

**Validation of Sample Extraction and Analysis Techniques
for Simultaneous Determination of Legacy and IM
Constituents**

ESTCP ER19-5078

USACE ERDC-EL¹, -CRREL², and USACE EM CX³

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List of Acronyms

μg	microgram
μL	microliter
μm	micrometer
ACN	acetonitrile
AEC	anion exchange capacity
2-Am-4,6-DNT	2-amino-4,6-dinitrotoluene
4-Am-2,6-DNT	4-amino-2,6-dinitrotoluene
APCI	atmospheric pressure chemical ionization
ASTM	American Society for Testing and Materials
BGE	background electrolyte
CE	capillary electrophoresis
CEC	cation exchange capacity
CH ₃	methyl
cm	centimeter
Comp-B	Composition-B
CRREL	Cold Regions Research and Engineering Laboratory
DAD	diode array detector
3,5-DNA	3,5-dinitroaniline
DNAN	2,4-dinitroanisole
1,2-DNB	1,2-dinitrobenzene
1,3-DNB	1,3-dinitrobenzene
2,4-DNP	2,4-dinitrophenol
2,4-DNT	2,4-dinitrotoluene
2,6-DNT	2,6-dinitrotoluene

DoD	Department of Defense
EL	Environmental Laboratory
EOF	electroosmotic flow
ER	Environmental Restoration
ERDC	Engineer Research and Development Center
ESTCP	Environmental Security Technology Certification Program
FA	formic acid
GAC	granular activated carbon
H	hydrogen atom, proton
HCl	hydrochloric acid
HMX	octahydro-1,3,5-7-tetranitro-1,3,5,7-tetrazocine
HOAc	acetic acid
HPLC	high-performance liquid chromatography
Hz	hertz
IM	insensitive munition
JBCC	Joint Base Cape Cod
L	liter
LCL	lower control limit
LCS	laboratory control sample
LDR	linear dynamic range
M	molar (M = moles/liter)
MDL	method detection limit
MEKC	micellar electrokinetic chromatography
MeOH	methanol
meq	mole equivalent
mg	milligram

min	minute
mm	millimeter
mM	millimolar
MS	mass spectrometer, mass spectrometry
MS	matrix spike
NaOH	sodium hydroxide
NB	nitrobenzene
ND	not detected
NG	nitroglycerine
nL	nanoliter
nm	nanometer
2-NP	2-nitrophenol
4-NP	4-nitrophenol
NQ	nitroguanidine
NRL	Naval Research Laboratory
2-NT	2-nitrotoluene
3-NT	3-nitrotoluene
4-NT	4-nitrotoluene
NTO	3-nitro-1,2,4-triazol-5-one
<i>o</i> -NBA	<i>ortho</i> -nitrobenzoic acid (2-nitrobenzoic acid)
PA	picric acid
PETN	pentaerythritol tetranitrate
PSI	pounds per square inch
PTFE	polytetrafluoroethylene
QC	quality control
QSM	quality systems manual

RP	reverse phase
RPD	relative percent difference
RDX	hexahydro-1,3,5-trinitro- <i>s</i> -triazine
SE	solvent extraction
sec	second
SERDP	Strategic Environmental Research and Development Program
SM	sample matrix
SPE	solid phase extraction
SQ	single quadrupole
TDS	total dissolved solids
Tetryl	<i>N</i> -methyl- <i>N</i> -(2,4,6-trinitrophenyl)nitramide
TFA	trifluoroacetic acid
1,3,5-TNB	1,3,5-trinitrobenzene
TNT	trinitrotoluene
2,4,6-TNT	2,4,6-trinitrotoluene
TOC	total organic carbon
TRIS	tris(hydroxymethyl)aminomethane
UCL	upper control limit
UL	upper limit
USEPA	United States Environmental Protection Agency
UV	ultraviolet
UXO	unexploded ordnance
v/v	volume/volume
vol/vol	volume/volume
W	Watt

Keywords

Insensitive munition (IM compounds, legacy munitions, energetic compounds, U.S. Environmental Protection Agency (USEPA) Method 8330B, USEPA 8330B Addendum. High performance liquid chromatography (HPLC), solid phase extraction (SPE), solvent extraction (SE), environmental matrices

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Validation of Sample Extraction and Analysis Techniques for Simultaneous Determination of Legacy and IM Constituents

Abstract

Introduction and Objectives: Currently there exists no standardized method for the analysis of insensitive munitions (IM) in environmental matrices such as water, soils, and tissues. However, standardized methods for legacy munitions exist for water and soil matrices, i.e., United States Environmental Protection Agency (USEPA) 8330B. The lack of standardized methods for IM analysis results in a wide variety of incomplete and overlapping analytical methodologies being modified from USEPA 8330B for IM or use of methods developed for neat munitions compound analysis. The overall project's first phase, Strategic Environmental Research and Development Program (SERDP) ER-2722, was to develop and optimize methods to address these methodological gaps by creating analytical methods for simultaneous analysis of IM and legacy munitions in water, soil and tissue matrices. The main objective of the current project phase, Environmental Security Technology Certification Program (ESTCP) ER19-5078, builds upon the previous work and is focused on the validation of the previously developed methods. The methods were validated and submitted to USEPA for inclusion as a possible addendum to USEPA 8330B. Lastly, these combined insensitive and legacy munitions methods have been published as a peer-reviewed journal article and the validation study results reported here will also be submitted for publication.

Technical Approach: Inter-laboratory batch studies were used during method validation consisting of a series of round-robin studies with participating laboratories from the Department of Defense (DoD) (United States Army Engineer Research and Development Center (ERDC) Environmental Laboratory and ERDC Cold Regions Research and Engineering Laboratory), a non-DoD federal laboratory (US Geological Survey Water Mission Area), a DoD contract laboratory (American Ordinance limited liability company (LLC)), a commercial laboratory (Agriculture & Priority Pollutants Laboratories Inc), and an academic laboratory (Jackson State University). Aqueous matrix studies were performed in 3 round robin format studies (detailed in the Inter-laboratory Testing Methods section) separated by sample preparation technique: Round-robin 1 (direct injection), Round-robin 2 (SPE), Round-robin 3 (low level SPE). The first aqueous round-robin study had 8 participating laboratories, the second round-robin study had 5 participating laboratories, and the third round-robin study had 4 participating laboratories. Soil and tissue matrix round robins were conducted separately to ensure laboratories (3 participants for soil matrices and 4 participants for tissues) had the ability to execute the number of samples generated. Soil and tissue round robin studies had 3 and 4 laboratories participating, respectively. Detailed methods developed through SERDP ER-2722 were provided to participant laboratories alongside troubleshooting support and ample time to implement methods (6 months minimum) on their specific instrumentation. ERDC-EL was responsible for preparing and distributing the samples in a "double-blind" manner, with sample pre-processing following USEPA Method 8330B.

Results: The round robin testing structure produced distinct groupings of data within each extraction method. The first was a synthetic group used to establish recovery ranges (in reagent water, ASTM Fat Clay, or clean earthworm and ryegrass tissue) at a given tolerance interval (TI). The first group was accompanied by spiked natural matrices (groundwater, surface water, and clean field collected soils) to assess whether natural matrices behaved similarly and recovered within the recovery ranges. The second group, an aqueous semisynthetic group, was generated by diluting waters from Iowa Army Ammunition Plant (IAAAP) known to contain legacy and IM compounds. These IAAAP waters were fortified prior to dilution with the compounds that are included in ESTCP-ER19-5078 but weren't detected in the waters.

Lastly, a set of low-level samples were created by spiking reagent waters or ASTM Fat Clay to determine method performance near regulatory limits.

The calculated recovery ranges are generally wider than the control limits for USEPA 8330B found in DoD QSM 5.4 (2021). However, the ranges are still within reason and likely reflect the increased difficulty in execution of these complex methods. The spiked natural matrices and the semi-synthetic results support this assessment and are also satisfactory, with infrequent issues occurring on a per-laboratory or per-analyte basis. Method performance degrades, as expected, as analytes are near instrument detection limits, however the calculated recoveries are broadly acceptable, albeit some analytes exhibit bias toward > 100% recovery. A broad view of method performance, with laboratory data aggregated, reveals that the methods are effective, and that inter-laboratory variability is the main driver of varying analyte recoveries.

Benefits: The validation of these methodologies is necessary to ensure that the techniques can be successfully implemented by other laboratories with varying instrumentation and analysts. Validation studies were used to assess method repeatability and reproducibility and are a requirement prior to their addition to USEPA Method 8330B. These methods add the following benefits to USEPA Method 8330B: (1) addition of 7 IM / IM degradation products, (2) addition of a surrogate (o-NBA), (3) separate extraction procedures for soil and tissues, and (4) combined extraction and analysis of legacy and IM. These additions provide greater applicability and efficiency to monitoring efforts at firing ranges, demilitarization, and manufacturing facilities, and at any site where munitions are tested, manufactured, or detected (Felt et al., 2013; Walsh et al., 2014). Analytical methods like these will also prove useful in environmental fate and transport studies requiring the analysis of both IM and legacy munitions.

Validation of Sample Extraction and Analysis Techniques for Simultaneous Determination of Legacy and IM Constituents

Executive Summary

Introduction

Currently there exists no standardized method for the combined analysis of legacy and insensitive munitions (IM) in environmental matrices such as water, soils, and tissues. The main goal of this project was the validation of a series of methods developed under Strategic Environmental Research and Development Program (SERDP) Project ER-2722 for the combined analysis of legacy and insensitive munitions in waters, soils, and tissues. Formal method validation was performed through a series of round robin studies used to determine the repeatability and reproducibility of the methods prior to their submission, as an addendum to the United States Environmental Protection Agency (USEPA) Method 8330B.

Objectives

The primary objective of the project was the completion of a multi-laboratory formal method validation of recently developed methods of extraction, and analysis of 26 compounds of interest (legacy munitions, IM, IM degradation products, and other munitions compounds). Methods validated included primary and secondary high performance liquid chromatography-ultraviolet (HPLC-UV) methods and an alternate LC-mass spectrometry (MS) method. Additionally, preparatory methods for the analysis of waters, soils and tissues were validated, as they were modified to suit the added analytes and sample matrices. Further objectives of this project were submission of an addendum to USEPA for inclusion in USEPA method 8330B and academic publication of the results of the validation study.

Technical Approach

To validate the methods, samples containing known concentrations of the various munitions compounds were distributed to participating laboratories during multiple round-robin style studies, wherein laboratories extracted and analyzed aliquots of the same bulk sample. A wide variety of participating laboratories were selected to ensure that the developed methods could be implemented successfully regardless of analyst, local laboratory environment, and specific instrumentation. Due to the large numbers of samples shipped throughout the project, the samples were delivered / analyzed in batches. The samples were prepared prior to shipment in either of 2 ways: (1) spiking uncontaminated reagent water / soil / tissue, or (2) spiking into a sample matrix from a site known to have munitions waste (with previous analysis identifying and quantifying the munitions). The participant laboratories performed their own sample processing.

Results and Discussion

High-level Waters (Direct-Injection)

For aqueous samples with suspected high concentration of analyte(s), extraction is unnecessary, and samples can be analyzed directly after pH adjustment and dilution. Owing to the relatively high analyte concentration and simple sample preparation the direct injection results show both the tightest recovery ranges at a 91% tolerance interval (TI), and the best analyte recoveries seen in the round robin testing. The calculated recovery ranges produced during ESTCP ER19-5078 are comparable or even superior to

those found in Department of Defense Quality Systems Manual (DoD QSM) 5.4 (2021). Analyte recoveries in groundwater and surface water are, as expected, very good with most analytes recovering within 80 – 120%. On aggregate, analyte recoveries were within 90 – 110%. This demonstrates a core competency of the direct injection preparation method, the instrumental analysis method, and the participating laboratories / analysts' capabilities.

Low-level Waters (SPE)

In contrast to direct injection, method performance for solid phase extraction (SPE) waters shows increased variability, due, in large part, to the increased sample preparation complexity. Recovery ranges at a 95% TI were generally wider, typically within the range of 50 – 160%. Most analyte recoveries for spiked natural matrices fall within the calculated recovery ranges. Only one laboratory, Laboratory H, had consistently low recoveries, with approx. 40% of analytes outside of the established recovery ranges. For the low level SPE waters (~2 µg/L) most analytes recover well with acceptable ranges, although with greater variability.

Soils (2 stage Solvent Extraction)

Soil recovery ranges calculated at an 82% TI are generally between the method performance of direct injection and SPE waters, with soils showing recovery ranges that are generally comparable to those found in DoD QSM Ver 5.4 (2021). Nearly all analyte recoveries for test samples fall within the calculated recovery ranges. At lower analyte concentrations (~2 mg/kg), method performance is lower, with more analytes outside of calculated recovery ranges, and relatively high variability.

Tissues (Single Stage Solvent Extraction)

Tissues represent the most complex and challenging matrix tested, and the method performance observed reflects this. Generally, the calculated recovery ranges at a 95% TI were wider than soils, although the recovery ranges produced by the highest performing laboratory were markedly closer to the control limits from DoD QSM 5.4 (2021). Additionally, some analytes 1,3,5-trinitrobenzene (1,3,5-TNB), nitroguanidine (NQ), and 3-nitro-1,2,4-triazol-5-one (NTO) display possible issues with interferences, causing elevated recoveries by certain laboratories, and subsequently wider recovery ranges. These interferences are not observed by every participating laboratory or even every sample analyzed by a particular laboratory.

