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Preface

This study was conducted for the U.S. Army Corps of Engineers under the Department of Defense (DoD) Strategic Environmental Research Development Program (SERDP) Project ER-2722.

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The Commander of ERDC was COL Teresa A. Schlosser and the Director was Dr. David W. Pittman.

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Methods for simultaneous determination of legacy and insensitive munition (IM) constituents in aqueous, soil/sediment, and tissue matrices

ABSTRACT

Currently, no standard method exists for analyzing insensitive munition (IM) compounds in environmental matrices, with or without concurrent legacy munition compounds, resulting in potentially inaccurate determinations. The primary objective of this work was to develop new methods of extraction, pre-concentration, and analytical separation/quantitation of 17 legacy munition compounds along with several additional IM compounds, IM breakdown products, and other munition compounds that are not currently included in U. S. Environmental Protection Agency (EPA) Method 8330B. The eight additional compounds included were nitroguanidine, 3-nitro-1,2,4-triazol-5-one, picric acid, 2,4-dinitroanisole, 2,4-dinitrophenol, 2-nitrophenol, 4-nitrophenol, and new surrogate *ortho*-nitrobenzoic acid (*o*-NBA). Analytical methods were developed to enable sensitive, simultaneous detection and quantitation of the 24 IM and legacy compounds, including two orthogonal high-performance liquid chromatography (HPLC) column separations with either ultraviolet (UV) or mass spectrometric (MS) detection. Procedures were developed for simultaneous extraction of all 24 analytes and two surrogates (1,2-dinitrobenzene, 1,2-DNB; *o*-NBA) from high- and low-level aqueous matrices and solid matrices, using acidification, solid phase extraction (SPE), or solvent extraction (SE), respectively. For low-level aqueous samples extracted by SPE, all compounds were recovered within current Department of Defense Quality Systems Manual (DoD QSM) Ver5.3 accepted limits for aqueous samples analyzed by EPA Method 8330B (57–135%), except NQ, which was consistently recovered at approximately 50%. Likewise, all compounds were recovered from six geographically/geochemically unique soil types within current QSM accepted limits for solid samples analyzed by EPA Method 8330B (64–135%). Further, the majority of compounds were recovered from four tissue types within current limits for solids, with generally low recovery only for Tetryl (from 4 to 62%). A preparatory chromatographic interference removal procedure was adapted for tissue extracts, as various analytical interferences were observed for all studied tissue types.

1. Introduction

As an effort to improve warfighter safety, the U. S. Army is seeking to replace conventional munition constituents, also called legacy munitions, with safer insensitive munition (IM) compounds. However, with increased IM use comes the need for improved detection methods for IM compounds and their daughter products at training ranges, demilitarization and manufacturing facilities, and environmental sites, where they are produced and tested [1,2]. Currently, no standard method exists for analyzing IM compounds in environmental matrices,

with or without concurrent legacy compounds. Current standardized methods for extraction of legacy compounds, such as U. S. Environmental Protection Agency (EPA) Method 8330B, fail to extract some IM compounds and their breakdown products. Moreover, some IM compounds are not well-resolved using current standardized analytical detection methods. Lacking established methods for IM determinations, contract and research laboratories either 1) do not measure IM compounds at all; 2) quantify some but not all IM components, using methods developed for legacy compounds, leading to inaccuracies for certain IM compounds and degradation products [3]; or, 3) use

methods originally developed for individual neat materials, with no proven performance metrics in complex matrices.

In the current work, two orthogonal high-performance liquid chromatography-ultraviolet (HPLC-UV) methods (C18, biphenyl) and one LC-mass spectrometric (MS) method were developed for simultaneous analysis of legacy and IM compounds in environmental matrices. The LC-MS method was developed using an Agilent 6120B single-quadrupole (SQ) MS detector equipped with an atmospheric pressure chemical ionization (APCI) source, located downstream of the primary column HPLC-UV system. The IM, breakdown products, and other additional compounds included were nitroguanidine (NQ), 3-nitro-1,2,4-triazole-5-one (NTO), picric acid (PA), 2,4-dinitroanisole (DNAN), 2,4-dinitrophenol (2,4-DNP), 2-nitrophenol (2-NP), and 4-nitrophenol (4-NP). In addition, one new surrogate (*ortho*-nitrobenzoic acid, *o*-NBA) and one current EPA Method 8330B surrogate (1,2-dinitrobenzene, 1,2-DNB) were included. Extraction methods were developed to simultaneously extract IM and legacy compounds from aqueous (direct-injection and solid-phase extraction, SPE), soil (2-stage solvent extraction, SE), and tissue (1-stage SE) matrices. An analytical interference reduction procedure was modified for tissue extracts.

It is estimated that the simultaneous analysis of IM and legacy compounds using the developed primary and secondary HPLC-UV methods may reduce total analysis time by approximately 50% versus using at least four different analytical methods as would otherwise be required. Moreover, when the primary HPLC-UV method is coupled with downstream MS detection, both UV and MS data are collected during each analysis, reducing analysis time even further. In addition, the expected labor and supply cost savings due to co-extraction and sample preparation is at least 50%, as only a single extraction procedure and analytical sample preparation are required versus at least four (including confirmation analyses and associated quality control (QC) samples), as the sample preparation for the primary and secondary methods are the same. Overall, these savings may lead to greatly reduced laboratory costs, and more rapid data acquisition and processing. The products of this work will contribute toward the ability to conduct fate and transport studies for IM compounds, by providing a standardized method for co-extraction and quantitation of IM and legacy materials that is needed to assess long-term effects.

2. Experimental

2.1. Chemicals, supplies, and source materials

2.1.1. Chemicals

Most of the analyte and surrogate (Table 1) reference standards were obtained from Restek (Bellefonte, PA, USA) and AccuStandard (New Haven, CT, USA). Neat *o*-NBA, DNAN, and NTO were obtained from Sigma Aldrich (St. Louis, MO, USA), Alfa Aesar (Tewksbury, MA, USA), and BAE Systems/Holston Army Ammunition Plant (HAAP)(Kingsport, TN, USA), respectively. Stock solutions of neat materials were prepared in methanol (MeOH). Complete mixed standards containing all analytes and surrogates were prepared at 100 mg/L in a mixture of 1:9 acetonitrile (ACN)-MeOH. LC-MS grade MeOH, ACN, formic acid (FA), and trifluoroacetic acid (TFA) were purchased from Thermo Fisher Scientific (Hampton, NH, USA). All chemicals were used without further purification. Reagent water (18.23 M Ω -cm) was produced at the U. S. Army Engineer Research and Development Center Environmental Laboratory (ERDC-EL) using a Millipore water purifier.

