was controlled by GC ChemStation software Rev. A. 10.02 (Agilent Technologies). The organic explosives were detected based on their GC/ECD retention times, which had to be within ± 0.01 min of the retention time of the reference standard to be screened positive. An additional requirement for detection was that the signal-to-noise (S:N) ratio of a given peak needed to be 3 or greater. Noise values, used for this calculation, were taken manually immediately before or after a peak of interest in a range approximately equal to the peak's width.

2.4. Method validation

Because this study aims to validate a qualitative method for identifying trace explosives in soils, the performance characteristics evaluated included: limit of detection (LOD), explosives extract sample stability, and interferences.

2.4.1. Limit of detection

The LOD was estimated by analyzing explosives-fortified soil samples at three different concentration levels in triplicate over three consecutive days. Potting soil, sand, and loam were used as the matrices and processed separately. The sand represented a very simple matrix, whereas the potting soil represented a more

complex, high organic matrix. The CRM loam sample was selected as one of the matrices for the LOD study to facilitate comparison of results between laboratories. Five gram samples of each of the three soils were spiked with 10 µg of the internal standard and the explosive standard mixture at three levels, 0.1 µg, 0.01 µg, or 0.001 µg for the potting soil and sand, and 1 µg, 0.1 µg, or 0.01 µg for the more retentive loam. The loam extracts were centrifuged for 2.5 min at 3500 rpm to remove bulk particulate prior to dilution with water and then processing by SPE. The triplicate signals were evaluated independently for LOD pass/fail criteria. Each matrix was also spiked at a high concentration (10 µg of each explosive and the internal standard) for comparison purposes. Two matrix blanks, one with and one without the internal standard added, were prepared for each soil to help differentiate between peaks belonging to the explosives of interest and peaks resulting from matrix components. A negative control without matrix or explosives was prepared and processed by SPE to determine if the sample processing steps contributed to co-eluting signals of any of the explosives peaks. In addition, acetone blanks were analyzed between samples in order to ensure that no carryover contributed to explosive peaks for low concentrations. The LOD was considered the lowest concentration that yielded a reproducible instrument response greater than or equal to three times the noise level of the background signal. Lastly, the peak height for the explosive at the LOD had to be greater than two times any co-eluting background peak detected in a matrix blank.

2.4.2. SPE processed sample stability

Processed sample stability was investigated to determine the length of time a SPE processed sample can be maintained before it undergoes unacceptable changes. The CRM loam from Sigma-Aldrich was utilized as the sample matrix for the stability study, as it was judged to be the most difficult matrix and estimated that both potting soil and sand stabilities would be greater or equal. Loam samples were fortified with the explosives mixture at low (0.2 µg/g) and high (2 µg/g) concentrations and with 2 µg/g of the internal standard. Following SPE processing, final extracts of the same explosives concentration were combined and split among several autosampler vials with inserts to ensure that 33 individual samples were available to complete the evaluation. The first aliquots of each concentration were immediately analyzed in triplicate to establish the time zero response. All remaining vials were stored in the autosampler tray at room temperature and analyzed in triplicate at different time intervals: 4, 8, 16, 24, 32, 40, 48, 56, 64, 72, and 168 h. Average responses for the explosives and internal standard at each time interval were then compared to the time zero response. The analyte was considered as stable until the average signal decreased below 80% or increased above 120% of the average time zero response.

2.4.3. Interferences

To evaluate matrix interferences, two studies were conducted. Thirteen matrix blanks from different sources were collected to evaluate interferences in the matrix without the addition of the internal standard: the three soils used in the LOD studies and 10 natural soils representing a wide range of physical and chemical characteristics. Of these 10 soils, four came from different locations in Virginia and six came from other states/U.S. territories. One blank potting soil sample was spiked with the internal standard and monitored to determine if the internal standard interferes with detection of the analytes of interest. Likewise, one blank potting soil sample was spiked with a high analyte concentration (10 µg of each explosive) and analyzed without the internal standard to determine if any peaks were detected at the same retention time as the internal standard.

3. Results and discussion

3.1. SPE cartridge testing and selection

In order to compare the three SPE cartridges, four samples of potting soil were fortified with 10 µg of each explosive and 10 µg of the internal standard. One of these four samples was spiked with 2% spent motor oil to evaluate the efficacy of the SPE cartridges at removing non-explosive contaminants. All of the samples were extracted with the appropriate solvent and processed using a SPE method from the literature (Table 2). It is important to note that the Bond Elut NEXUS cartridges were previously named ABS ELUT Nexus, which is the designation referenced by Song-im et al. [12].

3.1.1. Oasis HLB SPE cartridges

The Oasis HLB cartridges provided the highest initial average recovery (35%) of the three methods for the fortified potting soil, but had the longest processing times, with the total SPE time ranging from 1.5 to 3 h. The longer processing times may be attributed to the large conditioning volumes and inconsistent flows due to clogging, the last of which accounts for the range of SPE times per set. Soil particulate present in the acetone extract and the small particle size and pore size of the sorbent are the likely culprits for the clogging observed with HLB cartridges. In an attempt to decrease SPE processing times, particles within the acetone extracts were sedimented out by flocculation for 15 min with 5 mL of 10 g/L aqueous calcium chloride [18]. Despite this, some of the HLB cartridges still had inconsistent flows and long processing times. The oil-contaminated soil sample had an average recovery for the 12 explosives of 32%, similar to the potting soil samples without oil. The combined cost of the HLB cartridges (\$2.69 USD/cartridge) and large solvent volumes required for conditioning, in addition to the long processing times, make it less desirable for sample cleanup of soil extracts.

