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Methods for Simultaneous Determination of 29 Legacy and Insensitive Munition (IM) Constituents in Aqueous, Soil-Sediment, and Tissue Matrices by High-Performance Liquid Chromatography (HPLC)

Bobbi Stromer, Rebecca Crouch, Katrinka Wayne,
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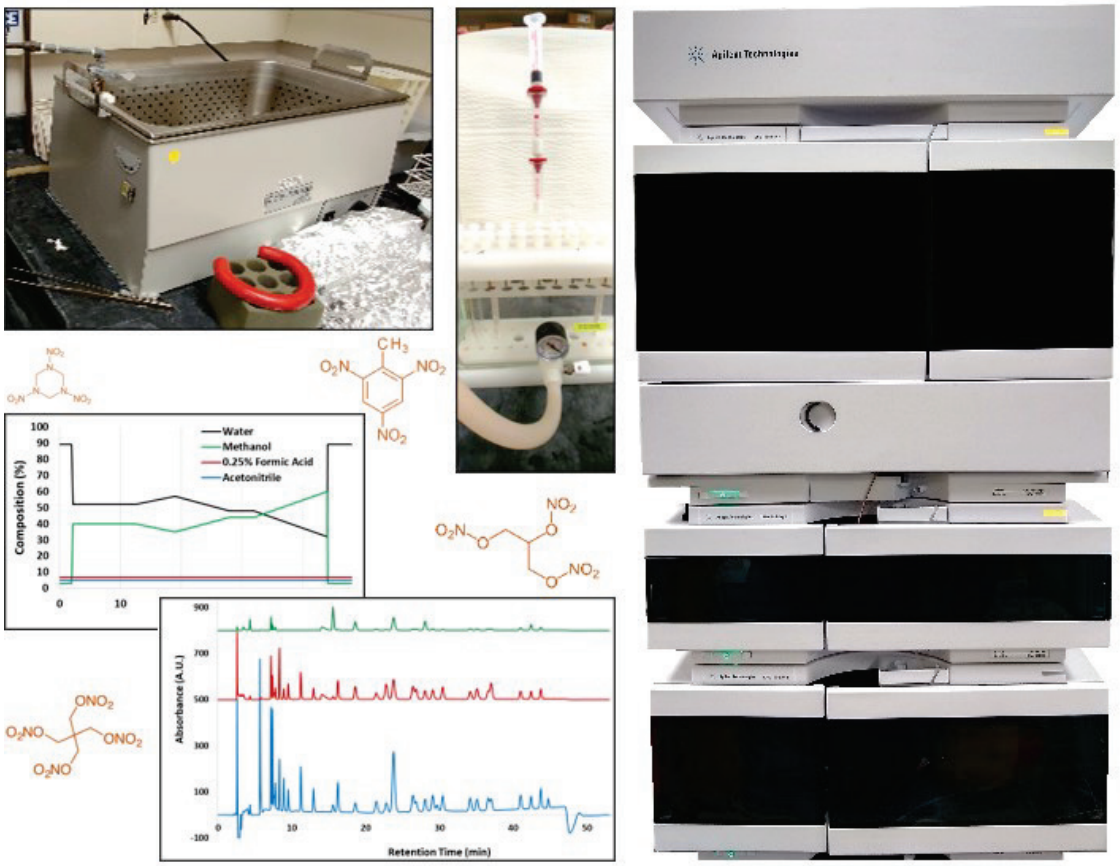
September 2021



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Abstract

Standard methods are in place for analysis of 17 legacy munitions compounds and one surrogate in water and soil matrices; however, several insensitive munition (IM) and degradation products are not part of these analytical procedures. This lack could lead to inaccurate determinations of munitions in environmental samples by either not measuring for IM compounds or using methods not designed for IM and other legacy compounds. This work seeks to continue expanding the list of target analytes currently included in the US Environmental Protection Agency (EPA) Method 8330B. This technical report presents three methods capable of detecting 29 legacy, IM, and degradation products in a single High Performance Liquid Chromatography (HPLC) method with either ultraviolet (UV)-visible absorbance detection or mass spectrometric detection. Procedures were developed from previously published works and include the addition of hexahydro-1-nitroso-3,5-dinitro-1,3,5-triazine (MNX); hexahydro-1,3-dinitroso-5-nitro-1,3,5-triazine (DNX); hexahydro-1,3,5-trinitroso-1,3,5-triazine (TNX); 2,4-diamino-6-nitrotoluene (2,4-DANT); and 2,6-diamino-4-nitrotoluene (2,6-DANT). One primary analytical method and two secondary (confirmation) methods were developed capable of detecting 29 analytes and two surrogates. Methods for high water concentrations (direct injection), low-level water concentrations (solid phase extraction), soil (solvent extraction), and tissue (solvent extraction) were tested for analyte recovery of the new compounds.

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Preface

This study was conducted for the US Army Environmental Quality Technology under the "Environmental Sensors for Explosives" program under PE 633728/Project 03F/Task 19. The technical monitor was Dr. Elizabeth Ferguson.

The work was performed by the Environmental Chemistry Branch (EPC) of the Environmental Processes Division (EP), US Army Engineer Research and Development Center, Environmental Laboratory (ERDC-EL). At the time of publication, Amber L. Russell was Chief, EPC; Warren P. Lorentz was Chief, EP; and Elizabeth Ferguson, was the Technical Director for Installations and Operation Environment. The Deputy Director of ERDC-EL was Dr. Brandon Lafferty, and the Director was Dr. Edmund Russo.

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

1 Introduction

The ability to detect trace amounts of explosives and their degradation products accurately with known data quality and in complex environmental media is important for a variety of military and domestic security applications. This report attempts to broaden the analytical capabilities of standard methods for analyzing munitions in environmental matrices.

1.1 Background

Recently, the military has been replacing legacy munitions with insensitive munitions (IM) to protect warfighters while still maintaining the same level of performance (Powell 2016, 409–413; Isler 1998, 283–291). Training ranges, demilitarization facilities, manufacturing facilities, and sites where munitions have been tested and produced can contain legacy (Comfort et al. 1995, 1174–1182) and IM residues (Taylor, Dontsova, and Walsh 2017, 407–443). The environmental fate of these munitions includes dilution, dispersion, biotic and abiotic transformations, and sorption to environmental matrices, resulting in many different degradation products.

