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1 **Methods for Simultaneous Determination of Legacy and Insensitive Munition (IM) Constituents in**
2 **Aqueous, Soil/Sediment, and Tissue Matrices**
3

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14

15 **Abstract**
16 Currently, no standard method exists for analyzing insensitive munition (IM) compounds in
17 environmental matrices, with or without concurrent legacy munition compounds, resulting in potentially
18 inaccurate determinations. The primary objective of this work was to develop new methods of extraction,
19 pre-concentration, and analytical separation/quantitation of 17 legacy munition compounds along with
20 several additional IM compounds, IM breakdown products, and other munition compounds that are not
21 currently included in U. S. Environmental Protection Agency (EPA) Method 8330B. The eight additional
22 compounds included were nitroguanidine, 3-nitro-1,2,4-triazol-5-one, picric acid, 2,4-dinitroanisole, 2,4-
23 dinitrophenol, 2-nitrophenol, 4-nitrophenol, and new surrogate *ortho*-nitrobenzoic acid (*o*-NBA).
24 Analytical methods were developed to enable sensitive, simultaneous detection and quantitation of the 24
25 IM and legacy compounds, including two orthogonal high-performance liquid chromatography (HPLC)
26 column separations with either ultraviolet (UV) or mass spectrometric (MS) detection. Procedures were
27 developed for simultaneous extraction of all 24 analytes and two surrogates (1,2-dinitrobenzene, 1,2-
28 DNB; *o*-NBA) from high- and low-level aqueous matrices and solid matrices, using acidification, solid
29 phase extraction (SPE), or solvent extraction (SE), respectively. For low-level aqueous samples extracted
30 by SPE, all compounds were recovered within current Department of Defense Quality Systems Manual
31 (DoD QSM) Ver5.3 accepted limits for aqueous samples analyzed by EPA Method 8330B (57-135%),
32 except NQ, which was consistently recovered at approximately 50%. Likewise, all compounds were
33 recovered from six geographically/geochemically unique soil types within current QSM accepted limits
34 for solid samples analyzed by EPA Method 8330B (64-135%). Further, the majority of compounds were
35 recovered from four tissue types within current limits for solids, with generally low recovery only for
36 Tetryl (from 4-62%). A preparatory chromatographic interference removal procedure was adapted for
37 tissue extracts, as various analytical interferences were observed for all studied tissue types.
38

39 **Keywords**
40 legacy munition constituents, insensitive munition constituents (IM), EPA Method 8330B, high-
41 performance liquid chromatography (HPLC), solid phase extraction (SPE), solvent extraction (SE)
42

43 ## Introduction
44
45 As an effort to improve warfighter safety, the U. S. Army is seeking to replace conventional munition
46 constituents, also called legacy munitions, with safer insensitive munition (IM) compounds. However,
47 with increased IM use comes the need for improved detection methods for IM compounds and their
48 daughter products at training ranges, demilitarization and manufacturing facilities, and environmental
49 sites, where they are produced and tested [1,2]. Currently, no standard method exists for analyzing IM
50 compounds in environmental matrices, with or without concurrent legacy compounds. Current
51 standardized methods for extraction of legacy compounds, such as U. S. Environmental Protection

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52 Agency (EPA) Method 8330B, fail to extract some IM compounds and their breakdown products.
53 Moreover, some IM compounds are not well-resolved using current standardized analytical detection
54 methods. Lacking established methods for IM determinations, contract and research laboratories either 1)
55 do not measure IM compounds at all; 2) quantify some but not all IM components, using methods
56 developed for legacy compounds, leading to inaccuracies for certain IM compounds and degradation
57 products [3]; or, 3) use methods originally developed for individual neat materials, with no proven
58 performance metrics in complex matrices.
59
60 In the current work, two orthogonal high-performance liquid chromatography-ultraviolet (HPLC-UV)
61 methods (C18, biphenyl) and one LC-mass spectrometric (MS) method were developed for simultaneous
62 analysis of legacy and IM compounds in environmental matrices. The LC-MS method was developed
63 using an Agilent 6120B single-quadrupole (SQ) MS detector equipped with an atmospheric pressure
64 chemical ionization (APCI) source, located downstream of the primary column HPLC-UV system. The
65 IM, breakdown products, and other additional compounds included were nitroguanidine (NQ), 3-nitro-
66 1,2,4-triazole-5-one (NTO), picric acid (PA), 2,4-dinitroanisole (DNAN), 2,4-dinitrophenol (2,4-DNP), 2-
67 nitrophenol (2-NP), and 4-nitrophenol (4-NP). In addition, one new surrogate (*ortho*-nitrobenzoic acid, *o*-
68 NBA) and one current EPA Method 8330B surrogate (1,2-dinitrobenzene, 1,2-DNB) were included.
69 Extraction methods were developed to simultaneously extract IM and legacy compounds from aqueous
70 (direct-injection and solid-phase extraction, SPE), soil (2-stage solvent extraction, SE), and tissue (1-stage
71 SE) matrices. An analytical interference reduction procedure was modified for tissue extracts.
72
73 It is estimated that the simultaneous analysis of IM and legacy compounds using the developed primary
74 and secondary HPLC-UV methods may reduce total analysis time by approximately 50% versus using at
75 least four different analytical methods as would otherwise be required. Moreover, when the primary
76 HPLC-UV method is coupled with downstream MS detection, both UV and MS data are collected during
77 each analysis, reducing analysis time even further. In addition, the expected labor and supply cost savings
78 due to co-extraction and sample preparation is at least 50%, as only a single extraction procedure and
79 analytical sample preparation are required versus at least four (including confirmation analyses and
80 associated quality control (QC) samples), as the sample preparation for the primary and secondary
81 methods are the same. Overall, these savings may lead to greatly reduced laboratory costs, and more rapid
82 data acquisition and processing. The products of this work will contribute toward the ability to conduct
83 fate and transport studies for IM compounds, by providing a standardized method for co-extraction and
84 quantitation of IM and legacy materials that is needed to assess long-term effects.
85