2.1.2. Analysis and extraction supplies

Strata X-A (polymeric strong anion exchange, 500 mg/6 mL) and Strata X (polymeric reverse phase, 500 mg/6 mL) SPE cartridges; Synergi 4 μ m Hydro-RP, 80 Å, 250 $\times$ 4.6mm HPLC columns; and, Security Guard AQ C18 pre-column guard cartridges were purchased from Phenomenex (Torrance, CA, USA). ENVI-Carb™ (granular activated carbon, GAC, 500 mg/6 mL) SPE cartridges and SPE adapters

Table 1

Compounds included in the extraction and analysis methods developed in the current work.

Compound	Acronym	CAS
2-Amino-4,6-dinitrotoluene	2-Am-4,6-DNT	35572-78-2
4-Amino-2,6-dinitrotoluene	4-Am-2,6-DNT	19406-51-0
3,5-Dinitroaniline	3,5-DNA	618-87-1
2,4-Dinitroanisole	DNAN	119-27-7
1,2-Dinitrobenzene (<i>surrogate</i>)	1,2-DNB (<i>surr.</i>)	528-29-0
1,3-Dinitrobenzene	1,3-DNB	99-65-0
2,4-Dinitrophenol	2,4-DNP	51-28-5
2,4-Dinitrotoluene	2,4-DNT	121-14-2
2,6-Dinitrotoluene	2,6-DNT	606-20-2
Hexahydro-1,3,5-trinitro-1,3,5-triazine	RDX	121-82-4
<i>N</i> -Methyl- <i>N</i> -(2,4,6-trinitrophenyl)nitramide	Tetryl	479-45-8
Nitrobenzene	NB	98-95-3
<i>ortho</i>-Nitrobenzoic acid (<i>surrogate</i>)	<i>o</i>-NBA (<i>surr.</i>)	552-16-9
Nitroglycerine	NG	55-63-0
Nitroguanidine	NQ	556-88-7
2-Nitrophenol	2-NP	88-75-5
4-Nitrophenol	4-NP	100-02-7
2-Nitrotoluene	2-NT	88-72-2
3-Nitrotoluene	3-NT	99-08-1
4-Nitrotoluene	4-NT	99-99-0
3-Nitro-1,2,4-triazol-5-one	NTO	932-64-9
Octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine	HMX	2691-41-0
Pentaerythritol tetranitrate	PETN	78-11-5
Picric acid	PA	88-89-1
1,3,5-Trinitrobenzene	1,3,5-TNB	99-35-4
2,4,6-Trinitrotoluene	2,4,6-TNT	118-96-7

Bold, italicized are compounds added to the new methods (compared to EPA Method 8330B).

(Supelco) were purchased from Millipore Sigma (St. Louis, MO, USA). Pinnacle II Biphenyl, 110 Å, 5 μ m, 150 $\times$ 4.6 mm HPLC columns were purchased from Restek (Bellefonte, PA, USA). The 0.45 μ m polytetrafluoroethylene (PTFE) disk filters used for solid matrix extracts were purchased from Fisher Scientific (Pittsburgh, PA, USA).

2.1.3. Aqueous sample sources

Five aqueous sample sources were obtained and tested using the developed extraction and analysis methods. Some characteristic properties are listed in Table 2. The tap water was collected from a laboratory faucet at ERDC-EL connected to the municipal water supply (Vicksburg, MS, USA). Sea water was collected from Houston shipping channel, north of Morgan's Point. All samples were collected in 2018, filtered through 2.7 μ m and 0.7 μ m filters using a Büchner vacuum filtration apparatus, and then stored at 4 °C in amber bottles.

2.1.4. Soil sample sources

Six soil sample sources were obtained and tested using the developed extraction and analysis methods. Some geochemical properties for each are listed in Table 3. The fat clay soil (CH-1) was obtained from the American Society for Testing and Materials (ASTM) Institute for Standards Research (ISR) program. Soils were dried at 25 °C before being ground using a mortar and pestle. The fraction collected by sieving using a standard #20 sieve (< 850 μ m) was stored at 4 °C in amber bottles.

2.1.5. Tissue sample sources

Four tissue types were tested in the current work, including 1) earthworm (*Eisenia fetida*, a soil-dwelling invertebrate), 2) fathead minnow (*Pimephales promelas*, a freshwater vertebrate), 3) polychaete worm (*Alitta virens*, a marine invertebrate), and 4) perennial ryegrass (*Lolium perenne* L., a perennial plant). Earthworms were obtained from The Worm Farm (Durham, CA), polychaete worms were obtained from Aquatic Research Organisms (Hampton, NH), and adult fathead minnows were obtained from Aquatic Biosystems (Fort Collins, CO). Earthworms underwent a 48 h purge period to remove gastrointestinal

Table 2
Characteristic properties of the five aqueous sample sources tested.

Water Source	PH	conductivity ($\mu\text{S}/\text{cm}$)	Total Organic Carbon (TOC) (mg/L)		Total Dissolved Solids (TDS) (mg/L)	
			Average (n = 3)	Std. Dev.	Average (n = 3)	Std. Dev.
Reagent (ERDC-EL)	6.30	3.53	ND	0.00	ND	1.2
Tap (Vicksburg, MS)	9.16	208.1	1.11	0.12	125	1.2
Ground (Rayville, LA)	8.10	456.6	1.31	0.33	264	3.3
Yazoo River (Vicksburg, MS)	8.22	254.2	5.62	0.03	198	2.0
Sea (Houston Shipping Channel)	7.84	13,350	5.14	0.05	8910	7.2

*ND: not detected (< reporting limit value).

contents. Perennial ryegrass Palmer III cultivar seeds (94% germination, 98% pure live seed, 2% inert material) were obtained from La Crosse Seed (La Crosse, WI) and grown in non-contaminated, sandy soil for 28 days.

After harvest, all tissues were carefully cleaned with reagent water, blotted dry, and stored in sample bags at $-20\text{ }^{\circ}\text{C}$ degrees. Prior to use in extraction method development, whole tissues were ground, using either a stainless steel blender mill or a mortar-and-pestle, with liquid nitrogen, to a fine particle size or paste.

2.2. Extraction Methods and Preparation of Extracts for Analysis

Extraction methods were developed to enable simultaneous extraction of the IM and legacy compounds of interest, including methods for high-level aqueous samples, low-level aqueous samples, soil samples, and tissue samples. Care was taken to avoid exposure of all samples to light and elevated temperatures during sample processing, spiking, and extraction.