Validation of Sample Extraction and Analysis Techniques for Simultaneous Determination of Legacy and IM Constituents Final Report

Objective

The main objective of this project was the validation of the methods previously developed in SERDP Project ER-2722. To accomplish this a formal interlaboratory validation study was conducted alongside 8 participating laboratories (including DoD laboratories, other governmental laboratories, commercial laboratories and an academic laboratory). Each participating laboratory abided by the sample hold times and conditions from existing standard methods (EPA 8330B and EPA 8095) as well as the recommendations included in SERDP ER-2722. The participating laboratories also performed necessary quality control (QC) such as laboratory control samples (LCS). The data produced by these laboratories were subjected to statistical analysis which provides insight into the methods' interlaboratory reproducibility and transferability. This insight is crucial for broad adoption and regulatory acceptance of the new methods and is a prerequisite to the methods' submission as an addendum to USEPA 8330B. The main products of the statistical analysis were 4 sets of recovery ranges (1 for each preparation method for specific matrices) and average analyte recoveries.

Background

There is no accepted standard method for analysis of insensitive munitions in environmental matrices. Without an established method, laboratories must use methods that do not measure IM compounds at all; quantify some but not all using USEPA Method 8330B (which is intended for legacy munitions only); or use methods originally developed for neat materials with no proven performance metrics.

IM compounds are high-energy compounds that were designed to perform similarly to legacy munitions, however they are less sensitive to shock, heat and sympathetic detonation (Barrie, 2013). Development of IM began in the 1980s after accidents caused the U.S. Army to seek ways to improve soldier safety and resource management (Newman, 2010). Two of the most common legacy munitions to be replaced by IM compounds are 2,4,6-trinitrotoluene (TNT) and Composition B, which is a blend of TNT and Hexahydro-1,3,5-trinitro-s-triazine (RDX). The prospective IM compounds must be as effective as legacy munitions, they must show reduced sensitivity compared to legacy munitions, they must be inexpensive, and they must be scalable for production (Roos, 2007). Three IM compounds have met these criteria and have begun testing and usage, they are 3-nitro-1,2,4-triazol-5-one (NTO), nitroguanidine (NQ), and 2,4-dinitroanisole (DNAN).

Although these compounds are subject to rigorous monitoring and handling procedures, their release into the environment is inevitable given their presence in manufacturing facilities, test ranges, operational theater, and demilitarization facilities. Unintended releases could occur due to leaks at manufacturing facilities and wastewater treatment plants, or from unintentional spills during transport. IM release also occurs at test ranges and in the operational theater where low order detonations can spread munitions near the target and at the point from which the shot was fired in the form of particles ranging from mm to cm (Hewitt et al. 2009; Jenkins, et al., 2005; Jenkins, et al., 2006; Pennington, et al., 2001, 2002, 2003, 2004, 2005, 2006; Taylor, et al., 2004). Corrosion and breaching of the casings of unexploded ordnance (UXO)

can be a long-term source of IM release as IM compounds leach through the compromised casing (Chendorain, et al., 2005; Lewis et al., 2009).

The wide range of chemical and physical properties of legacy and IM compounds poses a complex analytical challenge. Furthermore, the unique degradation products of these compounds and the various interferences encountered in environmental matrices further complicate their analysis. These factors are critical because, invariably, IM and legacy munitions will be encountered alongside each other in the vast majority of cases and degradation products of IM readily form as the IM degrades (i.e., 2 and 4-nitrophenols or 2,4-dinitrophenol formation as DNAN degrades) (Hawari et al., 2015). Aminotoluenes and nitrotoluenes from the degradation of TNT are examples of degradation products that might also be present if legacy munitions had previously been used at the site (Walsh, et al. 1990). Alkaline degradation is a potential remediation technology for TNT and RDX as they degrade at pH > 11 (Johnson et al., 2011).

Currently used high-performance liquid chromatography (HPLC) methods require solid-phase extraction to pre-concentrate analytes and lower detection limits in an aqueous matrix. SPE methods have seen extensive use with legacy munitions analysis, however outside of that only nitrophenols and NTO have been developed (Walsh, 2016). SPE method development is a crucial step in creating a comprehensive legacy and IM analytical method as, without it, analysis is limited to direct injection and relatively high detection limits.

Additionally, the acetonitrile extraction found in USEPA Method 8330B was designed for extraction of legacy munitions; accordingly, the extraction solvent must be adjusted to accommodate the addition of IM compounds. These issues are addressed with the method development completed under SERDP ER-2722. The implementation of those methods requires rigorous validation, which is the subject of this report and should result in cost savings, and faster sample analysis time.

Interlaboratory Comparison Study

Materials and Methods

HPLC-UV and HPLC-MS Methods

Primary HPLC-UV Method

The Primary HPLC-UV method was developed based on previous munitions compound analytical methods developed internally (Crouch et al., 2020; Russell et al., 2014) as well as established methods found in USEPA 8330B. Separation utilized a Phenomenex Synergi 4 μ m Hydro-RP column (80Å, 250 x 4.6 mm) or a comparable reverse phase (RP) column. The method mobile phase gradient and method parameters are shown in Table 1, while analyte retention times for both the primary and secondary HPLC-UV methods are shown in Table 2. The 0.1% trifluoroacetic acid (TFA) mobile phase can be replaced with 0.25% formic acid (FA) (vol/vol) with no impact on method performance. Additionally, guard columns like the Phenomenex SecurityGuard AQ C18 pre-column guard cartridge can be used with this method.

Table 1: Primary HPLC-UV Mobile Phase Gradient and Method Conditions

Method Run Time: 48 min			Detector Wavelengths: 210, 254 and 310 nm	
Column Temperature: 25°C			Flow Rate 1 ml / min	
Injection Volume: 50 µL				
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

Table 2: Primary and Secondary HPLC-UV Method Analyte Retention Times

Compound	Abbreviation	Primary Method Retention Time (min)	Secondary Method Retention Time (min)
Nitroguanidine	NQ	3.51	2.33
3-Nitro-1,2,4-triazol-5-one	NTO	4.29	2.67
Ortho-Nitrobenzoic Acid	o-NBA (surr.)	7.45	5.86
Octahydro-1,3,5,7-tetranitro-1,3,5,7-triazine	HMX	8.96	8.98
Hexahydro-1,3,5-trinitro-1,3,5-triazine	RDX	12.81	9.22
Picric Acid	PA	14.64	5.86
4-Nitrophenol	4-NP	15.31	8.82
1,3,5-Trinitrobenzene	1,3,5-TNB	16.17	26.63
2,4-Dinitrophenol	2,4-DNP	17.86	13.55
1,2-Dinitrobenzene	1,2-DNB (surr.)	20.35	13.89
1,3-Dinitrobenzene	1.3-DNB	21.80	17.13
2-Nitrophenol	2-NP	22.91	11.54
Nitrobenzene	NB	25.42	12.68
3,5-Dinitroaniline	3,5-DNA	26.16	12.90
2,4-Dinitroanisole	DNAN	26.89	22.53
N-Methyl-N-(2,4,6-trinitrophenyl) nitramide	Tetryl	28.43	28.67
Nitroglycerine	NG	29.30	12.94
2,4,6-Trinitrotoluene	2,4,6-TNT	30.06	30.59
4-Amino-2,6-dinitrotoluene	4-Am-2,6-DNT	32.60	15.08
2-Amino-4,6-dinitrotoluene	2-Am-4,6-DNT	33.43	15.62

2,6-Dinitrotoluene	2,6-DNT	35.25	23.37
2,4-Dinitrotoluene	2,4-DNT	35.57	25.62
2-Nitrotoluene	2-NT	40.18	18.56
4-Nitrotoluene	4-NT	41.42	20.47
3-Nitrotoluene	3-NT	42.84	20.92
Pentaerythritol Tetranitrate	PETN	44.38	24.51

Secondary HPLC-UV Method

The secondary HPLC-UV method was developed parallel to the primary method as a confirmatory method and utilized a Restek Pinnacle II Biphenyl 5 μ m (150 x 4.6 mm) or comparable column. An optional guard column, the Phenomenex SecurityGuard AQ C18 pre-column guard cartridge, was also used. The secondary method mobile phase gradient and method parameters are shown in Table 3.

Table 3: Secondary HPLC-UV Mobile Phase Gradient and Method Conditions

Method Run Time: 43 min			Detector Wavelengths: 210, 254, and 310 nm	
Column Temperature: 25°C			Flow Rate 0.8 ml / min	
Injection Volume: 50 µL				
Time (min)	Reagent Water %	MeOH %	0.1 % TFA/Water (%)	ACN %
0.00	82	10	10	5
2.50	82	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	35	50	10	5
15.1	43	42	10	5
33.0	30	55	10	5
33.10	82	10	10	5
43.0	82	10	10	5

HPLC-MS Method

The MS method was developed with the primary method and utilized an Agilent 6120B single quadrupole (SQ) instrument with an atmospheric pressure chemical ionization source as the sample introduction method. The MS detector was operated in scanning mode for the entirety of the 48 min run time. The atmospheric pressure chemical ionization (APCI) and detector parameters are included in Table 4. A large majority of the analytes included in the primary method produced ion masses suitable for confirmatory analysis, except for nitroglycerine (which is covered extensively in USEPA method 8332) and 2-,3-, and 4- nitrotoluene compounds. Ions were identified where possible and are listed in Table 5 by decreasing abundance. Electrospray ionization (ESI) was originally investigated as an ionization source for this method but resulted in poor ion production because of the HPLC mobile phase, therefore, all work was performed with APCI.

Table 4: APCI and Detector Parameters

Parameter	Value
Ionization Source	APCI
Ionization Mode	Negative
Drying Gas Temperature (°C)	350
Vaporizer Temperature (°C)	325
Drying Gas Flow (L/min)	4.0
Nebulizer Pressure (psig)	40
Corona Current (μA)	10
Capillary Potential (V)	1500
Mass Range	40 – 400
Fragmentor	100
Gain	1
Threshold	0
Step Size	0.20
Speed (μ/sec)	743
Peak Width (min)	0.06
Cycle Time (sec/cycle)	0.57

Table 5: MS Method Retention Times, Ion Masses and Ion Identities

Retention Time (min)	Compound	Abbreviation	CAS	Ion Mass (m/z)	Ion Identity
3.78	Nitroguanidine	NQ	556-88-7	203.6	
				103.0	[M]-H
4.50	3-Nitro-1,2,4-triazol-5-one	NTO	932-64-9	129.0	[M]-H
				55.0	
7.64	Ortho-Nitrobenzoic acid	o-NBA (surr.)	552-16-9	122.0	
				167.0	[M]-H
9.21	Octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine	HMX	2691-41-0	341.0	[M]-H+FA
				147.0	[M]/2-H
13.40	Hexahydro-1,3,5-trinitro-1,3,5-triazine	RDX	121-82-4	267.0	[M]-H+FA
				335.0	[M]-H+TFA
16.00	4-Nitrophenol	4-NP	100-02-7	108.0	
				138.0	[M]-H
16.80	1,3,5-Trinitrobenzene	1,3,5-TNB	99-35-4	213.0	[M]-H
				183.0	
18.20	Picric Acid	PA	88-89-1	228.0	[M]-H
				211.9	
18.80	2,4-Dinitrophenol	2,4-DNP	51-28-5	183.0	[M]-H
				153.0	
22.70	1,2-Dinitrobenzene	1,2-DNB (surr.)	528-29-0	138.0	
				168.0	[M]-H
23.55	1,3-Dinitrobenzene	1,3-DNB	99-65-0	168.0	[M]-H
24.60	2-Nitrophenol	2-NP	88-75-5	138.0	[M]-H
				122.0	[M]-H ₂ O-H
27.10	3,5-Dinitroaniline	3,5-DNA	618-87-1	182.0	[M]-H
				138.0	
27.80	Nitrobenzene	NB	98-95-3	182.0	[M]-H+HAc
				135.0	
29.00	2,4-Dinitroanisole	DNAN	119-27-7	183.0	[M]-CH ₃
				152.9	
30.00	N-Methyl-N-(2,4,6-trinitrophenyl)nitramide	Tetryl	479-45-8	241.0	[M]-H+NO ₂ ⁻ *
				288.0	[M]-H
	Nitroglycerine	NG	55-63-0	226.0	[M]-H (not obsv.)
31.30	2,4,6-Trinitrotoluene	2,4,6-TNT	118-96-7	226.0	[M]-H
				210.0	[M]-H ₂ O-H
35.10	4-Amino-2,6-dinitrotoluene	4-Am-2,6-DNT	19406-51-0	196.9	[M]-H
				167.0	
35.95	2-Amino-4,6-dinitrotoluene	2-Am-4,6-DNT	35572-78-2	196.0	[M]-H
				179.9	[M]-H ₂ O-H
37.54	2,6-Dinitrotoluene	2,6-DNT	606-20-2	152.0	[M]-30*
				122.1	
37.87	2,4-Dinitrotoluene	2,4-DNT	121-14-2	165.0	[M]-H ₂ O-H
				181.0	[M]-H
42.00 – 45.50	2-,3-, and 4-Nitrotoluene	2-NT / 3-NT / 4-NT	88-72-2 / 99-08-1	136.1	[M]-H (not obsv.)

			99-99-0		
45.10	Pentaerythritol Tetranitrate	PETN	78-11-5	62.0	NO ₃ ^{-*}
				270.9	

* indicates ion identity reported in Avci, et al., 2017; Gapeev, et al., 2003; Jiang, 2010; Kinghorn, et al., 2005; Thurman, et al., 2012; Xu, et al., 2004; respectively.

Preparation Methods

The methods described in this section do not require any modification of the sample storage or hold times stated in USEPA Method 8330B. However, care should still be taken to avoid exposure of samples to light (including use of amber glassware for sample storage) and elevated temperatures during sample preparation and extraction.