3.1.2. Empore SDB-XC SPE cartridges

For the SDB-XC cartridges, it was necessary to change the elution solvent from the previously published conditions of 90:10 methanol:water to 100% methanol so that the final extracts were in a solvent compatible with GC analysis. Injecting a solution of 10% water into the GC can lead to backflash in the injection port liner, damage of the stationary phase, and decreased ECD sensitivity. For consistency with the other SPE methods, 1 mL was used as the final extract volume instead of 0.5 mL. Under these conditions, the SDB-XC cartridges provided similar average recoveries to the HLB cartridges for the potting soil (34%) and oil-contaminated soil (36%) extracts, but had faster processing times (1–2 h). Using a higher vacuum pressure did not reduce processing times significantly and recoveries decreased for the explosives. In addition, the original SDB-XC SPE method required five different solvent systems, acetone, acetonitrile, methanol, water, and 90:10 methanol:water, which makes it more complicated than the other two cartridge methods. The cost of the SDB-XC cartridges was the highest overall, at \$5.80 USD per cartridge. Because of these issues, the SDB-XC cartridges were not further investigated for sample cleanup of soils.

3.1.3. Bond Elut NEXUS SPE cartridges

Soil extracts processed with NEXUS cartridges had processing times of less than an hour using the previously published method, but recoveries were very low, with an average of 15% for the 12 explosives in potting soil and 14% in oil-contaminated soil. In order to examine recoveries for the NEXUS cartridges, the volume of water in the acetone extract was increased so that the final volumes prior to SPE were 15 mL, 25 mL, 50 mL, or 100 mL, which equated to approximately 33:66, 20:80, 10:90, and 5:95 organic:

water respectively. The 25 mL and 50 mL aqueous dilutions provided the highest recoveries for all of the explosives, averaging between 34 and 45%, but follow-up studies showed that the 50 mL dilution was slightly higher. The processing times were reduced to less than 30 min by performing SPE with vacuum elution at 5 in. of mercury instead of using gravity flow. The larger particle and pore sizes of the NEXUS cartridge also facilitated less clogging and therefore shorter processing times, which contrast what was observed with the HLB cartridges. Because of its low cost (\$2.35 USD/cartridge) and potential to rapidly recover all of the organic explosives in a timely manner, method development continued with only the NEXUS cartridges.

3.2. Performance improvement of Bond Elut NEXUS SPE cartridges

Improvements to the method using NEXUS cartridges were explored in keeping with the goal of developing a quick and simple cleanup procedure for the recovery of organic explosives residue in soils. The initial NEXUS method involved the use of four different solvent systems: 60:40 methanol:water for extraction and methanol, water, and acetonitrile for SPE. To simplify the method, acetone was used as a potential substitute for the extraction step, conditioning of the SPE cartridge, and elution of the final extract. Acetone was selected because it is less toxic than other organic solvents like acetonitrile and methanol. It has also been widely used in the explosives community for the extraction of organic explosives [11,12,17,19]. Changing the extraction solvent from 60:40 methanol:water to acetone showed similar or better recoveries when paired with a higher volume dilution compared to the original method. For SPE, changing the conditioning solvents to acetone and water and the SPE elution solvent to only acetone produced no significant positive or negative change in the recoveries. Because of minimal performance effects with these modifications, the method continued with only acetone and water to reduce the number of solvents needed and simplify the process further (Table 3). This simplified method provided an average recovery and RSD for the 12 explosives in potting soil of 48% and 6.8% (see Table 5 in Section 3.6), respectively. Additionally, an attempt was made to eliminate the conditioning steps in the SPE process to reduce processing times even more; however, recoveries were found to be lower by about 5–10% without conditioning. As a result, conditioning was kept as part of the final SPE method.

Several authors have reported methods that utilize acetonitrile for the extraction of organic explosives in soil [9,20,21]. In general, the explosives are very soluble in acetonitrile and will partition into the solvent instead of remaining in the matrix. If the final extracts are in acetonitrile after sample cleanup and analysis is performed on a GC/ECD, it is possible to observe backflash following injection of the acetonitrile extracts, as the expansion volume of acetonitrile is near the capacity of the injection liner at the conditions specified for the inlet under instrumentation. Backflash can produce ghost peaks, carryover, and baseline problems in the chromatogram. Another drawback of acetonitrile is its high toxicity and cost. Despite these disadvantages, acetonitrile is still commonly used for the extraction of organic explosives, especially soils, and a study was included in this

Table 3

Final Bond Elut NEXUS protocol for the cleanup of soil extracts containing organic explosives.

Condition	3 mL acetone 3 mL water
Load	50 mL sample ^a
Wash	0.5 mL water
Elute	1 mL acetone

^a Vacuum: ~5 in. of Hg.

research comparing the recoveries of acetonitrile and acetone as the extraction solvents. The goal was to determine if there were any significant advantages of using acetonitrile for extraction of the explosives in soil.

To compare the efficacy of explosives extraction with acetonitrile, five potting soil samples were fortified with a mixture of the 12 explosives at 2 µg/g, extracted with acetonitrile, diluted to 50 mL with water, and then processed by SPE using the method described in Table 3. The average recovery and RSD were similar for the potting soil extracts at 47% and 6.2%, respectively, for acetonitrile compared to 48% and 6.8% for acetone (Table 5). The percentages were estimated from a calibration curve generated using standards of the explosives mixture. With the exception of ETN and R-salt, the difference in percent recovery of the individual explosives was within the RSD of the two extraction methods. The average percent recovery for R-salt was 10% higher with acetone extraction versus acetonitrile extraction, but 9% lower for ETN. Regardless of the extraction solvent or matrix, R-salt had the lowest recovery of all the explosives when processed by SPE. Unlike the other explosives, it is a nitroso-compound and as a result, has different binding properties for the SPE sorbent. R-salt is the smallest of the nitramines included in this study and more polar than its nitro-analogue RDX; as a result, it is likely not well retained on the sorbent and may come off during the loading and/or wash steps. Further studies would be necessary to evaluate the binding of R-salt and to determine if recoveries can be improved for the NEXUS SPE method. Because it was possible to obtain comparable recoveries for the majority of the explosives by both extraction methods, validation proceeded as originally intended with acetone as the extraction solvent. Using acetone allows for a streamlined cleanup method for processing complex matrices in a more environmentally friendly manner with little to no loss in analytical performance.