Use of IM compounds at sites where legacy munitions are present requires improved detection methods capable of identification and quantitation of both legacy and IM compounds concurrently. The standard method for munitions constituents' extraction and analysis is the United States Environmental Protection Agency's Method 8330 (SW-846): Nitroaromatics, Nitramines, and Nitrate Esters by High Performance Liquid Chromatography (HPLC) and a subsequent addendum (USEPA 2006) that targets a select list of legacy explosive compounds. While there is no standard method for IM analysis, some recent literature details analytical methods for IM compounds (Russell et al. 2014, 524–530; Walsh 2016, 76–91). This lack of a standard method requires at least two separate approaches to analyze samples that contain both legacy and IM compounds, which can cost analysts time and money. Recent efforts aimed to standardize methods of analysis for USEPA 8330B analytes along with seven additional legacy, IM, or degradation products and one new surrogate (Crouch et al. 2020, 121008). However, several known legacy, IM, and degradation products are not included in these updated methods.

1.2 Objective

The current work builds capabilities of simultaneous munitions constituent and degradation product analysis by adding an additional five degradation compounds to the previously reported method (Crouch et al. 2020, 121008).

Hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) is an abundantly used munitions compound that is biodegraded under anaerobic conditions into three degradation products: Hexahydro-1-nitroso-3,5-dinitro-1,3,5-triazine (MNX); Hexahydro-1,3-dinitroso-5-nitro-1,3,5-triazine (DNX); and Hexahydro-1,3,5-trinitroso-1,3,5-triazine (TNX) (Adrian and Arnett 2004, 332–340; Hawari et al. 2000, 605–618). The well-known legacy munition 2,4,6-trinitrotoluene (TNT) has been used for decades by the military, mining operations, and several other industries and is degradable by many organisms through aerobic and anaerobic pathways, resulting in several degradation intermediates and final products (Hawari et al. 1998, 2200–2206; Gorontzy et al. 1994, 265–284). Some of these degradates are detected by the USEPA 8330B method (including 2,4-dinitrotoluene; 2,6-dinitrotoluene; 2-amino-4,6-dinitrotoluene; and 4-amino-2,6-dinitrotoluene, among others). However, to the authors' knowledge, TNT degradates 2,4-diamino-6-nitrotoluene (2,4-DANT) and 2,6-diamino-4-nitrotoluene (2,6-DANT) are not detected by a comprehensive method.

1.3 Approach

The analytical methods presented herein are based on previous work (Crouch et al. 2020, 121008; Crouch et al. 2021), in which eight new compounds were added to the USEPA 8330B target list of compounds. That work resulted in one primary and two secondary analytical methods capable of identifying and quantifying 24 legacy, IM, and degradation products in a single HPLC analysis. It also provided a modified solid phase extraction, soil-sediment solvent extraction, and tissue solvent extraction with interference reduction procedure. For the current work, previous analytical methods (Russell et al. 2014, 524–530; Crouch et al. 2020, 121008; Crouch et al. 2021) were modified to enable chromatographic separation and quantitation of 29 legacy, IM, and degradation compounds in a single HPLC analysis. Methods of direct-injection water analysis, solid phase extraction, and solvent extraction of soil and tissues methods were tested to determine extraction efficiencies for the five additional compounds of interest. Method detection limits for each extraction

method were determined on the primary and both secondary methods, and they are reported herein.

2 Materials and Methods

2.1 Materials

A list of all analytes, acronyms, and Chemical Abstract Service (CAS) numbers is provided in Table 1. Concentrated standards for nitroguanidine (NQ); picric acid (PA); 2,4-dinitrophenol (2,4-DNP); 4-nitrophenol (4-NP); 1,2-dinitrobenzene (1,2-DNB); and the 17-compound mixture of the nitroaromatic and nitramine compounds included in USEPA 8330B and Pinnacle II Biphenyl 5 µm[1], 150 × 4.6 mm analytical column were purchased from Restek (Bellefonte, PA, USA). The 2-nitrophenol (2-NP); 2,4-DANT; and 2,6-DANT were obtained from AccuStandard (New Haven, CT, USA). The ortho-nitrobenzoic acid (o-NBA) was obtained from Sigma Aldrich (St. Louis, MO, USA), 2,4-dinitroanisol (DNAN) was obtained from Alfa Aesar (Tewksbury, MA, USA), and 3-nitro-1,2,4-triazol-5-one (NTO) was supplied by BAE Systems/Holston Army Ammunition Plant (HAAP) as neat powder (Kingsport, TN, USA). Powders of MNX, DNX, and TNX were acquired from SRI International (Menlo Park, CA, USA). Stock solutions of neat material were prepared in methanol. Standards containing all legacy, IM, and surrogate compounds were prepared using individual (or 17-compound mix) stock solutions of 1000 mg/L to yield complete mixed-reference standards of 65 mg/L in 10% acetonitrile (ACN)—90% methanol (MeOH) solution.[2] Because of the low purity of MNX, DNX, and TNX standards, concentrations for RDX in the stock solution was 68.7 mg/L as determined by individual standard analysis prior to creating the mixed standard.

1. For a full list of the spelled-out forms of the units of measure used in this document, please refer to *US Government Publishing Office Style Manual*, 31st ed. (Washington, DC: US Government Publishing Office, 2016), 248–52, https://www.govinfo.gov/content/pkg/GPO-STYLEMANUAL-2016/pdf/GPO-STYLEMANUAL-2016.pdf.

2 For a full list of the spelled-out forms of the chemical elements used in this document, please refer to *US Government Publishing Office Style Manual*, 31st ed. (Washington, DC: US Government Publishing Office, 2016), 265, https://www.govinfo.gov/content/pkg/GPO-STYLEMANUAL-2016/pdf/GPO-STYLEMANUAL-2016.pdf.

Table 1. Compounds included in the extraction and analysis methods developed in the current work. Bold compounds are new to the method.