Direct-Injection Method for Natural Waters

Aqueous samples suspected to contain the compounds of interest at levels detectable without any extraction/pre-concentration are suitable for analysis by direct injection. The method differs from the method found in USEPA Method 8330B in the addition of a pH adjustment step and use of MeOH rather than acetonitrile (ACN) for dilution. Throughout the methods presented here ACN is replaced with methanol for extractions and dilutions as the use of ACN results in poor NTO and NQ recovery (Walsh et al., 2016; Felt et al., 2016). The pH adjustment is needed for any alkaline samples to ensure reproducible chromatographic separation of ionic compounds (e.g., NTO or PA). A solution of 1% HCl/MeOH is used to acidify and dilute the samples to a final acid concentration of 0.5% (vol/vol) and a final solvent ratio of 1:1 MeOH/H₂O. Alternative acid concentrations can be used, depending on the buffering capacity of the mobile phase and the sample(s) to be analyzed, but should be tested for method reproducibility.

SPE Method for Natural Waters

Aqueous samples containing the compounds of interest at low levels require extraction and pre-concentration using solid phase extraction (SPE). The SPE method here differs from the procedure found in USEPA Method 3535A in a few ways. The most significant difference is the use of a triple SPE cartridge setup using cartridge adapters, while in Method 3535A only a single cartridge is used. The solvents used for column conditioning and elution are altered to facilitate the triple cartridge SPE as well. The cartridges used during method development were a Strata X (Phenomenex Inc., Torrance, CA, USA), Strata X-A (Phenomenex Inc., Torrance, CA, USA) and Envi-CarbTM (MilliporeSigma, Burlington, MA, USA), however any comparable SPE cartridge chemistries (anion, strong anion, and carbon) should be sufficient. The SPE cartridges were first conditioned with two 5-mL aliquots of MeOH and equilibrated using two 5 -mL aliquots of reagent water. Then approximately 2 mL of reagent water should be added to each conditioned cartridge to prevent drying of the substrate before stacking the cartridges in the following order: (1) Strata X on top, (2) Strata X-A in the middle, and (3) Envi-CarbTM on the bottom. Sample is then loaded onto the triple cartridge stack. After allowing the sample laden SPE cartridges to air-dry under vacuum for approximately 10 min, the order of the cartridges should be reversed before elution. The elution is two stages, the first elution used 5 mL of MeOH, and the second stage used 5 mL 2% HCl/MeOH. Storing the two extracts separately was explored, however showed no clear benefit, therefore they are combined after collection and stored for analysis.

Solvent Extraction Method for Soils

Soil collection, storage, drying and grinding procedures are unchanged from the USEPA Method 8330B procedures; however, the solvent extraction (SE) procedure differs in number of sonication steps, sample mass and solvent used. Sample sizes were approximately 2 g and a mass to volume ratio of 1:5 (g:mL) were used for extraction. The first stage of sonication was carried out in MeOH (6 h), followed by a second sonication in 1:1 MeOH:H₂O solution (14 h). This timing allowed the procedure to fit within an 8-hour workday without sacrificing extraction efficiency or reproducibility. The soil-MeOH mixtures were placed in a cooled ultrasonic bath for 6 h, centrifuged, and the supernatant collected via syringe filtration through a 0.45 µm hydrophobic PTFE disk filter. After centrifugation, 5 mL of the 1:1 MeOH/H₂O solution was added per g of soil to the sample container for the second sonication step. After the 14 h sonication step, centrifugation and filtration were performed to collect the 1:1 MeOH/H₂O extract. The two extract solutions were combined and stored for analysis.

A complementary study evaluating and comparing two soil grinding methods (mortar and pestle, and puck mill grinding) was performed during the early stages of the project. This comparison is useful as puck mill grinding is the standard EPA 8330B grinding method, while mortar and pestle grinding is a practical approach but isn't included in EPA 8330B. This study utilized the same ERDC soil used during soil recovery experiments. Soil samples were prepared by both spiking at approximately 10 mg/kg (with all 26 analytes and surrogates) and leaving un-spiked (blank) soil. Each of these preparations were performed, extracted (using the method above) and analyzed in triplicate. This study followed guidance in USEPA Method 8330B and DoD QSM Ver5.4 including soil processing, subsampling procedures, and QA/QC requirements.

Solvent Extraction Method for Tissues

During development of the method, homogenized frozen tilapia tissue was used to replicate a typical tissue analyzed via USEPA Method 8330B. These 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 µm PTFE disk filter. At cold temperatures (e.g. during storage), some tissue extracts are observed to develop a precipitate. These samples can be filtered using either 0.45 µm PTFE hydrophobic, 0.20 µm Anotop 10 inorganic membrane disk filter, or equivalent.

An interference reduction method for tissue extracts was adapted from previous work (Larson et al., 1999). Small-scale chromatography columns were prepared using 5 ¾" borosilicate pipettes plugged with 0.5 – 1 cm of dichloromethane treated glass wool and loaded with 0.2 g activated silica gel (100–200 mesh). Chromatography columns were wetted with MeOH before placing into a separate amber collection vial. The columns remained in the collection vials during loading and elution of the column. Then, each column is loaded with 1 mL of tissue MeOH extract. Once the 1 mL MeOH extract has completely passed through the column, 1 mL of MeOH was used for elution, followed by 1 mL 2% HCl/MeOH (vol/ vol). Each resulting 3 mL aliquot of silica-treated MeOH extract was vortexed to mix and stored prior to analysis. On the day of analysis, each sample should be diluted 1:1 with reagent water, resulting in a 1:1 MeOH: H₂O sample solution for analysis.

Inter-laboratory Testing Methods

The inter-laboratory evaluation studies were a critical step in assessing the legacy and IM extraction and analysis methods' transferability and applicability in laboratories throughout the US. All samples used in the studies were extracted according to the methods previously described and encompassed each sample

matrix (water, soil, and tissues). Participating laboratories were provided samples as shown in Table 6. In total, 8 laboratories participated in the aqueous matrix studies using full-synthetic samples for direct injection and SPE, 5 laboratories participated in studies using semi-synthetic samples extracted by SPE, and 4 laboratories participated in the low level SPE study. Three laboratories participated in the final soil study and 4 laboratories participated in the tissue study. No participating laboratories reported any deviations from the method. Each participant in the study generated peak integration data, calculated amount data, and surrogate percent recovery data, based on external calibration, for all compounds (both analytes and surrogates) shown in Table 5. The laboratory identities are as follows: Cold Regions Research and Engineering Laboratory (Laboratory A), American Ordinance LLC (Laboratory B), United States Geological Survey (Laboratory C), ERDC - Environmental Chemistry Branch (Laboratory E), ERDC – Environmental Engineering Branch (Laboratory F), APPL Inc. (Laboratory G), Jackson State University (Laboratory H), and ERDC – Environmental Processes Branch (Laboratory I). Statistical analysis of the data produced recovery ranges for each analyte (in clean matrices) and was used for comparison with the DoD Quality Systems Manual Ver5.4 (2021) limits for USEPA Method 8830B.

Table 6: Number of Sample Replicates Provided to Participating Laboratories

	Lab A	Lab B	Lab C	Lab E (1)	Lab E (2)	Lab F	Lab G	Lab H	Lab I
Direct Injection Subtotal	100	100	100	100	50	50	100	50	50
reagent water	10	10	10	10	10	10	10	10	10
surface water	20	20	20	20	20	20	20	20	20
groundwater	20	20	20	20	20	20	20	20	20
semi-synthetic (field collected) waters	50	50	50	50			50		
SPE Subtotal	90	50	90	90		50	90	90	50
reagent water	10	10	10	10		10	10	10	10
surface water	20	20	20	20		20	20	20	20
groundwater	20	20	20	20		20	20	20	20
semi-synthetic (field collected) waters	40		40	40			40	40	
Low Level SPE Subtotal	24			24			24		24
reagent water	8			8			8		8
surface water	8			8			8		8
groundwater	8			8			8		8
Soils Subtotal	40			40	40				
High-Level Fat Clay	10			10	10				
Low-Level Fat Clay	10			10	10				
ERDC Soil	10			10	10				
JBCC Soil	10			10	10				
Tissues Subtotal			40	40				40	40
earthworm			20	20				20	20
ryegrass			20	20				20	20
Total Samples Provided	254	150	190	294	90	100	214	180	164

Each grouping of synthetic samples was prepared by spiking reference standard solutions containing each analyte found in Table 5 into a test matrix confirmed to have nondetectable analyte concentrations. Optimal test matrices like reagent water or ASTM Fat Clay were used to produce the recovery ranges. Laboratory grown earthworms and ryegrass were used to determine recovery ranges and were used in all tissue studies. In addition, spiked natural matrices were also prepared to compare analyte recoveries and determine whether the recoveries generally fell within the recovery ranges. These spiked natural matrices were comparable to those commonly used with USEPA Method 8330B. Surface waters from 2 sources, Yazoo River (Vicksburg, MS) and the Ross Barnett Reservoir (Brandon, MS) and groundwaters from 2 sources Rayville, LA and Salem, AR were used as natural water matrices for direct injection and SPE water testing. Soils from 3 sources, ASTM Fat Clay CH-1, ERDC in Vicksburg, MS and Cape Cod, MA were used for the soil testing. Two different tissues representing relevant animal (common earthworm) and plant (ryegrass) tissues were used for the tissue testing. These samples mimic real-world samples containing all analytes included in the method. Low and high analyte levels were prepared to determine any potential bias of individual laboratory results owing to relatively high or low analyte concentration. Samples were batch prepared and distributed to each laboratory according to previously established hold times in USEPA Method 8330B and USEPA SW-846 Chapter 4 Organic Analytes, Table 4.1 Semi-volatiles. The sub-sampling procedures used were established in USEPA Method 8330B and the QC criteria was established in DoD QSM Ver5.4 (2021).

Initial statistical testing for soils showed major inconsistencies for most analyte recoveries across all participating laboratories (originally N=7), ranging from 0 to 100,000%. This was hypothesized to be the result of either heterogeneous soil spiking or a compromised spiking solution. These initial recoveries had no obvious trend based on analyte, sample, soil source, or laboratory. As a result, this portion of the study was redone, however due to time constraints only 3 participating laboratories were used for the repeat round robin (CRREL, and 2 separate laboratories at ERDC). Additionally, only the spiked ASTM Fat Clay and spiked natural soils were analyzed again. The semisynthetic fortified soils deteriorated, and recollection wasn't possible due to time restraints. The soils were spiked at two levels to produce additional data due to the lower number of performing laboratories. The soils used were the same clean soils (ASTM Fat Clay (Soil #1), ERDC soil (Soil #2), and Joint Base Cape Cod (JBCC) soil (Soil #3)) and were prepared by sieving, drying and spiking with analytes diluted in MeOH followed by a rigorous ~72 h homogenization on a sample roller. Throughout the process sample hold times and storage conditions described in USEPA Method 8330B and USEPA SW-846 Semi-volatiles were adhered to.

To provide data on method applicability to more realistic samples, and inherent interferences they may contain, the semi-synthetic study was conducted wherein water samples were collected from sites where IM compounds are likely to be found (Middletown, IAAAP) and used to create semisynthetic samples. This study originally included soils collected from IAAAP, however, due to spiking issues these semisynthetic soil analyses produced unusable data in similar fashion to the synthetic samples. This semi-synthetic study allows for a comparison of analyte recoveries and determining whether the recoveries generally fell within the recovery ranges from full-synthetic testing. Process waters from ammunition production facilities have previously been shown to contain IM constituents at mg/L concentrations. Sample collection from the plant included pre-treatment process waters and manhole sumps. Samples were collected and transported back to the ERDC where they were pre-screened for legacy and IM compounds using the methods previously developed during SERDP ER-2722 efforts. The pre-screening determined the presence of DNAN, NTO, TNT and NQ (1 – 250 mg/L); 2,4-DNP and RDX (1 – 20 mg/L); NG, 3,5-DNA, 4-Am-2,6-DNT, 2-Am-4,6-DNT, 1,3,5-TNB, 2,6-DNT, 2,4-DNT (1 – 5 mg/L) and PA, 2-NP, and HMX (~ 1 mg/L). The test samples for this semisynthetic study were created by diluting the field collected waters containing IM compounds above into the ground waters and surface waters previously described and fortifying with analytes not present in the field collected waters. At participating laboratories, the samples were prepared and processed as with the previous synthetic samples. The semi-

synthetic field samples generated in this manner closely (more so than the full-synthetic samples) mimic real-world field activities that would release IM into aqueous matrices.

Results and Discussion

Participant Laboratory Feedback

During the round robin studies comments and observations from the various participating analysts were noted. This feedback is consolidated here since similar themes were observed at the various participant laboratories during project execution. Quaternary pumps are preferable to binary pump HPLC systems as it allows fine-tuning of solvent gradient programming. This solvent programming is particularly critical for these methods as subtle changes in mobile phase pH can cause peak shifting and potential coelution. In addition to use of a quaternary pump HPLC, ensuring that mobile phases are freshly and correctly prepared is a high priority. The method may also need to be adjusted slightly and should be tested prior to use to ensure that the chromatographic parameters result in optimal analyte separation. This adjustment may need to occur more often, depending on how often mobile phases are remade and how often chromatographic columns are changed. Optimization of the method will improve chromatographic separation and therefore improve integration accuracy and reproducibility. It is necessary to manually integrate peaks on most systems, which is a labor-intensive process, but produces the highest quality data. The SPE extraction of water matrices is a lengthy process that requires analyst attention, particularly for large volumes of water in order to minimize SPE cartridges clogging and drying out while loading cartridges. The loading of cartridges here, contrary to single-stack SPE extractions, is not routine and may require some adjustments (i.e., swapping vacuum ports, tightening SPE adapters, etc.) throughout.