2.2.1. Method for preparation of high-level aqueous samples for direct-injection analysis

Aqueous samples prepared to contain the compounds of interest at levels detectable without extraction/pre-concentration were analyzed by direct injection. In order to ensure the accurate analysis of ionic species (including NTO and PA), an acidification step was included for high-level aqueous samples with an observed pH $> \sim 6.5$. A solution of 1% HCl/MeOH (vol/vol) was used to acidify and dilute aqueous samples, for a final acid concentration of 0.5% (vol/vol) and final solvent ratio of 1:1 MeOH/water. Samples were stored at $\leq 4\text{ }^{\circ}\text{C}$ prior to analysis for a maximum of 40 days.

2.2.2. SPE method for low-level aqueous samples

Aqueous samples prepared to contain the compounds of interest at low levels were extracted/pre-concentrated using SPE. The SPE cartridges were conditioned with two 5-mL aliquots of MeOH, and then equilibrated with two 5-mL aliquots of reagent water. Approximately 2 mL of reagent water was then added to each conditioned cartridge (to prevent drying) before the cartridges were stacked in the following order for sample loading: 1) Strata X on the top, 2) Strata X-A in the middle, and 3) ENVI-Carb™ on the bottom. Each triple-stacked SPE unit was loaded with 100 mL laboratory-spiked water sample containing all 24 compounds of interest and two surrogates. After allowing sample-loaded SPE cartridges to air-dry under vacuum for approximately 10 min to remove all remaining water, the stacking order of the cartridges was reversed so that 1) ENVI-Carb™ was on the top, 2) Strata X-A was in the middle, and 3) Strata X was on the bottom. Each triple-stacked SPE unit was eluted first with 5 mL MeOH, followed by 5 mL 2% HCl/MeOH. Extracts were collected separately and were stored at $\leq 4\text{ }^{\circ}\text{C}$ prior to analysis for a maximum of 40 days. Samples were prepared for analysis as described below on the same day as analysis.

2.2.3. SE method for soil samples

Soil sample collection, storage, drying, and grinding procedures

were not modified from current EPA Method 8330B procedures. Soil sub-samples were spiked either with a mixed reference standard containing all 24 compounds of interest and two surrogates, and vortexed for 1 min to mix. Samples were allowed to dry again in a convection oven at $25\text{ }^{\circ}\text{C}$ for a maximum of 3 h. Dried samples were homogenized using a horizontal sample roller for up to 18 h at $4\text{ }^{\circ}\text{C}$. Laboratory-spiked soil samples were extracted using a two-stage ultrasonication procedure. Each sample was either 1 g or 2 g, and a mass-to-volume ratio of 1:5 was used. The first sonication stage was carried out using MeOH (6 h), and the second using a 1:1 MeOH/water solution (14 h). Tightly-capped soil-MeOH mixtures were placed in a cooled ultrasonic bath for 6 h, centrifuged, and the supernatant (MeOH extract) collected by syringe filtering through a $0.45\text{ }\mu\text{m}$ hydrophobic PTFE disk filter. Next, 5 mL of 1:1 MeOH/water per gram of soil was added to the same sample container, and the ultrasonication (14 h), centrifugation, and filtering procedures were repeated to collect the 1:1 MeOH/water extracts. Each MeOH and MeOH/water extract was collected separately and were stored at $\leq 4\text{ }^{\circ}\text{C}$ prior to analysis for a maximum of 40 days. Samples were prepared for analysis as described below on the same day as analysis.

2.2.4. SE method for tissue samples

Tissue sub-samples were spiked with a mixed reference standard containing all 24 compounds of interest and two surrogates, and vortexed for 1 min to mix. Laboratory-spiked tissue samples were extracted using a ratio of 1 g wet tissue to 5 mL MeOH via a single 18-h ultrasonication in a cooled ultrasonic bath. Tissue-MeOH mixtures were then centrifuged and the supernatant (MeOH extract) collected by syringe filtering through a $0.45\text{ }\mu\text{m}$ PTFE disk filter. Extracts were stored at $\leq 4\text{ }^{\circ}\text{C}$ prior to analysis for a maximum of 40 days. Samples were prepared for analysis as described below on the same day as analysis. (At cold temperatures, some tissue extracts were observed to develop a precipitate. These samples were filtered further using either $0.45\text{ }\mu\text{m}$ PTFE hydrophobic or $0.20\text{ }\mu\text{m}$ Anotop 10 inorganic membrane disk filters.)

2.2.5. Chromatographic interference reduction method for tissue extracts

An interference reduction method for tissue extracts was adapted from Larson et al., 1999 [4]. Small-scale chromatography columns were prepared using $5\text{ }\frac{3}{4}$ " borosilicate pipettes, loaded with 0.2 g activated silica gel (100–200 mesh). Chromatography columns were wetted with MeOH just before setting each into a separate amber collection vial. The columns remained in their collection vials during column loading and eluting. Onto each column was loaded 1 mL tissue MeOH extract. After the 1 mL MeOH extract had completely passed through the column, 1 mL of MeOH was used to elute, followed by 1 mL 2% HCl/MeOH (vol/vol). Each resulting 3 mL of silica-treated MeOH extract was vortexed to mix. Treated extracts were returned to $\leq 4\text{ }^{\circ}\text{C}$ storage prior to analysis for a maximum of 40 days following the original extraction event. Samples were prepared for analysis as described below on the same day as analysis.

2.2.6. Preparation of extracts for analysis

High-level aqueous samples that had been diluted either with MeOH

Table 3
Characteristic geochemical properties of the six soil sample sources tested.

Name	Collection Site	Region	Classification	% Gravel	% Sand	% Fines	pH	CEC (meq/100g)	AEC (meq/100g)	TOC (mg/kg)	Solids (%)
Aberdeen	Aberdeen Proving Ground (Aberdeen, MD)	Mid Atlantic, Southeast	Clay (CL) with sand	2.9	25.4	71.7	7.12	20.6	51.2	6700	97.9
Jefferson	Jefferson Proving Ground (Madison, IN)	Great Lakes, Midwest	Clay (CL) with sand	0.0	19.7	80.3	4.62	26.2	60.9	1400	97.9
Memphis	Memphis, TN	Mid-South	Silt (ML)	0.0	0.5	99.5	7.56	11.8	45.3	610	98.9
Riley	Ft. Riley (Riley, KS)	Central Plains, Midwest	Clay, trace of sand	0.0	3.3	96.7	5.96	32.9	53.0	15000	98.6
Yuma	Yuma Proving Ground (Yuma, AZ)	Pacific, Southwest	Sand with gravel	35	53.2	11.8	6.98	14.7	47.9	ND ^a	98.4
ASTM Fat Clay (CH-1)	(ASTM-ISR Reference Soils Program)		Clay (CH)	0.0	0.7	99.3	7.45	24.5	24.4	3600	94.1

^a (Reporting limit: 250 mg/kg).

or with HCl/MeOH and stored at ≤ 4 °C as described above were analyzed without further preparation, within 40 days of the dilution event.

