

**Development and Optimization of Analytical  
Methods for Simultaneous Determination of IM and Legacy  
Explosive Compounds**

**SERDP ER-2722**

**USACE ERDC-EL<sup>1</sup>, -CRREL<sup>2</sup>, and NRL<sup>3</sup>**

**Rebecca A. Crouch<sup>1</sup>, Jared C. Smith<sup>1</sup>, Bobbi S. Stromer<sup>1</sup>,  
Christian T. Hubley<sup>1</sup>, Samuel Beal<sup>2</sup>, Guilherme R. Lotufo<sup>1</sup>,  
Afrachanna D. Butler<sup>1</sup>, Michelle T. Wynter<sup>1</sup>, Dale A. Ro-  
sado<sup>1</sup>, Amber L. Russell<sup>1</sup>, Jessica G. Coleman<sup>1</sup>, Jay L.  
Clausen<sup>2</sup>, Braden C. Giordano<sup>3</sup>, Michael T. Montgomery<sup>3</sup>,  
and Anthony J. Bednar<sup>1</sup>**

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**Final Report**

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| <b>6. AUTHOR(S)</b><br>Anthony Bednar, Rebecca A. Crouch, Jared C. Smith, Bobbi S. Stromer, Christian T. Hubley, Guilherme R. Lotufo, Afrachanna D. Butler, Michelle T. Wynter, Dale A. Rosado, Amber L. Russell, Jessica G. Coleman (USACE ERDC-EL)<br>Samuel Beal and Jay L. Clausen (SACE ERDC-CRREL)<br>Braden C. Giordano and Michael T. Montgomery (NRL)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             |                    |                                             |                                   | <b>5d. PROJECT NUMBER</b><br>ER-2722 |                                                                  |  |
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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                       |
| ASTM            | American Society for Testing and Materials       |
| BGE             | background electrolyte                           |
| CE              | capillary electrophoresis                        |
| CEC             | cation exchange capacity                         |
| CH <sub>3</sub> | 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                                    |
| L     | liter                                                   |
| LCL   | lower control limit                                     |
| 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                     |
| 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                                  |
| QSM           | quality systems manual                                   |
| RDX           | hexahydro-1,3,5-trinitro-s-triazine                      |
| SE            | solvent extraction                                       |
| sec           | second                                                   |
| SERDP         | Strategic Environmental Research and Development Program |
| SM            | sample matrix                                            |

|           |                                                             |
|-----------|-------------------------------------------------------------|
| SPE       | solid phase extraction                                      |
| 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

legacy munition compounds, insensitive munition (IM) compounds, energetic compounds, U.S. Environmental Protection Agency (USEPA) Method 8330B, high-performance liquid chromatography (HPLC), micellar electrokinetic chromatography (MEKC), solid phase extraction (SPE), solvent extraction (SE), environmental matrices

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# Development and Optimization of Analytical Methods for Simultaneous Determination of IM and Legacy Explosive Compounds

## Abstract

**Introduction and Objectives:** Currently, no standard method for analyzing IM compounds in environmental matrices exists (with or without concurrent legacy compounds). Lacking established methods, contract and research laboratories either do not measure IM compounds at all; quantify some but not all, using USEPA Method 8330B developed for legacy compounds, leading to inaccuracies for certain IM compounds (Felt et al., 2016) and degradation products; or, use methods originally developed for neat materials with no proven performance metrics. The primary objective of this project was to fulfill the requirements of SON ERSON-17-02 by developing new methods of extraction, pre-concentration, and analytical separation/quantitation of 17 legacy and seven IM compounds, daughter products of IM, and other munition compounds absent from USEPA Method 8330B, to yield a single standardized method for simultaneous analysis in environmental matrices, leading to cost savings with minimal increase in environmental monitoring and no change to existing sample collection procedures.

**Technical Approach:** In order to develop new methods of extraction, pre-concentration, and analytical separation/quantitation of the proposed list to yield a single standardized method for simultaneous analysis in environmental matrices, currently accepted and utilized methods of analysis (HPLC, MEKC, and CE) and extraction (solid phase extraction and solvent extraction) were modified for applicability to the proposed list of 24 analytes, and were then verified in laboratory-spiked environmental water, soil, and tissue samples. In addition, stability studies were performed to investigate applicability of currently accepted extraction and analysis hold times for water and solid matrices. Furthermore, inter-laboratory batch analysis studies were performed using laboratory-spiked water, soil, and tissue samples to investigate extraction and analytical method transfer from the originating laboratory.

**Results:** Two HPLC separation methods with UV detection have been developed which allow the detection and quantitation of 24 legacy and IM compounds (plus two surrogates), with  $\mu\text{g/L}$  detection limits; in addition, an LC-MS method has been developed for confirmatory analysis. Alternate analytical methods using MEKC and CE have been developed to detect compounds of interest present at higher concentrations. Extraction methods for environmental water and solid matrices were developed by modification of existing USEPA Method 8330B and previously-developed ERDC methods. The developed SPE method was tested on five water sources, including river, sea, and ground water, with a majority of compounds recovered within current DoD QSM Ver5.2 limits. A 2-step solvent extraction method was developed for soils, which included testing of five field soils. Recoveries of all 24 compounds of interest (plus two surrogates) for all soils were within QSM limits. A single 18-h solvent extraction method was developed for tissues, including a soil-dwelling invertebrate, freshwater vertebrate, marine invertebrate, and perennial plant. For most tissues, at least 20 compounds of interest, were recovered within current QSM limits. In order to address observed analytical interferences, a post-extraction preparative chromatographic method was developed, which allowed for improved recoveries of several compounds in tissues, including NQ and NTO.

**Benefits:** The development of methods to simultaneously analyze legacy and IM compounds is necessary to enable monitoring of each component at firing ranges, demilitarization facilities, manufacturing facilities, and environmental sites, where munition compounds are tested, produced, and detected (Felt et al., 2013; Walsh et al., 2014). New methods of extraction and analytical separation and quantitation (by HPLC, MEKC, and CE) of 17 legacy and seven IM compounds, daughter products of IM, and other munition compounds absent from USEPA Method 8330B have been developed. New HPLC methods reduce total analytical run time by  $\sim 25\%$  versus the use of separate legacy and IM methods. In addition, the ability to carry out simultaneous extractions and analytical sample preparation could reduce labor and supply costs by 50%. Products of this work will assist in conducting fate and transport studies for IM compounds by providing a standardized method for quantitation of concurrent IM and legacy materials, which is needed to determine long-term environmental impacts.

# **Development and Optimization of Analytical Methods for Simultaneous Determination of IM and Legacy Explosive Compounds**

## **Executive Summary**

### **Introduction**

Currently, no standard method for analyzing IM compounds in environmental matrices exists (with or without concurrent legacy compounds). Lacking established methods, contract and research laboratories either do not measure IM compounds at all; quantify some but not all, using USEPA Method 8330B developed for legacy compounds, leading to inaccuracies for certain IM compounds (Felt et al., 2016) and degradation products; or, use methods originally developed for neat materials with no proven performance metrics.

### **Objectives**

The primary objective of this project was to fulfill the requirements of SON ERSON-17-02 by developing new methods of extraction, pre-concentration, and analytical separation/quantitation of 17 legacy and seven IM compounds, daughter products of IM, and other munition compounds absent from USEPA Method 8330B, to yield a single standardized method for simultaneous analysis in environmental matrices, leading to cost savings with minimal increase in environmental monitoring and no change to existing sample collection procedures.

### **Technical Approach**

In order to develop new methods of extraction, pre-concentration, and analytical separation/quantitation of the proposed list to yield a single standardized method for simultaneous analysis in environmental matrices, currently accepted and utilized methods of analysis (HPLC, MEKC, and CE) and extraction (solid phase extraction [SPE] and solvent extraction [SE]) were modified for applicability to the proposed list of 24 analytes, and were then verified in laboratory-spiked environmental water, soil, and tissue samples. In addition, stability studies were performed to investigate applicability of currently accepted extraction and analysis hold times for water and solid matrices. Furthermore, inter-laboratory batch analysis studies were performed using laboratory-spiked water, soil, and tissue samples to investigate extraction and analytical method transfer from the originating laboratory.

### **Results and Discussion**

#### *MEKC and CE Methods*

In this work, two distinct capillary electrophoresis (CE)-based separations were developed to compliment HPLC-based analyses. One was a separation of all components *via* micellar electrokinetic chromatography (MEKC), and the second was a “total extraction efficiency” method. The MEKC separation was based upon a combination of charge-to-size ratio of the compounds and their affinity for negatively charged cholate micelles included in the background electrolyte (BGE). The “total extraction efficiency” method allow for rapid evaluation of the overall extraction performance. MEKC method development focused on an analytical method capable of resolving all of the 24 compounds of interest and the two surrogates with a separation time of less than 60 min. The inherent difficulty in developing this method was the inclusion of both neutral and negatively charged analytes. The approach was to widen the separation window associated with the neutral molecules as much as possible, while ensuring that negatively charged species remained in the capillary column throughout the separation. At the point where all neutral components were detected, a slight pressure was applied to the inlet side of the capillary to push the negatively charged compounds to the detector in order to minimize total separation time.

## Primary HPLC-UV Method

Previously developed (Russell et al., 2014) and currently employed munition compound analysis methods (USEPA 8330B, in-house IM method) were used as references in the development of the primary HPLC-UV method. The method parameters and solvent gradient scheme are shown in Table 1. Method development was carried out using Phenomenex Synergi 4 $\mu$ m 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 environmentally matrices. As indicated in Table 1, either a 0.1% TFA or 0.25% FA (vol/vol) solution in water can be used, which was tested during HPLC-MS method development. An example chromatogram is shown in Figure 1. Peak identities (obtained by analysis of individual reference standards), method detection limits (MDLs), and linear dynamic ranges (LDRs) are provided in Table 2. In the table, LDR values represent the highest spiked standard analyzed within  $\pm 10\%$  of the known spike concentration, with 80 mg/L being the highest tested concentration.

Table 1. Primary HPLC-UV separation solvent gradient scheme and other method conditions.

| Total Run Time: 48.0 min |                   | Detection Wavelengths: 210, 254, 315 nm |                     |                              |
|--------------------------|-------------------|-----------------------------------------|---------------------|------------------------------|
| Column Temperature: 25°C |                   | Flow Rate: 1.0 mL/min                   |                     | Injection Volume: 50 $\mu$ L |
| 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

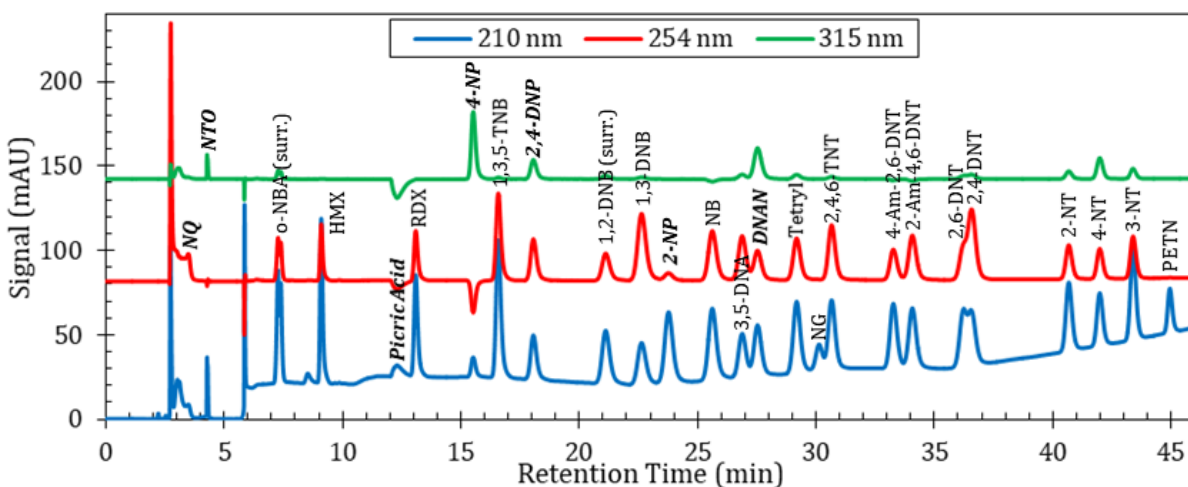


Figure 1. HPLC chromatograms of a mixed standard containing 5 mg/L of 24 legacy and IM compounds and two surrogates analyzed by the developed primary HPLC-UV method.

Table 2. Retention times (min), MDLs and LDR Upper Limits (ULs) for the 24 compounds of interest and two surrogates, determined in direct-injection water (“Estimated”), reagent water that underwent SPE pre-concentration, and Ottawa sand that underwent SE, analyzed by the primary HPLC-UV method.

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

***Bold, italicized*** are IM compounds and degradation products.

†co-elute at concentrations >30 mg/L

‡RDX not detected appreciably at 315 nm

### 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 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 3, and an example chromatogram is shown in Figure 2 (with peak assignments determined using individual reference standards). As indicated again, either a 0.1% TFA or 0.25% FA (vol/vol) solution in water can be used.

Table 3. Secondary HPLC-UV separation solvent gradient scheme and other method conditions.

| Total Run Time: 43.0 min |                   | Detection Wavelengths: 210, 254, 315 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                       |
| 33.1                     | 82                | 10                                      | 10                  | 5                       |
| 43.0                     | 82                | 10                                      | 10                  | 5                       |

\*or 0.25% FA/water

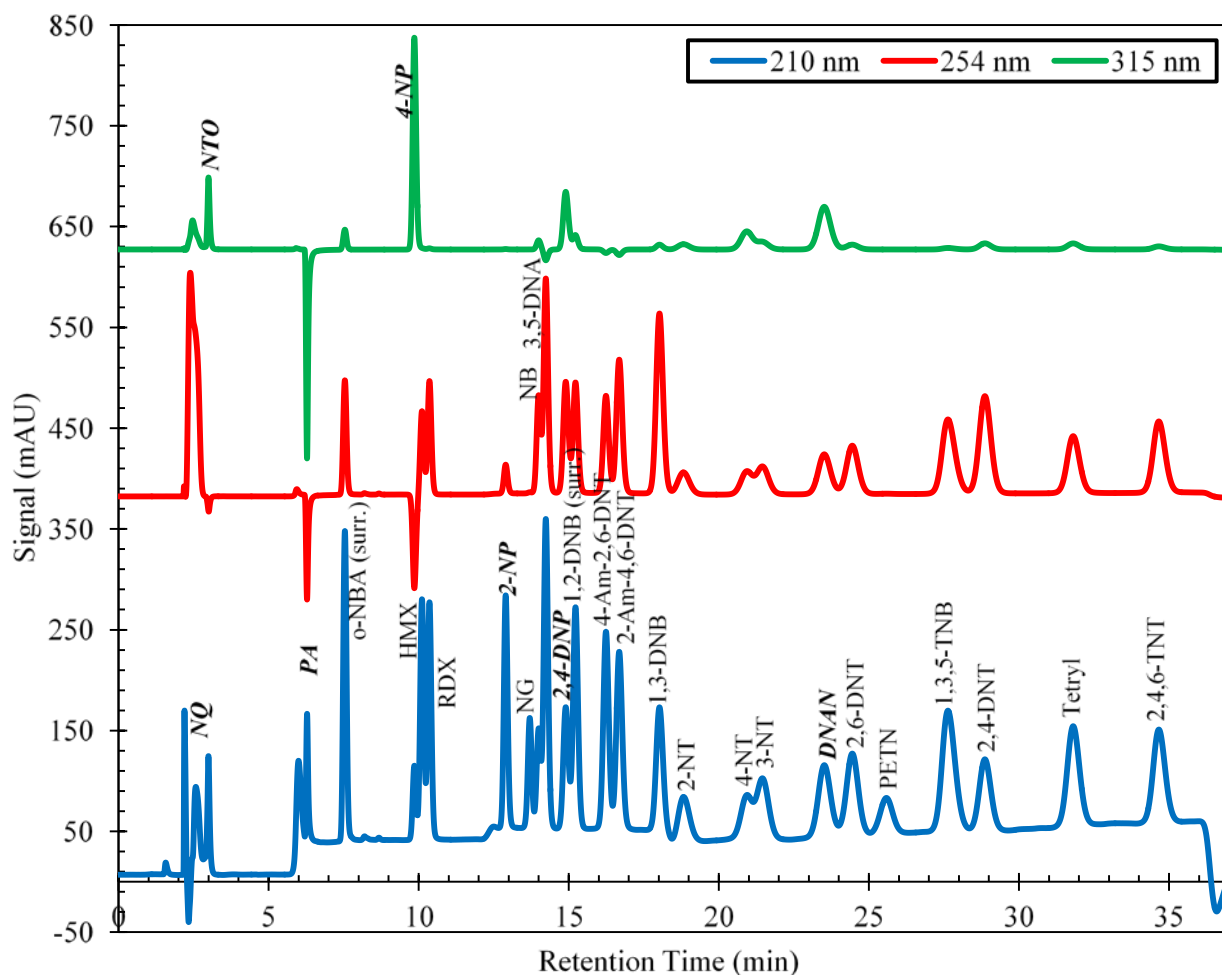


Figure 2. HPLC chromatograms of a mixed standard containing 10 mg/L of 24 legacy and IM compounds and two surrogates analyzed by the developed secondary HPLC-UV method. ***Bold, italicized*** are IM compounds and degradation products.

### HPLC-MS Method

An HPLC-MS scan mode method was developed using an Agilent 6120B single quadrupole (SQ) system equipped with an atmospheric-pressure chemical ionization (APCI) source for confirmatory analysis of the 24 compounds of interest and two surrogates. Spray chamber and MS detector parameters for the scan mode method are provided in Table 4. Chromatographic separation was effected by the primary HPLC-UV method described above. An example MS total ion chromatogram (TIC) is shown in Figure 3. Several ion masses were observed for each of the 26 compounds analyzed (shown in Table 5), with the exception of nitroglycerine (NG) and 2-/3-/4-nitrotoluene (2-/3-/4-NT), which did not produce any detectable MS signal under the developed spray chamber and detector conditions, and 1,3-dinitrobenzene (1,3-DNB), for which just a single ion was observed. Ions were identified where possible. Observed ions are listed in order of abundance for each compound.

Table 4. Spray chamber and detector parameters for the developed HPLC-MS scan mode method.

| Parameter              | Value    |
|------------------------|----------|
| Source                 | APCI     |
| Ionization Mode        | Negative |
| Drying Gas (°C)        | 350      |
| Vaporizer (°C)         | 325      |
| Drying Gas (L/min)     | 4.0      |
| Nebulizer (psig)       | 40       |
| Corona (μA)            | 10       |
| Capillary (V)          | 1500     |
| Mass Range             | 40-400   |
| Fragmentor             | 100      |
| Gain                   | 1.00     |
| Threshold              | 0        |
| Step Size              | 0.20     |
| Speed (μ/sec)          | 743      |
| Peak Width (min)       | 0.060    |
| Cycle Time (sec/cycle) | 0.57     |

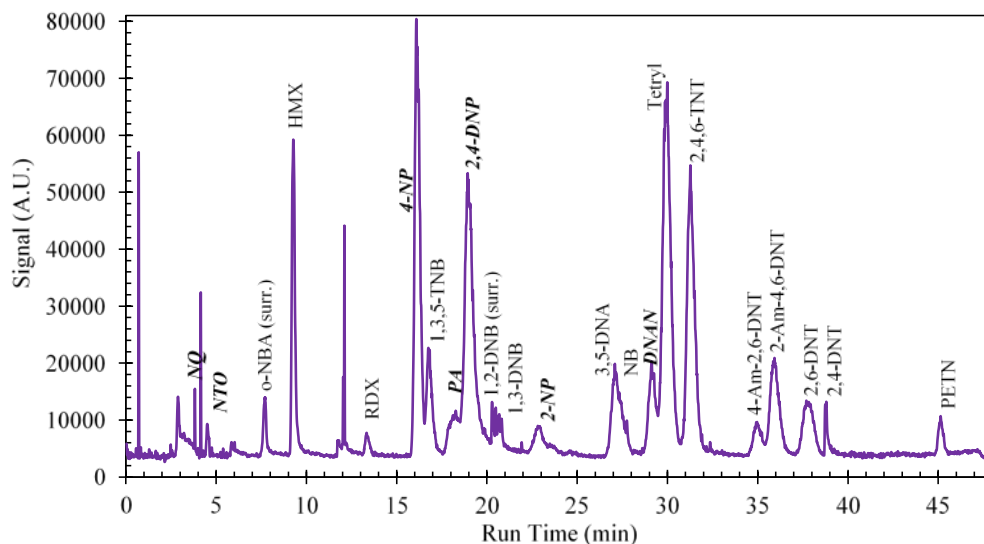


Figure 3. TIC for a mixed standard containing 10 mg/L of 24 legacy and IM compounds and two surrogates analyzed by the developed confirmatory HPLC-MS scan mode method. **Bold, italicized** are IM compounds and degradation products.

Table 5. 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 HPLC-MS scan mode method.

| Retention Time (min) | Compound            | Ion Mass (m/z) | Ion Identity                         |
|----------------------|---------------------|----------------|--------------------------------------|
| 3.78                 | <b>NQ</b>           | 203.6          |                                      |
|                      |                     | 103.0          | [M]-H                                |
| 4.50                 | <b>NTO</b>          | 129.0          | [M]-H                                |
|                      |                     | 55.0           |                                      |
| 7.64                 | o-NBA (surrogate)   | 122.0          |                                      |
|                      |                     | 167.0          | [M]-H                                |
| 9.21                 | HMX                 | 341.0          | [M]-H+FA                             |
|                      |                     | 147.0          | [M]/2-H                              |
| 13.40                | RDX                 | 267.0          | [M]-H+FA                             |
|                      |                     | 335.0          | [M]-H+TFA                            |
| 16.00                | <b>4-NP</b>         | 108.0          |                                      |
|                      |                     | 138.0          | [M]-H                                |
| 16.80                | 1,3,5-TNB           | 213.0          | [M]-H                                |
|                      |                     | 183.0          |                                      |
| 18.20                | <b>PA</b>           | 228.0          | [M]-H                                |
|                      |                     | 211.9          |                                      |
| 18.80                | <b>2,4-DNP</b>      | 183.0          | [M]-H                                |
|                      |                     | 153.0          |                                      |
| 22.70                | 1,2-DNB (surrogate) | 138.0          |                                      |
|                      |                     | 168.0          | [M]-H                                |
| 23.55                | 1,3-DNB             | 168.0          | [M]-H                                |
| 24.60                | <b>2-NP</b>         | 138.0          | [M]-H                                |
|                      |                     | 122.0          | [M]-H <sub>2</sub> O-H               |
| 27.10                | 3,5-DNA             | 182.0          | [M]-H                                |
|                      |                     | 138.0          |                                      |
| 27.80                | NB                  | 182.0          | [M]-H+HAc                            |
|                      |                     | 153.0          |                                      |
| 29.00                | <b>DNAN</b>         | 183.0          | [M]-CH <sub>3</sub>                  |
|                      |                     | 152.9          |                                      |
| 30.00                | Tetryl              | 241.0          | [M]-H+NO <sub>2</sub> * <sup>*</sup> |
|                      |                     | 288.0          | [M]-H                                |
|                      | NG                  | 226.1          | [M]-H (not obsv.)                    |
| 31.30                | 2,4,6-TNT           | 226.0          | [M]-H                                |
|                      |                     | 210.0          | [M]-H <sub>2</sub> O-H               |
| 35.10                | 4-Am-2,6-DNT        | 196.9          | [M]-H                                |
|                      |                     | 167.0          |                                      |
| 35.95                | 2-Am-4,6-DNT        | 196.0          | [M]-H                                |
|                      |                     | 179.9          | [M]-H <sub>2</sub> O-H               |
| 37.54                | 2,6-DNT             | 152.0          | [M]-30*                              |
|                      |                     | 122.1          |                                      |
| 37.87                | 2,4-DNT             | 165.0          | [M]-H <sub>2</sub> O-H               |
|                      |                     | 181.0          | [M]-H                                |
| 42.00 - 44.50        | 2-NT/3-NT/4-NT      | 136.1          | [M]-H (not obsv.)                    |
| 45.10                | PETN                | 62.0           | NO <sub>3</sub> * <sup>*</sup>       |
|                      |                     | 270.9          |                                      |

**Bold, italicized** are IM compounds and degradation products.

\*Avci, et al., 2017; Gapeev, et al., 2003; Jiang, 2010; Kinghorn, et al., 2005; Thurman, et al., 2012; Xu, et al., 2004.

## SPE Method for Extraction and Pre-Concentration of Environmental Waters

Current USEPA Method 8330B and ERDC-EL SPE methods were modified to enable extraction and pre-concentration of all 24 compounds of interest (and two surrogates) from environmental water samples. A series of cation-exchange, anion-exchange, reverse phase, and granular-activated carbon (GAC)-based SPE cartridges were evaluated by comparing their ability individually to recover the 24 compounds of interest and two surrogates from laboratory-spiked reagent water samples, making slight modifications to existing SPE methods for environmental water extraction and pre-concentration. These included, among others, polymeric reverse phase (PRP, 500 mg) and polymeric strong anion (PSA, 500 mg) from Phenomenex, and C18 (1 g) and GAC (250 or 500 mg) from Supelco.

Based on results with individual cartridges, several double- and triple-stacked options were tested, in order to identify a sequential option that yielded acceptable recoveries for all compounds. The stacking order for sample loading and elution was reversed to prevent irreversible binding of analytes to incompatible SPE cartridge matrices, which was observed during evaluation of individual cartridges. Results for six stacked options are shown in Table 6, where loading-stage stacking order from top to bottom is indicated, including A) PRP500-PSA500-GAC250, B) PRP500-PSA500-GAC500, C) C181G-PSA500-GAC250,

Table 6. Compound recoveries (%) from laboratory-spiked reagent water samples using stacked SPE cartridges for extraction/pre-concentration of the 24 compounds of interest and 1,2-DNB surrogate.

| Compound            | Recoveries (%)                            |                                           |                                          |                                          |                                          |                            |
|---------------------|-------------------------------------------|-------------------------------------------|------------------------------------------|------------------------------------------|------------------------------------------|----------------------------|
|                     | A<br>PRP500<br> <br>PSA500<br> <br>GAC250 | B<br>PRP500<br> <br>PSA500<br> <br>GAC500 | C<br>C181G<br> <br>PSA500<br> <br>GAC250 | D<br>C181G<br> <br>PSA500<br> <br>GAC500 | E<br>PSA500<br> <br>C181G<br> <br>GAC250 | F<br>PSA500<br> <br>GAC250 |
| NQ                  | 2                                         | 19                                        | 48                                       | 4                                        | 43                                       | 38                         |
| NTO (210nm)         | 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 (254nm)     | 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                        |

**Bold, italicized** are IM compounds and degradation products.

Green: 75-125%; Light Green: 57-74% or 126-135%; Red: <57% or >135%  
(DoD QSM Ver5.2 for Waters – Lowest LCL: MNX, 57%; Highest UCL: HMX, 135%)

D) C181G-PSA500-GAC500, E) PSA500-C181G-GAC250, and F) PSA500-GAC250. Options B and C from Table 6 were selected for further tests. Based on results of stability studies in water and soil (data not shown), option B was selected as the best multi-cartridge option for recovery of all compounds, with NQ recovery consistently near 50%. However, when NQ is known/likely to be absent or not of interest, a single PSA500 cartridge may be sufficient.

### *SE Method for Extraction of Environmental Soils*

Current USEPA Method 8330B and ERDC-EL SE methods were modified to enable extraction of all 24 compounds of interest (and two surrogates) from environmental soil samples. Preliminary SE trials indicated that traditional USEPA Method 8330B solid matrix extraction methods (ie, extraction using ACN for a single 18-h ultrasonication period) would be insufficient to extract all 24 compounds of interest, supported by previous studies (Walsh et al., 2016; Felt et al., 2016). Therefore, several different solvents, ultrasonication times, and multi-stage extraction procedures were investigated.

SE method development trials were carried out using six geochemically diverse soils (Table 7). Several 2-stage and 3-stage sequential ultrasonication options were tested (data not shown). Many options were successful for each soil type. These tests confirmed that including water as an extraction solvent resulted in improved recoveries for NQ, NTO, HMX, and RDX across a broad range of soil types. Ultimately, a 6 h MeOH extraction, followed by a 14 h 50/50 MeOH/water extraction was selected (Table 8). For cases in which NQ, NTO, HMX, and RDX are known to be absent or are not of interest, an extraction procedure which includes water as an extraction solvent may not be necessary; in such cases, a single MeOH extraction may be sufficient.

Table 7. Characteristic properties for six soils used in SE method development and testing for soils.

| Name                 | Collection Site                        | Region                   | Classification      | % Gravel | % Sand | % Fines | pH   | CEC (meq Na/100g) | AEC (meq S/100g) | TOC (mg/kg) | Solids (%) |
|----------------------|----------------------------------------|--------------------------|---------------------|----------|--------|---------|------|-------------------|------------------|-------------|------------|
| Aberdeen             | Aberdeen Proving Ground (Aberdeen, MD) | Mid Atlantic, South-east | 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, South-west      | 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       |

\*ND: not detected (Reporting limit: 250 mg/kg)

Table 8. Recoveries for six different soils spiked at 20 mg/kg of the 24 compounds and two surrogates.

|                     | Recoveries (%) |            |              |            |              |            |              |            |              |            |               |            |
|---------------------|----------------|------------|--------------|------------|--------------|------------|--------------|------------|--------------|------------|---------------|------------|
|                     | Aberdeen       |            | Jefferson    |            | Memphis      |            | Riley        |            | Yuma         |            | ASTM Fat Clay |            |
|                     | Avg<br>(n=3)   | Std<br>Dev | Avg<br>(n=3) | Std<br>Dev | Avg<br>(n=3) | Std<br>Dev | Avg<br>(n=3) | Std<br>Dev | Avg<br>(n=3) | Std<br>Dev | Avg<br>(n=3)  | Std<br>Dev |
| <i>NQ</i>           | 90             | 1.6        | 85           | 0.7        | 93           | 1.7        | 79           | 4.0        | 98           | 1.4        | 82            | 1.8        |
| <i>NTO (210nm)</i>  | 76             | 0.8        | 72           | 1.8        | 105          | 2.2        | 129          | 3.1        | 104          | 0.9        | 97            | 0.5        |
| <i>NTO (315nm)</i>  | 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        |
| <i>PA (210nm)</i>   | 84             | 1.5        | 87           | 7.5        | 90           | 4.4        | 82           | 4.7        | 99           | 6.2        | 82            | 2.4        |
| <i>PA (315nm)</i>   | 88             | 2.3        | 85           | 1.2        | 94           | 2.0        | 79           | 2.9        | 98           | 1.4        | 89            | 0.9        |
| <i>4-NP</i>         | 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        |
| <i>2,4-DNP</i>      | 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        |
| <i>2-NP</i>         | 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 (210nm)     | 93             | 2.7        | 92           | 1.3        | 77           | 3.8        | 93           | 3.2        | 90           | 0.5        | 92            | 0.9        |
| 3,5-DNA (254nm)     | 93             | 0.3        | 91           | 0.9        | 76           | 1.6        | 93           | 1.5        | 88           | 0.7        | 91            | 1.0        |
| <i>DNAN</i>         | 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        |

**Bold, italicized** are IM compounds and degradation products.

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

(DoD QSM Ver5.2 for Solids – Lowest LCL: 4-Am-2,6-DNT, 64%; Highest UCL: Tetryl, 135%)

### SE Method for Extraction of Environmental Tissues

Current USEPA Method 8330B and ERDC-EL SE methods were modified to enable extraction of all 24 compounds of interest (and two surrogates) from environmental tissue samples. Experiments led to the adoption of a single-step 18-h MeOH extraction, which was shown to efficiently extract the majority of compounds (17 from earthworm, 22 from polychaete worm, 24 from minnow, and 23 from ryegrass) within the accepted range for solids according to DoD QSM Ver5.2 (data not shown). All of the tissues (fathead minnow [*Pimephales promelas*], polychaete worm [*Alitta virens*], earthworm [*Eisenia fetida*], and ryegrass [*Lolium perenne*]) suffered from varying degrees of analytical interference due to co-eluting non-target extracted components.

### Interference Reduction Method for Environmental Tissue Extracts

Based on the chromatographic results obtained during tissue extraction method development, an interference reduction procedure for tissue matrix interferences was necessary. Several materials were tested (adapted from Larson, et al., 1999). Five different column packing schemes were used: 1) 0.1 g neutral alumina layered on top of 0.1 g florisil, 2) 0.1 g florisil layered on top of 0.1 g neutral alumina, 3) 1:1 mixed neutral alumina and florisil, 4), basic alumina and 5) silica gel. Based on results in post-extraction spiked tissues (data not shown), the silica gel option was selected. Finally, pre-extraction

spiked tissue sample MeOH extracts were treated with the silica gel. Overall, the results (Table 9) showed using a single 18-h MeOH extraction in combination with silica gel treatment was sufficient to yield recoveries for most compounds within current DoD QSM limits.

Table 9. Recoveries (%) from pre-extraction spiked tissue MeOH extracts, following chromatographic treatment with silica gel for matrix-related analytical interference reduction.

| Compound            | Recoveries (%) |                 |           |          |
|---------------------|----------------|-----------------|-----------|----------|
|                     | Fathead Minnow | Polychaete Worm | Earthworm | Ryegrass |
| <i>NQ</i>           | 113            | 73              | 86        | 68       |
| <i>NTO (210)</i>    | 8477           | 5318            | 4503      | 149      |
| <i>NTO (315)</i>    | 87             | 113             | 98        | 133      |
| o-NBA (surrogate)   | 101            | 82              | 111       | 67       |
| HMX                 | 81             | 66              | 79        | 99       |
| RDX                 | 86             | 66              | 93        | 85       |
| <i>PA (210)</i>     | 79             | 73              | 37        | 79       |
| <i>PA (315)</i>     | 66             | 66              | 68        | 71       |
| <i>4-NP</i>         | 83             | 71              | 72        | 84       |
| 1,3,5-TNB           | 84             | 52              | 3670      | 83       |
| <i>2,4-DNP</i>      | 110            | 72              | 81        | 93       |
| 1,2-DNB (surrogate) | 93             | 72              | 82        | 73       |
| 1,3-DNB             | 76             | 65              | 65        | 86       |
| <i>2-NP</i>         | 105            | 81              | 74        | 94       |
| NB                  | 84             | 94              | 83        | 90       |
| 3,5-DNA (210)       | 183            | 139             | 73        | 90       |
| 3,5-DNA (254)       | 69             | 75              | 80        | 73       |
| <i>DNAN</i>         | 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       |

***Bold, italicized*** are IM compounds and degradation products.

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

(DoD QSM Ver5.2 for Solids – Lowest LCL: 4-Am-2,6-DNT, 64%; Highest UCL: Tetryl, 135%)

### Implications for Future Research and Benefits

The development of these methods to simultaneously analyze environmentally co-present legacy and insensitive munition will be necessary to enable future monitoring of co-occurring legacy and IM components at firing ranges, demilitarization facilities, manufacturing facilities, and environmental sites, where munitions are tested, produced, and detected (Felt et al., 2013; Walsh et al., 2014). The optimized primary HPLC method reduces total analysis time by ~25% (48 min) versus using two different analytical methods (62 min) for legacy and for IM. In addition, the labor and supply savings due to co-extraction and sample preparation is approximately 50%, requiring one extraction procedure and analytical sample preparation versus two. Furthermore, the products of this work will contribute to the ability to conduct fate and transport studies for IM compounds by providing a standardized method for quantitation of IM and legacy materials that is needed to determine long-term environmental impacts.

# **Development and Optimization of Analytical Methods for Simultaneous Determination of IM and Legacy Explosive Compounds**

## **Final Report**

### **Objective**

The primary objective of this project was to fulfill the requirements of SON ERSON-17-02 by developing new methods of extraction, pre-concentration, and analytical separation and quantitation of 17 legacy munition compounds described in U.S. Environmental Protection Agency (USEPA) Method 8330B, as well as seven insensitive munition (IM) compounds, daughter products of IM, and other munition compounds absent from USEPA Method 8330B, the current standard method for energetic compound determinations by high-performance liquid chromatography (HPLC) with ultraviolet (UV) detection. The aim was to yield standardized methods for simultaneous extraction of legacy and IM compounds from various environmental matrices, as well as simultaneous analysis of extracted legacy and IM compounds by HPLC-UV and HPLC-mass spectrometry (MS), leading to cost savings with a minimal increase in environmental monitoring efforts and no modifications to existing sample collection procedures. Additionally, micellar electrokinetic chromatography (MEKC) and capillary electrophoresis (CE) were investigated as alternative separation techniques to complement HPLC separations. The development of such standardized methods allows for the monitoring of each legacy and IM compound at environmental sites, at manufacturing facilities, on firing ranges, and at demilitarization facilities, with the ability to conduct fate and transport studies for IM compounds that are needed to determine the scope of long-term effects.

This report includes a summary of the methods developed and results obtained, including optimized analytical methods to separate and quantify all 24 compounds of interests (and two surrogates) by HPLC, MEKC and CE, as well as solid-phase extraction (SPE) and solvent extraction (SE) methods, for extraction and pre-concentration of munition compounds from environmental water, soil, and tissue matrices. In addition, analyte stability studies and hold time studies (extraction and analysis) were performed. Inter-laboratory tests were carried out using batch-spiked water (5) and soil (6) matrices. All of the proposed extraction and analytical methods were developed and are reported herein. As a continuation of this work, a 3-year project has been funded by the Environmental Security Technology Certification Program (ESTCP) to perform validation studies of the extraction/(pre-concentration) and HPLC-UV/-MS analytical methods developed under SERDP ER-2722 in order to transfer the methods to USEPA as an addendum to USEPA Method 8330B.

### **Background**

No standard method currently exists for analyzing insensitive munition (IM) compounds in environmental matrices (with or without concurrent legacy compounds). Lacking established methods, contract and research laboratories either do not measure IM compounds at all; quantify some but not all, using USEPA Method 8330B developed for legacy compounds (leading to inaccuracies for certain IM compounds and degradation products (Felt, et al., 2016); or, use methods originally developed for neat materials with no proven performance metrics.

IM compounds are high-energy compounds that, while designed to match the performance of legacy materials, are less likely to detonate when exposed to unintended stimuli, including shock, heat, and adjacent detonating munitions (Barrie, 2013). The development of IM was initiated in the 1980s upon review of several unintended detonations at U.S. Army facilities as a means to improve soldier safety and reduce resource loss (Newman, 2010). The primary goal of the IM Program is to oversee the development and

production of safer alternatives to replace trinitrotoluene (TNT) and Composition-B (Comp-B) in U.S. Military ordnance (Kiebler, et al., 2010). Comp-B contains TNT and hexahydro-1,3,5-trinitro-s-triazine (RDX). The compounds and formulations developed under the IM Program are required to meet certain criteria. They 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). Of the numerous compounds and mixtures that have been evaluated for use in IM formulations, three compounds, 3-nitro-1,2,4-triazol-5-one (NTO), nitroguanidine (NQ), and 2,4-dinitroanisole (DNAN) are the most promising as legacy replacements. IMX-101 (NTO, NQ, and DNAN) is the current frontrunner for replacement of TNT in U.S. munitions packing (Picatinny Public Affairs, 2010), and IMX-104 (NTO, DNAN, and RDX) is set to replace Comp-B (Mainiero, 2015). Other compositions, such as PAX-21 (RDX, DNAN, and ammonium perchlorate) and PAX-41 (DNAN and RDX) have already seen use in theater (Fung, et al., 2012).

Though care may be taken to follow strict munition handling guidelines, it is possible that unintentional release of munition compounds into the environment could occur at any point during the manufacturing, testing, operation, or demilitarization processes. Contamination could occur due to leaky plumbing at manufacturing facilities or at wastewater treatment plants, or from unintentional spills during transport. On the battlefield and on testing ranges, unconsumed munition compounds are dispersed at the target and at the point from which the shot was fired (from propellant) as small particles (Jenkins, et al., 2001; Jenkins, et al., 2005; Jenkins, et al., 2006; Pennington, et al., 2001, 2002, 2003, 2004, 2005, 2006; Taylor, et al., 2004). Corrosion and leakage of unexploded ordnance (UXO) can be a major long-term point source on impact areas (Brannon, et al., 2000; Chendorain, et al., 2005).

Contamination found on munitions ranges is complex. The various chemical and physical properties of each munition compound, their transformation products, along with interferences arising from the environmental matrix, make extraction and quantitation a challenge. Two factors complicate the assessment of environmental impacts from munitions contamination. Firstly, new IM formulations will be used on training ranges where legacy munition contamination may be present. Secondly, daughter products of IM components, such as 2-/4-nitrophenol (2-/4-NP) or 2,4-dinitrophenol (2,4-DNP) (Hawari, et al. 2015) from the degradation of DNAN, appear as IM compounds degrade. 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). Other legacy munitions, such as picric acid (PA) may be also be present on training ranges, but are not included in U.S. Environmental Protection Agency (USEPA) Method 8330 (Goerlitz, 1979).

Current high-performance liquid chromatography (HPLC)- and gas chromatography (GC)-based analytical methods require pre-concentration by solid-phase extraction (SPE) prior to analysis in order to achieve sub- $\mu\text{g/L}$  detection limits in water samples. While methods of extracting legacy munitions from water by SPE have been standardized, and several SPE methods are available for the extraction of nitrophenols, only one has been developed for NTO (Walsh, 2016). To date, no unified SPE method has been developed that allows for co-extraction of NTO, NQ, DNAN, PA, 2,4-DNP, and 2-/4-NP. Lack of such a method means that detection limits are significantly elevated because IM-containing waters must undergo direct-injection analysis by HPLC.

The traditional acetonitrile (ACN) extraction method for explosives in soils described in USEPA Method 8330B results in poor extraction efficiency of NTO, NQ, PA, 2,4-DNP, and 2-/4-NP. The compounds are only slightly soluble in acetonitrile, but have relatively high solubility in water at 13 g/L for NTO, 2.6 g/L for NQ, 17.6 g/L for PA, 5.6 g/L for 2,4-DNP, 2.1 g/L for 2-NP, and 16 g/L for 4-NP. Furthermore, PA, NTO, and 2,4-DNP exist as anions at neutral pH due to their acidic nature, with  $\text{pK}_a = 0.38, 3.76,$  and  $4.11$ , respectively. Alternatively, NQ ( $\text{pK}_a 12.2$ ) can exist as either a nitroimine (predominant at environmental pH) or nitroamine, and at very low pH could become protonated to form a cation (De Vries, et

al., 1953; Kaplan, et al., 1982). DNAN is the exception of the IM compounds, with water solubility comparable to TNT (approximately 200 mg/L) (Lide, 2005). Indeed, DNAN has been extracted *via* the standard SPE procedure outlined in USEPA Method 3535 (USEPA, 2007).

HPLC separations, coupled with ultraviolet (UV) absorbance or mass spectrometric (MS) detection and quantitation, are the gold standard for analysis of munition compounds (USEPA, 2006). Many munition compounds are nitroaromatics (e.g. nitrotoluenes and nitrobenzenes) or nitramines (e.g. RDX) and are analyzed using USEPA Method 8330. Other energetic compounds, including NTO, DNAN, NQ, and PA, can also be determined using HPLC separations, though the mobile and stationary phases may be modified from the conditions reported in USEPA Method 8330B (USEPA, 1996; Goerlitz, 1979; Russell, et al., 2014). The chemical properties of some of these compounds preclude direct application of the extraction, pre-concentration, and analysis methods described in USEPA Methods 3535 and 8330B protocols without modification. Therefore, when analysis of samples containing IM and legacy compounds is required, multiple analytical procedures would have to be employed, leading to increased costs and delays in data availability.

Much research is underway to determine the effects of NTO, DNAN, and NQ in the environment. The lack of uniform methods for extraction of munition compounds in water, soil, and tissue is a barrier to determining the long-term environmental impact of IM compounds. The wide-ranging solubilities and ionic properties of NTO, NQ, DNAN, 2,4-DNP, 2-/4-NP, and legacy munition compounds necessitated the development and optimization of methods to effectively extract/(pre-concentrate) all of the IM and legacy compounds studied. This work serves to fill current gaps in energetics determination methodology by developing a direct-injection and SPE method for waters, solvent extraction (SE) methods for soils and tissues, and two HPLC-UV and one HPLC-MS method for analysis of IM, IM transformation products, and legacy munition compounds. The implementation of these methods for simultaneous determination of IM and legacy munition compounds should lead to resource and labor cost savings, with a minimal increase in environmental monitoring efforts and no modification to existing sample collection procedures.

Three main analytical approaches (HPLC, MEKC and CE) were used to develop methods for simultaneous separation, detection, and quantitation of 24 legacy and IM compounds of interest. Primary- and secondary-column HPLC-UV methods, as well as a confirmatory HPLC-MS method, were developed by the Environmental Laboratory (EL) at the U.S. Army Corps of Engineers (USACE) Engineer Research and Development Center (ERDC), and MEKC and CE methods were developed by the Naval Research Laboratory (NRL). The compounds of interest were 17 legacy compounds included in USEPA Method 8330B and seven insensitive munition compounds, daughter products, and others absent from USEPA Method 8330B, specifically, nitroguanidine (NQ), 3-nitro-1,2,4-triazol-5-one (NTO), picric acid (PA), 2,4-dinitroanisole (DNAN), 2,4-dinitrophenol (2,4-DNP), 2-nitrophenol (2-NP), and 4-nitrophenol (4-NP). The linear dynamic ranges (LDRs), method detection limits (MDLs), and matrix effects for IM compounds, IM daughter products, and legacy munition compounds have been studied, using the developed extraction/(pre-concentration) and analytical methods in aqueous and solid matrices.

SPE is a method utilized for extraction and pre-concentration of explosive compounds from environmental water samples in both USEPA Method 8330B and in ERDC in-house IM methods. An SPE method was developed to extract and pre-concentrate all 24 compounds of interest (and two surrogates, 1,2-dinitrobenzene, 1,2-DNB, and *ortho*-nitrobenzoic acid, *o*-NBA) by evaluating a series of cation-exchange, anion-exchange, reverse phase (polymeric and traditional C-18), and activated carbon-based SPE cartridges. Methods developed using reagent water samples were applied to four additional water sources: 1) tap water (ERDC-EL), 2) ground water (well water from Rayville, LA), 3) river water (Yazoo River near Vicksburg, MS), and 4) sea water (Houston Shipping Channel). The developed water extraction method

efficiently extracted all 24 compounds of interest and two surrogates within acceptable Department of Defense (DoD) Quality Systems Manual (QSM) Ver5.2 levels, except NQ (approx. 50% recovery), from each water source, with slight pH-related variability, particularly with NTO.