Compound	Acronym	CAS
2-Amino-4,6-dinitrotoluene	2-Am-4,6-DNT	35572-78-2
4-Amino-2,6-dinitrotoluene	4-Am-2,6-DNT	19406-51-0
3,5-Dinitroaniline	3,5-DNA	618-87-1
2,4-Diamino-6-nitrotoluene	**2,4-DANT**	**6629-29-4**
2,6-Diamino-4-nitrotoluene	**2,6-DANT**	**59229-75-3**
2,4-Dinitroanisole	DNAN	119-27-7
1,2-Dinitrobenzene (*surrogate*)	1,2-DNB (surr.)	528-29-0
1,3-Dinitrobenzene	1,3-DNB	99-65-0
2,4-Dinitrophenol	2,4-DNP	51-28-5
2,4-Dinitrotoluene	2,4-DNT	121-14-2
2,6-Dinitrotoluene	2,6-DNT	606-20-2
Hexahydro-1,3,5-trinitro-1,3,5-triazine	RDX	121-82-4
Hexahydro-1,3,5-trinitroso-1,3,5-triazine	**TNX**	**13980-04-6**
Hexahydro-1,3-dinitroso-5-nitro-1,3,5-triazine	**DNX**	**80251-29-2**
Hexahydro-1-nitroso-3,5-dinitro-1,3,5-triazine	**MNX**	**5755-27-1**
N-Methyl-*N*-(2,4,6-trinitrophenyl)nitramide	Tetryl	479-45-8
Nitrobenzene	NB	98-95-3
ortho-Nitrobenzoic acid (*surrogate*)	*o*-NBA (surr.)	552-16-9
Nitroglycerine	NG	55-63-0
Nitroguanidine	NQ	556-88-7
2-Nitrophenol	2-NP	88-75-5
4-Nitrophenol	4-NP	100-02-7
2-Nitrotoluene	2-NT	88-72-2
3-Nitrotoluene	3-NT	99-08-1
4-Nitrotoluene	4-NT	99-99-0
3-Nitro-1,2,4-triazol-5-one	NTO	932-64-9
Octahydro-1,3,5-7-tetranitro-1,3,5,7-tetrazocine	HMX	2691-41-0
Pentaerythritol tetranitrate	PETN	78-11-5
Picric acid	PA	88-89-1
1,3,5-Trinitrobenzene	1,3,5-TNB	99-35-4
2,4,6-Trinitrotoluene	2,4,6-TNT	118-96-7

2.2 Analytical methods

2.2.1 Primary liquid chromatography–UV-visible (LC-UV) method

Previous methods (Comfort et al. 1995, 1174–1182; US EPA 2006; Russell et al. 2014, 524–530; Crouch et al. 2020, 121008; Crouch et al. 2021) were used as references for the modification of a primary HPLC-UV method capable of separating and quantifying 29 analytes and 2 surrogates in a single injection. Separation was achieved on a Synergi Hydro-RP 4 µm Hydro-RP, 80 Å, 250 × 4.6 mm HPLC column. Method parameters and mobile phase gradient are shown in Figure 1, Table 1, and Table 2.

Figure 1. Graph showing gradient scheme for primary method separation.

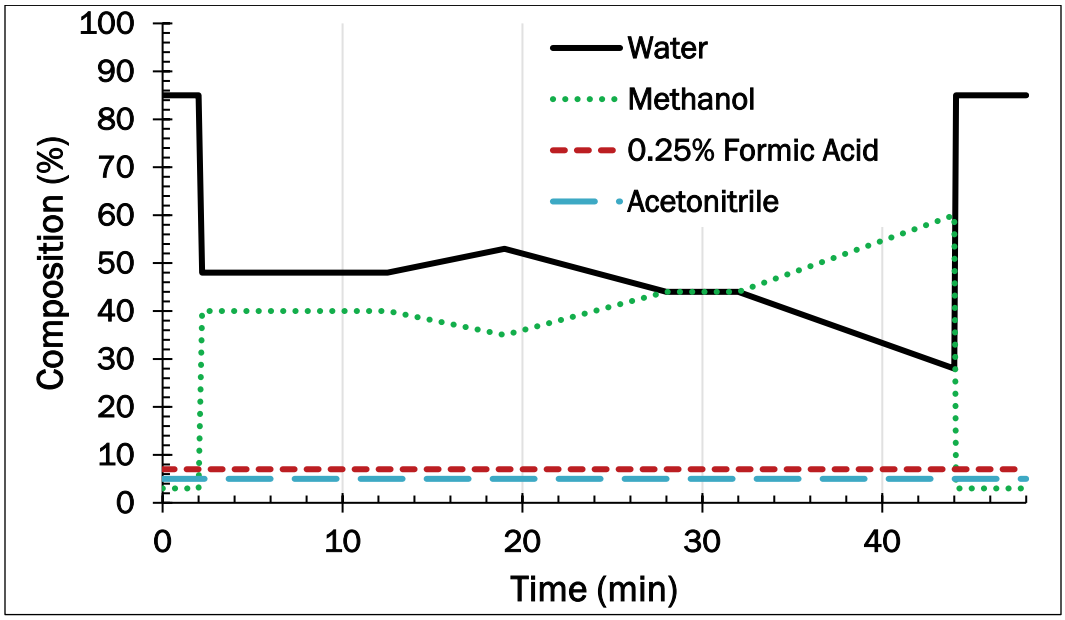


Table 2. Primary high-performance liquid chromatography–UV (HPLC-UV) parameters and mobile phase gradient.

Total run time: 48.0 min		Column temperature: 25 °C		
Injection volume: 50 µL		Flow rate: 1.0 mL/min		
Detection wavelengths: 210 nm (ref 360 nm), 254 and 315 nm (ref 500 nm)				
Time (min)	Reagent water (%)	Methanol (%)	0.25% Formic acid (FA) in reagent water (%)	Acetonitrile (%)
0.0	85	3	7	5
2.0	85	3	7	5
2.2	48	40	7	5
12.5	48	40	7	5
19.0	53	35	7	5
28.0	44	44	7	5
32.0	44	44	7	5
44.0	28	60	7	5
44.1	85	3	7	5
48.0	85	3	7	5

2.2.2 Secondary LC/UV-Vis method

The secondary HPLC-UV method previously developed (Russell et al. 2014, 524–530; Walsh 2016, 76–91; Crouch et al. 2020, 121008; Crouch et al. 2021) was modified for confirmation of the 29 analytes and 2 surrogates. Separation was achieved on a Pinnacle II Biphenyl, 5 µm, 150 × 4.6 mm HPLC column by Restek. Mobile phase gradient and other instrument conditions are listed in Figure 2 and Table 3.

Figure 2. Graph showing gradient scheme for secondary method using Pinnacle II Biphenyl HPLC column.