Soil Milling Study

The milling procedure comparison study between puck mill, and mortar and pestle preparations utilized lab spiked soils split into aliquots prior to puck mill or mortar and pestle preparations. The results of this comparison study confirmed comparable performance between the two methods, with both achieving recoveries and relative percent differences (RPDs) that meet or surpass DoD QSM Ver5.4 criteria (recovery between 64% and 135% and $RPD < 40\%$). However, 3,5-DNA and NB, had lower performance following mortar and pestle milling that were slightly below the DoD QSM Ver5.4 criteria. 3,5-DNA had lower recovery using the primary HPLC-UV method (62%) while NB had lower recovery with the secondary HPLC-UV method (61%). 3,5-DNA recovery improved to 103% (50% RPD) with the secondary HPLC-UV method, while NB recovery improved to 102% (5.7% RPD) with the primary HPLC-UV method. Low recovery seems to be unique to these analytes, as the same trend is observed in the data from use of a puck mill, although in that case the recoveries and RPDs still fell within the DoD QSM Ver5.4 criteria. This may indicate an issue with spiking or the original spike solutions, or just random analytical error. Comparisons between the two milling methods are shown in Table 7 and Table 8. These tables include the measured concentration (concentration detected by the instrument), calculated concentration (concentration in soil prior to extraction), percent recoveries, standard deviation, and relative percent difference. Results from the triplicate unspiked (blank) soils are not shown as all analytes were below detection, except for surrogates, which were all within DoD QSM Ver5.4 criteria regardless of grinding method.

Table 7: Recoveries and RPDs for Primary HPLC-UV Analysis of Spiked Soil Samples

Puck mill Spiked (Primary)					Mortar-Pestle Spiked (Primary)					RPD
Compound	Measured (mg/L)	Calculated (mg/kg)	Recovery (%)	SD (N=3)	Compound	Measured (mg/L)	Calculated (mg/kg)	Recovery (%)	SD (N=3)	
1,2-DNB (surrogate)	1.049	4.996	105	± 0.5	1,2-DNB (surrogate)	1.064	5.131	106	± 5.1	1
1,3,5-TNB	1.895	9.028	95	± 1.3	1,3,5-TNB	1.992	9.590	100	± 6.2	5
1,3-DNB	2.008	9.564	100	± 2.1	1,3-DNB	2.020	9.729	101	± 5.0	1
2,4,6-TNT	2.032	9.679	102	± 3.4	2,4,6-TNT	2.070	9.968	103	± 5.7	2
2,4-DNP	2.016	9.603	101	± 2.1	2,4-DNP	2.102	10.117	105	± 6.4	4
2,4-DNT	1.962	9.344	98	± 1.5	2,4-DNT	2.036	9.797	102	± 7.9	4
2,6-DNT	2.016	9.605	101	± 2.0	2,6-DNT	1.977	9.549	99	± 3.4	2
2-Am-4,6-DNT	1.888	8.992	94	± 2.8	2-Am-4,6-DNT	2.018	9.715	101	± 5.5	7
2-NP	8.162	7.769	82	± 5.1	2-NP	8.618	8.308	86	± 2.8	5
2-NT	1.541	7.345	77	± 1.7	2-NT	1.429	6.874	71	± 5.9	8
3,5-DNA	1.375	6.548	69	± 2.7	3,5-DNA	1.236	5.954	62	± 2.9	11
3-NT	1.617	7.706	81	± 4.9	3-NT	1.518	7.309	76	± 5.1	6
4-Am-2,6-DNT	1.686	8.033	84	± 1.3	4-Am-2,6-DNT	1.984	9.553	99	± 5.1	16
4-NP	2.024	9.641	101	± 2.4	4-NP	2.051	9.876	103	± 4.6	1
4-NT	1.736	8.272	87	± 3.8	4-NT	1.679	8.073	84	± 7.8	3
DNAN	1.952	9.300	98	± 2.6	DNAN	1.979	9.527	99	± 5.5	1
HMX	1.794	8.542	90	± 2.1	HMX	1.900	9.151	95	± 4.7	6
NB	1.744	8.307	87	± 2.0	NB	2.044	9.842	102	± 5.7	16
NG	1.886	8.984	94	± 5.1	NG	1.583	7.633	79	± 11.2	17
NQ	1.490	7.099	75	± 1.5	NQ	1.644	7.911	82	± 6.6	10
NTO	1.552	7.389	78	± 1.9	NTO	1.975	9.523	99	± 4.4	24
o-NBA (surrogate)	0.847	4.036	85	± 2.2	o-NBA (surrogate)	0.858	4.139	86	± 3.6	1
PA (315 nm)	2.049	9.759	102	± 2.2	PA (315 nm)	2.157	10.389	108	± 4.5	5
PETN	1.907	9.088	95	± 4.6	PETN	1.730	8.348	87	± 3.2	10
RDX	1.898	9.039	95	± 2.5	RDX	1.999	9.629	100	± 5.0	5
Tetryl	1.776	8.463	89	± 2.2	Tetryl	1.943	9.361	97	± 4.4	9

Table 8: Recoveries and RPDs for Secondary HPLC-UV Analysis of Spiked Soil Samples

Puck mill Spiked (Secondary)					Mortar-Pestle Spiked (Secondary)					RPD
Compound	Measured (mg/L)	Calculated (mg/kg)	Recovery (%)	SD (N=3)	Compound	Measured (mg/L)	Calculated (mg/kg)	Recovery (%)	SD (N=3)	
1,2-DNB (surrogate)	1.188	5.662	119	± 1.1	1,2-DNB (surrogate)	0.943	4.549	94	± 6.2	23
1,3,5-TNB	1.990	9.478	100	± 3.7	1,3,5-TNB	2.010	9.677	101	± 6.2	1
1,3-DNB	1.994	9.495	100	± 2.9	1,3-DNB	2.017	9.715	101	± 5.0	1
2,4,6-TNT	2.010	9.576	101	± 2.2	2,4,6-TNT	2.043	9.840	102	± 4.9	2
2,4-DNP	1.986	9.461	99	± 4.3	2,4-DNP	2.346	11.294	117	± 7.4	17
2,4-DNT	1.882	8.963	94	± 3.3	2,4-DNT	1.976	9.513	99	± 6.3	5
2,6-DNT	2.091	9.960	105	± 1.6	2,6-DNT	1.965	9.461	98	± 4.9	6
2-Am-4,6-DNT	1.924	9.165	96	± 2.8	2-Am-4,6-DNT	2.051	9.875	103	± 5.2	6
2-NP	8.131	7.741	81	± 4.4	2-NP	8.404	8.091	84	± 5.3	3
2-NT	1.547	7.365	77	± 3.1	2-NT	1.398	6.735	70	± 2.6	10
3,5-DNA	1.751	8.339	88	± 2.7	3,5-DNA	2.057	9.905	103	± 5.3	16
3-NT	1.791	8.526	90	± 5.1	3-NT	1.499	7.211	75	± 6.7	18
4-Am-2,6-DNT	1.603	7.638	80	± 1.4	4-Am-2,6-DNT	1.977	9.523	99	± 4.9	21
4-NP	2.000	9.524	100	± 2.9	4-NP	2.072	9.976	104	± 5.6	4
4-NT	2.035	9.700	102	± 3.3	4-NT	1.723	8.300	86	± 3.4	17
DNAN	1.959	9.332	98	± 1.5	DNAN	2.004	9.651	100	± 4.9	2
HMX	1.734	8.258	87	± 2.0	HMX	1.895	9.123	95	± 6.0	9
NB	1.378	6.561	69	± 2.0	NB	1.214	5.845	61	± 3.4	13
NG	2.010	9.577	101	± 0.5	NG	2.055	9.881	103	± 9.9	2
NQ	2.030	9.666	101	± 2.4	NQ	2.126	10.237	106	± 5.3	5
NTO	1.495	7.124	75	± 1.4	NTO	1.602	7.713	80	± 4.0	7
o-NBA (surrogate)	0.819	3.905	82	± 2.2	o-NBA (surrogate)	0.828	3.993	83	± 1.3	1
PA (315 nm)	1.973	9.396	99	± 4.4	PA (315 nm)	2.002	9.642	100	± 4.0	1
PETN	2.311	11.013	116	± 3.7	PETN	2.023	9.760	101	± 0.9	13
RDX	1.808	8.615	90	± 2.0	RDX	1.903	9.164	95	± 5.1	5
Tetryl	1.742	8.296	87	± 2.3	Tetryl	1.940	9.342	97	± 5.5	11

Direct-Injection Method for Waters

Generally, the direct injection data produced the tightest recovery ranges (91% tolerance interval (TI)) with most analyte recoveries within $\pm 10\%$ of 100% recovery. This is almost certainly attributable to the relatively straightforward sample preparation procedure, and the higher analyte concentrations used to test the direct-injection method. The direct injection multi laboratory recovery ranges are shown in Table 9 along with the lower and upper control limits from USEPA Method 8330B included for relative comparison. Direct Injection recoveries for surface waters and groundwaters, respectively, are shown in Tables 10 and 11. Semi-synthetic direct injection recoveries of field collected waters are shown in Table 12 and represent method performance under the most realistic conditions. Table 12 also includes the recovery of legacy explosives in these waters by USEPA Method 8330B for comparison.

Table 9: Direct Injection Reagent Water Recovery Ranges and USEPA 8330B Lower and Upper Control Limits

Analyte	Recovery Range at 91% TI (N=7)	Lower and Upper Control Limit (8330B)
1,2-DNB (surr.)	74 – 112	83 – 119
1,3-DNB	88 – 119	78 – 120
1,3,5-TNB	95 – 125	73 – 125
1,4-DNB (surr.)	95 – 140 (26% TI)	
2-Am-4,6-DNT	88 – 115	79 – 120
2-NP	88 – 121	
2-NT	86 – 118	70 – 127
2,4-DNP	96 – 131	
2,4-DNT	86 – 116	78 – 120
Picric Acid (PA)	93 – 147 (90% TI)	
2,4,6-TNT	95 – 151	71 – 123
2,6-DNT	86 – 132	77 – 127
3-NT	88 – 168	73 – 125
3,5-DNA	87 – 154	71 – 117
4-Am-2,6-DNT	87 – 113	76 – 125
4-NP	93 – 145	
4-NT	87 – 125	71 – 127
DNAN	93 – 134	
HMX	96 – 122	65 – 135
NB	88 – 113	65 – 134
NG	72 – 189	74 – 127
NQ	55 – 145	
NTO	72 – 124	
o-NBA (surr.)	68 – 140 (87% TI)	
PETN	86 – 133	73 – 127
RDX	93 – 125	68 – 130
Tetryl	84 – 144	64 – 128

*Entries in **bold** are additional analytes not included in USEPA Method 8330B*

Table 10: Direct Injection Surface Water Percent Recoveries

Direct Injection Recoveries Surface Water (1.64 and 5.11 mg/L spike concentration in 2 separate waters)												
Analyte	Lab A	Lab B	Lab C	Lab E (1)	Lab E (2)	Lab F	Lab G	Lab H	Lab I	Multi-Lab		
										AVG	SD	N
1,2-DNB (surr.)	99.9	86.1	89.0	115.8	Not used	88.3	102.6	85.9		95.4	± 13.4	138
1,3-DNB	105.0	102.4	111.6	99.9	98.3	90.6	95.6	89.7	100.2	99.4	± 9.8	178
1,3,5-TNB	104.9	103.4	114.4	100.7	97.8	90.3	98.4	101.8	108.9	102.3	± 10.7	178
1,4-DNB (surr.)	Not used	Not used	Not used	Not used	119.8	Not used	Not used	Not used	98.1	108.9	± 18.4	60
2-Am-4,6-DNT	103.8	102.2	112.3	99.3	97.4	90.1	93.2	96.2	100.1	99.4	± 9.8	178
2-NP	99.7	99.3	117.6	97.8	104.4	97.0	85.7	106.5	95.2	100.3	± 10.5	178
2-NT	102.6	96.6	90.4	95.6	93.8	88.2	91.8	88.9	100.7	94.3	± 9.3	178
2,4-DNP	98.8	107.4	124.2	103.8	101.7	94.4	95.0	102.9	96.3	102.7	± 11.2	178
2,4-DNT	101.9	102.7	109.8	98.6	98.9	90.9	101.9	89.0	99.4	99.2	± 10.1	178
Picric Acid (PA)	120.8	106.0	112.6	98.8	91.7	93.7	95.6	93.4	107.8	101.7	± 14.5	178
2,4,6-TNT	105.4	103.1	111.3	100.1	98.4	89.7	95.6	97.9	100.9	100.3	± 9.8	178
2,6-DNT	107.6	102.4	118.1	102.7	94.9	90.6	105.9	89.1	109.5	102.3	± 26.8	178
3-NT	103.2	98.4	94.7	95.9	94.4	88.6	91.6	97.3	100.0	96.0	± 12.3	178
3,5-DNA	103.9	103.5	90.1	104.2	92.4	92.4	95.1	96.8	98.4	97.4	± 12.5	178
4-Am-2,6-DNT	101.4	103.1	112.3	98.0	97.8	90.9	93.8	94.3	99.1	99.0	± 10.0	178
4-NP	92.4	108.5	143.3	103.7	103.1	95.9	95.0	106.1	100.0	105.3	± 16.1	178
4-NT	103.8	97.3	99.1	93.7	93.4	89.7	89.9	96.8	109.8	97.0	± 13.5	178
DNAN	100.5	105.8	123.0	101.9	97.9	88.6	94.6	101.5	94.7	101.0	± 11.2	178
HMX	106.5	102.8	117.8	100.3	97.9	89.7	92.8	98.3	99.2	100.6	± 11.0	178
NB	102.7	102.6	111.3	98.4	97.2	92.6	93.5	92.6	97.9	98.8	± 7.6	178
NG	103.6	112.7	98.9	99.1	96.9	111.0	89.3	99.9	139.1	105.6	± 43.0	178
NQ	96.5	109.8	120.5	78.2	83.6	75.4	118.2	85.1	116.1	98.2	± 22.6	178
NTO	91.1	99.4	118.7	102.6	94.4	92.8	87.2	94.4	111.0	99.1	± 11.4	178
o-NBA (surr.)	113.1	85.3	108.7	110.9	120.6	87.6		94.5	97.4	102.3	± 16.0	158
PETN	99.2	123.3	111.5		101.8	91.5	96.2	94.9	106.8	103.1	± 15.5	178
RDX	108.7	104.2	113.1	103.5	97.9	92.2	94.7	93.1	99.2	100.7	± 10.2	178
Tetryl	104.2	102.6	110.8	96.6	88.7	88.4	90.0	99.3	100.9	98.0	± 10.6	178