Current USEPA Method 8330B solvent extraction methods were modified to enable extraction of all 24 legacy and IM compounds of interest (and two surrogates, 1,2-DNB and *o*-NBA) from soil samples, by adjusting the solvent system and using a 2-stage extraction procedure. The developed soil extraction method efficiently extracted all 24 compounds of interest and two surrogates within acceptable DoD QSM Ver5.2 levels, using six different soil sources, with widely varying organic matter, pH, and other properties: 1) ASTM fat clay (CH-1), 2) Aberdeen clay (Aberdeen, MD), 3) Jefferson clay (Madison, IN), 4) Memphis silt (Memphis, TN), 5) Ft. Riley clay (Riley, KS), and 6) Yuma sand (Yuma, AZ).

A tissue extraction method was also developed by slight modification to existing USEPA Method 8330B processes, with the majority of the 24 compounds and two surrogates being extracted within acceptable DoD QSM Ver5.2 ranges via a single 18-h MeOH (rather than ACN) extraction from four different tissue matrices: 1) earthworms (*Eisenia fetida*, soil-dwelling invertebrate), 2) fathead minnows (*Pimephales promelas*, freshwater vertebrate), 3) polychaete worms (*Alitta virens*, marine invertebrate), and 4) perennial ryegrass (*Lolium perenne*, perennial plant). Varying degrees of analytical interference arising from co-extracted non-target matrix components were observed for each tissue type, with the most interference occurring in earthworm tissue. Chromatographic interference reduction methods, using common organic laboratory chromatography materials, were developed to remove these matrix-associated interferences.

Stability studies were carried out using laboratory-spiked reagent water and three laboratory-spiked soils (Riely, Yuma, and ASTM fat clay), in order to verify whether current standard pre-extraction and post-extraction hold times of seven or 14 days and 40 days for semi-volatile organics (USEPA, 2014) would remain valid for mixtures of the 24 compounds of interest and two surrogates. Samples were held in triplicate under three different conditions: 1) cold/dark (4°C/amber vials), 2) room temperature/dark (25°C/amber vials), and 3) room temperature/light (25°C/clear vials) in order to distinguish photo- vs thermal-related degradation of each compound. In addition, the developed SPE and SE methods were applied to laboratory-spiked water (5) and soil (6) samples for verification by inter-laboratory extraction and analysis. For these tests, samples were extracted and analyzed within current standard hold times for semi-volatile organics.

## Section I. HPLC-UV and HPLC-MS Methods

### Materials and Methods

#### HPLC-UV and HPLC-MS Methods

The HPLC-UV and -MS methods developed under SERDP ER-2722 were modified from existing methods and established laboratory techniques. Previously developed (Russell et al., 2014) and currently used explosives analysis methods (USEPA 8330B, in-house IM method; see Figure 4, Results and Discussion) were used as a starting point and reference in the development of the primary and secondary HPLC-UV methods for SERDP ER-2722.

##### *Primary HPLC-UV Method*

The primary HPLC-UV method for simultaneous separation and quantitation of 17 legacy and seven IM compounds, daughter products, and other munition compounds not included in USEPA Method 8330B was developed by adapting previous methods. Method parameters and solvent gradient scheme are shown

in Table 1. Method development was carried out using Phenomenex Synergi 4 $\mu$ m Hydro-RP, 80Å, 250 x 4.6 mm HPLC columns. An optional Phenomenex SecurityGuard AQ C18 pre-column guard cartridge was included, which could extend column lifetime, especially when analyzing samples containing complex environmental matrices. As indicated in Table 1, either a 0.1% TFA or 0.25% FA (v/v) solution in water can be used. (This was investigated during HPLC-MS method development (discussed below), due to ion suppression effects caused by TFA.) An example chromatogram is shown in Figure 1 (acquired using TFA). All chromatographic peaks were identified using individual reference standards.

Table 1. Primary HPLC-UV separation solvent gradient scheme and other method conditions.

| Total Run Time: 48.0 min<br>Column Temperature: 25°C |                   | Detection Wavelengths: 210, 254, 315 nm<br>Flow Rate: 1.0 mL/min    Injection Volume: 50 $\mu$ L |                     |         |
|------------------------------------------------------|-------------------|--------------------------------------------------------------------------------------------------|---------------------|---------|
| 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

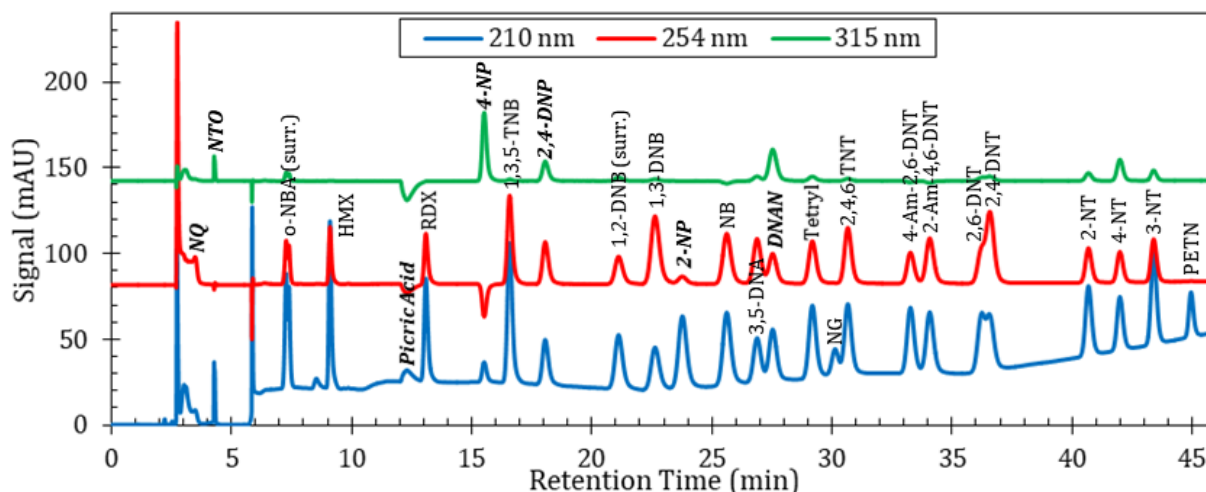


Figure 1. HPLC chromatograms of a mixed standard containing 5 mg/L of 24 legacy and IM compounds and two surrogates analyzed by the developed primary HPLC-UV method. ***Bold, italicized*** are IM compounds and degradation products.

### Secondary HPLC-UV Method

A secondary HPLC-UV method was developed for confirmatory analysis using Restek Pinnacle II Biphenyl, 5  $\mu$ m, 150 X 4.6 mm HPLC columns. Again, an optional Phenomenex SecurityGuard AQ C18 pre-column guard cartridge was included. The method parameters are shown in Table 2. As indicated, again either a 0.1% TFA or 0.25% FA (v/v) solution in water can be used to acidify the mobile phase for optimal chromatographic resolution. An example chromatogram is shown in Figure 2 (acquired using FA). All chromatographic peaks were identified using individual reference standards.

Table 2. Secondary HPLC-UV separation solvent gradient scheme and other method conditions.

| Total Run Time: 35.0 min |                   | Detection Wavelengths: 210, 254, 315 nm |                              |         |
|--------------------------|-------------------|-----------------------------------------|------------------------------|---------|
| Column Temperature: 25°C |                   | Flow Rate: 0.9 mL/min                   | Injection Volume: 50 $\mu$ L |         |
| 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

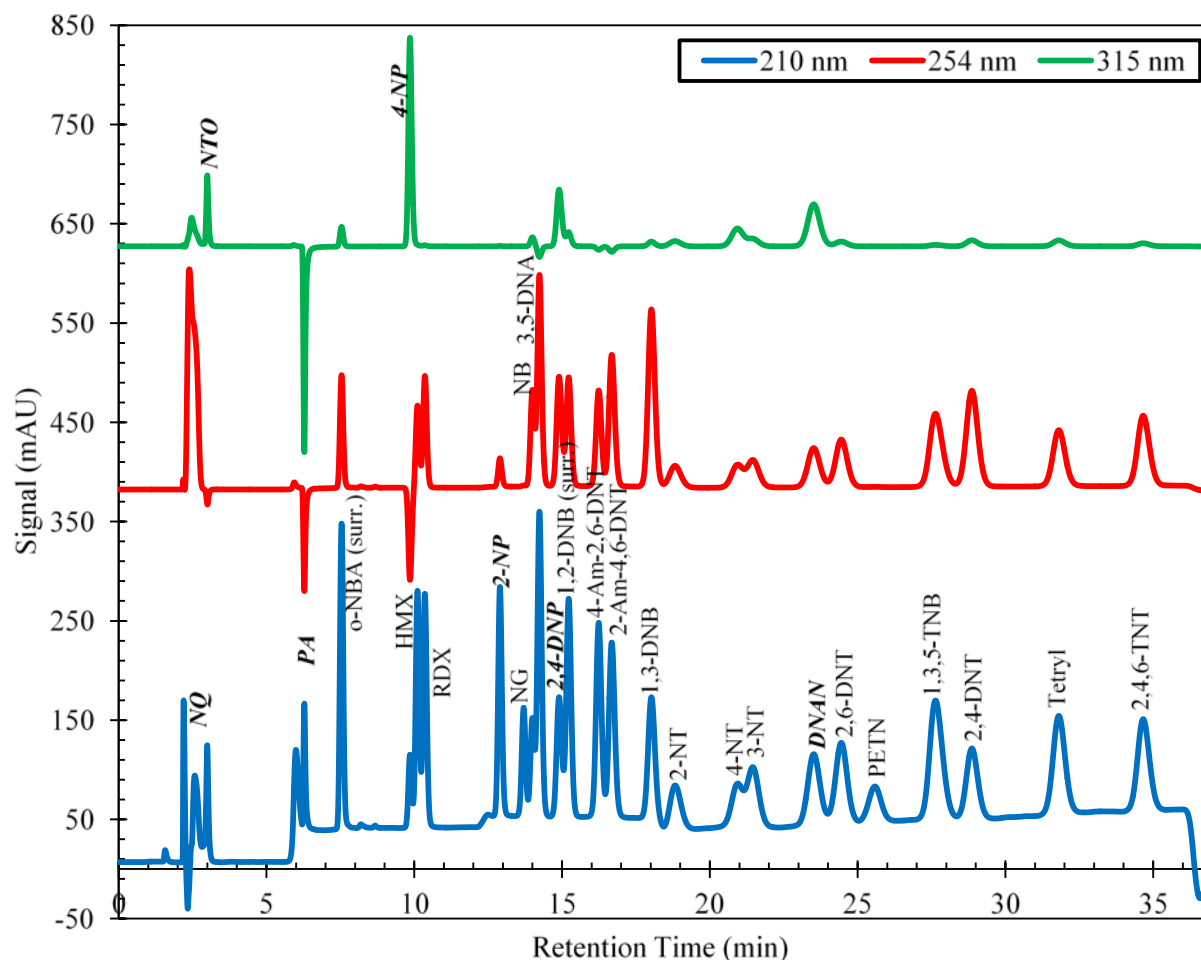


Figure 2. HPLC chromatograms of a mixed standard containing 10 mg/L of 24 legacy and IM compounds and two surrogates analyzed by the developed secondary HPLC-UV method. ***Bold, italicized*** are IM compounds and degradation products.

### HPLC-MS Method

An HPLC-MS scan mode method was developed using an Agilent 6120B single quadrupole (SQ) system equipped with an atmospheric-pressure chemical ionization (APCI) source for confirmatory analysis of the 24 compounds of interest and two surrogates. Spray chamber and MS detector parameters for the scan mode method are provided in Table 3. Chromatographic separation was effected by the primary HPLC-UV method described above. An example MS total ion chromatogram (TIC) is shown in Figure 3. Several ion masses were observed for each of the 26 compounds analyzed (shown in Table 4), with the exception of nitroglycerine (NG) and 2-, 3-, or 4-nitrotoluene (2-NT, 3-NT, 4-NT), which did not produce any detectable MS signal under the developed spray chamber and detector conditions. In addition, 1,3-dinitrobenzene (1,3-DNB) yielded only a single observed ion. Ions were identified where possible. Observed ions are listed in order of abundance for each compound.

Table 3. Spray chamber and detector parameters for the developed HPLC-MS scan mode method.

| Parameter              | Value    |
|------------------------|----------|
| Source                 | APCI     |
| Ionization Mode        | Negative |
| Drying Gas (°C)        | 350      |
| Vaporizer (°C)         | 325      |
| Drying Gas (L/min)     | 4.0      |
| Nebulizer (psig)       | 40       |
| Corona (μA)            | 10       |
| Capillary (V)          | 1500     |
| Mass Range             | 40-400   |
| Fragmentor             | 100      |
| Gain                   | 1.00     |
| Threshold              | 0        |
| Step Size              | 0.20     |
| Speed (μ/sec)          | 743      |
| Peak Width (min)       | 0.060    |
| Cycle Time (sec/cycle) | 0.57     |

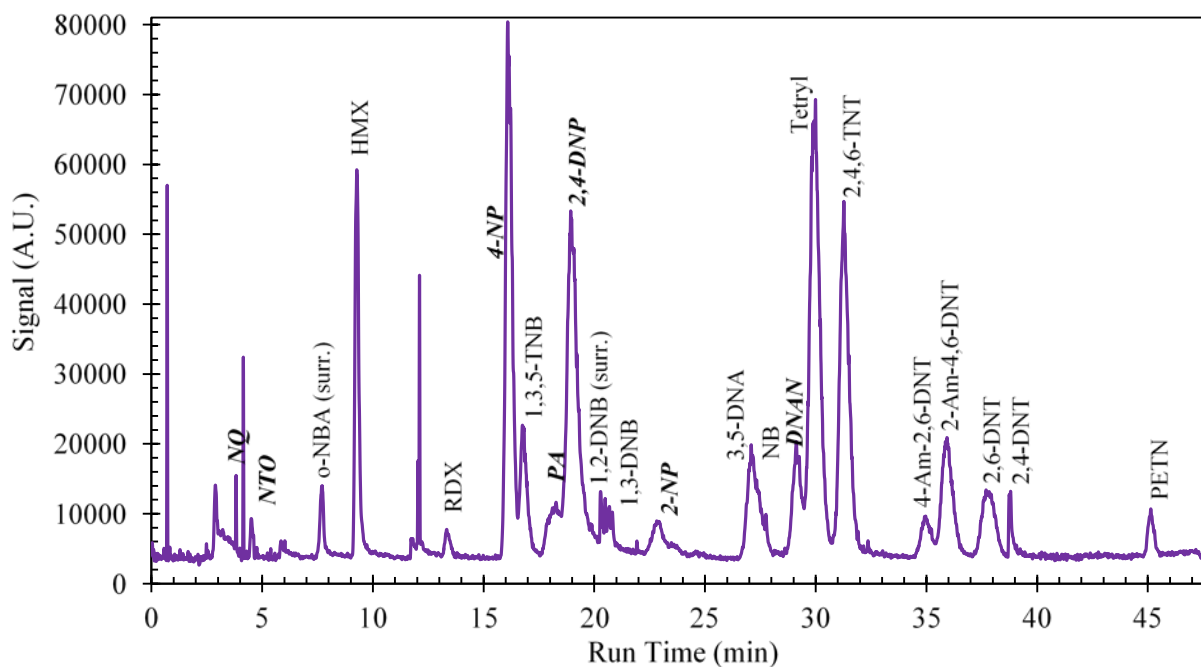


Figure 3. TIC for a mixed standard containing 10 mg/L of 24 legacy and IM compounds and two surrogates analyzed by the developed confirmatory HPLC-MS scan mode method. ***Bold, italicized*** are IM compounds and degradation products.

Table 4. 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 HPLC-MS scan mode method.

| Retention Time (min) | Compound            | Ion Mass (m/z) | Ion Identity                         |
|----------------------|---------------------|----------------|--------------------------------------|
| 3.78                 | <b>NQ</b>           | 203.6          |                                      |
|                      |                     | 103.0          | [M]-H                                |
| 4.50                 | <b>NTO</b>          | 129.0          | [M]-H                                |
|                      |                     | 55.0           |                                      |
| 7.64                 | o-NBA (surrogate)   | 122.0          |                                      |
|                      |                     | 167.0          | [M]-H                                |
| 9.21                 | HMX                 | 341.0          | [M]-H+FA                             |
|                      |                     | 147.0          | [M]/2-H                              |
| 13.40                | RDX                 | 267.0          | [M]-H+FA                             |
|                      |                     | 335.0          | [M]-H+TFA                            |
| 16.00                | <b>4-NP</b>         | 108.0          |                                      |
|                      |                     | 138.0          | [M]-H                                |
| 16.80                | 1,3,5-TNB           | 213.0          | [M]-H                                |
|                      |                     | 183.0          |                                      |
| 18.20                | <b>PA</b>           | 228.0          | [M]-H                                |
|                      |                     | 211.9          |                                      |
| 18.80                | <b>2,4-DNP</b>      | 183.0          | [M]-H                                |
|                      |                     | 153.0          |                                      |
| 22.70                | 1,2-DNB (surrogate) | 138.0          |                                      |
|                      |                     | 168.0          | [M]-H                                |
| 23.55                | 1,3-DNB             | 168.0          | [M]-H                                |
| 24.60                | <b>2-NP</b>         | 138.0          | [M]-H                                |
|                      |                     | 122.0          | [M]-H <sub>2</sub> O-H               |
| 27.10                | 3,5-DNA             | 182.0          | [M]-H                                |
|                      |                     | 138.0          |                                      |
| 27.80                | NB                  | 182.0          | [M]-H+HAc                            |
|                      |                     | 153.0          |                                      |
| 29.00                | <b>DNAN</b>         | 183.0          | [M]-CH <sub>3</sub>                  |
|                      |                     | 152.9          |                                      |
| 30.00                | Tetryl              | 241.0          | [M]-H+NO <sub>2</sub> * <sup>*</sup> |
|                      |                     | 288.0          | [M]-H                                |
|                      | NG                  | 226.1          | [M]-H (not obsv.)                    |
| 31.30                | 2,4,6-TNT           | 226.0          | [M]-H                                |
|                      |                     | 210.0          | [M]-H <sub>2</sub> O-H               |
| 35.10                | 4-Am-2,6-DNT        | 196.9          | [M]-H                                |
|                      |                     | 167.0          |                                      |
| 35.95                | 2-Am-4,6-DNT        | 196.0          | [M]-H                                |
|                      |                     | 179.9          | [M]-H <sub>2</sub> O-H               |
| 37.54                | 2,6-DNT             | 152.0          | [M]-30*                              |
|                      |                     | 122.1          |                                      |
| 37.87                | 2,4-DNT             | 165.0          | [M]-H <sub>2</sub> O-H               |
|                      |                     | 181.0          | [M]-H                                |
| 42.00 - 44.50        | 2-NT/3-NT/4-NT      | 136.1          | [M]-H (not obsv.)                    |
| 45.10                | PETN                | 62.0           | NO <sub>3</sub> * <sup>*</sup>       |
|                      |                     | 270.9          |                                      |

**Bold, italicized** are IM compounds and degradation products.

\*Avci, et al., 2017; Gapeev, et al., 2003; Jiang, 2010; Kinghorn, et al., 2005; Thurman, et al., 2012; Xu, et al., 2004.

## Extraction Methods for Samples Analyzed by HPLC-UV and HPLC-MS

### *Method for Preparation of Waters for Direct-Injection Analysis*

Water samples known or suspected to contain the compounds of interest at levels detectable without extraction/pre-concentration can be analyzed by direct injection using the developed analytical HPLC-UV/-MS methods. In order to ensure the accurate analysis of ionic species (including NTO), an acidification step was added for direct-injection water samples. A solution of 1% HCl/MeOH was used to acidify and dilute water samples, for final acid concentration of 0.5% (v/v) and final solvent ratio of 50/50 MeOH/water.

### *SPE Method for Waters*

The SPE method developed under SERDP ER-2722 involved the sequential stacking of three different commercially-available SPE cartridges: 1) Strata X (polymeric reverse phase, 500 mg/6 mL, Phenomenex), 2) Strata X-A (polymeric strong anion exchange, 500 mg/6 mL, Phenomenex), and 3) Supelclean™ ENVI-Carb™ (granular activated carbon, GAC, 500 mg/6 mL, Supelco). The SPE cartridge conditioning, loading, and elution procedures were developed using common SPE methods for environmental water extraction and pre-concentration. Specifically, 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 loading using SPE tube adapters (Supelco): 1) Strata X at 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 26 compounds of interest (including two surrogates) or blank (unspiked) water sample. In order to allow for mass balance analysis, a small amount of pass-through water was collected (e.g. breakthrough determinations).

After allowing 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 at the top, 2) Strata X-A was in the middle, and 3) Strata X was on the bottom. Each sample-loaded SPE unit was eluted first with 5 mL MeOH, followed by 5 mL 2% HCl/MeOH. Extracts were collected separately, stored at  $\leq 4^{\circ}\text{C}$ , and later prepared for analysis. MeOH and acidified MeOH extracts were analyzed individually, or by combining known ratios of each, and diluting with reagent water, for a final solvent ratio of 50/50 MeOH/water.

### *SE Method for Soils*

The SE method developed under SERDP ER-2722 for soils was a two-stage extraction procedure, modified from the current USEPA Method 8330B. Sample collection, storage, drying, and grinding procedures were not modified. Specifically, soils were ground to  $<0.85$  mm (US Standard sieve #20) particle diameter using a ceramic mortar-and-pestle, dried at  $25^{\circ}\text{C}$  in a forced-air convection oven in the absence of light. Aliquoted soil samples were spiked (varying concentrations) with a mixed reference standard containing all 26 compounds of interest (including two surrogates) in a mixture of MeOH/ACN or with unspiked MeOH/ACN, and vortexed for 1 min to mix. Samples were allowed to dry again in the oven at  $25^{\circ}\text{C}$ , and then mixed using a horizontal sample roller for up to 18 h at  $4^{\circ}\text{C}$  in the absence of light.

Samples were then extracted using a two-stage ultrasonication procedure. For each sample, 5 mL of MeOH per gram of soil was added, and soil/solvent mixtures were placed in a cooled (by constant water replacement) ultrasonic bath for 6 h in the absence of light. Soil/solvent mixtures were centrifuged, and the supernatant (MeOH extract) was collected by syringe filtering through a  $0.45\ \mu\text{m}$  PTFE hydrophobic

disk filter. Next, 5 mL of 50/50 MeOH/water per gram of soil was added, and the soil/solvent mixtures were placed in the cooled ultrasonic bath for another 14 h in the absence of light. Again, soil/solvent mixtures were centrifuged, and the supernatant (MeOH/water extract) was collected by syringe filtering through a new 0.45  $\mu\text{m}$  PTFE hydrophobic disk filter. Extracts were collected separately, stored at  $\leq 4^{\circ}\text{C}$ , and later prepared for analysis. Extracts were analyzed individually, or by combining known ratios of each, and diluting for a final solvent ratio of 50/50 MeOH/water.

### *SE Method for Tissues*

The SE method developed under SERDP ER-2722 for tissues was a single 18-h ultrasonic extraction procedure, modified from the current USEPA Method 8330B. Briefly, whole tissues were ground without lyophilization (wet) under liquid nitrogen, using either a mill or stainless steel mortar-and-pestle, to as fine a particle size or paste as possible. Aliquotted samples were spiked with a mixed reference standard containing all 26 compounds of interest (including two surrogates) in a mixture of MeOH/ACN or with unspiked MeOH/ACN, and vortexed for 1 min and were homogenized well prior to extraction. No post-spike drying step was performed for tissue samples. Homogenized samples were extracted using a single 18-h ultrasonication with MeOH. For each sample, 5 mL of MeOH per gram of wet sample was added, and samples were placed in a cooled (by constant water replacement) ultrasonic bath for 18 h in the absence of light. Tissue/solvent mixtures were centrifuged, and the supernatant (MeOH extract) was collected by syringe filtering through a 0.45  $\mu\text{m}$  PTFE hydrophobic disk filter. Extracts were stored at  $\leq 4^{\circ}\text{C}$  and later prepared for analysis. MeOH extracts were analyzed after diluting with water for a final solvent ratio of 50/50 MeOH/water. (At cold temperatures, some tissue extracts were observed to develop a precipitate. These samples were filtered further using either 0.45  $\mu\text{m}$  PTFE hydrophobic or 0.20  $\mu\text{m}$  Anotop 10 inorganic membrane disk filters.)

### **Chromatographic Interference Reduction Method for Tissue Extracts**

The tissue interference reduction method developed under SERDP ER-2722 was a modified chromatographic method (Larson, et al., 1999). Other common organic laboratory procedures were tested, including storage at low temperature or acidification to induce complete precipitation, followed by filtration. However, these methods were not as effective as chromatographic methods. Initial tests were performed using either neutral alumina or florisil, and later tests included combinations of these, basic alumina, or silica. In later tests, MeOH extracts of unspiked tissues were spiked for a final concentration of 6 mg/L of the 24 compounds of interest and two surrogates. Small-scale chromatography columns were prepared using 5  $\frac{3}{4}$ " borosilicate pipettes, loaded with a total of 0.2 g packing material. Five different column packing schemes were used: 1) 0.1 g neutral alumina layered on top of 0.1 g florisil, 2) 0.1 g florisil layered on top of 0.1 g neutral alumina, 3) 1:1 mixed neutral alumina and florisil, 4), basic alumina and 5) silica gel. Chromatography columns were wetted with MeOH just before loading 1 mL tissue MeOH extract. After the 1 mL MeOH extract had completely passed through the column, 1 mL of MeOH was used to elute, followed by 1 mL 2% HCl/MeOH. The resulting 3 mL of column-treated MeOH extract was vortexed to mix. Treated extracts were again stored at  $\leq 4^{\circ}\text{C}$  and later prepared for analysis by diluting with water for a final solvent ratio of 50/50 MeOH/water. Several of the treatment options were effective at reducing the analytical interferences observed for the tissues. However, the silica gel option proved most effective for the majority of compounds in the majority of tissue types.

### **Stability Study Methods**

Photo-/thermal stability was investigated for each of the 24 compounds of interest and two surrogates by testing the current pre-/post-extraction hold times for water and soils, which are 7/14 days for extraction,

respectively, and 40 days for analysis for munition compound determinations performed using USEPA Method 8330B. Extraction/analysis hold times were not previously reported for tissues in Method 8330B.

Reagent water was batch-spiked, aliquotted, extracted (using the developed SPE method), and analyzed (using the primary HPLC-UV method) on Day 0. Portions of laboratory-spiked water were stored under three different conditions until Day 7. The three storage conditions were: 1) cold (4°C), dark (amber containers), 2) room temperature (25°C), dark, and 3) room temperature, light (clear containers). On Day 7, the stored water samples were extracted and analyzed. In addition to storing portions of laboratory-spiked water on Day 0, aliquots of Day 0 MeOH/acidified MeOH extracts were also stored under the same three conditions until Day 40. On Day 40, the stored MeOH/acidified MeOH extracts were analyzed using the primary HPLC-UV method. Included as part of the stability studies in water were direct-injection (no SPE) water samples, which were spiked at a higher level to enable detection without pre-concentration.

Three soils were batch-spiked and extracted (using the methods described in 2.6 SE Method for Soils), and analyzed (using the primary HPLC-UV method) on Day 0/1 (due to the 2-day extraction procedure). Portions of the laboratory-spiked soils were stored under the same three conditions as for water samples until Day 14. On Day 14, the stored soil samples were extracted (Day 14/15) and analyzed (Day 15). Again, in addition to storing portions of laboratory-spiked soils on Day 0, aliquots of Day 0/1 MeOH and MeOH/water extracts (combined) were also stored under the same three conditions until Day 40. On Day 40, the stored extracts were analyzed using the primary HPLC-UV method.

## **Inter-Laboratory Batch Analysis Methods**

An inter-laboratory batch study was carried out at ERDC-EL and ERDC-CRREL. Briefly, five water sources (reagent, tap, river, sea, and ground) and six soil sources (Aberdeen, Jefferson, Memphis, Riley, Yuma, and ASTM fat clay) were batch-spiked, homogenized, aliquotted, and distributed to each laboratory for extraction using the developed SPE and SE methods, followed by analysis using the developed primary HPLC-UV method. Samples were spiked with the 24 compounds of interest and two surrogates. High-level direct-injection water samples were spiked at 2 mg/L, and low-level water samples for SPE extraction/pre-concentration were spiked at 0.10 mg/L. Soils were spiked at 20 mg/kg. Each laboratory received identical protocols for sample extraction, analytical sample preparation, and analysis.

During cross-laboratory studies using the direct injection method for waters, much lower recoveries for NTO, and in some cases *o*-NBA surrogate, were observed for field water samples than for reagent water. Therefore, further experiments were carried out in which the pH of samples to be analyzed by direct injection was adjusted using 2% HCl/MeOH (the same used for stage two of SPE elution), so that the final acid concentration was the same as in SPE extracted/pre-concentrated samples, or double. Briefly, reagent water, Vicksburg municipal water (tap), and three field water samples were spiked with the 24 compounds of interest and two surrogates for a final concentration of 1 mg/L and either 0.5% or 1.0% HCl (v/v). The acidified laboratory-spiked water samples were diluted 1:1 with MeOH and analyzed using the developed primary HPLC-UV method.

## **Results and Discussion**

### **Primary HPLC-UV Method**

Previously developed (Russell, et al., 2014) and currently employed munition compound analysis methods (USEPA 8330B, in-house IM method) were used as references in the development of the primary HPLC-UV method. Shown in Figure 4 are the isocratic or gradient methods referenced. The separation solvent gradient scheme and other method conditions for the primary HPLC-UV method itself are shown in Table 1 in above. Peak identities (obtained by analysis of individual reference standards), method detection limits (MDLs), and linear dynamic ranges (LDRs) are provided in Table 5.

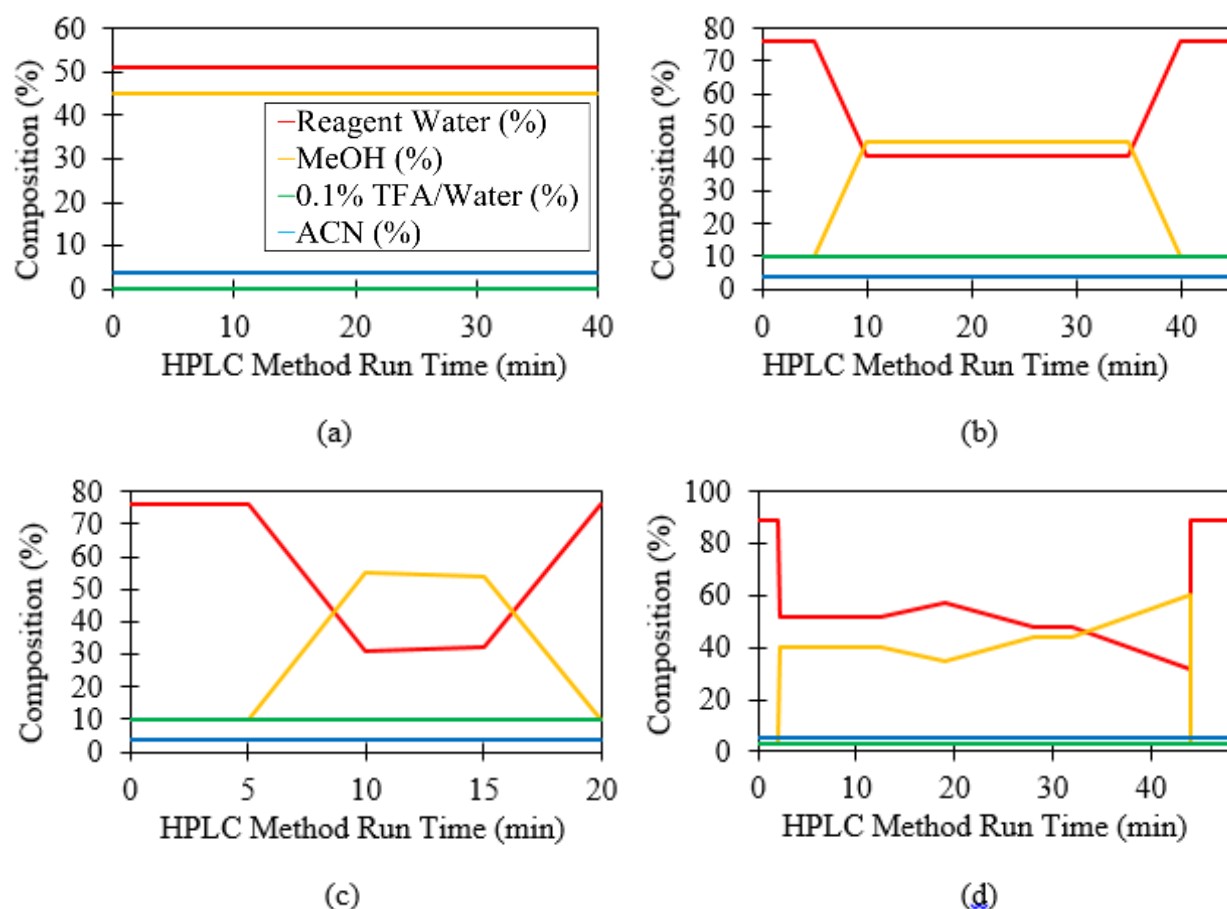


Figure 4. Previous HPLC methods used for munition compound determinations, including a) current USEPA Method 8330B employed at ERDC-EL for HPLC-UV analysis of legacy compounds, b) method developed at ERDC-EL for HPLC-MS analysis of IM compounds (Russell et al., 2014), c) current method employed at ERDC-EL for HPLC-UV analysis of IM compounds, and d) primary column HPLC-UV method developed for simultaneous analysis of legacy and IM compounds for SERDP ER-2722.

In the table, LDR values represent the highest concentration of spiked standard analyzed within  $\pm 10\%$  of the known spike concentration, using a complete mixed reference standard of the 24 compounds of interest and two surrogates. Because most of the commercially-available individual reference standards and mixes were acquired at 1000 mg/L in MeOH and/or ACN, solutions at higher concentrations ( $>50$  mg/L) contained greater than 1:1 MeOH/water ratios, which was determined to be the optimal analytical sample solvent mixture (see Figure 9). Because previous studies indicated that MeOH/water ratios of 9:1 caused chromatographic deterioration (see Figure 9), 80 mg/L with 8:1 MeOH/water content was the highest concentration tested. The upper limit of the linear range for some compounds may be higher than 80 mg/L. Results for multiple wavelengths were included for NTO, PA, and 3,5-DNA, in order to allow for quantitation using either wavelength. In some cases, detection of NTO and PA at 315 nm may be preferred in order to minimize the effects of analytical interferences, for example, with tissue samples. The PA chromatographic peak at 315 nm is inverted (versus at 210 nm) and lies below the baseline. For 3,5-DNA, the detection limits obtained for 210 nm and 254 nm were typically very similar, and either wavelength could be used for quantitation. MDLs were determined for direct-injection water samples (“Estimated”), SPE water samples (“MDL in Reagent Water”), and SE solid samples with Ottawa sand (“MDL in Ottawa Sand”).

Table 5. Retention times (min), MDLs and LDR Upper Limits (ULs) for the 24 compounds of interest and two surrogates, determined in direct-injection water (“Estimated”), reagent water that underwent SPE pre-concentration, and Ottawa sand that underwent SE, analyzed by the primary HPLC-UV method.

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

***Bold, italicized*** are IM compounds and degradation products. †co-elute at concentrations >30 mg/L; ‡RDX not detected appreciably at 315 nm

For simultaneous legacy and IM compound analysis, it was clear that a more complex gradient of the major aqueous (water) and organic phases (MeOH) would be required, with the final method incorporating elements of all three of the methods referenced (Figure 4a-c). Specifically, the primary HPLC-UV method gradient started with very low % MeOH which increased sharply after 2 min, initiating a period of approximately 30 min during which the % water was to varying degrees slightly greater than the % MeOH, followed by a period of approximately 10 min during which the % MeOH surpassed the % water and continued to increase up to 60%. A brief re-equilibration period was placed at the end of the gradient method in order to allow the system time between sample injections to re-equilibrate to initial conditions. The small decrease in MeOH from 40% at 12.5 min to 35% at 19 min (Figure 4d) was introduced in order to improve separation of the hydrophobic compounds that elute between 20-30 min, as neither isocratic conditions nor a linear increase in % MeOH from 12.5 to 28 min resulted in well-resolved, stable chromatography. The developed primary HPLC-UV method incorporated conditions favorable for separation of the 24 chemically-disparate compounds of interest (and two surrogates). Further details on the optimization of the initial % MeOH, as well as the isocratic % 0.1% TFA (or 0.25% FA) and % ACN throughout the method are discussed below.

Throughout primary column HPLC-UV method development, NQ and 2,6-/2,4-DNT resolution remained the most challenging. Resolution of 2,6-DNT and 2,4-DNT was observed to vary greatly across manufacturer production lots and individual parts; however, resolution tended to be improved for well-conditioned, newer columns. Therefore, a decrease in the resolution of these two compounds could be a signal to the user that a column has degraded and a new column could improve analyses. Meanwhile, NQ, as anticipated, seemed to suffer the most from solvent effects, specifically changes in MeOH concentration, resulting in peak splitting and migration, so that the optimal initial % MeOH determined for the primary HPLC-UV separation served to minimize these negative effects, but did not completely eliminate them. In fact, NQ resolution (retention time and peak shape) was greatly dependent upon the aqueous-to-organic ratio in both sample solvent matrix and elution solvent gradient, with comprehensive studies discussed below. Therefore, secondary confirmation of NQ by the secondary HPLC-UV or -MS method may be necessary to confirm results obtained using the primary column.

As previously mentioned, solvent effects on the gradient method, as well as on sample preparation, were studied. Preliminary method development results indicated that a complex multi-stage gradient would be required for separation of all compounds of interest and that the most effective initial % MeOH concentration was likely to be <10%. It was, thus, necessary to determine the initial % MeOH which would yield the most stable retention times and peak shapes for NQ and NTO, the most polar and earliest-eluting compounds of interest, so that reliable quantitation could be ensured. The effect of varying the solvent gradient initial % MeOH from 2% to 15% was studied, while holding the isocratic 0.1% TFA and ACN constant at 3% and 5%, respectively, using a 5 mg/L mixed standard containing all 24 compounds of interest and 1,2-DNB (surrogate) prepared in 50/50 MeOH/water. Little effect was observed for any compounds other than NQ and NTO (data not shown). Both NQ and NTO underwent large retention time shifts (>0.5 min) and changes in peak shape, including reduced intensity and splitting of NTO at >10% MeOH (Figure 5). Meanwhile, NQ shifted almost entirely into the void peak, but did become sharper as the MeOH concentration increased. Overall, it was determined that 3% MeOH yielded the best NTO peak shape and intensity, as well as NQ distance from both the void peak and NTO, with sufficient baseline resolution for reliable quantitation.

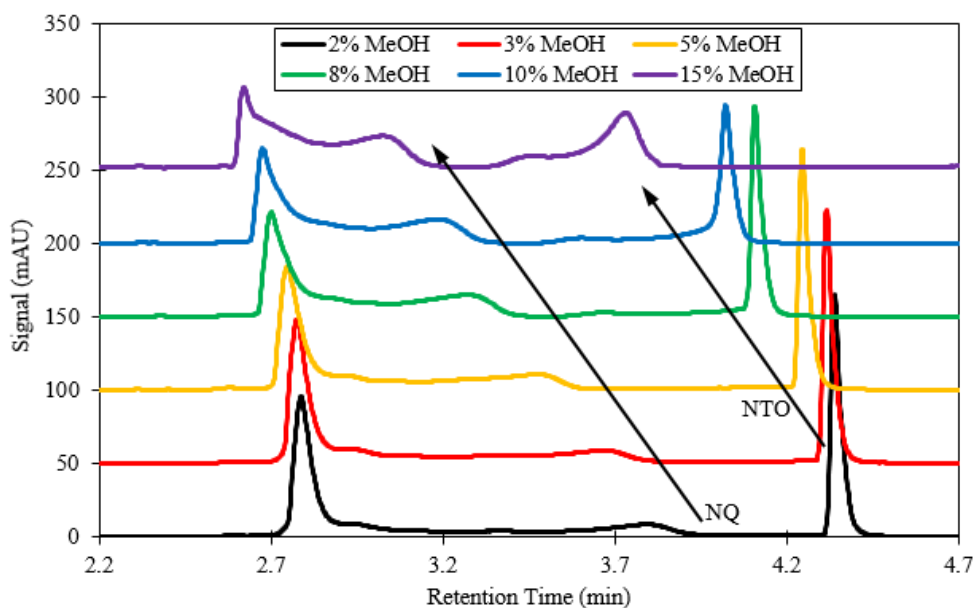


Figure 5. Effect of varying the solvent gradient initial MeOH from 2% to 15%, while holding the 0.1% TFA/water and ACN constant, using 5 mg/L mixed standard containing all 24 compounds of interest and 1,2-DNB surrogate prepared in 50/50 MeOH/water. The detection wavelength was 210 nm.

The effect of varying the isocratic mobile phase component 0.1% TFA/water from 2% to 15% was studied, while holding the initial MeOH and isocratic ACN constant at 3% and 5%, respectively, using a 5 mg/L mixed standard containing all 24 compounds of interest and 1,2-DNB (surrogate) prepared in 50/50 MeOH/water. Little effect was observed for compounds with retention times greater than 20 min (data not shown), which were generally non-ionic. NQ was for the most part unaffected by changes in % TFA (Figure 6a). The most significant effects were observed for PA, NTO, and 2,4-DNP, which were the most acidic compounds studied ( $pK_a = 0.38$ ,  $3.76$ , and  $4.11$ , respectively). Meanwhile, NTO underwent a great deal of peak broadening (from a peak width of  $0.1$  min to  $0.3$  min) and retention time shift of approximately  $0.5$  min (Figure 6a) as 0.1% TFA/water increased from 2% to 15%.

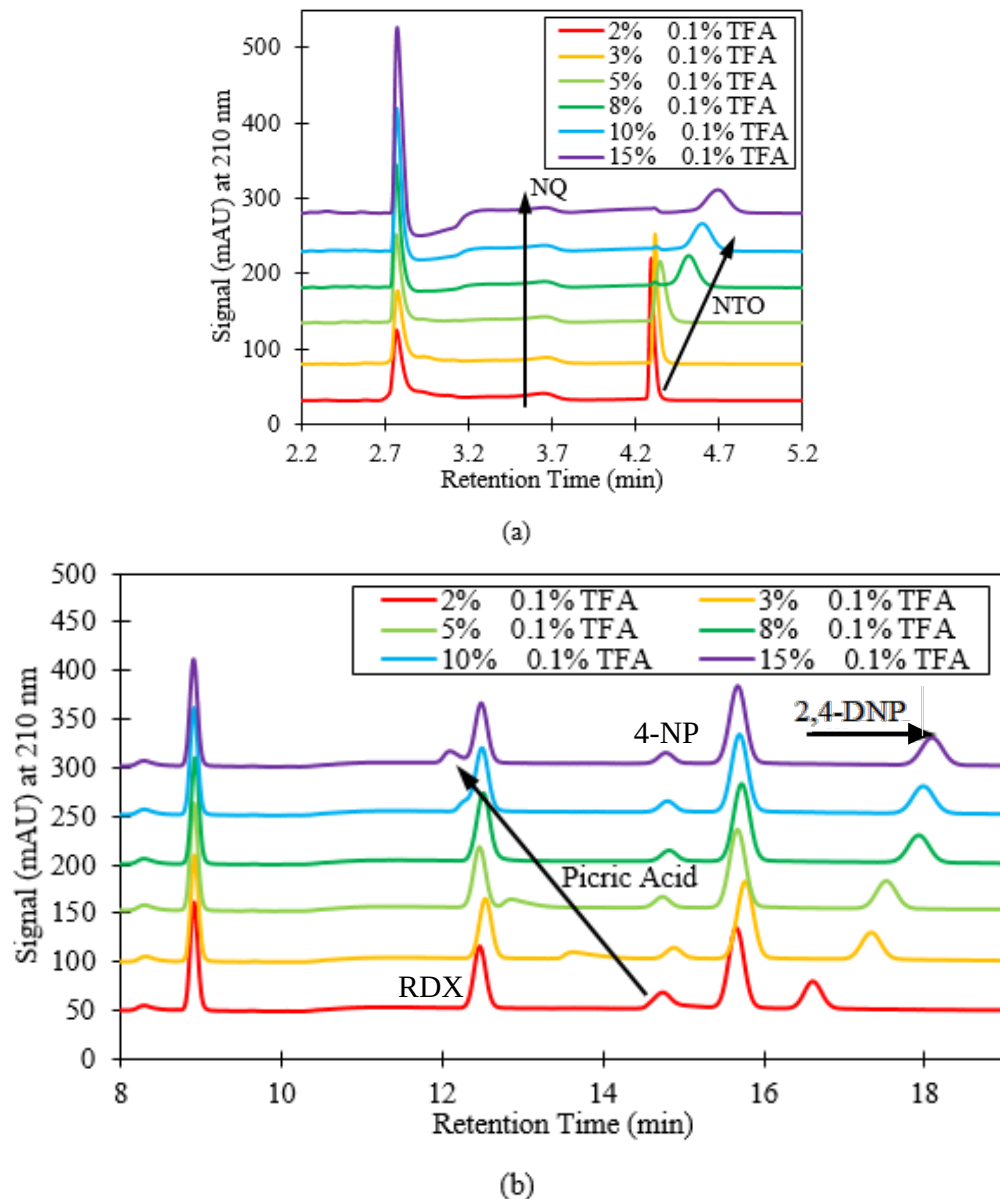


Figure 6. Effect of varying the solvent gradient isocratic 0.1% TFA/water from 2% to 15%, while setting the initial MeOH at 3% and holding ACN constant at 5%, using 5 mg/L mixed standards containing all 24 compounds of interest and 1,2-DNB surrogate were prepared in 50/50 MeOH/water. (a) Effect on NQ and NTO, (b) Effect on PA and 2,4-DNP retention times (min) and peak shapes.

In addition, both PA and 2,4-DNP underwent extreme peak shifting (ranging from 12-15 min and 16.5-18 min, respectively), and shifted inversely with respect to one another, based on the TFA concentration. At lower pH, PA shifted to earlier retention times, while 2,4-DNP shifted to later retention times. No analytical (co-elution) challenges arose because of 2,4-DNP peak shifting.