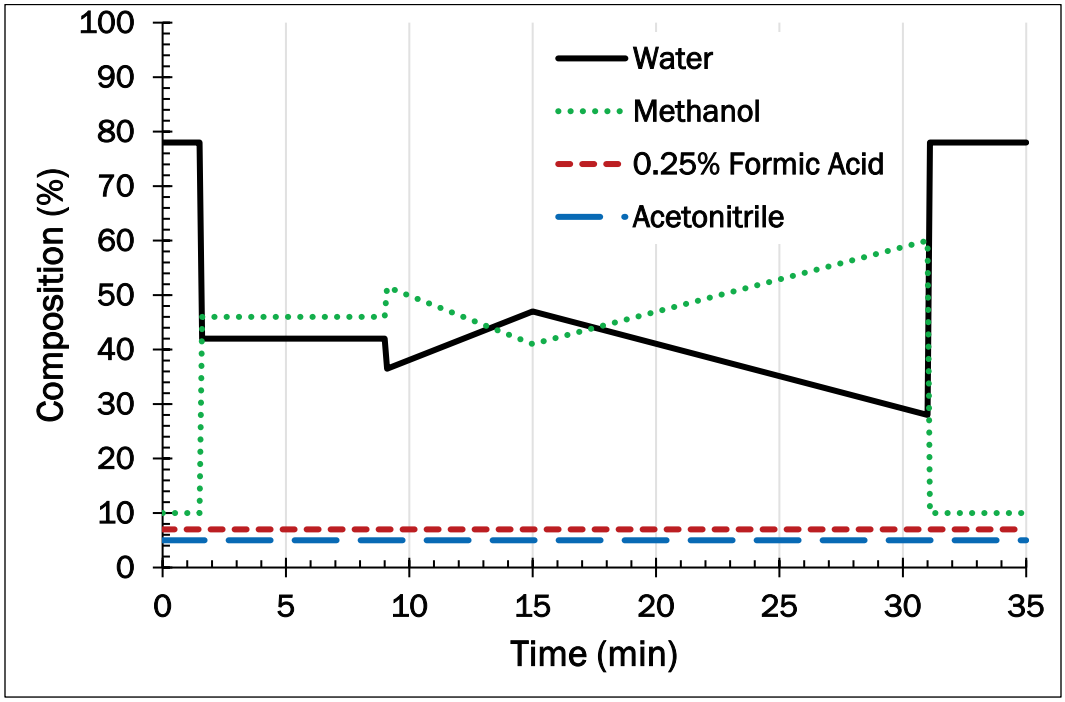


Table 3. Secondary HPLC-UV parameters and mobile phase gradient.

Total run time: 35.0 min		Column temperature: 20°C		
Injection volume: 50 µL		Flow rate: 0.9 mL/min		
Detection wavelengths: 210 nm (ref 360 nm), 254 and 315 nm (ref 500nm)				
Time (min)	Reagent water (%)	Methanol (%)	0.25 % FA in reagent water (%)	Acetonitrile (%)
0.0	78	10	7	5
1.50	78	10	7	5
1.60	42	46	7	5
9.0	42	46	7	5
9.1	36.5	51.5	7	5
15.0	47	41	7	5
31.0	28	60	7	5
31.1	78	10	7	5
35.0	78	10	7	5

2.2.3 Secondary mass spectrometer (MS) method

An additional confirmation method using an Agilent 6120B single quadrupole mass spectrometer equipped with an atmospheric pressure chemical ionization (APCI) source (Crouch et al. 2020, 121008; Crouch et al. 2021) was developed in addition to the secondary HPLC-UV method described above. Spray chamber and MS parameters were as follows: drying

gas flow at 4 L/min, nebulizer pressure at 40 psig (1 psig = 6894.76 Pa), drying gas temperature at 350°C, vaporizer temperature at 325°C, negative capillary voltage at 1500 V, negative corona current at 10 μA, and fragmentor for all ions was set between 50 and 100. Analytes were chromatographically separated by the primary LC method described in section 2.2.1, and analytes were detected in selective ion monitoring (SIM) mode. FA adducts of MNX, DNX, and TNX were identified, while 2,4-DANT and 2,6-DANT were not observed in the mass spectrum.

2.3 Extraction methods

2.3.1 Analysis and extraction supplies

LC-MS grade MeOH, ACN, and FA were purchased from Thermo Fisher Scientific (Hampton, NH, USA). In-house reagent water from Millipore water purifier was 18.23 MΩ-cm. Fat clay (CH-1) was obtained from American Society for Testing and Materials (ASTM) Institute for Standards Research (ISR) program, and frozen tilapia was purchased from a local supermarket. Strata-X-A (500 mg/6 mL) and Strata-X (500 mg/6 mL) Solid Phase Extraction (SPE) cartridges, Synergi Hydro RP, 80 Å, 250 × 4.6 mm HPLC column and Security Guard AQ C18 precolumn guard were purchased from Phenomenex (Torrance, CA, USA). Pinnacle II Biphenyl, 110 Å, 5 μm, 150 × 4.6 mm column was purchased from Restek (Bellefonte, PA, USA). ENVI-Carb (500 mg/6mL) SPE cartridges and SPE adaptors (by Supelco) were purchased from Millipore Sigma (St. Louis, MO, USA). Syringe filters (0.45 μm, Polytetrafluoroethylene (PTFE) disks) were purchased from Fisher Scientific (Pittsburg, PA, USA).

2.3.2 Direct-injection preparation

Direct-injection samples representative of high-concentration water samples were prepared by diluting stock solutions in DI water before diluting 2× with methanol to a final concentration of 86 μg/L for all analytes except RDX, which had concentrations of 91.6 μg/L because of the previously described contamination from the MNX, DNX, and TNX standards. Final samples were in 50/50 methanol/reagent water.

2.3.3 Solid phase extraction procedure

Low-level water samples were extracted following the procedures previously published (Crouch et al. 2020, 121008; Crouch et al. 2021). A brief summary of the method follows: reagent water (250 mL) in a glass bottle

was spiked with stock solution containing 29 analytes and 2 surrogates so that the final concentration was RDX (13 µg/L) and 12 µg/L for all other analytes. Conditioned Strata-X (top), Strata-X-A (middle), and ENVI-Carb (bottom) were loaded with sample using PTFE tubes to draw the sample from the reagent bottle onto the bed of the Strata-X (top) cartridge by vacuum. Sample flowed through the stacked cartridges at a rate of approximately 3 mL/min until the entire sample was loaded. SPE cartridges were left under vacuum for 5 min to dry. All liquid passing through the SPE cartridges from the sample loading process was discarded.