Highlighted entries are outside calculated recovery ranges, but within 8330B Control Limits

Highlighted entries are outside both calculated recovery ranges and 8330B Control Limits

Table 11: Direct Injection Groundwater Percent Recoveries

Direct Injection Recoveries Groundwater (2.96 and 4.36 mg/L spike concentration in 2 separate waters)												
Analyte	Lab A	Lab B	Lab C	Lab E (1)	Lab E (2)	Lab F	Lab G	Lab H	Lab I	Multi-Lab		
										AVG	SD	N
1,2-DNB (surr.)	99.1	89.5	86.1	101.8	Not used	84.1	103.5	84.6		92.7	± 10.6	140
1,3-DNB	109.1	93.5	111.0	103.6	97.9	87.8	101.4	90.1	100.8	99.5	± 9.6	180
1,3,5-TNB	108.3	94.4	113.6	104.3	97.5	86.9	102.3	102.1	101.0	101.1	± 9.5	180
1,4-DNB (surr.)	Not used	Not used	Not used	Not used	130.1	Not used	Not used	Not used	96.7	113.4	± 20.9	40
2-Am-4,6-DNT	107.4	93.3	112.2	102.9	97.5	87.1	99.6	93.6	100.0	99.3	± 9.4	180
2-NP	103.4	92.0	116.7	102.7	103.9	100.7	92.9	109.3	91.3	101.4	± 9.7	180
2-NT	105.6	91.7	88.5	99.9	91.1	81.9	98.2	92.7	100.1	94.4	± 8.4	180
2,4-DNP	102.7	98.1	120.8	107.8	101.6	93.6	100.1	100.5	97.3	102.5	± 10.3	180
2,4-DNT	107.0	93.9	110.4	103.5	99.1	87.5	95.0	91.5	100.4	98.7	± 10.7	180
Picric Acid (PA)	110.4	96.7	93.3	102.7	95.6	92.9	102.6	95.1	103.1	99.6	± 9.6	167
2,4,6-TNT	108.6	94.1	110.6	103.5	98.1	86.1	100.1	111.9	101.3	101.6	± 11.8	180
2,6-DNT	110.7	93.5	119.9	105.6	93.9	88.4	96.5	91.1	109.2	101.1	± 13.5	180
3-NT	106.3	92.6	93.1	99.8	92.3	83.3	98.7	95.5	89.6	94.6	± 8.6	180
3,5-DNA	107.7	94.3	105.3	103.8	90.2	85.8	102.8	113.2	100.5	98.4	± 13.7	180
4-Am-2,6-DNT	106.4	93.8	113.7	103.5	98.2	87.5	98.6	90.2	98.2	98.9	± 9.9	180
4-NP	100.4	99.5	136.4	107.2	103.3	95.0	100.7	108.3	100.4	105.7	± 14.2	180
4-NT	106.8	91.0	97.9	97.6	91.6	83.9	96.6	95.7	115.1	97.4	± 10.6	180
DNAN	105.2	96.5	121.2	105.7	98.4	83.1	100.0	115.9	92.7	102.1	± 13.5	180
HMX	111.2	94.9	116.7	103.9	97.6	87.1	104.5	108.7	102.0	102.8	± 10.8	180
NB	106.2	101.1	110.7	102.7	97.3	86.7	103.8	101.9	99.8	101.1	± 7.4	180
NG	107.7	103.8	96.4	104.4	96.7	99.9	94.2	138.1	173.9	112.8	± 53.2	180
NQ	101.2	98.5	122.2	79.3	82.3	70.9	111.2	67.7	112.5	94.0	± 20.5	180
NTO	96.4	92.7	117.7	104.4	92.9	90.1	91.8	97.0	102.9	98.4	± 11.2	180
o-NBA (surr.)	108.9	88.9	112.3	97.0	131.1	84.1		95.0	98.6	102.0	± 16.7	160
PETN	103.0	118.6	111.4		95.8	92.5	100.9	96.0	103.1	102.7	± 17.1	180
RDX	112.1	95.0	112.4	107.3	97.8	87.1	99.7	93.8	100.5	100.6	± 10.0	180
Tetryl	107.2	93.6	110.3	99.3	89.4	85.4	96.4	119.8	101.6	100.3	± 12.9	180

Highlighted entries are outside calculated recovery ranges, but within 8330B Control Limits

Highlighted entries are outside both calculated recovery ranges and 8330B Control Limits

Table 12: Direct Injection Field Collected Waters Percent Recoveries

Direct Injection Recoveries Field collected waters (varying spike concentration from 3.85 to 35.78 mg/L across 4 waters)									
	ESTCP Methods					Multi-Lab			8330B
Analyte	Lab A	Lab B	Lab C	Lab E	Lab G	AVG	SD	N	Lab G
1,2-DNB (surr.)	86.3	105.0	93.2	Not used	88.9	93.4	12.8	200	100.3
1,3-DNB	103.8	97.9	119.8	85.6	98.9	101.2	12.3	200	89.9
1,3,5-TNB	99.0	93.4	110.4	83.1	96.6	96.5	8.9	200	96.4
1,4-DNB (surr.)	Not used	Not used	Not used	93.8	Not used	93.8	31.6	50	
2-Am-4,6-DNT	70.2	66.5	90.2	57.0	92.9	74.4	29.3	190	99.1
2-NP	88.7	91.4	129.1	83.9	99.6	98.5	15.0	200	
2-NT	94.1	87.3	109.6	82.8	94.4	93.7	45.8	200	79.2
2,4-DNP	4.1	3.9	4.1	3.8	4.2	4.0	44.6	200	
2,4-DNT	93.3	90.3	102.7	80.2	94.0	92.1	11.8	200	90.1
Picric Acid (PA)	97.1	98.9	76.1	85.1	102.9	92.0	11.0	200	
2,4,6-TNT	93.6	89.0	103.4	85.2	92.8	92.8	10.4	200	86.8
2,6-DNT	101.9	94.3	116.2	79.7	95.8	97.3	12.2	190	93.1
3-NT	91.3	81.8	103.3	77.6	93.0	89.4	17.6	200	80.3
3,5-DNA	88.5	63.5	104.7	84.4	86.4	85.3	27.8	180	93.2
4-Am-2,6-DNT	83.3	54.5	88.1	45.4	70.8	67.2	28.4	180	120.9
4-NP	99.7	96.7	128.0	82.3	95.7	100.5	14.0	200	
4-NT	89.6	83.9	103.0	79.3	89.9	89.1	37.7	200	81.8
DNAN	27.7	30.7	37.4	36.3	32.7	32.9	32.2	200	
HMX	50.6	42.0	40.9	37.2	45.0	43.1	31.6	200	39.8
NB	91.1	79.3	98.5	71.4	92.5	86.7	16.3	180	92.7
NG	93.2	89.6	107.5	85.4	97.9	94.7	37.2	200	88.0
NQ	34.6	43.2	49.1	28.0	39.3	38.8	28.5	200	
NTO	42.5	60.5	59.9	51.4	48.4	52.5	31.4	200	
o-NBA (surr.)	96.4	111.2	114.0	95.1	96.6	102.7	41.4	250	
PETN	32.1	32.2	31.1	46.3	38.3	36.0	45.9	200	35.2
RDX	97.2	122.8	105.5	86.1	96.3	101.6	23.4	200	93.1
Tetryl	94.5	83.9	97.8	78.7	90.7	89.1	13.3	200	81.4

Highlighted entries are outside calculated recovery ranges, but within 8330B Control Limits

Highlighted entries are outside both calculated recovery ranges and 8330B Control Limits

Solid Phase Extraction Method for Aqueous Matrices

The recovery ranges presented in this section are split into 3 different groups: (1) results from full-synthetic testing (spiked reagent water and natural waters), (2) results from semi-synthetic testing (process waters known to contain legacy and IM compounds, (3) results from low level testing (reagent waters spiked approximately at USEPA drinking water limits. The full-synthetic SPE multi laboratory recovery ranges (95% TI) and SPE high performing laboratory recovery ranges (95% TI) are shown in Table 13, along with the lower and upper control limits from USEPA Method 8330B included for relative comparison. Percent recoveries for surface waters and groundwaters, respectively, are shown in Tables 14 and 15. Lower concentration SPE recoveries are observed, as shown in Table 16 and demonstrate method performance at $\sim 2 \mu\text{g/L}$. Semi-synthetic SPE recoveries for field collected waters are shown in Table 17 and shows practical method performance.

Table 13: Full-synthetic SPE Reagent Water Recovery Ranges and USEPA 8330B Lower and Upper Control Limits

Analyte	Recovery Range at 95% TI (N = 6)	Recovery Range at 95% TI (Highest performing lab) (N=1)	Lower and Upper Control Limit (8330B)
1,2-DNB (surr.)	69 – 119	88 – 106	83 – 119
1,3-DNB	39 – 130	81 – 113	78 – 120
1,3,5-TNB	69 – 166	91 – 109 (9% TI)	73 – 125
1,4-DNB (surr.)	47 – 132	Not used	
2-Am-4,6-DNT	37 – 137	84 – 109	79 – 120
2-NP	58 – 182	76 – 106	
2-NT	46 – 200	66 – 103	70 – 127
2,4-DNP	81 – 163	83 – 105	
2,4-DNT	50 – 123	73 – 119	78 – 120
Picric Acid (PA)	57 – 127	75 – 104	
2,4,6-TNT	51 – 142	85 – 112	71 – 123
2,6-DNT	37 – 126	84 – 121	77 – 127
3-NT	47 – 280	80 – 127	73 – 125
3,5-DNA	49 – 200	85 – 105	71 – 117
4-Am-2,6-DNT	50 – 161	86 – 107	76 – 125
4-NP	64 – 139	85 – 100	
4-NT	44 – 169	67 – 111	71 – 127
DNAN	47 – 131	76 – 114	
HMX	65 – 163	117 – 162	65 – 135
NB	60 – 131	74 – 105	65 – 134
NG	58 – 177	81 – 115	74 – 127
NQ	0 – 257	81 – 115	
NTO	11 – 160	57 – 114	
o-NBA (surr.)	41 – 110	68 – 127	
PETN	25 – 164	83 – 103	73 – 127
RDX	13 – 157	97 – 110	68 – 130
2Tetryl	47 – 152	87 – 101	64 – 128

*Entries in **bold** are additional analytes not included in USEPA Method 8330B*

Table 14: SPE Surface Water Percent Recoveries

SPE Waters Synthetic Surface Water Percent Recoveries (0.023 and 0.041 mg/L spike concentration in 2 separate waters)									
Analyte	Lab A	Lab C	Lab E	Lab F	Lab G	Lab H	Multi-Lab		
							AVG	SD	N
1,2-DNB (surr.)	84.9	91.8	Not used	88.8	73.2	54.7	77.2	± 26.7	90
1,3-DNB	89.3	103.0	94.3	89.3	90.2	49.2	85.9	± 22.9	120
1,3,5-TNB	62.2	47.0	92.5	76.0	65.9	30.3	62.2	± 25.6	117
1,4-DNB (surr.)	Not used	Not used	106.5	Not used	Not used	Not used	106.5	± 14.4	20
2-Am-4,6-DNT	91.5	105.2	90.0	87.4	97.0	44.4	85.9	± 27.7	120
2-NP	80.4	138.1	88.5	98.4	88.3	48.5	90.4	± 32.3	120
2-NT	83.5	139.1	88.6	81.8	85.8	56.1	89.1	± 32.6	120
2,4-DNP	92.4	123.5	93.2	101.6	99.6	64.3	95.8	± 25.1	120
2,4-DNT	89.2	102.3	94.6	87.9	86.5	63.4	87.3	± 19.4	120
Picric Acid (PA)	87.6		96.5	84.0	77.7	67.1	82.6	± 17.5	99
2,4,6-TNT	77.4	75.0	92.2	86.1	69.9	46.6	74.5	± 21.7	120
2,6-DNT	99.0	109.7		90.5	92.5	58.6	90.1	± 25.9	100
3-NT	95.2	148.8	93.3	81.0	94.7	71.8	97.5	± 31.7	119
3,5-DNA	95.5	142.5	90.1	95.1	97.6	43.8	94.1	± 36.6	120
4-Am-2,6-DNT	87.7	100.2	83.6	79.7	86.9	38.4	79.4	± 23.5	120
4-NP	90.6	114.4	94.2	92.9	98.6	52.5	90.5	± 23.9	120
4-NT	87.2	125.9	89.0	84.1	87.0	67.5	90.1	± 25.5	120
DNAN	91.8	113.6	93.4	92.4	91.7	61.7	90.8	± 24.4	120
HMX	126.8	112.1	91.6	88.7	98.5	56.9	95.7	± 23.6	120
NB	86.2	117.1	91.7	85.7	91.6	82.0	90.8	± 21.4	119
NG	95.0	110.4	74.8	106.2	89.2	58.9	89.1	± 28.8	120
NQ	2.1	20.4	45.3	24.3		0.0	17.9	± 42.5	95
NTO	114.7	115.1	117.7	86.1	128.8	80.0	107.1	± 27.1	120
o-NBA (surr.)	91.9	103.2	95.0	79.1	84.9	68.6	85.7	± 17.7	100
PETN	97.6	126.8	88.0	97.0	192.7	45.8	107.3	± 161.2	119
RDX	105.1	112.8	91.5	90.0	97.1	52.0	91.4	± 32.6	119
Tetryl	45.7	31.5	71.1	62.1	34.2	34.2	46.5	± 21.1	120

