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Method development and laboratory intercomparison of an RP-HPLC-UV method for energetic chemicals in marine tissues

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Abstract

Experiments were conducted to develop a method for the determination of a set of 17 military-relevant energetic compounds (including nitroaromatics, nitramines, and nitrate esters) in 5 types of marine tissues (Dungeness crab, Manila clam, starry flounder, sea cucumber, and geoduck) using reversed-phase high performance liquid chromatography with a UV detector (RP-HPLC-UV). Dry-ice grinding was evaluated and found to be an excellent method of sample homogenization prior to sample extraction and determination. An extract cleanup procedure based on solid-phase extraction was assessed. A cleanup procedure using solid phase extraction was adequate for the removal of interferences prior to HPLC analysis for the five marine tissue matrices tested. Mean method detection limits (MDLs) were estimated using two columns at two wavelengths (254 and 210 nm) and ranged from 17 to 293μg/kg for the five tissue matrices tested. A six-laboratory intercomparison test was conducted to evaluate the performance of the method, each analyzing five marine tissue matrices fortified at three levels. The same marine tissues were used in the laboratory intercomparison study except Pacific halibut was substituted for starry flounder. Overall, USEPA Method 8330B modified for tissue analysis showed suitable detection capability, analytical accuracy, precision, sensitivity, linear range, and robustness for sixteen (16) of the seventeen (17) analytes, for all five (5) of the marine tissue matrices studied. The exception was tetryl that proved to be unstable for all matrices as has been found for soils and sediments.

Keywords

EPA Method 8330; Marine tissue; Munitions-related compounds; HPLC-UV; Underwater munitions

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1. Introduction

The presence of conventional munitions in the underwater environment occurs worldwide due to 1) use in military training and conflicts, 2) accidents, 3) loss and mishandling, and 4) intentional sea disposal of conventional munitions. The two World Wars left a legacy of enormous quantities of munitions requiring disposal. Internationally, sea disposal was adopted by most nations at the time as an acceptable means of disposal of munitions and other surplus materials, and was continued until 1972 [1].

Off the coast of Germany, it is estimated that 1.6 million metric tons were dumped in the North Sea, and 300,000 tons were dumped in the Baltic Sea [2]. In the northeastern Atlantic, estimates suggest that in excess of 1 million tons of conventional munitions were dumped in Beaufort's Dyke (Irish Sea) and 168,000 tons in the Skagerrak Fjord. At least 148 dumpsites are spread from Iceland to Gibraltar, where conventional munitions were dumped at 78% of the sites. In North America, more than 400 sites totaling more than 10 million acres are estimated to contain underwater munitions in the United States [3], and more than 1 million tons of munitions in Canadian waters, including a shipwreck with an estimated 80,000 tons [4]. In the Pacific Islands a number of islands have legacy underwater conventional munitions contamination remaining from combat operations during World War II and post war dumping [5].

More than 70 years after World War II, undersea conventional munitions remain subject to corrosion effects on metal casings that may increase the rate of release of explosive and propellant compounds in the future. In the northeastern Atlantic, of the 1879 munitions encountered from April 1999 to October 2008 primarily by fishermen, 786 cases (42%) were reported to be in various stages of corrosion, from partly to completely corroded [6]. In the Pacific, munitions disposal sites south of the island of Oahu were known to contain both conventional and chemical munitions disposed of between 1920 and 1951. Digital images and video reconnaissance logs were used to classify the integrity and state of corrosion of 1842 discarded military munitions (DMM) objects. Of these, 5% (or 90 individual DMM objects) were found to exhibit mild-moderate degrees of corrosion, the majority 66% (or 1222 DMM objects) were observed to be significantly corroded, but visually intact on the seafloor. The remaining 29% of DMM encountered were found to be severely corroded and breached, with their contents exposed [7].

The rates of metal casing corrosion and dissolution kinetics of conventional munitions compounds are not well understood, but preliminary modeling suggests dissolution timeframes from 25 to 2700 years for explosives compounds such at TNT, RDX, and HMX [8]. Initial screening studies show evidence of explosives and propellant compounds in marine sediments [9–11] and in marine tissues [12,13] due to leakage from undersea conventional munitions. A recent study by Taylor et al. [14] indicated that even in a terrestrial environment, eight out of 42 UXO items were leaking energetic compounds at three military training sites.

Increased demand for living and non-living resources and diminished or exhausted reserves on land and in shallow water are pushing human activities ever deeper into the world's

oceans [15]. The exploitation of fisheries resources in the vicinity of conventional munitions disposal sites may present potential human health and ecological risks from ingestion of munitions compounds from utilized marine species [16]. The mammalian toxicity of exposure to nitroaromatic, nitramine, and nitrate ester conventional munitions compounds is well established from the early 20th century [17–23] and aquatic toxicity from the early 21st century [24–26]. Risk based carcinogenic and noncarcinogenic human health screening levels for consumption of fish tissues have been developed by the U.S. Environmental Protection Agency (USEPA) for a number of conventional munitions compounds [27], and ecological No Observed Adverse Effect Levels (NOAELs) for pore waters [28] and sediments [29].

1.1. Objective

At present there are no well-established methods for the determination of munitions-related energetic compounds in marine tissue. It is the object of this work to determine if a method based on Method 8330B [30] and modified for the marine tissue matrix would be adequate for the analysis of marine tissue for these compounds.

A major challenge for the determination of contaminants in tissue samples is the difficulty in obtaining a representative subsample for Target analytes, their abbreviations, CAS numbers, and military relevance. extraction and analysis. Common methods of homogenization such as passing the tissue repeatedly through a meat grinder or using a blender have been found to be inadequate [31]. Commonly, tissue samples are lyophilized (freeze-dried) to remove the water from the tissue prior to some form of homogenization such as blending. For energetic compounds, the stability of the normal suite of 17 energetic compounds [30] through the freeze-drying step has not been thoroughly investigated, although some preliminary work suggested it was an acceptable approach for drying soil samples containing TNT, RDX, and HMX prior to homogenization, subsampling, and extraction [32]. The research presented here adapted a dry-ice grinding method that the USFDA used for preparing shrimp tissue for analysis [31].