Conversely, PA co-eluted with 4-NP at 2% 0.1% TFA/water, shifted left to co-elute with RDX with the 0.1% TFA/water set to 8% of the mobile phase composition, and eluted before RDX by 15% 0.1% TFA/water. In other studies (data not shown), PA was also observed to undergo retention time shifts over a range of approximately 1 min as a result of increased or decreased sample pH arising from changes in the concentration of PA itself. Higher concentrations of PA resulted in earlier elution (~13 min), and lower concentrations of PA resulted in later elution (~14 min), similar to the TFA-dependent shifting observed in Figure 6b. This wide variability of PA retention time was due to its very low pKa, which was not buffered well by the TFA in the mobile phase. Over the course of the current effort, PA concentration-dependent PA peak shifting was observed over a range of concentrations, or due to sample matrix pH variability or prepared 0.1% TFA/water mobile phase variability from batch-to-batch. Considering all effects of TFA concentration on the primary HPLC-UV method chromatography, a 3% isocratic mobile phase composition of 0.1% TFA/water was chosen, in order to enable efficient and consistent quantitation of NTO and PA. As a technical note, the extreme pH sensitivity observed for PA highlighted the importance of careful preparation of the 0.1%TFA/water solvent to ensure chromatographic stability.

The effect of varying the solvent gradient isocratic % ACN from 5% to 8% was studied, while holding the initial MeOH and isocratic 0.1% TFA/water constant at 3%, using a 5 mg/L mixed standard containing all 24 compounds of interest and 1,2-DNB (surrogate) prepared in 50/50 MeOH/water (Figure 7). While retention time shifts for NQ and NTO from 3.6 min to 3.5 min and 4.3 min to 3.9 min, respectively, as % ACN increased from 5% to 8%, peak shape, resolution, and intensity were not affected by changes in % ACN (Figure 7a). However, ACN concentration was shown to impact the resolution of middle- and late-eluting compounds, including most of the legacy munition compounds (present in the current USEPA Method 8330B), resulting in an overall decrease of 2-3 min method run time, and one or more co-elutions at >6% ACN (Figure 7b). Because there were no co-elutions at either 5% or 6% ACN and because some compounds were better resolved either at 5% (1,3-DNB/2-NP) or at 6% ACN (Tetryl/NG), the use of either 5% or 6% isocratic ACN would be appropriate.

Cursory experiments were carried out to investigate whether the gradient method could be shortened by ramping up the % ACN during the final 20 min of the method (Figure 8). For this test, 6% ACN was used from 0-28 min, then was increased from 6% to 20% from 28-44 min. Total run time was shortened by 5 min; however, the introduction of an ACN gradient would only be recommended in cases where the additional complication to the method resulted in significant benefit to the user.

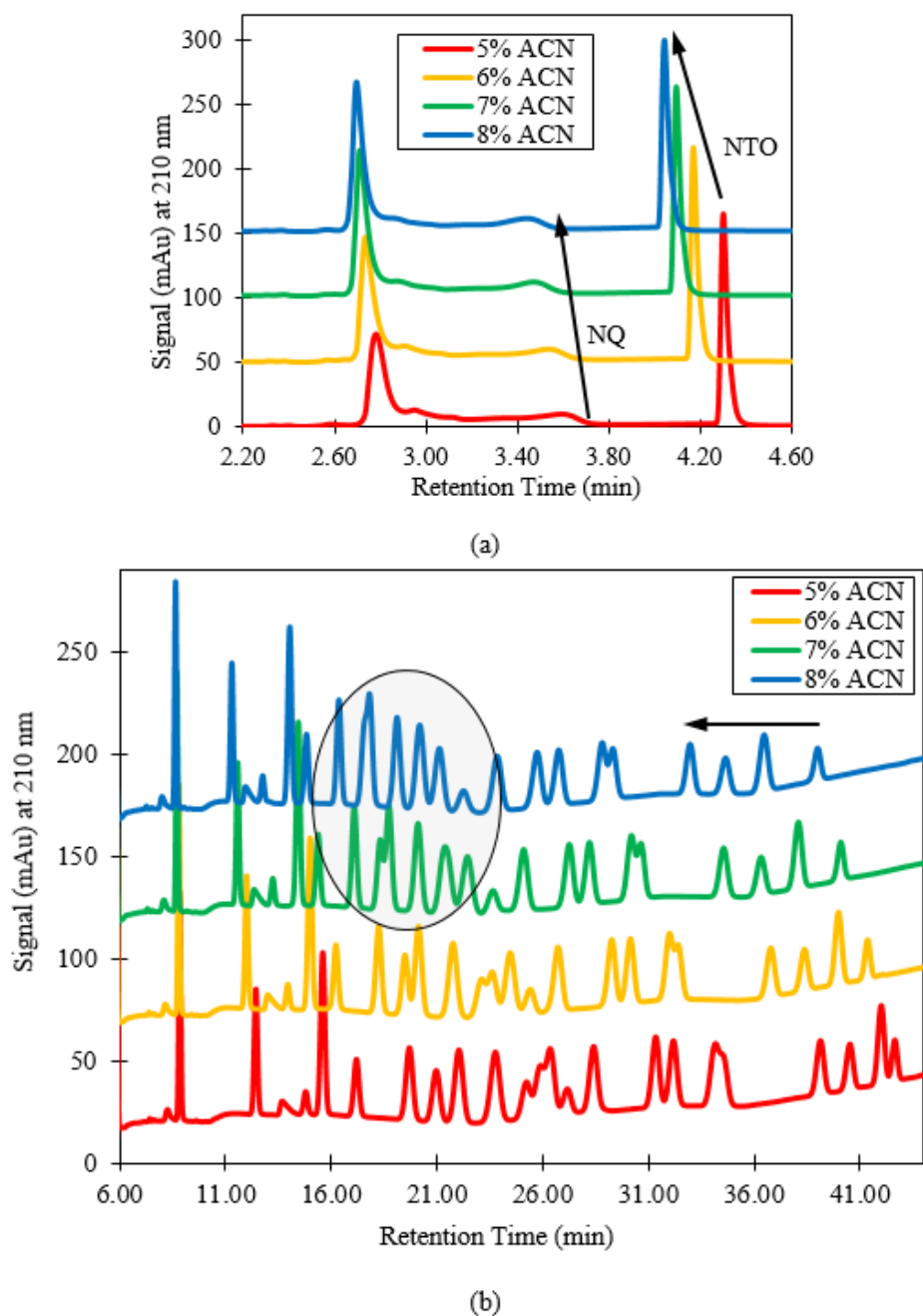


Figure 7. Effect of varying the solvent gradient isocratic % ACN from 5% to 8%, while setting the initial % MeOH at 3% and holding % 0.1% TFA/water constant at 3%, using 5 mg/L mixed standards containing all 24 compounds of interest and 1,2-DNB surrogate were prepared in 50/50 MeOH/water. (a) Effect on NQ and NTO, (b) Effect on middle- and late-eluting compounds.

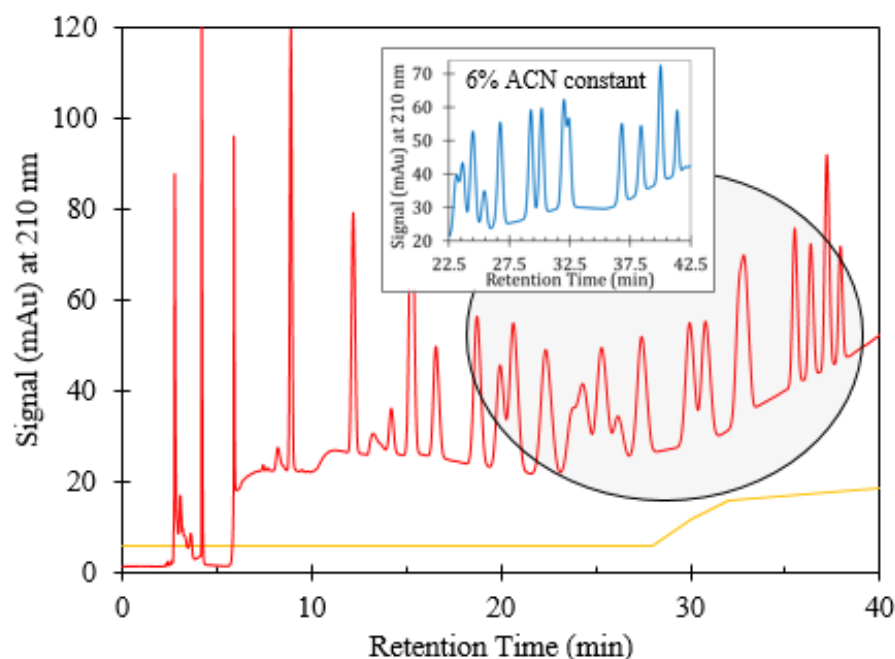


Figure 8. Effect of introducing an ACN gradient from 6-20% over the period between 28-44 min. Red: HPLC-UV chromatogram resulting from ACN gradient method; Gold: ACN composition (%); Blue: HPLC-UV chromatogram resulting from 6% isocratic ACN (without ACN gradient).

Further experiments showed that NQ underwent substantial changes in retention time (from 3.3 min to 3.9 min) and peak shape, including splitting and broadening, resulting in several small peaks that appeared near (and possibly included within) the void peak (2.7 min) and one main peak which shifted closer to the NTO peak as % MeOH composition increased (Figure 9a). Overall, a 50/50 MeOH/water sample solvent composition was determined to be optimal. Neither the NTO nor any of the later eluting analytes were negatively affected by a 50% MeOH composition, and adequate baseline resolution enabled analysis using the main NQ peak (3.65 min), which yielded a linear calibration curve from 0.020 mg/L to 20 mg/L ( $R^2 \geq 0.97$ ) with MDL of 16  $\mu\text{g/L}$  (Table 5). For the majority of included compounds, no peak broadening or shifting was observed up to 75% MeOH, with the first signs of negative chromatographic effects appearing at 90% MeOH between 8-28 min (Figure 9b). Overall, NQ posed the greatest analytical challenges, due to its highly polar character. The solvent gradient and sample solvent composition conditions developed for the primary HPLC-UV method served to minimize negative effects on NQ retention time and peak shape, but did not eliminate them, so that a confirmation method (such as the secondary HPLC-UV or -MS method described) should be used to confirm NQ determinations.

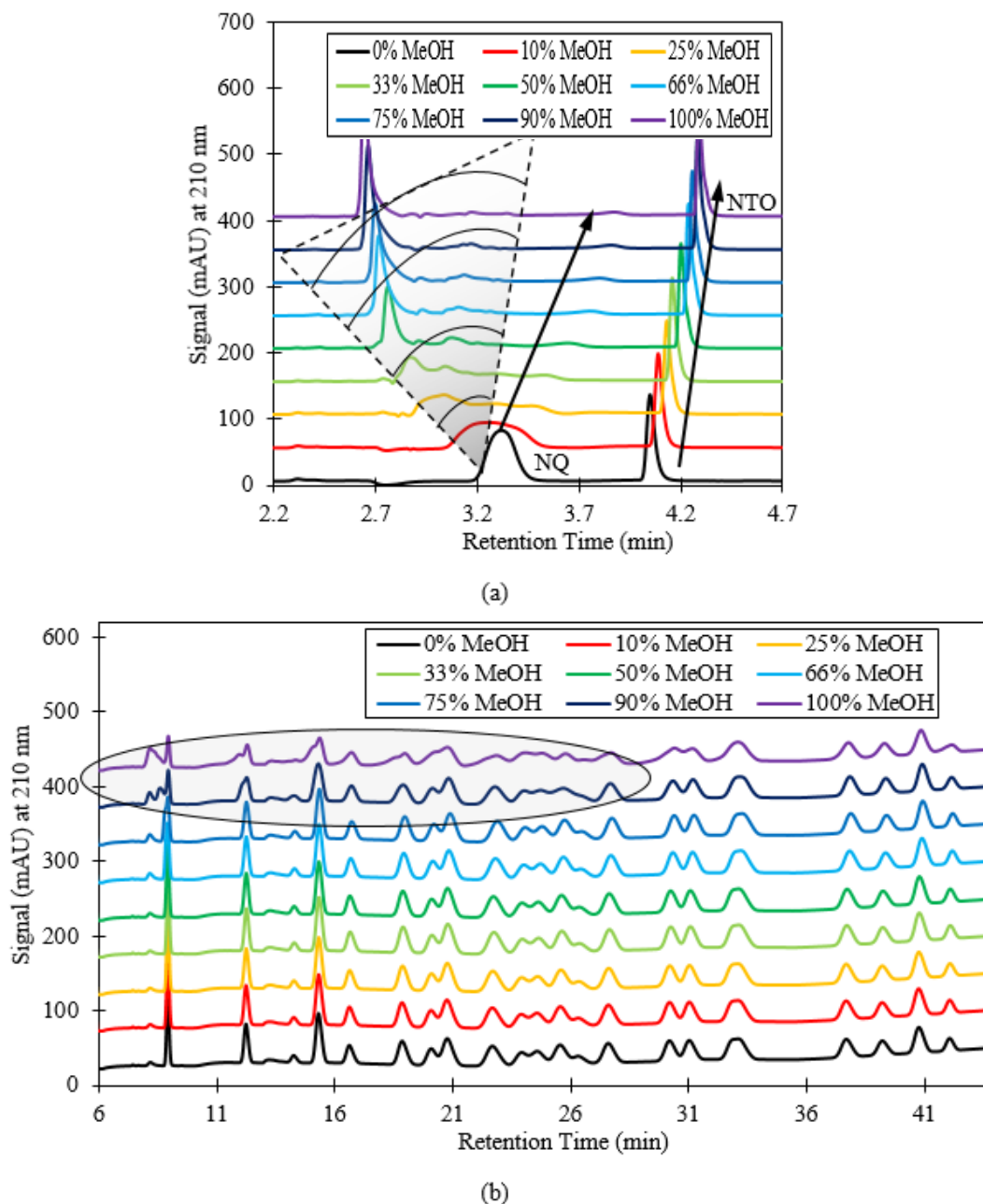


Figure 9. Effect of varying the sample solvent composition % MeOH from 0% to 100%, using the developed primary HPLC-UV method to analyze 5 mg/L mixed standards containing all 24 compounds of interest and 1,2-DNB surrogate prepared with indicated MeOH/water solvent ratios. (a) Effect on NQ and NTO, (b) Effect on middle- and late-eluting compounds.

### 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. The solvent gradient scheme and other method parameters are shown in Table 2, and an example chromatogram is shown in Figure 2 (with peak assignments determined using individual reference standards), shown above.

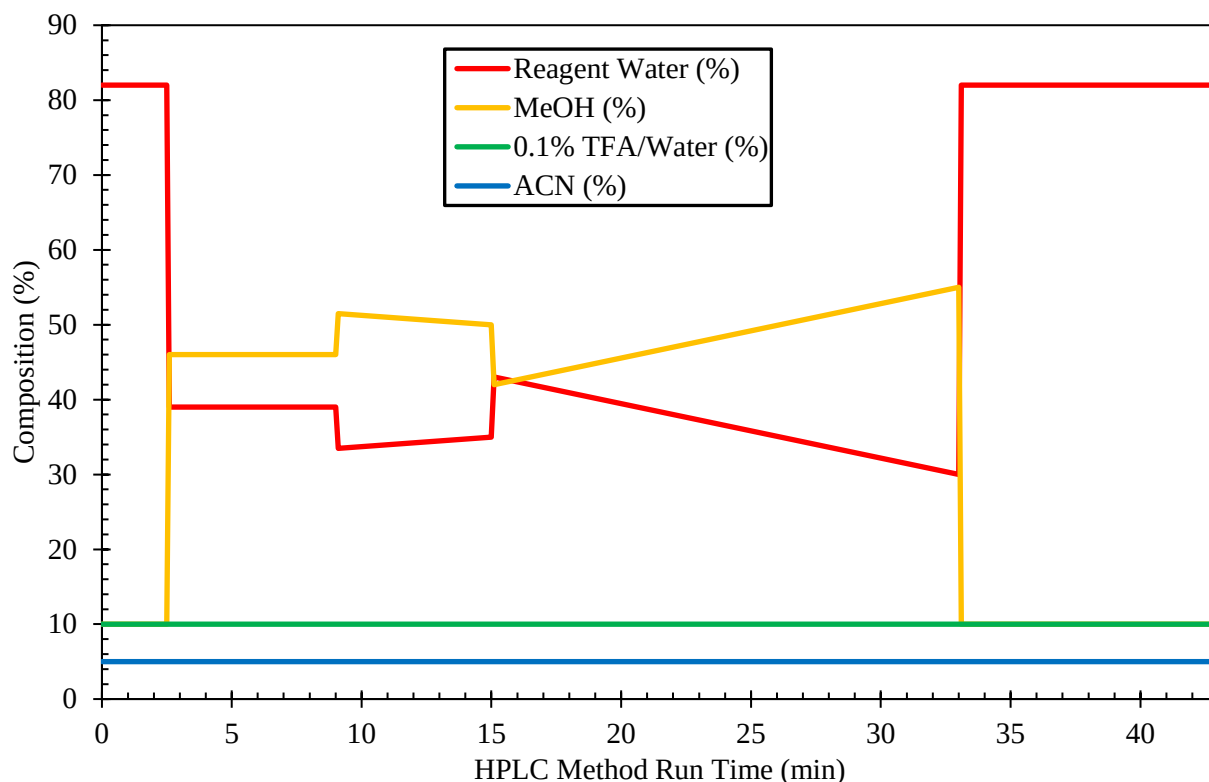


Figure 10. Secondary HPLC-UV method solvent gradient scheme developed for simultaneous confirmatory analysis of legacy and IM compounds.

A graphical representation of the solvent gradient scheme for the secondary HPLC-UV method is shown in Figure 10. The initial portion of the gradient (0-2.5 min) was designed to elute the highly polar NQ and NTO. The next stage consists of a brief isocratic period (2.5-9 min), followed by a spike and then a gradual decrease of % MeOH (9-15 min), was designed to elute and resolve the more hydrophobic compounds. The third stage of the gradient (15-29 min), with an increase to 60% MeOH composition, was designed to elute the most hydrophobic compounds, decrease their retention times, and reduce overall method run time. A brief period at the end of the method returned the system to initial conditions to re-equilibrate before the next sample injection.

Three difficulties were addressed during the development of this method. Initially, 1) PA eluted within a void peak (6.5 min) resulting from a sharp shift in aqueous-to-organic ratio at 4.1 min (rather than the final 2.6 min), visible at 210 nm; 2) HMX, 4-NP, and RDX co-eluted with each other; and, 3) NQ was poorly resolved at 210 nm. To mitigate these issues, the isocratic % 0.1% TFA/water and % ACN were optimized, and small adjustments to solvent gradient timing and % MeOH shifts were tested. By making slight changes in the % MeOH gradient, the HMX, 4-NP, and RDX became well resolved. While NQ and PA became better resolved through solvent gradient method optimization, the issues with their chromatography were not eliminated. Therefore, an alternative option for improved chromatography could be to use alternate detection wavelengths, such as 254 nm for NQ and 315 nm for PA, rather than using 210 nm, where void peaks did not appear, as these wavelengths were distant enough from the MeOH UV cut-off (205 nm) and should result in acceptable detection limits.

## HPLC-MS Method

An HPLC-MS scan mode method was developed to enable confirmatory analysis of the 24 legacy and IM compounds of interest and two surrogates. The solvent gradient scheme and other HPLC method parameters were unchanged from the primary HPLC-UV method described above. Efforts were made to transition the LC-MS scan method to a selective-ion monitoring (SIM) method in order to improve detection limits by selectively detecting either one or two of the ion masses observed for each compound in scan mode experiments. The experimentally-acquired ion masses obtained using the developed scan mode method, described above (Table 4 and Figure 3), were used to create ion/time windows for a SIM method. Several iterations with varying numbers of windows and times were attempted, with the most successful shown in Table 6 and Figure 11. The only other parameter that was changed from the scan mode method for the chromatogram shown in Figure 11 was the gain (2.0), which was increased to boost the signal. The peak width setting of 0.06 min resulted in total dwell times of between 330-345 msec per SIM Group, which was equally divided among the SIM ions in each group, as a fixed function of the system's software. Retention times were approximately the same as those for the scan mode method (Table 4). The compounds in SIM Group 1 (NQ, NTO, *o*-NBA, and HMX) were not observed. Most compounds in Groups 2 and 4 were detected well, except for PA, 2,4-DNP, NB, and NG, which were not observed. Meanwhile, all the compounds in Group 3 were observed. For Groups 5 and 6, just one compound each was observed, 4-Am-2,6-DNT and PETN, respectively. Due to the issues encountered during SIM mode method development, confirmatory analysis should be performed using the scan mode method.

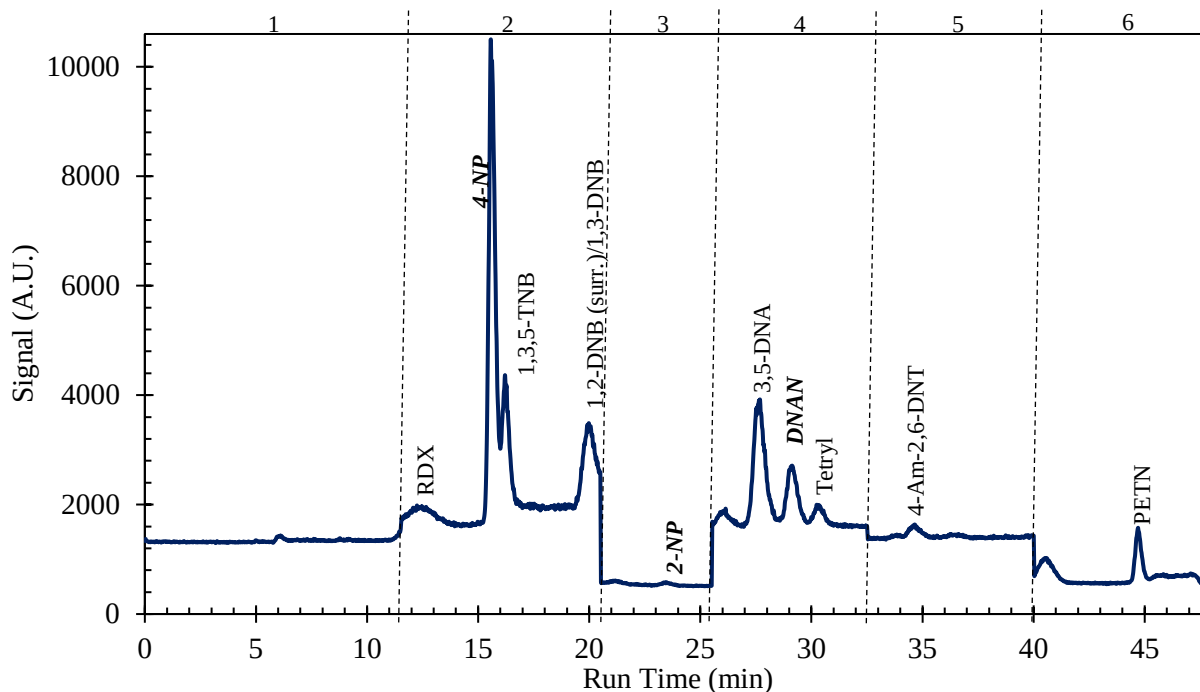


Figure 11. HPLC-MS TIC acquired by SIM mode method. SIM window start times are indicated by dotted lines, and SIM group numbers are noted above the plot area. ***Bold, italicized*** are IM compounds and degradation products.

Table 6. SIM windows developed from UV peak retention times (min) and ion masses (m/z) observed using the developed scan mode method.

| SIM Window Start Time (min) | SIM Window Group # | Compound                        | SIM Ion (m/z) | Dwell Time (ms) |
|-----------------------------|--------------------|---------------------------------|---------------|-----------------|
| 0.00                        | 1                  | <i>NQ (1)</i>                   | 203.6         | 42              |
|                             |                    | <i>NQ (2)</i>                   | 103.0         | 42              |
|                             |                    | <i>NTO (1)</i>                  | 129.0         | 42              |
|                             |                    | <i>NTO (2)</i>                  | 55.0          | 42              |
|                             |                    | o-NBA (1) (surrogate)           | 122.0         | 42              |
|                             |                    | o-NBA (2) (surrogate)           | 167.0         | 42              |
|                             |                    | HMX (1)                         | 341.0         | 42              |
|                             |                    | HMX (2)                         | 147.0         | 42              |
| 11.50                       | 2                  | RDX (1)                         | 267.0         | 37              |
|                             |                    | RDX (2)                         | 335.0         | 37              |
|                             |                    | <i>4-NP (1)</i>                 | 138.0         | 37              |
|                             |                    | <i>4-NP (2)</i>                 | 108.0         | 37              |
|                             |                    | 1,3,5-TNB (1)                   | 213.0         | 37              |
|                             |                    | 1,3,5 - TNB (2)/2,4-DNP (1)     | 183.0         | 37              |
|                             |                    | <i>2,4-DNP (2)</i>              | 153.0         | 37              |
|                             |                    | <i>PA (1)</i>                   | 228.0         | 37              |
|                             |                    | <i>PA (2)</i>                   | 211.9         | 37              |
| 20.50                       | 3                  | 1,2-DNB (1) (surr.)/2-NP (1)    | 138.0         | 115             |
|                             |                    | 1,3-DNB (1)/1,2-DNB (2) (surr.) | 168.0         | 115             |
|                             |                    | <i>2-NP (2)</i>                 | 122.1         | 115             |
| 25.50                       | 4                  | NB (1)/3,5-DNA (1)              | 182.0         | 37              |
|                             |                    | NB (2)                          | 153.0         | 37              |
|                             |                    | 3,5-DNA (2)                     | 138.0         | 37              |
|                             |                    | <i>DNAN (1)</i>                 | 183.0         | 37              |
|                             |                    | <i>DNAN (2)</i>                 | 152.9         | 37              |
|                             |                    | Tetryl (1)                      | 241.0         | 37              |
|                             |                    | Tetryl (2)                      | 288.0         | 37              |
|                             |                    | 2,4,6-TNT (1)/NG (not obsv.)    | 226.0         | 37              |
|                             |                    | 2,4,6-TNT (2)                   | 210.0         | 37              |
| 32.50                       | 5                  | 4-Am-2,6-DNT (1)                | 196.9         | 42              |
|                             |                    | 4-Am-2,6-DNT (2)                | 167.0         | 42              |
|                             |                    | 2-Am-4,6-DNT (1)                | 196.0         | 42              |
|                             |                    | 2-Am-4,6-DNT (2)                | 179.9         | 42              |
|                             |                    | 2,6-DNT (1)                     | 152.0         | 42              |
|                             |                    | 2,6-DNT (2)                     | 122.1         | 42              |
|                             |                    | 2,4-DNT (1)                     | 165.0         | 42              |
|                             |                    | 2,4-DNT (2)                     | 181.0         | 42              |
| 40.00                       | 6                  | 2-NT/3-NT/4-NT (not observed)   | 136.1         | 115             |
|                             |                    | PETN (1)                        | 62.0          | 115             |
|                             |                    | PETN (2)                        | 270.9         | 115             |

\*SIM window start time = LC-MS Method run time (no MS delay)

***Bold, italicized*** are IM compounds and degradation products.

## Method for Preparation of Waters for Direct-Injection Analysis

During the direct-injection water portion of the inter-laboratory study (discussed below), much lower recoveries for NTO, and in some cases *o*-NBA surrogate, were observed for field water samples than for reagent water. As this trend appeared to be pH-related, with NTO recovery being lower for higher-pH water samples (Table 7), further experiments were carried out in which the pH of samples to be analyzed by direct injection was adjusted. A solution of 2% HCl/MeOH (the same used for stage two of SPE elution) was used, for final acid concentrations equal (0.5%, v/v) or double (1%, v/v) that of corresponding SPE extracted/pre-concentrated samples. Recovery results for the acidified direct-injection water samples are shown in Table 8. For each field water source, acidification with HCl resulted in near-100% recovery for NTO and *o*-NBA. Moreover, an acid concentration of 0.5% was sufficient, and 1% acid was not necessary. Therefore, for water samples with pH  $\geq 6.5$  (approximately) to be analyzed by direct injection, acidification is recommended. Furthermore, for higher-pH samples extracted and pre-concentrated by SPE that are known or suspected to contain NTO, additional acidification may also be necessary (as for the pH 9.2 tap water used in the current study).

Table 7. Characteristic properties of five water sources used in inter-laboratory batch studies.

| Water Source                      | pH   | conductivity<br>( $\mu\text{S}/\text{cm}$ ) | Total Organic Carbon<br>(TOC) (mg/L) |         | Total Dissolved Solids<br>(TDS) (mg/L) |         |
|-----------------------------------|------|---------------------------------------------|--------------------------------------|---------|----------------------------------------|---------|
|                                   |      |                                             | Average (n=3)                        | Std Dev | Average (n=3)                          | Std Dev |
| Reagent (ERDC-EL)                 | 6.30 | 3.53                                        | 0.00                                 | 0.00    | -11                                    | 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<br>(Vicksburg, MS)    | 8.22 | 254.2                                       | 5.62                                 | 0.03    | 198                                    | 2.0     |
| Sea (Houston Shipping<br>Channel) | 7.84 | 13,350                                      | 5.14                                 | 0.05    | 8910                                   | 7.2     |

Table 8. Recoveries (%) for five water samples acidified using 2% HCl/MeOH and analyzed by direct injection without undergoing SPE.

| Compound            | Recoveries (%)     |         |               |         |                   |         |                                |         |               |         |                  |         |               |         |                   |         |                                |         |               |         |
|---------------------|--------------------|---------|---------------|---------|-------------------|---------|--------------------------------|---------|---------------|---------|------------------|---------|---------------|---------|-------------------|---------|--------------------------------|---------|---------------|---------|
|                     | 0.5% HCl (vol/vol) |         |               |         |                   |         |                                |         |               |         | 1% HCl (vol/vol) |         |               |         |                   |         |                                |         |               |         |
|                     | Reagent Water      |         | Tap Water     |         | Yazoo River Water |         | Houston Shipping Channel Water |         | Well Water    |         | Reagent Water    |         | Tap Water     |         | Yazoo River Water |         | Houston Shipping Channel Water |         | Well Water    |         |
|                     | Average (n=2)      | Std Dev | Average (n=3) | Std Dev | Average (n=3)     | Std Dev | Average (n=3)                  | Std Dev | Average (n=3) | Std Dev | Average (n=3)    | Std Dev | Average (n=3) | Std Dev | Average (n=3)     | Std Dev | Average (n=3)                  | Std Dev | Average (n=3) | Std Dev |
| NQ                  | 99                 | 5.4     | 110           | 2.2     | 101               | 2.2     | 107                            | 7.4     | 101           | 6.4     | 109              | 6.1     | 108           | 12.2    | 120               | 14.4    | 112                            | 17.6    | 106           | 19.0    |
| NTO (210nm)         | 111                | 10.4    | 114           | 3.9     | 117               | 7.0     | 119                            | 2.4     | 111           | 8.0     | 118              | 9.9     | 108           | 6.5     | 132               | 15.6    | 125                            | 21.4    | 112           | 11.0    |
| NTO (315nm)         | 110                | 14.7    | 112           | 6.2     | 120               | 3.9     | 121                            | 3.8     | 114           | 4.6     | 121              | 10.2    | 113           | 6.2     | 131               | 15.6    | 121                            | 20.4    | 117           | 11.2    |
| o-NBA (surrogate)   | 94                 | 9.0     | 98            | 5.8     | 98                | 5.8     | 99                             | 1.9     | 96            | 7.1     | 104              | 4.1     | 104           | 8.5     | 112               | 11.9    | 102                            | 17.2    | 102           | 11.6    |
| HMX                 | 94                 | 9.1     | 97            | 5.4     | 95                | 5.9     | 98                             | 4.2     | 94            | 0.9     | 101              | 10.1    | 98            | 8.1     | 106               | 16.0    | 97                             | 17.3    | 94            | 10.2    |
| RDX                 | 96                 | 12.3    | 100           | 4.0     | 95                | 6.7     | 100                            | 1.1     | 96            | 6.2     | 101              | 6.7     | 102           | 10.4    | 109               | 17.7    | 102                            | 17.5    | 99            | 9.6     |
| PA (210nm)          | 83                 | 5.4     | 93            | 4.5     | 87                | 6.3     | 87                             | 8.6     | 89            | 6.3     | 95               | 10.8    | 98            | 7.2     | 104               | 23.7    | 105                            | 23.6    | 97            | 8.7     |
| PA (315nm)          | 91                 | 8.0     | 96            | 5.2     | 93                | 7.0     | 93                             | 5.1     | 90            | 7.8     | 97               | 6.0     | 97            | 8.6     | 106               | 14.0    | 98                             | 18.8    | 95            | 10.0    |
| 4-NP                | 95                 | 12.0    | 98            | 5.1     | 99                | 6.1     | 99                             | 4.8     | 94            | 6.3     | 99               | 6.7     | 95            | 8.9     | 108               | 14.1    | 100                            | 17.2    | 96            | 10.5    |
| 1,3,5-TNB           | 92                 | 10.9    | 98            | 3.0     | 96                | 4.8     | 96                             | 3.3     | 92            | 4.5     | 97               | 6.1     | 98            | 5.3     | 105               | 15.4    | 98                             | 17.9    | 94            | 8.9     |
| 2,4-DNP             | 86                 | 9.6     | 93            | 7.7     | 92                | 5.9     | 94                             | 7.3     | 87            | 3.8     | 100              | 3.2     | 92            | 11.2    | 107               | 13.6    | 94                             | 18.2    | 92            | 10.6    |
| 1,2-DNB (surrogate) | 91                 | 11.0    | 98            | 6.5     | 92                | 5.0     | 96                             | 3.6     | 93            | 2.9     | 97               | 9.0     | 98            | 10.1    | 110               | 13.8    | 100                            | 18.6    | 94            | 14.0    |
| 1,3-DNB             | 92                 | 11.2    | 100           | 7.4     | 95                | 4.5     | 96                             | 3.4     | 91            | 4.6     | 103              | 12.1    | 99            | 7.2     | 107               | 13.5    | 99                             | 17.5    | 96            | 10.8    |
| 2-NP                | 88                 | 13.5    | 92            | 6.8     | 92                | 5.9     | 92                             | 4.1     | 88            | 2.4     | 90               | 4.5     | 84            | 9.6     | 99                | 17.7    | 95                             | 13.1    | 91            | 9.7     |
| NB                  | 88                 | 107.0   | 85            | 6.8     | 90                | 7.4     | 92                             | 2.0     | 88            | 5.1     | 94               | 5.2     | 82            | 6.0     | 103               | 14.7    | 96                             | 19.4    | 93            | 9.9     |
| 3,5-DNA (210nm)     | 106                | 12.4    | 109           | 6.6     | 101               | 4.9     | 106                            | 3.1     | 98            | 3.7     | 102              | 1.1     | 103           | 11.4    | 115               | 17.1    | 106                            | 18.1    | 102           | 10.8    |
| 3,5-DNA (254nm)     | 107                | 11.8    | 111           | 8.5     | 106               | 5.7     | 109                            | 1.9     | 104           | 2.1     | 108              | 4.6     | 107           | 9.1     | 119               | 18.0    | 109                            | 17.5    | 105           | 10.1    |
| DNAN                | 87                 | 12.3    | 92            | 5.0     | 86                | 3.9     | 88                             | 2.1     | 83            | 3.8     | 89               | 3.6     | 89            | 6.8     | 96                | 16.6    | 95                             | 15.4    | 88            | 11.2    |
| Tetryl              | 94                 | 7.5     | 99            | 3.5     | 96                | 5.1     | 97                             | 5.6     | 95            | 7.1     | 98               | 4.4     | 104           | 15.7    | 111               | 13.9    | 101                            | 18.5    | 97            | 9.4     |
| NG                  | 106                | 5.0     | 103           | 8.3     | 112               | 2.8     | 99                             | 8.3     | 109           | 4.6     | 112              | 11.5    | 113           | 18.9    | 127               | 9.7     | 118                            | 15.4    | 108           | 9.6     |
| 2,4,6-TNT           | 92                 | 6.7     | 99            | 6.8     | 94                | 8.0     | 93                             | 3.9     | 94            | 0.4     | 95               | 6.0     | 100           | 15.5    | 110               | 10.5    | 100                            | 20.3    | 93            | 10.1    |
| 4-Am-2,6-DNT        | 93                 | 11.0    | 80            | 4.4     | 95                | 7.0     | 98                             | 6.4     | 94            | 7.6     | 96               | 8.3     | 76            | 9.2     | 108               | 14.5    | 100                            | 18.6    | 95            | 11.8    |
| 2-Am-4,6-DNT        | 93                 | 11.4    | 83            | 2.3     | 95                | 5.6     | 102                            | 4.6     | 99            | 1.8     | 99               | 8.7     | 85            | 9.5     | 108               | 16.1    | 101                            | 20.0    | 94            | 11.1    |
| 2,6-DNT             | 92                 | 8.4     | 93            | 5.8     | 95                | 2.1     | 102                            | 7.1     | 89            | 4.4     | 95               | 5.1     | 96            | 7.0     | 109               | 17.0    | 102                            | 21.2    | 95            | 10.2    |
| 2,4-DNT             | 88                 | 9.5     | 97            | 4.3     | 96                | 6.6     | 98                             | 6.3     | 94            | 4.8     | 103              | 6.5     | 98            | 8.8     | 107               | 13.1    | 99                             | 19.2    | 96            | 12.0    |
| 2-NT                | 98                 | 8.9     | 104           | 1.2     | 98                | 6.3     | 101                            | 2.4     | 95            | 2.2     | 101              | 4.5     | 98            | 6.5     | 103               | 27.0    | 100                            | 16.2    | 95            | 11.4    |
| 4-NT                | 91                 | 8.2     | 97            | 4.6     | 91                | 5.8     | 95                             | 5.7     | 92            | 6.4     | 96               | 7.3     | 91            | 8.7     | 114               | 12.7    | 95                             | 14.1    | 94            | 10.6    |
| 3-NT                | 97                 | 13.8    | 101           | 3.3     | 95                | 6.1     | 99                             | 6.3     | 92            | 4.3     | 96               | 1.1     | 98            | 4.7     | 107               | 15.8    | 97                             | 14.8    | 95            | 9.5     |
| PETN                | 99                 | 13.3    | 106           | 7.7     | 101               | 6.6     | 98                             | 7.5     | 94            | 7.2     | 97               | 12.7    | 110           | 4.8     | 110               | 11.8    | 100                            | 19.0    | 94            | 9.1     |

***Bold, italicized*** are IM compounds and degradation products.

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

(DoD QSM Ver5.2 for Waters – Lowest LCL: MNX, 57%; Highest UCL: HMX, 135%)

## SPE Method for Waters

Current USEPA Method 8330B and ERDC-EL SPE methods were modified to enable extraction and pre-concentration of all 24 compounds of interest (and two surrogates) from environmental water samples. A series of cation-exchange, anion-exchange, reverse phase, and GAC-based SPE cartridges were evaluated using slightly modified SPE methods for environmental water extraction and pre-concentration. The ability of each cartridge, individually or sequentially, to recover the 24 compounds of interest and two surrogates from laboratory-spiked reagent water samples was compared.

The results in Table 9 show the varying abilities of seven individual SPE cartridges to extract and pre-concentrate 25 compounds (prior to addition of the second surrogate, *o*-NBA). The individual cartridges compared in this experiment were (from left to right in the table): 1) Strata X (polymeric reverse phase, 500 mg/6 mL, Phenomenex, PRP500), 2) ENVI-18 (traditional C-18, 1 g/6 mL, Supelclean, C181G), 3) Strata X-A (polymeric strong anion exchange, 200 mg/6 mL, Phenomenex, PSA200), 4) Strata X-A (polymeric strong anion exchange, 500 mg/6 mL, Phenomenex, PSA500), 5) ENVI-Carb (GAC, 250 mg/3 mL, Supelclean, GAC250), 6) Strata ABW (mixed mode strong cation exchange (4-ethylbenzene sulfonate) and weak anion exchange (propylamine), 200 mg/3 mL, Phenomenex, ABW200), and 7) a new Phenomenex GAC cartridge undergoing product testing (500 mg/6 mL, ACP500).

Overall, the reverse phase (PRP500 and C181G) and anion exchange (PSA200 and PSA500) tested were able to extract most compounds within DoD QSM Ver5.2 limits. The GAC250 was also able to extract many compounds, but fewer than the reverse phase and anion exchange. In some cases, particularly for the acidic compounds, when recoveries were low, mass balance analysis indicated that the compounds were effectively adsorbed onto cartridges but failed to elute. On the other hand, NQ tended to be the only compound that was poorly adsorbed onto any individual cartridge. Of the seven individual cartridges, ABW200 performed best for NQ with 61% recovery; however, this result proved to be irreproducible.

The ABW200 and ACP500 cartridges did not recover many analytes within accepted ranges, and were not included in further studies. Aside from ABW200, GAC250 performed best for NQ with 30% recovery. Meanwhile, PSA500 was twice as effective as PSA200 for NQ recovery (11% vs 6%), and PRP500 was better than C181G for NQ (10% vs 5%), NTO (3% vs 1%), and PA (102% vs 11%) recovery. Furthermore, both of the PSA cartridges were able to recover all of the remaining compounds within DoD QSM Ver5.2 limits (apart from the anomalously high NG recoveries for this experiment). The PRP500 and C181G failed only to recover NTO or NTO and PA, respectively, indicating that inclusion of an anion exchange cartridge would be important for extraction of the highly acidic compounds.

Based on results with individual cartridges, several double- and triple-stacked options were tested, in order to identify a sequential option that yielded acceptable recoveries for all compounds. The stacking order for sample loading and elution was reversed to prevent irreversible binding of analytes to incompatible SPE cartridge matrices, which was observed during evaluation of individual cartridges. In addition, two sizes of ENVI-Carb cartridges were tested, the 250 mg/3 mL (GAC250) and a larger 500 mg/6 mL (GAC500). Results for six stacked options are shown in Table 10, where loading-stage stacking order from top to bottom is indicated, including A) PRP500-PSA500-GAC250, B) PRP500-PSA500-GAC500, C) C181G-PSA500-GAC250, D) C181G-PSA500-GAC500, E) PSA500-C181G-GAC250, and F) PSA500-GAC250.

NQ recovery for each option was expected to be  $\geq 50\%$ , based on the recoveries for individual cartridges noted in Table 9 and the assumption of an additive relationship. While NQ recoveries for this particular experiment were not as high as expected, options B and C from Table 10 were selected for further tests. Although option F (PSA500-GAC250) also yielded higher recovery of NQ (38%), triple-stacked options which included a reverse phase cartridge were preferred for further studies as an added mechanism to ensure prevention of analyte break-through in more concentrated, larger volume, or more complex matrix

Table 9. Compound recoveries from laboratory-spiked reagent water samples using individual SPE cartridges for extraction/pre-concentration of the 24 compounds of interest and 1,2-DNB surrogate.

| Compound                  | Recoveries (%) |            |            |            |            |           |            |
|---------------------------|----------------|------------|------------|------------|------------|-----------|------------|
|                           | PRP500         | C181G      | PSA200     | PSA500     | GAC250     | ABW200    | ACP500     |
| <b><i>NQ</i></b>          | <b>10</b>      | <b>5</b>   | <b>6</b>   | <b>11</b>  | <b>30</b>  | <b>61</b> | <b>2</b>   |
| <b><i>NTO (210nm)</i></b> | <b>3</b>       | <b>1</b>   | <b>120</b> | <b>118</b> | <b>123</b> | <b>2</b>  | <b>235</b> |
| HMX                       | 113            | 104        | 118        | 118        | 118        | 88        | 27         |
| RDX                       | 112            | 115        | 113        | 113        | 116        | 102       | 1          |
| <b><i>PA (210nm)</i></b>  | <b>102</b>     | <b>11</b>  | <b>119</b> | <b>113</b> | <b>1</b>   | <b>ND</b> | <b>102</b> |
| <b><i>4-NP</i></b>        | <b>108</b>     | <b>112</b> | <b>112</b> | <b>110</b> | <b>121</b> | <b>16</b> | <b>65</b>  |
| 1,3,5-TNB                 | 114            | 114        | 112        | 112        | 88         | 3         | 15         |
| <b><i>2,4-DNP</i></b>     | <b>108</b>     | <b>106</b> | <b>126</b> | <b>117</b> | <b>4</b>   | <b>ND</b> | <b>102</b> |
| 1,2-DNB (surrogate)       | 100            | 102        | 100        | 101        | 100        | 99        | 4          |
| 1,3-DNB                   | 111            | 114        | 112        | 112        | 112        | 21        | 52         |
| <b><i>2-NP</i></b>        | <b>95</b>      | <b>97</b>  | <b>95</b>  | <b>96</b>  | <b>95</b>  | <b>56</b> | <b>49</b>  |
| NB                        | 112            | 114        | 112        | 113        | 19         | 9         | ND         |
| 3,5-DNA (254nm)           | 111            | 114        | 111        | 114        | 113        | 110       | ND         |
| <b><i>DNAN</i></b>        | <b>106</b>     | <b>108</b> | <b>108</b> | <b>107</b> | <b>108</b> | <b>1</b>  | <b>10</b>  |
| Tetryl                    | 112            | 115        | 81         | 81         | 105        | 86        | 6          |
| NG                        | 114            | 115        | 148        | 175        | 105        | 111       | ND         |
| 2,4,6-TNT                 | 110            | 113        | 104        | 105        | 79         | 8         | 28         |
| 4-Am-2,6-DNT              | 117            | 114        | 112        | 112        | 118        | 53        | 52         |
| 2-Am-4,6-DNT              | 115            | 115        | 112        | 113        | 6          | 13        | ND         |
| 2,6-DNT                   | 113            | 118        | 115        | 108        | 115        | 100       | 24         |
| 2,4-DNT                   | 105            | 98         | 100        | 119        | 113        | 2         | 21         |
| 2-NT                      | 109            | 111        | 108        | 109        | 109        | 85        | 3          |
| 4-NT                      | 109            | 113        | 110        | 109        | 109        | 59        | 31         |
| 3-NT                      | 109            | 112        | 110        | 110        | 109        | 75        | 14         |
| PETN                      | 126            | 110        | 109        | 111        | 110        | 102       | 2          |

***Bold, italicized*** are IM compounds and degradation products. ND: not detected.

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

(DoD QSM Ver5.2 for Waters – Lowest LCL: MNX, 57%; Highest UCL: HMX, 135%)

Table 10. Compound recoveries (%) from laboratory-spiked reagent water samples using stacked SPE cartridges for extraction/pre-concentration of the 24 compounds of interest and 1,2-DNB surrogate.