86 Experimental

87

88 *Chemicals, Supplies, and Source Materials*

89

90 Chemicals

91

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

102 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 (*surrogate*)	1,2-DNB (surr.)	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
N-Methyl-*N*-(2,4,6-trinitrophenyl)nitramide	Tetryl	479-45-8
Nitrobenzene	NB	98-95-3
ortho-Nitrobenzoic acid (surrogate)	**o-*NBA (surr.)***	***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

103 ***Bold, italicized*** are compounds added to the new methods (compared to EPA Method 8330B).
104
105
106 **Analysis and Extraction Supplies**
107
108 Strata X-A (polymeric strong anion exchange, 500 mg/6 mL) and Strata X (polymeric reverse phase, 500
109 mg/6 mL) SPE cartridges; Synergi 4µm Hydro-RP, 80Å, 250 x 4.6mm HPLC columns; and, Security
110 Guard AQ C18 pre-column guard cartridges were purchased from Phenomenex (Torrance, CA, USA).
111 ENVI-Carb™ (granular activated carbon, GAC, 500 mg/6 mL) SPE cartridges and SPE adapters
112 (Supelco) were purchased from Millipore Sigma (St. Louis, MO, USA). Pinnacle II Biphenyl, 110Å,
113 5µm, 150 X 4.6 mm HPLC columns were purchased from Restek (Bellefonte, PA, USA). The 0.45 µm
114 polytetrafluoroethylene (PTFE) disk filters used for solid matrix extracts were purchased from Fisher
115 Scientific (Pittsburgh, PA, USA).
116
117 **Aqueous Sample Sources**
118
119 Five aqueous sample sources were obtained and tested using the developed extraction and analysis
120 methods. Some characteristic properties are listed in Table 2. The tap water was collected from a
121 laboratory faucet at ERDC-EL connected to the municipal water supply (Vicksburg, MS, USA). Sea
122 water was collected from Houston shipping channel, north of Morgan's Point. All samples were collected
123 in 2018, filtered through 2.7 µm and 0.7 µm filters using a Büchner vacuum filtration apparatus, and then
124 stored at 4ºC in amber bottles.

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

Water Source	pH	conductivity (µS/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

126 *ND: not detected (<reporting limit value)
127
128
129
130 **Soil Sample Sources**
131
132 Six soil sample sources were obtained and tested using the developed extraction and analysis methods.
133 Some geochemical properties for each are listed in Table 3. The fat clay soil (CH-1) was obtained from
134 the American Society for Testing and Materials (ASTM) Institute for Standards Research (ISR) program.
135 Soils were dried at 25°C before being ground using a mortar and pestle. The fraction collected by sieving
136 using a standard #20 sieve (<850 µm) was stored at 4°C in amber bottles.
137
138 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*	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

139 *(Reporting limit: 250 mg/kg)
140
141
142
143 **Tissue Sample Sources**
144
145 Four tissue types were tested in the current work, including 1) earthworm (*Eisenia fetida*, a soil-dwelling
146 invertebrate), 2) fathead minnow (*Pimephales promelas*, a freshwater vertebrate), 3) polychaete worm
147 (*Alitta virens*, a marine invertebrate), and 4) perennial ryegrass (*Lolium perenne* L., a perennial plant).
148 Earthworms were obtained from The Worm Farm (Durham, CA), polychaete worms were obtained from
149 Aquatic Research Organisms (Hampton, NH), and adult fathead minnows were obtained from Aquatic
150 Biosystems (Fort Collins, CO). Earthworms underwent a 48 h purge period to remove gastrointestinal
151 contents. Perennial ryegrass Palmer III cultivar seeds (94% germination, 98% pure live seed, 2% inert
152 material) were obtained from La Crosse Seed (La Crosse, WI) and grown in non-contaminated, sandy soil
153 for 28 days.
154
155 After harvest, all tissues were carefully cleaned with reagent water, blotted dry, and stored in sample bags
156 at -20°C degrees. Prior to use in extraction method development, whole tissues were ground, using either
157 a stainless steel blender mill or a mortar-and-pestle, with liquid nitrogen, to a fine particle size or paste.

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.

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 >~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 ≤4°C prior to analysis for a maximum of 40 days.

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 ≤4°C prior to analysis for a maximum of 40 days. Samples were prepared for analysis as described below on the same day as analysis.

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°C for a maximum of 3 hours. Dried samples were homogenized using a horizontal sample roller for up to 18 h at 4°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 µm 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 ≤4°C prior to analysis for a maximum of 40 days. Samples were prepared for analysis as described below on the same day as analysis.