Highlighted entries are outside calculated recovery ranges, but within 8330B Control Limits

Highlighted entries are outside both calculated recovery ranges and 8330B Control Limits

Table 15: SPE Groundwater Percent Recoveries

SPE Waters Synthetic Groundwater Percent Recoveries (0.035 and 0.046 mg/L spike concentration in 2 separate waters)									
Analyte	Lab A	Lab C	Lab E	Lab F	Lab G	Lab H	Multi-Lab		
							AVG	SD	N
1,2-DNB (surr.)	95.9	96.3	Not used	104.8	106.7	68.9	94.5	± 18.2	99
1,3-DNB	94.8	110.1	96.3	97.3	94.6	65.1	92.9	± 16.3	119
1,3,5-TNB	90.8	112.8	99.8	102.1	101.0	41.2	91.1	± 28.1	119
1,4-DNB (surr.)	Not used	Not used	103.3	Not used	Not used	Not used	103.3	± 8.0	20
2-Am-4,6-DNT	94.8	96.0	93.8	100.4	95.0	54.8	89.1	± 20.4	119
2-NP	89.8	124.3	94.7	120.1	93.8	54.9	96.0	± 27.1	119
2-NT	84.0	107.1	90.6	98.8	88.6	64.1	88.7	± 16.0	119
2,4-DNP	95.0	118.7	95.4	114.2	103.4	58.9	97.4	± 26.8	119
2,4-DNT	92.7	107.5	100.9	93.4	89.8	81.6	94.2	± 10.6	119
Picric Acid (PA)	89.9		100.5	104.1	98.9	95.2	97.7	± 14.0	100
2,4,6-TNT	102.3	117.2	97.0	97.7	95.7	54.9	93.9	± 22.4	119
2,6-DNT	99.7	110.9		97.4	90.0	72.6	93.9	± 15.0	99
3-NT	87.2	122.0	96.7	105.8	93.3	81.7	97.6	± 15.5	119
3,5-DNA	95.3	110.7	95.9	108.8	96.3	42.2	91.4	± 26.0	119
4-Am-2,6-DNT	96.0	85.8	92.6	105.5	93.7	42.7	86.1	± 25.6	119
4-NP	96.2	114.6	95.4	137.5	101.6	44.0	98.1	± 34.2	119
4-NT	86.9	103.4	90.0	95.9	88.9	73.5	89.7	± 12.0	119
DNAN	94.5	108.7	94.8	104.8	95.9	52.5	91.7	± 22.0	119
HMX	131.8	106.2	96.2	117.4	94.4	44.8	98.4	± 32.8	119
NB	89.4	105.2	96.3	104.2	94.7	76.4	94.3	± 15.9	119
NG	89.5	129.5	59.3	123.5	103.0	40.8	90.6	± 39.3	119
NQ	7.3	14.6	39.7	16.6		0.0	15.6	± 13.9	99
NTO	86.6	92.4	77.9	82.6	75.8	73.3	81.3	± 9.4	119
o-NBA (surr.)	84.3	110.6	99.2	82.8	100.0	85.0	93.5	± 13.8	119
PETN	100.8	110.2	100.2	127.2	106.9	33.1	96.3	± 35.7	119
RDX	102.2	113.6	95.0	128.1	100.5	34.3	95.5	± 35.8	119
Tetryl	58.3	58.3	75.6	101.8	69.6	30.6	65.8	± 27.3	119

Highlighted entries are outside calculated recovery ranges, but within 8330B Control Limits

Highlighted entries are outside both calculated recovery ranges and 8330B Control Limits

Table 16: Low Level SPE Surface Water Recoveries

Low-level SPE Percent Recoveries Surface Water (1.88 µg/L spike)					
			Multi-Lab		
Analyte	Lab A	Lab E	AVG	SD	N
1,2-DNB (surr.)	79.2		79.2	13.0	7
1,3-DNB	85.7	111.7	98.7	16.6	15
1,3,5-TNB	23.9	69.2	46.5	25.4	15
1,4-DNB (surr.)		102.1	102.1	7.4	8
2-Am-4,6-DNT	97.1	105.5	101.3	10.2	15
2-NP	46.1	99.2	72.7	29.6	15
2-NT	83.4	95.6	89.5	10.5	15
2,4-DNP	97.6	116.3	106.9	11.0	15
2,4-DNT	83.6	142.5	113.1	32.3	15
Picric Acid (PA)	92.1	74.2	83.2	17.2	15
2,4,6-TNT	27.3	105.1	66.2	45.8	15
2,6-DNT	124.3	84.2	104.2	22.6	15
3-NT	120.4	165.5	143.0	74.1	15
3,5-DNA	101.3	106.2	103.8	9.5	15
4-Am-2,6-DNT	113.8	103.9	108.9	12.1	15
4-NP	96.9	128.4	112.6	18.7	15
4-NT	84.4	91.9	88.2	13.1	15
DNAN	84.5	131.6	108.1	26.5	15
HMX	92.6	117.7	105.1	16.1	15
NB	100.4	102.4	101.4	7.4	15
NG	91.7	84.3	88.0	31.7	15
NQ		52.4	52.4	26.4	8
NTO	505.6	110.7	308.1	218.1	15
o-NBA (surr.)	79.9	88.7	84.3	12.8	15
PETN	95.1	170.5	132.8	76.6	15
RDX	93.7	122.3	108.0	16.5	15
Tetryl	12.9	27.0	20.0	13.2	15

Highlighted entries are outside calculated recovery ranges, but within 8330B Control Limits

Highlighted entries are outside both calculated recovery ranges and 8330B Control Limits

Table 17: Low level SPE Groundwater Recoveries

Low-level SPE Recoveries Groundwater (1.88 µg/L spike)					
Analyte	Lab A	Lab E	Multi-Lab		
			AVG	SD	N
1,2-DNB (surr.)	96.7	Not used	96.7	5.0	8
1,3-DNB	99.0	126.6	112.8	20.0	16
1,3,5-TNB	98.0	162.6	130.3	38.3	16
1,4-DNB (surr.)	Not used	117.5	117.5	16.4	8
2-Am-4,6-DNT	103.4	137.0	120.2	21.1	16
2-NP	77.1	108.6	92.9	19.1	16
2-NT	85.9	107.7	96.8	16.7	16
2,4-DNP	104.8	114.5	109.7	7.6	16
2,4-DNT	95.8	164.9	130.4	43.9	16
Picric Acid (PA)	99.9	131.1	115.5	21.2	16
2,4,6-TNT	138.0	203.4	170.7	42.9	16
2,6-DNT	122.0	88.6	105.3	22.5	16
3-NT	124.4	1005.8	565.1	570.3	16
3,5-DNA	95.5	106.6	101.0	9.1	16
4-Am-2,6-DNT	110.7	148.9	129.8	22.9	16
4-NP	102.2	111.1	106.6	8.1	16
4-NT	106.6	112.8	109.7	14.3	16
DNAN	75.5	121.4	98.4	29.1	16
HMX	142.5	138.5	140.5	18.4	16
NB	99.7	114.1	106.9	13.5	16
NG	90.5	101.3	95.9	47.7	8
NQ		45.8	45.8	19.6	16
NTO	79.1	95.6	87.3	23.8	16
o-NBA (surr.)	73.0	95.9	84.4	22.4	16
PETN	184.9	145.5	165.2	55.4	16
RDX	103.1	119.1	111.1	12.9	16
Tetryl	45.1	72.2	58.6	20.0	16

Highlighted entries are outside calculated recovery ranges, but within 8330B Control Limits

Highlighted entries are outside both calculated recovery ranges and 8330B Control Limits

Table 18: SPE Field Collected Semisynthetic Waters Percent Recoveries

SPE Recoveries Field collected waters (0.07 and 0.12 mg/L spike concentration for 2 separate waters)								
Analyte	ESTCP Methods				Multi-Lab			8330B
	Lab A	Lab C	Lab E	Lab G	AVG	SD	N	Lab G
1,2-DNB (surr.)	108.7	110.0	Not used	97.7	104.6	11.4	48	99.2
1,3-DNB	101.1	82.5	85.8	136.1	101.2	99.1	77	88.4
1,3,5-TNB	94.5	81.1	81.1	139.4	98.8	88.9	77	86.6
1,4-DNB (surr.)	Not used	Not used	102.7	Not used	102.7	17.9	20	
2-Am-4,6-DNT	100.2	92.1	82.9	139.8	103.5	97.0	77	88.0
2-NP	85.8	108.1	84.6	126.8	101.1	90.4	77	
2-NT	92.9	111.2	81.4	130.8	103.8	97.2	77	77.9
2,4-DNP	19.7	17.9	70.7	20.4	40.7	26.8	48	
2,4-DNT	134.5	77.6	82.0	126.7	104.9	93.7	79	95.0
Picric Acid (PA)	113.9	68.9	88.1	136.8	101.7	88.3	77	
2,4,6-TNT	95.8	75.7	50.2	93.2	78.3	36.8	77	84.2
2,6-DNT	97.7	79.6	0.0	137.7	77.7	114.7	77	88.7
3-NT	95.5	109.7	84.5	134.4	105.8	102.2	77	78.8
3,5-DNA	101.3	121.5	84.5	132.5	109.6	96.5	77	81.7
4-Am-2,6-DNT	97.8	97.2	78.9	140.5	103.3	91.5	77	89.8
4-NP	101.9	101.2	86.9	141.3	107.6	98.6	77	
4-NT	96.1	97.0	80.4	128.7	100.3	87.5	77	80.7
DNAN	26.2	21.3	60.7	35.9	41.7	23.0	48	
HMX	114.8	84.8	85.6	143.4	106.8	89.6	77	83.5
NB	100.0	94.5	84.7	876.0	208.1	279.9	68	85.9
NG	115.7	120.2	55.5	132.6	105.3	95.3	77	89.2
NQ	5.7	8.8	9.5	6.4	7.6	4.1	77	
NTO	68.4	106.0	7.6	85.8	66.2	38.5	77	
o-NBA (surr.)	110.6	132.2	184.5	98.9	132.7	39.1	67	
PETN	99.4	107.2	86.2	141.2	108.2	97.6	77	82.6
RDX	108.0	110.0	71.3	118.8	101.6	40.8	77	89.7
Tetryl	94.3	67.9	68.4	135.1	91.1	88.9	77	91.7

Highlighted entries are outside calculated recovery ranges, but within 8330B Control Limits

Highlighted entries are outside both calculated recovery ranges and 8330B Control Limits

Solvent Extraction Method for Soils

Initially, the soil testing included several additional laboratories; however inconsistent analyte recoveries regardless of laboratory, analyte, or sample source led to unusable data and therefore a repeat of the experiment. Due to the short notice only 3 laboratories were available. The inconsistent recoveries were hypothesized to be the result of heterogenous soil spiking or a compromised spiking solution prior to sample distribution, rather than some post-spiking treatment or event. Initial recoveries ranged from zero to several thousand percent regardless of analyte, soil source, or laboratory. As a result, the decision was made to redo this portion of the experiment. The soils used were the same original soils (ASTM Fat Clay, ERDC soil, and Joint Base Cape Cod (JBCC) soil) and were prepared by sieving, drying, and spiking with analytes diluted in MeOH (using a higher MeOH volume than previous) followed by a rigorous ~72 h homogenization on a sample roller. While soils were on the sample roller they appeared to clump along the outside edges of the containers, so hand mixing with plastic sampling spatulas was performed every 12 hours approx. Ultimately, the independently prepared, homogenized soils were distributed and analyzed as previously described and resulted in considerably more reasonable recovery results for the soils tested.

The recovery ranges (82% TI) shown below in Table 19 were calculated using the full synthetic percent from 3 participating laboratories. Each laboratory analyzed 3 different soils: ASTM Fat Clay (Soil #1), soil from ERDC located in Vicksburg, MS (Soil #2), and JBCC located in Cape Cod, MA (Soil #3). Soil drying, sieving, homogenization and spiking occurred prior to sample distribution. Recovery ranges were calculated from the ASTM Fat Clay, as a standardized soil matrix, whereas the average percent recoveries are reported from the two soils (2 and 3) as shown in Tables 20 and 21, respectively. Recoveries for ASTM Fat Clay spiked at lower concentration, ~ 2 mg/kg, are shown in Table 22. The reported percent recoveries generally fall within the calculated recovery ranges; however, method performance is somewhat poorer at lower concentrations as would be expected for any analytical technique.