3.3. Validation

3.3.1. Limit of detection

The limit of detection was estimated by spiking blank matrices with a mixture of the 12 explosives at decreasing concentrations, between 0.2 µg/g and 0.0002 µg/g, and processing the acetone extracts by SPE with Bond Elut NEXUS cartridges. The S:N ratios were then calculated at each concentration for all three matrices. The S:N ratios of each replicate sample were evaluated independently, but the average ratio is given in Table 4 for brevity's sake. The LOD for most of the explosives was determined to be between 0.0002 and 0.002 µg explosive/gram soil. All of the explosives were detectable at 0.02 µg/g in sand, potting soil, and loam. The matrix affected recovery of the explosives, with loam being the most difficult to extract of the three. Average recoveries for loam processed by SPE were the lowest of the three matrices, at 38% with a 16% RSD. The lower recoveries may be a result of the preferential adsorption of the explosives to the clay fraction of the loam due to its high surface area with strong binding sites [3,4].

3.3.2. SPE processed sample stability

For the stability study, Sigma loam was spiked with a mixture of the 12 explosives at either a low concentration (0.2 µg explosive/gram soil) or high concentration (2 µg explosive/gram soil). The explosives were found to be more stable at higher concentrations, with seven out of the 12 explosives detectable within specifications up to seven days. These explosives included EGDN, DMDNB, 4-NT, R-salt, DNT, TNT, and RDX. Conversely, NG, ETN, PETN, tetryl, and HMX had a reduction in signal to less than 80% of the original response between 4 and 16 h.

Unlike the high concentration study, only three of the 12 explosives (DMDNB, 4-NT, and DNT) were stable up to seven

Table 4

Signal-to-noise ratios at each concentration level tested in triplicate over three days in the LOD study for sand, potting soil, and Sigma loam. Dashes represent concentration levels that did not pass criteria set forth in the study and blank cells represent levels that were not tested.

Average signal-to-noise ratios (n=9)					
Matrix	Spike level	0.0002 µg/g	0.002 µg/g	0.02 µg/g	0.2 µg/g
Sand	EGDN	12	109	954	
	DMDNB	17	259	2822	
	4-NT	–	–	61	
	NG	33	301	2684	
	R-Salt	–	62	999	
	DNT	21	189	1555	
	ETN	14	134	1316	
	TNT	10	297	2711	
	PETN	–	99	1297	
	RDX	30	234	2807	
	Tetryl	–	37	493	
	HMX	–	–	77	
Potting soil	EGDN	6	75	652	
	DMDNB	10	172	1832	
	4-NT	–	–	36	
	NG	23	251	1889	
	R-Salt	–	59	805	
	DNT	19	157	1125	
	ETN	20	123	1141	
	TNT	33	246	2356	
	PETN	–	90	1114	
	RDX	–	167	2378	
	Tetryl	–	79	824	
	HMX	–	–	104	
Loam	EGDN		76	858	9619
	DMDNB		168	2180	23,761
	4-NT		–	46	405
	NG		131	1651	20,619
	R-Salt		130	990	11,015
	DNT		–	1158	9647
	ETN		55	697	9863
	TNT		113	1155	14,624
	PETN		37	462	6810
	RDX		93	1547	21,327
	Tetryl		–	336	6058
	HMX		–	54	797

days at the lower concentration. Nitroglycerin, ETN, PETN, tetryl, and HMX at an original concentration of 0.2 µg/g of soil dropped below 80% within 8 h, TNT dropped out within 24 h, and EGDN and R-salt dropped out within 48 h. Even though TNT stayed between 80 and 88% for the entire study except at the 24-h mark, where it fell to 76%, it was designated a fail for the stability study. Results indicate that the stability of the explosives is greatly affected by concentration. Losses at lower concentrations and matrix effects may be more noticeable when working near the detection limit of the method. DeTata et al. attributed the instability observed in lower concentration standards over time to adsorption onto glassware and analyte decomposition and emphasized the need for making fresh dilutions [20]. Walsh et al. also found that there was a noticeable loss of tri-nitroaromatic explosives like TNT and tetryl in acetonitrile at lower concentrations when left at room temperature in the autosampler tray near the GC injection port and with different brands of acetonitrile [22]. Cold storage of processed samples in amber vials, in the event of being unable to analyze them immediately, is a potential way to ameliorate the low survival times of certain explosives [22,23].

3.3.3. Interferences

For the interference study, a collection of 10 natural soil samples from across the United States and its territories were obtained and analyzed to evaluate interferences that may hinder detection of the explosives of interest. These locations included

Colorado, Nevada, North Carolina, Virginia, Washington, and the U.S. Virgin Islands, which provided a diverse group of soil types with varying percentages of clay, sand, silt, and organic matter as well as different pH, cation-exchange capacity, and electrical conductivity values, even for samples obtained within the same state or territory (additional information available in the Supplementary section). These soil type characteristics were not determined experimentally, but derived from the official soil series descriptions at the collection locations accessed on two websites: the Web Soil Survey from the United States Department of Agriculture (USDA) [24] and the SoilWeb survey map developed by the California Soil Resource Lab [25]. The three purchased soils (loam, potting soil, and sand) were also evaluated for interferences.