209 **SE Method for Tissue Samples**
210
211 Tissue sub-samples were spiked either with a mixed reference standard containing all 24 compounds of
212 interest and two surrogates, and vortexed for 1 min to mix. Laboratory-spiked tissue samples were
213 extracted using a ratio of 1 g wet tissue to 5 mL MeOH via a single 18-h ultrasonication in a cooled
214 ultrasonic bath. Tissue-MeOH mixtures were then centrifuged and the supernatant (MeOH extract)
215 collected by syringe filtering through a 0.45 µm PTFE disk filter. Extracts were stored at ≤4°C prior to
216 analysis for a maximum of 40 days. Samples were prepared for analysis as described below on the same
217 day as analysis. (At cold temperatures, some tissue extracts were observed to develop a precipitate. These
218 samples were filtered further using either 0.45 µm PTFE hydrophobic or 0.20 µm Anotop 10 inorganic
219 membrane disk filters.)
220
221 **Chromatographic Interference Reduction Method for Tissue Extracts**
222
223 An interference reduction method for tissue extracts was adapted from Larson, et al., 1999 [4]. Small-
224 scale chromatography columns were prepared using 5 ¾" borosilicate pipettes, loaded with 0.2 g
225 activated silica gel (100-200 mesh). Chromatography columns were wetted with MeOH just before setting
226 each into a separate amber collection vial. The columns remained in their collection vials during column
227 loading and eluting. Onto each column was loaded 1 mL tissue MeOH extract. After the 1 mL MeOH
228 extract had completely passed through the column, 1 ml of MeOH was used to elute, followed by 1 mL
229 2% HCl/MeOH (vol/vol). Each resulting 3 mL of silica-treated MeOH extract was vortexed to mix.
230 Treated extracts were returned to ≤4°C storage prior to analysis for a maximum of 40 days following the
231 original extraction event. Samples were prepared for analysis as described below on the same day as
232 analysis.
233
234 **Preparation of Extracts for Analysis**
235
236 High-level aqueous samples that had been diluted either with MeOH or with HCl/MeOH and stored at
237 ≤4ºC as described above were analyzed without further preparation, within 40 days of the dilution event.
238
239 For low-level aqueous samples that had been extracted/pre-concentrated by SPE and stored at ≤4ºC as
240 described above, the MeOH and acidified MeOH extracts may be analyzed individually, or by combining
241 known ratios of each, and diluting with reagent water, for a final solvent ratio of 1:1 MeOH/water.
242
243 For soil samples that had been extracted by the 2-stage SE method and stored at ≤4ºC as described above,
244 the MeOH and 1:1 MeOH/water extracts were analyzed either 1) individually, by diluting with reagent
245 water or 1:1 MeOH/water, respectively; or, 2) by combining known ratios of each associated extract and
246 diluting with a 1:3 mixture of MeOH/water, for a final solvent ratio of 1:1 MeOH/water.
247
248 For tissue samples that had been extracted using MeOH and stored at ≤4ºC as described above, the MeOH
249 extracts were analyzed either before or after silica gel column treatment, by diluting with water for a final
250 solvent ratio of 1:1 MeOH/water.
251
252
253
254 *Analytical Methods*
255
256 Chromatographic separations were achieved using a MeOH:water gradient over either a modified reverse
257 phase C18 (primary) or biphenyl (secondary) standard particle-size HPLC column, described below.
258
259

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 4µm Hydro-RP, 80Å, 250 x 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.

Table 4. Primary HPLC-UV separation solvent gradient scheme and other method conditions.

Total Run Time: 48.0 min Column Temperature: 25°C		Detection Wavelengths: 210, 254, 315 nm Injection Volume: 50 µL		Flow Rate: 1.0 mL/min
Time (min)	Reagent Water (%)	MeOH (%)	0.1% TFA/Water (%)*	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

*or 0.25% FA/water

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 µm, 150 X 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, either a 0.1% TFA or 0.25% FA (vol/vol) solution in water can be used.

Table 5. Secondary HPLC-UV separation solvent gradient scheme and other method conditions.

Total Run Time: 35.0 min Column Temperature: 25°C		Detection Wavelengths: 210, 254, 315 nm Injection Volume: 50 µL		Flow Rate: 0.9 mL/min
Time (min)	Reagent Water (%)	MeOH (%)	0.1% TFA/Water (%)*	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

*or 0.25% FA/water

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 350ºC and 4.0 L/min, vaporizer at 325ºC, 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 sec/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.

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]

Results and Discussion

Analytical Methods

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 Figure 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 Figure 2.