The cartridges were removed from the manifold and the order of stack reversed: ENVI-Carb (top), Strata-X-A (middle), Strata-X (bottom) to prevent irreversible binding of analytes to the GAC cartridge during elution. Methanol (5 mL) was added to the top and allowed to pass through all three cartridges before being collected in a 10 mL amber vial. Next, 5 mL of acidified methanol (2% HCl in methanol, v/v) was used to elute any remaining analytes from the cartridges and collected in a separate 10 mL amber vial. Extracts were stored at −20°C while awaiting preparation for injection on the HPLC. Before injecting onto the HPLC, the two extracted fractions were combined (1:1) and diluted by 2× using reagent water (that is, 250 µL MeOH eluent + 250 µL HCl/MeOH eluent + 500 µL reagent water).

2.3.4 Soil extraction procedure

Soil extraction methods were used as previously published (Crouch et al. 2020, 121008; Crouch et al. 2021). In summary, soil (1.0 g, ASTM Fat Clay, passed through an ASTM #40 sieve) was weighed into a 40 mL amber volatile organic analysis (VOA) vial and spiked with a stock solution containing 29 analytes and 2 surrogates so that the final concentrations were RDX (2.1 mg/kg) and 2.0 mg/kg for all other analytes and surrogates. Spiked soils were dried at 25°C in a convection oven for 30 min followed by the addition of 5 mL of LC-MS grade methanol. Samples in methanol were sonicated for 6 h in a continuous flow-cooled (<30°C) sonication bath. Samples were centrifuged at 190g to settle any suspended particles. Supernatant was removed and filtered through a 0.45 µm PTFE syringe disk filter, 5 mL of 50/50 methanol/reagent water was added, and the vials vortexed to resuspend the soil. Samples were sonicated 14 h in a continuous flow-cooled sonicator, filtered through a 0.45 µm PTFE syringe disk filter and collected in clean 10 mL amber vials. The two fractions were stored at −20°C until ready to inject on the instrument. Samples were

prepared for HPLC analysis by combining the two fractions (1:1) and diluting 2× with 1:3 methanol/water (that is, 250 µL methanol extract + 250 µL methanol/water extract + 500 µL 1:3 methanol/water), so that the final sample is in 50/50 water/methanol.

2.3.5 Tissue extraction procedure

Ground tilapia (1.0 g) was weighed into a 40 mL VOA amber vial and spiked with a stock solution containing 29 analytes and 2 surrogates so that the final concentrations were RDX (4.7 mg/kg) and 4.5 mg/kg for all other analytes and surrogates. Methanol (5 mL) was added to the tissues immediately after spiking, and the samples sonicated for 18 h in a continuous flow cooled (<30°C) sonication bath. Samples were centrifuged, and the supernatant filtered through 0.45 µm PTFE syringe filter, collected in a clean amber vial, and stored at −20°C.

Because of the presence of lipids and other interfering components from the tissues, a cleanup method was employed to improve analysis. This method was adapted from Larson et al. (1999) and modified by Crouch et al. (2020) (Crouch et al. 2021). Small-scale chromatography columns were prepared in a 5 ¾" borosilicate pipette that was loaded with 0.2 g of activated silica using glass wool as the frit. Columns were wetted with methanol that was discarded as it passed through the column. Methanol extract (1 mL) was loaded onto the column, allowed to flow through (gravity), and collected in a 5 mL amber vial. The column was then eluted with 1 mL methanol and 1 mL acidified methanol (2 %), which were also collected in the same vial. While awaiting analysis, cleaned extracts were stored at −20°C. Samples were prepared for analysis by diluting 2× with reagent-grade water (that is, 500 µL sample + 500 µL reagent water) so that the final sample was 50/50 methanol/water.