2. Materials and methods

2.1. Marine tissue matrices

The five types of marine tissues selected for study during method development were Manila clams (*Venerupis japonica*), starry flounder (*Platichthys stellatus*), Dungeness crab (*Cancer magister*), sea cucumber (*Parastichopus californicus*), and geoduck (*Panopea generosa*). These species were selected based on target marine species for the Pacific Northwest, USA [33]. The Manila clams were collected locally along the south end of Oakland Bay within Puget Sound, Washington (USA). The starry flounders were caught in Yukon harbor off Blake Island within Puget Sound. Dungeness crab, sea cucumber, and geoduck were purchased from the Suquamish tribe and were obtained from Puget Sound as well.

The flounders were filleted within 12 h of collection and then frozen. The clams were soaked in salt water, rinsed with reagent grade water, shucked, and frozen. The sea cucumbers were rinsed with reagent grade water and frozen. The crabs were rinsed and the

edible tissue removed from the shells and frozen. The geoducks were shucked, rinsed with reagent grade water, the gut balls removed, and frozen.

2.2. Preparation of standards and spiking solutions

Multi-analyte stock standards were obtained from Restek Corporation at 1000 μg/mL, containing 1,3-DNB, 2,4-DNT, HMX, NB, RDX, 1,3,5-TNB, TNT, 2-Am-DNT, 4-Am-DNT, 2,6-DNT, 2-NT, 3-NT, 4-NT, tetryl, PETN, NG, and 3,5-DNA. An individual stock standard of the surrogate 1,2-dinitrobenzene (1,2-DNB) at 1000 μg/mL was also obtained from Restek Corporation. A list of the chemicals, their abbreviations, CAS numbers and military relevance is presented in Table 1.

An intermediate calibration standard was prepared by combining aliquots from the multi-analyte stock standard and surrogate stock standard and diluting with acetonitrile to a concentration of 10,000 μg/ L. Standards were refrigerated and stored in the dark.

Working calibration standards were prepared at eight concentration levels ranging from 50 to 10,000 μg/L by dilution of the intermediate calibration solution with acetonitrile. Standards were refrigerated and stored in the dark. Calibration standards were prepared on the day of calibration by dilution of an aliquot of the working calibration solutions 1:1 with reagent grade water.

2.3. Dry-ice grinding of tissues

In order to obtain a representative sample of the marine tissue prior to subsampling, a homogenization step is required. Simple addition of the tissue to a blender at room temperature and subsequent blending was not successful due to the nature of the tissue and resulting consistency of the tissue. Instead, the tissue was frozen at ≤ −20 °C, placed in a blender at the same temperature, dry ice added, and blended until a powder-like consistency was obtained [31], thus allowing representative subsampling.

Bulky frozen tissue samples were cut into pieces no greater than 2.5 cm cubes using a hacksaw. For clams the unfrozen tissue was placed into ice cube trays and frozen. Approximately 2.5 cm pieces of dry ice were placed in a blender for a minute to adequately cool the surfaces before adding the frozen tissue. The cut tissues were added (1:1 dry ice to tissue) to a Waring Model TB-14 industrial blender, and the blender was pulsed on and off for about a minute or until the larger pieces were ground. The blender was then run at its lowest, medium, and highest speed, progressing to the next speed once the noise of hard chunks hitting the sides was eliminated. The ground tissues were then taken into a walk-in freezer (≤−20 °C) and placed in a chilled shallow tray inside a sealable plastic bag, but the bag was left open to allow the dry ice to sublime overnight. Any unground chunks of tissue were ground in a pre-chilled mortar and pestle. The ground tissues were then transferred to sealable plastic bags and the contents thoroughly mixed by kneading to give the loose, powdery snow-like samples. The tissues were maintained frozen until used for various experiments.

2.4. Tissue spiking

For tissue spiking experiments, 10 g of each dry-ice ground tissue was placed in an amber 40 mL VOA vial and a 1.00 mL aliquot of the multi-analyte spiking solution and 1.00 mL of surrogate solution were added on top of the frozen ground tissue. The spike was allowed to interact with each tissue for a minimum of 5 min prior to extraction.

2.5. Freeze-drying

Freeze-drying experiments utilized dry-ice ground material that was spiked as described above. The mouth of each vial was covered with a laboratory wipe that was attached with a rubber band. The vials were placed in a vial rack in the chamber of a Virtis Freezemobile 12XL freeze drying unit. The vials were freeze-dried for 18 h. Once dry, the tissue samples were warmed to room temperature and extracted as described below.

2.6. Tissue extraction

Tissues (10 g) that were freeze-dried were extracted by adding 20 mL of acetonitrile containing 0.1% acetic acid to each amber 40 mL VOA vial and placing the vials in a temperature-controlled ultrasonic bath at 4 °C for 18 h as described in Method 8330B [30]. Tissues that were not freeze-dried were extracted with 18 mL of acetonitrile instead of 20 mL because the acetonitrile used in the spiked solvent and surrogate addition was not evaporated as in the freeze-dried tissues prior to extraction. After sonication, the extracts were centrifuged at 500 RCF and decanted into a disposable syringe equipped with a 0.45 μm PTFE syringe filter. The extracts were filtered, the first 1.0 mL of filtered extract was discarded and the remaining extract retained in a clean amber VOA vial. Prior to HPLC analysis, the extracts were diluted 1:1 with reagent grade water. Tissues that were dry-ice ground, but not freeze-dried, were extracted in an identical manner.