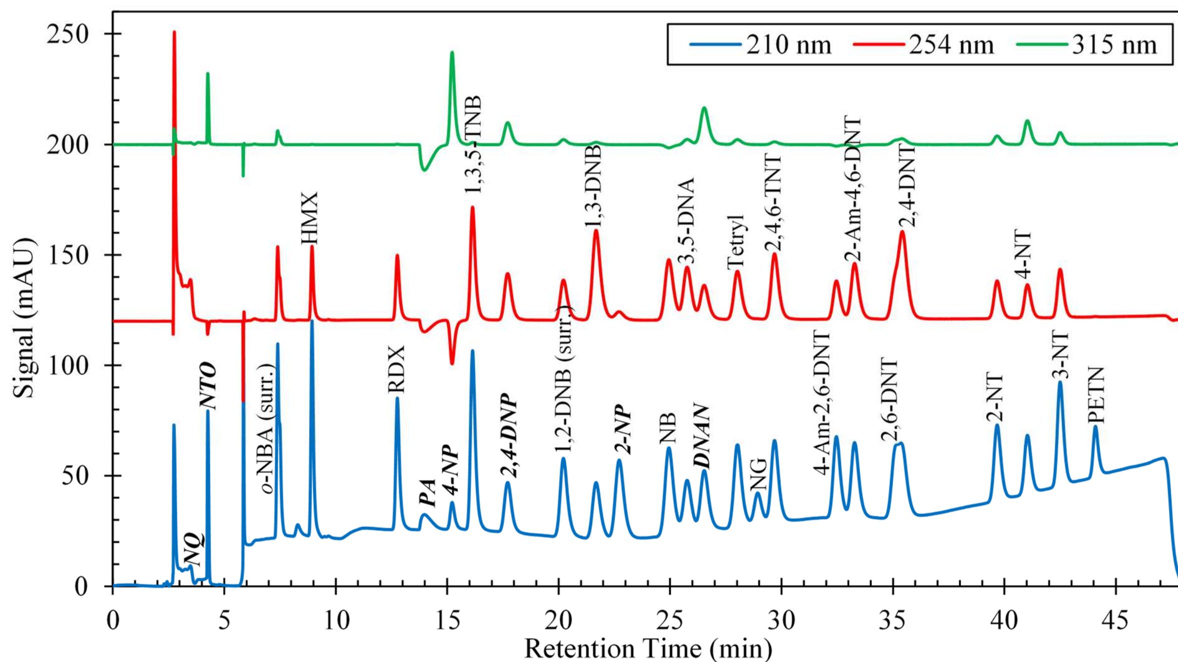


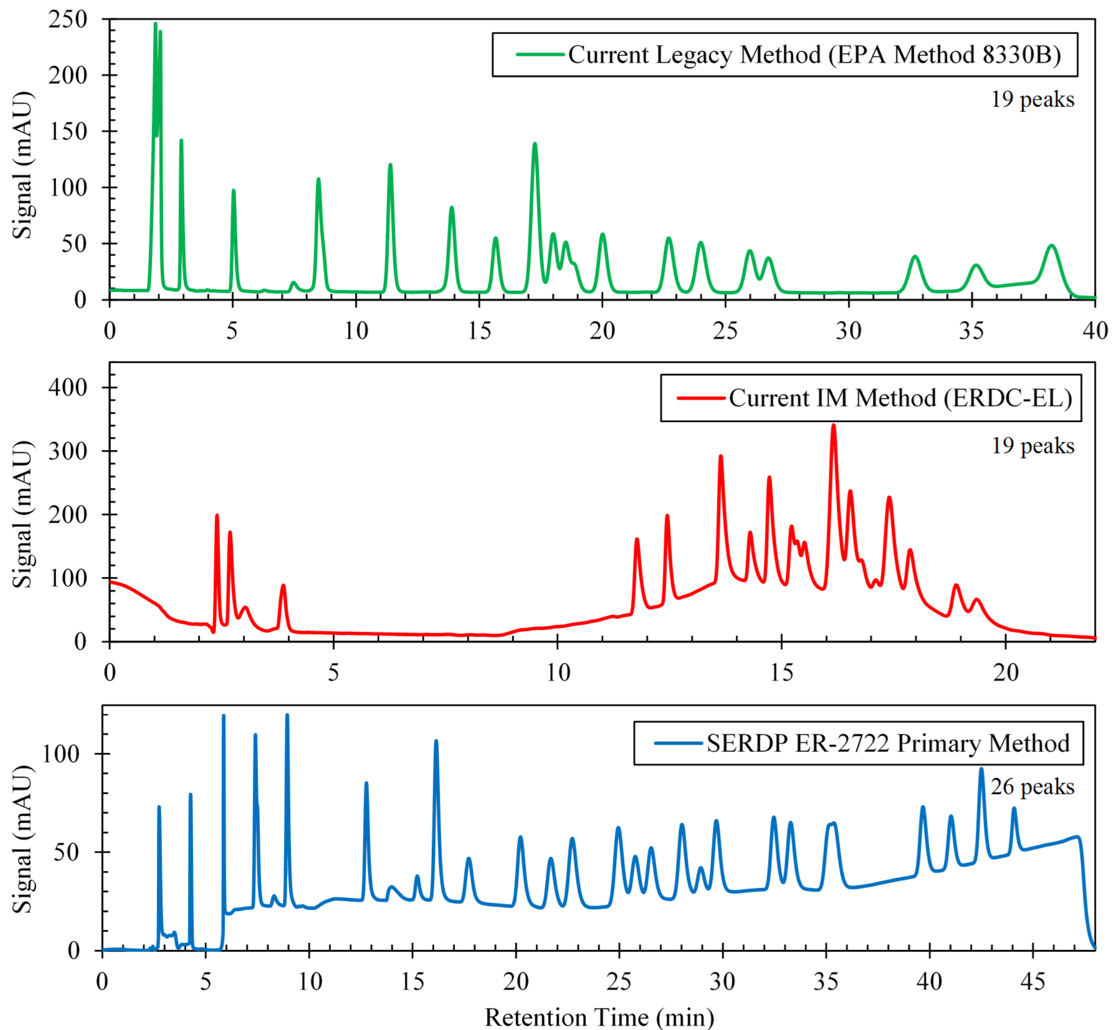
Figure 1. HPLC chromatograms of a mixed standard containing 5 mg/L of 24 legacy and IM compounds and two surrogates analyzed by the developed primary HPLC-UV method. ***Bold, italicized*** are compounds added to the new method (compared to EPA Method 8330B).