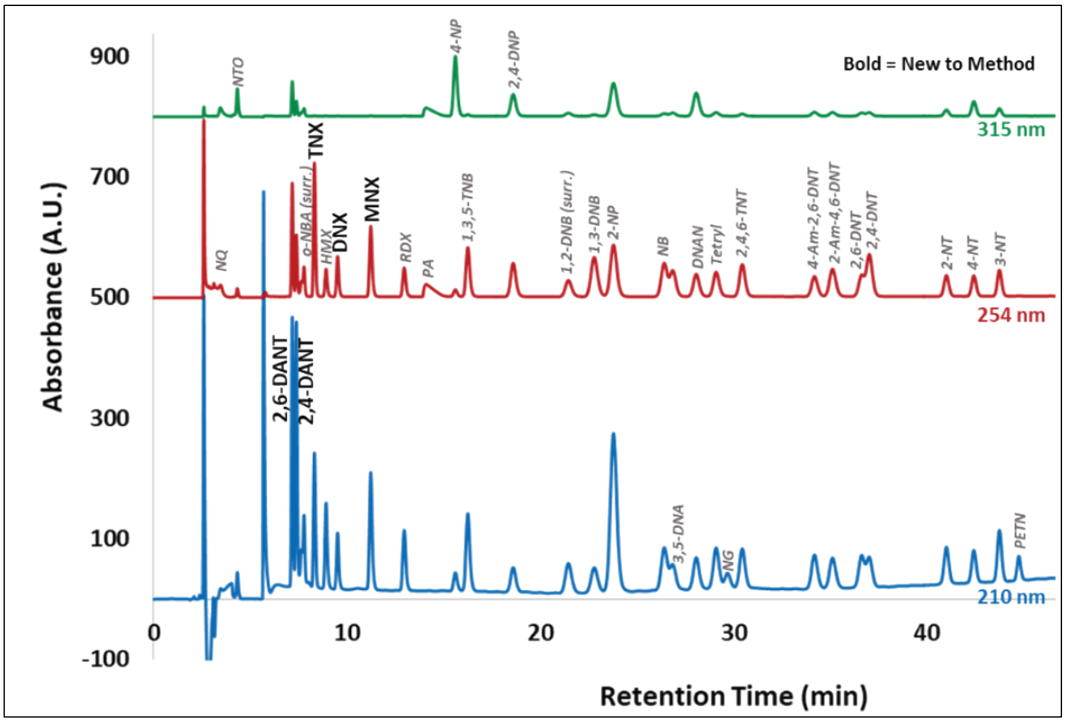
3 Results

3.1 Analytical methods

3.1.1 Primary HPLC-UV method

The primary analysis method developed in this work was based on several existing published methods (US EPA 2006; Russell et al. 2014, 524–530; Crouch et al. 2020, 121008; Crouch et al. 2021). The separation mobile phase gradient employed in this work is shown in Table 2, as are typical chromatograms for separation of the 29 analytes and 2 surrogates (Figure 3). All five of the additional analytes (MNX, DNX, TNX, 2,4-DANT, and 2,6-DANT) eluted between 7 and 12 min and were observed at 210 nm and 254 nm, while only 2,4-DANT and 2,6-DANT had weak responses at 315 nm. Correlation coefficient (R^2) calculated by Agilent OpenLab software for a 10-point calibration curve using peak area from 0.039 mg/L to 20.0 mg/L were >0.999 for all analytes at 254 and 210 nm, with the exception of 2,6-DANT, which had a fit of 0.9579 when detected at 254 nm. Lower-limit, upper-limit, and linear-response fits were compared for each analyte when detected at 210 and 254 nm. Upper limits (mg/L) were determined to be 20.0 (2,6-DANT and 2,4-DANT), 18.1 (TNX), 9.7 (DNX), and 34.7 (MNX), and were the same for all analytes at both 210 and 254 nm. Optimal linear response of 2,6-DANT, in addition to improved sensitivity at low concentrations of 2,4-DANT, indicated that the 210 nm wavelength was optimal for analysis of these two analytes. Improved sensitivity indicated that MNX, DNX, and TNX are best detected at 254 nm.

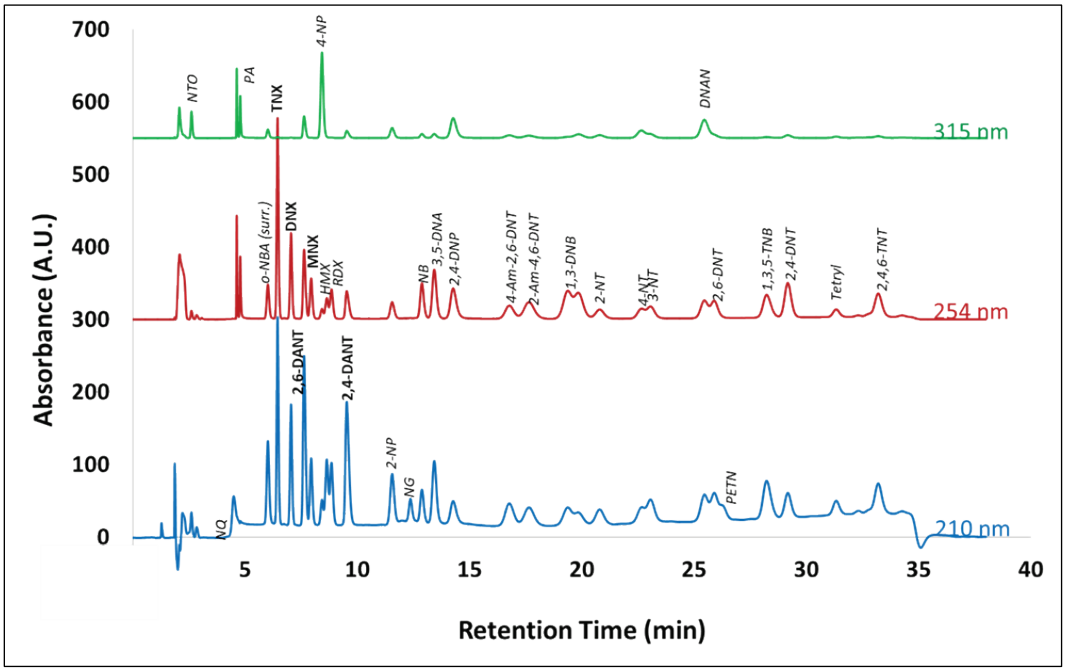
Figure 3. Chromatographic separation of 29 analytes and 2 surrogates using the reported primary method. Bolded analytes are new to the method; all other analytes and surrogates were previously reported under different separation conditions (Crouch et al. 2020). All analytes are at 5 mg/L in 50/50 methanol/water.



3.1.2 Secondary HPLC-UV method

A secondary HPLC-UV method was modified as an analyte confirmation method. This method results in different chromatographic separations compared to the primary method but shows comparable resolution, sensitivities, and quantitation. Method conditions are presented in Table 3, and an example chromatogram showing separation of the 29 analytes and 2 surrogates is shown in Figure 4. All additional analytes eluted between 6- and 10-min. Upper limits (mg/L) were determined to be 32.5 mg/L for all new analytes for both the 210 and 254 nm wavelengths. The lower limit of detection (mg/L) was determined to be 0.016 for TNX, DNX, and MNX at 254 nm; 0.032 mg/L for TNX, DNX, and MNX at 210 nm; and 0.016 mg/L for 2,4-DANT and 2,6-DANT at 210 and 254 nm. Correlation coefficient (R^2) calculated by Agilent OpenLab software for 12-point calibrations curves were determined to be >0.999 for all five analytes at both 210 and 254 nm.