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

***Bold, italicized*** are IM compounds and degradation products.

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

(DoD QSM Ver5.2 for Waters – Lowest LCL: MNX, 57%; Highest UCL: HMX, 135%)

samples; however, this option (or PSA500-GAC500) may be acceptable in some cases to reduce method complexity. Furthermore, in cases where NQ is known or likely to be absent or not of interest, it may be acceptable to use a single PSA500 cartridge (Table 9).

Studies of the 24 compounds' and two surrogates' pre- and post-extraction stability in laboratory-spiked reagent water samples were carried out using both option B and C from Table 10. Based on the slightly better results for option B in the stability studies (discussed below), the PRP500-PSA500-GAC500 option was selected as the best multi-cartridge option for recovery of all compounds, with NQ recovery consistently approximately 50%. There was also a laboratory process-based preference for GAC500 vs. GAC250 in stacked options, as the reduced surface area and volume capacity of the GAC250 (3 mL) resulted in an increased time requirement for sample loading and complicated SPE conditioning and extract elution procedures. Therefore, option B was chosen for use in cross-laboratory batch tests of five different laboratory-spiked water samples (Table 28 in Inter-Laboratory Batch Analysis below).

## **SE Method for Soils**

Current USEPA Method 8330B and ERDC-EL SE methods were modified to enable extraction of all 24 compounds of interest (and two surrogates) from environmental soil samples. Preliminary SE trials indicated that traditional USEPA Method 8330B solid matrix extraction methods (i.e., extraction using ACN for a single 18-h ultrasonication period) would be insufficient to extract all 24 compounds of interest, supported by previous studies (Walsh, et al., 2016; Felt, et al., 2016). Therefore, several different solvents, ultrasonication times, and multi-stage extraction procedures were investigated.

Early trials compared single 18-h extractions of laboratory-spiked samples using either MeOH or ACN, according to USEPA Method 8330B procedures, including spiking, drying, and ultrasonication. The majority of the method development was carried out using a standard soil, ASTM fat clay (CH-1). Based on results of early trials, it became clear that using either ACN or MeOH alone would be insufficient to extract all compounds from soils at acceptable recovery rates. Table 11 compares compound recoveries for laboratory-spiked ASTM fat clay soil samples using either MeOH or ACN for either 1 x 18-h or 2 x 9-h ultrasonication periods, with no other modifications to USEPA Method 8330B procedures of drying, sonicating, and filtering. For both MeOH and ACN, splitting the ultrasonication period into two 9-h stages, rather than using a single 18-h period, improved recoveries, especially for some of the IM compounds and daughter products. For NTO, PA, 4-NP, 2,4-DNP, and DNAN, higher recoveries were observed when MeOH was the extraction solvent, while higher recoveries were observed for 2-NP and NQ with ACN. Interestingly, HMX and RDX recoveries were consistently poor in MeOH extractions, while with ACN, these two legacy compounds were recovered at near 100%. Since the majority of IM compounds and daughter products were recovered at higher rates by MeOH than ACN, and since a multi-stage ultrasonication procedure was shown to increase recovery rates overall, further experiments were modified from the 2 x 9h MeOH option, and were designed mainly to improve recoveries of NQ, NTO, HMX, and RDX.

Several 2- and 3-stage ultrasonication sequences using various combinations of extraction solvents (including MeOH, 50/50 MeOH/water, and water) were tested, with a summary of the results shown in Table 12. Options A-D were 3-stage sequences, where stage one and two were carried out during extraction "Day 1", and stage three was carried out overnight from "Day 1" into "Day 2". Options E-J were 2-stage sequences, where stage one of option E-G was carried out during extraction "Day 1", and stage two was carried out overnight from "Day 1" into "Day 2", as for option A-D. Meanwhile, for option H-J, extraction stage one was carried out on "Day 1" and stage two was carried out on "Day 2", with overnight storage at 4°C in the absence of light. Several multi-stage ultrasonication sequences resulted in recovery rates for all compounds within the DoD QSM Ver5.2 accepted range (64-135%) for soils analyzed by USEPA Method 8330B, including option A, B, F, G, I, and J. Each of these options employed a combination of

Table 11. Recoveries (%) from spiked ASTM fat clay using either MeOH or ACN to perform SE for either 1 x 18 h or 2 x 9 h ultrasonication periods

| Compound                  | Recoveries (%) |                 |             |                |
|---------------------------|----------------|-----------------|-------------|----------------|
|                           | 18 h<br>MeOH   | 2 x 9 h<br>MeOH | 18 h<br>ACN | 2 x 9 h<br>ACN |
| <b><i>NQ</i></b>          | <b>30</b>      | <b>41</b>       | <b>36</b>   | <b>50</b>      |
| <b><i>NTO (210nm)</i></b> | <b>22</b>      | <b>58</b>       | <b>15</b>   | <b>30</b>      |
| HMX                       | 15             | 37              | 84          | 103            |
| RDX                       | 15             | 35              | 78          | 95             |
| <b><i>PA (210nm)</i></b>  | <b>75</b>      | <b>92</b>       | <b>64</b>   | <b>76</b>      |
| <b><i>4-NP</i></b>        | <b>84</b>      | <b>104</b>      | <b>82</b>   | <b>93</b>      |
| 1,3,5-TNB                 | 87             | 108             | 90          | 110            |
| <b><i>2,4-DNP</i></b>     | <b>48</b>      | <b>75</b>       | <b>24</b>   | <b>21</b>      |
| 1,2-DNB (surrogate)       | 81             | 103             | 82          | 101            |
| 1,3-DNB                   | 86             | 106             | 76          | 97             |
| <b><i>2-NP</i></b>        | <b>45</b>      | <b>67</b>       | <b>64</b>   | <b>80</b>      |
| NB                        | 84             | 105             | 78          | 97             |
| 3,5-DNA (254nm)           | 93             | 113             | 87          | 104            |
| <b><i>DNAN</i></b>        | <b>84</b>      | <b>105</b>      | <b>75</b>   | <b>89</b>      |
| Tetryl                    | 82             | 102             | 68          | 98             |
| NG                        | 85             | 106             | 45          | 121            |
| 2,4,6-TNT                 | 87             | 107             | 86          | 119            |
| 4-Am-2,6-DNT              | 88             | 111             | 109         | 165            |
| 2-Am-4,6-DNT              | 88             | 114             | 120         | 172            |
| 2,6-DNT                   | 84             | 99              | 86          | 114            |
| 2,4-DNT                   | 90             | 116             | 92          | 108            |
| 2-NT                      | 92             | 112             | 79          | 114            |
| 4-NT                      | 88             | 107             | 90          | 115            |
| 3-NT                      | 88             | 110             | 89          | 128            |
| PETN                      | 90             | 111             | 87          | 118            |

***Bold, italicized*** are IM compounds and degradation products.

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

(DoD QSM Ver5.2 for Solids – Lowest LCL: 4-Am-2,6-DNT, 64%; Highest UCL: Tetryl, 135%)

Table 12. Recoveries (%) from spiked ASTM fat clay using several multi-stage SE sequences with ultrasonication for either 3-h – 3-h – 14-h, 6-h – 14-h, or 8-h – 8-h with various solvent combinations.

| Compound                  | Recoveries (%)                                 |                                                            |                                                     |                                                                 |                         |                                          |                                     |                         |                                     |                                          |
|---------------------------|------------------------------------------------|------------------------------------------------------------|-----------------------------------------------------|-----------------------------------------------------------------|-------------------------|------------------------------------------|-------------------------------------|-------------------------|-------------------------------------|------------------------------------------|
|                           | 3 h - 3 h - 14 h                               |                                                            |                                                     |                                                                 | 6 h - 14 h              |                                          |                                     | 8 h - 8 h               |                                     |                                          |
|                           | A<br>1. MeOH<br>2. MeOH<br>3. H <sub>2</sub> O | B<br>1. MeOH<br>2. H <sub>2</sub> O<br>3. H <sub>2</sub> O | C<br>1. MeOH<br>2. MeOH<br>3. MeOH/H <sub>2</sub> O | D<br>1. MeOH<br>2. MeOH/H <sub>2</sub> O<br>3. H <sub>2</sub> O | E<br>1. MeOH<br>2. MeOH | F<br>1. MeOH<br>2. MeOH/H <sub>2</sub> O | G<br>1. MeOH<br>2. H <sub>2</sub> O | H<br>1. MeOH<br>2. MeOH | I<br>1. MeOH<br>2. H <sub>2</sub> O | J<br>1. MeOH<br>2. MeOH/H <sub>2</sub> O |
| <b><i>NQ</i></b>          | 69                                             | 88                                                         | 54                                                  | 69                                                              | 53                      | 69                                       | 79                                  | 52                      | 74                                  | 66                                       |
| <b><i>NTO (210nm)</i></b> | 77                                             | 94                                                         | 91                                                  | 107                                                             | 37                      | 97                                       | 86                                  | 36                      | 81                                  | 82                                       |
| HMX                       | 65                                             | 92                                                         | 68                                                  | 92                                                              | 31                      | 72                                       | 71                                  | 32                      | 69                                  | 67                                       |
| RDX                       | 69                                             | 99                                                         | 72                                                  | 96                                                              | 32                      | 76                                       | 77                                  | 33                      | 74                                  | 71                                       |
| <b><i>PA (210nm)</i></b>  | 104                                            | 126                                                        | 107                                                 | 125                                                             | 116                     | 121                                      | 123                                 | 110                     | 114                                 | 115                                      |
| <b><i>4-NP</i></b>        | 103                                            | 119                                                        | 107                                                 | 125                                                             | 120                     | 122                                      | 124                                 | 116                     | 119                                 | 119                                      |
| 1,3,5-TNB                 | 105                                            | 110                                                        | 110                                                 | 124                                                             | 125                     | 122                                      | 115                                 | 121                     | 110                                 | 121                                      |
| <b><i>2,4-DNP</i></b>     | 92                                             | 116                                                        | 95                                                  | 118                                                             | 81                      | 110                                      | 111                                 | 79                      | 104                                 | 102                                      |
| 1,2-DNB (surrogate)       | 104                                            | 117                                                        | 106                                                 | 124                                                             | 120                     | 121                                      | 122                                 | 115                     | 117                                 | 118                                      |
| 1,3-DNB                   | 105                                            | 120                                                        | 108                                                 | 128                                                             | 124                     | 125                                      | 125                                 | 120                     | 121                                 | 123                                      |
| <b><i>2-NP</i></b>        | 77                                             | 107                                                        | 79                                                  | 109                                                             | 69                      | 103                                      | 104                                 | 69                      | 95                                  | 93                                       |
| NB                        | 104                                            | 116                                                        | 104                                                 | 124                                                             | 119                     | 118                                      | 116                                 | 117                     | 112                                 | 114                                      |
| 3,5-DNA (254nm)           | 99                                             | 113                                                        | 99                                                  | 116                                                             | 110                     | 111                                      | 112                                 | 107                     | 107                                 | 108                                      |
| <b><i>DNAN</i></b>        | 104                                            | 115                                                        | 112                                                 | 127                                                             | 128                     | 120                                      | 121                                 | 118                     | 115                                 | 120                                      |
| Tetryl                    | 102                                            | 114                                                        | 103                                                 | 123                                                             | 119                     | 117                                      | 117                                 | 115                     | 111                                 | 119                                      |
| NG                        | 94                                             | 102                                                        | 86                                                  | 105                                                             | 98                      | 99                                       | 101                                 | 98                      | 97                                  | 98                                       |
| 2,4,6-TNT                 | 99                                             | 109                                                        | 100                                                 | 119                                                             | 117                     | 118                                      | 113                                 | 113                     | 110                                 | 114                                      |
| 4-Am-2,6-DNT              | 106                                            | 119                                                        | 113                                                 | 130                                                             | 122                     | 123                                      | 122                                 | 119                     | 118                                 | 120                                      |
| 2-Am-4,6-DNT              | 108                                            | 123                                                        | 116                                                 | 137                                                             | 131                     | 132                                      | 130                                 | 127                     | 126                                 | 133                                      |
| 2,6-DNT                   | 110                                            | 116                                                        | 105                                                 | 124                                                             | 117                     | 117                                      | 124                                 | 133                     | 121                                 | 117                                      |
| 2,4-DNT                   | 104                                            | 119                                                        | 111                                                 | 128                                                             | 126                     | 126                                      | 123                                 | 117                     | 119                                 | 124                                      |
| 2-NT                      | 105                                            | 120                                                        | 108                                                 | 127                                                             | 127                     | 127                                      | 125                                 | 123                     | 120                                 | 126                                      |
| 4-NT                      | 105                                            | 116                                                        | 108                                                 | 124                                                             | 126                     | 127                                      | 124                                 | 122                     | 119                                 | 125                                      |
| 3-NT                      | 103                                            | 116                                                        | 110                                                 | 126                                                             | 122                     | 123                                      | 121                                 | 118                     | 117                                 | 118                                      |
| PETN                      | 113                                            | 132                                                        | 109                                                 | 128                                                             | 130                     | 131                                      | 135                                 | 125                     | 132                                 | 135                                      |

**Bold, italicized** are IM compounds and degradation products.

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

MeOH and water extraction solvents. Only options E and H, which consisted of sequential (6-h – 14-h or 8-h – 8-h, respectively) MeOH extractions, resulted in low recoveries of NQ, NTO, HMX, and RDX, as expected based on previous results (Table 11). Of the six multi-stage options that showed acceptable recoveries for all compounds, option F or G was preferred in order to simplify the method and to enable users to perform the procedure within the time constraints of a normal workday.

Overall, the results shown in Table 12 indicated that water (a more polar solvent than either ACN or MeOH) effectively improved recoveries of NQ, NTO, HMX, and RDX from ASTM fat clay spiked samples, and also aided recovery of some other compounds, including 2,4-DNP and 2-NP. While NQ and NTO are highly water-soluble and higher recoveries due to extracting with MeOH/water or water were expected for them, the superior ability of water versus MeOH to extract HMX and RDX was not predicted. However, it may likely be attributed to the observed ability of the water physically to disperse the soil much better than the MeOH, possibly preventing the entrapment of the hydrophobic compounds, and resulting in higher observed recoveries.

Due to the unexpected nature of the results when using water as the extraction solvent or co-solvent with MeOH, a similar experiment was carried out to confirm the effects (data not shown). In addition, a comparison of 18-h ultrasonic extractions using either ACN, MeOH, or 50/50 MeOH/water in several different soil types was performed. Six soils with varying physicochemical characteristics were used, described in Table 13, including five field soils (Aberdeen, Jefferson, Memphis, Riley, and Yuma), and the ASTM fat clay used previously. Soil classification is provided, including gravel, sand and fines percentages.

Chemical properties include pH, cation-exchange capacity (CEC), anion-exchange capacity (AEC), total organic carbon (TOC), and percent solids. The results shown in Table 14 indicated that extraction of the compounds of interest depended upon compound chemical properties and soil characteristics, and reinforced previous results that showed that extraction using any single solvent or co-solvent mixture would not be sufficient to extract the 24 compounds (and 1,2-DNB surrogate) from any of the six tested soils. (For the ASTM soil, a water only option was also tested, with mixed results.)

Table 13. Characteristic properties for six soils used in SE method development and testing for soils.

| Name                 | Collection Site                        | Region                  | Classification      | % Gravel | % Sand | % Fines | pH   | CEC (meq Na/100g) | AEC (meq S/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       |

\*ND: not detected (Reporting limit: 250 mg/kg)

Table 14. Recoveries (%) from six laboratory-spiked soils using an 18-h ultrasonication period with ACN, MeOH, 50/50 MeOH/water, or water.

| Compound            | Recoveries (%) |      |                           |           |      |                           |         |      |                           |
|---------------------|----------------|------|---------------------------|-----------|------|---------------------------|---------|------|---------------------------|
|                     | Aberdeen       |      |                           | Jefferson |      |                           | Memphis |      |                           |
|                     | ACN            | MeOH | MeOH/<br>H <sub>2</sub> O | ACN       | MeOH | MeOH/<br>H <sub>2</sub> O | ACN     | MeOH | MeOH/<br>H <sub>2</sub> O |
| <i>NQ</i>           | 67             | 72   | 108                       | 67        | 81   | 106                       | 38      | 91   | 123                       |
| <i>NTO (210nm)</i>  | 22             | 14   | 88                        | 22        | 8    | 108                       | 24      | 50   | 101                       |
| HMX                 | 109            | 40   | 96                        | 112       | 39   | 96                        | 107     | 42   | 87                        |
| RDX                 | 110            | 45   | 108                       | 103       | 49   | 103                       | 105     | 48   | 97                        |
| <i>PA (210nm)</i>   | 58             | 98   | 113                       | 11        | 100  | 120                       | 84      | 108  | 128                       |
| <i>4-NP</i>         | 98             | 99   | 105                       | 100       | 109  | 97                        | 94      | 103  | 104                       |
| 1,3,5-TNB           | 109            | 99   | 85                        | 106       | 104  | 84                        | 104     | 100  | 55                        |
| <i>2,4-DNP</i>      | 21             | 82   | 105                       | 60        | 51   | 105                       | 15      | 85   | 103                       |
| 1,2-DNB (surrogate) | 95             | 87   | 87                        | 92        | 96   | 84                        | 85      | 89   | 91                        |
| 1,3-DNB             | 92             | 87   | 88                        | 92        | 98   | 85                        | 91      | 90   | 89                        |
| <i>2-NP</i>         | 43             | 33   | 45                        | 33        | 44   | 6                         | 11      | 15   | 38                        |
| NB                  | 108            | 105  | 100                       | 97        | 103  | 99                        | 105     | 104  | 92                        |
| 3,5-DNA (254nm)     | 15             | 11   | 15                        | 20        | 25   | ND                        | 63      | 4    | 24                        |
| <i>DNAN</i>         | 102            | 103  | 99                        | 103       | 108  | 93                        | 104     | 98   | 94                        |
| Tetryl              | 99             | 94   | 72                        | 98        | 102  | 31                        | 104     | 49   | 97                        |
| NG                  | 97             | 92   | 97                        | 64        | 90   | 86                        | 83      | 90   | 86                        |
| 2,4,6-TNT           | 106            | 102  | 102                       | 101       | 108  | 115                       | 96      | 118  | 88                        |
| 4-Am-2,6-DNT        | 108            | 108  | 105                       | 97        | 102  | 102                       | 102     | 106  | 92                        |
| 2-Am-4,6-DNT        | 111            | 107  | 101                       | 100       | 103  | 101                       | 109     | 104  | 95                        |
| 2,6-DNT             | 96             | 88   | 86                        | 93        | 89   | 80                        | 85      | 88   | 89                        |
| 2,4-DNT             | 96             | 87   | 117                       | 84        | 94   | 83                        | 82      | 91   | 86                        |
| 2-NT                | 51             | 29   | 33                        | 43        | 45   | 4                         | 16      | 15   | 43                        |
| 4-NT                | 61             | 56   | 58                        | 69        | 71   | 24                        | 51      | 44   | 67                        |
| 3-NT                | 58             | 44   | 48                        | 54        | 61   | 12                        | 20      | 30   | 55                        |
| PETN                | 112            | 110  | 103                       | 106       | 111  | 99                        | 97      | 106  | 103                       |

***Bold, italicized*** are IM compounds and degradation products. ND: not detected.

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

(DoD QSM Ver5.2 for Solids – Lowest LCL: 4-Am-2,6-DNT, 64%; Highest UCL: Tetryl, 135%)

Table 14 (cont.)

| Compound                  | Recoveries (%) |      |                           |      |      |                           |               |      |                           |                  |
|---------------------------|----------------|------|---------------------------|------|------|---------------------------|---------------|------|---------------------------|------------------|
|                           | Riley          |      |                           | Yuma |      |                           | ASTM Fat Clay |      |                           |                  |
|                           | ACN            | MeOH | MeOH/<br>H <sub>2</sub> O | ACN  | MeOH | MeOH/<br>H <sub>2</sub> O | ACN           | MeOH | MeOH/<br>H <sub>2</sub> O | H <sub>2</sub> O |
| <b><i>NQ</i></b>          | 35             | 65   | 100                       | 52   | 79   | 84                        | 62            | 66   | 103                       | 91               |
| <b><i>NTO (210nm)</i></b> | 23             | 21   | 64                        | 24   | 43   | 95                        | 8             | 17   | 107                       | 105              |
| HMX                       | 94             | 26   | 79                        | 105  | 34   | 84                        | 92            | 19   | 75                        | 70               |
| RDX                       | 97             | 32   | 88                        | 99   | 33   | 94                        | 92            | 23   | 86                        | 99               |
| <b><i>PA (210nm)</i></b>  | 38             | 99   | 108                       | 92   | 106  | 130                       | 96            | 109  | 122                       | 129              |
| <b><i>4-NP</i></b>        | 86             | 101  | 98                        | 99   | 104  | 97                        | 97            | 123  | 114                       | 120              |
| 1,3,5-TNB                 | 109            | 103  | 55                        | 99   | 105  | 86                        | 93            | 121  | 84                        | 37               |
| <b><i>2,4-DNP</i></b>     | 42             | 51   | 99                        | 23   | 56   | 97                        | 32            | 51   | 92                        | 96               |
| 1,2-DNB (surrogate)       | 94             | 93   | 93                        | 94   | 87   | 84                        | 95            | 113  | 92                        | 101              |
| 1,3-DNB                   | 97             | 97   | 89                        | 91   | 90   | 83                        | 91            | 117  | 96                        | 87               |
| <b><i>2-NP</i></b>        | 56             | 65   | 69                        | 36   | 23   | 37                        | 59            | 53   | ND                        | 77               |
| NB                        | 85             | 86   | 76                        | 101  | 105  | 95                        | 92            | 117  | 101                       | 71               |
| 3,5-DNA (254nm)           | 57             | 54   | 59                        | 11   | 8    | 9                         | 45            | 52   | ND                        | 33               |
| <b><i>DNAN</i></b>        | 97             | 101  | 86                        | 99   | 101  | 94                        | 94            | 113  | 96                        | 55               |
| Tetryl                    | 97             | 93   | 79                        | 107  | 70   | 31                        | 17            | 65   | 66                        | 35               |
| NG                        | 84             | 100  | 92                        | 101  | 95   | 85                        | 104           | 124  | 95                        | 109              |
| 2,4,6-TNT                 | 95             | 105  | 84                        | 95   | 116  | 112                       | 109           | 138  | 106                       | 70               |
| 4-Am-2,6-DNT              | 80             | 85   | 78                        | 103  | 106  | 113                       | 18            | 121  | 110                       | 6                |
| 2-Am-4,6-DNT              | 91             | 93   | 81                        | 103  | 104  | 95                        | 93            | 117  | 101                       | 68               |
| 2,6-DNT                   | 87             | 106  | 97                        | 71   | 91   | 84                        | 87            | 138  | 81                        | 107              |
| 2,4-DNT                   | 96             | 92   | 84                        | 89   | 90   | 84                        | 95            | 118  | 94                        | 69               |
| 2-NT                      | 73             | 69   | 68                        | 35   | 20   | 20                        | 55            | 64   | ND                        | 43               |
| 4-NT                      | 94             | 85   | 78                        | 62   | 45   | 44                        | 62            | 80   | ND                        | 58               |
| 3-NT                      | 76             | 79   | 74                        | 38   | 32   | 32                        | 67            | 71   | ND                        | 50               |
| PETN                      | 97             | 108  | 99                        | 100  | 105  | 95                        | 100           | 122  | 102                       | 31               |

**Bold, italicized** are IM compounds and degradation products. ND: not detected.

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

(DoD QSM Ver5.2 for Solids – Lowest LCL: 4-Am-2,6-DNT, 64%; Highest UCL: Tetryl, 135%)

Because differences were observed for different soil types, further SE method development trials were carried out using all six soils. Based on results from the ASTM fat clay trials (Table 12), two 2-stage and two 3-stage sequential ultrasonication options were tested (Table 15). All four options were successful for each soil type. Only three outliers occurred, for the Yuma sand using option C (3-h MeOH – 3-h water – 14-h water), which were 1-3% higher than the accepted 135% (DoD QSM Ver5.2). Thus, it was confirmed that including water as an extraction solvent resulted in improved recoveries for NQ, NTO, HMX, and RDX across a broad range of soil types.

Overall, it was shown that a 2-stage SE procedure including water as an extraction solvent (Table 15, option A and B) was necessary and sufficient to result in recovery of all compounds of interest (and two surrogates) within current accepted limits (64-135%) for all soils tested. Results for further tests carried out using option A (for the compounds of interest and two surrogates) appear in the Stability Study and Inter-Laboratory Batch Analysis sections below. Option A was selected over option B so that both stages in the extraction procedure would include organic extraction solvent (MeOH), in order to minimize deviation

Table 15. Recoveries (%) from six laboratory-spiked soils using four different 2-/3-stage ultrasonication sequences. A: 6-h MeOH – 14-h MeOH/water; B: 6-h MeOH – 14-h water; C: 3-h MeOH – 3-h water – 14-h water; D: 3-h MeOH – 3-h MeOH – 14-h water.

| Compound                  | Recoveries (%)   |            |                  |              |                  |            |                  |            |                  |            |                  |            |                  |            |                  |            |
|---------------------------|------------------|------------|------------------|--------------|------------------|------------|------------------|------------|------------------|------------|------------------|------------|------------------|------------|------------------|------------|
|                           | Aberdeen         |            |                  |              |                  |            |                  |            | Jefferson        |            |                  |            |                  |            |                  |            |
|                           | A                |            | B                |              | C                |            | D                |            | A                |            | B                |            | C                |            | D                |            |
|                           | Average<br>(n=3) | Std<br>Dev | Average<br>(n=3) | Std<br>Dev   | Average<br>(n=3) | Std<br>Dev | Average<br>(n=3) | Std<br>Dev | Average<br>(n=3) | Std<br>Dev | Average<br>(n=3) | Std<br>Dev | Average<br>(n=3) | Std<br>Dev | Average<br>(n=3) | Std<br>Dev |
| <b><i>NQ</i></b>          | <b>98</b>        | <b>10</b>  | <b>102</b>       | <b>5</b>     | <b>120</b>       | <b>3</b>   | <b>117</b>       | <b>8</b>   | <b>106</b>       | <b>10</b>  | <b>104</b>       | <b>4</b>   | <b>119</b>       | <b>1</b>   | <b>109</b>       | <b>6</b>   |
| <b><i>NTO (210nm)</i></b> | <b>81</b>        | <b>10</b>  | <b>80</b>        | <b>3</b>     | <b>96</b>        | <b>2</b>   | <b>86</b>        | <b>2</b>   | <b>83</b>        | <b>8</b>   | <b>88</b>        | <b>1</b>   | <b>113</b>       | <b>2</b>   | <b>96</b>        | <b>1</b>   |
| HMX                       | 87               | 5          | 80               | 2            | 107              | 1          | 91               | 3          | 83               | 3          | 78               | 2          | 100              | 3          | 87               | 3          |
| RDX                       | 93               | 6          | 88               | 4            | 113              | 2          | 103              | 1          | 94               | 3          | 89               | 2          | 110              | 2          | 99               | 1          |
| <b><i>PA (210nm)</i></b>  | <b>105</b>       | <b>7</b>   | <b>106</b>       | <b>8</b>     | <b>120</b>       | <b>8</b>   | <b>117</b>       | <b>4</b>   | <b>109</b>       | <b>5</b>   | <b>101</b>       | <b>4</b>   | <b>114</b>       | <b>8</b>   | <b>115</b>       | <b>9</b>   |
| <b><i>4-NP</i></b>        | <b>115</b>       | <b>8</b>   | <b>110</b>       | <b>5</b>     | <b>123</b>       | <b>7</b>   | <b>126</b>       | <b>2</b>   | <b>121</b>       | <b>4</b>   | <b>113</b>       | <b>5</b>   | <b>122</b>       | <b>6</b>   | <b>124</b>       | <b>7</b>   |
| 1,3,5-TNB                 | 112              | 8          | 100              | 2            | 114              | 6          | 120              | 6          | 111              | 6          | 96               | 3          | 132              | 3          | 119              | 2          |
| <b><i>2,4-DNP</i></b>     | <b>104</b>       | <b>7</b>   | <b>99</b>        | <b>&lt;1</b> | <b>120</b>       | <b>3</b>   | <b>114</b>       | <b>4</b>   | <b>95</b>        | <b>4</b>   | <b>88</b>        | <b>3</b>   | <b>107</b>       | <b>6</b>   | <b>96</b>        | <b>2</b>   |
| 1,2-DNB (surrogate)       | 123              | 6          | 118              | 4            | 125              | 6          | 128              | 1          | 121              | 10         | 117              | 6          | 127              | 3          | 123              | 8          |
| 1,3-DNB                   | 119              | 9          | 112              | 4            | 125              | 8          | 128              | 4          | 123              | 5          | 115              | 4          | 124              | 3          | 126              | 3          |
| <b><i>2-NP</i></b>        | <b>103</b>       | <b>11</b>  | <b>96</b>        | <b>3</b>     | <b>108</b>       | <b>6</b>   | <b>107</b>       | <b>3</b>   | <b>100</b>       | <b>4</b>   | <b>95</b>        | <b>3</b>   | <b>105</b>       | <b>2</b>   | <b>105</b>       | <b>1</b>   |
| NB                        | 119              | 9          | 111              | 4            | 125              | 8          | 128              | 4          | 120              | 6          | 110              | 3          | 119              | 3          | 122              | 3          |
| 3,5-DNA (254nm)           | 89               | 7          | 86               | 4            | 92               | 6          | 96               | 3          | 101              | 6          | 96               | 3          | 101              | 3          | 102              | 5          |
| <b><i>DNAN</i></b>        | <b>117</b>       | <b>10</b>  | <b>111</b>       | <b>5</b>     | <b>123</b>       | <b>10</b>  | <b>126</b>       | <b>6</b>   | <b>121</b>       | <b>6</b>   | <b>109</b>       | <b>2</b>   | <b>115</b>       | <b>3</b>   | <b>124</b>       | <b>3</b>   |
| Tetryl                    | 103              | 10         | 99               | 7            | 113              | 4          | 115              | 3          | 115              | 6          | 109              | 7          | 120              | 5          | 121              | 7          |
| NG                        | 113              | 16         | 105              | 10           | 115              | 3          | 119              | 10         | 107              | 11         | 105              | 12         | 112              | 10         | 115              | 14         |
| 2,4,6-TNT                 | 116              | 8          | 106              | 6            | 120              | 7          | 123              | 5          | 117              | 1          | 105              | 3          | 112              | 3          | 120              | 5          |
| 4-Am-2,6-DNT              | 124              | 10         | 112              | 4            | 126              | 9          | 129              | 3          | 120              | 6          | 112              | 3          | 121              | 3          | 123              | 2          |
| 2-Am-4,6-DNT              | 120              | 9          | 111              | 4            | 125              | 8          | 128              | 3          | 123              | 5          | 114              | 3          | 123              | 3          | 126              | 3          |
| 2,6-DNT                   | 116              | 5          | 107              | 4            | 118              | 11         | 124              | 6          | 120              | 4          | 112              | 3          | 119              | 3          | 119              | 3          |
| 2,4-DNT                   | 118              | 6          | 110              | 4            | 125              | 9          | 126              | 6          | 123              | 8          | 111              | 6          | 117              | 7          | 124              | 6          |
| 2-NT                      | 103              | 7          | 96               | 1            | 103              | 6          | 107              | 4          | 108              | 9          | 101              | 3          | 108              | 4          | 111              | 4          |
| 4-NT                      | 110              | 6          | 98               | 5            | 110              | 9          | 118              | 5          | 115              | 10         | 106              | 2          | 113              | 4          | 114              | 5          |
| 3-NT                      | 103              | 7          | 98               | 3            | 107              | 6          | 112              | 4          | 110              | 7          | 103              | 4          | 110              | 5          | 112              | 5          |
| PETN                      | 114              | 8          | 109              | 3            | 124              | 8          | 123              | 3          | 121              | 8          | 114              | 3          | 125              | 4          | 124              | 5          |

***Bold, italicized*** are IM compounds and degradation products. ND: not detected.

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

(DoD QSM Ver5.2 for Solids – Lowest LCL: 4-Am-2,6-DNT, 64%; Highest UCL: Tetryl, 135%)

Table 15 (cont.)

| Compound                  | Recoveries (%)   |            |                  |            |                  |            |                  |            |                  |            |                  |            |                  |            |                  |              |
|---------------------------|------------------|------------|------------------|------------|------------------|------------|------------------|------------|------------------|------------|------------------|------------|------------------|------------|------------------|--------------|
|                           | Memphis          |            |                  |            |                  |            |                  |            | Riley            |            |                  |            |                  |            |                  |              |
|                           | A                |            | B                |            | C                |            | D                |            | A                |            | B                |            | C                |            | D                |              |
|                           | Average<br>(n=3) | Std<br>Dev | Average<br>(n=3) | Std<br>Dev | Average<br>(n=3) | Std<br>Dev | Average<br>(n=3) | Std<br>Dev | Average<br>(n=3) | Std<br>Dev | Average<br>(n=3) | Std<br>Dev | Average<br>(n=3) | Std<br>Dev | Average<br>(n=3) | Std<br>Dev   |
| <b><i>NQ</i></b>          | <b>107</b>       | <b>9</b>   | <b>111</b>       | <b>1</b>   | <b>118</b>       | <b>9</b>   | <b>115</b>       | <b>9</b>   | <b>90</b>        | <b>6</b>   | <b>98</b>        | <b>9</b>   | <b>111</b>       | <b>2</b>   | <b>109</b>       | <b>5</b>     |
| <b><i>NTO (210nm)</i></b> | <b>115</b>       | <b>15</b>  | <b>106</b>       | <b>2</b>   | <b>125</b>       | <b>2</b>   | <b>119</b>       | <b>2</b>   | <b>100</b>       | <b>8</b>   | <b>112</b>       | <b>3</b>   | <b>128</b>       | <b>2</b>   | <b>119</b>       | <b>4</b>     |
| HMX                       | 98               | 7          | 90               | 1          | 109              | 3          | 103              | 1          | 67               | 2          | 66               | 4          | 84               | 2          | 72               | 3            |
| RDX                       | 99               | 4          | 94               | <1         | 114              | 3          | 106              | 2          | 81               | 1          | 78               | 1          | 101              | 3          | 88               | 1            |
| <b><i>PA (210nm)</i></b>  | <b>121</b>       | <b>6</b>   | <b>111</b>       | <b>5</b>   | <b>126</b>       | <b>7</b>   | <b>121</b>       | <b>2</b>   | <b>111</b>       | <b>5</b>   | <b>103</b>       | <b>4</b>   | <b>124</b>       | <b>3</b>   | <b>124</b>       | <b>2</b>     |
| <b><i>4-NP</i></b>        | <b>120</b>       | <b>4</b>   | <b>112</b>       | <b>4</b>   | <b>126</b>       | <b>5</b>   | <b>123</b>       | <b>2</b>   | <b>115</b>       | <b>4</b>   | <b>103</b>       | <b>3</b>   | <b>118</b>       | <b>5</b>   | <b>120</b>       | <b>&lt;1</b> |
| 1,3,5-TNB                 | 122              | 4          | 108              | <1         | 121              | 4          | 125              | 4          | 113              | 4          | 96               | 1          | 104              | 2          | 120              | <1           |
| <b><i>2,4-DNP</i></b>     | <b>117</b>       | <b>7</b>   | <b>106</b>       | <b>2</b>   | <b>123</b>       | <b>6</b>   | <b>118</b>       | <b>1</b>   | <b>95</b>        | <b>4</b>   | <b>89</b>        | <b>3</b>   | <b>108</b>       | <b>1</b>   | <b>97</b>        | <b>3</b>     |
| 1,2-DNB (surrogate)       | 121              | 7          | 118              | 6          | 126              | 6          | 127              | 4          | 121              | 5          | 111              | 8          | 126              | 5          | 124              | 4            |
| 1,3-DNB                   | 123              | 3          | 115              | 2          | 127              | 5          | 126              | 3          | 121              | 4          | 108              | 1          | 120              | 4          | 125              | 1            |
| <b><i>2-NP</i></b>        | <b>91</b>        | <b>4</b>   | <b>88</b>        | <b>1</b>   | <b>101</b>       | <b>5</b>   | <b>96</b>        | <b>4</b>   | <b>106</b>       | <b>4</b>   | <b>96</b>        | <b>2</b>   | <b>105</b>       | <b>5</b>   | <b>108</b>       | <b>2</b>     |
| NB                        | 124              | 3          | 116              | 1          | 128              | 4          | 127              | 3          | 110              | 3          | 93               | <1         | 103              | 3          | 111              | <1           |
| 3,5-DNA (254nm)           | 86               | 4          | 81               | 1          | 88               | 6          | 85               | 3          | 108              | 3          | 100              | 2          | 108              | 5          | 112              | 2            |
| <b><i>DNAN</i></b>        | <b>122</b>       | <b>3</b>   | <b>112</b>       | <b>2</b>   | <b>126</b>       | <b>5</b>   | <b>125</b>       | <b>1</b>   | <b>119</b>       | <b>3</b>   | <b>102</b>       | <b>1</b>   | <b>115</b>       | <b>3</b>   | <b>123</b>       | <b>1</b>     |
| Tetryl                    | 86               | 14         | 82               | 5          | 102              | 4          | 97               | 7          | 112              | 8          | 100              | 4          | 112              | 5          | 117              | 5            |
| NG                        | 118              | 11         | 113              | 9          | 117              | 13         | 118              | 8          | 111              | 15         | 103              | 11         | 111              | 10         | 118              | 13           |
| 2,4,6-TNT                 | 130              | 1          | 119              | 1          | 129              | 4          | 129              | 4          | 117              | 7          | 100              | 2          | 111              | 1          | 122              | 2            |
| 4-Am-2,6-DNT              | 127              | 3          | 119              | 1          | 130              | 4          | 129              | 2          | 109              | 2          | 93               | 1          | 103              | 2          | 110              | 2            |
| 2-Am-4,6-DNT              | 125              | 3          | 117              | 1          | 128              | 4          | 127              | 2          | 116              | 3          | 99               | <1         | 109              | 3          | 118              | 1            |
| 2,6-DNT                   | 121              | 4          | 109              | 2          | 124              | 2          | 121              | 4          | 117              | 10         | 100              | 2          | 113              | 6          | 125              | 6            |
| 2,4-DNT                   | 123              | 4          | 115              | 3          | 125              | 5          | 126              | 2          | 119              | 2          | 106              | 2          | 115              | 6          | 126              | 4            |
| 2-NT                      | 99               | 4          | 91               | 2          | 101              | 5          | 100              | 6          | 112              | 5          | 103              | 1          | 112              | 5          | 119              | 2            |
| 4-NT                      | 106              | 4          | 99               | 2          | 110              | 2          | 108              | 4          | 114              | 4          | 104              | 2          | 112              | 3          | 121              | 1            |
| 3-NT                      | 101              | 5          | 93               | <1         | 103              | 5          | 101              | 3          | 114              | 3          | 104              | 2          | 112              | 7          | 116              | 4            |
| PETN                      | 115              | 5          | 109              | 7          | 120              | 7          | 117              | 1          | 121              | 7          | 112              | 3          | 122              | 5          | 125              | 5            |

***Bold, italicized*** are IM compounds and degradation products. ND: not detected.

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

(DoD QSM Ver5.2 for Solids – Lowest LCL: 4-Am-2,6-DNT, 64%; Highest UCL: Tetryl, 135%)

Table 15 (cont.)

| Compound            | Recoveries (%)   |            |                  |            |                  |            |                  |            |                  |            |                  |            |                  |            |                  |            |
|---------------------|------------------|------------|------------------|------------|------------------|------------|------------------|------------|------------------|------------|------------------|------------|------------------|------------|------------------|------------|
|                     | Yuma             |            |                  |            |                  |            |                  |            | ASTM Fat Clay    |            |                  |            |                  |            |                  |            |
|                     | A                |            | B                |            | C                |            | D                |            | A                |            | B                |            | C                |            | D                |            |
|                     | Average<br>(n=3) | Std<br>Dev | Average<br>(n=3) | Std<br>Dev | Average<br>(n=3) | Std<br>Dev | Average<br>(n=3) | Std<br>Dev | Average<br>(n=3) | Std<br>Dev | Average<br>(n=3) | Std<br>Dev | Average<br>(n=3) | Std<br>Dev | Average<br>(n=3) | Std<br>Dev |
| <b>NQ</b>           | <b>109</b>       | 7          | <b>106</b>       | 2          | <b>122</b>       | 4          | <b>115</b>       | 4          | <b>101</b>       | 7          | <b>100</b>       | 5          | <b>116</b>       | 3          | <b>113</b>       | 5          |
| <b>NTO (210nm)</b>  | <b>103</b>       | 3          | <b>88</b>        | 2          | <b>111</b>       | 5          | <b>98</b>        | 1          | <b>90</b>        | 1          | <b>92</b>        | 2          | <b>118</b>       | 1          | <b>101</b>       | 3          |
| HMX                 | 86               | 4          | 84               | 3          | 105              | 2          | 93               | 2          | 66               | 1          | 65               | 1          | 92               | 2          | 75               | 2          |
| RDX                 | 92               | 3          | 89               | 2          | 111              | 2          | 98               | <1         | 72               | 1          | 72               | 1          | 101              | 1          | 82               | 2          |
| <b>PA (210nm)</b>   | <b>117</b>       | 6          | <b>107</b>       | 4          | <b>127</b>       | 6          | <b>123</b>       | 3          | <b>106</b>       | 5          | <b>103</b>       | 7          | <b>119</b>       | 5          | <b>120</b>       | 2          |
| <b>4-NP</b>         | <b>119</b>       | 5          | <b>110</b>       | 3          | <b>132</b>       | 10         | <b>125</b>       | 2          | <b>115</b>       | 6          | <b>112</b>       | 3          | <b>124</b>       | 1          | <b>125</b>       | 1          |
| 1,3,5-TNB           | 120              | 5          | 111              | 1          | 130              | 7          | 127              | 2          | 114              | 4          | 103              | 1          | 110              | 1          | 124              | 4          |
| <b>2,4-DNP</b>      | <b>102</b>       | 4          | <b>98</b>        | 1          | <b>117</b>       | 3          | <b>105</b>       | 1          | <b>92</b>        | 2          | <b>91</b>        | 3          | <b>110</b>       | 1          | <b>100</b>       | 3          |
| 1,2-DNB (surrogate) | 122              | 7          | 116              | 7          | 134              | 16         | 124              | 5          | 121              | 10         | 115              | 7          | 125              | 3          | 125              | 1          |
| 1,3-DNB             | 121              | 4          | 114              | 1          | 134              | 9          | 127              | 2          | 119              | 4          | 115              | 2          | 125              | 1          | 129              | 5          |
| <b>2-NP</b>         | <b>85</b>        | 5          | <b>81</b>        | 4          | <b>99</b>        | 6          | <b>93</b>        | 1          | <b>89</b>        | 2          | <b>88</b>        | 2          | <b>102</b>       | 3          | <b>93</b>        | 5          |
| NB                  | 122              | 4          | 116              | 1          | 135              | 9          | 128              | 2          | 118              | 5          | 113              | 2          | 122              | <0         | 127              | 5          |
| 3,5-DNA (254nm)     | 75               | 3          | 72               | 4          | 84               | 9          | 79               | 2          | 96               | 5          | 93               | 1          | 101              | 1          | 103              | 3          |
| <b>DNAN</b>         | <b>120</b>       | 4          | <b>114</b>       | 2          | <b>133</b>       | 7          | <b>129</b>       | 1          | <b>117</b>       | 4          | <b>110</b>       | 2          | <b>119</b>       | 2          | <b>127</b>       | 4          |
| Tetryl              | 93               | 11         | 93               | 5          | 118              | 6          | 113              | 6          | 99               | 4          | 98               | 2          | 111              | 3          | 111              | 1          |
| NG                  | 114              | 11         | 106              | 8          | 125              | 17         | 118              | 13         | 106              | 7          | 108              | 9          | 116              | 10         | 114              | 5          |
| 2,4,6-TNT           | 128              | 5          | 119              | 2          | 138              | 12         | 130              | 5          | 116              | 2          | 112              | 4          | 119              | 2          | 124              | 3          |
| 4-Am-2,6-DNT        | 126              | 4          | 118              | 1          | 137              | 9          | 130              | 2          | 121              | 4          | 117              | 4          | 126              | 1          | 127              | 2          |
| 2-Am-4,6-DNT        | 123              | 4          | 117              | 1          | 136              | 9          | 129              | 2          | 119              | 4          | 113              | 2          | 122              | 1          | 126              | 2          |
| 2,6-DNT             | 116              | 11         | 109              | 6          | 132              | 4          | 121              | 3          | 118              | 4          | 109              | 6          | 123              | 5          | 124              | 4          |
| 2,4-DNT             | 123              | 2          | 115              | 2          | 135              | 8          | 127              | 1          | 114              | 4          | 112              | 3          | 122              | 2          | 125              | 3          |
| 2-NT                | 88               | 5          | 83               | 3          | 98               | 9          | 92               | 2          | 105              | 4          | 101              | 1          | 110              | 4          | 111              | 3          |
| 4-NT                | 97               | 2          | 93               | 3          | 110              | 10         | 104              | 1          | 110              | 6          | 105              | 3          | 114              | 2          | 117              | 5          |
| 3-NT                | 94               | 4          | 88               | 4          | 101              | 10         | 97               | 1          | 103              | 6          | 101              | 2          | 108              | <1         | 111              | 1          |
| PETN                | 120              | 7          | 113              | 1          | 130              | 11         | 123              | 2          | 117              | 5          | 115              | 3          | 123              | 1          | 126              | 3          |

**Bold, italicized** are IM compounds and degradation products.

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

(DoD QSM Ver5.2 for Solids – Lowest LCL: 4-Am-2,6-DNT, 64%; Highest UCL: Tetryl, 135%)

from current USEPA Method 8330B procedures. A 3-stage extraction sequence was shown to be unnecessary, but may be employed, if desired. For cases in which NQ, NTO, HMX, and RDX are known to be absent or are not of interest in soils similar to those tested, an extraction procedure that includes water as an extraction solvent may not be necessary. In such cases, a one-step ultrasonication may be sufficient, and a 2-step procedure may not enhance recovery at a statistically-significant or cost-effective rate.

## SE Method for Tissues

Current USEPA Method 8330B and ERDC-EL SE methods were modified to enable extraction of all 24 compounds of interest (and two surrogates) from environmental tissue samples. A single ultrasonication period using MeOH was predicted to be sufficient for extraction of the 26 compounds, as the tissues utilized during method development experiments were not previously lyophilized, and, therefore, contained a significant portion of water. Initial tests led to the adoption of a single-step 18-h MeOH extraction. Additionally, an extract interference reduction method was needed (adapted from Larson, et al., 1999), as all of the tissues (fathead minnow [*Pimephales promelas*], polychaete worm [*Alitta virens*], earthworm [*Eisenia fetida*], and ryegrass [*Lolium perenne*]) suffered from varying degrees of analytical interference due to co-eluting non-target extracted components.