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†
6	***PA (210 nm)***	14.64	10	23	3.88	≥70†
6	***PA (315 nm)***	14.58	12	11	1.44	≥80‡
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

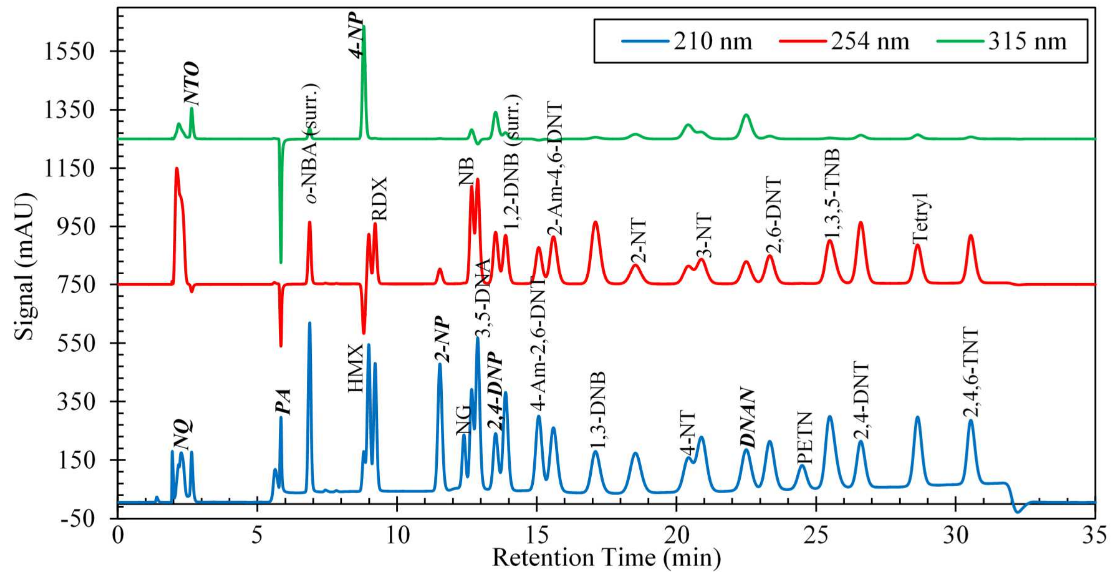
†co-elute at concentrations >30 mg/L; ‡RDX not detected appreciably at 315 nm

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

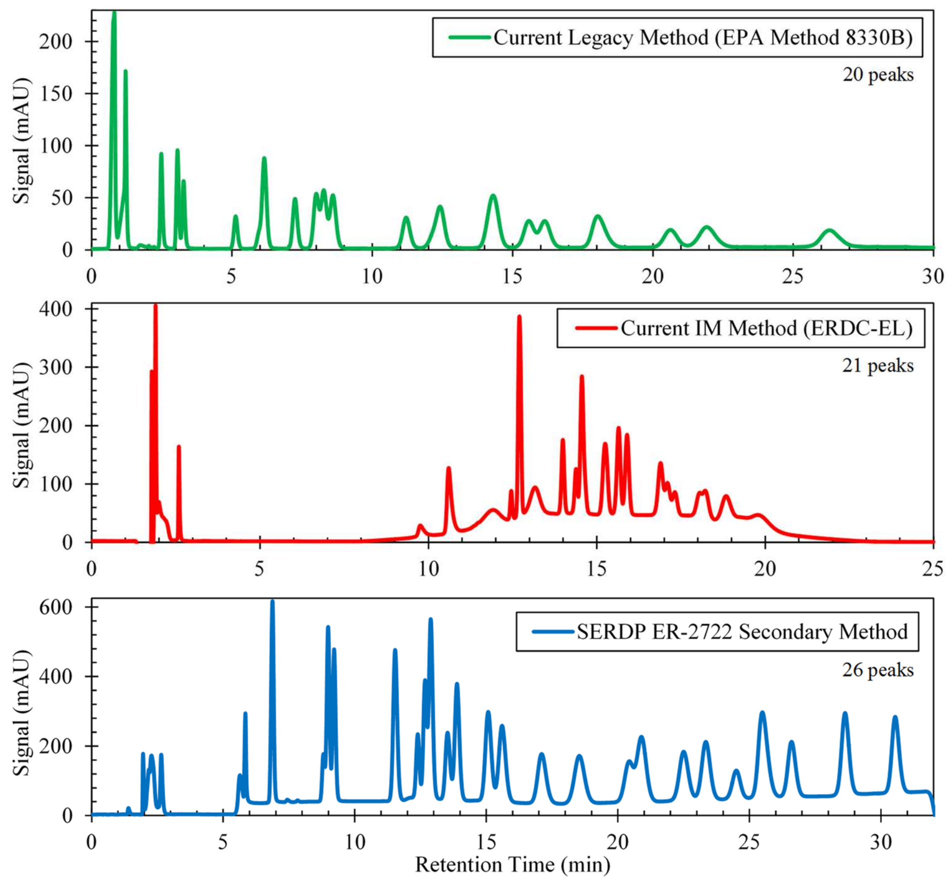


341
342 Figure 2. HPLC chromatograms of a mixed standard containing 5 mg/L of 24 legacy and IM compounds
343 and two surrogates analyzed by the current or new primary methods.