2.7. Solid phase extraction cleanup of tissue extracts

Blank tissue for each tissue type was fortified with the surrogate and extracted as described above. The extracts were analyzed using the E1 and E2 columns. The background varied for each marine tissue type, but overall there were sufficient background interferences in the chromatograms that warranted some form of cleanup prior to HPLC analysis.

Because of this concern, the use of an efficient cleanup step was investigated. Solid phase extraction (SPE) using organic resins has been successful in retaining HMX and RDX during preconcentration of water samples and the retained analytes are easily eluted with acetonitrile [34].

SPE cartridges (Waters Oasis® HLB) were used to clean up extracts prior to HPLC analysis. Each cartridge was preconditioned by passing 2 cartridge volumes of acetonitrile, methanol, and water through the cartridges, not allowing the cartridges to dry out. A 2.00 mL aliquot of each tissue extract was diluted with 10 mL reagent grade water and passed though the SPE cartridges at approximately 1–3 mL/min. A 95:5 ratio of water: methanol was then passed through the cartridge to remove a portion of the most polar interferences. The cartridges were then vacuum dried for 5 min and the target analytes were eluted from the cartridges using 1 mL of acetonitrile. The resulting extracts were then brought to a final

volume of 1.00 mL. The final extracts were refrigerated and stored in the dark. (Note, based on stability checks, the holding time of 40 days used for semi-volatile organic compounds in EPA Method 8270, will work for the analytes in this method). An aliquot of the final extract was diluted 1:1 with reagent grade water just prior to analysis.

As discussed in Section 4.2 and would be deduced from the denominators in Eq. (2) of Section 2.11, a larger amount of tissue extract (10 mL instead of 2 mL) can be passed through the SPE cartridge with a resulting improvement in the detection limit, however the limit of the cartridges to remove interferents may be reached on some tissue types that are more complex or contain more lipids. Clean-up of 2 mL of extract from 10 g tissue was always consistent for the tissues studied. This is an area for further study and improvements.

2.8. Reagents

Reagent grade water was obtained from a Milli-Q® system. HPLC grade acetonitrile was obtained from J.T. Baker, HPLC grade methanol was obtained from Honeywell Burdick & Jackson, and HPLC grade acetic acid was obtained from Fisher Scientific.

2.9. Analytical instrumentation

The HPLC unit used for this work was a Thermo Scientific UltiMate 3000 series system equipped with an UltiMate 3000 Pump module, an UltiMate 3000 autosampler module, and a 100 μL variable volume sampling loop injector. The system was equipped with a dual column, dual UV detector system.

Analysis at 210 and 254 nm was conducted using two 4.6 × 250 mm, 5 μm reversed phase HPLC columns. The column used as the primary was a Thermo Scientific Acclaim™ Explosives E1 and the column used for confirmation was a Thermo Scientific Acclaim™ Explosives E2. Both columns have a proprietary stationary phase chemistry with an average pore size of 120 Å and surface area of 300 m^2/g. The E1 was selected as the primary due to having superior resolution for all 18 analytes (17 targets and 1 surrogate) compared to the E2. These two columns provided adequate separations for the 17 target analytes for this method within a retention time of 60 min under isocratic conditions. The mobile phase used for the E1 column was 64:33:3 water:methanol:acetonitrile using a flow rate of 1.25 mL/min at 36 °C. The mobile phase used for the E2 column was 52:48 water:-methanol using a flow rate of 1.0 mL/min at 30 °C.

2.10. Instrument calibration

The initial calibration was conducted by injection of eight (8) different concentrations ranging from 25 to 5000 μg/L (concentration after 1:1 dilution with water). The linear calibration relationship for the various target analytes had correlation coefficients of 0.995 or greater. A mid-level calibration standard was prepared from a different source, and was analyzed prior to analysis of any sample extract.

2.11. Calculation of results

Results were calculated on a fresh weight of tissue basis according to Eqs. (1) and (2):

$$V_{total}(mL) = V_{es} + V_{ss} + V_{ms} + \left(\left(W_s x\ \%Moisture\ \right)/100\right) \quad (1)$$

where:

V_{es} = Initial volume (mL) of extraction solvent

V_{ss} = Volume (mL) of surrogate spike solution added

V_{ms} = Volume (mL) of matrix spike solution added

W_s = Weight (g) of sample extracted (nominal = 10), for moisture

1 g = 1 mL

$$Final\ conc.\ (\mu g/Kg) = \left(C_e\ x\ \ V_{total} x\ \ V_{cf}\ x\ \ df\right)/\left(W_s\ x\ \ V_{ci}\right) \quad (2)$$

where:

C_e = On-column compound concentration (ng/mL)

V_{total} = Total volume (mL) of extract from Eq. (1)

V_{cf} = Final volume (mL) after SPE cleanup (nominal = 1)

df = Dilution Factor (dimensionless) (nominal = 2)

W_s = Initial weight of sample extracted (g) (nominal = 10)

V_{ci} = Initial volume (mL) of aliquot taken for SPE cleanup (nominal = 2)

2.12. Laboratory intercomparison test

2.12.1. Conduct of the laboratory intercomparison test—The laboratory intercomparison results reported here were conducted by the CB&I Federal Services LLC (CB&I) Quality Assurance Technical Support (QATS) Program in Las Vegas, NV under the guidance of USEPA Analytical Services Branch (ASB) and USEPA Region 10, Manchester, WA. The results presented here were obtained after an Initial Demonstration of Proficiency (IDP) study at six commercial and research laboratories in the United States and Canada. Results are presented for the analysis of five different marine tissue matrices unspiked and spiked with two concentration levels of 17 nitroaromatic, nitramine, and nitrate ester analytes [35]. The analytical results from one (1) laboratory were eliminated from the Study due to an instrument malfunction, and therefore the results from five (5) laboratories are presented here.