Compound recoveries prior to application of the interference reduction procedure are shown in Table 16 for all four tissue types. The DoD QSM Ver5.2 UCL and LCL accepted limits for solid (soil) matrices analyzed by USEPA Method 8330B were used as a benchmark (64-135%), as they were during soil SE method development described previously, since limits have not been established specifically for tissues matrices. A single ultrasonication period of 18 hours using MeOH was shown to be sufficient to extract the majority of compounds (17 from earthworm, 22 from polychaete worm, 24 from minnow, and 23 from ryegrass) within the accepted range for solids according to DoD QSM Ver5.2. Tetryl was the only compound that was generally recovered poorly from all three tested animal tissue types, at <5%. The scientific literature highlights the matrix- and temperature-dependent stability of Tetryl (Harvey, et al., 1992; Jenkins, 1994; Jenkins, et al., 1994; Boopathy, 2000; Douglas, et al., 2009), which was consistent with current and previous results obtained at ERDC-EL. For this reason, it may be beneficial to investigate reduced ultrasonication periods, to determine if the full 18-h period is necessary for efficient recovery of the other compounds of interest, or whether Tetryl recovery could be improved by having a shorter time period during which degradation could occur. While no compound stability studies were performed in tissue matrices for the current work, results for water and soil matrices are shown and discussed in the Stability Study section below, with results for Tetryl consistent with referenced literature.

UV chromatograms of both unspiked and spiked tissue samples (not shown) revealed the presence of non-target matrix components for each tissue type, with the majority occurring toward the beginning of the chromatographic separation (0-16 min), which indicated more polar interferents. This resulted in observed positive interferences for the more polar compounds of interest (mainly NQ, NTO, and PA), with the worst interference observed for earthworm tissue. Matrix-based analytical interferences occurred in three chromatographic regions for earthworm tissue, which resulted in large positive interference and very high recovery rates for NTO, PA, and 1,3,5-TNB. In addition, NQ and o-NBA surrogate were recovered, but not quite within current QSM accepted limits. Compound recovery from polychaete worm tissue was similar to earthworm tissue. However, positive interference was observed for NQ, PA recovery was slightly low, and 1,3,5-TNB analysis was not affected by interference. Positive interference was still observed for NTO, but to a lesser extent. For the fathead minnows, positive interference again inhibited accurate determination of NTO. No positive chromatographic interference was observed for ryegrass tissue; however, neither NTO (12%) nor o-NBA (24%) was recovered within current accepted QSM limits. The analytical interferences could have been due to co-elution or co-extraction of non-target compounds that interfered with NTO/o-NBA detection during the HPLC analytical method, arising from pH-related or other interac-

tions. Therefore, it was predicted that subjecting plant tissue samples to an appropriate interference reduction procedure might eliminate these interferences, as well. Interestingly, Tetryl recovery was approximately 50% from plant tissue, whereas it was <5% for all animal tissue types.

Table 16. Recoveries (%) from four laboratory-spiked tissues using one 18-h ultrasonic extraction with MeOH, prior to undergoing chromatographic treatment to reduce analytical interference.

| Compound            | Recoveries (%) |         |               |         |                 |         |               |         |
|---------------------|----------------|---------|---------------|---------|-----------------|---------|---------------|---------|
|                     | Fathead Minnow |         | Earthworm     |         | Polychaete Worm |         | Ryegrass      |         |
|                     | Average (n=4)  | Std Dev | Average (n=2) | Std Dev | Average (n=5)   | Std Dev | Average (n=3) | Std Dev |
| <i>NQ</i>           | 73             | 18.0    | 59            | 5.1     | 140             | 134.2   | 99            | 6.0     |
| <i>NTO (210nm)</i>  | 1112           | 325.3   | 668           | 133.7   | 395             | 122.7   | 12            | 1.4     |
| o-NBA (surrogate)   | 85             | 6.7     | 54            | 35.3    | 119             | 14.5    | 24            | 1.8     |
| HMX                 | 76             | 2.5     | 89            | 27.6    | 72              | 2.9     | 81            | 7.2     |
| RDX                 | 73             | 1.1     | 84            | 18.8    | 74              | 1.4     | 86            | 9.9     |
| <i>PA (210nm)</i>   | 77             | 20.7    | 419           | 335.8   | 60              | 4.5     | 85            | 9.1     |
| <i>4-NP</i>         | 102            | 15.4    | 110           | 34.4    | 79              | 1.0     | 89            | 5.6     |
| 1,3,5-TNB           | 72             | 4.0     | 234           | 192.7   | 74              | 2.4     | 87            | 6.6     |
| <i>2,4-DNP</i>      | 79             | 1.7     | 96            | 21.6    | 77              | 2.1     | 110           | 6.9     |
| 1,2-DNB (surrogate) | 82             | 0.6     | 83            | 2.4     | 82              | 0.8     | 86            | 7.5     |
| 1,3-DNB             | 74             | 0.6     | 76            | 2.9     | 74              | 0.8     | 87            | 7.0     |
| <i>2-NP</i>         | 68             | 1.5     | 69            | 2.2     | 68              | 1.8     | 86            | 6.7     |
| NB                  | 75             | 0.8     | 79            | 1.3     | 74              | 1.7     | 87            | 7.6     |
| 3,5-DNA (254nm)     | 83             | 2.2     | 85            | 2.6     | 83              | 1.0     | 90            | 7.0     |
| <i>DNAN</i>         | 70             | 6.8     | 73            | 3.8     | 70              | 2.5     | 90            | 6.4     |
| Tetryl              | 3              | 3.2     | 2             | 2.1     | 4               | 1.8     | 51            | 16.5    |
| NG                  | 73             | 3.7     | 74            | 0.1     | 72              | 0.6     | 85            | 5.5     |
| 2,4,6-TNT           | 73             | 3.9     | 77            | 1.3     | 74              | 1.3     | 87            | 6.7     |
| 4-Am-2,6-DNT        | 75             | 3.4     | 78            | 0.8     | 73              | 0.8     | 93            | 8.2     |
| 2-Am-4,6-DNT        | 73             | 2.0     | 77            | 1.9     | 74              | 1.3     | 94            | 7.3     |
| 2,6-DNT             | 91             | 17.3    | 85            | 5.0     | 82              | 2.4     | 88            | 7.2     |
| 2,4-DNT             | 74             | 7.3     | 72            | 4.3     | 71              | 2.4     | 87            | 7.4     |
| 2-NT                | 79             | 1.0     | 82            | 0.1     | 76              | 2.3     | 87            | 7.1     |
| 4-NT                | 76             | 1.9     | 80            | 3.6     | 70              | 3.7     | 89            | 5.4     |
| 3-NT                | 79             | 2.0     | 80            | 3.5     | 80              | 3.6     | 89            | 6.8     |
| PETN                | 72             | 3.2     | 73            | 2.7     | 83              | 10.2    | 92            | 5.1     |

***Bold, italicized*** are IM compounds and degradation products.

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

(DoD QSM Ver5.2 for Solids – Lowest LCL: 4-Am-2,6-DNT, 64%; Highest UCL: Tetryl, 135%)

## Chromatographic Interference Reduction Method for Tissue Extracts

Based on the chromatographic results obtained during tissue extraction method development, an interference reduction procedure for tissue matrix interferences was necessary in order to obtain accurate and reliable results for tissue extract analysis, especially extracts known or suspected of containing the more polar IM compounds, like NQ, NTO, and PA. Furthermore, as mentioned briefly in Materials and Methods above, at cold temperatures, some tissue MeOH extracts were observed to develop a precipitate. This also occurred for certain extracts that had been prepared for analysis to a ratio of 50/50 MeOH/water. The majority of the precipitation issue was observed for earthworm extracts, to a lesser extent for polychaete worm and minnow extracts, and not at all for ryegrass extracts. In the case of precipitation, samples underwent additional filtering at the point of collection or following dilution with reagent water in preparation for analysis. Additional filtering was performed using either a second 0.45  $\mu\text{m}$  PTFE hydrophobic or a 0.20  $\mu\text{m}$  Anotop 10 inorganic membrane disk filter. When HPLC-UV data for samples that had undergone additional filtering showed persistent chromatographic interference, further experiments were carried out to determine an effective interference reduction method.

As an alternative to additional filtration alone, either 0.1% or 1% TFA (final v/v) was added to MeOH extracts or 50/50 MeOH/water-prepared analytical samples to encourage “complete” precipitation, followed by additional filtering (if needed). The addition of acid yielded no further benefit than a second filtration step alone (data not shown). Therefore, a chromatographic interference reduction method was adapted from Larson, et al, (1999). Some changes were made to the method described in the original publication. The original ACN and basic alumina were changed to MeOH and neutral alumina, respectively. Instead of using a combination of alumina and florisil, either alumina or florisil was used initially to enable determination of the responsible component for the effect on HPLC-UV chromatography for each compound of interest. Initial trials of the adapted chromatographic method were performed only on polychaete worms (Table 17). For the experiment shown, treatment with alumina removed all of the analytical interferences, and even resulted in improved recoveries for many other compounds; however, it also caused a reduction in the detected *o*-NBA surrogate to 39%. Meanwhile, treatment with florisil proved similar; however, both NTO and *o*-NBA recoveries were reduced to levels below the accepted DoD QSM Ver5.2 lower limit of 64%.

Due to preliminary success with the alumina and florisil methods, the same tests were repeated, using all four tissue matrices, shown in Table 18, with similar results. Further interference reduction methods were investigated, including mixed combinations of neutral alumina and florisil, as well as basic alumina, and silica. For these tests, MeOH extracts were obtained *via* 18-h ultrasonication of unspiked tissue. Post-extraction spiked MeOH extracts underwent chromatographic treatment as described in Materials and Methods above. Compound recoveries (%) for each tissue type using each of the five chromatographic methods are shown in Tables 19-22.

Each chromatographic treatment option was effective for fathead minnow tissue (Table 19). A reduction of NQ recovery was observed for neutral alumina-florisil combinations (to 77-81%), compared to a positive NQ interference introduced by basic alumina (150% recovery). With the exception of a slightly higher than acceptable recovery for NTO using the silica gel option (141%), NTO detected at 315 nm (detection wavelength selection discussed below) was within DoD QSM Ver5.2 accepted limits (64-135%) for each treatment option.

For earthworm tissue (Table 20), again several chromatographic treatment options were successful at reducing or eliminating matrix-related interference. For the three neutral alumina-florisil combinations, all compounds were recovered within current DoD QSM Ver5.2 accepted limits. The silica gel option resulted in acceptable recoveries for all compounds as well, with the exception of NTO, which was slightly

high at 138%. Treatment with basic alumina resulted in three out-of-spec recoveries, including *o*-NBA surrogate (146%), Tetryl (58%), and NG (169%).

For polychaete worm tissue (Table 21), analytical interferences were previously observed for NQ, NTO, and *o*-NBA surrogate. All five chromatographic interference reduction options resulted in *o*-NBA recoveries of approximately 100%. NTO interference was also greatly reduced. NTO recoveries were only slightly above the accepted limit (135%) for mixed neutral alumina-florisil (136%) and silica gel (140%), and within limits for two stacked alumina-florisil options (132-134%). Unlike for other tissue types, polychaete worm tissue also suffered from analytical interference of NQ. All five chromatographic treatments were able to reduce this interference as well, with the silica gel option yielding the best NQ recovery result (92%), followed by basic alumina (80%), and mixed neutral alumina-florisil (72%). Neither stacked neutral alumina-florisil option was able to remove completely the NQ interference. However, because each chromatographic interference removal procedure tested had specific successes and failures, all remain options for specific applications if needed.

For ryegrass plant tissue (Table 22), analytical interferences were observed for NQ, NTO, *o*-NBA surrogate, and 2,4-DNP in the original untreated extracts. With each of the five chromatographic options, the NQ and the 2,4-DNP interferences were completely removed. In addition, all but the mixed neutral alumina-florisil were able to remove the *o*-NBA interference to yield recoveries within or just above the accepted DoD QSM Ver5.2 limit. Meanwhile, NTO interference was reduced by all but the basic alumina option. Based on results from all four tissue types using each of the five chromatographic options, the silica gel option was chosen as the preferred option. This was due to 1) the ability to remove the majority of interferences from the majority of tissue matrices, 2) the generally higher recoveries observed for this option, and 3) the simplicity versus a combination of neutral alumina-florisil, which typically yielded similar recovery results.

During method development for tissue matrix-related interference reduction, comparative analysis of NTO and of PA using detection wavelengths of 210 nm and 315 nm showed that much of the analytical interferences observed at 210 nm were absent at 315 nm; therefore, use of 315 nm was preferred for determinations of NTO and PA in tissue extracts. A detection wavelength of 315 nm may also be preferred for NTO and PA in water and soil matrices, as a measure to combat the contribution of interferent compounds in munition compound determinations. In addition, lower MDLs were obtained for both NTO and PA in water and soil by detecting at 315 nm rather than 210 nm (Table 5 above).

Finally, pre-extraction spiked tissue sample MeOH extracts were treated with the silica gel interference reduction option. Overall, the results (Table 23) showed that using a single 18-h MeOH extraction in combination with silica gel treatment was sufficient to yield recoveries for most compounds within current DoD QSM limits. The only compound recovered poorly from all four tissue matrices was Tetryl, with the highest recovery obtain for plant tissue, which again may point to the matrix-dependent stability of this compound. The silica gel treatment was able to remove or reduce the majority of interferences that were previously observed for each tissue type. Non-target chromatographic peaks remained in the chromatographic region surrounding NTO, *o*-NBA surrogate, and 1,3,5-TNB (for earthworm). Of these remaining observed interferences, only the 1,3,5-TNB determination was affected. For polychaete worm, the 1,3,5-TNB and 4-Am-2,6-DNT recoveries were somewhat low, but had been shown in previous experiments to be recovered within QSM Ver5.2 accepted limits. Therefore, 18-h MeOH extraction in combination with silica gel chromatographic treatment for interference reduction was selected for future work.

Table 17. Recoveries (%) from laboratory-spiked polychaete worm tissue MeOH extracts following either neutral alumina or florisil chromatographic treatment for interference reduction.

| Compound            | Polychaete Worm - Recoveries (%) |             |                 |             |
|---------------------|----------------------------------|-------------|-----------------|-------------|
|                     | Alumina Column                   |             | Florisil Column |             |
|                     | Average (n=2)                    | Std Dev     | Average (n=2)   | Std Dev     |
| <i>NQ</i>           | <b>117</b>                       | <b>5.0</b>  | <b>129</b>      | <b>13.7</b> |
| <i>NTO (210nm)</i>  | <b>93</b>                        | <b>16.2</b> | <b>26</b>       | <b>4.2</b>  |
| o-NBA (surrogate)   | <b>39</b>                        | 1.4         | <b>24</b>       | 27.9        |
| HMX                 | <b>82</b>                        | 2.0         | <b>90</b>       | 3.3         |
| RDX                 | <b>109</b>                       | 5.7         | <b>95</b>       | 16.3        |
| <i>PA (210nm)</i>   | <b>88</b>                        | <b>2.7</b>  | <b>90</b>       | <b>3.9</b>  |
| <i>4-NP</i>         | <b>92</b>                        | <b>2.1</b>  | <b>97</b>       | <b>4.1</b>  |
| 1,3,5-TNB           | <b>90</b>                        | 0.7         | <b>96</b>       | 1.4         |
| <i>2,4-DNP</i>      | <b>95</b>                        | <b>0.5</b>  | <b>96</b>       | <b>4.6</b>  |
| 1,2-DNB (surrogate) | <b>91</b>                        | 2.3         | <b>96</b>       | 2.4         |
| 1,3-DNB             | <b>91</b>                        | 1.1         | <b>96</b>       | 2.1         |
| <i>2-NP</i>         | <b>70</b>                        | <b>3.4</b>  | <b>71</b>       | <b>2.6</b>  |
| NB                  | <b>88</b>                        | 1.4         | <b>94</b>       | 2.7         |
| 3,5-DNA (254nm)     | <b>97</b>                        | 2.7         | <b>97</b>       | 3.4         |
| <i>DNAN</i>         | <b>78</b>                        | <b>1.5</b>  | <b>85</b>       | <b>0.6</b>  |
| Tetryl              | <b>4</b>                         | 0.3         | <b>5</b>        | 1.6         |
| NG                  | <b>95</b>                        | 3.6         | <b>96</b>       | 9.1         |
| 2,4,6-TNT           | <b>92</b>                        | 0.1         | <b>97</b>       | 2.1         |
| 4-Am-2,6-DNT        | <b>87</b>                        | 0.7         | <b>91</b>       | 4.3         |
| 2-Am-4,6-DNT        | <b>90</b>                        | 0.6         | <b>92</b>       | 4.9         |
| 2,6-DNT             | <b>88</b>                        | 1.3         | <b>95</b>       | 1.4         |
| 2,4-DNT             | <b>88</b>                        | 0.2         | <b>90</b>       | 2.5         |
| 2-NT                | <b>98</b>                        | 4.1         | <b>94</b>       | 13.9        |
| 4-NT                | <b>89</b>                        | 2.9         | <b>91</b>       | 1.6         |
| 3-NT                | <b>98</b>                        | 3.6         | <b>103</b>      | 2.6         |
| PETN                | <b>84</b>                        | 4.5         | <b>85</b>       | 1.4         |

**Bold, italicized** are IM compounds and degradation products.

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

Table 18. Recoveries (%) from laboratory-spiked tissues MeOH extract, following no interference reduction treatment, or treatment with either neutral alumina or florisil.

| Compound            | Recoveries (%)                            |         |                |         |                 |         |                                           |         |                |         |                 |         |
|---------------------|-------------------------------------------|---------|----------------|---------|-----------------|---------|-------------------------------------------|---------|----------------|---------|-----------------|---------|
|                     | Fathead Minnow                            |         |                |         |                 |         | Earthworm                                 |         |                |         |                 |         |
|                     | No Chromatographic Interference Reduction |         | Alumina Column |         | Florisil Column |         | No Chromatographic Interference Reduction |         | Alumina Column |         | Florisil Column |         |
|                     | Average (n=3)                             | Std Dev | Average (n=3)  | Std Dev | Average (n=3)   | Std Dev | Average (n=3)                             | Std Dev | Average (n=3)  | Std Dev | Average (n=3)   | Std Dev |
| <i>NQ</i>           | 398                                       | 33.6    | 238            | 20.3    | 280             | 30.1    | 88                                        | 4.0     | 203            | 77.8    | 162             | 21.9    |
| <i>NTO (210nm)</i>  | 4363                                      | 422.6   | 4441           | 81.1    | 5659            | 723.5   | 3095                                      | 346.1   | 3532           | 1845.6  | 5994            | 1205.5  |
| <i>NTO (315nm)</i>  | 21                                        | 8.7     | 114            | 10.6    | 140             | 8.0     | 16                                        | 3.0     | 129            | 10.1    | 162             | 5.1     |
| o-NBA (surrogate)   | 93                                        | 4.3     | 82             | 4.3     | 98              | 4.0     | 52                                        | 3.8     | 79             | 6.8     | 82              | 1.8     |
| HMX                 | 87                                        | 2.6     | 88             | 5.7     | 96              | 1.6     | 102                                       | 5.0     | 60             | 6.0     | 54              | 2.1     |
| RDX                 | 88                                        | 2.3     | 93             | 2.5     | 96              | 5.2     | 94                                        | 6.3     | 108            | 4.4     | 88              | 3.2     |
| <i>PA (210nm)</i>   | 70                                        | 7.4     | 78             | 3.5     | 81              | 6.6     | 719                                       | 36.8    | 165            | 135.6   | 360             | 44.2    |
| <i>PA (315nm)</i>   | 71                                        | 1.7     | 68             | 2.4     | 73              | 2.3     | 51                                        | 2.0     | 0              | 0.0     | 7               | 0.4     |
| <i>4-NP</i>         | 101                                       | 1.9     | 97             | 3.1     | 104             | 4.5     | 57                                        | 2.4     | 48             | 6.3     | 48              | 3.5     |
| 1,3,5-TNB           | 52                                        | 0.6     | 64             | 6.1     | 54              | 1.5     | 53                                        | 1.5     | 59             | 2.9     | 57              | 3.4     |
| <i>2,4-DNP</i>      | 66                                        | 1.9     | 62             | 3.0     | 73              | 4.2     | 63                                        | 5.7     | 71             | 4.9     | 67              | 5.0     |
| 1,2-DNB (surrogate) | 80                                        | 0.1     | 81             | 3.7     | 82              | 4.3     | 76                                        | 0.6     | 78             | 6.6     | 84              | 6.3     |
| 1,3-DNB             | 89                                        | 1.4     | 90             | 3.5     | 94              | 1.4     | 76                                        | 1.3     | 79             | 0.9     | 78              | 2.4     |
| <i>2-NP</i>         | 92                                        | 1.6     | 80             | 2.8     | 87              | 1.6     | 73                                        | 1.9     | 67             | 1.6     | 72              | 4.1     |
| NB                  | 78                                        | 1.2     | 75             | 6.4     | 77              | 0.7     | 73                                        | 1.2     | 77             | 3.8     | 77              | 3.7     |
| 3,5-DNA (210nm)     | 92                                        | 1.7     | 92             | 3.8     | 99              | 7.3     | 79                                        | 4.6     | 82             | 1.3     | 84              | 7.4     |
| 3,5-DNA (254nm)     | 97                                        | 2.2     | 104            | 2.3     | 104             | 3.5     | 85                                        | 2.0     | 89             | 3.9     | 93              | 5.5     |
| <i>DNAN</i>         | 107                                       | 3.2     | 107            | 3.9     | 109             | 6.1     | 76                                        | 2.4     | 89             | 2.9     | 92              | 2.2     |
| Tetryl              | 2                                         | 2.8     | 0              | 0.0     | 0               | 0.0     | 7                                         | 6.4     | 11             | 2.1     | 12              | 2.3     |
| NG                  | 42                                        | 3.5     | 36             | 7.2     | 44              | 4.5     | 39                                        | 4.6     | 41             | 2.8     | 45              | 2.7     |
| 2,4,6-TNT           | 78                                        | 2.1     | 90             | 3.4     | 87              | 4.6     | 71                                        | 2.7     | 87             | 1.6     | 86              | 2.8     |
| 4-Am-2,6-DNT        | 67                                        | 0.5     | 66             | 3.3     | 64              | 5.5     | 64                                        | 2.3     | 69             | 4.2     | 68              | 3.0     |
| 2-Am-4,6-DNT        | 83                                        | 2.2     | 82             | 3.7     | 86              | 1.8     | 73                                        | 2.5     | 79             | 2.8     | 77              | 9.5     |
| 2,6-DNT             | 88                                        | 6.0     | 96             | 4.3     | 100             | 0.4     | 72                                        | 4.3     | 74             | 2.7     | 72              | 2.7     |
| 2,4-DNT             | 88                                        | 4.8     | 88             | 1.4     | 94              | 3.0     | 72                                        | 2.7     | 75             | 1.3     | 76              | 2.3     |
| 2-NT                | 91                                        | 3.2     | 91             | 5.0     | 97              | 6.8     | 77                                        | 5.7     | 75             | 3.1     | 94              | 9.1     |
| 4-NT                | 86                                        | 1.2     | 78             | 7.5     | 88              | 3.4     | 71                                        | 2.9     | 73             | 7.8     | 70              | 3.4     |
| 3-NT                | 85                                        | 2.4     | 88             | 2.1     | 97              | 1.3     | 71                                        | 1.6     | 75             | 3.3     | 74              | 2.7     |
| PETN                | 67                                        | 5.6     | 59             | 1.6     | 65              | 6.0     | 52                                        | 7.5     | 60             | 5.9     | 60              | 4.0     |

***Bold, italicized*** are IM compounds and degradation products.

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

Table 18 (cont.)

| Compound            | Recoveries (%)                            |         |                |         |                 |         |                                           |         |                |         |                 |         |
|---------------------|-------------------------------------------|---------|----------------|---------|-----------------|---------|-------------------------------------------|---------|----------------|---------|-----------------|---------|
|                     | Polychaete Worm                           |         |                |         |                 |         | Ryegrass                                  |         |                |         |                 |         |
|                     | No Chromatographic Interference Reduction |         | Alumina Column |         | Florisol Column |         | No Chromatographic Interference Reduction |         | Alumina Column |         | Florisol Column |         |
|                     | Average (n=3)                             | Std Dev | Average (n=3)  | Std Dev | Average (n=3)   | Std Dev | Average (n=3)                             | Std Dev | Average (n=3)  | Std Dev | Average (n=3)   | Std Dev |
| <i>NQ</i>           | 2135                                      | 188.2   | 324            | 206.0   | 889             | 7.4     | 159                                       | 4.1     | 88             | 6.0     | 53              | 29.3    |
| <i>NTO (210nm)</i>  | 1613                                      | 202.6   | 1955           | 372.4   | 3060            | 99.2    | 1612                                      | 92.2    | 984            | 258.5   | 1084            | 1094.1  |
| <i>NTO (315nm)</i>  | 20                                        | 1.9     | 109            | 11.6    | 174             | 11.2    | 8                                         | 5.7     | 86             | 27.9    | 363             | 493.2   |
| o-NBA (surrogate)   | 24                                        | 19.4    | 78             | 6.9     | 90              | 5.9     | 29                                        | 8.4     | 137            | 43.2    | 113             | 96.0    |
| HMX                 | 68                                        | 6.7     | 71             | 2.0     | 71              | 2.2     | 75                                        | 6.4     | 85             | 5.0     | 68              | 23.1    |
| RDX                 | 77                                        | 0.9     | 77             | 2.6     | 76              | 1.4     | 97                                        | 3.3     | 88             | 6.8     | 89              | 18.8    |
| <i>PA (210nm)</i>   | 75                                        | 5.0     | 83             | 8.5     | 106             | 5.1     | 141                                       | 27.4    | 57             | 7.0     | 89              | 52.8    |
| <i>PA (315nm)</i>   | 67                                        | 0.4     | 65             | 3.7     | 64              | 4.8     | 78                                        | 4.4     | 76             | 14.2    | 65              | 21.5    |
| <i>4-NP</i>         | 75                                        | 0.6     | 75             | 1.3     | 78              | 1.2     | 83                                        | 4.5     | 81             | 3.5     | 78              | 12.3    |
| 1,3,5-TNB           | 47                                        | 0.8     | 47             | 0.4     | 46              | 5.8     | 75                                        | 3.6     | 86             | 6.6     | 75              | 20.2    |
| <i>2,4-DNP</i>      | 65                                        | 2.7     | 59             | 13.0    | 69              | 5.1     | 37                                        | 9.6     | 109            | 19.3    | 92              | 11.9    |
| 1,2-DNB (surrogate) | 70                                        | 8.6     | 74             | 3.4     | 72              | 5.3     | 69                                        | 1.1     | 74             | 5.1     | 70              | 9.2     |
| 1,3-DNB             | 70                                        | 2.4     | 75             | 1.5     | 73              | 2.2     | 78                                        | 2.0     | 78             | 2.8     | 72              | 12.7    |
| <i>2-NP</i>         | 75                                        | 3.4     | 66             | 4.5     | 67              | 1.8     | 73                                        | 1.8     | 63             | 3.5     | 66              | 11.4    |
| NB                  | 60                                        | 1.5     | 59             | 1.2     | 62              | 4.1     | 74                                        | 3.7     | 66             | 4.3     | 66              | 16.8    |
| 3,5-DNA (210nm)     | 78                                        | 1.2     | 82             | 1.5     | 85              | 7.1     | 72                                        | 1.8     | 70             | 2.8     | 67              | 15.5    |
| 3,5-DNA (254nm)     | 81                                        | 3.0     | 81             | 4.6     | 86              | 5.9     | 72                                        | 0.4     | 74             | 5.1     | 71              | 16.2    |
| <i>DNAN</i>         | 79                                        | 3.2     | 73             | 1.6     | 77              | 5.0     | 83                                        | 1.1     | 82             | 8.1     | 69              | 9.8     |
| Tetryl              | 0                                         | 0.0     | 0              | 0.0     | 0               | 0.0     | 20                                        | 2.6     | 17             | 2.2     | 18              | 0.9     |
| NG                  | 68                                        | 4.9     | 67             | 7.4     | 63              | 2.4     | 104                                       | 4.9     | 104            | 9.1     | 86              | 19.1    |
| 2,4,6-TNT           | 64                                        | 1.7     | 64             | 5.6     | 60              | 5.9     | 75                                        | 4.6     | 75             | 3.5     | 70              | 13.0    |
| 4-Am-2,6-DNT        | 44                                        | 0.4     | 44             | 5.6     | 48              | 1.2     | 61                                        | 16.8    | 45             | 4.8     | 42              | 9.4     |
| 2-Am-4,6-DNT        | 65                                        | 1.2     | 64             | 6.6     | 68              | 2.8     | 72                                        | 7.8     | 73             | 4.2     | 65              | 11.9    |
| 2,6-DNT             | 80                                        | 1.5     | 75             | 4.1     | 79              | 3.7     | 75                                        | 3.7     | 72             | 3.1     | 67              | 20.2    |
| 2,4-DNT             | 70                                        | 1.2     | 73             | 3.9     | 75              | 2.8     | 76                                        | 4.0     | 75             | 3.4     | 73              | 14.6    |
| 2-NT                | 90                                        | 2.2     | 77             | 6.1     | 93              | 5.0     | 77                                        | 2.5     | 84             | 6.7     | 71              | 13.4    |
| 4-NT                | 86                                        | 4.8     | 69             | 6.1     | 75              | 3.8     | 73                                        | 6.7     | 75             | 4.2     | 62              | 12.1    |
| 3-NT                | 81                                        | 4.4     | 79             | 3.5     | 82              | 1.9     | 68                                        | 2.9     | 73             | 1.4     | 64              | 9.1     |
| PETN                | 61                                        | 2.2     | 57             | 2.0     | 59              | 2.5     | 82                                        | 7.4     | 74             | 7.2     | 72              | 10.7    |

***Bold, italicized*** are IM compounds and degradation products.

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

(DoD QSM Ver5.2 for Solids – Lowest LCL: 4-Am-2,6-DNT, 64%; Highest UCL: Tetryl, 135%)

Table 19. Recoveries (%) from post-extraction spiked fathead minnow tissue MeOH extract, following five different chromatographic treatment options for matrix-related analytical interference reduction.

| Compound            | Fathead Minnow - Recoveries (%)  |         |                                  |             |                                     |              |                  |         |                  |              |
|---------------------|----------------------------------|---------|----------------------------------|-------------|-------------------------------------|--------------|------------------|---------|------------------|--------------|
|                     | Neutral Alumina<br> <br>Florisil |         | Florisil<br> <br>Neutral Alumina |             | Mixed Neutral<br>Alumina - Florisil |              | Basic Alumina    |         | Silica Gel       |              |
|                     | Average<br>(n=3)                 | Std Dev | Average<br>(n=2)                 | Std Dev     | Average<br>(n=3)                    | Std Dev      | Average<br>(n=3) | Std Dev | Average<br>(n=3) | Std Dev      |
| <i>NQ</i>           | <b>81</b>                        | 6.7     | <b>81</b>                        | <b>0.7</b>  | <b>77</b>                           | <b>9.8</b>   | <b>150</b>       | 8.7     | <b>92</b>        | 4.7          |
| <i>NTO (210nm)</i>  | <b>2280</b>                      | 747.3   | <b>1766</b>                      | <b>41.0</b> | <b>1797</b>                         | <b>157.0</b> | <b>3924</b>      | 189.3   | <b>2054</b>      | <b>179.0</b> |
| <i>NTO (315nm)</i>  | <b>130</b>                       | 1.7     | <b>116</b>                       | <b>2.0</b>  | <b>119</b>                          | <b>9.4</b>   | <b>127</b>       | 10.6    | <b>141</b>       | 5.3          |
| o-NBA (surrogate)   | 100                              | 3.6     | 93                               | 0.1         | 96                                  | 5.3          | 105              | 2.8     | 95               | 5.4          |
| HMX                 | 104                              | 9.9     | 94                               | 1.5         | 94                                  | 6.3          | 100              | 4.1     | 98               | 3.3          |
| RDX                 | 99                               | 3.5     | 97                               | 1.5         | 94                                  | 9.7          | 106              | 1.1     | 99               | 2.9          |
| <i>PA (210nm)</i>   | <b>119</b>                       | 29.2    | <b>115</b>                       | <b>31.0</b> | <b>127</b>                          | <b>60.9</b>  | <b>101</b>       | 3.7     | <b>124</b>       | <b>14.1</b>  |
| <i>PA (315nm)</i>   | <b>96</b>                        | 3.3     | <b>97</b>                        | <b>0.7</b>  | <b>99</b>                           | <b>2.5</b>   | <b>121</b>       | 7.2     | <b>99</b>        | <b>3.6</b>   |
| <i>4-NP</i>         | <b>108</b>                       | 2.2     | <b>107</b>                       | <b>0.5</b>  | <b>110</b>                          | <b>1.7</b>   | <b>106</b>       | 4.3     | <b>112</b>       | <b>2.0</b>   |
| 1,3,5-TNB           | 102                              | 4.5     | 97                               | 4.6         | 97                                  | 3.4          | 97               | 4.5     | 106              | 4.1          |
| <i>2,4-DNP</i>      | <b>106</b>                       | 9.7     | <b>91</b>                        | <b>2.7</b>  | <b>98</b>                           | <b>7.6</b>   | <b>96</b>        | 1.5     | <b>94</b>        | <b>4.2</b>   |
| 1,2-DNB (surrogate) | 101                              | 3.3     | 97                               | 3.7         | 97                                  | 6.8          | 102              | 4.6     | 100              | 4.6          |
| 1,3-DNB             | 100                              | 6.5     | 96                               | 0.8         | 101                                 | 4.9          | 99               | 3.2     | 98               | 2.6          |
| <i>2-NP</i>         | <b>106</b>                       | 25.8    | <b>90</b>                        | <b>0.9</b>  | <b>104</b>                          | <b>18.5</b>  | <b>97</b>        | 2.5     | <b>92</b>        | <b>4.3</b>   |
| NB                  | 95                               | 4.8     | 96                               | 5.1         | 93                                  | 11.3         | 97               | 4.1     | 96               | 1.0          |
| 3,5-DNA (210nm)     | 88                               | 7.1     | 91                               | 8.3         | 89                                  | 11.5         | 111              | 3.8     | 91               | 3.2          |
| 3,5-DNA (254nm)     | 94                               | 0.9     | 94                               | 1.0         | 96                                  | 2.1          | 106              | 8.1     | 97               | 1.7          |
| <i>DNAN</i>         | <b>104</b>                       | 3.5     | <b>100</b>                       | <b>10.8</b> | <b>98</b>                           | <b>10.8</b>  | <b>101</b>       | 8.1     | <b>104</b>       | <b>3.7</b>   |
| Tetryl              | 92                               | 3.8     | 102                              | 2.4         | 102                                 | 7.8          | 89               | 2.7     | 96               | 3.6          |
| NG                  | 91                               | 5.5     | 100                              | 3.9         | 104                                 | 9.0          | 111              | 5.7     | 88               | 11.6         |
| 2,4,6-TNT           | 98                               | 8.6     | 100                              | 7.1         | 102                                 | 4.8          | 99               | 7.0     | 95               | 6.5          |
| 4-Am-2,6-DNT        | 94                               | 7.1     | 93                               | 0.5         | 97                                  | 3.5          | 92               | 3.9     | 88               | 3.8          |
| 2-Am-4,6-DNT        | 102                              | 10.5    | 97                               | 3.9         | 104                                 | 6.4          | 97               | 5.6     | 99               | 7.4          |
| 2,6-DNT             | 106                              | 12.6    | 120                              | 2.2         | 98                                  | 8.5          | 104              | 5.8     | 96               | 6.2          |
| 2,4-DNT             | 97                               | 1.7     | 98                               | 2.2         | 99                                  | 5.9          | 97               | 7.1     | 101              | 3.8          |
| 2-NT                | 94                               | 0.8     | 95                               | 0.6         | 100                                 | 1.3          | 99               | 5.8     | 99               | 2.6          |
| 4-NT                | 101                              | 14.0    | 94                               | 0.3         | 100                                 | 5.0          | 96               | 7.3     | 97               | 4.6          |
| 3-NT                | 96                               | 3.7     | 98                               | 6.1         | 101                                 | 2.6          | 98               | 8.9     | 97               | 5.8          |
| PETN                | 101                              | 7.7     | 91                               | 0.4         | 98                                  | 6.0          | 96               | 6.9     | 94               | 8.2          |

***Bold, italicized*** are IM compounds and degradation products.

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

(DoD QSM Ver5.2 for Solids – Lowest LCL: 4-Am-2,6-DNT, 64%; Highest UCL: Tetryl, 135%)

Table 20. Recoveries (%) from post-extraction spiked earthworm tissue MeOH extract, following five different chromatographic treatment options for matrix-related analytical interference reduction.

| Compound            | Earthworm - Recoveries (%)       |              |                                  |              |                                     |             |                  |              |                  |             |
|---------------------|----------------------------------|--------------|----------------------------------|--------------|-------------------------------------|-------------|------------------|--------------|------------------|-------------|
|                     | Neutral Alumina<br> <br>Florisil |              | Florisil<br> <br>Neutral Alumina |              | Mixed Neutral<br>Alumina - Florisil |             | Basic Alumina    |              | Silica Gel       |             |
|                     | Average<br>(n=3)                 | Std Dev      | Average<br>(n=3)                 | Std Dev      | Average<br>(n=3)                    | Std Dev     | Average<br>(n=3) | Std Dev      | Average<br>(n=3) | Std Dev     |
| <i>NQ</i>           | <b>95</b>                        | <b>1.2</b>   | <b>67</b>                        | <b>1.2</b>   | <b>84</b>                           | <b>9.7</b>  | <b>98</b>        | <b>1.0</b>   | <b>116</b>       | <b>4.9</b>  |
| <i>NTO (210nm)</i>  | <b>1814</b>                      | <b>19.8</b>  | <b>2081</b>                      | <b>180.4</b> | <b>1971</b>                         | <b>69.4</b> | <b>746</b>       | <b>255.7</b> | <b>2244</b>      | <b>54.6</b> |
| <i>NTO (315nm)</i>  | <b>121</b>                       | <b>5.7</b>   | <b>115</b>                       | <b>10.5</b>  | <b>117</b>                          | <b>2.6</b>  | <b>82</b>        | <b>8.7</b>   | <b>138</b>       | <b>6.2</b>  |
| o-NBA (surrogate)   | 86                               | 15.9         | 89                               | 12.8         | 76                                  | 7.3         | 146              | 17.0         | 78               | 5.5         |
| HMX                 | 89                               | 9.6          | 91                               | 11.8         | 86                                  | 7.8         | 91               | 2.8          | 81               | 7.1         |
| RDX                 | 76                               | 33.7         | 96                               | 3.2          | 96                                  | 3.0         | 107              | 7.4          | 99               | 0.1         |
| <i>PA (210nm)</i>   | <b>170</b>                       | <b>103.3</b> | <b>175</b>                       | <b>82.1</b>  | <b>149</b>                          | <b>70.8</b> | <b>86</b>        | <b>10.4</b>  | <b>112</b>       | <b>63.4</b> |
| <i>PA (315nm)</i>   | <b>93</b>                        | <b>6.9</b>   | <b>84</b>                        | <b>16.9</b>  | <b>80</b>                           | <b>9.7</b>  | <b>87</b>        | <b>23.2</b>  | <b>67</b>        | <b>14.8</b> |
| <i>4-NP</i>         | <b>113</b>                       | <b>27.3</b>  | <b>92</b>                        | <b>9.2</b>   | <b>90</b>                           | <b>0.7</b>  | <b>101</b>       | <b>0.7</b>   | <b>89</b>        | <b>10.4</b> |
| 1,3,5-TNB           | 96                               | 10.9         | 92                               | 3.7          | 92                                  | 4.4         | 98               | 0.4          | 89               | 8.5         |
| <i>2,4-DNP</i>      | <b>95</b>                        | <b>1.5</b>   | <b>96</b>                        | <b>2.4</b>   | <b>96</b>                           | <b>1.5</b>  | <b>104</b>       | <b>12.3</b>  | <b>95</b>        | <b>4.1</b>  |
| 1,2-DNB (surrogate) | 97                               | 2.8          | 100                              | 0.7          | 98                                  | 1.0         | 104              | 9.6          | 101              | 2.8         |
| 1,3-DNB             | 95                               | 1.5          | 98                               | 1.2          | 96                                  | 2.3         | 100              | 0.4          | 96               | 2.0         |
| <i>2-NP</i>         | <b>101</b>                       | <b>14.4</b>  | <b>91</b>                        | <b>3.1</b>   | <b>91</b>                           | <b>1.5</b>  | <b>85</b>        | <b>3.7</b>   | <b>88</b>        | <b>3.6</b>  |
| NB                  | 99                               | 2.0          | 98                               | 6.4          | 98                                  | 3.0         | 131              | 15.3         | 98               | 2.7         |
| 3,5-DNA (210nm)     | 97                               | 14.3         | 94                               | 4.4          | 88                                  | 3.0         | 100              | 0.6          | 90               | 4.4         |
| 3,5-DNA (254nm)     | 94                               | 2.6          | 96                               | 1.6          | 94                                  | 2.2         | 112              | 5.5          | 94               | 2.6         |
| <i>DNAN</i>         | <b>104</b>                       | <b>14.2</b>  | <b>102</b>                       | <b>6.2</b>   | <b>95</b>                           | <b>3.2</b>  | <b>113</b>       | <b>8.2</b>   | <b>98</b>        | <b>6.4</b>  |
| Tetryl              | 89                               | 13.0         | 103                              | 11.4         | 106                                 | 4.2         | 58               | 2.7          | 104              | 6.1         |
| NG                  | 70                               | 32.3         | 111                              | 16.7         | 107                                 | 20.8        | 169              | 11.6         | 116              | 19.2        |
| 2,4,6-TNT           | 95                               | 5.5          | 99                               | 5.1          | 103                                 | 7.0         | 108              | 1.4          | 104              | 8.3         |
| 4-Am-2,6-DNT        | 104                              | 10.5         | 98                               | 5.0          | 97                                  | 1.6         | 104              | 7.8          | 95               | 0.9         |
| 2-Am-4,6-DNT        | 105                              | 7.4          | 97                               | 1.3          | 98                                  | 3.0         | 104              | 9.5          | 100              | 2.7         |
| 2,6-DNT             | 96                               | 8.9          | 90                               | 2.5          | 93                                  | 6.7         | 102              | 2.4          | 85               | 1.0         |
| 2,4-DNT             | 99                               | 4.8          | 99                               | 3.2          | 96                                  | 3.6         | 101              | 1.1          | 97               | 2.9         |
| 2-NT                | 98                               | 4.1          | 101                              | 4.1          | 101                                 | 1.1         | 117              | 2.1          | 98               | 2.8         |
| 4-NT                | 97                               | 2.9          | 104                              | 9.7          | 94                                  | 5.9         | 99               | 4.6          | 100              | 0.8         |
| 3-NT                | 99                               | 5.1          | 100                              | 3.7          | 100                                 | 4.0         | 109              | 9.0          | 97               | 2.1         |
| PETN                | 99                               | 1.4          | 89                               | 5.4          | 89                                  | 6.5         | 95               | 2.0          | 95               | 6.4         |

***Bold, italicized*** are IM compounds and degradation products.

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

(DoD QSM Ver5.2 for Solids – Lowest LCL: 4-Am-2,6-DNT, 64%; Highest UCL: Tetryl, 135%)

Table 21. Recoveries (%) from post-extraction spiked polychaete worm tissue MeOH extract, following five different chromatographic treatment options for matrix-related analytical interference reduction.