An interference was defined as any peak that eluted at the same retention time as one of the 12 target explosives with a S:N ratio greater than the S:N determined for the explosive in potting soil at the LOD. The potting soil LOD was chosen as the interference threshold because it has intermediate LODs for the explosives among the soils tested. After processing, it was determined that four of the 13 natural soil samples had interference peaks with S:N ratios above the LOD of the potting soil. Among the 13 soils and 12 explosives, only five interference peaks out of 156 possibilities were detected, signifying a 3.2% false positive rate for the validated NEXUS SPE method with GC/ECD detection. The largest interference peaks detected were for tetryl in the two Virginia soils. However, the S:N ratios for these peaks (S:N 82 and 119) were only slightly larger than the value for tetryl at the LOD of potting soil (S:N 79). The extract of the potting soil spiked with only the internal standard did not exhibit interference peaks near the retention times of the explosives of interest. Likewise, none of the explosives showed an impurity near the retention time of the internal standard.

3.3.4. Post-blast residue testing

The SPE method described in Section 3.2 was tested using two soils containing organic explosives to evaluate its potential to detect residues from field samples. Matrix 8 was collected in Africa

after an improvised explosive device (IED) detonation with an unknown explosive. The sample from that area has been mapped as sandy clay loam using SoilGrids [26] and the soil texture triangle [2]. The determination was consistent with microscopic observations. Screening of the extract obtained from Matrix 8 showed ECD peaks corresponding to the following explosives: NG, DNT, TNT, and RDX (Fig. 2). Small explosives peaks were sometimes detected in the matrix extracts and are denoted with a dot in the chromatograms in this paper. Other unlabeled peaks are unknown compounds.

The other soil sample that was tested, Matrix 23, came from a crater at the demolition range on MCBQ and is classified as a sandy loam based on the USDA's web soil survey [24] for the location of sample collection. A TNT-based explosive was detonated on the range just preceding soil collection, and TNT was one of the dominant peaks detected in the chromatogram for Matrix 23 (Fig. 3). Additional peaks corresponding to other explosives were also observed in Matrix 23. The presence of other explosives, including 2,4-DNT, PETN, RDX, and HMX, is due to the site being previously used for explosives training exercises. Nevertheless, several organic explosives were detected in Matrix 8 and Matrix 23, which demonstrate the successful application of the validated method to soils containing post-blast residue.

3.4. Cost and efficiency of filtration versus SPE

As mentioned previously, syringe filtration has been used to filter soil or swab extracts containing organic explosives prior to analysis. This method is quick, simple, and inexpensive, but may lead to instrument contamination. The cost, complexity, and processing times were compared for fortified potting soil extracted by acetone and processed by either syringe filtration or SPE with Bond Elut NEXUS cartridges. The NEXUS cartridge was less expensive (\$2.35 USD) than the syringe (\$0.67 USD) and filter (\$3.00 USD) combined, with the cartridge being significantly cheaper if multiple filters are used during filtration due to clogging. In terms of complexity, syringe filtration and SPE are similar.

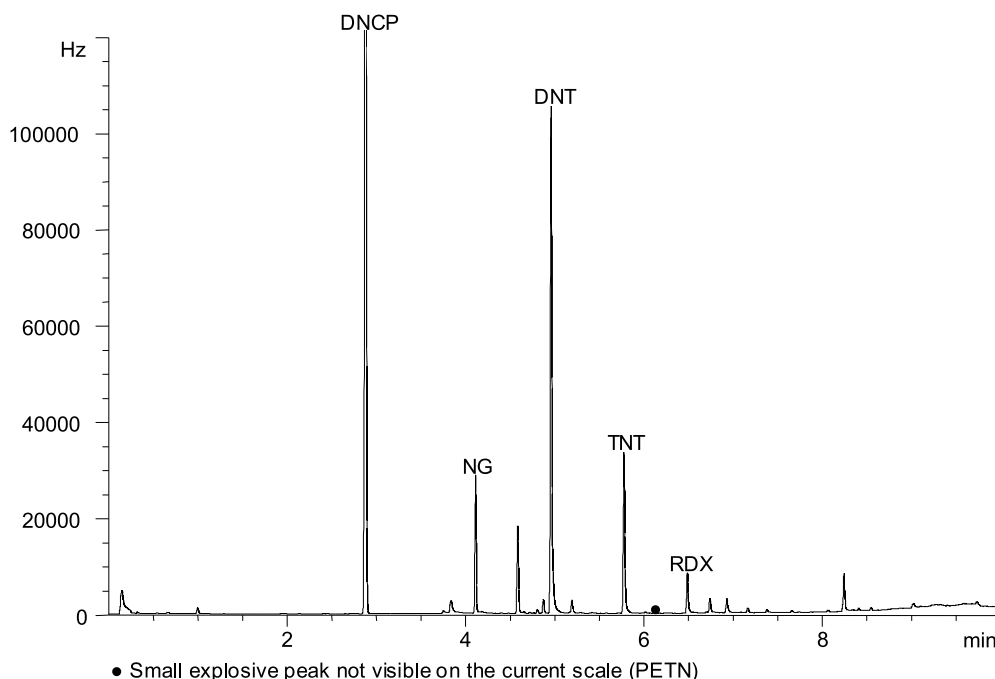


Fig. 2. GC/ECD chromatogram of Matrix 8, a sandy clay loam post-blast residue sample from Africa, following extraction with acetone and cleanup using the validated NEXUS SPE method. The DNCP peak exceeds the scale presented.