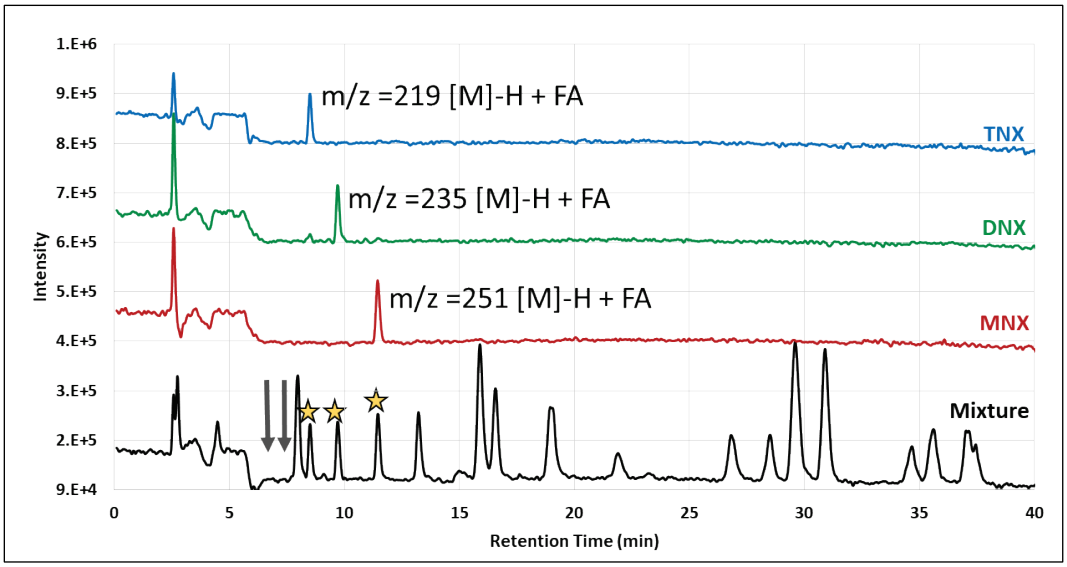
Figure 4. Secondary light chromatography–UV (LC-UV) method for separation of 29 munitions analytes and 2 surrogates by the reported secondary method. All analytes are at 5 mg/L in 50/50 methanol/water.



3.1.3 MS detection method

The secondary confirmation method based on mass spectrometry was modified to sequentially detect the analytes in line after the primary chromatography separation technique. The MS method from previously reported techniques (Hawari et al. 2000, 605–618; Crouch et al. 2020, 121008; Crouch et al. 2021) was modified to include ions for MNX, DNX, and TNX. Source and detector parameters were optimized for detection of the greatest number of analytes possible with high sensitivity. No ions for 2,4-DANT and 2,6-DANT were observed, while MNX, DNX, and TNX were detected in SIM mode as FA adducts (Figure 5). Lower limits of detection were 16 µg/L for MNX, DNX, and TNX and were linear up to 32.5 mg/L for MNX, DNX, and TNX. All three-calibration curves had correlation coefficients of 0.999 or higher.

Figure 5. Mass spectrometric (MS) secondary method detection of MNX, DNX, and TNX in scan mode. Ion (m/z) peaks of MNX, DNX, and TNX are indicated with yellow stars. Arrows indicate where 2,6-DANT and 2,4-DANT elute but are not observed by the MS.



3.2 Extraction methods

Extraction methods developed previously (Crouch et al. 2020, 121008; Crouch et al. 2021) were tested to determine their efficiency in extraction (% recovery) of five additional compounds of interest. Method detection limits (MDLs) were also determined for each analyte by each extraction method using the reported primary LC-UV method.

3.2.1 Direct-injection method

High-level water samples were analyzed using the direct-injection method. Waters were diluted 1:1 with methanol and separated by the primary chromatography method with UV-absorbance detection. Calculated percent recoveries and MDLs are listed in Table 4.

Table 4. Percent recoveries and method detection limits (MDLs) for direct injection of each analyte when analyzed by primary, UV-secondary, and MS-secondary methods.

Method	Analyte	Wavelength or Ion	MDL (ug/L)	Recovery (%)
Primary method	2,6-DANT	210 nm	28	98 ± 5
	2,4-DANT	210 nm	30	132 ± 5
	TNX	254 nm	16	97 ± 3
	DNX	254 nm	19	97 ± 3
	MNX	254 nm	12	95 ± 4
UV-secondary method	2,6-DANT	210 nm	95	108 ± 19
	2,4-DANT	210 nm	97	118 ± 20
	TNX	254 nm	101	117 ± 20
	DNX	254 nm	99	117 ± 20
	MNX	254 nm	101	116± 20
MS-secondary method	TNX	219 m/z	143	135 ± 29
	DNX	235 m/z	125	148 ± 25
	MNX	251 m/z	147	138 ± 30

All recoveries of the primary method were within DoD QSM Ver5.3 water limits (lowest LCL: MNX, 57 %; highest UCL: HMX, 135 %), confirming acceptable extraction performance. Recoveries calculated from the UV-secondary method were also within DoD QSM Ver5.3 limits; however, error for the method was higher compared to the primary method. Recoveries calculated from the MS-secondary method were all slightly higher than the uppermost limit outlined by the DoD QSM Ver5.3.

3.2.2 Solid phase extraction method

Solid phase extraction was performed on reagent water spiked with the 29 analytes and 2 surrogates. Extraction recoveries and MDL values results for each method were calculated and are shown in Table 5.

Table 5. Determined MDL (µg/L) based on an extraction ratio of 250:10 and percent-recovery values for solid phase extraction analysis of each analyte using the primary, UV-secondary, and MS-secondary methods.

Method	Analyte	Wavelength or ion	MDL (µg/L)	Recovery (%)
Primary method	2,6-DANT	210 nm	6	94 ± 20
	2,4-DANT	210 nm	6	122 ± 22
	TNX	254 nm	7	94 ± 25
	DNX	254 nm	12	106 ± 25
	MNX	254 nm	8	97 ± 28
UV-secondary method	2,6-DANT	210 nm	3	89 ± 32
	2,4-DANT	210 nm	5	93 ± 14
	TNX	254 nm	2	92 ± 7
	DNX	254 nm	3	94 ± 9
	MNX	254 nm	4	94 ± 11
MS-secondary method	TNX	219 m/z	1	70 ± 11
	DNX	235 m/z	1	90 ± 9
	MNX	251 m/z	1	92 ± 9

Extraction efficiencies of all five analytes as analyzed by the primary method were within the DoD QSM Ver5.3 limits (lowest LCL: MNX, 57 %; highest UCL: HMX, 135 %), confirming acceptable method recoveries. Recoveries for both UV-secondary and MS-secondary methods were also within DoD QSM Ver5.3 limits and had MDLs close to those of the primary method.

3.2.3 Solvent extraction of soil

ASTM fat clay was used to determine the efficiency of soil extraction of the five additional compounds. A two-step solvent extraction method developed previously (Crouch et al. 2020, 121008; Crouch et al. 2021) was used, with analyte percent recoveries and MDLs calculated shown in Table 6.

Table 6. Determined MDL (mg/kg) and percent-recovery values for each analyte extracted from soil via solvent extraction and analyzed by the primary, UV-secondary, and MS-secondary methods.