| Compound            | Polychaete Worm - Recoveries (%) |              |                                  |              |                                     |             |                  |              |                  |             |
|---------------------|----------------------------------|--------------|----------------------------------|--------------|-------------------------------------|-------------|------------------|--------------|------------------|-------------|
|                     | Neutral Alumina<br> <br>Florisil |              | Florisil<br> <br>Neutral Alumina |              | Mixed Neutral<br>Alumina - Florisil |             | Basic Alumina    |              | Silica Gel       |             |
|                     | Average<br>(n=3)                 | Std Dev      | Average<br>(n=3)                 | Std Dev      | Average<br>(n=3)                    | Std Dev     | Average<br>(n=3) | Std Dev      | Average<br>(n=3) | Std Dev     |
| <i>NQ</i>           | <b>140</b>                       | <b>100.4</b> | <b>183</b>                       | <b>123.6</b> | <b>72</b>                           | <b>5.4</b>  | <b>80</b>        | <b>5.3</b>   | <b>92</b>        | <b>6.3</b>  |
| <i>NTO (210nm)</i>  | <b>977</b>                       | <b>218.1</b> | <b>848</b>                       | <b>49.0</b>  | <b>836</b>                          | <b>25.5</b> | <b>1860</b>      | <b>642.5</b> | <b>939</b>       | <b>61.5</b> |
| <i>NTO (315nm)</i>  | <b>134</b>                       | <b>2.1</b>   | <b>132</b>                       | <b>7.6</b>   | <b>136</b>                          | <b>5.1</b>  | <b>148</b>       | <b>5.2</b>   | <b>140</b>       | <b>7.3</b>  |
| o-NBA (surrogate)   | 102                              | 2.0          | 100                              | 0.7          | 102                                 | 3.6         | 105              | 6.2          | 98               | 6.8         |
| HMX                 | 98                               | 3.2          | 95                               | 2.5          | 97                                  | 3.5         | 92               | 1.7          | 91               | 8.2         |
| RDX                 | 99                               | 3.7          | 101                              | 3.1          | 100                                 | 3.5         | 95               | 1.8          | 95               | 6.7         |
| <i>PA (210nm)</i>   | <b>98</b>                        | <b>5.2</b>   | <b>102</b>                       | <b>11.9</b>  | <b>122</b>                          | <b>7.1</b>  | <b>93</b>        | <b>1.7</b>   | <b>101</b>       | <b>8.9</b>  |
| <i>PA (315nm)</i>   | <b>98</b>                        | <b>1.3</b>   | <b>99</b>                        | <b>0.7</b>   | <b>99</b>                           | <b>2.4</b>  | <b>95</b>        | <b>6.6</b>   | <b>94</b>        | <b>7.3</b>  |
| <i>4-NP</i>         | <b>99</b>                        | <b>1.7</b>   | <b>99</b>                        | <b>0.4</b>   | <b>100</b>                          | <b>3.5</b>  | <b>96</b>        | <b>1.6</b>   | <b>95</b>        | <b>6.3</b>  |
| 1,3,5-TNB           | 97                               | 2.8          | 96                               | 1.5          | 107                                 | 16.0        | 93               | 2.0          | 94               | 8.3         |
| <i>2,4-DNP</i>      | <b>98</b>                        | <b>3.9</b>   | <b>97</b>                        | <b>2.3</b>   | <b>90</b>                           | <b>9.5</b>  | <b>94</b>        | <b>1.9</b>   | <b>99</b>        | <b>8.0</b>  |
| 1,2-DNB (surrogate) | 104                              | 3.9          | 102                              | 1.5          | 92                                  | 9.4         | 97               | 2.3          | 101              | 7.9         |
| 1,3-DNB             | 99                               | 1.1          | 99                               | 2.2          | 100                                 | 3.4         | 97               | 5.1          | 96               | 8.0         |
| <i>2-NP</i>         | <b>91</b>                        | <b>4.3</b>   | <b>90</b>                        | <b>4.7</b>   | <b>89</b>                           | <b>4.1</b>  | <b>103</b>       | <b>7.6</b>   | <b>89</b>        | <b>5.2</b>  |
| NB                  | 98                               | 2.6          | 99                               | 0.4          | 98                                  | 5.1         | 91               | 2.5          | 97               | 9.9         |
| 3,5-DNA (210nm)     | 96                               | 7.9          | 100                              | 5.5          | 99                                  | 8.7         | 108              | 0.1          | 90               | 9.1         |
| 3,5-DNA (254nm)     | 103                              | 3.9          | 102                              | 2.6          | 104                                 | 7.1         | 105              | 11.1         | 93               | 8.6         |
| <i>DNAN</i>         | <b>98</b>                        | <b>9.3</b>   | <b>100</b>                       | <b>6.5</b>   | <b>92</b>                           | <b>1.4</b>  | <b>91</b>        | <b>4.8</b>   | <b>94</b>        | <b>8.6</b>  |
| Tetryl              | 101                              | 2.6          | 97                               | 0.7          | 97                                  | 3.6         | 89               | 0.4          | 95               | 8.4         |
| NG                  | 106                              | 5.6          | 95                               | 3.7          | 98                                  | 16.7        | 103              | 2.0          | 98               | 13.6        |
| 2,4,6-TNT           | 101                              | 1.5          | 98                               | 1.0          | 103                                 | 7.1         | 95               | 1.3          | 95               | 6.7         |
| 4-Am-2,6-DNT        | 99                               | 2.7          | 98                               | 5.5          | 101                                 | 9.5         | 91               | 1.6          | 93               | 9.2         |
| 2-Am-4,6-DNT        | 97                               | 3.3          | 95                               | 7.4          | 96                                  | 0.8         | 95               | 2.1          | 91               | 8.0         |
| 2,6-DNT             | 97                               | 2.4          | 100                              | 3.9          | 96                                  | 4.3         | 105              | 6.0          | 103              | 15.2        |
| 2,4-DNT             | 99                               | 3.3          | 94                               | 3.4          | 99                                  | 5.5         | 94               | 2.9          | 96               | 7.3         |
| 2-NT                | 100                              | 3.0          | 99                               | 4.1          | 102                                 | 8.8         | 103              | 3.3          | 92               | 6.8         |
| 4-NT                | 99                               | 1.4          | 96                               | 9.5          | 99                                  | 1.1         | 94               | 0.9          | 93               | 6.8         |
| 3-NT                | 106                              | 4.7          | 104                              | 4.2          | 106                                 | 5.3         | 101              | 1.8          | 96               | 8.6         |
| PETN                | 100                              | 0.5          | 95                               | 4.8          | 99                                  | 2.9         | 95               | 7.8          | 90               | 8.5         |

***Bold, italicized*** are IM compounds and degradation products.

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

(DoD QSM Ver5.2 for Solids – Lowest LCL: 4-Am-2,6-DNT, 64%; Highest UCL: Tetryl, 135%)

Table 22. Recoveries (%) from post-extraction spiked ryegrass tissue MeOH extract, following five different chromatographic treatment options for matrix-related analytical interference reduction.

| Compound            | Ryegrass - Recoveries (%)        |              |                                  |             |                                     |             |                  |               |                  |             |
|---------------------|----------------------------------|--------------|----------------------------------|-------------|-------------------------------------|-------------|------------------|---------------|------------------|-------------|
|                     | Neutral Alumina<br> <br>Florisil |              | Florisil<br> <br>Neutral Alumina |             | Mixed Neutral<br>Alumina - Florisil |             | Basic Alumina    |               | Silica Gel       |             |
|                     | Average<br>(n=3)                 | Std Dev      | Average<br>(n=3)                 | Std Dev     | Average<br>(n=3)                    | Std Dev     | Average<br>(n=3) | Std Dev       | Average<br>(n=3) | Std Dev     |
| <i>NQ</i>           | <b>97</b>                        | <b>4.7</b>   | <b>82</b>                        | <b>7.6</b>  | <b>88</b>                           | <b>0.7</b>  | <b>100</b>       | <b>3.5</b>    | <b>90</b>        | <b>7.0</b>  |
| <i>NTO (210nm)</i>  | <b>573</b>                       | <b>147.7</b> | <b>576</b>                       | <b>63.6</b> | <b>500</b>                          | <b>45.4</b> | <b>1081</b>      | <b>1272.6</b> | <b>595</b>       | <b>29.7</b> |
| <i>NTO (315nm)</i>  | <b>114</b>                       | <b>3.2</b>   | <b>112</b>                       | <b>3.0</b>  | <b>113</b>                          | <b>3.9</b>  | <b>174</b>       | <b>146.1</b>  | <b>130</b>       | <b>4.9</b>  |
| o-NBA (surrogate)   | 138                              | 29.6         | 127                              | 15.0        | 171                                 | 6.3         | 107              | 8.4           | 146              | 8.0         |
| HMX                 | 98                               | 2.7          | 93                               | 5.5         | 101                                 | 4.0         | 99               | 5.0           | 96               | 9.9         |
| RDX                 | 100                              | 5.2          | 100                              | 2.7         | 101                                 | 3.1         | 101              | 2.9           | 99               | 2.8         |
| <i>PA (210nm)</i>   | <b>96</b>                        | <b>12.1</b>  | <b>84</b>                        | <b>3.1</b>  | <b>101</b>                          | <b>2.8</b>  | <b>90</b>        | <b>15.6</b>   | <b>96</b>        | <b>5.4</b>  |
| <i>PA (315nm)</i>   | <b>100</b>                       | <b>3.5</b>   | <b>101</b>                       | <b>1.8</b>  | <b>101</b>                          | <b>2.5</b>  | <b>87</b>        | <b>12.5</b>   | <b>93</b>        | <b>3.4</b>  |
| <i>4-NP</i>         | <b>101</b>                       | <b>1.0</b>   | <b>100</b>                       | <b>3.1</b>  | <b>98</b>                           | <b>1.8</b>  | <b>98</b>        | <b>4.2</b>    | <b>96</b>        | <b>3.9</b>  |
| 1,3,5-TNB           | 99                               | 3.6          | 100                              | 6.1         | 98                                  | 2.7         | 96               | 3.4           | 96               | 5.8         |
| <i>2,4-DNP</i>      | <b>98</b>                        | <b>2.4</b>   | <b>106</b>                       | <b>10.8</b> | <b>104</b>                          | <b>4.3</b>  | <b>95</b>        | <b>6.1</b>    | <b>96</b>        | <b>4.8</b>  |
| 1,2-DNB (surrogate) | 108                              | 1.3          | 112                              | 5.8         | 106                                 | 2.4         | 104              | 1.7           | 103              | 9.3         |
| 1,3-DNB             | 100                              | 2.4          | 101                              | 1.4         | 99                                  | 2.1         | 99               | 2.1           | 97               | 4.2         |
| <i>2-NP</i>         | <b>92</b>                        | <b>4.2</b>   | <b>84</b>                        | <b>3.6</b>  | <b>95</b>                           | <b>4.7</b>  | <b>84</b>        | <b>15.1</b>   | <b>88</b>        | <b>4.1</b>  |
| NB                  | 103                              | 1.4          | 103                              | 4.0         | 100                                 | 0.5         | 97               | 3.9           | 103              | 2.6         |
| 3,5-DNA (210nm)     | 101                              | 2.9          | 113                              | 6.2         | 96                                  | 1.2         | 98               | 20.6          | 97               | 4.0         |
| 3,5-DNA (254nm)     | 101                              | 1.4          | 103                              | 3.3         | 97                                  | 4.1         | 99               | 25.7          | 94               | 1.6         |
| <i>DNAN</i>         | <b>102</b>                       | <b>4.7</b>   | <b>115</b>                       | <b>7.3</b>  | <b>96</b>                           | <b>1.9</b>  | <b>92</b>        | <b>5.0</b>    | <b>100</b>       | <b>3.7</b>  |
| Tetryl              | 100                              | 3.6          | 97                               | 3.6         | 94                                  | 2.8         | 95               | 8.4           | 96               | 7.5         |
| NG                  | 109                              | 8.3          | 100                              | 2.1         | 94                                  | 2.4         | 109              | 7.3           | 86               | 13.3        |
| 2,4,6-TNT           | 110                              | 8.2          | 105                              | 8.5         | 98                                  | 4.1         | 100              | 2.8           | 97               | 3.3         |
| 4-Am-2,6-DNT        | 98                               | 1.7          | 99                               | 3.7         | 98                                  | 2.6         | 96               | 5.4           | 104              | 9.9         |
| 2-Am-4,6-DNT        | 98                               | 0.4          | 100                              | 6.0         | 97                                  | 2.6         | 99               | 2.0           | 95               | 6.1         |
| 2,6-DNT             | 98                               | 3.1          | 97                               | 8.2         | 96                                  | 2.0         | 93               | 9.8           | 108              | 8.5         |
| 2,4-DNT             | 98                               | 2.0          | 101                              | 1.7         | 101                                 | 5.5         | 99               | 2.3           | 95               | 3.8         |
| 2-NT                | 99                               | 3.0          | 101                              | 5.7         | 102                                 | 3.3         | 93               | 23.3          | 97               | 2.6         |
| 4-NT                | 101                              | 0.3          | 99                               | 1.1         | 99                                  | 4.2         | 97               | 7.3           | 96               | 2.7         |
| 3-NT                | 107                              | 6.1          | 98                               | 7.1         | 99                                  | 9.8         | 92               | 16.4          | 95               | 7.0         |
| PETN                | 100                              | 2.3          | 97                               | 3.8         | 101                                 | 5.4         | 107              | 7.3           | 99               | 1.1         |

***Bold, italicized*** are IM compounds and degradation products.

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

(DoD QSM Ver5.2 for Solids – Lowest LCL: 4-Am-2,6-DNT, 64%; Highest UCL: Tetryl, 135%)

Table 23. Recoveries (%) from pre-extraction spiked tissue MeOH extracts, following chromatographic treatment with silica gel for matrix-related analytical interference reduction.

| Compound                | Recoveries (%) |                 |             |            |
|-------------------------|----------------|-----------------|-------------|------------|
|                         | Fathead Minnow | Polychaete Worm | Earthworm   | Ryegrass   |
| <b><i>NQ</i></b>        | <b>113</b>     | <b>73</b>       | <b>86</b>   | <b>68</b>  |
| <b><i>NTO (210)</i></b> | <b>8477</b>    | <b>5318</b>     | <b>4503</b> | <b>149</b> |
| <b><i>NTO (315)</i></b> | <b>87</b>      | <b>113</b>      | <b>98</b>   | <b>133</b> |
| o-NBA (surrogate)       | 101            | 82              | 111         | 67         |
| HMX                     | 81             | 66              | 79          | 99         |
| RDX                     | 86             | 66              | 93          | 85         |
| <b><i>PA (210)</i></b>  | <b>79</b>      | <b>73</b>       | <b>37</b>   | <b>79</b>  |
| <b><i>PA (315)</i></b>  | <b>66</b>      | <b>66</b>       | <b>68</b>   | <b>71</b>  |
| <b><i>4-NP</i></b>      | <b>83</b>      | <b>71</b>       | <b>72</b>   | <b>84</b>  |
| 1,3,5-TNB               | 84             | 52              | 3670        | 83         |
| <b><i>2,4-DNP</i></b>   | <b>110</b>     | <b>72</b>       | <b>81</b>   | <b>93</b>  |
| 1,2-DNB (surrogate)     | 93             | 72              | 82          | 73         |
| 1,3-DNB                 | 76             | 65              | 65          | 86         |
| <b><i>2-NP</i></b>      | <b>105</b>     | <b>81</b>       | <b>74</b>   | <b>94</b>  |
| NB                      | 84             | 94              | 83          | 90         |
| 3,5-DNA (210)           | 183            | 139             | 73          | 90         |
| 3,5-DNA (254)           | 69             | 75              | 80          | 73         |
| <b><i>DNAN</i></b>      | <b>123</b>     | <b>72</b>       | <b>86</b>   | <b>76</b>  |
| 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         |

***Bold, italicized*** are IM compounds and degradation products.

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

(DoD QSM Ver5.2 for Solids – Lowest LCL: 4-Am-2,6-DNT, 64%; Highest UCL: Tetryl, 135%)

## Stability Study

Extraction and analysis hold time studies were carried out in reagent water (direct-injection and SPE) and three soils (Riley, Yuma, and ASTM fat clay, described in Table 13) spiked with the 17 legacy and seven IM compounds of interest and two surrogates. Currently accepted pre-extraction hold times are seven days for waters and 14 days for solid matrices, and post-extraction hold time for analysis is 40 days for samples analyzed by USEPA Method 8330B, which references USEPA SW-846 Chapter 4.1 guidelines for semi-volatile organics (USEPA, 2014). As these hold times were adopted for methods including only legacy munition compounds, experiments were carried out to test whether the limits would also be appropriate for the seven compounds of interest and *o*-NBA surrogate added under SERDP-ER2722. Soils with varying physicochemical properties were tested in order to observe any potential matrix-related variability.

Results for the pre-extraction hold time experiments for water are shown in Table 24. For direct-injection water samples, the majority of compounds were recovered within current DoD QSM limits on Day 0 and Day 7, so most compounds were stable under all three storage conditions for at least seven days. However, NTO recovery was <57% on Day 7 for samples stored at room temperature in clear containers. For unknown reasons, NTO determinations were generally very high in this study, possibly suffering from analytical interference not observed elsewhere in the current project. Still, the recoveries for NTO stored at room temperature in clear containers were extremely low compared to those for samples stored under the other two conditions. Therefore, NTO determinations in samples stored in light for extended periods might not be reliable.

In general, SPE option B was more effective at NQ recovery than option A, and option B was selected during SPE method finalization. Apart from possible NTO instability, only NQ showed possible instability at room temperature. NQ recoveries were lower for room temperature than for cold samples, and were lower in samples stored in clear containers than those stored in amber containers. Thus, NQ determinations in samples stored at room temperature (especially in light) for extended periods might not be reliable.

Results for the post-extraction hold time experiments for water are shown in Table 25. Again, for unknown reasons, NTO determinations were generally very high in this study, possibly due to analytical interference. Overall, though, NTO recoveries decreased from Day 0 to Day 40, and were lower in room temperature samples, especially those stored in clear containers. However, all of the NTO recoveries were nominally within current DoD QSM limits. Therefore, determination of NTO might be affected by possible instability issues.

On the other hand, NQ determinations in SPE samples were quite different depending upon the SPE cartridge option used to extract/pre-concentrate the water sample. For SPE option A, recoveries for NQ were much higher (about 100%) in Day 40 samples, whereas the average recovery on Day 0 was very low (19%). It is suspected that there could be a positive analytical interferent, such as a degradation product, accumulating over the course of the 40-day storage period, and enhancing the NQ recovery determination. Alternately, for SPE option B, recoveries for NQ were fairly stable over the 40-day period.

In addition, instability of the three NTs was observed in direct-injection samples and SPE samples, with recoveries for each of them generally decreasing with increased temperature and light exposure. Recoveries of these compounds were within current DoD QSM limits for Day 40 samples stored in cold temperatures, indicating the instability could be reduced by avoiding elevated temperatures, and keeping with current recommendations to store at  $\leq 4^{\circ}\text{C}$ .

Table 24. Extraction hold time stability for laboratory-spiked reagent water samples over seven days.

| Compound            | Recoveries (%)                       |         |               |              |               |         |                  |         |               |                                      |         |               |                       |         |               |                        |               |         |
|---------------------|--------------------------------------|---------|---------------|--------------|---------------|---------|------------------|---------|---------------|--------------------------------------|---------|---------------|-----------------------|---------|---------------|------------------------|---------------|---------|
|                     | Waters Day 0 Extraction and Analysis |         |               |              |               |         |                  |         |               | Waters Day 7 Extraction and Analysis |         |               |                       |         |               |                        |               |         |
|                     | SPE A (evap)                         |         |               | SPE B (evap) |               |         | Direct Injection |         |               | Cold/Dark                            |         |               | Room Temperature/Dark |         |               | Room Temperature/Light |               |         |
|                     | Average (n=3)                        | Std Dev | Average (n=3) | Std Dev      | Average (n=3) | Std Dev | Average (n=3)    | Std Dev | Average (n=3) | Average (n=3)                        | Std Dev | Average (n=3) | Average (n=3)         | Std Dev | Average (n=3) | Std Dev                | Average (n=3) | Std Dev |
| NQ                  | 19                                   | 1.4     | 42            | 1.9          | 74            | 13.6    | 14               | 2.9     | 34            | 4.3                                  | 75      | 20.8          | 15                    | 2.1     | 26            | 1.1                    | 88            | 9.8     |
| NTQ (210nm)         | 261                                  | 2.5     | 286           | 14.1         | 138           | 2.8     | 305              | 37.8    | 311           | 4.2                                  | 79      | 19.2          | 293                   | 17.7    | 303           | 6.3                    | 80            | 3.8     |
| NTQ (315nm)         | 270                                  | 2.4     | 292           | 15.2         | 99            | 14.2    | 306              | 40.9    | 307           | 2.4                                  | 78      | 25.0          | 291                   | 16.4    | 299           | 2.3                    | 68            | 17.1    |
| o-NBA (surrogate)   | 107                                  | 0.9     | 112           | 7.0          | 106           | 10.6    | 106              | 16.1    | 104           | 4.7                                  | 100     | 23.0          | 99                    | 5.3     | 99            | 2.1                    | 101           | 4.2     |
| HMN                 | 91                                   | 0.8     | 98            | 5.1          | 88            | 9.6     | 104              | 10.2    | 108           | 2.3                                  | 95      | 0.6           | 100                   | 3.9     | 104           | 0.7                    | 100           | 4.7     |
| RDX                 | 77                                   | 4.3     | 88            | 7.8          | 68            | 10.3    | 93               | 20.9    | 102           | 2.4                                  | 91      | 3.1           | 78                    | 19.0    | 101           | 3.3                    | 90            | 0.3     |
| PA (210nm)          | 81                                   | 1.5     | 93            | 5.4          | 84            | 5.8     | 101              | 21.7    | 111           | 0.3                                  | 97      | 5.6           | 88                    | 21.6    | 107           | 5.4                    | 116           | 6.5     |
| PA (315nm)          | 93                                   | 1.1     | 100           | 5.4          | 83            | 4.9     | 107              | 6.9     | 109           | 0.9                                  | 97      | 0.3           | 101                   | 6.0     | 107           | 2.1                    | 103           | 7.6     |
| 4-NP                | 90                                   | 0.8     | 97            | 5.0          | 92            | 6.3     | 110              | 7.0     | 114           | 1.0                                  | 109     | 1.0           | 105                   | 6.4     | 111           | 0.3                    | 105           | 5.2     |
| 1,3,5-TNB           | 89                                   | 1.3     | 95            | 4.9          | 87            | 7.0     | 104              | 6.5     | 105           | 1.2                                  | 102     | 1.2           | 99                    | 6.1     | 102           | 0.8                    | 104           | 1.0     |
| 2,4-DNP             | 85                                   | 0.6     | 90            | 4.3          | 96            | 2.5     | 106              | 18.7    | 114           | 0.8                                  | 119     | 7.9           | 95                    | 19.5    | 112           | 0.7                    | 136           | 4.7     |
| 1,2-DNB (surrogate) | 87                                   | 0.8     | 79            | 7.0          | 97            | 5.3     | 114              | 8.9     | 115           | 3.6                                  | 116     | 10.3          | 109                   | 5.4     | 113           | 1.6                    | 115           | 3.7     |
| 1,3-DNB             | 84                                   | 0.8     | 77            | 6.7          | 87            | 6.2     | 104              | 5.6     | 106           | 1.0                                  | 104     | 3.1           | 100                   | 5.5     | 104           | 1.6                    | 104           | 1.7     |
| 2-NP                | 5                                    | 3.0     | 0             | 0.0          | 87            | 0.8     | 88               | 5.9     | 93            | 1.9                                  | 87      | 5.6           | 82                    | 5.5     | 89            | 0.6                    | 92            | 5.7     |
| NB                  | 90                                   | 2.3     | 97            | 7.9          | 79            | 9.7     | 104              | 7.1     | 106           | 0.6                                  | 106     | 5.1           | 98                    | 6.1     | 104           | 0.2                    | 108           | 6.0     |
| 3,5-DNA (210nm)     | 1                                    | 1.1     | 0             | 0.0          | 88            | 9.4     | 93               | 8.0     | 101           | 1.4                                  | 107     | 12.2          | 81                    | 0.1     | 98            | 1.9                    | 101           | 6.7     |
| 3,5-DNA (254nm)     | 1                                    | 0.8     | 0             | 0.0          | 87            | 10.8    | 93               | 7.5     | 102           | 2.2                                  | 98      | 3.6           | 86                    | 5.3     | 99            | 1.6                    | 96            | 2.0     |
| DNAN                | 88                                   | 0.5     | 92            | 6.5          | 72            | 5.4     | 98               | 5.2     | 98            | 1.4                                  | 109     | 0.1           | 92                    | 6.3     | 96            | 1.7                    | 100           | 2.5     |
| Tetryl              | 89                                   | 1.8     | 95            | 4.6          | 86            | 16.1    | 100              | 5.7     | 103           | 1.0                                  | 96      | 8.0           | 95                    | 5.5     | 100           | 0.7                    | 86            | 6.7     |
| NG                  | 86                                   | 0.6     | 85            | 5.9          | 39            | 10.3    | 97               | 5.7     | 97            | 1.0                                  | 93      | 10.5          | 91                    | 4.8     | 95            | 3.2                    | 92            | 4.4     |
| 2,4,6-TNT           | 87                                   | 0.9     | 91            | 5.5          | 60            | 12.2    | 103              | 6.2     | 103           | 1.8                                  | 101     | 2.2           | 98                    | 5.8     | 100           | 1.7                    | 96            | 5.3     |
| 4-Am-2,6-DNT        | 90                                   | 0.2     | 97            | 4.9          | 85            | 8.0     | 101              | 7.8     | 106           | 1.0                                  | 104     | 10.7          | 97                    | 6.6     | 102           | 0.3                    | 110           | 2.7     |
| 2-Am-4,6-DNT        | 92                                   | 2.4     | 99            | 4.9          | 88            | 6.9     | 101              | 6.6     | 105           | 1.6                                  | 108     | 15.4          | 97                    | 6.5     | 102           | 1.7                    | 109           | 2.8     |
| 2,6-DNT             | 74                                   | 2.6     | 38            | 10.0         | 100           | 11.8    | 96               | 4.8     | 96            | 1.8                                  | 68      | 6.4           | 92                    | 6.3     | 95            | 1.5                    | 71            | 1.6     |
| 2,4-DNT             | 84                                   | 0.8     | 70            | 7.2          | 91            | 13.5    | 100              | 6.6     | 99            | 1.9                                  | 78      | 7.5           | 94                    | 6.4     | 96            | 3.2                    | 69            | 13.4    |
| 2-NT                | 1                                    | 0.5     | 1             | 0.0          | 65            | 19.5    | 86               | 8.5     | 96            | 2.6                                  | 81      | 17.8          | 78                    | 4.5     | 93            | 1.1                    | 84            | 6.7     |
| 4-NT                | 4                                    | 1.8     | 1             | 0.3          | 88            | 9.9     | 92               | 6.4     | 100           | 4.8                                  | 99      | 5.5           | 88                    | 6.7     | 95            | 3.3                    | 94            | 11.7    |
| 3-NT                | 2                                    | 1.3     | 1             | 0.1          | 75            | 11.7    | 91               | 7.6     | 103           | 2.3                                  | 92      | 10.2          | 86                    | 5.8     | 99            | 3.2                    | 96            | 1.7     |
| PETN                | 92                                   | 1.4     | 88            | 14.7         | 73            | 12.8    | 103              | 7.4     | 107           | 0.8                                  | 108     | 24.7          | 98                    | 7.9     | 102           | 5.7                    | 101           | 8.3     |

**Bold, italicized** are IM compounds and degradation products.

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

(DoD QSM Ver5.2 for Waters – Lowest LCL: MNX, 57%; Highest UCL: HMX, 135%)

Table 25. Analysis hold time stability for laboratory-spiked reagent water samples over 40 days.

| Compound            | Recoveries (%)                       |         |         |               |               |      |               |         |                  |               |         |      |                                           |         |         |               |                        |      |               |         |                         |               |         |      |               |         |         |      |
|---------------------|--------------------------------------|---------|---------|---------------|---------------|------|---------------|---------|------------------|---------------|---------|------|-------------------------------------------|---------|---------|---------------|------------------------|------|---------------|---------|-------------------------|---------------|---------|------|---------------|---------|---------|------|
|                     | Waters Day 0 Extraction and Analysis |         |         |               |               |      |               |         |                  |               |         |      | Waters Day 40 Extraction, Day 40 Analysis |         |         |               |                        |      |               |         |                         |               |         |      |               |         |         |      |
|                     | SPE A (evap)                         |         |         |               | SPE B (evap)  |      |               |         | Direct Injection |               |         |      | Cold/Dark                                 |         |         |               | Room Temperature, Dark |      |               |         | Room Temperature, Light |               |         |      |               |         |         |      |
|                     | Average (n=3)                        |         | Std Dev |               | Average (n=3) |      | Std Dev       |         | Average (n=3)    |               | Std Dev |      | Average (n=3)                             |         | Std Dev |               | Average (n=3)          |      | Std Dev       |         | Average (n=3)           |               | Std Dev |      | Average (n=3) |         | Std Dev |      |
|                     | Average (n=3)                        | Std Dev |         | Average (n=3) | Std Dev       |      | Average (n=3) | Std Dev |                  | Average (n=3) | Std Dev |      | Average (n=3)                             | Std Dev |         | Average (n=3) | Std Dev                |      | Average (n=3) | Std Dev |                         | Average (n=3) | Std Dev |      | Average (n=3) | Std Dev |         |      |
| NP                  | 19                                   | 1.4     | 42      | 1.9           | 74            | 13.6 | 102           | 16.8    | 17               | 2.9           | 37      | 5.0  | 38                                        | 1.7     | 89      | 20.0          | 100                    | 14.3 | 42            | 4.1     | 83                      | 12.9          | 98      | 21.0 | 39            | 4.5     | 77      | 3.9  |
| NT0 (210nm)         | 261                                  | 2.5     | 286     | 14.1          | 138           | 2.8  | 126           | 2.0     | 123              | 1.2           | 134     | 5.1  | 134                                       | 9.0     | 136     | 3.3           | 128                    | 2.3  | 142           | 0.0     | 136                     | 3.3           | 127     | 2.9  | 139           | 3.2     | 120     | 2.7  |
| NT0 (315nm)         | 270                                  | 2.4     | 292     | 15.2          | 99            | 14.2 | 128           | 2.6     | 123              | 0.8           | 133     | 4.9  | 131                                       | 9.0     | 130     | 4.5           | 130                    | 3.0  | 142           | 0.0     | 131                     | 3.1           | 128     | 3.2  | 140           | 3.0     | 126     | 1.2  |
| c-NBA (HMX)         | 107                                  | 0.9     | 112     | 7.0           | 106           | 10.6 | 109           | 1.1     | 111              | 1.9           | 112     | 1.5  | 118                                       | 4.9     | 117     | 4.6           | 106                    | 0.5  | 109           | 5.1     | 117                     | 5.9           | 105     | 3.3  | 108           | 4.3     | 118     | 1.4  |
| HMX                 | 91                                   | 0.8     | 98      | 5.1           | 88            | 9.6  | 81            | 30.6    | 108              | 0.8           | 117     | 4.3  | 116                                       | 7.8     | 96      | 2.4           | 113                    | 2.4  | 125           | 1.9     | 99                      | 2.4           | 112     | 2.7  | 123           | 2.6     | 98      | 3.1  |
| PDN                 | 77                                   | 4.3     | 88      | 7.8           | 68            | 10.3 | 116           | 2.4     | 113              | 1.7           | 120     | 4.7  | 120                                       | 8.4     | 106     | 3.3           | 118                    | 2.7  | 127           | 2.4     | 107                     | 5.4           | 117     | 3.3  | 126           | 2.6     | 105     | 3.5  |
| PA (210nm)          | 81                                   | 1.5     | 92      | 5.4           | 84            | 5.8  | 88            | 2.1     | 81               | 1.6           | 93      | 5.0  | 88                                        | 1.7     | 114     | 10.6          | 89                     | 1.1  | 99            | 2.0     | 111                     | 4.0           | 88      | 2.2  | 99            | 2.1     | 139     | 7.0  |
| PA (315nm)          | 93                                   | 1.1     | 100     | 5.4           | 83            | 4.9  | 94            | 1.6     | 83               | 1.5           | 102     | 1.6  | 92                                        | 2.6     | 97      | 3.1           | 96                     | 1.4  | 108           | 2.2     | 113                     | 3.7           | 95      | 2.2  | 107           | 1.8     | 135     | 4.0  |
| 4-NP                | 90                                   | 0.8     | 97      | 5.0           | 92            | 6.3  | 106           | 1.6     | 100              | 1.1           | 111     | 4.5  | 108                                       | 6.9     | 107     | 3.2           | 108                    | 2.3  | 119           | 2.2     | 109                     | 1.2           | 107     | 2.7  | 117           | 2.3     | 109     | 1.5  |
| 1,3,5-TNB           | 89                                   | 1.3     | 95      | 4.9           | 87            | 7.0  | 109           | 2.2     | 109              | 1.4           | 111     | 5.8  | 115                                       | 7.2     | 111     | 3.7           | 110                    | 1.6  | 116           | 0.9     | 115                     | 0.3           | 108     | 2.0  | 113           | 1.9     | 116     | 3.1  |
| 2,4-DNP             | 85                                   | 0.6     | 90      | 4.3           | 96            | 2.5  | 110           | 1.5     | 108              | 1.9           | 112     | 5.8  | 119                                       | 5.3     | 99      | 8.9           | 111                    | 2.4  | 120           | 4.1     | 100                     | 10.4          | 110     | 2.1  | 118           | 4.5     | 106     | 12.0 |
| 1,2-DNB (surrogate) | 87                                   | 0.8     | 79      | 7.0           | 97            | 5.3  | 108           | 1.2     | 114              | 2.1           | 97      | 7.6  | 120                                       | 9.6     | 110     | 3.0           | 106                    | 2.2  | 99            | 8.3     | 102                     | 7.8           | 105     | 1.7  | 98            | 8.0     | 107     | 10.8 |
| 1,3-DNB             | 84                                   | 0.8     | 77      | 6.7           | 87            | 6.2  | 106           | 2.1     | 110              | 0.4           | 102     | 19.7 | 116                                       | 6.3     | 104     | 3.6           | 109                    | 2.2  | 104           | 0.2     | 101                     | 6.8           | 109     | 3.0  | 99            | 5.8     | 103     | 3.5  |
| 2-NP                | 5                                    | 3.0     | 0       | 0.0           | 87            | 0.8  | 7             | 3.8     | 103              | 0.6           | 0       | 0.0  | 106                                       | 3.1     | 91      | 40.7          | 6                      | 3.3  | 0             | 0.0     | 82                      | 52.9          | 6       | 3.5  | 0             | 0.0     | 92      | 42.0 |
| NB                  | 90                                   | 2.3     | 97      | 7.9           | 79            | 9.7  | 116           | 2.8     | 105              | 0.2           | 116     | 0.1  | 112                                       | 7.0     | 111     | 6.1           | 116                    | 2.4  | 121           | 7.5     | 127                     | 0.1           | 110     | 1.4  | 123           | 0.2     | 120     | 3.6  |
| 3,5-DNA (210nm)     | 1                                    | 1.1     | 0       | 0.0           | 88            | 9.4  | 0             | 0.0     | 102              | 0.8           | 0       | 0.0  | 104                                       | 3.0     | 89      | 68.7          | 0                      | 0.0  | 0             | 0.0     | 75                      | 63.1          | 0       | 0.0  | 0             | 0.0     | 129     | 1.6  |
| 3,5-DNA (254nm)     | 1                                    | 0.8     | 0       | 0.0           | 87            | 10.8 | 0             | 0.0     | 117              | 0.7           | 0       | 0.0  | 119                                       | 4.0     | 81      | 66.7          | 0                      | 0.0  | 0             | 0.0     | 111                     | 2.0           | 0       | 0.0  | 0             | 0.0     | 74      | 63.7 |
| DNAI                | 88                                   | 0.5     | 92      | 6.5           | 72            | 5.4  | 104           | 2       | 89               | 0             | 104     | 1    | 94                                        | 4       | 92      | 6             | 106                    | 2    | 110           | 2       | 93                      | 4             | 102     | 2    | 109           | 2       | 91      | 2.6  |
| Tetrol              | 89                                   | 1.8     | 95      | 4.6           | 86            | 16.1 | 110           | 2       | 111              | 1             | 113     | 4    | 117                                       | 7       | 101     | 4             | 114                    | 2    | 117           | 2       | 78                      | 9             | 111     | 3    | 111           | 3       | 89      | 7.1  |
| NG                  | 86                                   | 0.6     | 85      | 5.9           | 39            | 10.3 | 115           | 1.6     | 121              | 4.2           | 112     | 6.5  | 129                                       | 8.4     | 101     | 5.7           | 107                    | 3.6  | 117           | 4.5     | 96                      | 10.3          | 105     | 1.3  | 115           | 4.8     | 94      | 8.2  |
| 2,4,6-TNT           | 87                                   | 0.9     | 91      | 5.5           | 60            | 12.2 | 113           | 2.2     | 112              | 3.6           | 115     | 4.0  | 120                                       | 7.4     | 110     | 4.2           | 114                    | 2.3  | 122           | 2.0     | 107                     | 4.3           | 112     | 2.4  | 120           | 2.1     | 109     | 2.9  |
| 4-Am-2,6-DNT        | 90                                   | 0.2     | 97      | 4.9           | 85            | 8.0  | 110           | 2.1     | 106              | 2.4           | 114     | 4.9  | 116                                       | 8.5     | 100     | 7.2           | 111                    | 1.9  | 108           | 13.1    | 98                      | 9.8           | 107     | 3.2  | 102           | 15.6    | 106     | 7.1  |
| 2-Am-4,6-DNT        | 92                                   | 2.4     | 99      | 4.9           | 88            | 6.9  | 112           | 2.3     | 106              | 2.2           | 116     | 4.8  | 117                                       | 9.0     | 107     | 6.6           | 112                    | 4.1  | 114           | 11.7    | 102                     | 11.2          | 110     | 2.2  | 117           | 0.1     | 116     | 7.3  |
| 2,6-DNT             | 74                                   | 2.6     | 38      | 10.0          | 100           | 11.8 | 99            | 1.0     | 111              | 3.3           | 49      | 11.1 | 113                                       | 1.2     | 80      | 19.9          | 103                    | 1.5  | 57            | 1.0     | 68                      | 15.4          | 104     | 6.8  | 59            | 0.4     | 70      | 14.4 |
| 2,4-DNT             | 84                                   | 0.8     | 70      | 7.2           | 91            | 13.5 | 94            | 4.8     | 104              | 4.7           | 80      | 3.3  | 106                                       | 7.5     | 102     | 3.1           | 99                     | 4.7  | 73            | 40.4    | 93                      | 12.2          | 97      | 1.3  | 79            | 28.0    | 103     | 7.5  |
| 2-NI                | 1                                    | 0.5     | 1       | 0.0           | 65            | 19.5 | 0             | 0.0     | 110              | 2.1           | 0       | 0.0  | 110                                       | 3.8     | 74      | 65.1          | 0                      | 0.0  | 1             | 1.5     | 73                      | 63.2          | 0       | 0.0  | 1             | 1.7     | 108     | 1.8  |
| 4-NI                | 4                                    | 1.8     | 1       | 0.3           | 88            | 9.9  | 4             | 2.9     | 109              | 2.6           | 0       | 0.0  | 108                                       | 4.1     | 67      | 58.7          | 2                      | 0.0  | 1             | 0.3     | 52                      | 47.0          | 2       | 0.0  | 1             | 1.0     | 54      | 47.5 |
| 3-NI                | 2                                    | 1.3     | 1       | 0.1           | 75            | 11.7 | 2             | 1.3     | 108              | 1.3           | 0       | 0.0  | 104                                       | 6.5     | 65      | 56.4          | 2                      | 1.2  | 0             | 0.2     | 59                      | 51.7          | 2       | 1.2  | 0             | 0.2     | 57      | 49.4 |
| PETN                | 92                                   | 1.4     | 88      | 14.7          | 73            | 12.8 | 110           | 2.5     | 112              | 3.8           | 109     | 15.8 | 117                                       | 8.2     | 117     | 14.0          | 99                     | 5.7  | 132           | 30.2    | 115                     | 7.8           | 97      | 3.6  | 134           | 31.4    | 120     | 11.2 |

**Bold, italicized** are IM compounds and degradation products.

Green: 75-125%; Light Green: 57-74% or 126-135%; Red: <57% or >135%  
(DoD QSM Ver5.2 for Waters – Lowest LCL: MNX, 57%; Highest UCL: HMX, 135%)

Results for the pre-extraction hold time experiments for soil are shown in Table 26. For unknown reasons, Day 0 soil samples, a few compounds were recovered at lower than usual levels, especially for the Riley field soil. In addition, NTO, HMX, and RDX recoveries were recovered at levels below current limits (<64%) at Day 14. This was observed for two of the soils for HMX and RDX, and for all three soils for NTO, indicating that pre-extraction holding time limits might need to be shortened for soils where these compounds are known or suspected to be present. Overall, though, the majority of compounds were recovered within current DoD QSM limits at both Day 0 and Day 14, including all other IM compounds tested.

Results for the post-extraction hold time experiments for soil are shown in Table 27. Again, for unknown reasons, NTO determinations were generally very high in this study, possibly due to analytical interference. However, NTO recoveries were fairly stable from Day 0 to Day 40, with no storage condition-dependent trends. Only three compounds were detected at reduced levels at Day 40, including HMX, RDX, and Tetryl. HMX recoveries did not seem to depend upon storage condition, while RDX recoveries were slightly lower for room temperature samples than those stored at colder temperatures. Tetryl recoveries were much lower for room temperature samples than for cold samples; however, even the cold samples were detected at 50% of the levels observed on Day 0. As in the soil pre-extraction stability study, the majority of compounds in the analysis time stability study were recovered within current DoD QSM limits at both Day 0 and Day 40, including all other IM compounds tested.

Compound recovery results obtained for Day 0 of the water stability study also yielded information on compound volatility, as several compounds, specifically 2-NP, 3,5-DNA, 2,6-DNT and 2-/3-/4-NT, were recovered at lower levels on Day 0 than on Day 7. The compounds were detected at their usual levels within current DoD QSM limits on Day 7, but were nearly absent in samples from Day 0. Since samples prepared on Day 0 were stored for extraction on Day 7, if the compounds were lost from the original water samples, they would not have been detected in the Day 7 extracts. Because they were, the loss of these more volatile compounds from Day 0 extracts likely occurred when the extracts obtained by SPE on Day 0 underwent evaporative concentration under nitrogen. This phenomenon was also observed during SPE method development. Therefore, in general, and especially when any of these compounds are known or suspected to be present in a sample, evaporative concentration of extracts is not recommended

Table 26. Extraction hold time stability for three laboratory-spiked soil samples over 14 days.

| Compound            | Recoveries (%)                     |         |  |               |         |  |               |         |  |                                      |         |  |                       |         |  |                       |         |  |                        |         |  |               |         |  |     |     |  |     |      |  |     |      |  |     |     |
|---------------------|------------------------------------|---------|--|---------------|---------|--|---------------|---------|--|--------------------------------------|---------|--|-----------------------|---------|--|-----------------------|---------|--|------------------------|---------|--|---------------|---------|--|-----|-----|--|-----|------|--|-----|------|--|-----|-----|
|                     | Soils Day0 Extraction and Analysis |         |  |               |         |  |               |         |  | Soils Day 14 Extraction and Analysis |         |  |                       |         |  |                       |         |  |                        |         |  |               |         |  |     |     |  |     |      |  |     |      |  |     |     |
|                     | Riley                              |         |  | Yuma          |         |  | ASTMFC        |         |  | Cold/Dark                            |         |  | Room Temperature/Dark |         |  | Room Temperature/Dark |         |  | Room Temperature/Light |         |  | ASTMFC        |         |  |     |     |  |     |      |  |     |      |  |     |     |
|                     | Average (n=3)                      | Std Dev |  | Average (n=3) | Std Dev |  | Average (n=3) | Std Dev |  | Average (n=3)                        | Std Dev |  | Average (n=3)         | Std Dev |  | Average (n=3)         | Std Dev |  | Average (n=3)          | Std Dev |  | Average (n=3) | Std Dev |  |     |     |  |     |      |  |     |      |  |     |     |
| NQ                  | 65                                 | 4.7     |  | 65            | 4.1     |  | 64            | 4.1     |  | 98                                   | 8.3     |  | 147                   | 10.1    |  | 102                   | 6.9     |  | 90                     | 6.9     |  | 116           | 10.0    |  | 95  | 3.3 |  | 98  | 4.9  |  | 119 | 14.0 |  | 103 | 8.2 |
| NFO (210nm)         | 260                                | 7.4     |  | 73            | 1.5     |  | 78            | 4.9     |  | 33                                   | 3.3     |  | 35                    | 1.4     |  | 34                    | 2.7     |  | 31                     | 2.2     |  | 35            | 2.6     |  | 35  | 1.5 |  | 31  | 1.8  |  | 38  | 3.8  |  | 30  | 1.3 |
| o-NBA (surrogate)   | 108                                | 1.0     |  | 100           | 1.7     |  | 97            | 4.1     |  | 91                                   | 6.2     |  | 99                    | 1.2     |  | 95                    | 1.8     |  | 90                     | 3.2     |  | 96            | 2.0     |  | 93  | 0.8 |  | 90  | 7.9  |  | 101 | 1.0  |  | 97  | 3.4 |
| HMX                 | 41                                 | 0.6     |  | 71            | 3.2     |  | 57            | 14.2    |  | 43                                   | 1.2     |  | 64                    | 2.3     |  | 39                    | 2.1     |  | 38                     | 4.6     |  | 63            | 1.7     |  | 38  | 2.9 |  | 47  | 1.9  |  | 68  | 2.3  |  | 41  | 4.1 |
| RDX                 | 91                                 | 1.4     |  | 113           | 3.4     |  | 112           | 2.3     |  | 53                                   | 4.1     |  | 64                    | 7.7     |  | 44                    | 1.3     |  | 53                     | 1.0     |  | 63            | 3.9     |  | 42  | 3.0 |  | 56  | 4.9  |  | 65  | 5.9  |  | 44  | 7.9 |
| PA (210nm)          | 56                                 | 0.5     |  | 81            | 3.3     |  | 59            | 0.4     |  | 83                                   | 8.0     |  | 119                   | 1.3     |  | 111                   | 0.6     |  | 67                     | 12.2    |  | 125           | 1.5     |  | 109 | 2.6 |  | 69  | 12.3 |  | 131 | 0.6  |  | 114 | 0.6 |
| 4-NP                | 125                                | 1.9     |  | 125           | 1.0     |  | 127           | 1.4     |  | 115                                  | 0.7     |  | 119                   | 0.9     |  | 120                   | 0.9     |  | 103                    | 5.8     |  | 114           | 0.7     |  | 117 | 0.8 |  | 113 | 3.0  |  | 121 | 0.4  |  | 122 | 2.1 |
| 1,3,5-TNB           | 102                                | 2.7     |  | 115           | 0.7     |  | 112           | 3.2     |  | 97                                   | 0.6     |  | 109                   | 0.7     |  | 103                   | 0.3     |  | 87                     | 3.9     |  | 107           | 1.4     |  | 98  | 1.8 |  | 89  | 4.5  |  | 114 | 0.6  |  | 103 | 1.1 |
| 2,4-DNP             | 92                                 | 4.0     |  | 102           | 5.3     |  | 103           | 1.0     |  | 90                                   | 3.7     |  | 103                   | 1.5     |  | 101                   | 0.5     |  | 75                     | 3.7     |  | 100           | 3.0     |  | 96  | 1.6 |  | 83  | 1.3  |  | 105 | 1.4  |  | 102 | 2.4 |
| 1,2-DNB (surrogate) | 125                                | 4.3     |  | 124           | 2.0     |  | 124           | 5.1     |  | 121                                  | 2.7     |  | 122                   | 3.8     |  | 119                   | 0.7     |  | 110                    | 5.8     |  | 115           | 1.3     |  | 118 | 2.8 |  | 117 | 4.0  |  | 122 | 2.8  |  | 122 | 2.0 |
| 1,3-DNB             | 117                                | 1.7     |  | 118           | 0.6     |  | 120           | 1.2     |  | 108                                  | 0.3     |  | 111                   | 1.0     |  | 112                   | 0.6     |  | 95                     | 4.4     |  | 106           | 1.0     |  | 110 | 1.3 |  | 104 | 2.4  |  | 112 | 0.3  |  | 113 | 2.0 |
| 2-NP                | 98                                 | 3.9     |  | 80            | 0.3     |  | 81            | 1.3     |  | 90                                   | 0.4     |  | 82                    | 2.2     |  | 80                    | 0.4     |  | 81                     | 3.3     |  | 78            | 1.4     |  | 76  | 0.7 |  | 89  | 1.5  |  | 85  | 1.9  |  | 81  | 1.4 |
| NB                  | 101                                | 1.2     |  | 113           | 2.3     |  | 115           | 0.7     |  | 87                                   | 1.4     |  | 110                   | 0.2     |  | 110                   | 0.6     |  | 64                     | 4.7     |  | 105           | 0.9     |  | 102 | 2.2 |  | 67  | 1.9  |  | 112 | 1.2  |  | 107 | 1.6 |
| 3,5-DNA (210nm)     | 114                                | 1.7     |  | 91            | 0.6     |  | 103           | 9.8     |  | 99                                   | 3.1     |  | 96                    | 4.8     |  | 98                    | 4.4     |  | 92                     | 5.1     |  | 89            | 1.7     |  | 95  | 2.9 |  | 103 | 8.0  |  | 96  | 4.9  |  | 101 | 2.3 |
| DNAN                | 103                                | 2.8     |  | 104           | 1.0     |  | 109           | 3.3     |  | 102                                  | 2.6     |  | 112                   | 1.4     |  | 107                   | 4.1     |  | 89                     | 5.7     |  | 107           | 1.5     |  | 102 | 2.5 |  | 98  | 0.2  |  | 113 | 3.0  |  | 105 | 2.6 |
| Tetryl              | 114                                | 3.9     |  | 111           | 0.6     |  | 112           | 0.7     |  | 100                                  | 1.3     |  | 104                   | 4.4     |  | 104                   | 3.5     |  | 68                     | 7.6     |  | 66            | 3.2     |  | 75  | 2.7 |  | 63  | 4.7  |  | 70  | 2.1  |  | 78  | 2.0 |
| NG                  | 123                                | 4.2     |  | 118           | 3.5     |  | 120           | 6.1     |  | 101                                  | 0.9     |  | 105                   | 4.7     |  | 106                   | 1.2     |  | 67                     | 5.2     |  | 102           | 3.7     |  | 106 | 2.1 |  | 71  | 3.0  |  | 106 | 3.9  |  | 109 | 2.6 |
| 2,4,6-TNT           | 118                                | 2.0     |  | 121           | 2.7     |  | 122           | 3.6     |  | 104                                  | 1.6     |  | 108                   | 2.1     |  | 108                   | 0.7     |  | 82                     | 4.0     |  | 91            | 2.3     |  | 90  | 3.3 |  | 84  | 3.7  |  | 97  | 0.8  |  | 94  | 2.6 |
| 4-Am-2,6-DNT        | 105                                | 1.9     |  | 115           | 1.8     |  | 117           | 1.4     |  | 87                                   | 2.1     |  | 111                   | 1.8     |  | 112                   | 1.8     |  | 61                     | 4.0     |  | 100           | 0.6     |  | 105 | 2.8 |  | 64  | 3.2  |  | 112 | 10.9 |  | 108 | 1.6 |
| 2-Am-4,6-DNT        | 108                                | 2.4     |  | 114           | 3.3     |  | 115           | 0.8     |  | 100                                  | 1.3     |  | 111                   | 1.5     |  | 111                   | 1.1     |  | 82                     | 4.1     |  | 104           | 1.2     |  | 106 | 1.9 |  | 88  | 2.2  |  | 114 | 5.7  |  | 111 | 3.4 |
| 2,6-DNT             | 116                                | 0.6     |  | 113           | 1.6     |  | 116           | 1.8     |  | 115                                  | 0.2     |  | 113                   | 2.5     |  | 115                   | 0.6     |  | 74                     | 6.4     |  | 107           | 0.6     |  | 114 | 2.1 |  | 115 | 3.1  |  | 114 | 0.4  |  | 118 | 1.4 |
| 2,4-DNT             | 112                                | 0.7     |  | 115           | 1.0     |  | 117           | 1.8     |  | 106                                  | 1.6     |  | 112                   | 2.1     |  | 113                   | 1.3     |  | 92                     | 2.0     |  | 107           | 1.1     |  | 108 | 2.1 |  | 100 | 3.1  |  | 113 | 2.0  |  | 113 | 2.6 |
| 2-NT                | 112                                | 2.0     |  | 95            | 3.3     |  | 102           | 9.1     |  | 107                                  | 0.7     |  | 102                   | 2.1     |  | 108                   | 1.5     |  | 100                    | 5.1     |  | 99            | 0.3     |  | 106 | 0.6 |  | 108 | 2.6  |  | 103 | 2.0  |  | 109 | 2.7 |
| 4-NT                | 113                                | 3.1     |  | 96            | 4.1     |  | 102           | 9.3     |  | 109                                  | 1.3     |  | 103                   | 2.9     |  | 109                   | 1.3     |  | 96                     | 5.5     |  | 99            | 1.3     |  | 107 | 1.0 |  | 105 | 1.5  |  | 102 | 1.6  |  | 108 | 2.3 |
| 3-NT                | 113                                | 0.9     |  | 96            | 1.5     |  | 105           | 9.2     |  | 107                                  | 0.8     |  | 101                   | 3.9     |  | 107                   | 0.7     |  | 97                     | 5.5     |  | 98            | 1.2     |  | 105 | 1.5 |  | 105 | 2.6  |  | 103 | 1.1  |  | 110 | 2.0 |
| PETN                | 116                                | 4.6     |  | 111           | 4.6     |  | 118           | 3.9     |  | 109                                  | 1.9     |  | 109                   | 2.4     |  | 110                   | 1.9     |  | 91                     | 5.5     |  | 102           | 2.5     |  | 111 | 5.4 |  | 99  | 2.8  |  | 110 | 2.4  |  | 115 | 1.9 |