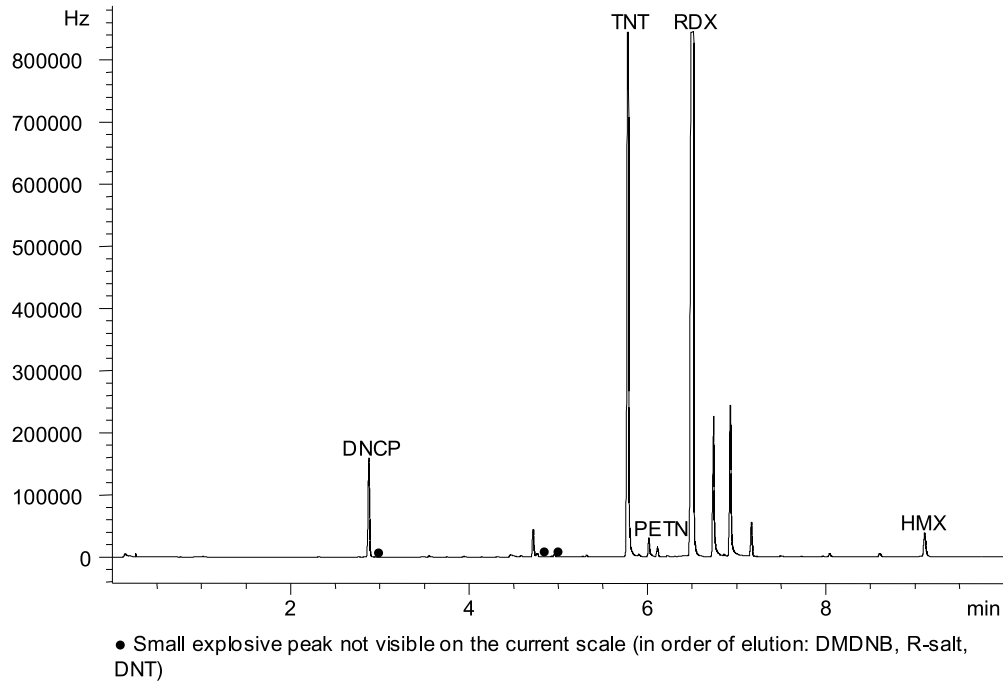


Fig. 3. GC/ECD chromatogram of Matrix 23, a sandy loam from a demolition range, following extraction with acetone and cleanup using the validated NEXUS SPE method. The peaks for TNT and RDX exceed the scale presented.

Syringe filtration requires a single solvent, syringe/filter, and evaporator for completion whereas SPE requires two solvents for the whole process and utilizes a vacuum manifold for sample cleanup. After extraction of the organic explosives with acetone, it can take under 30 min to process a few (1–4) samples by either method. Overall, the cost and efficiency for the two methods are comparable.

3.5. Mitigation of oil-contamination by SPE versus filtration

To evaluate matrix rejection, the potting soil was spiked with 2% (0.1 g) spent motor oil, extracted with acetone, and then processed by both syringe filtration and NEXUS SPE (Section 3.2). The final extracts for syringe filtration were viscous, yellow in color, and cloudier than the extracts for SPE, which were generally clear and

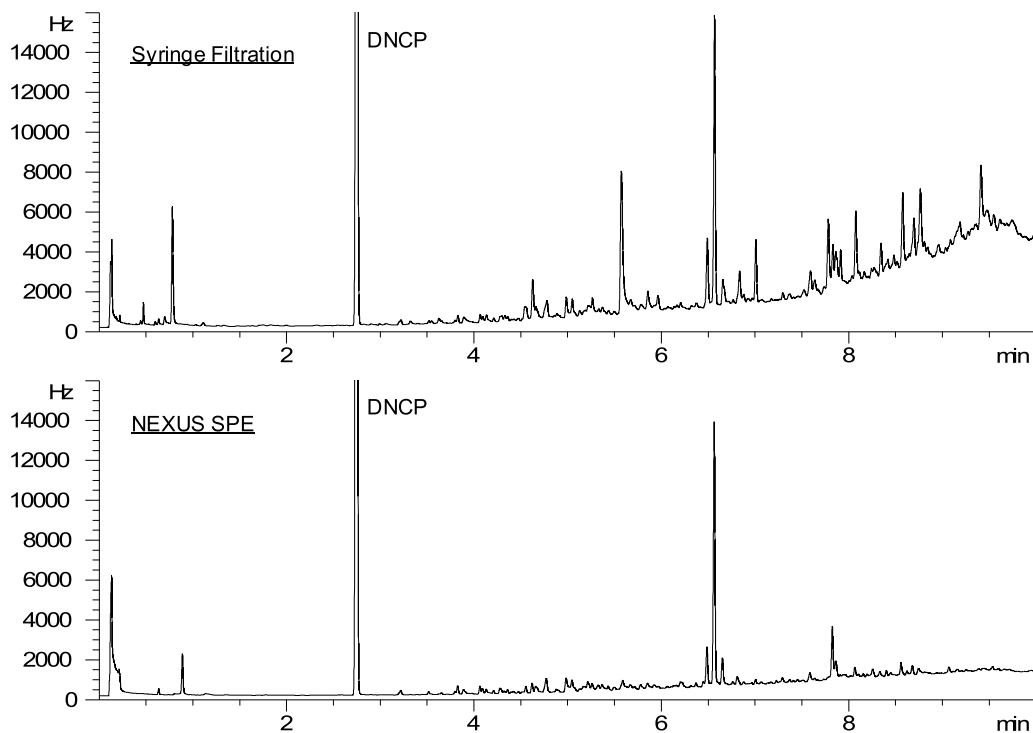


Fig. 4. Comparison of the background present in an unspiked oil-contaminated potting soil sample processed by syringe filtration and the validated NEXUS SPE method. The DNCP peaks exceed the scale presented.

colorless liquids. The chromatograms of SPE soil extracts were observed to have a lower intensity baseline than those done by syringe filtration, particularly between 6 and 10 min (Fig. 4). This trend was present when comparing four replicate samples done for both processes, with the syringe baseline's height exceeding the SPE baseline by up to 3,200 Hz towards the end of the chromatogram. In addition, fewer and smaller background peaks were found in the samples processed by SPE when compared to syringe filtration (on average 72 peaks for SPE vs. 96 peaks for syringe). The cumulative area of all matrix peaks per run found by SPE was less than half of its equivalent in syringe filtration (85,000 Hz·sec ± 25,000 Hz·sec vs. 190,000 Hz·sec ± 15,000 Hz·sec). Examining interference peaks that co-elute with explosives, syringe filtration showed two (DMDNB and ETN) whereas SPE only showed one (ETN), both consistently through four trials. However, taking the large differences in the number of matrix peaks/areas and the baseline into account, it is clear that fewer contaminants are being injected with samples processed by SPE, which helps to minimize the frequency of replacing consumables like the column and injection liner.