344 **Secondary HPLC-UV Method**
345
346 The secondary HPLC-UV method employs a biphenyl column to achieve analyte separation, resulting in
347 a significantly different elution order versus the primary column (modified C18) method for analyte
348 confirmation. An example chromatogram of a mixed standard containing 10 mg/L of the 24 compounds
349 of interest and two surrogates analyzed by the secondary HPLC-UV method is shown in Figure 3. The
350 mobile phase gradient was adjusted for optimal resolution of some closely-eluting analytes, including
351 octahydro-1,3,5-7-tetranitro-1,3,5,7-tetrazocine (HMX), Hexa-hydro-1,3,5-trinitro-1,3,5-triazine (RDX),
352 and 4-NP [6]. While NQ and PA became better resolved through mobile phase gradient method
353 optimization, the chromatography challenges with these two analytes were not entirely eliminated.
354 Therefore, an additional option for improved chromatography was to utilize alternate detection
355 wavelengths, such as 254 nm for NQ and 315 nm for PA, where void peaks do not interfere with analyte
356 peaks, as the longer wavelengths were sufficiently distant from the MeOH UV cutoff (205 nm). The same
357 26-compound standard was analyzed using current secondary methods employed at ERDC-EL for
358 analysis of munition compounds. Not all compounds were resolved by either of the current methods, as
359 shown in Figure 4.



360
361 Figure 3. HPLC chromatograms of a mixed standard containing 10 mg/L of 24 legacy and IM compounds
362 and two surrogates analyzed by the developed secondary HPLC-UV method. ***Bold, italicized*** are
363 compounds added to the new method (compared to EPA Method 8330B).



364
365 Figure 4. HPLC chromatograms of a mixed standard containing 5 mg/L of 24 legacy and IM compounds
366 and two surrogates analyzed by the current or new primary methods.

367 **LC-MS Method**
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369 The MS detector used for method development was located downstream of the primary HPLC-UV
370 system, so that upon separation by the primary column method, compounds were detected by UV and
371 then MS. The mobile phase gradient and other HPLC method parameters were unchanged from the
372 primary method description above, except that FA was highly preferred in MS detection versus TFA, due
373 to the extreme ionization suppression observed when TFA was used. The MS scan mode method was
374 adapted into a SIM mode method in order to improve sensitivity by selectively detecting the most
375 abundant ion masses observed in scan mode experiments. The experimentally-acquired ion masses (Table
376 6), were used to create ion/time windows for the SIM method, shown in Table 8. The SIM windows were
377 optimized, based upon precise retention times, sometimes requiring slight adjustment, such as when the
378 HPLC column was changed. Fragmentor settings were optimized for each ion. Depending upon the ion,
379 signals in SIM mode were between 1.5x – 4.5x higher than those observed in scan mode. Further
380 adaptation/optimization of the MS parameters may be necessary, especially in different instrument
381 models/software systems.
382
383 Table 8. SIM mode method windows.

SIM Window Start Time* (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 (surrogate)***	166	50	294
		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 (surrogate), ***2-NP***	138	70	83
		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, 2-Am-4,6-DNT	196	80	195
39.00	6	PETN	62	40	294
		2-NT, 3-NT, 4-NT	136	100	294

384 *SIM window start time = LC-MS Method run time (no MS delay)
385 ***Bold, italicized*** are compounds added to the new method (compared to EPA Method 8330B).
386
387 *Extraction Methods*
388
389 **Method for Preparation of Aqueous Samples for Direct-Injection Analysis**
390
391 During experiments with high-level aqueous samples, much lower recoveries for NTO and other ionic
392 compounds were observed for field waters than for reagent water (data not shown). This phenomenon
393 appeared to be pH-related, with lower recoveries for higher pH samples (Table 2). Further experiments
394 were performed in which the pH was adjusted using a solution of 2% HCl/MeOH, the same used for stage
395 two of SPE elution. Samples were diluted, for final acid concentrations either equal to (0.5%, vol/vol) or
396 double (1%, vol/vol) that of corresponding SPE extracted/pre-concentrated samples. For each field water
397 source, acidification with HCl resulted in near-100% recovery for the ionic compounds. Moreover, an