**Bold, italicized** are IM compounds and degradation products.

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

Table 27. Analysis hold time stability for three laboratory-spiked soil samples over 40 days.

| Compound            | Recoveries (%)                      |         |               |         |               |         |                                        |         |               |         |               |         |                       |         |               |         |               |         |                        |         |               |         |               |         |
|---------------------|-------------------------------------|---------|---------------|---------|---------------|---------|----------------------------------------|---------|---------------|---------|---------------|---------|-----------------------|---------|---------------|---------|---------------|---------|------------------------|---------|---------------|---------|---------------|---------|
|                     | Soils Day 0 Extraction and Analysis |         |               |         |               |         | Soils Day 0 Extraction Day 40 Analysis |         |               |         |               |         |                       |         |               |         |               |         |                        |         |               |         |               |         |
|                     |                                     |         |               |         |               |         | Cold Dark                              |         |               |         |               |         | Room Temperature Dark |         |               |         |               |         | Room Temperature Light |         |               |         |               |         |
|                     | Riley                               |         | Yuma          |         | ASTMFC        |         | Riley                                  |         | Yuma          |         | ASTMFC        |         | Riley                 |         | Yuma          |         | ASTMFC        |         | Riley                  |         | Yuma          |         | ASTMFC        |         |
|                     | Average (n=3)                       | Std Dev | Average (n=3) | Std Dev | Average (n=3) | Std Dev | Average (n=3)                          | Std Dev | Average (n=3) | Std Dev | Average (n=3) | Std Dev | Average (n=3)         | Std Dev | Average (n=3) | Std Dev | Average (n=3) | Std Dev | Average (n=3)          | Std Dev | Average (n=3) | Std Dev | Average (n=3) | Std Dev |
| NQ                  | 65                                  | 4.7     | 65            | 4.1     | 64            | 4.1     | 85                                     | 4.1     | 104           | 3.9     | 94            | 0.8     | 84                    | 4.0     | 102           | 3.1     | 92            | 2.2     | 83                     | 4.0     | 102           | 8.9     | 93            | 2.2     |
| NTO (210nm)         | 260                                 | 7.4     | 73            | 1.5     | 78            | 4.9     | 148                                    | 4.7     | 149           | 1.4     | 143           | 3.5     | 149                   | 5.9     | 153           | 0.9     | 146           | 4.1     | 144                    | 4.0     | 150           | 1.7     | 144           | 3.1     |
| o-NBA (surrogate)   | 108                                 | 1.0     | 100           | 1.7     | 97            | 4.1     | 87                                     | 8.8     | 97            | 0.9     | 91            | 1.1     | 87                    | 8.3     | 99            | 2.9     | 90            | 0.9     | 86                     | 8.6     | 97            | 1.3     | 91            | 2.0     |
| HMX                 | 41                                  | 0.6     | 71            | 3.2     | 57            | 14.2    | 35                                     | 2.5     | 60            | 2.5     | 39            | 1.0     | 36                    | 3.8     | 60            | 3.5     | 39            | 0.9     | 35                     | 3.5     | 61            | 2.5     | 39            | 2.0     |
| RDX                 | 91                                  | 1.4     | 113           | 3.4     | 112           | 2.3     | 60                                     | 1.7     | 74            | 2.5     | 98            | 4.7     | 60                    | 1.9     | 72            | 3.2     | 51            | 0.9     | 57                     | 6.7     | 71            | 2.8     | 50            | 0.4     |
| PA (210nm)          | 56                                  | 0.5     | 81            | 3.3     | 59            | 0.4     | 88                                     | 2.3     | 104           | 3.4     | 98            | 4.7     | 111                   | 4.5     | 152           | 3.1     | 143           | 5.9     | 120                    | 3.1     | 153           | 3.6     | 142           | 6.2     |
| 4-NP                | 125                                 | 1.9     | 125           | 1.0     | 127           | 1.4     | 107                                    | 1.2     | 108           | 2.9     | 108           | 4.0     | 107                   | 1.5     | 107           | 1.9     | 106           | 4.1     | 106                    | 0.8     | 107           | 2.1     | 107           | 3.9     |
| 1,3,5-TNB           | 102                                 | 2.7     | 115           | 0.7     | 112           | 3.2     | 96                                     | 1.8     | 109           | 1.0     | 102           | 1.9     | 96                    | 0.9     | 109           | 1.7     | 103           | 1.8     | 95                     | 2.0     | 109           | 0.4     | 103           | 1.7     |
| 2,4-DNP             | 92                                  | 4.0     | 102           | 5.3     | 103           | 1.0     | 87                                     | 3.3     | 98            | 0.7     | 96            | 2.7     | 86                    | 2.7     | 100           | 2.4     | 96            | 2.0     | 88                     | 2.0     | 99            | 1.2     | 95            | 2.0     |
| 1,2-DNB (surrogate) | 125                                 | 4.3     | 124           | 2.0     | 124           | 5.1     | 115                                    | 1.3     | 117           | 1.7     | 115           | 2.6     | 119                   | 0.3     | 117           | 0.7     | 119           | 1.8     | 118                    | 0.2     | 119           | 0.9     | 118           | 2.0     |
| 1,3-DNB             | 117                                 | 1.7     | 118           | 0.6     | 120           | 1.2     | 106                                    | 2.2     | 109           | 1.0     | 110           | 1.9     | 107                   | 0.8     | 110           | 1.7     | 110           | 1.9     | 106                    | 1.5     | 110           | 0.6     | 110           | 1.3     |
| 2-NP                | 98                                  | 3.9     | 80            | 0.3     | 81            | 1.3     | 85                                     | 1.6     | 71            | 2.1     | 71            | 2.0     | 85                    | 1.9     | 72            | 1.4     | 71            | 1.4     | 85                     | 1.4     | 71            | 0.3     | 71            | 1.7     |
| NB                  | 101                                 | 1.2     | 113           | 2.3     | 115           | 0.7     | 89                                     | 1.6     | 103           | 0.9     | 102           | 2.1     | 92                    | 0.3     | 105           | 2.1     | 102           | 2.4     | 91                     | 0.7     | 105           | 0.4     | 103           | 1.3     |
| 3,5-DNA (210nm)     | 114                                 | 1.7     | 91            | 0.6     | 103           | 9.8     | 112                                    | 1.5     | 93            | 1.9     | 110           | 1.0     | 113                   | 0.8     | 94            | 1.2     | 111           | 1.1     | 112                    | 1.7     | 94            | 0.7     | 110           | 0.7     |
| DNAN                | 103                                 | 2.8     | 104           | 1.0     | 109           | 3.3     | 76                                     | 0.3     | 82            | 1.0     | 78            | 3.0     | 77                    | 0.8     | 82            | 1.3     | 79            | 1.6     | 77                     | 0.6     | 82            | 0.9     | 80            | 1.9     |
| Tetryl              | 114                                 | 3.9     | 111           | 0.6     | 112           | 0.7     | 94                                     | 2.4     | 52            | 2.6     | 55            | 1.4     | 35                    | 7.5     | 0             | 0.0     | 0             | 0.0     | 4                      | 0.3     | 0             | 0.0     | 0             | 0.0     |
| NG                  | 123                                 | 4.2     | 118           | 3.5     | 120           | 6.1     | 86                                     | 5.6     | 98            | 2.7     | 97            | 2.5     | 101                   | 2.7     | 94            | 7.3     | 99            | 5.4     | 109                    | 0.2     | 78            | 3.8     | 84            | 7.8     |
| 2,4,6-TNT           | 118                                 | 2.0     | 121           | 2.7     | 122           | 3.6     | 98                                     | 2.9     | 135           | 4.7     | 130           | 3.4     | 106                   | 0.9     | 112           | 0.3     | 110           | 1.7     | 98                     | 0.9     | 109           | 0.3     | 105           | 4.6     |
| 4-Am-2,6-DNT        | 105                                 | 1.9     | 115           | 1.8     | 117           | 1.4     | 94                                     | 1.4     | 112           | 1.1     | 113           | 1.9     | 87                    | 1.3     | 99            | 1.1     | 105           | 10.0    | 97                     | 9.1     | 99            | 1.1     | 99            | 0.6     |
| 2-Am-4,6-DNT        | 108                                 | 2.4     | 114           | 3.3     | 115           | 0.8     | 98                                     | 1.7     | 109           | 1.1     | 107           | 1.5     | 87                    | 0.5     | 93            | 1.2     | 97            | 10.4    | 96                     | 8.2     | 93            | 0.9     | 92            | 1.0     |
| 2,6-DNT             | 116                                 | 0.6     | 113           | 1.6     | 116           | 1.8     | 96                                     | 1.7     | 89            | 3.4     | 91            | 7.1     | 100                   | 8.1     | 95            | 2.1     | 94            | 4.9     | 95                     | 6.2     | 96            | 2.7     | 95            | 1.4     |
| 2,4-DNT             | 112                                 | 0.7     | 115           | 1.0     | 117           | 1.8     | 100                                    | 4.5     | 111           | 2.5     | 110           | 5.1     | 99                    | 4.2     | 107           | 4.0     | 109           | 8.4     | 103                    | 2.8     | 106           | 1.2     | 108           | 2.3     |
| 2-NT                | 112                                 | 2.0     | 95            | 3.3     | 102           | 9.1     | 104                                    | 1.9     | 92            | 1.5     | 104           | 1.5     | 107                   | 5.5     | 91            | 1.8     | 103           | 1.5     | 106                    | 2.1     | 93            | 2.3     | 103           | 1.1     |
| 4-NT                | 113                                 | 3.1     | 96            | 4.1     | 102           | 9.3     | 104                                    | 2.3     | 95            | 2.2     | 105           | 2.9     | 106                   | 4.6     | 95            | 2.0     | 105           | 1.7     | 106                    | 1.3     | 94            | 0.9     | 105           | 0.6     |
| 3-NT                | 113                                 | 0.9     | 96            | 1.5     | 105           | 9.2     | 106                                    | 3.2     | 92            | 1.0     | 103           | 0.9     | 106                   | 2.2     | 92            | 1.1     | 104           | 0.7     | 105                    | 2.4     | 92            | 1.4     | 104           | 0.4     |
| PETN                | 116                                 | 4.6     | 111           | 4.6     | 118           | 3.9     | 106                                    | 1.9     | 110           | 1.0     | 110           | 3.8     | 110                   | 2.1     | 110           | 2.6     | 110           | 2.0     | 111                    | 2.3     | 111           | 3.5     | 111           | 2.6     |

**Bold, italicized** are IM compounds and degradation products.

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

(DoD QSM Ver5.2 for Solids – Lowest LCL: 4-Am-2,6-DNT, 64%; Highest UCL: Tetryl, 135%)

## Inter-Laboratory Batch Analysis

A cross-laboratory batch study was carried out at ERDC-EL and ERDC-CRREL using five water sources (Table 7) and six soil sources (Table 13). Multiple source samples were used to observe any potential matrix-related variability of extraction and analysis method efficiency. Both high-level (direct-injection) and low-level (SPE) water samples were used. Samples were extracted using the extraction methods and primary HPLC-UV method described in Materials and Methods above.

Compound recovery results for direct-injection and SPE water extracts are shown in Table 28. For samples that underwent extraction/pre-concentration by SPE, the majority of compounds were recovered from all five water source samples at similar levels to those observed during SPE method development (Table 10, option B). As expected, NQ recoveries averaged approximately 50% for SPE extracts at ERDC-EL; however, much higher recoveries were obtained for NQ at ERDC-CRREL, so further investigation may be needed. In general, at ERDC-EL, recoveries from reagent water tended to be slightly higher than usual, and at ERDC-CRREL, recovery of Tetryl from all water sources tended to be lower than usual. At both laboratories, it became apparent that using a detection wavelength of 315 nm rather than 210 nm for NTO might be preferred, in order to avoid positive interferences that appear at 210 nm for field samples. In addition, both laboratories observed low recoveries for NTO, 4-NP, 2-NP, NB (ERDC-EL only), 3,5-DNA and Tetryl (ERDC-CRREL only), and 4-Am-2,6-DNT in tap water samples (pH 9.2) that underwent SPE. Due to the consistency between laboratories, the high pH of the water samples, and the chemical properties of the compounds affected (acidic or unstable at high pH), the low recoveries may have been due to insufficient pH reduction during the second SPE elution step (using 2% HCl/MeOH).

In the same manner, during the direct injection cross-lab study, much lower recoveries for NTO, and in some cases *o*-NBA surrogate (ERDC-CRREL), were observed for field water samples than for reagent water. As this trend appeared to be pH related, with NTO recovery being lower for higher-pH water samples (see Table 26), further experiments were carried out in which the pH of samples to be analyzed by direct injection was adjusted, as discussed in Results and Discussion above.

Compound recovery results for cross-laboratory soil studies are shown in Table 29. The majority of compounds were recovered within current DoD QSM limits, with some compounds having inconsistent recoveries, from one soil to another, especially for NQ, NTO, *o*-NBA, and HMX. Some discrepancies were observed between the recoveries obtained by ERDC-EL and ERDC-CRREL, but for the most part, all compounds were recovered within current limits from most soils.

Table 28. Cross-laboratory batch analysis tests using five different water sources and the SPE method developed under SERDP ER-2722.

| ERDC-EL             |                       |                             |                     | Recoveries (%)                |                 |         |               |         |               |         |               |         |                                                    |         |               |         |                           |         |               |         |                   |         |               |         |                                                    |         |               |         |                   |         |               |         |                   |  |  |  |
|---------------------|-----------------------|-----------------------------|---------------------|-------------------------------|-----------------|---------|---------------|---------|---------------|---------|---------------|---------|----------------------------------------------------|---------|---------------|---------|---------------------------|---------|---------------|---------|-------------------|---------|---------------|---------|----------------------------------------------------|---------|---------------|---------|-------------------|---------|---------------|---------|-------------------|--|--|--|
| Compound            | Blank (Reagent Water) | Blank Spike (Reagent Water) | Matrix Spike (HSCN) | Matrix Spike Duplicate (HSCN) | SPE (0.10 mg/L) |         |               |         |               |         |               |         |                                                    |         |               |         | Direct Injection (2 mg/L) |         |               |         |                   |         |               |         |                                                    |         |               |         |                   |         |               |         |                   |  |  |  |
|                     |                       |                             |                     |                               | Reagent Water   |         |               |         | Tap Water     |         |               |         | Houston Shipping Channel (North of Morgan's Point) |         |               |         | Yazoo River Water         |         |               |         | Rayville, LA Well |         |               |         | Houston Shipping Channel (North of Morgan's Point) |         |               |         | Yazoo River Water |         |               |         | Rayville, LA Well |  |  |  |
|                     |                       |                             |                     |                               | Average (n=3)   |         | Std Dev       |         | Average (n=3) |         | Std Dev       |         | Average (n=3)                                      |         | Std Dev       |         | Average (n=3)             |         | Std Dev       |         | Average (n=3)     |         | Std Dev       |         | Average (n=3)                                      |         | Std Dev       |         | Average (n=3)     |         | Std Dev       |         |                   |  |  |  |
|                     |                       |                             |                     |                               | Average (n=3)   | Std Dev | Average (n=3) | Std Dev | Average (n=3) | Std Dev | Average (n=3) | Std Dev | Average (n=3)                                      | Std Dev | Average (n=3) | Std Dev | Average (n=3)             | Std Dev | Average (n=3) | Std Dev | Average (n=3)     | Std Dev | Average (n=3) | Std Dev | Average (n=3)                                      | Std Dev | Average (n=3) | Std Dev | Average (n=3)     | Std Dev | Average (n=3) | Std Dev |                   |  |  |  |
| NQ                  | 0                     | 23                          | 32                  | 36                            | 50              | 8.6     | 1             | 0.5     | 47            | 3.0     | 30            | 12.4    | 43                                                 | 9.4     | 99            | 3.3     | 92                        | 6.6     | 83            | 1.9     | 114               | 8.4     | 84            | 4.6     | 114                                                | 8.4     | 84            | 4.6     |                   |         |               |         |                   |  |  |  |
| NTD (210nm)         | 0                     | 127                         | 113                 | 136                           | 154             | 17.0    | 27            | 14.8    | 158           | 18.1    | 212           | 42.5    | 129                                                | 17.2    | 115           | 0.9     | 3                         | 0.2     | 11            | 0.5     | 15                | 0.7     | 2             | 0.1     | 15                                                 | 0.7     | 2             | 0.1     |                   |         |               |         |                   |  |  |  |
| NTD (315nm)         | 0                     | 124                         | 97                  | 120                           | 141             | 14.6    | 0             | 0.3     | 110           | 11.3    | 131           | 24.1    | 117                                                | 15.4    | 116           | 0.7     | 3                         | 0.5     | 10            | 0.6     | 14                | 0.8     | 2             | 0.3     | 14                                                 | 0.8     | 2             | 0.3     |                   |         |               |         |                   |  |  |  |
| o-NBA (surrogate)   | 133                   | 94                          | 93                  | 109                           | 113             | 10.7    | 111           | 29.1    | 96            | 11.2    | 105           | 10.5    | 112                                                | 10.7    | 102           | 0.3     | 100                       | 0.9     | 105           | 2.1     | 103               | 3.1     | 104           | 2.4     | 103                                                | 3.1     | 104           | 2.4     |                   |         |               |         |                   |  |  |  |
| HMX                 | 0                     | 89                          | 106                 | 107                           | 123             | 22.0    | 105           | 19.0    | 103           | 3.7     | 103           | 22.3    | 107                                                | 6.5     | 98            | 1.2     | 96                        | 1.0     | 100           | 0.9     | 100               | 1.2     | 103           | 5.3     | 100                                                | 1.2     | 103           | 5.3     |                   |         |               |         |                   |  |  |  |
| RDX                 | 0                     | 94                          | 109                 | 110                           | 123             | 19.4    | 109           | 12.8    | 106           | 4.9     | 111           | 19.5    | 109                                                | 8.2     | 102           | 2.6     | 99                        | 1.1     | 104           | 0.3     | 103               | 1.1     | 102           | 2.7     | 103                                                | 1.1     | 102           | 2.7     |                   |         |               |         |                   |  |  |  |
| PA (210nm)          | 0                     | 91                          | 94                  | 120                           | 126             | 15.2    | 107           | 28.0    | 109           | 3.7     | 107           | 18.8    | 114                                                | 8.2     | 88            | 5.8     | 96                        | 13.2    | 99            | 12.8    | 96                | 8.4     | 98            | 3.3     | 96                                                 | 8.4     | 98            | 3.3     |                   |         |               |         |                   |  |  |  |
| PA (315nm)          | 0                     | 86                          | 97                  | 114                           | 114             | 16.1    | 105           | 18.6    | 98            | 8.0     | 96            | 12.2    | 102                                                | 5.7     | 89            | 1.8     | 91                        | 0.9     | 94            | 0.4     | 92                | 1.1     | 92            | 1.3     | 92                                                 | 1.1     | 92            | 1.3     |                   |         |               |         |                   |  |  |  |
| 4-NP                | 0                     | 86                          | 103                 | 105                           | 123             | 23.6    | 39            | 5.3     | 103           | 0.4     | 106           | 17.2    | 105                                                | 4.1     | 94            | 1.2     | 94                        | 0.8     | 97            | 1.0     | 95                | 2.0     | 94            | 2.0     | 95                                                 | 2.0     | 94            | 2.0     |                   |         |               |         |                   |  |  |  |
| 1,3,5-TNB           | 0                     | 95                          | 103                 | 110                           | 140             | 22.8    | 116           | 23.1    | 109           | 1.2     | 104           | 19.0    | 118                                                | 8.8     | 100           | 1.4     | 100                       | 0.8     | 105           | 2.2     | 103               | 4.2     | 103           | 2.0     | 103                                                | 4.2     | 103           | 2.0     |                   |         |               |         |                   |  |  |  |
| 2,4-DNP             | 0                     | 89                          | 104                 | 101                           | 122             | 20.6    | 104           | 22.8    | 101           | 6.1     | 100           | 15.6    | 108                                                | 5.7     | 101           | 2.5     | 101                       | 1.4     | 103           | 1.8     | 104               | 3.6     | 100           | 2.2     | 104                                                | 3.6     | 100           | 2.2     |                   |         |               |         |                   |  |  |  |
| 1,2-DNB (surrogate) | 110                   | 89                          | 106                 | 107                           | 124             | 27.6    | 97            | 23.3    | 104           | 5.6     | 108           | 21.1    | 113                                                | 9.1     | 97            | 1.9     | 96                        | 0.5     | 99            | 2.3     | 99                | 1.0     | 99            | 2.5     | 99                                                 | 1.0     | 99            | 2.5     |                   |         |               |         |                   |  |  |  |
| 1,3-DNB             | 0                     | 97                          | 100                 | 114                           | 132             | 12.5    | 114           | 25.0    | 109           | 8.0     | 105           | 10.7    | 118                                                | 13.5    | 100           | 0.8     | 100                       | 1.2     | 103           | 0.3     | 102               | 0.5     | 101           | 1.1     | 102                                                | 0.5     | 101           | 1.1     |                   |         |               |         |                   |  |  |  |
| 2-NP                | 0                     | 93                          | 100                 | 105                           | 131             | 17.4    | 20            | 3.3     | 109           | 2.1     | 106           | 10.4    | 114                                                | 2.9     | 104           | 0.2     | 97                        | 1.0     | 108           | 0.9     | 107               | 0.6     | 103           | 0.9     | 107                                                | 0.6     | 103           | 0.9     |                   |         |               |         |                   |  |  |  |
| NB                  | 0                     | 91                          | 101                 | 108                           | 133             | 24.1    | 52            | 7.2     | 105           | 1.9     | 113           | 21.0    | 114                                                | 9.5     | 99            | 1.3     | 99                        | 0.9     | 102           | 0.9     | 101               | 0.5     | 99            | 0.2     | 101                                                | 0.5     | 99            | 0.2     |                   |         |               |         |                   |  |  |  |
| 3,5-DNA (210nm)     | 0                     | 95                          | 103                 | 114                           | 128             | 9.5     | 90            | 10.9    | 106           | 5.1     | 102           | 3.8     | 117                                                | 8.6     | 103           | 1.0     | 104                       | 2.2     | 108           | 1.3     | 104               | 1.3     | 104           | 0.3     | 104                                                | 1.3     | 104           | 0.3     |                   |         |               |         |                   |  |  |  |
| 3,5-DNA (254nm)     | 0                     | 96                          | 102                 | 110                           | 130             | 9.4     | 108           | 16.6    | 110           | 5.9     | 103           | 2.3     | 117                                                | 10.1    | 104           | 0.7     | 104                       | 1.1     | 107           | 0.9     | 106               | 0.2     | 105           | 0.3     | 106                                                | 0.2     | 105           | 0.3     |                   |         |               |         |                   |  |  |  |
| DNAN                | 0                     | 104                         | 102                 | 121                           | 132             | 1.6     | 109           | 30.0    | 107           | 8.7     | 106           | 4.8     | 118                                                | 8.0     | 104           | 2.8     | 104                       | 2.3     | 109           | 1.4     | 105               | 0.6     | 107           | 3.4     | 105                                                | 0.6     | 107           | 3.4     |                   |         |               |         |                   |  |  |  |
| Tetrad              | 0                     | 95                          | 91                  | 93                            | 132             | 23.3    | 75            | 15.5    | 88            | 3.4     | 85            | 19.6    | 96                                                 | 10.8    | 91            | 1.9     | 85                        | 5.9     | 97            | 1.0     | 94                | 1.8     | 98            | 2.1     | 94                                                 | 1.8     | 98            | 2.1     |                   |         |               |         |                   |  |  |  |
| NG                  | 0                     | 94                          | 105                 | 104                           | 129             | 25.0    | 127           | 34.8    | 100           | 7.5     | 113           | 21.4    | 141                                                | 7.3     | 94            | 2.7     | 97                        | 6.9     | 101           | 5.4     | 96                | 2.4     | 98            | 3.3     | 96                                                 | 2.4     | 98            | 3.3     |                   |         |               |         |                   |  |  |  |
| 2,4,6-TNT           | 0                     | 94                          | 101                 | 108                           | 129             | 19.3    | 109           | 29.2    | 99            | 4.9     | 95            | 15.1    | 122                                                | 12.9    | 100           | 4.8     | 103                       | 3.6     | 105           | 2.8     | 102               | 3.5     | 100           | 1.0     | 102                                                | 3.5     | 100           | 1.0     |                   |         |               |         |                   |  |  |  |
| 4-Am-2,6-DNT        | 0                     | 92                          | 96                  | 97                            | 143             | 31.1    | 5             | 1.4     | 95            | 6.1     | 116           | 34.3    | 112                                                | 12.1    | 97            | 1.3     | 95                        | 1.5     | 103           | 1.5     | 100               | 0.2     | 99            | 2.2     | 100                                                | 0.2     | 99            | 2.2     |                   |         |               |         |                   |  |  |  |
| 2-Am-4,6-DNT        | 0                     | 96                          | 99                  | 106                           | 136             | 22.2    | 106           | 19.7    | 100           | 8.3     | 109           | 15.2    | 118                                                | 15.0    | 97            | 2.0     | 99                        | 1.2     | 102           | 3.0     | 99                | 1.6     | 98            | 3.1     | 99                                                 | 1.6     | 98            | 3.1     |                   |         |               |         |                   |  |  |  |
| 2,6-DNT             | 0                     | 92                          | 96                  | 113                           | 117             | 6.0     | 91            | 23.9    | 94            | 4.9     | 89            | 8.3     | 103                                                | 11.2    | 97            | 1.3     | 96                        | 2.7     | 104           | 1.9     | 100               | 3.2     | 96            | 1.0     | 100                                                | 3.2     | 96            | 1.0     |                   |         |               |         |                   |  |  |  |
| 2,4-DNT             | 0                     | 103                         | 99                  | 114                           | 121             | 9.5     | 113           | 22.4    | 113           | 6.9     | 100           | 1.3     | 112                                                | 9.9     | 101           | 1.7     | 102                       | 1.6     | 105           | 1.7     | 104               | 0.9     | 103           | 0.8     | 104                                                | 0.9     | 103           | 0.8     |                   |         |               |         |                   |  |  |  |
| 2-NP                | 0                     | 95                          | 96                  | 106                           | 112             | 4.2     | 105           | 15.1    | 104           | 7.1     | 94            | 6.8     | 114                                                | 11.7    | 102           | 0.4     | 104                       | 2.8     | 105           | 1.1     | 104               | 0.8     | 100           | 1.4     | 104                                                | 0.8     | 100           | 1.4     |                   |         |               |         |                   |  |  |  |
| 4-NP                | 0                     | 96                          | 93                  | 106                           | 108             | 13.9    | 95            | 24.6    | 107           | 7.3     | 86            | 10.6    | 109                                                | 11.4    | 100           | 0.7     | 101                       | 1.7     | 104           | 5.0     | 104               | 1.8     | 101           | 4.3     | 104                                                | 1.8     | 101           | 4.3     |                   |         |               |         |                   |  |  |  |
| 3-NP                | 0                     | 92                          | 92                  | 101                           | 110             | 10.9    | 102           | 27.3    | 108           | 8.0     | 94            | 5.9     | 110                                                | 13.4    | 99            | 1.4     | 99                        | 3.0     | 101           | 0.2     | 99                | 0.7     | 100           | 2.7     | 99                                                 | 0.7     | 100           | 2.7     |                   |         |               |         |                   |  |  |  |
| PETN                | 0                     | 87                          | 101                 | 102                           | 117             | 20.5    | 94            | 14.6    | 101           | 11.2    | 100           | 26.7    | 97                                                 | 8.6     | 94            | 3.5     | 95                        | 4.3     | 101           | 2.6     | 96                | 5.6     | 95            | 3.8     | 96                                                 | 5.6     | 95            | 3.8     |                   |         |               |         |                   |  |  |  |

| ERDC-CRREL          |                       |                             |                      | Recoveries (%)                 |                 |         |               |         |               |         |               |         |                                                    |         |               |         |                           |         |               |         |                   |         |               |         |                                                    |         |               |         |                   |         |               |         |                   |  |  |  |
|---------------------|-----------------------|-----------------------------|----------------------|--------------------------------|-----------------|---------|---------------|---------|---------------|---------|---------------|---------|----------------------------------------------------|---------|---------------|---------|---------------------------|---------|---------------|---------|-------------------|---------|---------------|---------|----------------------------------------------------|---------|---------------|---------|-------------------|---------|---------------|---------|-------------------|--|--|--|
| Compound            | Blank (Reagent Water) | Blank Spike (Reagent Water) | Matrix Spike (Yazoo) | Matrix Spike Duplicate (Yazoo) | SPE (0.10 mg/L) |         |               |         |               |         |               |         |                                                    |         |               |         | Direct Injection (2 mg/L) |         |               |         |                   |         |               |         |                                                    |         |               |         |                   |         |               |         |                   |  |  |  |
|                     |                       |                             |                      |                                | Reagent Water   |         |               |         | Tap Water     |         |               |         | Houston Shipping Channel (North of Morgan's Point) |         |               |         | Yazoo River Water         |         |               |         | Rayville, LA Well |         |               |         | Houston Shipping Channel (North of Morgan's Point) |         |               |         | Yazoo River Water |         |               |         | Rayville, LA Well |  |  |  |
|                     |                       |                             |                      |                                | Average (n=3)   |         | Std Dev       |         | Average (n=3) |         | Std Dev       |         | Average (n=3)                                      |         | Std Dev       |         | Average (n=3)             |         | Std Dev       |         | Average (n=3)     |         | Std Dev       |         | Average (n=3)                                      |         | Std Dev       |         | Average (n=3)     |         | Std Dev       |         |                   |  |  |  |
|                     |                       |                             |                      |                                | Average (n=3)   | Std Dev | Average (n=3) | Std Dev | Average (n=3) | Std Dev | Average (n=3) | Std Dev | Average (n=3)                                      | Std Dev | Average (n=3) | Std Dev | Average (n=3)             | Std Dev | Average (n=3) | Std Dev | Average (n=3)     | Std Dev | Average (n=3) | Std Dev | Average (n=3)                                      | Std Dev | Average (n=3) | Std Dev | Average (n=3)     | Std Dev | Average (n=3) | Std Dev |                   |  |  |  |
| NQ                  | 0                     | 72                          | 72                   | 86                             | 7.6             | 0       | 0.0           | 83      | 4.7           | 67      | 10.4          | 62      | 3.0                                                | 76      | 15.6          | 88      | 2.2                       | 104     | 2.9           | 106     | 1.8               | 103     | 3.0           | 106     | 1.8                                                | 103     | 3.0           | 106     | 1.8               |         |               |         |                   |  |  |  |
| NTD                 | 0                     | 169                         | 161                  | 164                            | 124             | 2.4     | 0             | 0.0     | 140           | 17.4    | 144           | 12.6    | 138                                                | 6.4     | 120           | 26.2    | 2                         | 0.5     | 8             | 1.6     | 11                | 2.0     | 6             | 1.4     | 11                                                 | 2.0     | 6             | 1.4     | 11                | 2.0     |               |         |                   |  |  |  |
| o-NBA (surrogate)   | 117                   | 0                           | 0                    | 0                              | 0               | 0.0     | 0             | 0.0     | 0             | 0.0     | 0             | 0.0     | 0                                                  | 0.0     | 0             | 0.0     | 286                       | 159.3   | 30            | 24.7    | 62                | 50.6    | 23            | 19.4    | 16                                                 | 13.0    | 16            | 13.0    | 16                | 13.0    |               |         |                   |  |  |  |
| HMX                 | 0                     | 125                         | 108                  | 120                            | 115             | 5.4     | 113           | 3.1     | 119           | 4.9     | 110           | 9.6     | 122                                                | 6.2     | 100           | 3.4     | 98                        | 3.7     | 97            | 3.2     | 99                | 3.5     | 96            | 3.2     | 99                                                 | 3.5     | 96            | 3.2     | 99                | 3.5     |               |         |                   |  |  |  |
| RDX                 | 0                     | 118                         | 106                  | 117                            | 83              | 45.9    | 111           | 1.3     | 116           | 8.5     | 103           | 7.6     | 123                                                | 2.6     | 115           | 4.8     | 117                       | 4.9     | 107           | 6.8     | 105               | 6.6     | 106           | 7.9     | 105                                                | 6.6     | 106           | 7.9     | 105               | 6.6     |               |         |                   |  |  |  |
| PA                  | 0                     | 116                         | 109                  | 118                            | 103             | 3.5     | 109           | 0.4     | 119           | 12.4    | 100           | 10.3    | 124                                                | 4.6     | 98            | 0.9     | 99                        | 1.6     | 97            | 0.0     | 98                | 0.5     | 96            | 0.3     | 98                                                 | 0.5     | 96            | 0.3     | 98                | 0.5     |               |         |                   |  |  |  |
| 4-NP                | 0                     | 116                         | 96                   | 110                            | 107             | 5.0     | 33            | 1.2     | 112           | 0.9     | 105           | 2.2     | 114                                                | 2.2     | 101           | 3.1     | 97                        | 3.3     | 98            | 2.8     | 99                | 3.1     | 96            | 2.9     | 99                                                 | 3.1     | 96            | 2.9     | 99                | 3.1     |               |         |                   |  |  |  |
| 1,3,5-TNB           | 0                     | 116                         | 89                   | 108                            | 107             | 5.9     | 108           | 4.7     | 101           | 5.4     | 65            | 11.0    | 115                                                | 6.1     | 102           | 4.9     | 99                        | 5.1     | 98            | 4.6     | 100               | 4.8     | 97            | 4.6     | 100                                                | 4.8     | 97            | 4.6     | 100               | 4.8     |               |         |                   |  |  |  |
| 2,4-DNP             | 0                     | 126                         | 104                  | 119                            | 122             | 9.6     | 126           | 2.8     | 122           | 1.4     | 113           | 2.7     | 125                                                | 2.5     | 99            | 8.0     | 71                        | 4.6     | 91            | 5.7     | 71                | 4.5     | 68            | 3.9     | 71                                                 | 4.5     | 68            | 3.9     | 71                | 4.5     |               |         |                   |  |  |  |
| 1,2-DNB (surrogate) | 84                    | 113                         | 100                  | 111                            | 103             | 6.5     | 99            | 3.2     | 102           | 4.8     | 93            | 9.2     | 108                                                | 5.4     | 231           | 117.1   | 164                       | 115.8   | 162           | 113.4   | 163               | 115.0   | 159           | 113.0   | 163                                                | 115.0   | 159           | 113.0   | 163               | 115.0   |               |         |                   |  |  |  |
| 1,3-DNB             | 0                     | 116                         | 105                  | 110                            | 101             | 5.1     | 97            | 4.0     | 109           | 6.6     | 95            | 10.4    | 103                                                | 5.1     | 101           | 3.3     | 99                        | 3.2     | 98            | 2.8     | 99                | 3.2     | 96            | 3.1     | 99                                                 | 3.2     | 96            | 3.1     | 99                | 3.2     |               |         |                   |  |  |  |
| 2-NP                | 0                     | 112                         | 102                  | 108                            | 100             | 5.6     | 10            | 0.2     | 107           | 5.0     | 96            | 9.6     | 107                                                | 3.6     | 113           | 13.4    | 99                        | 13.0    | 104           | 13.1    | 103               | 13.4    | 103           | 13.3    | 103                                                | 13.4    | 103           | 13.3    | 103               | 13.4    |               |         |                   |  |  |  |
| NB                  | 0                     | 113                         | 105                  | 107                            | 99              | 4.9     | 96            | 4.0     | 106           | 5.3     | 95            | 8.8     | 101                                                | 3.7     | 101           | 2.8     | 99                        | 3.2     | 98            | 2.6     | 99                | 2.7     | 97            | 2.9     | 97                                                 | 2.7     | 97            | 2.9     | 97                | 2.9     |               |         |                   |  |  |  |
| 3,5-DNA             | 0                     | 115                         | 104                  | 108                            | 103             | 6.0     | 44            | 2.0     | 105           | 5.4     | 107           | 9.3     | 106                                                | 5.9     | 100           | 2.8     | 98                        | 3.0     | 98            | 2.6     | 99                | 2.9     | 96            | 2.4     | 99                                                 | 2.9     | 96            | 2.4     | 99                | 2.9     |               |         |                   |  |  |  |
| DNAN                | 0                     | 117                         | 106                  | 109                            | 100             | 4.5     | 98            | 3.9     | 108           | 7.2     | 96            | 10.4    | 102                                                | 3.0     | 99            | 1.7     | 98                        | 1.4     | 97            | 1.2     | 98                | 1.7     | 96            | 1.6     | 98                                                 | 1.7     | 96            | 1.6     | 98                | 1.7     |               |         |                   |  |  |  |
| Tetrad              | 0                     | 118                         | 64                   | 78                             | 108             | 11.1    | 55            | 3.6     | 60            | 18.8    | 44            | 3.4     | 42                                                 | 28.1    | 131           | 54.4    | 83                        | 42.1    | 102           | 39.1    | 101               | 38.2    | 102           | 39.0    | 101                                                | 38.2    | 102           | 39.0    | 101               | 38.2    |               |         |                   |  |  |  |
| NG                  | 0                     | 124                         | 95                   | 112                            | 104             | 64      | 113           | 11.6    | 115           | 13.3    | 117           | 10.6    | 128                                                | 14.7    | 98            | 20.4    | 88                        | 8.6     | 90            | 5.7     | 98                | 15.2    | 107           | 13.5    | 98                                                 | 15.2    | 107           | 13.5    | 98                | 15.2    |               |         |                   |  |  |  |
| 2,4,6-TNT           | 0                     | 114                         | 99                   | 111                            | 100             | 4.2     | 96            | 3.7     | 103           | 8.4     | 78            | 10.5    | 114                                                | 4.4     | 103           | 0.9     | 103                       | 2.7     | 1             |         |                   |         |               |         |                                                    |         |               |         |                   |         |               |         |                   |  |  |  |

Table 29. Cross-laboratory batch analysis tests using six different soil sources and the 2-stage SE method developed under SERDP ER-2722.