Matrix	Analyte	Wavelength or ion	MDL (mg/kg)	Recovery (%)
Primary method	2,6-DANT	210 nm	0.47	116 ± 8
	2,4-DANT	210 nm	0.48	147 ± 9
	TNX	254 nm	0.31	89 ± 5
	DNX	254 nm	0.31	88 ± 5
	MNX	254 nm	0.31	86 ± 6
UV-secondary method	2,6-DANT	210 nm	0.47	94 ± 8
	2,4-DANT	210 nm	0.87	93 ± 15
	TNX	254 nm	0.20	75 ± 4
	DNX	254 nm	0.30	60 ± 5
	MNX	254 nm	0.43	57 ± 8
MS-secondary method	TNX	219 m/z	0.45	78 ± 8
	DNX	235 m/z	0.49	77 ± 9
	MNX	251 m/z	0.33	69 ± 7

Recoveries calculated from the primary method for 2,6-DANT, TNX, DNX, and MNX were within the limits found in the DoD QSM Ver5.2 for solids (lowest LCL: 4-Am-2,6-DNT, 64%; highest UCL: Tetryl, 135%). Recovery for 2,4 DANT for the primary method was slightly high. Recoveries for 2,6-DANT, 2,4-DANT, and TNX for the secondary UV method were also within the DoD QSM Ver5.3 limits; however, recoveries of DNX and MNX were slightly low (60% and 57%, respectively). All recoveries for the MS secondary method were within the DoD QSM Ver5.3 limits.

3.2.4 Solvent extraction and cleanup of tissue

A single-step solvent extraction procedure for tissues and subsequent interference reduction technique was tested to determine the recoveries and MDLs for new analytes in a fish tissue matrix (Table 7).

Table 7. Calculated MDL (mg/kg) and percent-recovery values for each analyte extracted from tissue via solvent extraction analyzed by the primary, UV-secondary and MS-secondary methods.

Matrix	Analyte	Wavelength or ion	MDL (mg/kg)	Recovery (%)
Primary method	2,6-DANT	210 nm	2.0	65 ± 36
	2,4-DANT	210 nm	3.1	100 ± 23
	TNX	254 nm	2.9	91 ± 23
	DNX	254 nm	2.1	66 ±24
	MNX	254 nm	3.0	96 ± 21
UV-secondary method	2,6-DANT	210 nm	0.50	29 ± 11
	2,4-DANT	210 nm	2.1	44 ± 46
	TNX	254 nm	1.8	80 ± 42
	DNX	254 nm	1.7	89 ± 39
	MNX	254 nm	1.3	104 ± 29
MS-secondary method	TNX	219 m/z	0.65	39 ± 15
	DNX	235 m/z	0.87	52 ± 21
	MNX	251 m/z	0.63	45 ± 15

Recoveries for all analytes for the primary method analysis were all within DoD QSM Ver5.2 limits for solids (lowest LCL: 4-Am-2,6-DNT, 64%; highest UCL: Tetryl, 135%). Most recoveries for the secondary UV method were within the limits, with exception of 2,6-DANT and 2,4-DANT, which were below the minimum recovery of 64%. Recoveries for all analytes via the MS-secondary method were below the minimum recovery. Lower recoveries of the secondary UV and MS secondary method may be due to remaining interferences in the samples. Chromatograms of the primary method analysis maintained a flat baseline, while secondary methods did not maintain a steady baseline. Several additional peaks, most likely interferences, were also noted in the secondary chromatogram. Interferences could also lead to ion suppression, which would explain the decreased recovery in the MS method as well.

4 Conclusion

This report details new analytical methods capable of quantifying 29 legacy and IM analytes and 2 surrogates. This work was executed as part of an ongoing effort to improve detection of munitions in environmental matrices. A new primary method based on HPLC-UC separation and detection was shown capable of detecting all five new analytes. Secondary methods based on UV detection after separation on a different type of HPLC column and MS detection in tandem with the primary method separations were able to resolve all 29 analytes and 2 surrogates. Detection limits of the five new compounds were acceptable for all three methods, with all analytes' LDL falling between 0.016 and 0.032 mg/L, while linear dynamic ranges were found to be 20.0 (2,6-DANT and 2,4-DANT), 18.1 (TNX), 9.7 (DNX), and 34.7 (MNX) for the primary method and greater than 32.5 mg/L for both secondary methods. Analyte recovery from all three matrices calculated from the primary method were mostly found to be within DOD QSM Ver5.3 limits, with the exception of 2,4-DANT recovered from soil matrix. Analyte recovery from all three matrices calculated from the UV-secondary method were all within DOD QSM Ver5.3 limits, with the exception of DNX and MNX extracted from soil matrix and 2,4-DANT and 2,6-DANT extracted from tissue, all of which showed low recovery. Lastly, recoveries calculated by the MS-secondary method were high for all analytes from the direct-inject water matrix and low for all analytes extracted from tissue matrix, possibly due to ion suppression. Overall, the developed analytical methods proved robust detection limits and showed promise for analyzing munitions, while extraction methods developed previously proved to be efficient for recovering new munitions compounds.

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14. ABSTRACT

Standard methods are in place for analysis of 17 legacy munitions compounds and one surrogate in water and soil matrices; however, several insensitive munition (IM) and degradation products are not part of these analytical procedures. This lack could lead to inaccurate determinations of munitions in environmental samples by either not measuring for IM compounds or using methods not designed for IM and other legacy compounds. This work seeks to continue expanding the list of target analytes currently included in the US Environmental Protection Agency (EPA) Method 8330B. This technical report presents three methods capable of detecting 29 legacy, IM, and degradation products in a single High Performance Liquid Chromatography (HPLC) method with either ultraviolet (UV)-visible absorbance detection or mass spectrometric detection. Procedures were developed from previously published works and include the addition of hexahydro-1-nitroso-3,5-dinitro-1,3,5-triazine (MNX); hexahydro-1,3-dinitroso-5-nitro-1,3,5-triazine (DNX); hexahydro-1,3,5-trinitroso-1,3,5-triazine (TNX); 2,4-diamino-6-nitrotoluene (2,4-DANT); and 2,6-diamino-4-nitrotoluene (2,6-DANT). One primary analytical method and two secondary (confirmation) methods were developed capable of detecting 29 analytes and two surrogates. Methods for high water concentrations (direct injection), low-level water concentrations (solid phase extraction), soil (solvent extraction), and tissue (solvent extraction) were tested for analyte recovery of the new compounds.

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