398 acid concentration of 0.5% was sufficient, and 1% acid was not necessary. Overall, for aqueous samples
399 to be analyzed by direct injection with pH ≥6.5 (approximately), acidification was beneficial.
400
401 **SPE Method for Aqueous Samples**
402
403 EPA Method 8330B and current ERDC-EL SPE procedures were modified to enable extraction and pre-
404 concentration of all 24 compounds of interest (and two surrogates) from low-level aqueous samples. A
405 series of cation-exchange, anion-exchange, reverse phase, and GAC-based SPE cartridges were evaluated
406 for their ability individually to recover the 26 compounds from laboratory-spiked reagent water samples.
407 Cartridges with widely differing chemistries were investigated due to the widely varying chemistries of
408 the analytes of interest. These included, among others, the 1) Phenomenex Strata X polymeric reverse
409 phase 500 mg (PRP500), 2) Phenomenex Strata X-A polymeric strong anion exchanger 500 mg
410 (PSA500), 3) Supelco C18 1 g (C181G), and 4) Supelco GAC 250 mg and 500 mg (GAC250, GAC500).
411 It was expected that the majority of analytes would be extracted by a reverse phase material, while the
412 anionic analytes (especially PA and NTO) would require the use of an anionic exchange resin. Additional
413 materials, such as GAC and modified GAC were investigated in an effort to improve NQ extraction
414 efficiency. Based on results with individual SPE cartridges [10], several double- and triple-stacked
415 options were tested, in order to identify a sequential option that yielded acceptable recoveries for all
416 compounds.
417
418 The stacking order for sample loading and elution was reversed to prevent irreversible binding of analytes
419 to incompatible SPE cartridge matrices, which was observed during evaluation of individual cartridges.
420 Results for six stacked options (A-F) are listed in Table 9, where loading-stage stacking order from top to
421 bottom is indicated. Based on results of stability studies in water and soil (data not shown), option B was
422 selected as the best multi-cartridge option for recovery of all compounds, with NQ recovery consistently
423 near 50%. However, when NQ is likely absent or not of interest, inclusion of a GAC cartridge is not
424 necessary. In some cases, using a single PRP500 (Strata X) or PSA500 (Strata X-A) cartridge may be
425 sufficient, and may be investigated, based on the analytes of interest for a particular project or laboratory.
426
427 Table 9. Compound recoveries (%) from laboratory-spiked reagent water samples using stacked SPE
428 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**
NQ	2	19	48	4	43	38
NTO (210 nm)	107	98	109	103	107	111
HMX	97	95	117	102	113	110
RDX	91	92	114	99	107	109
PA (210nm)	94	86	96	67	102	101
4-NP	87	90	110	97	103	104
1,3,5-TNB	93	91	113	100	106	109
2,4-DNP	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
2-NP	76	76	95	85	91	91
NB	92	90	114	100	107	109
3,5-DNA (254 nm)	79	84	111	80	105	107
DNAN	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

429 Green: 75-125%; Light Green: 57-74% or 126-135%; Red: <57% or >135%
430 (DoD QSM Ver5.3 for Waters – Lowest LCL: MNX, 57%; Highest UCL: HMX, 135%)
431 ***Bold, italicized*** are compounds added to the new method (compared to EPA Method 8330B).
432
433 **SE Method for Soils**
434
435 EPA Method 8330B and current ERDC-EL SE methods were modified to enable extraction of all 24
436 compounds of interest (and two surrogates) from soil samples. Preliminary trials indicated that traditional
437 EPA Method 8330B solid matrix extraction methods (i.e., a single 18-h ultrasonication period using
438 ACN) would be insufficient to extract all compounds, supported by previous studies [3,11]. Thus, several
439 different solvents, ultrasonication times, and multi-stage extraction procedures were investigated.
440
441 Method development was carried out using six geochemically/geographically diverse soils (Table 3).
442 Various 2- or 3-stage ultrasonication options were tested [10]. Several schemes were successful for each
443 soil. Including water as an extraction solvent resulted in improved recoveries for NQ, NTO, HMX, and
444 RDX across a broad range of soil types, which is thought to be due, at least in part, to an increased degree
445 of physical dispersion of the soil in water versus MeOH. Ultimately, a 6 h MeOH extraction, followed by
446 a 14 h 1:1 MeOH/water extraction was selected (data shown in Table 10). For cases in which NQ, NTO,
447 HMX, and RDX are likely absent or not of interest, including water as an extraction solvent may not be
448 necessary; in such cases, a single MeOH extraction may be sufficient.
449
450 Table 10. Recoveries for six soils spiked at 20 mg/kg for each of the 24 compounds and two surrogates.

	Recoveries (%)											
	Aberdeen		**Jefferson**		**Memphis**		**Riley**		**Yuma**		**ASTM Fat Clay**	
Compound	**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).

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 extract solvent.

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

	Recoveries (%)			
Compound	**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