2.12.2. Explosive standards and spiking materials—All target analyte and surrogate calibration standards were obtained commercially from Restek Corporation. Target analytes used for the preparation of the second source initial calibration verification (ICV) were obtained from Absolute Standards. The surrogate analyte used for the preparation of

the ICV was obtained using a different lot from Restek Corporation. Target analytes and surrogate used for the preparation of spiking solutions were obtained from Restek Corporation, with the exception of 2-Am-DNT which was obtained from Supelco.

2.12.3. Blank marine tissue matrices—Five marine tissue types were used for the laboratory inter-comparison test: Dungeness crab, Manila clam, Pacific halibut (*Hippoglossus stenolepis*), geoduck, and sea cucumber. Four of the matrices (except sea cucumber) were purchased live from seafood providers in the Pacific Northwest (USA). The sea cucumber was obtained fresh frozen from the EPA Region 10 Laboratory.

The crab was shelled, cut into small portions, and frozen at ≤ −20 °C. The clam shells were removed, and the flesh was frozen at ≤ −20 °C. The geoduck shells were removed, and the flesh, minus the visceral mass (gut ball), was cut into small pieces and frozen at ≤ −20 °C. The Pacific halibut skin was removed, the sea cucumbers were cut into small pieces, and both were kept frozen at ≤ −20 °C. All five matrices were stored at ≤ −20 °C and homogenized by dry ice grinding as discussed in Section 2.3 above. Moisture contents of five types ranged from 72.9% to 89.8%.

2.12.4. Materials supplied to intercomparison laboratories—The following was provided to each of the six laboratories conducting the intercomparison tests:

- An e-mail containing:
  - Instruction sheets for the multi-laboratory study (MLS)
  - An electronic copy of USEPA Method 8330B modified for marine tissue analysis.
  - Electronic Excel spreadsheet templates for reporting results.
- One (1) ampule of mixed Method 8330B standard solution @ 1000 μg/mL, for calibration standards and continuing calibration verification (CCV) preparation. The midpoint initial calibration curve standard @ 5000 μg/L is used as the CCV.
- One (1) ampule of the surrogate 1,2-dinitrobenzene (1,2-DNB) @ 1000 μg/mL for standards preparation.
- One (1) 12 mL screw-cap vial containing 10 mL of a combined low-level spiking solution.
- One (1) 12 mL screw-cap vial containing 10 mL of a combined high-level spiking solution.
- Thermo Scientific, Acclaim™ E1 and E2 reversed-phase HPLC columns (4.6 × 250 mm, 5 μm), with guard columns.
- Oasis® HLB Extraction Clean-Up Cartridge, 3cc/60 mg. A total of sixty (60) cartridges (Waters part number: WAT094226).
- Nine (9) 40 mL VOA vials for each of the five tissue types, each containing approximately 10 g of unspiked marine tissue.

2.12.5. Protocol for laboratory intercomparison test—The analytical work was conducted in two steps. First, analysts became familiar with the method by establishing calibration curves for the 17 analytes. The responses at 254 nm were used for all analytes, except NG and PETN where the responses at 210 nm were used because these analytes do not absorb at 254 nm. Limits of quantitation (LOQ) were estimated for all 17 analytes in acetonitrile/water and converted to LOQ for tissue using the mass of tissue and the volume of acetonitrile extractant. Samples of blank tissue were provided to evaluate potential interferences.

For the laboratory intercomparison test, nine replicate 10 g blank tissue samples for each of the five marine tissue types were provided to each laboratory. Samples analyzed in the test were prepared at each participating laboratory by spiking three replicate 10 g samples of each of the five tissues with 1.00 mL of the low-level spiking solution and three replicates with 1.00 mL of the high-level spiking solutions. An additional three replicates were extracted unspiked. Samples were extracted and subjected to SPE cleanup as discussed in Sections 2.6 and 2.7 (respectively) above. Analysis was conducted using the HPLC conditions described in Section 2.9 above.

The concentrations of the 17 target analytes in the two spiking solutions are presented in Table 2. The concentrations shown are on a wet weight of tissue basis. The QATS Laboratory conducted a statistical evaluation of the results reported by the participants using guidance from USEPA [36].

3. Results and discussion

3.1. Selection of HPLC primary and secondary columns

Generally, water and soil analysis for energetic compounds using RP-HPLC have relied on a primary HPLC column for quantification and a secondary column for analyte confirmation. Method 8330 [37] recommends a LC-18 column for the primary column and a LC-CN column is recommended for analyte confirmation because the elution order is very different than obtained on the LC-18 column. Additional columns were recommended in Method 8330B [30] to include a LC-8 for the primary column and a phenylhexyl column for confirmation. The retention order for the phenylhexyl column is less different than for the LC-CN column, but it provides much better resolution for the 17 target analytes than the LC-CN column.

Several different column pairs and eluents were evaluated for this application and a 4.6 × 250 mm, 5 μm, Thermo Scientific, Acclaim™ E1 column was selected as the primary column, and a 4.6 × 250 mm, 5 μm, Thermo Scientific, Acclaim™ E2 column was chosen as the confirmation column. Eluents were adjusted to provide adequate separation for each column (Fig. 1). Analyte separation was adequate for quantitation on the primary column (top) and for confirmation on the secondary column (bottom).

3.2. Analyte recovery after freeze-drying

A tissue-spiking experiment was conducted to assess whether freeze-drying was useful in removing water prior to tissue extraction and analysis. Significant losses of NB and NTs (<

10% recovery), DNBs (approximately 30% recovery), 2,6-DNT (27% recovery), 2,4-DNT (42% recovery), and NG (50% recovery) were found, thus the freeze-drying approach was abandoned

3.3. Interferences and cleanup of extract prior to HPLC analysis

SPE cleanup discussed in Section 2.7 was used with extracts from the various marine tissue types. These extracts were analyzed before and after SPE cleanup. Fig. 2 presents the before and after chromatograms for the Manila clam tissue spiked with the low target analyte spiking solution and the surrogate. The SPE step reduced interferences in all cases, particularly in the early portion of the chromatograms near where HMX and RDX elute. The SPE step had an additional benefit by equalizing the solvent strength of the extract with that of the standard.