For low-level aqueous samples that had been extracted/pre-concentrated by SPE and stored at ≤ 4 °C as described above, the MeOH and acidified MeOH extracts may be analyzed individually, or by combining known ratios of each, and diluting with reagent water, for a final solvent ratio of 1:1 MeOH/water.

For soil samples that had been extracted by the 2-stage SE method and stored at ≤ 4 °C as described above, the MeOH and 1:1 MeOH/water extracts were analyzed either 1) individually, by diluting with reagent water or 1:1 MeOH/water, respectively; or, 2) by combining known ratios of each associated extract and diluting with a 1:3 mixture of MeOH/water, for a final solvent ratio of 1:1 MeOH/water.

For tissue samples that had been extracted using MeOH and stored at ≤ 4 °C as described above, the MeOH extracts were analyzed either before or after silica gel column treatment, by diluting with water for a final solvent ratio of 1:1 MeOH/water.

2.3. Analytical methods

Chromatographic separations were achieved using a MeOH:water gradient over either a modified reverse phase C18 (primary) or bi-phenyl (secondary) standard particle-size HPLC column, described below.

2.3.1. Primary HPLC-UV method

Previously developed [5] and currently employed munition compound analysis methods (EPA Method 8330B, in-house IM methods) were used as references in the development of the primary HPLC-UV method for analysis of the 24 legacy and IM compounds of interest and two surrogates,. The method parameters and solvent gradient scheme are shown in Table 4. Method development was carried out using Phenomenex Synergi 4 μ m Hydro-RP, 80 Å, 250 $\times$ 4.6 mm HPLC columns. An optional Phenomenex SecurityGuard AQ C18 pre-column guard cartridge was included, which extends column lifetime, especially when analyzing complex environmental matrices. As indicated in Table 4, either a 0.1% TFA or 0.25% FA (vol/vol) solution in water can be used, which was tested during LC-MS method development.

2.3.2. Secondary HPLC-UV method

A secondary HPLC-UV method was developed to enable confirmatory analysis of the 24 legacy and IM compounds of interest and two surrogates, using Restek Pinnacle II Biphenyl, 5 μ m, 150 $\times$ 4.6 mm HPLC columns. Again, an optional Phenomenex SecurityGuard AQ C18 pre-column guard cartridge was included. The solvent gradient scheme and other method parameters are shown in Table 5. As indicated again,

Table 4

Primary HPLC-UV separation solvent gradient scheme and other method conditions.

Total Run Time: 48.0 min Detection Wavelengths: 210, 254, 315 nm Column Temperature: 25 °C Injection Volume: 50 μ L Flow Rate: 1.0 mL/min.				
Time (min)	Reagent Water (%)	MeOH (%)	0.1% TFA/Water (%) ^a	ACN (%)
0.00	89	3	3	5
2.00	89	3	3	5
2.20	52	40	3	5
12.5	52	40	3	5
19.0	57	35	3	5
28.0	48	44	3	5
32.0	48	44	3	5
44.0	32	60	3	5
44.1	89	3	3	5
48.0	89	3	3	5

^a Or 0.25% FA/water.

Table 5

Secondary HPLC-UV separation solvent gradient scheme and other method conditions.

Total Run Time: 35.0 min Detection Wavelengths: 210, 254, 315 nm

Column Temperature: 25 °C Injection Volume: 50 µL Flow Rate: 0.9 mL/min.

Time (min)	Reagent Water (%)	MeOH (%)	0.1% TFA/Water (%) ^a	ACN (%)
0.00	75	10	10	5
2.50	75	10	10	5
2.60	39	46	10	5
9.00	39	46	10	5
9.10	33.5	51.5	10	5
15.0	44	41	10	5
29.0	25	60	10	5
29.1	75	10	10	5
35.0	75	10	10	5

^a Or 0.25% FA/water.

either a 0.1% TFA or 0.25% FA (vol/vol) solution in water can be used.

2.3.3. LC-MS method

An LC-MS method was developed using an Agilent 6120B SQ MS detector equipped with an APCI source, located downstream of the primary column HPLC-UV system, for confirmatory analysis of the 24 compounds of interest and two surrogates. Chromatographic separation was effected by the primary HPLC method described above. A scan mode method was developed first and then adapted into a selective ion monitoring (SIM) mode method to increase sensitivity. The APCI source was used in negative mode, with drying gas at 350 °C and 4.0 L/min, vaporizer at 325 °C, nebulizer at 40 psig, corona at 10 µA, capillary at -1500 V, mass range of 40–500 m/z, fragmentor at 50, gain at 1.00, threshold at 150, step size at 0.10, speed at 473 µ/sec, peak width at 0.20 min, and cycle time at 1.60 s/cycle. Several ion masses were observed for each of the 26 compounds, except nitrobenzene (NB), nitroglycerin (NG), and the nitrotoluenes (2-NT, 3-NT, and 4-NT), which did not produce any detectable MS signal. For 1,3-dinitrobenzene (1,3-DNB), just a single ion was observed. Ions were identified where possible. The most abundant ions are listed in Table 6.

3. Results and discussion

3.1. Analytical methods

3.1.1. Primary HPLC-UV method

The primary HPLC-UV method mobile phase gradient, modifier (acid and ACN) concentrations, and sample preparation protocols were methodically investigated for optimal method performance [10]. An example chromatogram of a mixed standard containing 5 mg/L of the 24 compounds of interest and two surrogates analyzed by the primary HPLC-UV method is shown in Fig. 1. Compound identities (obtained by analysis of individual reference standards), method detection limits (MDLs), and linear dynamic ranges (LDRs) are provided in Table 7. LDR values represent the highest standard analyzed within ± 10% of the nominal concentration, with 80 mg/L being the highest tested concentration. Some compounds were detected at multiple wavelengths. In some cases, detection of NTO and PA at 315 nm was preferred to minimize analytical interferences, especially in tissue matrices. The PA chromatographic peak at 315 nm is inverted and lies below the baseline when a reference wavelength of 360 nm is used, due to its broad UV absorption below 500 nm; however, a reference wavelength of approximately 500–600 nm results in a peak above the baseline. The same 26-compound standard was analyzed using current primary methods employed at ERDC-EL for analysis of legacy munition compounds (based on EPA Method 8330B) and IM compounds [5]. Not all compounds were resolved by either of the current methods, as shown in Fig. 2.