3.6. Percent recoveries by SPE and filtration

Another metric for comparing SPE and syringe filtration is to quantitatively determine the percent recovery of each explosive across various matrices. Recoveries for the organic explosives in sand, potting soil, and potting soil contaminated with spent motor oil were calculated for acetone extracts processed by the validated NEXUS SPE method and syringe filtration (Table 5). There is also a column in Table 5 with percent recoveries for positive controls (no matrix) to assess the recovery biases of each sample preparation method for the explosives. Replicate samples were processed for each method and soil type in order to obtain RSD values. Additional information for individual explosives is available in the Supplementary section.

3.6.1. Positive control (no matrix) extracts

As anticipated, the positive control extracts for both syringe filtration and SPE gave the highest average and individual recoveries for the explosives overall (Table 5). It is also not surprising that the individual explosives recoveries for the positive controls processed via syringe filtration were generally high, as the explosives are expected to pass through the filter without any major losses. For example, the average recovery for positive

Table 5
Average percent recoveries for positive controls, sand, potting soil, and oil-contaminated potting soil fortified at 2 µg/g with the 12 organic explosives and processed by the syringe filtration (SYR) and validated NEXUS SPE methods (n = 3–10).

Explosive	Control		Sand		Potting soil		Soil + oil	
	SYR	SPE	SYR	SPE	SYR	SPE	SYR	SPE
EGDN	91%	42%	34%	34%	39%	35%	27%	29%
DMDNB	87%	82%	43%	60%	35%	55%	24%	37%
4-NT	100%	96%	36%	62%	56%	61%	27%	51%
NG	90%	97%	53%	68%	34%	56%	23%	37%
R-salt	96%	32%	62%	27%	43%	26%	34%	22%
2,4-DNT	90%	93%	50%	66%	31%	55%	26%	38%
ETN	91%	90%	67%	68%	33%	50%	24%	43%
2,4,6-TNT	43% ^a	100%	35%	75%	34%	61%	33%	45%
PETN	91%	92%	62%	71%	30%	49%	22%	41%
RDX	96%	84%	72%	65%	35%	43%	38%	40%
Tetryl	100%	75%	68%	38%	30%	53%	40%	55%
HMX	86%	52%	83%	36%	24%	35%	71%	50%
Average recovery	89%	78%	55%	56%	35%	48%	33%	41%
Average RSD	16%	9.5%	17%	7.2%	19%	6.8%	15%	11%

^a TNT recovery by itself via syringe filtration was 79%.

controls processed by syringe filtration was 92% for the nitramines versus 56% for SPE. In contrast, the average recoveries for the nitroaromatics, nitroesters, and nitroalkane were similar for the positive controls processed by both methods. One anomaly observed was the low recovery of TNT (43%) with explosives standard mixtures for the syringe filtration method.

Because of the observed lower TNT recoveries, other experiments were conducted to explore this issue. It was determined that the low TNT percent recovery for the positive controls processed by syringe filtration could be attributed to multiple factors, including loss on the nylon filter due to adsorption and/or partitioning (~8%) [27,28], loss during the evaporation step (~14%) [11,14], and loss through interactions with other explosives when processed as a mixture (~41%) [20]. This final loss factor occurred only when TNT and tetryl were processed together, independent of the presence of the other explosives. For comparison, TNT had a 79% recovery when processed without tetryl, which is consistent with a combined loss from the filter and evaporation step. It was also observed that only the extract containing both TNT and tetryl processed by syringe filtration was pink in color, compared to the colorless SPE solution, which may indicate a chemical change. However, no additional significant peaks were detected in the chromatogram of the soil extracts or standards to suggest the typical analyte breakdown products of TNT were present. Studies by DeTata et al. using high performance liquid chromatography with ultraviolet detection also reported a lack of transformation or breakdown products for a standard mixture containing TNT and tetryl, even though large decreases in peak areas were observed for these explosives after solvent evaporation (specifically acetone) or extended refrigeration at lower concentrations [14,20]. Because a full recovery was attainable for tetryl when processed together with TNT by syringe filtration, it doesn't appear that tetryl is lost through mechanisms such as decomposition or adsorption onto the glassware. Mixtures of TNT with other explosives, that excluded tetryl, did not produce a color change or result in large losses in recovery for TNT when processed by syringe filtration.

SPE has been suggested as an alternative sample cleanup method to avoid the issues described in the paragraph above [27]. In the validated method, SPE extracts are not filtered through a nylon medium or subjected to an evaporation step, which have both been shown to reduce analyte recoveries. SPE gave a full recovery for TNT even though the explosives were processed as a mixture. It has been reported by Jenkins et al. [29] that water may help to stabilize nitroaromatics such as TNT. Additionally, Walsh et al. noted that SPE extracts of water samples containing trinitroaromatic explosives did not exhibit instability, but experiments to confirm this link were inconclusive [22]. One of the major differences between SPE and syringe filtration is that the SPE acetone extracts are immediately diluted with deionized water (pH = 5.88) after removal from the matrix. Future experiments are necessary to determine the exact mechanism of action causing the significant loss of TNT when processed with tetryl by syringe filtration.