Table 19: Full-synthetic Soil (ASTM Fat Clay) Recovery Ranges and USEPA 8330B Lower and Upper Control Limits

Analyte	Recovery Range at 82% TI (N = 3)	Lower and Upper Control Limit (8330B)
1,2-DNB (surr.)	77 – 94	83 – 119
1,3-DNB	65 – 119	78 – 120
1,3,5-TNB	74 – 130	73 – 125
1,4-DNB (surr.)	57 – 127	
2-Am-4,6-DNT	81 – 118	79 – 120
2-NP	56 – 101	
2-NT	94 – 186	70 – 127
2,4-DNP	90 – 131	
2,4-DNT	103 – 150	78 – 120
Picric Acid (PA)	69 – 108	
2,4,6-TNT	97 – 137	71 – 123
2,6-DNT	97 – 137	77 – 127
3-NT	89 – 142	73 – 125
3,5-DNA	82 – 108	71 – 117
4-Am-2,6-DNT	72 – 123	76 – 125
4-NP	63 – 108	
4-NT	82 – 136	71 – 127
DNAN	77 – 119	
HMX	66 – 140	65 – 135
NB	93 – 126	65 – 134
NG	0 – 119	74 – 127
NQ	58 – 138	
NTO	83 – 119	
o-NBA (surr.)	56 – 89	
PETN	85 – 131	73 – 127
RDX	63 – 131	68 – 130
Tetryl	66 – 121	64 – 128

Entries in **bold** are additional analytes not included in USEPA Method 8330B

Table 20: Soil (ERDC) Recoveries

Average Soil Recoveries (ERDC) (3.75 mg/kg spike)						
Analyte				Multi-Lab		
	Lab A	Lab E (1)	Lab E (2)	AVG	SD	N
1,2-DNB (surr.)	95.7	Not used	Not used	95.7	± 3.2	10
1,3-DNB	105.3	96.2	114.1	105.2	± 9.8	30
1,3,5-TNB	101.8	97.6	110.4	103.3	± 7.8	30
1,4-DNB (surr.)	Not used	105.3	95.4	100.3	± 12.7	30
2-Am-4,6-DNT	106.4	104.5	118.1	109.6	± 10.1	30
2-NP	70.5	58.9	78.6	68.0	± 13.4	30
2-NT	81.4	128.7	116.7	108.9	± 22.9	30
2,4-DNP	103.3	102.8	106.2	104.1	± 6.0	30
2,4-DNT	103.3	139.8	106.2	116.4	± 17.7	30
Picric Acid (PA)	104.7	106.2	85.5	98.8	± 11.2	30
2,4,6-TNT	111.4	122.8	117.1	117.1	± 9.8	30
2,6-DNT	113.0	106.3	128.4	115.9	± 10.7	30
3-NT	82.5	112.2	79.3	91.3	± 16.0	30
3,5-DNA	108.4	99.3	103.0	103.6	± 8.7	30
4-Am-2,6-DNT	105.7	89.7	117.2	104.2	± 14.2	30
4-NP	104.5	85.5	98.3	96.1	± 10.3	30
4-NT	86.6	104.2	85.1	92.0	± 10.9	30
DNAN	80.9	103.1	110.5	98.2	± 14.4	30
HMX	102.4	103.1	91.9	99.1	± 6.9	30
NB	76.5	118.6	54.2	83.1	± 28.5	30
NG	96.9	110.3	102.7	103.3	± 13.4	30
NQ	107.5	115.4	88.7	103.9	± 15.8	30
NTO	26.9	59.7	89.9	58.8	± 26.5	30
o-NBA (surr.)	92.3	87.5	93.1	91.0	± 5.8	30
PETN	102.1	104.8	106.5	104.5	± 6.7	30
RDX	98.7	96.1	94.7	96.5	± 6.4	30
Tetryl	85.2	96.1	112.1	97.8	± 13.3	30

Highlighted entries are outside calculated recovery ranges, but within 8330B Control Limits

Highlighted entries are outside both calculated recovery ranges and 8330B Control Limits

Table 21: Soil (JBCC) Recoveries

Average Soil Recoveries (JBCC) (3.73 mg/kg spike)						
Analyte	Lab A	Lab E (1)	Lab E (2)	Multi-Lab		
				AVG	SD	N
1,2-DNB (surr.)	91.8	Not used	Not used	91.8	± 1.7	10
1,3-DNB	105.7	106.5	103.8	105.3	± 5.6	30
1,3,5-TNB	105.1	109.9	98.1	104.4	± 7.8	30
1,4-DNB (surr.)	Not used	111.3	90.2	100.8	± 13.7	20
2-Am-4,6-DNT	105.0	108.8	102.7	105.5	± 6.6	30
2-NP	83.0	86.8	79.8	83.2	± 9.2	30
2-NT	102.2	172.3	126.5	133.7	± 30.9	30
2,4-DNP	104.0	101.9	93.9	99.9	± 6.2	30
2,4-DNT	103.4	174.6	100.0	126.0	± 36.1	30
Picric Acid (PA)	104.6	108.8	82.0	98.5	± 14.6	30
2,4,6-TNT	109.1	134.2	115.2	119.5	± 12.3	30
2,6-DNT	115.6	118.3	126.4	120.1	± 9.9	30
3-NT	103.0	136.7	84.6	108.1	± 22.4	30
3,5-DNA	100.9	105.2	84.6	96.9	± 11.1	30
4-Am-2,6-DNT	98.0	89.2	96.4	94.5	± 7.3	30
4-NP	104.1	95.1	85.3	94.8	± 10.0	30
4-NT	103.4	129.1	86.6	106.3	± 18.6	30
DNAN	81.2	106.2	107.5	98.3	± 13.0	30
HMX	110.8	118.4	102.6	110.6	± 9.0	30
NB	101.7	121.3	67.6	96.9	± 23.0	30
NG	109.4	105.1	100.1	104.9	± 23.1	30
NQ	104.2	140.5	89.2	111.3	± 22.6	30
NTO	30.7	60.3	87.7	59.6	± 24.1	30
o-NBA (surr.)	89.1	77.6	79.3	82.0	± 7.9	30
PETN	102.7	113.3	95.0	103.7	± 14.8	30
RDX	108.9	113.6	99.8	107.4	± 8.7	30
Tetryl	75.6	113.4	94.0	94.3	± 16.5	30

Highlighted entries are outside calculated recovery ranges, but within 8330B Control Limits

Highlighted entries are outside both calculated recovery ranges and 8330B Control Limit

Table 22: Low level Soil (ASTM Fat Clay) Recoveries

Low Level Soil Recoveries (ASTM Fat Clay) (1.72 mg/kg spike)						
Analyte	Lab A	Lab E (1)	Lab E (2)	Multi-Lab		
				AVG	SD	N
1,2-DNB (surr.)	94.2	Not used	Not used	94.2	± 2.2	10
1,3-DNB	102.7	92.4	122.2	105.7	± 13.4	30
1,3,5-TNB	102.4	91.9	120.9	105.1	± 13.4	30
1,4-DNB (surr.)	Not used	106.1	89.4	97.8	± 10.6	20
2-Am-4,6-DNT	103.2	71.9	125.8	100.3	± 23.2	30
2-NP	70.9	41.4	97.0	127.5	± 69.1	30
2-NT	101.3	182.9	177.3	153.8	± 41.7	30
2,4-DNP	98.8	104.9	117.9	107.2	± 10.2	30
2,4-DNT	101.7	186.3	117.8	135.3	± 53.1	30
Picric Acid (PA)	102.0	101.6	87.6	97.1	± 10.8	30
2,4,6-TNT	103.2	131.4	136.4	123.7	± 15.1	30
2,6-DNT	115.5	162.6	160.8	146.3	± 23.2	30
3-NT	101.0	159.9	115.9	125.6	± 25.8	30
3,5-DNA	103.2	112.6	97.0	104.3	± 7.3	30
4-Am-2,6-DNT	104.3	64.9	116.1	95.1	± 23.8	30
4-NP	106.8	69.0	104.2	93.4	± 17.7	30
4-NT	101.8	144.3	98.0	114.7	± 25.1	30
DNAN	81.5	150.4	123.2	118.4	± 29.4	30
HMX	69.4	128.0	98.2	88.4	17.0	30
NB	101.0	144.1	85.2	110.1	25.5	30
NG	95.1	106.0	103.7	101.6	± 45.4	30
NQ	83.0	303.5	102.3	162.9	± 102.2	30
NTO	34.7	74.6	139.0	82.8	± 44.7	30
o-NBA (surr.)	94.0	91.0	85.4	90.1	± 6.1	30
PETN	102.4	112.1	127.2	113.9	± 18.4	30
RDX	71.9	97.5	87.4	85.6	± 11.8	30
Tetryl	76.1	144.4	130.1	101.3	± 23.0	30

Highlighted entries are outside calculated recovery ranges, but within 8330B Control Limits

Highlighted entries are outside both calculated recovery ranges and 8330B Control Limit

Solvent Extraction Method for Tissues

The recovery ranges (95% TI) shown below in Table 23 were calculated using recovery data from 4 participating laboratories. The average tissue percent recoveries for the two matrices, earthworm and ryegrass, are shown in Tables 24 and 25, respectively. Laboratories H and I encountered challenges with certain analytes in the tissue matrices (leading to elevated recovery ranges), whereas laboratories C and E demonstrated generally higher method performance (though still had some poor recoveries). This demonstrates the importance of prior experience with both the method and dealing with complex matrices, such as tissues. Earthworm and ryegrass tissues were selected as representative of both an animal and plant matrix that would be relevant to laboratory research studies as well as field-collection site investigation studies.

Table 23: Tissue (Ryegrass and Earthworm) Recovery Ranges and USEPA 8330B Lower and Upper Limits

Analyte	Recovery Range at 95% TI (N=4)	Recovery Range at 57% TI (Highest performing lab) (N=1)	Lower and Upper Control Limit (8330B)
1,2-DNB (surr.)	43 – 100 (59% TI)	Not used	83 – 119
1,3-DNB	44 – 118	46 – 109	78 – 120
1,3,5-TNB	26 – 3480	40 – 99	73 – 125
1,4-DNB (surr.)	54 – 120	54 – 87	
2-Am-4,6-DNT	33 – 164	40 – 143	79 – 120
2-NP	41 – 219	26 – 147 (95% TI)	
2-NT	45 – 198	45 – 116	70 – 127
2,4-DNP	38 – 338	41 – 113	
2,4-DNT	21 – 118	32 – 142 (95% TI)	78 – 120
Picric Acid (PA)	14 – 704	51 – 107	
2,4,6-TNT	36 – 134	50 – 115	71 – 123
2,6-DNT	38 – 274	13 – 127 (95% TI)	77 – 127
3-NT	48 – 189	48 – 116	73 – 125
3,5-DNA	46 – 204	44 – 117	71 – 117
4-Am-2,6-DNT	29 – 138	38 – 93	76 – 125
4-NP	5 – 289	14 – 136 (95% TI)	
4-NT	83 – 119	46 – 120	71 – 127
DNAN	46 – 175	41 – 271	
HMX	28 – 194	20 – 148 (95% TI)	65 – 135
NB	33 – 155	43 – 106	65 – 134
NG	0 – 588	2 – 161 (95% TI)	74 – 127
NQ	0 – 2800	23 – 128	
NTO	34 – 6380	46 – 112	
o-NBA (surr.)	13 – 271	13 – 122	
PETN	21 – 215	8 – 132 (95% TI)	73 – 127
RDX	19 – 291	19 – 197	68 – 130
Tetryl	0 – 64	7 – 36	64 – 128

*Entries in **bold** are additional analytes not included in USEPA Method 8330B*

Table 24: Tissue (Earthworm) Recoveries

Average Tissue Recoveries (Earthworm) (14.16 and 26.04 mg/kg spike concentration for 2 separate tissues)							
Analyte	Lab C	Lab E	Lab H	Lab I	Multi-Lab		
					AVG	SD	N
1,2-DNB (surr.)	63.8		81.3		72.3	19.1	40
1,3-DNB	71.2	68.9		78.3	72.6	20.7	60
1,3,5-TNB	59.6	64.1	2606.1	78.8	694.4	1174.9	80
1,4-DNB (surr.)		62.2	79.5	78.7	73.1	10.3	60
2-Am-4,6-DNT	64.0	65.0	85.9	68.5	71.1	23.7	80
2-NP	84.4	62.9	75.3	99.5	80.7	25.6	80
2-NT	89.4	67.0	82.7	107.7	89.2	24.4	80
2,4-DNP	66.4	65.2	69.5	246.4	111.0	89.8	80
2,4-DNT	54.4	61.9	71.6	40.6	58.8	24.7	80
Picric Acid (PA)	280.9	79.4	99.8	166.1	142.2	119.5	80
2,4,6-TNT	64.6	72.8	86.9	76.5	75.6	22.2	80
2,6-DNT	81.8	65.7	74.3	159.0	95.2	46.7	80
3-NT	88.3	69.4	87.3	100.1	86.3	22.1	80
3,5-DNA	94.2	65.8	72.5	98.4	82.3	24.6	80
4-Am-2,6-DNT	58.0	60.5	81.5	67.4	70.0	22.5	80
4-NP	99.9	63.3	106.5	203.1	117.9	70.1	80
4-NT	82.9	69.0	78.2	97.4	81.7	23.0	80
DNAN	62.4	78.9	71.3	75.3	71.9	35.9	80
HMX	90.5	53.2	79.6	86.1	77.3	31.9	80
NB	59.5	64.9	79.8	80.5	71.1	21.4	80
NG	31.4	39.5	69.0	158.0	105.7	75.5	80
NQ	73.7	52.3	0.0	2114.4	706.5	998.8	70
NTO	76.7	68.1	3948.8	5119.7	2276.1	2410.9	80
o-NBA (surr.)	211.9	51.3	155.3	61.3	119.1	77.9	80
PETN	60.4	64.0	77.3	85.2	71.9	24.6	80
RDX	69.2	54.5	91.2	80.9	73.2	23.6	80
Tetryl		0.2	26.0	9.3	11.8	13.0	60

Highlighted entries are outside calculated recovery ranges, but within 8330B Control Limits

Highlighted entries are outside both calculated recovery ranges and 8330B Control Limits

Table 25: Tissue (Ryegrass) Recoveries

Average Tissue Recoveries (Ryegrass) (9.72 and 30.24 mg/kg spike concentration for 2 separate tissues)							
Analyte	Lab C	Lab E	Lab H	Lab I	Multi-Lab		
					AVG	SD	N
1,2-DNB (surr.)	77.3		91.0		84.2	16.3	38
1,3-DNB	84.6	82.7		96.9	88.0	21.5	55
1,3,5-TNB	56.4	68.5	75.0	96.6	73.9	23.1	74
1,4-DNB (surr.)		60.5	84.0	83.3	76.0	13.2	55
2-Am-4,6-DNT	103.0	100.0	118.2	92.5	103.6	32.8	74
2-NP	139.7	83.6	94.7	129.8	112.1	44.1	74
2-NT	141.9	84.6	104.1	138.3	117.4	44.1	74
2,4-DNP	118.7	84.4	89.8	99.1	98.2	30.7	74
2,4-DNT	84.6	79.2	87.1	58.4	77.5	22.1	74
Picric Acid (PA)	34.4	68.7	68.7	171.3	85.3	71.9	74
2,4,6-TNT	76.2	82.0	99.8	90.1	87.1	23.0	74
2,6-DNT	133.8	74.8	101.7	213.5	130.6	68.2	74
3-NT	138.8	86.2	108.4	133.4	116.9	40.1	74
3,5-DNA	146.6	84.6	85.2	129.1	111.5	46.1	74
4-Am-2,6-DNT	77.0	67.8	96.7	81.9	81.0	26.4	74
4-NP	139.7	88.4	68.8	141.4	109.0	47.0	74
4-NT	128.6	87.1	101.6	130.5	112.0	38.3	74
DNAN	92.9	78.6	84.6	130.5	96.4	41.5	74
HMX	120.0	82.5	111.1	111.7	106.6	33.9	74
NB	85.8	78.3	100.2	104.3	92.2	28.9	74
NG	67.8	44.2	124.1	191.0	107.0	96.3	74
NQ	80.0	41.7	53.8	978.9	282.6	465.1	74
NTO	157.6	84.5	606.7	1407.3	559.1	675.6	74
o-NBA (surr.)	141.5	114.2	92.2	83.9	108.2	30.6	74
PETN	101.5	76.3	121.4	150.9	112.5	47.0	74
RDX	140.0	123.2	194.7	136.0	149.0	62.0	74
Tetryl	14.4	17.2	38.7	30.1	25.4	14.6	72

Highlighted entries are outside calculated recovery ranges, but within 8330B Control Limits

Highlighted entries are outside both calculated recovery ranges and 8330B Control Limits

Mass Spectrometric Detection Method

The recoveries in Tables 26 - 32 display the mass spectrometric (MS) method recoveries, rather than primary UV absorbance method (though the chromatographic separation is the same), for the same samples analyzed in the Direct-Injection Method for aqueous matrices, SPE Method for aqueous matrices, SE Method for Soils, and SE Method for Tissues sections. However, few participating laboratories used HPLC systems equipped with MS detection capability.