3.4. MDL estimation

MDLs are affected by background intensity, interferences, analyte sensitivity, and variations during sample preparation, extraction, cleanup, and analysis. MDLs were estimated on each marine tissue matrix by fortifying (spiking) 8 replicate 10 g portions of each blank tissue with the 17 target analytes and extracting, cleanup, and analyzing the tissue extracts (the extracts contained 60 ng/mL of each analyte, which was roughly about 4 times the MDL). Results were computed according to the USEPA protocol [36] wherein at least 7 replicates of samples containing 3–5 times the expected MDL are analyzed and the standard deviation is calculated, then multiplied times three. This value was then converted to tissue sample concentration units of μg/kg using all dilution factors, nominal weights, and volumes as listed in Section 2.11. The minimum, maximum, and mean values across the five marine tissue types are presented in Table 3 along with EPA human health screening levels for fish tissue [38]. The minimum represents the lowest MDL that was seen among the tissue types studied, which was typically the crab tissue or clams for many of the analytes. The maximum represents the highest MDL that was seen among the tissue types, which was typically the flounder for many of the analytes. Mean values over the five tissue matrices varied from 39 μg/kg for 1,3,5-TNB to 173 μg/kg for NG. In most cases, the mean values were adequate for human health screening criteria.

4. Laboratory Intercomparison test results

A laboratory intercomparison test was conducted among six commercial and research laboratories in the United States and Canada. The results from one laboratory were rejected because the laboratory reported instrument malfunction during the test and hence, the data from that laboratory were subject to sources of error not experienced by the other labs.

4.1. Linearity of calibration curves

The linearity of the initial calibration curves of the participating laboratories was evaluated by examining the calibration information obtained using calibration solutions up to a concentration of 10,000 μg/L as suggested by Method 8330B [30]. All the linear regressions for the linear calibration relationships of all the explosive target analytes had associated Coefficients of Determination (R^2) greater than 0.990.

4.2. Estimation of Limit of Quantitation (LOQ) at each participating laboratory

Estimated MDLs were determined first by each laboratory by spiking the suite of 17 analytes into acetonitrile according to the EPA protocol [36]. Once each specific MDL was estimated, a typical rounded value that was higher but in the vicinity of the MDL was selected and called the LOQ. Any concentrations less than the LOQ would be considered estimated. For more information on determining and verifying LOQs or LLOQs see Section 9.7 of Method 8000D [39].

The LOQs were consistent with those obtained by Hewitt et al. [40]. The average LOQs for the 17 target analytes, converted to μg/kg using the mass of tissue extracted and the volume of extractant after cleanup, were all less than 150 μg/kg except for NG (252 μg/kg) and PETN (392 μg/kg). An improvement in LOQs can be attained by processing an increased volume of extract. This can be done by addition of 10 mL of the tissue extract to 50 mL of water prior to conducting solid phase extraction. However, the limits of the cleanup by SPE may be reached if using these amounts on more complex tissue types.

4.3. Assessment of interferences from extraction of unfortified tissues

Care was taken to collect marine tissue study samples well away from any potential sources of the analytes of concern. Therefore, any peaks that appear in the retention time window for an analyte in samples that were not fortified with analytes would be either an interference or the result of laboratory contamination. Blank analyses indicated laboratory contamination was not a factor. There was a total of 75 marine tissue samples for the laboratory intercomparison test that were not fortified with the suite of target analytes. There were small peaks that co-eluted with HMX in a few of the chromatograms from replicate samples of the manila clam tissue, but the low amounts detected for HMX did not result in concentration estimates above the respective LOQs.

RDX was detected and reported in one of the triplicate Manila clam samples by Laboratories B and D at concentrations above LOQs at 179 μg/kg and 460 μg/kg, respectively. The reported LOQs for RDX from Laboratories B and D are 35 μg/kg and 140 μg/kg, respectively, and the values reported from the confirmation column were very similar. RDX was not detected in the other two triplicate Manila clam samples from these three laboratories, therefore, the detected mean result for RDX from Laboratories B and D are 60 μg/kg and 153 μg/kg, respectively. Inspection of the chromatograms indicates that the peak reported as RDX eluted near the upper edge of the RDX retention time window. When RDX was present at about the same peak magnitude, there is generally a clear distinction between them and no contribution would be made to the reported value for RDX. In addition, this minor interference for RDX was only found for the Manila Clam matrix. If blank tissue is available for a given type, the peak acceptance window can be adjusted to reduce/eliminate interferences that are close to the retention times for target analytes.

Except for HMX and RDX, no other non-target compounds were extracted from the unfortified marine tissues that directly interfered with at the retention times for the remaining 15 target analytes.

4.4. Outlier removal

Results from the laboratory intercomparison test included data from 5 laboratories (the sixth laboratory was eliminated due to instrument malfunction). Each laboratory was given 5 matrices, within each matrix there were 9 samples supplied, and the results were obtained for 17 target analytes. Thus, a total of 3825 individual results for the target analytes make up the data set for this study. Data was also obtained for the surrogate (1,2-DNB) in each sample for another 255 data points for a grand total of 4080.