3.6.2. Sand extracts

The sand extracts showed similar average percent recoveries for both cleanup methods, although the RSD value for SPE was notably better than for syringe filtration. The similar and generally high recoveries when compared to other matrices are likely due to the texture and low organic content of the matrix. Sandy soil has large particles, a smaller surface area, fewer charge sites than clay, and a lower retention of liquids and chemicals as they pass through the sand. It is also a very simple matrix with fewer interferences; therefore, cleaner samples are attainable regardless of cleanup method. In total, percent recoveries were greater for six of the 12 explosives by SPE, greater for four of the 12 explosives by

syringe filtration, and similar (within 1%) for two of the 12 explosives. Higher individual recoveries were seen for the nitramines processed by syringe filtration, whereas the nitroaromatics showed higher percent recoveries when processed by SPE, with the exception of tetryl.

3.6.3. Potting soil extracts

For the potting soil extracts, the percent recovery of the individual explosives was greater for all but three of the explosives processed by SPE versus syringe filtration: EGDN, 4-NT, and R-salt. One advantage of SPE is that the water, added prior to SPE processing, may compete for sorption sites on the lingering soil particles in the acetone extracts, thereby decreasing sorption of nitroaromatics and increasing recoveries. The only explosive that had a significantly higher recovery for syringe filtration was R-salt, explained in Section 3.2. Two of the 12 explosives, EGDN and 4-NT, showed similar recoveries for both sample preparation methods, but syringe extracts showed a large interference peak that partially overlapped with 4-NT's retention time (Fig. 5), making it difficult to assess its recovery accurately. This interference peak is not present in the SPE extracts of potting soil.

3.6.4. Oil-contaminated potting soil extracts

For the oil-contaminated soil spiked with explosives, average percent recoveries were slightly higher again with SPE; however, the RSDs were high for both methods. Potting soil is less homogenous than sand and the addition of spent motor oil makes it a more complex matrix; therefore, higher RSDs are not unexpected. In total, eight of the 12 explosives had a higher recovery for SPE, two were higher for syringe filtration, and two were similar (within 2%). Another noticeable difference in Table 5 has to do with the recovery of HMX for syringe filtration. In sand, HMX's recovery was 83%, but it dropped to 24% in soil and then increased to 71% in oil-contaminated soil. HMX's recovery also

increased for SPE between the potting soil and the potting soil contaminated with oil, although to a smaller effect. The increased recovery observed for both processing methods could be a consequence of the order in which the explosives and oil were added to the soil. R-salt and HMX were the only explosives that showed higher recoveries for syringe filtration. Like the other matrices, EGDN showed similar recoveries for oil-contaminated soil processed by both cleanup methods. Across the three soils and positive controls tested, syringe filtration showed higher RSDs when compared to SPE. The consistency in detection of the explosives and improvement in the quality of the sample via SPE highlights advantages of the validated cleanup method over syringe filtration, especially for complex matrices like potting soil.

3.6.5. Comparison of limit of detection for SPE versus syringe filtration

In addition to percent recoveries, the limit of detection was determined via syringe filtration for potting soil fortified with 1.0 μg (0.2 $\mu\text{g/g}$), 0.1 μg (0.02 $\mu\text{g/g}$), 0.01 μg (0.002 $\mu\text{g/g}$), or 0.001 μg (0.0002 $\mu\text{g/g}$) of each explosive and 10 μg of the internal standard in triplicate over three consecutive days. The three lower concentration levels correspond to those selected for the NEXUS SPE validation study. The goal was to determine if higher, lower, or similar LODs for each explosive were attainable by syringe filtration versus SPE and to explore performance consistency and the need for maintenance over those three days. The results indicated higher LODs for samples processed by syringe filtration, as no explosives were detectable at 0.0002 $\mu\text{g/g}$, only 7 of 12 were detectable at 0.002 $\mu\text{g/g}$, 10 of 12 at 0.02 $\mu\text{g/g}$, and all 12 at 0.2 $\mu\text{g/g}$ (Table 6). Comparing these results to those achieved by SPE indicates lower LODs for 10 of the 12 explosives. The validated SPE method can detect half of the explosives at the lowest concentration, 0.0002 $\mu\text{g/g}$, and can detect all of them at a concentration of 0.02 $\mu\text{g/g}$. Additionally, it was noted that several explosives were detectable at lower levels on the first day of the three-day LOD