Table 6

Retention times (min), ion masses (m/z) and identities obtained for mixed standard containing 24 legacy and IM compounds and two surrogates using the developed LC-MS methods.

Retention Time (min)	Compound	Ion Mass (m/z)	Ion Identity
2.74	NQ	103	[M - H]-
4.28	NTO	129	[M - H]-
7.42	o-NBA (surrogate)	166	[M - H]-
8.74	HMX	341	[M-H + FA]-
12.33	RDX	267	[M-H + FA]-
14.25	PA	228	[M - H]-
14.58	4-NP	138	[M - H]-
15.52	1,3,5-TNB	213	[M]-
16.80	2,4-DNP	183	[M - H]-
19.53	1,2-DNB (surrogate)	168	[M]-
20.82	1,3-DNB	168	[M]-
22.00	2-NP	138	[M - H]- (weak)
-	NB	Not detected	
23.45	3,5-DNA	183	[M]-
25.56	DNAN	183	[M - CH ₃]-
26.84	Tetryl	241	[M-NO ₂]-
-	NG	Not detected	
28.68	2,4,6-TNT	226	[M - H]-
31.45	4-Am-2,6-DNT	196	[M - H]-
32.16	2-Am-4,6-DNT	196	[M - H]-
34.134	2,6-DNT	182	[M]-
34.75	2,4-DNT	181	[M - H]-
-	2-NT	Not detected	
-	4-NT		
-	3-NT		
43.87	PETN	62	NO ₃ ⁻

Bold, italicized are compounds added to the new method (compared to EPA Method 8330B). [6,7,8,9].

3.1.2. Secondary HPLC-UV method

The secondary HPLC-UV method employs a biphenyl column to achieve analyte separation, resulting in a significantly different elution order versus the primary column (modified C18) method for analyte confirmation. An example chromatogram of a mixed standard containing 10 mg/L of the 24 compounds of interest and two surrogates analyzed by the secondary HPLC-UV method is shown in Fig. 3. The mobile phase gradient was adjusted for optimal resolution of some closely-eluting analytes, including octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX), Hexa-hydro-1,3,5-trinitro-1,3,5-triazine (RDX), and 4-NP [6]. While NQ and PA became better resolved through mobile phase gradient method optimization, the chromatography challenges with these two analytes were not entirely eliminated. Therefore, an additional option for improved chromatography was to utilize alternate detection wavelengths, such as 254 nm for NQ and 315 nm for PA, where void peaks do not interfere with analyte peaks, as the longer wavelengths were sufficiently distant from the MeOH UV cutoff (205 nm). The same 26-compound standard was analyzed using current secondary methods employed at ERDC-EL for analysis of munition compounds. Not all compounds were resolved by either of the current methods, as shown in Fig. 4.

3.1.3. LC-MS method

The MS detector used for method development was located downstream of the primary HPLC-UV system, so that upon separation by the primary column method, compounds were detected by UV and then MS. The mobile phase gradient and other HPLC method parameters were unchanged from the primary method description above, except that FA was highly preferred in MS detection versus TFA, due to the extreme ionization suppression observed when TFA was used. The MS scan mode method was adapted into a SIM mode method in order to improve sensitivity by selectively detecting the most abundant ion masses observed in scan mode experiments. The experimentally-

Fig. 1. HPLC chromatograms of a mixed standard containing 5 mg/L of 24 legacy and IM compounds and two surrogates analyzed by the developed primary HPLC-UV method. **Bold, italicized** are compounds added to the new method (compared to EPA Method 8330B).

acquired ion masses (Table 6), were used to create ion/time windows for the SIM method, shown in Table 8. The SIM windows were optimized, based upon precise retention times, sometimes requiring slight adjustment, such as when the HPLC column was changed. Fragmentor settings were optimized for each ion. Depending upon the ion, signals in SIM mode were between $1.5 \times - 4.5 \times$ higher than those observed in scan mode. Further adaptation/optimization of the MS parameters may be necessary, especially in different instrument models/software systems.

3.2. Extraction methods

3.2.1. Method for preparation of aqueous samples for direct-injection analysis

During experiments with high-level aqueous samples, much lower recoveries for NTO and other ionic compounds were observed for field waters than for reagent water (data not shown). This phenomenon appeared to be pH-related, with lower recoveries for higher pH samples (Table 2). Further experiments were performed in which the pH was

Table 7

MDLs and LDR Upper Limits (ULs) for the 24 compounds of interest and two surrogates, analyzed by the primary HPLC-UV method.

Peak #	Compound	Retention Time (min)	Direct-Injection Water MDL (n = 10 at 40 µg/L) (µg/L)	MDL in Reagent Water with SPE (n = 10 at 40 µg/L) (µg/L)	MDL in Ottawa Sand (n = 10 at 4 mg/kg) (mg/kg)	LDR UL (mg/L)
1	NQ	3.51	16	12	2.64	≥80
2	NTO (210 nm)	4.29	6	20	1.26	≥50
2	NTO (315 nm)	4.29	9	15	0.82	≥60
3	o-NBA (surrogate)	7.45	12	49	0.32	≥80
4	HMX	8.96	6	11	1.18	≥70
5	RDX	12.81	6	9	0.66	≥70 ^a
6	PA (210 nm)	14.64	10	23	3.88	≥70 ^a
6	PA (315 nm)	14.58	12	11	1.44	≥80 ^b
7	4-NP	15.31	10	7	0.94	≥80
8	1,3,5-TNB	16.17	11	13	1.14	≥80
9	2,4-DNP	17.86	9	22	4.42	≥80
10	1,2-DNB (surrogate)	20.35	15	16	2.38	≥70
11	1,3-DNB	21.80	7	12	0.94	≥80
12	2-NP	22.91	12	14	1.26	≥80
13	NB	25.42	9	10	1.74	≥80
14	3,5-DNA (210 nm)	26.16	13	16	3.02	≥70
14	3,5-DNA (254 nm)	25.93	13	13	0.38	≥70
15	DNAN	26.89	11	16	1.58	≥70
16	Tetryl	28.43	12	8	2.08	≥70
17	NG	29.30	17	27	5.06	≥70
18	2,4,6-TNT	30.06	15	11	2.14	≥70
19	4-Am-2,6-DNT	32.60	11	17	2.56	≥80
20	2-Am-4,6-DNT	33.43	11	22	2.50	≥80
21	2,6-DNT	35.25	14	37	2.82	≥70
22	2,4-DNT	35.57	7	12	0.70	≥80
23	2-NT	40.18	13	14	1.08	≥80
24	4-NT	41.42	12	16	2.00	≥80
25	3-NT	42.84	14	21	0.84	≥80
26	PETN	44.38	16	22	3.46	≥80

Bold, italicized are compounds added to the new method (compared to EPA Method 8330B).