Outliers in the data set were identified using a generalized ESD (Extreme Studentized Deviate) test. The ESD is more appropriate than the Grubb's Test when it is suspected that more than one or two outliers may exist within a data set. The ESD test was used with alpha set at 0.05 for a two-tailed test [41]. Type I error using this test is initially set at alpha = 0.05 (0.95 percentile), but alpha is known to increase to approximately 0.10 (0.90 percentile) as the number of identified outliers increases from one to seven. Not all mathematically identified outliers were rejected from the data set. If the pooled RSD value for the data set was already considered reasonable for the matrix (RSD of 15%, for example), then the identified outlying result was retained. Overall, a total of 112 outliers were removed from the data set, zero for the unfortified samples. These rejected data points represent only 2.7% of the overall data, a value that is quite low compared with other laboratory intercomparison studies [42–45]. Laboratory B contributed 51 of these outliers or 45.5% of the total. Of the analytes, HMX had the largest number of outliers at 20. HMX elutes the earliest and is the most difficult to quantify because of the large peak from weakly retained interferences where the tail of the peak overlaps the HMX retention time.

4.5. Tetryl

The recovery of tetryl in both the high-level and low-level fortified tissues was uniformly unacceptable for all five tissue types, but was noticeably worse for the clam and halibut tissues. Mean recovery for tetryl across the five tissues was 11.9% for the low level sample and 29.5% for the high level samples.

Tetryl has been a difficult analyte to obtain good recovery for a variety of matrices including soil/sediment. An improvement in tetryl stability can be obtained by acidification of the matrix extracts to a pH of 2 using sodium bisulfate [30,45]. Acidification was not used in this study because it has an unpredictable effect on 4-Am-DNT and 2-AmDNT [46] that were thought to be of more importance because of the instability of tetryl in sea water [47]. When tetryl is thought to be an important analyte for a given site, acidification of the tissue extract using a sodium bisulfate solution should be used to improve the recovery of tetryl.

4.6. Overall recovery for the remaining 16 target analytes

Results for the remaining 16 target analytes were much better than for tetryl. Overall mean target analyte recovery was 96.1% for the low-level fortified tissues computed across all 16 analytes and 103% for the high-level fortified tissue types.

Recoveries for individual target analytes varied from lows of 60.3% and 95.2% for TNT in the low and high fortified samples to highs of 121% and 116% for 4-Am-DNT, and 113%

and 119% for 2,6-DNT in the low and high fortified samples (Fig. 3). Recoveries for other target analytes generally ranged from 86.2% to 113% for the low level fortified samples to 95.3–119% for high-level fortified samples.

4-Am-DNT is a major environmental transformation product of TNT [48]. When TNT is present in the water, soil, or sediment, 4-Am-DNT is nearly always detectable as well. Whether TNT had transformed partially to 4-Am-DNT in the spiking solution prior to fortification or transformed in contact with the marine matrices, it is likely the lower recovery found for TNT and the higher recovery for 4-Am-DNT are related. Similarly, the increased recoveries reported for 2,6-DNT was probably due to a contribution from one of the transformation products of tetryl that elutes at a similar retention time as 2,6-DNT. Another degradation product of tetryl may have co-eluted with 4-Am-DNT, further inflating the recovery reported for that analyte.

4.7. Recovery and variability for different tissue types

The recovery of the target analytes varied somewhat for the different tissue types (Table 4). For example, the mean recovery of the 16 analytes (excluding tetryl) varied from a low of 73% and 84% for halibut in the low and high level fortified samples over all five laboratories to a high of 117% for both the low and high level fortified samples for Manila clam. The recovery for halibut was the lowest for both spike levels and the recovery for clam and crab were the highest in both cases. The lower recovery from the halibut tissue may be related to the higher oil content of this tissue type. Interestingly, if all individual recovery values are normalized using the 1,2-DNB surrogate, the mean % recoveries for all sixteen analytes over all five tissue matrices varies only from 92% to 94% and the % recovery was nearly identical for both high and low spikes for all of the tissue types. The surrogate correction reduces the random error associated with imprecise volume additions and recoveries. In addition, it corrects for the actual amount of water extracted from the various tissue types. The calculations assume that the total water present in the tissues is extracted, but visually, it does not appear that this is true. Thus it appears that this method could benefit from the use of an internal standard.

4.8. Intralaboratory precision estimates (repeatability RSD_r)

Repeatability was evaluated from the laboratory intercomparison study results averaging the five single-laboratory precision RSD values for each marine tissue types. The study design provided for triplicate analyses of each of three concentration levels (high, low, and blank) for the five marine tissue types. A summary of the repeatability (RSD_r) estimates for five of the most important target analytes from each laboratory's triplicate low and high spiked sample results for each tissue type is presented in Table 5. The mean RSD_r estimates presented are averaged across laboratories.

Because of the removal of outliers, the number of values for each analyte data set ranged from 9 to 15. Overall the mean RSDr values for the five analytes shown in Table 5 ranged from 1.7% to 23.6% for the low level spiked samples and from 2.5% to 12.0% for the high level spiked samples. When the data was surrogate corrected, the RSD_r estimates for most of

the analytes improved indicating that random error due to volumetric measurements in the sample preparation or extract injection volume during determination was significant.

4.9. Interlaboratory precision estimates (reproducibility RSD_R)

Reproducibility was evaluated from the data in the laboratory intercomparison study using the pooled multi-laboratory precision RSD values for each tissue type. The study design provided for 5 sets of data (one from each lab) for each tissue type and concentration. Each data set had a maximum of 15 values depending on the number of outliers removed. Thus an estimate of interlaboratory precision (RSD_R) was computed for each analyte for each tissue type at the low and high spike levels.

Table 5 presents RSD_R values for the same five analytes discussed above. RSDR ranged from 6.2% to 41.4% for the low spike level and 9.9–29.8% for the high spike level, respectively. As with repeatability, surrogate correction improved these precision estimates.

4.10. Possible improvements

There are several improvements that might be made that should improve the performance of this method besides the use of an internal standard. Other separations that provide better resolution for these analytes are now available, including solid core or porous shell columns. Targeted wash solutions can be used to further reduce ionic interferences during solid phase extraction. This would impact the chromatograms particularly for HMX and RDX.