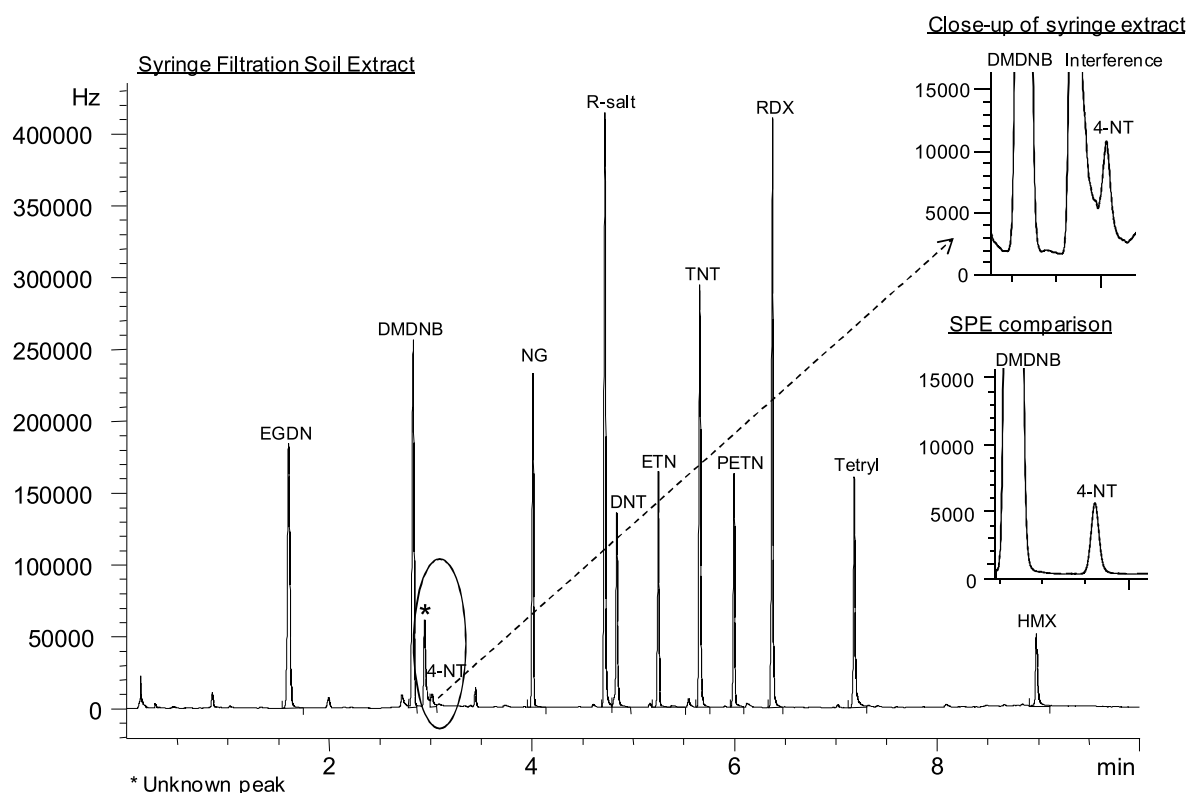


Fig. 5. Chromatogram of fortified potting soil (2 $\mu\text{g/g}$) extracted with acetone, processed by syringe filtration, and analyzed by GC/ECD. Close-ups of the syringe and NEXUS SPE extracts around the elution time of 4-NT are given to highlight the differences.

Table 6

Limits of detection for fortified potting soil processed via syringe filtration and the validated NEXUS SPE method. The lowest limit of detection for each explosive between the two methods is shaded in gray.

Explosive	Syringe LOD ($\mu\text{g/g}$)	SPE LOD ($\mu\text{g/g}$)
EGDN	0.002	0.0002
DMDNB	0.002	0.0002
4-NT	0.2	0.02
NG	0.002	0.0002
R-salt	0.002	0.002
2,4-DNT	0.002	0.0002
ETN	0.002	0.0002
2,4,6-TNT	0.02	0.0002
PETN	0.02	0.002
RDX	0.002	0.002
Tetryl	0.02	0.002
HMX	0.2	0.02

syringe filtration study than on subsequent days, indicating that instrument fouling occurred that decreased the sensitivity of the detector on days two and three. These indications are supported by observations, during routine maintenance, of dirtier septa and liners after a small number of runs with syringe filtration. Overall, the results suggest that it is beneficial to use SPE when analyzing trace explosives in soil, as lower LODs are achievable when compared to syringe filtration.

4. Conclusions

An improved cleanup procedure for processing soil samples containing trace levels of 12 nitro-organic explosives or explosive-related compounds was developed. Three different copolymer SPE cartridges using methods from the literature were evaluated in potting soil and with spent motor oil as a matrix interference. Each literature method presented challenges. The Oasis HLB cartridges originally had the highest recoveries, but also very long processing times due to high conditioning volumes, low flows, and frequent clogging, making it less practical in a forensic laboratory setting. These issues were not addressed adequately with flocculation and centrifugation. The Empore SDB-XC cartridges had processing times between 1 and 2 h, but recoveries and times could not be improved through the use of higher vacuum. The high cost of the SDB-XC cartridges also detracts from its appeal as a cleanup tool for soil extracts. The Bond Elut NEXUS cartridges had processing times under an hour and good explosives recovery when using a refined extraction method.

Once finalized, the NEXUS SPE method was validated, which included determining the method's limit of detection, common interferences, and stability of processed samples. All of the explosives were detectable at 0.02 μg explosive/gram of soil in potting soil, sand, and loam, while many of them were still detectable at concentrations ten- to one-hundredfold lower. Seven of the 12 explosives were stable in solvent extracted from loam up to 168 h (seven days) at high concentration. Five interference peaks were identified by GC/ECD in the 13 matrices collected from a variety of sources, which represented a 3.2% false positive rate. These interference peaks were very small and would likely not be confirmed as explosives by another type of instrumentation due to sensitivity issues. The oil-contaminated soil did have one interference peak slightly above the LOD of the method for ETN. The validated SPE method was then successfully applied to two field-contaminated soils containing post-blast residues, for which several explosives were detected in the extracts. The results highlight the potential usefulness and practicality of the method for recovery of trace levels of explosives in challenging matrices like soil. Future studies should be performed to evaluate a larger population of soils and additional post-blast residue samples.

Finally, syringe filtration was compared to the validated NEXUS SPE cleanup method based on cost of supplies, method complexity, percent recoveries of the explosives, processing times, limits of detection, and matrix rejection. The validated SPE method is simple, comparable in cost, requires only two solvents and no evaporation steps, and generally takes less than 30 min to process a few samples. One advantage of syringe filtration is that it involves minimal treatment of the extracts compared to the new SPE cleanup method. However, this study found that percent recoveries for complex explosives-fortified soil samples were on average 10% higher for SPE versus syringe filtration. Furthermore, a lower background and flatter baseline were observed for chromatograms of SPE processed samples. Additional peaks may be present in syringe extract chromatograms because filtration removes particulates, but does not remove chemical interferences. Extracts processed by SPE were cleaner and minimized introduction of contaminants onto the GC/ECD, allowing hundreds of samples to be processed without maintenance. Overall, the results showed that the NEXUS cartridges resulted in higher recoveries and maintained similar processing times compared to syringe filtration. This SPE procedure is a chemically selective cleanup method for a wide variety of nitro-organic explosives that reduces matrix interferences and as a result, allows users to maintain a cleaner instrument for longer periods of time versus alternate techniques like syringe filtration.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <https://doi.org/10.1016/j.forensic.2017.12.018>.

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