^a Co-elute at concentrations > 30 mg/L.

^b RDX not detected appreciably at 315 nm.

Fig. 2. HPLC chromatograms of a mixed standard containing 5 mg/L of 24 legacy and IM compounds and two surrogates analyzed by the current or new primary methods.

adjusted using a solution of 2% HCl/MeOH, the same used for stage two of SPE elution. Samples were diluted, for final acid concentrations either equal to (0.5%, vol/vol) or double (1%, vol/vol) that of corresponding SPE extracted/pre-concentrated samples. For each field water source, acidification with HCl resulted in near-100% recovery for the ionic compounds. Moreover, an acid concentration of 0.5% was sufficient, and 1% acid was not necessary. Overall, for aqueous samples to be analyzed by direct injection with pH $\geq$ 6.5 (approximately), acidification was beneficial.

3.2.2. SPE method for aqueous samples

EPA Method 8330B and current ERDC-EL SPE procedures were modified to enable extraction and pre-concentration of all 24 compounds of interest (and two surrogates) from low-level aqueous samples. A series of cation-exchange, anion-exchange, reverse phase, and GAC-based SPE cartridges were evaluated for their ability individually to recover the 26 compounds from laboratory-spiked reagent water samples. Cartridges with widely differing chemistries were investigated due to the widely varying chemistries of the analytes of interest. These

Fig. 3. HPLC chromatograms of a mixed standard containing 10 mg/L of 24 legacy and IM compounds and two surrogates analyzed by the developed secondary HPLC-UV method. **Bold, italicized** are compounds added to the new method (compared to EPA Method 8330B).

Fig. 4. HPLC chromatograms of a mixed standard containing 5 mg/L of 24 legacy and IM compounds and two surrogates analyzed by the current or new primary methods.

Table 8
SIM mode method windows.

SIM Window Start Time ^a (min)	SIM Window Group #	Compound	SIM Ion (m/z)	Fragmentor	Dwell Time (ms)
0.00	1	NQ	103	100	294
		NTO	129	100	294
6.00	2	o-NBA	166	50	294
		(surrogate)			
		HMX	341	50	294
11.00	3	4-NP	138	70	116
		2,4-DNP	183	70	116
		1,3,5-TNB	213	70	116
		PA	228	70	116
		RDX	267	50	116
19.00	4	1,2-DNB	138	70	83
		(surrogate), 2-NP			
		1,3-DNB	168	70	83
		3,5-DNA	182	70	83
		DNAN	183	70	83
		2,4,6-TNT	226	80	83
		Tetryl	241	80	83
31.00	5	2,4-DNT	181	100	195
		2,6-DNT	182	100	195
		4-Am-2,6-DNT,	196	80	195
		2-Am-4,6-DNT			
39.00	6	PETN	62	40	294
		2-NT, 3-NT, 4-NT	136	100	294

Bold, italicized are compounds added to the new method (compared to EPA Method 8330B).

^a SIM window start time = LC-MS Method run time (no MS delay).

included, among others, the 1) Phenomenex Strata X polymeric reverse phase 500 mg (PRP500), 2) Phenomenex Strata X-A polymeric strong anion exchanger 500 mg (PSA500), 3) Supelco C18 1 g (C181G), and 4) Supelco GAC 250 mg and 500 mg (GAC250, GAC500). It was expected that the majority of analytes would be extracted by a reverse phase material, while the anionic analytes (especially PA and NTO) would require the use of an anionic exchange resin. Additional materials, such as GAC and modified GAC were investigated in an effort to improve NQ extraction efficiency. Based on results with individual SPE cartridges [10], several double- and triple-stacked options were tested, in order to identify a sequential option that yielded acceptable recoveries for all compounds.

The stacking order for sample loading and elution was reversed to prevent irreversible binding of analytes to incompatible SPE cartridge matrices, which was observed during evaluation of individual cartridges. Results for six stacked options (A-F) are listed in Table 9, where loading-stage stacking order from top to bottom is indicated. Based on results of stability studies in water and soil (data not shown), option B was selected as the best multi-cartridge option for recovery of all compounds, with NQ recovery consistently near 50%. However, when NQ is likely absent or not of interest, inclusion of a GAC cartridge is not necessary. In some cases, using a single PRP500 (Strata X) or PSA500 (Strata X-A) cartridge may be sufficient, and may be investigated, based on the analytes of interest for a particular project or laboratory.

3.2.3. SE method for soils

EPA Method 8330B and current ERDC-EL SE methods were modified to enable extraction of all 24 compounds of interest (and two surrogates) from soil samples. Preliminary trials indicated that traditional EPA Method 8330B solid matrix extraction methods (i.e., a single 18-h ultrasonication period using ACN) would be insufficient to extract

Table 9

Compound recoveries (%) from laboratory-spiked reagent water samples using stacked SPE cartridges for extraction/pre-concentration of the 24 compounds of interest and 1,2-DNB surrogate.