5. Summary and conclusions

Dry-ice grinding is an acceptable means of tissue homogenization prior to subsampling and extraction. The chromatographic separations used in this study adequately resolved the seventeen target analyses and the potential interferences in all five tissue types tested. Cleanup of the tissue extracts using SPE reduced interferences particularly for the least retained analytes, HMX and RDX. The MDL for all 17 analytes are adequate in most cases and could be improved if necessary by processing a larger portion of the tissue extract. Analyte recovery was good for 16 of the 17 target analytes, recovery for tetryl was uniformly unacceptable. Repeatability and reproducibility estimates were marginally acceptable and could be improved by normalizing the results using the surrogate compound, 1,2-DNB. The method worked acceptably well for all five tissue types that represent a range of the types of marine tissues of interest from a human consumption standpoint.

Due to the significant transformation of tetryl in all species, development of a separate laboratory analytical method for nitrophenol transformation products in tissue samples is recommended.

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References

[1]. Bowles R, Beaufort's Dyke Background – DSC-Env1, Ministry of Defence, United Kingdom, 2006⟨http://webarchive.nationalarchives.gov.uk/20080206012606/http://www.mod.uk/NR/rdonlyres/52954E9D-A12E-4DB8-85D5BA5AC8EFB45C/0/bd_backgrd.doc⟩.

[2]. Bottcher C, Knobloch T, Ruhl N-P, Sternheim J, Wichert U, Wohler J, Munitions in German Marine Waters – Stocktaking and Recommendations (Effective 2011), German Marine Monitoring Programme, 2011 (https://portal.helcom.fi/meetings/SUBMERGED1-2014-164/MeetingDocuments/7-2-Att1 Munitions in German Marine Waters.pdf).

[3]. ESTCP, White Paper – Munitions in the Underwater Environment: State of the Science and Knowledge Gaps. ⟨https://www.serdp-estcp.org/Featured-Initiatives/Munitions-Response-Initiatives/Munitions-in-the-Underwater-Environment⟩, 6 2010.

[4]. Long TP, Growing A, International consensus of concern for chemical and conventional weapons abandoned in the marine environment, Mar. Technol. Soc. J. 45 (6) (2011) 8–11, 10.4031/MTSJ.45.6.13.

[5]. Francis S, Alama I, Kershaw L, WWII Unexploded Ordnance: A Study of UXO in Four Pacific Island Countries, Pacific Islands Forum Secretariat, 2011 ⟨http://www.forumsec.org/resources/uploads/attachments/documents/UXO⟩ final.pdf.

[6]. Nixon E, Assessment of the Impact of Dumped Conventional and Chemical Munitions (Update 2009), OSPAR Commission, Biodiversity Series, 2009 ⟨http://qsr2010.ospar.org/media/assessments/p00365_Munitions_assessment.pdf⟩.

[7]. Silva JAK, Chock T, Munitions integrity and corrosion features observed during the HUMMA deep-sea munitions disposal site investigations, Deep Sea Res. Part II: Top. Stud. Oceanogr 128 (2016) 14–24, 10.1016/j.dsr2.2015.09.001.

[8]. Wang PF, Liao Q, George R, Wild W, Release Rate and Transport of Munitions Constituents from Breached Shells in the Marine Environment, in: Chappell MA, Price CL, George RD (Eds.), Environmental Chemistry of Explosives and Propellant Compounds in Soils and Marine Systems: Distributed Source Characterization and Remedial Technologies, American Chemical Society (ACS) Symposium Series 1069, 2011, pp. 317–340 ⟨10.1021/bk-20111069.ch016⟩.

[9]. Darrach MR, Chutjian A, Plett GA, Trace explosives signature from world war II unexploded undersea ordnance, Environ. Sci. Technol 32 (9) (1998) 1354–1358 ⟨10.1021/es970992h⟩.

[10]. Rodacy PJ, Reber SD, Walker PK, Chemical Sensing of Explosive Targets in the Bedford Basin, Halifax Nova Scotia, Sandia National Laboratories, Albuquerque, NM, 2001 (Sandia Report SAND2001–3569), ⟨http://prod.sandia.gov/techlib/access-control.cgi/2001/013569.pdf⟩.

[11]. Monteil-Rivera F, Beaulieu C, Hawari J, Use of solid-phase microextraction/gas chromatography-electron capture detection for the determination of energetic chemicals in marine samples, J. Chromatogr. A 1066 (2005) 177–187, 10.1016/j.chroma.2005.01.049. [PubMed: 15794569]

[12]. Porter JW, Barton JV, Torres C, Ecological, Radiological, and Toxicological Effects of Naval Bombardment on the Coral Reefs of Isla de Vieques, Porter Rico, in: Machlis GE (Ed.), Warfare

Ecology: A New Synthesis for Peace and Security, NATO Science for Peace and Security Series C: Environmental Security, Springer Science +Business Media, 2011, pp. 65–122,, 10.1007/978-94-007-12140_8.

[13]. Koide S, Silva JAK, Dupra V, Edwards M, Bioaccumulation of chemical warfare agents, energetic materials, and metals in deep-sea shrimp from discarded military munitions sites off Pearl Harbor, Deep Sea Res. Part II: Top. Stud. Oceanogr 128 (2016) 53–62, 10.1016/j.dsr2.2015.03.002.

[14]. Taylor S, Bigl S, Packer B, Condition of in-situ unexploded ordnance, Sci. Total Environ 505 (2015) 762–769, 10.1016/j.scitotenv.2014.10.059. [PubMed: 25461079]

[15]. Benn AR, Weaver PP, Billet DSM, van den Hove S, Murdock AP, Doneghan GB, Le Bas T, Human activities on the deep seafloor in the North East Atlantic: an assessment of spatial extent, PLoS One 5 (9) (2010) 1–15, 10.1371/journal.pone.0012730.