| ERDC-EL            |                        | Recoveries (%)               |                            |                                         |                        |        |                  |        |                  |        |                  |        |                  |        |                  |        |
|--------------------|------------------------|------------------------------|----------------------------|-----------------------------------------|------------------------|--------|------------------|--------|------------------|--------|------------------|--------|------------------|--------|------------------|--------|
| Compound           | Blank<br>(Hydromatrix) | Blank Spike<br>(Hydromatrix) | Matrix Spike<br>(Aberdeen) | Matrix Spike<br>Duplicate<br>(Aberdeen) | Spiked Soil (20 mg/kg) |        |                  |        |                  |        |                  |        |                  |        |                  |        |
|                    |                        |                              |                            |                                         | Aberdeen               |        | Jefferson        |        | Memphis          |        | Riley            |        | Yuma             |        | ASTM Fat Clay    |        |
|                    |                        |                              |                            |                                         | Average<br>(n=3)       | StdDev | Average<br>(n=3) | StdDev | Average<br>(n=3) | StdDev | Average<br>(n=3) | StdDev | Average<br>(n=3) | StdDev | Average<br>(n=3) | StdDev |
| NQ                 | 0                      | 99                           | 100                        | 94                                      | 90                     | 1.6    | 85               | 0.7    | 93               | 1.7    | 79               | 4.0    | 98               | 1.4    | 82               | 1.8    |
| NTQ (210nm)        | 0                      | 124                          | 76                         | 74                                      | 76                     | 0.8    | 72               | 1.8    | 105              | 2.2    | 129              | 3.1    | 104              | 0.9    | 97               | 0.5    |
| NTQ (315nm)        | 0                      | 120                          | 69                         | 68                                      | 64                     | 0.9    | 66               | 1.7    | 98               | 1.5    | 84               | 2.0    | 100              | 1.2    | 90               | 0.7    |
| o-NBA (surrogate)  | 116                    | 108                          | 100                        | 97                                      | 95                     | 1.0    | 75               | 2.2    | 102              | 0.7    | 86               | 1.2    | 105              | 1.7    | 93               | 1.1    |
| HMX                | 0                      | 106                          | 90                         | 89                                      | 83                     | 2.6    | 70               | 1.0    | 91               | 1.0    | 55               | 2.7    | 86               | 2.6    | 56               | 1.0    |
| RDX                | 0                      | 106                          | 96                         | 94                                      | 89                     | 3.8    | 76               | 1.2    | 93               | 1.5    | 66               | 2.2    | 96               | 1.5    | 65               | 2.0    |
| PA (210nm)         | 0                      | 101                          | 92                         | 86                                      | 84                     | 1.5    | 87               | 7.5    | 90               | 4.4    | 82               | 4.7    | 99               | 6.2    | 82               | 2.4    |
| PA (315nm)         | 0                      | 98                           | 94                         | 92                                      | 88                     | 2.3    | 85               | 1.2    | 94               | 2.0    | 79               | 2.9    | 98               | 1.4    | 89               | 0.9    |
| 4-NP               | 0                      | 100                          | 97                         | 95                                      | 91                     | 1.4    | 90               | 2.7    | 94               | 1.5    | 86               | 1.0    | 99               | 2.0    | 92               | 2.0    |
| 1,3,5-TNB          | 0                      | 107                          | 98                         | 96                                      | 93                     | 1.1    | 84               | 1.0    | 100              | 1.1    | 82               | 0.4    | 106              | 1.5    | 92               | 1.5    |
| 2,4-DNP            | 0                      | 104                          | 100                        | 98                                      | 95                     | 1.6    | 82               | 1.8    | 99               | 1.1    | 80               | 2.0    | 103              | 6.7    | 91               | 2.7    |
| 12-DNB (surrogate) | 121                    | 103                          | 99                         | 98                                      | 98                     | 0.6    | 96               | 0.5    | 100              | 2.3    | 95               | 1.8    | 103              | 2.2    | 97               | 0.5    |
| 1,3-DNB            | 0                      | 105                          | 103                        | 100                                     | 99                     | 0.3    | 98               | 0.7    | 102              | 0.8    | 94               | 0.9    | 107              | 1.4    | 99               | 0.7    |
| 2-NP               | 0                      | 109                          | 103                        | 101                                     | 94                     | 0.4    | 90               | 0.1    | 84               | 1.0    | 91               | 1.7    | 93               | 1.6    | 85               | 0.9    |
| NB                 | 0                      | 107                          | 102                        | 100                                     | 97                     | 0.7    | 86               | 0.3    | 100              | 1.5    | 77               | 0.8    | 105              | 1.2    | 96               | 0.5    |
| 3,5-DNA (210nm)    | 0                      | 113                          | 102                        | 101                                     | 93                     | 2.7    | 92               | 1.3    | 77               | 3.8    | 93               | 3.2    | 90               | 0.5    | 92               | 0.9    |
| 3,5-DNA (254nm)    | 0                      | 111                          | 101                        | 99                                      | 93                     | 0.3    | 91               | 0.9    | 76               | 1.6    | 93               | 1.5    | 88               | 0.7    | 91               | 1.0    |
| DNAN               | 0                      | 113                          | 108                        | 104                                     | 101                    | 1.8    | 100              | 3.0    | 105              | 4.0    | 94               | 1.5    | 112              | 1.6    | 101              | 0.1    |
| Tetryl             | 0                      | 95                           | 96                         | 91                                      | 90                     | 2.8    | 97               | 0.8    | 89               | 1.7    | 84               | 1.0    | 99               | 1.1    | 89               | 3.4    |
| NG                 | 0                      | 110                          | 94                         | 90                                      | 96                     | 4.7    | 94               | 2.1    | 97               | 2.5    | 90               | 1.6    | 107              | 2.6    | 99               | 4.7    |
| 2,4,6-TNT          | 0                      | 113                          | 94                         | 94                                      | 95                     | 4.0    | 94               | 0.4    | 99               | 2.3    | 86               | 3.2    | 107              | 2.1    | 94               | 2.8    |
| 4-Am-2,6-DNT       | 0                      | 98                           | 101                        | 98                                      | 96                     | 1.5    | 87               | 1.7    | 99               | 1.0    | 73               | 0.8    | 103              | 1.3    | 95               | 1.2    |
| 2-Am-4,6-DNT       | 0                      | 101                          | 102                        | 99                                      | 98                     | 2.3    | 94               | 2.0    | 101              | 2.4    | 86               | 1.5    | 103              | 2.6    | 97               | 0.6    |
| 2,6-DNT            | 0                      | 104                          | 107                        | 102                                     | 102                    | 4.5    | 102              | 5.1    | 107              | 4.4    | 99               | 3.7    | 109              | 4.0    | 102              | 4.4    |
| 2,4-DNT            | 0                      | 105                          | 102                        | 95                                      | 96                     | 0.8    | 96               | 0.9    | 100              | 1.2    | 89               | 0.6    | 106              | 2.8    | 96               | 0.2    |
| 2-NT               | 0                      | 107                          | 101                        | 100                                     | 94                     | 1.2    | 92               | 0.3    | 80               | 1.7    | 93               | 1.2    | 89               | 0.8    | 92               | 1.4    |
| 4-NT               | 0                      | 108                          | 102                        | 94                                      | 94                     | 1.8    | 91               | 2.7    | 82               | 2.3    | 91               | 3.9    | 89               | 1.4    | 88               | 1.1    |
| 3-NT               | 0                      | 104                          | 99                         | 95                                      | 92                     | 1.2    | 91               | 1.6    | 76               | 1.7    | 90               | 2.8    | 86               | 1.8    | 88               | 1.0    |
| PETN               | 0                      | 99                           | 105                        | 92                                      | 94                     | 3.1    | 94               | 3.1    | 96               | 4.4    | 88               | 2.7    | 99               | 7.4    | 98               | 0.9    |

| ERDC-CRREL         |                        | Recoveries (%)               |                        |                                     |                        |        |                  |        |                  |        |                  |        |                  |        |                  |        |
|--------------------|------------------------|------------------------------|------------------------|-------------------------------------|------------------------|--------|------------------|--------|------------------|--------|------------------|--------|------------------|--------|------------------|--------|
| Compound           | Blank<br>(Hydromatrix) | Blank Spike<br>(Hydromatrix) | Matrix Spike<br>(Yuma) | Matrix Spike<br>Duplicate<br>(Yuma) | Spiked Soil (20 mg/kg) |        |                  |        |                  |        |                  |        |                  |        |                  |        |
|                    |                        |                              |                        |                                     | Aberdeen               |        | Jefferson        |        | Memphis          |        | Riley            |        | Yuma             |        | ASTM Fat Clay    |        |
|                    |                        |                              |                        |                                     | Average<br>(n=3)       | StdDev | Average<br>(n=3) | StdDev | Average<br>(n=3) | StdDev | Average<br>(n=3) | StdDev | Average<br>(n=3) | StdDev | Average<br>(n=3) | StdDev |
| NQ                 | 0                      | 186                          | 120                    | 117                                 | 148                    | 3.5    | 89               | 3.7    | 133              | 23.6   | 147              | 7.3    | 108              | 3.9    | 87               | 2.3    |
| NTQ                | 0                      | 58                           | 38                     | 81                                  | 29                     | 3.1    | 74               | 9.5    | 37               | 7.7    | 40               | 6.9    | 76               | 10.7   | 53               | 5.4    |
| o-NBA (surrogate)  | 119                    | 123                          | 90                     | 97                                  | 44                     | 2.6    | 92               | 5.3    | 90               | 41.4   | 44               | 3.1    | 98               | 2.6    | 95               | 2.6    |
| HMX                | 0                      | 126                          | 90                     | 90                                  | 96                     | 1.0    | 85               | 1.7    | 101              | 4.5    | 73               | 0.5    | 91               | 0.2    | 71               | 2.1    |
| RDX                | 0                      | 140                          | 115                    | 118                                 | 114                    | 3.5    | 103              | 2.7    | 120              | 7.5    | 99               | 2.0    | 112              | 0.9    | 97               | 2.5    |
| PA                 | 0                      | 141                          | 123                    | 127                                 | 117                    | 5.3    | 110              | 2.3    | 122              | 9.0    | 100              | 1.7    | 115              | 1.4    | 107              | 3.3    |
| 4-NP               | 0                      | 131                          | 122                    | 131                                 | 119                    | 5.7    | 111              | 2.6    | 120              | 10.3   | 113              | 1.7    | 117              | 1.4    | 110              | 3.9    |
| 1,3,5-TNB          | 0                      | 125                          | 120                    | 129                                 | 116                    | 5.3    | 100              | 2.4    | 119              | 10.5   | 102              | 1.8    | 117              | 1.4    | 105              | 3.9    |
| 2,4-DNP            | 0                      | 114                          | 97                     | 105                                 | 109                    | 4.0    | 94               | 3.7    | 103              | 6.2    | 88               | 0.7    | 103              | 1.4    | 92               | 2.2    |
| 12-DNB (surrogate) | 101                    | 128                          | 121                    | 129                                 | 121                    | 6.3    | 112              | 1.8    | 118              | 10.8   | 112              | 1.6    | 116              | 2.0    | 110              | 3.4    |
| 1,3-DNB            | 0                      | 125                          | 119                    | 128                                 | 118                    | 5.5    | 110              | 1.3    | 113              | 11.8   | 111              | 0.9    | 115              | 2.9    | 109              | 4.2    |
| 2-NP               | 0                      | 117                          | 98                     | 100                                 | 108                    | 3.2    | 98               | 0.2    | 87               | 7.6    | 103              | 0.2    | 95               | 3.3    | 88               | 1.1    |
| NB                 | 0                      | 133                          | 105                    | 112                                 | 109                    | 3.9    | 100              | 0.5    | 84               | 8.6    | 108              | 0.4    | 97               | 3.2    | 96               | 2.8    |
| 3,5-DNA            | 0                      | 127                          | 119                    | 130                                 | 117                    | 5.6    | 101              | 2.6    | 118              | 10.8   | 95               | 1.8    | 116              | 1.4    | 108              | 3.9    |
| DNAN               | 0                      | 121                          | 120                    | 130                                 | 119                    | 5.8    | 110              | 2.2    | 119              | 11.1   | 110              | 1.7    | 117              | 1.1    | 109              | 4.0    |
| Tetryl             | 0                      | 35                           | 58                     | 133                                 | 103                    | 20.0   | 119              | 16.3   | 73               | 33.3   | 121              | 4.7    | 99               | 14.4   | 75               | 4.7    |
| NG                 | 0                      | 152                          | 111                    | 121                                 | 114                    | 9.1    | 99               | 0.4    | 116              | 22.3   | 101              | 8.0    | 103              | 4.0    | 123              | 17.0   |
| 2,4,6-TNT          | 0                      | 137                          | 123                    | 123                                 | 113                    | 6.8    | 106              | 1.3    | 106              | 13.6   | 100              | 1.3    | 111              | 4.2    | 105              | 4.0    |
| 4-Am-2,6-DNT       | 0                      | 127                          | 120                    | 131                                 | 118                    | 5.9    | 103              | 2.2    | 120              | 10.6   | 92               | 2.4    | 116              | 1.3    | 111              | 4.0    |
| 2-Am-4,6-DNT       | 0                      | 124                          | 120                    | 130                                 | 120                    | 5.8    | 110              | 2.5    | 121              | 10.9   | 105              | 2.0    | 117              | 1.2    | 111              | 3.9    |
| 2,6-DNT            | 0                      | 0                            | 121                    | 131                                 | 117                    | 6.3    | 109              | 1.6    | 112              | 12.7   | 112              | 1.0    | 115              | 3.9    | 109              | 5.4    |
| 2,4-DNT            | 0                      | 111                          | 120                    | 131                                 | 117                    | 6.6    | 109              | 2.2    | 112              | 13.3   | 110              | 1.4    | 114              | 3.9    | 109              | 4.7    |
| 2-NT               | 0                      | 133                          | 108                    | 115                                 | 110                    | 3.9    | 102              | 0.1    | 88               | 10.6   | 109              | 0.2    | 99               | 4.2    | 97               | 3.8    |
| 4-NT               | 0                      | 129                          | 107                    | 113                                 | 114                    | 4.1    | 102              | 0.8    | 84               | 12.2   | 107              | 0.6    | 98               | 4.9    | 93               | 4.3    |
| 3-NT               | 0                      | 135                          | 106                    | 112                                 | 109                    | 4.0    | 100              | 1.0    | 81               | 11.3   | 105              | 0.8    | 95               | 5.1    | 92               | 3.3    |
| PETN               | 0                      | 123                          | 124                    | 132                                 | 120                    | 6.9    | 112              | 2.3    | 122              | 12.1   | 115              | 2.9    | 117              | 2.0    | 112              | 4.8    |

**Bold, italicized** are IM compounds and degradation products.

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

(DoD QSM Ver5.2 for Solids – Lowest LCL: 4-Am-2,6-DNT, 64%; Highest UCL: Tetryl, 135%)

## Section II. MEKC and CE Methods

### Materials and Methods

#### MEKC Methods

In this work, two distinct capillary electrophoresis-based separations were developed to facilitate analysis of explosives-containing samples. The primary mode of analysis is a micellar electrokinetic chromatography (MEKC) separation, and the secondary mode of analysis is a capillary electrophoresis (CE) method. This mode of separation is based upon a combination of charge-to-size ratio of the compounds and their affinity for negatively charged cholate micelles included in the background electrolyte (BGE). BGE, sample matrix (SM), and separation methodology are as follows:

*BGE 1: 25 mM Sodium Tetraborate, 50 mM Tris(hydroxymethyl)aminomethane (TRIS), 100 mM Sodium Cholate, 7.5% v/v MeOH*

*BGE 2: BGE 1, 3 mM diaminobutane (DAB)*

*SM: 300 mM Sodium Chloride with up to 20% v/v MeOH*

*Capillary: 60 cm (50 cm effective length), 50  $\mu$ m internal diameter*

*Capillary preconditioning: Rinse 1 min at 20 psi with 50/50 Ethanol:1 M NaOH; Rinse 1 min at 20 psi with 1 M NaOH; Rinse 1 min at 20 psi with water; Rinse 2 min at 20 psi with BGE 2*

*Sample Injection: Inject sample for 5 sec at 1 psi, producing a sample plug length of 5 mm and 10 nL volume. Inject BGE 2 for 40 sec at 1 psi, moving the sample plug 40 mm into the capillary*

*Separation Conditions: During separations, the inlet vial contains BGE 1, and the outlet vial contains BGE 2. A constant power of 0.7 W is applied across the capillary for 35 min, after which an additional 0.9 psi is applied to the inlet vial, while the constant power separation continues. Total separation time is 55 min. UV absorbance data is collected with a diode array detector (DAD) between 210-315 nm at a collection frequency of 4 Hz.*

The presence of 3 mM DAB in BGE 2 suppresses electroosmotic flow (EOF) in the capillary during the early part of the separation in order to improve separation performance of the least retained peaks. During the separation, the DAB migrates out of the capillary as BGE 2 is replaced with BGE 1. Careful consideration of DAB content is essential, as significant suppression of EOF will result in the cluster of six negatively charged compounds to migrate out of the capillary into the inlet vial in the early moments of the separation. The additional plug of BGE 2 after sample injection provides a “buffer” zone to ensure all sample remains in the capillary while DAB migrates out of the capillary, thus increasing EOF velocity. A representative separation of a 20 mg/L sample is shown in Figure 12 using a 225 nm detection wavelength. The separation can be broken down into three distinct regions, as shown in the figure. There are several examples where peaks co-elute; specifically, TNT/PETN, 2-NT/3-NT, and PA/o-NBA co-migrate under these separation conditions. We have noted that manipulation of the cholate and DAB concentrations will result in the ability to resolve the TNT/PETN and 2-NT/3-NT limiting pairs; however, all charged compounds are not retained in the capillary.

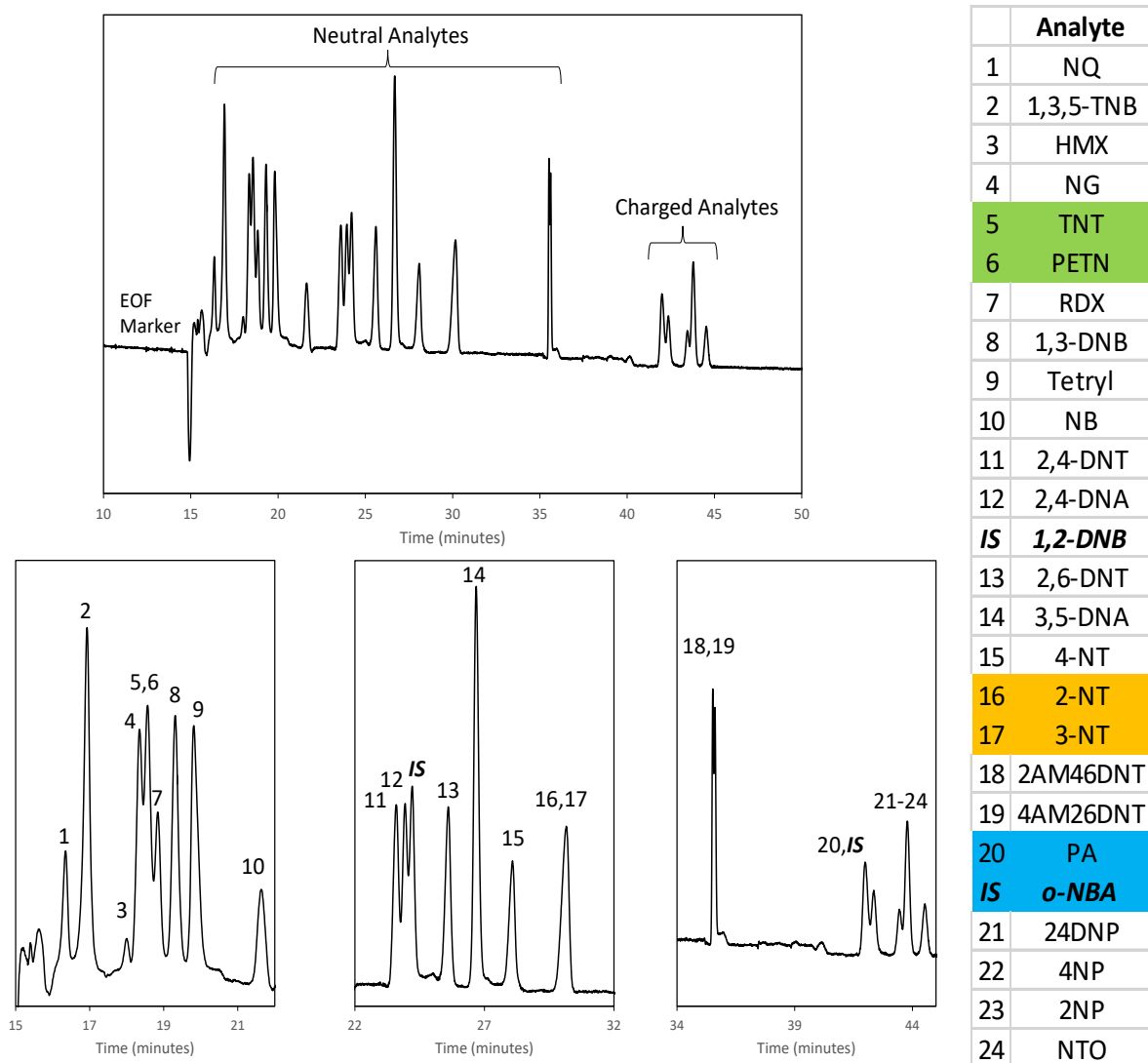


Figure 12. Representative separation of a standard solution of the 24 target analytes and surrogates 1,2-DNB and o-NBA. The sample concentration is 20 mg/L.

A secondary mode of analysis was developed to provide a “Total Extraction Efficiency” of samples to access rapidly the overall performance of compound extraction for soil, tissue, and water matrices. BGE, SM, and separation methodology are as follows:

*BGE: 25 mM Sodium Tetraborate, 50 mM TRIS*

*SM: 12.5 mM Sodium Tetraborate, 25 mM TRIS; SM may contain as much as 50% v/v MeOH*

*Pre-SM: 25 mM Sodium Tetraborate, 50 mM TRIS, 100 mM Sodium Cholate, 3 mM DAB, 7.5% v/v MeOH*

*Capillary: 60 cm (50 cm effective length), 50  $\mu$ m internal diameter*

*Capillary preconditioning: Rinse 1 min at 20 psi with 50/50 Ethanol:1 M NaOH; Rinse 1 min at 20 psi with 1 M NaOH; Rinse 1 min at 20 psi with water; Rinse 2 min at 20 psi with BGE 2*

*Sample Injection: Inject pre-SM for 10 sec at 1 psi, producing a 10 mm long sample plug. Inject sample for 5 sec at 1 psi, moving the sample plug 5 mm into the capillary with a 10 nL volume*

*Separation Conditions: Constant power of 0.7 W is applied across the capillary for 20 min. UV absorbance data is collected with a DAD between 210-315 nm at a collection frequency of 4 Hz.*

It should be noted that throughout this work, an underlying issue has been the limited sample volumes that are injectable in CE-based separations. A constant sample volume of 10 nL has been chosen, with a focus on resolving as many compounds as possible. Improved sensitivity can be realized for many of the compounds, but not without sacrificing the ability to resolve/detect others. For example, the addition of a larger concentration of DAB will improve the resolving power of the separation for the least retained compounds at the sacrifice of detecting the charged compounds.

Of course, another equally important goal is to determine the efficacy of compound extraction from the target matrices – this separation modality provides that capability. A representative electropherogram is shown in Figure 13. In this instance, all of the neutral target compounds and 1,2-DNB are detected as a single peak, while the negatively charged target compounds and *o*-NBA are resolvable peaks with PA and *o*-NBA co-eluting, as was the case in the MEKC-based separation. The use of the pre-SM injection of cholate containing BGE allows for temporal resolution between the EOF marker and the neutral target compounds.

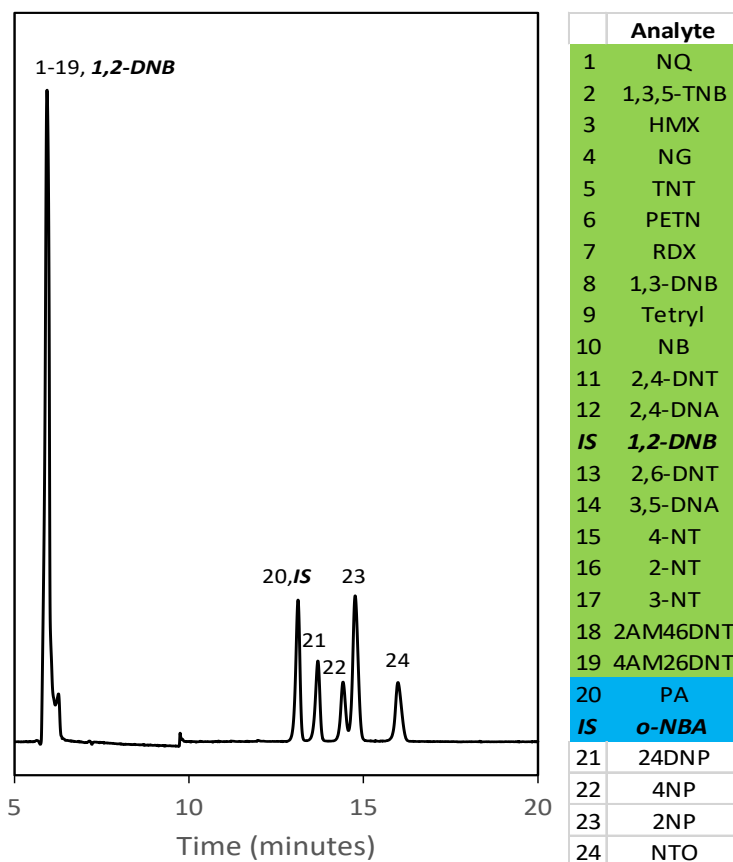


Figure 13. Representative separation of a standard solution of 24 target analytes and the internal standards 1,2-DNB and *o*-NBA. The sample concentration is 50 mg/L.

## Results and Discussion

### MEKC Analytical Method

MEKC method development focused on an analytical method capable of resolving all of the 24 compounds of interest and the two internal standards with a separation time less than 60 min. The inherent difficulty associated with the development of this method is that the analyte list includes both neutral and negatively charged compounds. The approach used in the optimization of the MEKC methods presented in this work was to widen the separation window associated with the neutral molecules as much as possible, while ensuring that negatively charged species remain in the capillary column throughout the separation. At the point where all neutral components were detected, a slight pressure was applied to the inlet side of the capillary to push the negatively charged compounds to the detector in order to minimize total separation time.

In order to improve peak capacity and selectivity for neutral compounds, the generalized approach was to increase surfactant concentration and modify EOF with an eye towards shifting the equilibrium of compound into the surfactant micelle serving as the pseudo-stationary phase in the separation. The consequence of these additions was an increase in separation time. Given that extracts from water, soil, and tissue samples were investigated, a cholate-based micelle was chosen as the pseudo-stationary phase to promote robustness against changes related to sample matrix composition. The effects of BGE, surfactant, and organic modifier concentration on separation performance were systematically studied in order to establish conditions under which all 26 target molecules (24 analytes, two surrogates) were resolved. Unfortunately, a single set of separation conditions was not achieved which allows for baseline resolution of all target compounds. Figure 12 illustrates the optimized conditions for the separation of all target compounds using the MEKC method. The resulting method was unable to resolve TNT/PETN, 2-NT/3-NT, and PA/*o*-NBA co-elutions. The separation can be considered in terms of three distinct regions spanning compounds 1-10, 11-17 (and 1,2-DNB), and 18-24 (and *o*-NBA). Each subset of the separation may be optimized to allow for complete resolution of a single sub-set of compounds, but resolution/detection of the compounds in other regions is compromised. For example, increasing surfactant micelle concentration allows for baseline resolution of the first 10 compounds; however, the resultant reduction in EOF causes negatively charged compounds to migrate into the inlet vial during the separation.

### Evaluation of Water Extraction Method

Utilizing the method developed for HPLC-analyzed water samples, MEKC samples were further diluted after solid phase extraction in order to be suitable for analysis. High organic content in MEKC sample matrices disrupts the boundary between the SM and the BGE in the capillary. This disruption results in significant broadening of injected compounds and an inability to resolve peaks, especially early in the separation window. SPE extracts were diluted 1:4 in 300 mM NaCl. The resultant sample was between 10-20% MeOH, a concentration that did not disrupt separation performance.

The results of water extractions proved inconclusive, due to the dilute nature of the samples when analyzed by MEKC. Figure 14 shows representative electropherograms of the MeOH and acidified MeOH extracts of the reagent water samples provided by ERDC-EL. As shown in Figure 14, more analytes of interest are observed in the acidified fraction; however, all responses were below the limits of quantitation of the MEKC technique.

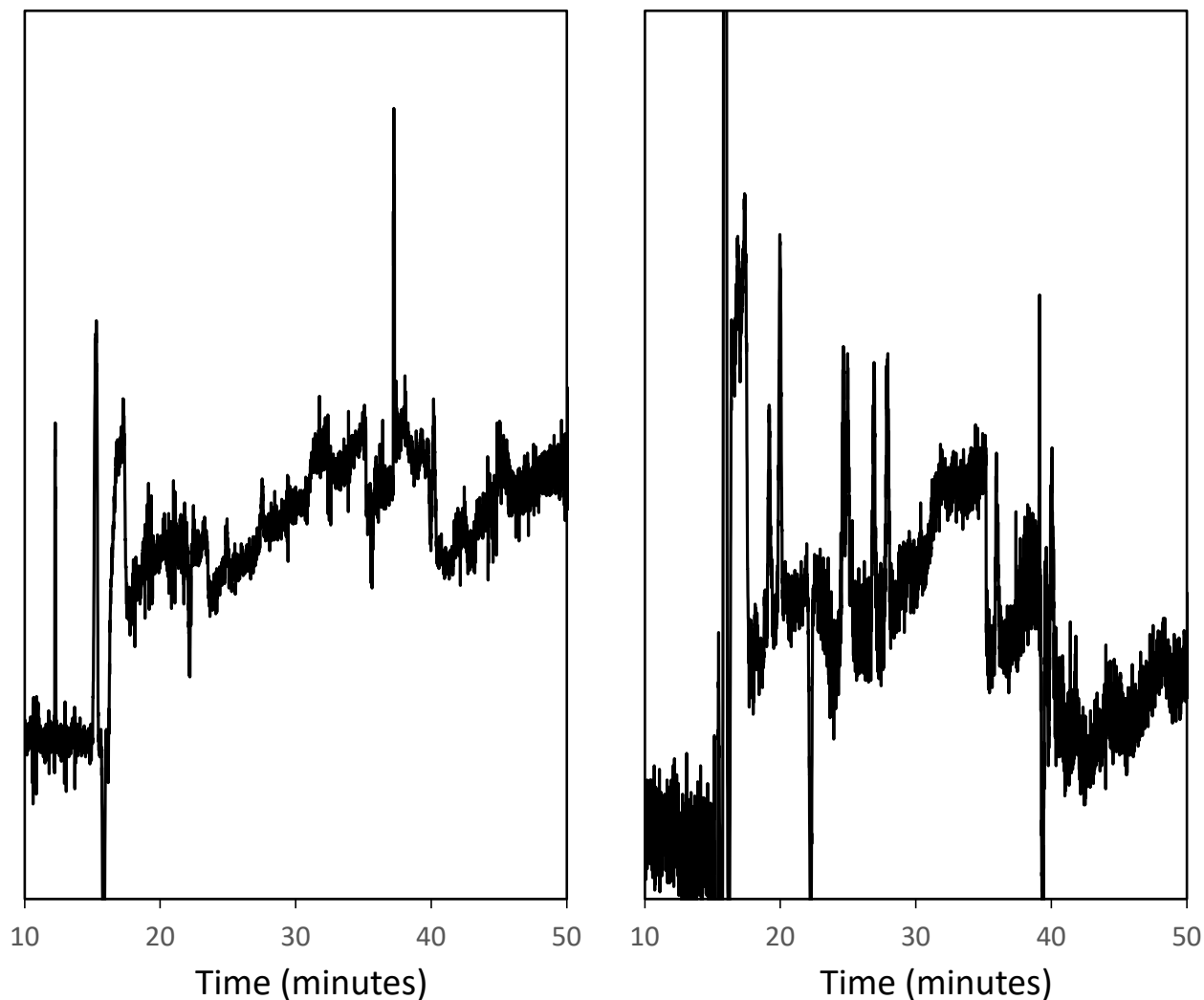


Figure 14. Representative electropherograms of reagent water extracts analyzed by MEKC method.  
Left: MeOH extract, Right: acidified MeOH extract.

### Evaluation of Soil Extraction Method

In all cases, samples were extracted per the method developed for HPLC-analyzed soil extracts. While HPLC protocols allowed for the combining of extract samples (i.e., combining the MeOH extract with the MeOH/water extract, followed by analysis of the combined sample), NRL chose to run sequential extracts separately. This allowed for the identification of any matrix effects on separation performance. In all cases, 10  $\mu$ L extract was added to 40  $\mu$ L 300 mM NaCl. The presence of NaCl in the SM allowed for sample stacking in the MEKC method, providing modest improvements in resolution and sensitivity with the 5 mm (10 nL) sample injection.

As with the water extractions, soil extraction results proved to be inconclusive using MEKC, due to final extract sample concentrations being lower than the limits of quantitation after preparing samples in the appropriate SM for MEKC analysis. Nominal extract concentration was 2 mg/L, with the MEKC method further reducing the sample concentration to 0.4 mg/L. Figure 15 shows a representative electropherogram of a soil extract, the MeOH extract of the Ft. Riley sample.

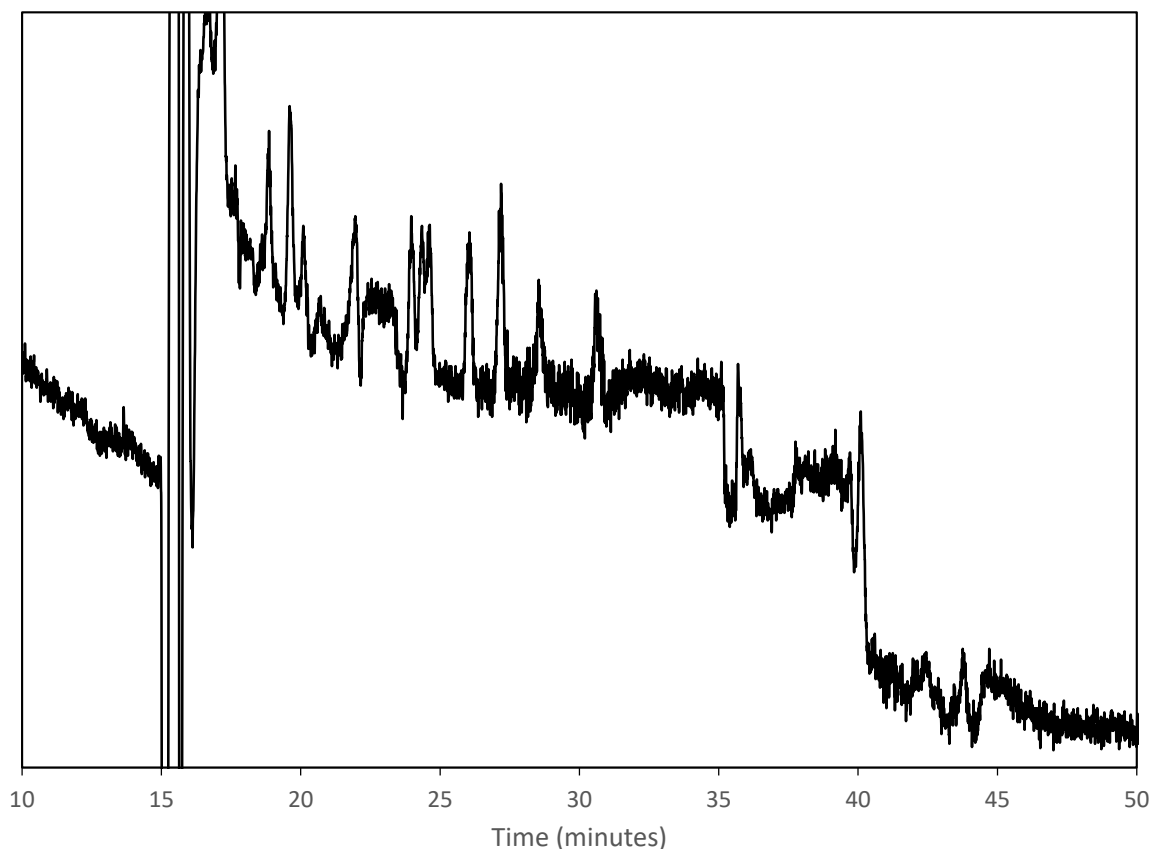


Figure 15. Example electropherogram of soil MeOH extract (Ft. Riley) analyzed by MEKC method.

### Evaluation of Tissue Extraction Method

For tissues, the same modifications of the extraction techniques developed for HPLC-analyzed samples were made, as for soils. Just as for waters and soils, tissue results proved to be inconclusive using MEKC due to final prepared sample concentrations being lower than the quantitation limits of the method. Figure 16 shows a representative electropherogram of the MeOH and acidified MeOH extracts that underwent interference reduction treatment. In this instance, the only detected compound in the MeOH fraction (left) was one of the amino-DNT's. Additional analytes were detected in the acidified MeOH fraction (right). While many analytes were observed, the response was well below quantitative limits.

### Capillary Electrophoresis for Total Extraction Efficiency

Given the dilute nature of the samples, which were below MEKC method detection limits, it was determined that a "Total Extraction Efficiency" separation would be more suitable, as sample concentrations would be reduced by only 50%, which would provide sufficient signal for detection and quantitation. Extraction efficiencies for water, soil, and tissue extractions are shown in Tables 30-32.

Of particular note in the soil samples is the extraction efficiency associated with 4-nitrophenol. In many cases, the efficiency exceeded 100%. As this method was only recently developed, potential matrix effects and soil-based interferences have not been fully investigated. It is possible that negative and/or positive interferences are co-extracted from soils, similar to those observed in the HPLC-UV analysis of tissue extracts. In the case of tissue samples, with the exception of the earthworm sample, the neutral compounds were not detected.

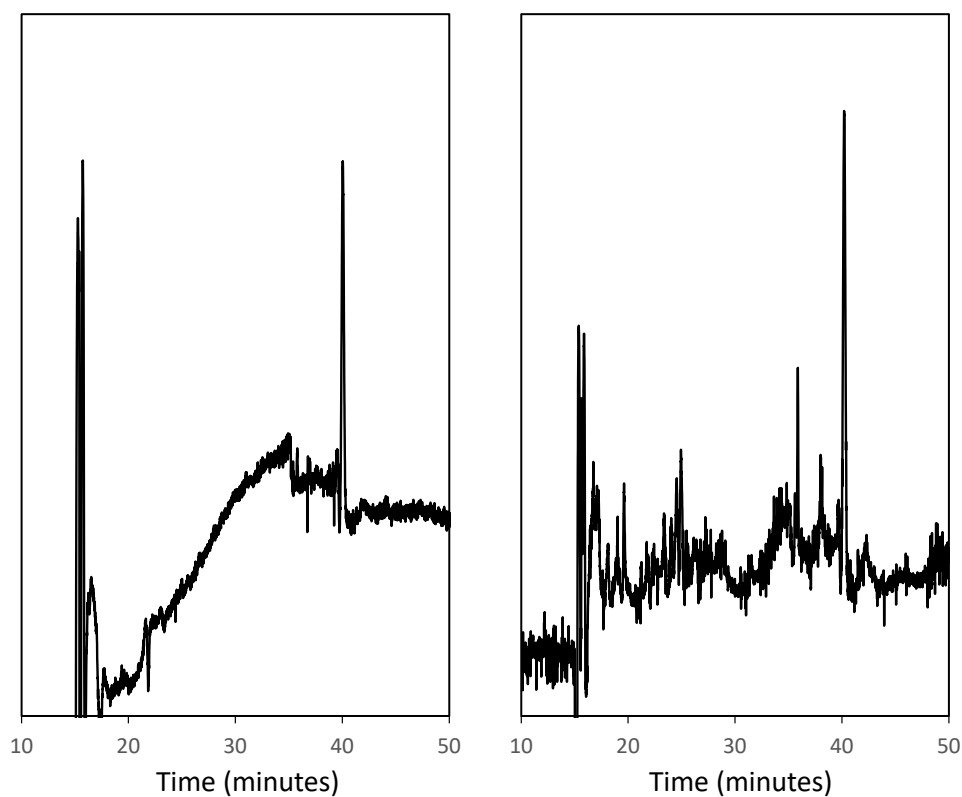


Figure 16. Example electropherograms of tissue extracts analyzed by MEKC method.  
Left: MeOH extract, Right: acidified MeOH extract.

Table 30. Summary of extraction efficiencies for water samples determined using the MEKC method.

| Compound            | Extraction Efficiency (%) |           |                                |                   |            |
|---------------------|---------------------------|-----------|--------------------------------|-------------------|------------|
|                     | Reagent Water             | Tap Water | Houston Shipping Channel Water | Yazoo River Water | Well Water |
| <i>NQ</i>           | 32                        | 0         | 9                              | 28                | 12         |
| 1,3,5-TNB           |                           |           |                                |                   |            |
| HMX                 |                           |           |                                |                   |            |
| NG                  |                           |           |                                |                   |            |
| 2,4,6-TNT           |                           |           |                                |                   |            |
| PETN                |                           |           |                                |                   |            |
| RDX                 |                           |           |                                |                   |            |
| 1,3-DNB             |                           |           |                                |                   |            |
| Tetryl              |                           |           |                                |                   |            |
| NB                  |                           |           |                                |                   |            |
| 2,4-DNT             |                           |           |                                |                   |            |
| <i>DNAN</i>         |                           |           |                                |                   |            |
| 1,2-DNB (surrogate) |                           |           |                                |                   |            |
| 2,6-DNT             |                           |           |                                |                   |            |
| 3,5-DNA             |                           |           |                                |                   |            |
| 4-NT                |                           |           |                                |                   |            |
| 2-NT                |                           |           |                                |                   |            |
| 3-NT                |                           |           |                                |                   |            |
| 2-Am-4,6-DNT        |                           |           |                                |                   |            |
| 4-Am-2,6-DNT        |                           |           |                                |                   |            |
| <i>PA</i>           | 60                        | 63        | 68                             | 68                | 15         |
| o-NBA (surrogate)   |                           |           |                                |                   |            |
| <i>2,4-DNP</i>      |                           |           |                                |                   |            |
| <i>4-NP</i>         |                           |           |                                |                   |            |
| <i>2-NP</i>         |                           |           |                                |                   |            |
| <i>NTO</i>          | 61                        | 0         | 39                             | 0                 | 0          |

***Bold, italicized are IM compounds and degradation products.***

Table 31. Summary of extraction efficiencies for soil samples determined using the MEKC method.

| Compound              | Extraction Efficiency (%) |           |         |       |      |               |
|-----------------------|---------------------------|-----------|---------|-------|------|---------------|
|                       | Aberdeen                  | Jefferson | Memphis | Riley | Yuma | ASTM Fat Clay |
| <b><i>NQ</i></b>      | 57                        | 94        | 53      | 48    | 60   | 42            |
| 1,3,5-TNB             |                           |           |         |       |      |               |
| HMX                   |                           |           |         |       |      |               |
| NG                    |                           |           |         |       |      |               |
| 2,4,6-TNT             |                           |           |         |       |      |               |
| PETN                  |                           |           |         |       |      |               |
| RDX                   |                           |           |         |       |      |               |
| 1,3-DNB               |                           |           |         |       |      |               |
| Tetryl                |                           |           |         |       |      |               |
| NB                    |                           |           |         |       |      |               |
| 2,4-DNT               |                           |           |         |       |      |               |
| <b><i>DNAN</i></b>    |                           |           |         |       |      |               |
| 1,2-DNB (surrogate)   |                           |           |         |       |      |               |
| 2,6-DNT               |                           |           |         |       |      |               |
| 3,5-DNA               |                           |           |         |       |      |               |
| 4-NT                  |                           |           |         |       |      |               |
| 2-NT                  |                           |           |         |       |      |               |
| 3-NT                  |                           |           |         |       |      |               |
| 2-Am-4,6-DNT          |                           |           |         |       |      |               |
| 4-Am-2,6-DNT          |                           |           |         |       |      |               |
| <b><i>PA</i></b>      | 114                       | 135       | 125     | 97    | 123  | 103           |
| o-NBA (surrogate)     |                           |           |         |       |      |               |
| <b><i>2,4-DNP</i></b> | 115                       | 130       | 121     | 58    | 118  | 112           |
| <b><i>4-NP</i></b>    | 144                       | 329       | 113     | 103   | 284  | 153           |
| <b><i>2-NP</i></b>    | 89                        | 105       | 82      | 76    | 89   | 82            |
| <b><i>NTO</i></b>     | 96                        | 0         | 126     | 77    | 102  | 100           |

***Bold, italicized*** are IM compounds and degradation products.

Table 32. Summary of extraction efficiencies for tissue samples determined using the MEKC method.

| Compound              | Extraction Efficiency (%) |           |                 |          |
|-----------------------|---------------------------|-----------|-----------------|----------|
|                       | Fathead Minnow            | Earthworm | Polychaete Worm | Ryegrass |
| <b><i>NQ</i></b>      | 0                         | 18        | 0               | 0        |
| 1,3,5-TNB             |                           |           |                 |          |
| HMX                   |                           |           |                 |          |
| NG                    |                           |           |                 |          |
| 2,4,6-TNT             |                           |           |                 |          |
| PETN                  |                           |           |                 |          |
| RDX                   |                           |           |                 |          |
| 1,3-DNB               |                           |           |                 |          |
| Tetryl                |                           |           |                 |          |
| NB                    |                           |           |                 |          |
| 2,4-DNT               |                           |           |                 |          |
| <b><i>DNAN</i></b>    |                           |           |                 |          |
| 1,2-DNB (surrogate)   |                           |           |                 |          |
| 2,6-DNT               |                           |           |                 |          |
| 3,5-DNA               |                           |           |                 |          |
| 4-NT                  |                           |           |                 |          |
| 2-NT                  |                           |           |                 |          |
| 3-NT                  |                           |           |                 |          |
| 2-Am-4,6-DNT          |                           |           |                 |          |
| 4-Am-2,6-DNT          |                           |           |                 |          |
| <b><i>PA</i></b>      | 39                        | 35        | 35              | 37       |
| o-NBA (surrogate)     |                           |           |                 |          |
| <b><i>2,4-DNP</i></b> | 30                        | 0         | 44              | 24       |
| <b><i>4-NP</i></b>    | 39                        | 117       | 104             | 81       |
| <b><i>2-NP</i></b>    | 29                        | 10        | 34              | 26       |
| <b><i>NTO</i></b>     | 47                        | 52        | 48              | 19       |

***Bold, italicized*** are IM compounds and degradation products.

## Conclusions and Implications for Future Research/Implementation

The primary objective of this project to fulfill the requirements of SON ERSON-17-02 by developing new methods of extraction, pre-concentration, and analytical separation and quantitation of 17 legacy as well as seven IM compounds, daughter products of IM, and other munition compounds absent from USEPA Method 8330B, the current standard method for energetic compound determinations, to yield a single standardized method for simultaneous analysis of legacy and IM compounds in environmental matrices has been fulfilled by developing several analytical method options (HPLC-UV, LC-MS, MEKC, and CE), as well as effective extraction methods for waters (SPE), soils (2-stage SE), and tissues (SE, along with accompanying interference reduction procedure). The development of these methods should, in turn, lead to cost savings with a minimal increase in environmental monitoring efforts and no modification of existing sample collection procedures.

During this project, two separate HPLC-UV column separation methods were developed, enabling orthogonal confirmation of analyte concentration down to low- $\mu\text{g/L}$  levels in water, soil/sediment, and tissue samples. Pre-concentration of water samples using solid phase extraction can lower analyte detection levels to sub- $\mu\text{g/L}$  levels with sufficient concentration factors applied. A less sensitive HPLC-MS scan mode method was developed, which can be used as an alternative to a secondary column confirmation method. Sample preparation and solvent gradients were fine-tuned for optimal method performance. Furthermore, the SPE and SE methods (and interference reduction method for tissues) developed for this project yield compound recoveries for all analytes of interest and surrogates within currently accepted limits, with the recoveries for many of the compounds consistently better than current methods.

The concurrently developed MEKC-based method proved to have limited utility in the analysis of extracts of laboratory-spiked environmental matrices, due to the requirement for further dilution of extracts in an appropriate sample matrix for analysis. An alternative method was proposed that allows for a rapid analysis and provides a total extraction efficiency measurement for the neutral compounds, while maintaining the ability to quantify the negatively charged compounds. It is important to note that extraction methods were developed with an eye towards compatibility with HPLC-based analysis. Future work focusing on the development of extraction methods that result in compounds eluted/extracted into more MEKC-compatible matrices would be beneficial.

The development of these methods to simultaneously analyze environmentally co-present legacy and insensitive munition compounds was necessary to enable future monitoring of co-occurring legacy and IM components at firing ranges, demilitarization facilities, manufacturing facilities, and environmental sites, where munitions are tested, produced, and detected (Felt et al., 2013; Walsh et al., 2014). The optimized primary and secondary HPLC methods reduce total analysis time by ~25% versus using four different analytical methods as has previously been required (two methods for legacy and two different methods for IM). In addition, the labor and supply cost savings due to co-extraction and sample preparation is at least 50%, as only a single extraction procedure and analytical sample preparation are required versus up to four (including confirmation analyses), since the sample preparation for the SERDP ER-2722 primary and secondary methods are the same. Furthermore, the products of this work will contribute to the ability to conduct fate and transport studies for IM compounds by providing a standardized method for quantitation of IM and legacy materials that is needed to determine long-term effects.

The method development work executed under SERDP ER-2722 will continue under ESTCP ER19-D1-5078 (start, Feb 2019) to validate the methods. The validation will consist of round-robin comparison studies, carried out with the cooperation of USEPA, with the end goal of producing an addendum to the current USEPA Method 8330B.

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