Compound	Recoveries (%)					
	A PRP500 PSA500 GAC250	B PRP500 PSA500 GAC500	C C181G PSA500 GAC250	D C181G PSA500 GAC500	E PSA500 C181G GAC250	F PSA500 GAC250
<i>NQ</i>	2	19	48	4	43	38
<i>NTO (210 nm)</i>	107	98	109	103	107	111
HMX	97	95	117	102	113	110
RDX	91	92	114	99	107	109
<i>PA (210nm)</i>	94	86	96	67	102	101
<i>4-NP</i>	87	90	110	97	103	104
1,3,5-TNB	93	91	113	100	106	109
<i>2,4-DNP</i>	93	94	110	93	112	115
1,2-DNB (surrogate)	90	88	101	97	95	98
1,3-DNB	90	87	113	97	106	109
<i>2-NP</i>	76	76	95	85	91	91
NB	92	90	114	100	107	109
3,5-DNA (254 nm)	79	84	111	80	105	107
<i>DNAN</i>	77	84	106	93	102	105
Tetryl	94	88	109	96	71	81
NG	94	103	114	101	184	108
2,4,6-TNT	93	89	113	100	96	99
4-Am-2,6-DNT	93	92	112	100	105	108
2-Am-4,6-DNT	95	92	113	99	106	108
2,6-DNT	85	86	115	99	109	113
2,4-DNT	97	89	105	96	99	95
2-NT	74	75	108	71	101	105
4-NT	78	77	109	77	102	104
3-NT	77	76	112	74	101	105
PETN	89	89	110	98	101	105

Green: 75–125%; Light Green: 57–74% or 126–135%; Red: < 57% or > 135%.

(DoD QSM Ver5.3 for Waters – Lowest LCL: MNX, 57%; Highest UCL: HMX, 135%).

Bold, italicized are compounds added to the new method (compared to EPA Method 8330B).

all compounds, supported by previous studies [3,11]. Thus, several different solvents, ultrasonication times, and multi-stage extraction procedures were investigated.

Method development was carried out using six geochemically/geographically diverse soils (Table 3). Various 2- or 3-stage ultrasonication options were tested [10]. Several schemes were successful for each soil. Including water as an extraction solvent resulted in improved recoveries for NQ, NTO, HMX, and RDX across a broad range of soil types, which is thought to be due, at least in part, to an increased degree of physical dispersion of the soil in water versus MeOH. Ultimately, a 6 h MeOH extraction, followed by a 14 h 1:1 MeOH/water extraction was selected (data shown in Table 10). For cases in which NQ, NTO, HMX, and RDX are likely absent or not of interest, including water as an extraction solvent may not be necessary; in such cases, a single MeOH extraction may be sufficient.

3.2.4. SE method for tissues

EPA Method 8330B and current ERDC-EL SE methods were modified to enable extraction of all 24 compounds of interest (and two surrogates) from tissue samples. Experiments led to the adoption of a single 18-h MeOH ultrasonication, with efficient extraction of the majority of compounds within the accepted range for EPA Method 8330B solid matrices, according to DoD QSM Ver5.3 [10]). It is thought that

the inherent water content of the wet tissue matrices eliminates the need for addition of water as an extraction solvent.

3.2.5. Chromatographic interference reduction method for tissue extracts

All four tissue types suffered from varying degrees of analytical interference due to co-eluting non-target extracted components, necessitating use of an interference reduction method adapted from Larson et al. [4]. Five column packing schemes were tested for their ability to remove interferents from post-extraction spiked extracts, including different combinations of neutral or basic alumina, florisil, and silica gel (data not shown). Silica gel was selected for further development, as it efficiently reduced the majority of interferences from all tissue types, and did not adversely affect analytes (especially, NQ), as observed for other materials. The MeOH extracts from pre-extraction spiked tissues were submitted to silica gel chromatography treatment, with results shown in Table 11. Recoveries for most compounds were within current DoD QSM limits, with consistently low recovery observed only for Tetryl. Interestingly, Tetryl recovery was significantly greater from plant than from animal tissue. Again, detection at higher wavelengths was preferred for some analytes, as much greater levels of interferences were observed at 210 nm, even following silica gel treatment.

Table 10

Recoveries for six soils spiked at 20 mg/kg for each of the 24 compounds and two surrogates.

Compound	Recoveries (%)											
	Aberdeen		Jefferson		Memphis		Riley		Yuma		ASTM Fat Clay	
	Avg (n = 3)	Std Dev	Avg (n = 3)	Std Dev	Avg (n = 3)	Std Dev	Avg (n = 3)	Std Dev	Avg (n = 3)	Std Dev	Avg (n = 3)	Std Dev
NQ	90	1.6	85	0.7	93	1.7	79	4.0	98	1.4	82	1.8
NTO (210 nm)	76	0.8	72	1.8	105	2.2	129	3.1	104	0.9	97	0.5
NTO (315 nm)	64	0.9	66	1.7	98	1.5	84	2.0	100	1.2	90	0.7
o-NBA (surrogate)	95	1.0	75	2.2	102	0.7	86	1.2	105	1.7	93	1.1
HMX	83	2.6	70	1.0	91	1.0	55	2.7	86	2.6	56	1.0
RDX	89	3.8	76	1.2	93	1.5	66	2.2	96	1.5	65	2.0
PA (210 nm)	84	1.5	87	7.5	90	4.4	82	4.7	99	6.2	82	2.4
PA (315 nm)	88	2.3	85	1.2	94	2.0	79	2.9	98	1.4	89	0.9
4-NP	91	1.4	90	2.7	94	1.5	86	1.0	99	2.0	92	2.0
1,3,5-TNB	93	1.1	84	1.0	100	1.1	82	0.4	106	1.5	92	1.5
2,4-DNP	95	1.6	82	1.8	99	1.1	80	2.0	103	6.7	91	2.7
1,2-DNB (surrogate)	98	0.6	96	0.5	100	2.3	95	1.8	103	2.2	97	0.5
1,3-DNB	99	0.3	98	0.7	102	0.8	94	0.9	107	1.4	99	0.7
2-NP	94	0.4	90	0.1	84	1.0	91	1.7	93	1.6	85	0.9
NB	97	0.7	86	0.3	100	1.5	77	0.8	105	1.2	96	0.5
3,5-DNA (210 nm)	93	2.7	92	1.3	77	3.8	93	3.2	90	0.5	92	0.9
3,5-DNA (254 nm)	93	0.3	91	0.9	76	1.6	93	1.5	88	0.7	91	1.0
DNAN	101	1.8	100	3.0	105	4.0	94	1.5	112	1.6	101	0.1
Tetryl	90	2.8	97	0.8	89	1.7	84	1.0	99	1.1	89	3.4
NG	96	4.7	94	2.1	97	2.5	90	1.6	107	2.6	99	4.7
2,4,6-TNT	95	4.0	94	0.4	99	2.3	86	3.2	107	2.1	94	2.8
4-Am-2,6-DNT	96	1.5	87	1.7	99	1.0	73	0.8	103	1.3	95	1.2
2-Am-4,6-DNT	98	2.3	94	2.0	101	2.4	86	1.5	103	2.6	97	0.6
2,6-DNT	102	4.5	102	5.1	107	4.4	99	3.7	109	4.0	102	4.4
2,4-DNT	96	0.8	96	0.9	100	1.2	89	0.6	106	2.8	96	0.2
2-NT	94	1.2	92	0.3	80	1.7	93	1.2	89	0.8	92	1.4
4-NT	94	1.8	91	2.7	82	2.3	91	3.9	89	1.4	88	1.1
3-NT	92	1.2	91	1.6	76	1.7	90	2.8	86	1.8	88	1.0
PETN	94	3.1	94	3.1	96	4.4	88	2.7	99	7.4	98	0.9

Green: 75–125%; Light Green: 64–74% or 126–135%; Red: < 64% or > 135%.