Recoveries generated by the MS method are generally reasonable, however a number of analytes are not detectable by APCI MS, likely due to the composition of the mobile phase (trifluoroacetic and formic acid are known to suppress ionization) and some compounds' high ionization potential or inability to form adducts under these mobile phase conditions (Furey et al. 2013). Laboratory E did not observe their 1,4-dinitrobenzene surrogate during the direct injection study, likely due to the aforementioned mobile phase composition.

Table 26: Direct Injection Surface Water MS Method Recoveries

Direct Injection Surface Water MS Method Recoveries (1.64 and 5.11 mg/L spike concentration for 2 separate waters)						
Analyte	Lab E (1)	Lab E (2)	Lab B	Multi-Lab		
				AVG	SD	N
1,2-DNB (surr.)	Not used	Not used	68.4	68.4	2.0	20
1,3-DNB	115.1	111.5	83.7	103.4	41.6	60
1,3,5-TNB	121.9	97.7	100.4	106.7	32.0	60
1,4-DNB (surr.)		82.2	Not used	82.2	7.1	20
2-Am-4,6-DNT	93.5	96.9	105.7	98.7	11.7	60
2-NP	90.8	94.7		92.7	12.5	40
2-NT		98.5		98.5	3.2	20
2,4-DNP	102.5	101.9	96.8	100.4	11.6	60
2,4-DNT	108.6	96.2	82.0	95.6	14.5	60
Picric Acid (PA)	87.4	102.6	90.3	93.4	16.0	60
2,4,6-TNT	108.3	98.2	94.2	100.2	14.7	60
2,6-DNT	149.6	96.2	83.5	109.8	58.7	60
3-NT		94.9		94.9	2.8	20
3,5-DNA	111.4	97.0	104.2	104.2	39.8	60
4-Am-2,6-DNT	41.4	95.8	102.7	79.9	31.4	60
4-NP	103.8	103.5	111.0	113.4	14.0	60
4-NT		95.1		95.1	2.3	20
DNAN	112.0	102.8	86.1	100.3	36.4	60
HMX		102.8	102.3	98.9	10.7	40
NB		94.7		94.7	1.7	20
NG		98.2	95.5	96.8	14.1	40
NQ	122.5	100.6	79.4	100.9	21.4	60
NTO	99.1	98.5	71.4	89.7	14.9	60
o-NBA (surr.)	205.0	89.0	67.2	120.4	66.1	60
PETN	122.2	93.3	104.4	106.6	34.9	60
RDX	103.4	98.5	102.7	101.5	12.3	60
Tetryl	104.7	97.4	93.8	98.6	11.7	60

Table 27: Direct Injection Groundwater MS Method Recoveries

Direct Injection Groundwater MS Method Recoveries (1.64 and 5.11 mg/L spike concentration for 2 separate waters)						
Analyte	Lab E (1)	Lab E (2)	Lab B	Multi-Lab		
				AVG	SD	N
1,2-DNB (surr.)	Not used	Not used	70.2	70.2	2.4	20
1,3-DNB	181.6	110.1	74.0	121.9	70.5	60
1,3,5-TNB	158.9	97.0	90.4	115.4	48.7	60
1,4-DNB (surr.)		82.0	Not used	82.0	10.2	20
2-Am-4,6-DNT	91.1	96.4	96.8	94.8	13.2	60
2-NP	91.8	94.1		93.0	15.9	40
2-NT		97.0		97.0	3.7	20
2,4-DNP	101.7	101.5	91.2	98.1	12.0	60
2,4-DNT	98.6	95.5	73.1	89.1	20.9	60
Picric Acid (PA)	89.6	101.5	87.1	92.7	13.4	60
2,4,6-TNT	102.0	97.5	85.6	95.0	12.6	60
2,6-DNT	247.0	95.6	72.6	138.4	110.8	60
3-NT		95.1		95.1	1.5	20
3,5-DNA	183.4	96.5	97.8	125.9	70.0	60
4-Am-2,6-DNT	33.2	95.5	92.6	73.8	30.6	60
4-NP	120.5	102.4	101.5	108.1	21.5	60
4-NT		93.9		93.9	1.9	20
DNAN	174.7	102.1	75.4	117.4	64.8	60
HMX		94.3	94.2	94.2	9.0	40
NB		93.4		93.4	1.2	20
NG		95.8	83.2	89.5	13.3	40
NQ	136.4	102.3	77.3	105.3	28.2	60
NTO	90.9	96.8	74.0	87.2	11.9	60
o-NBA (surr.)	170.8	91.5	74.6	112.3	48.5	60
PETN	199.0	95.7	93.3	129.3	74.3	60
RDX	116.7	97.5	92.5	102.3	14.6	60
Tetryl	110.5	96.5	87.8	98.3	12.6	60

Table 28: SPE Surface Water MS Method Recoveries

SPE Surface Water MS Method Recoveries (1.64 and 5.11 mg/L spike concentration for 2 separate waters)			
Analyte	Lab B	SD	N
1,2-DNB (surr.)	102.9	0.7	20
1,3-DNB	118.1	17.6	20
1,3,5-TNB	82.2	55.1	20
1,4-DNB (surr.)	Not used	Not used	Not used
2-Am-4,6-DNT	118.5	78.9	20
2-NP			
2-NT			
2,4-DNP	143.8	110.7	20
2,4-DNT	337.3	73.4	20
Picric Acid (PA)	147.1	37.3	20
2,4,6-TNT	103.3	44.2	20
2,6-DNT	194.9	26.1	20
3-NT			
3,5-DNA	125.7	99.7	20
4-Am-2,6-DNT	103.0	74.6	20
4-NP	131.9	98.5	20
4-NT			
DNAN	131.0	143.0	20
HMX	140.5	101.2	20
NB			
NG	309.6	58.4	20
NQ	26.9	28.7	20
NTO	151.9	59.1	20
o-NBA (surr.)	69.6	31.7	20
PETN	138.9	79.9	20
RDX	134.4	114.7	20
Tetryl	83.4	34.4	20

Table 29: SPE Groundwater MS Method Recoveries

SPE Groundwater MS Method Recoveries (1.64 and 5.11 mg/L spike concentration for 2 separate waters)			
Analyte	Lab B	SD	N
1,2-DNB (surr.)	103.0	0.3	20
1,3-DNB	136.9	14.1	20
1,3,5-TNB	172.3	47.7	20
1,4-DNB (surr.)	Not used	Not used	Not used
2-Am-4,6-DNT	202.0	37.5	20
2-NP			
2-NT			
2,4-DNP	258.6	37.8	20
2,4-DNT	264.4	69.0	20
Picric Acid (PA)	203.3	20.7	20
2,4,6-TNT	176.8	28.2	20
2,6-DNT	189.3	12.3	20
3-NT			
3,5-DNA	217.9	37.1	20
4-Am-2,6-DNT	178.8	33.4	20
4-NP	221.2	34.0	20
4-NT			
DNAN	238.0	14.1	20
HMX	205.5	44.2	20
NB			
NG	267.7	20.8	20
NQ	42.5	19.6	20
NTO	219.0	32.5	20
o-NBA (surr.)	93.3	18.4	20
PETN	213.2	32.7	20
RDX	220.8	48.5	20
Tetryl	142.5	60.1	20

Table 30: SPE Field Collected Semisynthetic Waters MS Method Percent Recoveries

SPE Recoveries Field collected waters MS Method (0.07 and 0.12 mg/L spike concentration for 2 separate waters)			
Analyte	Lab E	SD	N
1,2-DNB (surr.)	Not used	Not used	Not used
1,3-DNB	90.8	19.2	20
1,3,5-TNB	75.9	21.6	20
1,4-DNB (surr.)	103.4	19.0	20
2-Am-4,6-DNT	80.6	16.4	20
2-NP	70.2	17.1	20
2-NT			
2,4-DNP	60.1	11.7	20
2,4-DNT	115.1	39.0	20
Picric Acid (PA)	69.9	19.1	20
2,4,6-TNT	62.7	73.5	20
2,6-DNT	107.6	52.0	20
3-NT			
3,5-DNA	86.1	16.5	20
4-Am-2,6-DNT	76.7	12.6	20
4-NP	86.7	21.6	20
4-NT			
DNAN	66.2	22.9	20
HMX	67.7	15.0	20
NB			
NG			
NQ			
NTO	5.6	1.3	20
o-NBA (surr.)	154.0	26.5	20
PETN	61.5	26.2	20
RDX	69.8	13.5	20
Tetryl	56.4	16.8	20

Table 31: Tissue (Earthworm) MS Method Recoveries

Tissue Recoveries (Earthworm) MS Method (14.16 and 26.04 mg/kg spike concentration for 2 separate tissues)			
Analyte	Lab E	SD	N
1,2-DNB (surr.)	Not used	Not used	Not used
1,3-DNB	50.3	15.5	18
1,3,5-TNB	53.7	19.8	18
1,4-DNB (surr.)	109.5	182.2	18
2-Am-4,6-DNT	60.0	25.2	18
2-NP	63.1	19.1	18
2-NT			
2,4-DNP	53.0	18.8	18
2,4-DNT	69.5	19.2	18
Picric Acid (PA)	55.4	20.9	18
2,4,6-TNT			
2,6-DNT	69.5	19.2	18
3-NT			
3,5-DNA	53.6	19.0	18
4-Am-2,6-DNT	60.9	18.1	18
4-NP	114.8	126.1	18
4-NT			
DNAN	65.9	19.3	18
HMX	55.4	26.9	18
NB	54.8	16.0	18
NG			
NQ	40.2	41.8	18
NTO	107.2	147.1	18
o-NBA (surr.)			
PETN	41.9	10.5	18
RDX	53.6	16.9	18
Tetryl	12.1	22.5	18

Table 32: Tissue (Ryegrass) MS Method Recoveries

Tissue Recoveries (Ryegrass) MS Method (Ryegrass) (9.72 and 30.24 mg/kg spike concentration for 2 separate tissues)			
Analyte	Lab E	SD	N
1,2-DNB (surr.)	Not used	Not used	Not used
1,3-DNB	61.1	18.2	18
1,3,5-TNB	52.6	11.2	18
1,4-DNB (surr.)	69.1	15.2	18
2-Am-4,6-DNT	69.1	15.2	18
2-NP	86.2	21.5	18
2-NT			
2,4-DNP	79.7	22.6	18
2,4-DNT	94.7	24.7	18
Picric Acid (PA)	71.1	20.1	18
2,4,6-TNT			
2,6-DNT	139.0	58.1	18
3-NT			
3,5-DNA	70.8	20.6	18
4-Am-2,6-DNT	68.3	18.1	18
4-NP	93.4	25.5	18
4-NT			
DNAN	81.4	20.6	18
HMX	89.1	24.8	18
NB	71.1	23.7	18
NG			
NQ	93.7	56.0	18
NTO	95.6	112.7	18
o-NBA (surr.)			
PETN	58.7	17.7	18
RDX	78.6	22.6	18
Tetryl	20.9	6.3	18

Conclusions

The results of this study demonstrate the data quality obtainable by the methods developed under SERDP project 2722 and demonstrated and validated under ESTCP Project ER19-5078. Recovery ranges calculated are generally somewhat wider ($\pm 20\%$) than ranges outlined in the DOD QSM for USEPA Method 8330B, however, this is generally expected due to the significant increase in method complexity required to simultaneously detect and quantify IM and Legacy explosive compounds. The vastly different physiochemical properties of IM and legacy explosive compounds require substantial chemistry variations during extraction and separation processes and will inevitably introduce variability into the method. However, the ranges determined are not substantially different from Method 8330B, or indeed recovery ranges listed in the DOD QSM for other common organic analyte methods, such as USEPA Methods 8081, 8141, and 8270. Therefore, the results reported for the new method's performance are expected, considering operation by analysts and laboratories experienced in advanced organic analytical techniques.

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