481 Green: 75-125%; Light Green: 64-74% or 126-135%; Red: <64% or >135%
482 (DoD QSM Ver5.3 for Solids – Lowest LCL: 4-Am-2,6-DNT, 64%; Highest UCL: Tetryl, 135%)
483 ***Bold, italicized*** are compounds added to the new method (compared to EPA Method 8330B).
484
485
486
487 **Conclusion**
488
489 In the current work, new methods were developed for the extraction, pre-concentration, and analytical
490 separation and quantitation of 17 legacy and seven additional IM compounds, IM daughter products, and
491 other munition constituents absent from EPA Method 8330B. The objective was to produce a single
492 standardized method for simultaneous analysis of legacy and IM compounds in environmental matrices.
493 Extraction methods were developed for high- and low-level aqueous (direct-injection and SPE,
494 respectively), soil (2-stage SE), and tissue (1-stage SE) matrices, as well as an interference reduction
495 method for tissue extracts. HPLC-UV and LC-MS methods were developed, including two separate
496 HPLC column separation methods with UV detection, along with an MS detection option (scan and SIM),
497 enabling orthogonal confirmation of analyte concentrations down to low-µg/L, or sub-µg/L levels, for
498 samples pre-concentrated by SPE. Compound recoveries were within currently accepted limits for almost
499 all 24 analytes of interest and two surrogates in all tested matrices, with the recoveries for many of the
500 compounds consistently better than current methods. The few exceptions included low recovery of NQ by
501 SPE and low recovery of Tetryl from tissues.
502
503 Development of the new methods was necessary to assist in monitoring co-occurring components at
504 training ranges, demilitarization and manufacturing facilities, and environmental sites [1,2]. Implementing
505 the new methods should lead to cost savings with a minimal increase in environmental monitoring efforts
506 and no modification to existing sample collection or processing procedures. The simultaneous extraction
507 and subsequent analysis of all 24 analytes of interest may reduce total extraction, sample preparation, and
508 analysis time by 50% or more when compared to the current at least four separate methods for these
509 analytes.
510
511 The method development executed in the current work under Strategic Environmental Research and
512 Development Program (SERDP) project ER-2722 continues under ESTCP project ER19-5078 to validate
513 the new methods. The validation will consist of round-robin comparison studies, carried out with the
514 cooperation of EPA, with the end goal of producing an addendum to current EPA Method 8330B.
515 Participants will include government, commercial, academic, and manufacturing laboratories and
516 facilities. The standardization and publication of the validated methods will enable laboratories to perform
517 the standardized methodology on a broad scale, and it will facilitate the conduct of fate and transport
518 studies for IM compounds by providing a standardized method for co-extraction and quantitation of IM
519 and legacy materials.
520
521

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.

Declarations of Interest

The authors have no declarations of interest to report.

[1] Felt, D., Johnson, J.L., Larson, S., Hubbard, B., Henry, K., Nestler, C., and Ballard, J.H. (**2013**) Evaluation of Treatment Technologies for Wastewater from Insensitive Munitions Production: Phase 1: Technology Down-Selection. U.S. Army Corps of Engineers, Engineer Research and Development Center, Environmental Laboratory [Vicksburg, MS], ERDC/EL TR-13-20.
[2] Walsh, M.R., Walsh, M.E., Ramsey, C.A., Thiboutot, S., Ampleman, G., Diaz, E., and Zufelt, J.E. (**2014**) Energetic Residues from the Detonation of IMX-104 Insensitive Munitions. *Propellants, Explosives, Pyrotechnics*, ***39***, 243-250.
[3] Felt, D., Gurtowski, L., Nestler, C.C., Johnson, J., and Larson, S. (**2016**) A Two-Stage Extraction Procedure for Insensitive Munition (IM) Explosive Compounds in Soils. *Chemosphere*, ***165***, 18-26.
[4] Larson, S.L., Jones, R.P., Escalon, L., and Parker, D. (**1999**) Classification of Explosives Transformation Products in Plant Tissue. *Environ. Tox. & Chem.*, ***18(6)***, 1270-1276.
[5] Russell, A.L., Seiter, J.M., Coleman, J.G., Winstead, B., and Bednar, A.J. (**2014**) Analysis of Munitions Constituents in IMX Formulations by HPLC and HPLC-MS. *Talanta*, ***128***, 524-530.
[6] Gapeev, A.; Sigman, M.; Yinon, J. (**2003**) Liquid Chromatography/Mass Spectrometry Analysis of Explosives: RDX Adduct Ions. *Rapid Comm. Mass Spec.*, ***17***, 943-948.
[7] Jiang, G. (**2010**) Simultaneous UHPLC/MS Analyses of Explosive Compounds. *Thermo Fisher Scientific Application Note*, ***51879***.
[8] Kinghorn, R.; Milner, C.; Zweigenbaum, J. (**2005**) Analysis of Trace Residues of Explosive Materials by Time-of-Flight LC/MS. *Agilent Technologies Application Note*, ***5989-2449EN***.
[9] Thurman, E.M.; Ferrar, I. (**2012**) Adapting EPA Method 8330 for Analysis of Explosives in Water to SPE and LC/MS/MS. *Agilent Technologies Application Note*, ***5991-0676EN***.
[10] Crouch, R.A., Smith, J.C., Stromer, B.S., Hubley, C.T., Beal, S., Lotufo, G.R., Butler, A.D., Wynter, M.T., Rosado, D.A., Russell, A.L., Coleman, J.G., Clausen, J.L., and Bednar, A.J. (***submitted for editing Aug 2019***) Development and Optimization of Extraction and Analytical Methods for Simultaneous Determination of IM and Legacy Explosive Compounds. U.S. Army Corps of Engineers, Engineer Research and Development Center, Environmental Laboratory [Vicksburg, MS], ERDC/EL TR-20-xx.
[11] Walsh, M.E. (**2016**) Analytical Methods for Detonation Residues of Insensitive Munitions. *Journal of Energetic Materials*, ***34***, 76-91.

IM + EPA 8330 Analytes

Solvent Extraction

Solid Phase Extraction

Solvent Extraction

LC-UV & LC-MS