(DoD QSM Ver5.3 for Solid Samples – Lowest LCL: 4-Am-2,6-DNT, 64%; Highest UCL: Tetryl, 135%) Bold, italicized are compounds added to the new method (compared to EPA Method 8330B).

4. Conclusion

In the current work, new methods were developed for the extraction, pre-concentration, and analytical separation and quantitation of 17 legacy and seven additional IM compounds, IM daughter products, and other munition constituents absent from EPA Method 8330B. The objective was to produce a single, standardized method for simultaneous analysis of legacy and IM compounds in environmental matrices. Extraction methods were developed for high- and low-level aqueous (direct-injection and SPE, respectively), soil (2-stage SE), and tissue (1-stage SE) matrices, as well as an interference reduction method for tissue extracts. HPLC-UV and LC-MS methods were developed, including two separate HPLC column separation methods with UV detection, along with an MS detection option (scan and SIM), enabling orthogonal confirmation of analyte concentrations down to low- $\mu\text{g/L}$, or sub- $\mu\text{g/L}$ levels, for samples pre-concentrated by SPE. Compound recoveries were within currently accepted limits for almost all 24 analytes of interest and two surrogates in all tested matrices, with the recoveries for many of the compounds consistently better than current methods. The few exceptions included low recovery of NQ by SPE and low recovery of Tetryl from tissues.

Development of the new methods was necessary to assist in monitoring co-occurring components at training ranges, demilitarization and manufacturing facilities, and environmental sites [1,2]. Implementing the new methods should lead to cost savings with a minimal increase in environmental monitoring efforts and no modification to existing sample collection or processing procedures. The simultaneous extraction and subsequent analysis of all 24 analytes of interest may

reduce total extraction, sample preparation, and analysis time by 50% or more when compared to the current at least four separate methods for these analytes.

The method development executed in the current work under Strategic Environmental Research and Development Program (SERDP) project ER-2722 continues under ESTCP project ER19-5078 to validate the new methods. The validation will consist of round-robin comparison studies, carried out with the cooperation of EPA, with the end goal of producing an addendum to current EPA Method 8330B. Participants will include government, commercial, academic, and manufacturing laboratories and facilities. The standardization and publication of the validated methods will enable laboratories to perform the standardized methodology on a broad scale, and it will facilitate the conduct of fate and transport studies for IM compounds by providing a standardized method for co-extraction and quantitation of IM and legacy materials.

Table 11

Recoveries (%) from pre-extraction spiked tissue MeOH extracts, following chromatographic treatment with silica gel for matrix-related analytical interference reduction.

Compound	Recoveries (%)			
	Fathead Minnow	Polychaete Worm	Earthworm	Ryegrass
NQ	113	73	86	68
NTO (210 nm)	8477	5318	4503	149
NTO (315 nm)	87	113	98	133
o-NBA (surrogate)	101	82	111	67
HMX	81	66	79	99
RDX	86	66	93	85
PA (210 nm)	79	73	37	79
PA (315 nm)	66	66	68	71
4-NP	83	71	72	84
1,3,5-TNB	84	52	3670	83
2,4-DNP	110	72	81	93
1,2-DNB (surrogate)	93	72	82	73
1,3-DNB	76	65	65	86
2-NP	105	81	74	94
NB	84	94	83	90
3,5-DNA (210 nm)	183	139	73	90
3,5-DNA (254 nm)	69	75	80	73
DNAN	123	72	86	76
Tetryl	16	6	4	62
NG	116	132	87	125
2,4,6-TNT	119	100	80	80
4-Am-2,6-DNT	78	57	74	88
2-Am-4,6-DNT	102	67	88	85
2,6-DNT	110	71	79	85
2,4-DNT	108	67	69	88
2-NT	129	101	68	89
4-NT	95	73	69	80
3-NT	64	64	68	86
PETN	109	74	67	91

Green: 75–125%; Light Green: 64–74% or 126–135%; Red: < 64% or > 135%. (DoD QSM Ver5.3 for Solids – Lowest LCL: 4-Am-2,6-DNT, 64%; Highest UCL: Tetryl, 135%).

Bold, italicized are compounds added to the new method (compared to EPA Method 8330B).

Acknowledgments

The use of trade, product, or firm names in this report is for descriptive purposes only and does not imply endorsement by the U.S. Government. The tests described and the resulting data presented herein, unless otherwise noted, were obtained from research conducted under SERDP project ER-2722, a DoD program. Permission was granted to publish this information by the Chief of Engineers. The findings of this report are not to be construed as an official Department of the Army position unless so designated by other authorized documents. The authors thank Drs. Lee Moores and Brooke Stevens of ERDC-EL for their editorial comments.

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14. ABSTRACT Currently, no standard method exists for analyzing insensitive munition (IM) compounds in environmental matrices, with or without concurrent legacy munition compounds, resulting in potentially inaccurate determinations. The primary objective of this work was to develop new methods of extraction, pre-concentration, and analytical separation/quantitation of 17 legacy munition compounds along with several additional IM compounds, IM breakdown products, and other munition compounds that are not currently included in U.S. Environmental Protection Agency (EPA) Method 8330B. Analytical methods were developed to enable sensitive, simultaneous detection and quantitation of the 24 IM and legacy compounds, including two orthogonal high-performance liquid chromatography (HPLC) column separations with either ultraviolet (UV) or mass spectrometric (MS) detection. Procedures were developed for simultaneous extraction of all 24 analytes and two surrogates (1,2-dinitrobenzene, 1,2-DNB; o-NBA) from high- and low-level aqueous matrices and solid matrices, using acidification, solid phase extraction (SPE), or solvent extraction (SE), respectively. The majority of compounds were recovered from four tissue types within current limits for solids, with generally low recovery only for Tetraol (from 4 to 62%). A preparatory chromatographic interference removal procedure was adapted for tissue extracts, as various analytical interferences were observed for all studied tissue types.					
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