[16]. Voie ØA, Mariussen E, Risk assessment of sea dumped conventional munitions, Prop. Explos. Pyrotech 42 (2017) 98–105, 10.1002/prep.201600163.

[17]. Hamilton A, Industrial Poisons Used or Produced in the Manufacture of Explosives, Bulletin of the U.S. Bureau of Labor Statistics, Whole No.219, U.S. Government Printing Office, Washington, D.C., 1917⟨http://www.jstor.org/stable/41823282⟩.

[18]. Dacre JC, Rosenblatt DH, Mammalian Toxicology and Toxicity to Aquatic Organisms of Four Important Types of Waterborne Munitions Pollutants – An Extensive Literature Evaluation, U.S. Army Medical Bioengineering Research and Development Laboratory, Aberdeen Proving Ground, MD, AD-778 725, 3, 1974 ⟨http://www.dtic.mil/dtic/tr/fulltext/u2/778725.pdf⟩.

[19]. Rickert DE (Ed.), Toxicity of Nitroaromatic Compounds, Hemisphere Publishing Corporation, Washington, D.C., 1985.

[20]. Burrows EP, Rosenblatt DH, Mitchell WR, Parmer DL, Organic Explosives and Related Compounds: Environmental and Health Considerations, U.S. Army Biomedical Research and Development Laboratory, Ft. Detrick, Fredrick, MD, Technical Report-8901, 3, 1989 ⟨http://www.dtic.mil/dtic/tr/fulltext/u2/a210554.pdf⟩.

[21]. Yinon J, Toxicity and Metabolism of Explosives, CRC Press, Boca Raton, FL, 1990.

[22]. Roberts WC, Hartley WR, Drinking Water Health Advisory: Munitions, Lewis Publishers, Boca Raton, FL, 1992.

[23]. Bodeau DT, Military Energetic Materials: Explosives and Propellants, in: Deeter DP, Gaydos JC (Eds.), Occupational Health – The Soldier and the Industrial Base, Office of the Surgeon General, Walter Reed Medical Center, Washington, D.C., 1993.

[24]. Talmage SS, Opresko DM, Maxwell CJ, Welsh CJE, Cretella FM, Reno PH, Daniel FB, Nitroaromatic munitions compounds: environmental effects and screening values, Rev. Environ. Contam. Toxicol 161 (1999) 1–156, 10.1007/978-1-4757-6427-7_1. [PubMed: 10218448]

[25]. Sunahara GI, Lotufo G, Kuperman RG, Hawari J. (Eds.), Ecotoxicology of Explosives, CRC Press, Boca Raton, FL, 2009.

[26]. Lotufo GR, Rosen G, Wild W, Carton G, Summary Review of the Aquatic Toxicology of Munitions Constituents, U.S. Army Corps of Engineers, Engineering Research and Development Center, Vicksburg, MS, ERDC/EL TR-13–8, 6, 2013 ⟨http://hdl.handle.net/11681/7059⟩.

[27]. Craig HD, Taylor S, Framework for evaluating fate, transport, and risks from conventional munitions compounds in underwater environments, Mar. Technol. Soc. J. 45 (6) (2011) 35–46, 10.4031/MTSJ.45.6.10.

[28]. Nipper M, Carr RS, Biedenbach JM, Hooten RL, Miller K, Saepoff S, Development of marine toxicity data for ordnance compounds, Arch. Environ. Contam. Toxicol 41 (2001) 308–318, 10.1007/s002440010253. [PubMed: 11503067]

[29]. Pascoe GA, Kroeger K, Leisle D, Feldpausch RJ, Munitions constituents: preliminary sediment screening criteria for the protection of marine benthic invertebrates, Chemosphere 81 (2010) 807–816, 10.1016/j.chemosphere.2010.06.077. [PubMed: 20674958]

[30]. United States Environmental Protection Agency, Method 8330B, Nitroaromatics, Nitramines, and Nitrate Esters by High Performance Liquid Chromatography (HPLC), U.S. EPA Office of Solid Waste and Emergency Response, Washington, D.C, Revision 2, 10 ⟨https://www.epa.gov/sites/production/files/2015-12/documents/8330b.pdf⟩, 2006.

[31]. Bunch EA, Altwein DM, Johnson LE, Farley JR, Hammersmith AM, Homogeneous sample preparation of raw shrimp using dry ice, J. AOAC Intern 78 (1995) 883–887.

[32]. Cragin JH, Leggett DC, Foley BT, Schumacher PW. TNT, RDX and HMX Explosives in Soils and Sediments: Analysis Techniques and Drying Losses CRREL Report 85–15, Hanover, NH (USA), 1985 ⟨http://www.dtic.mil/dtic/tr/fulltext/u2/a163278.pdf⟩.

[33]. United States Environmental Protection Agency, Guidance for Assessing Chemical Contamination Data for Use in Fish Advisories, Vol.1, Fish Sampling and Analysis Third Edition, U.S. EPA Office of Water, Washington, D.C., EPA 823-B-00–007, 11 ⟨https://nepis.epa.gov/Exe/ZyPDF.cgi/20003OMP.PDF?Dockey=20003OMP.PDF⟩, 2000.

[34]. Jenkins TF, Miyares PH, Myers KF, McCormick EF, Strong AB, Comparison of solid phase extraction with salting-out solvent extraction for preconcentration of nitroaromatic and nitramine explosives from water, Anal. Chem. Acta 289 (1994) 69–78, 10.1016/0003-2670(94)80009-X.

[35]. United States Environmental Protection Agency, Explosives in Marine Tissue Multi-Laboratory Study, U.S. EPA Office of Superfund Remediation and Technical Innovation Analytical Services Branch Quality Assurance Technical Support (QATS) Program, Las Vegas, NV, Contract number EP-W-10–033, CB&I Federal Services, 8 ⟨https://www.dropbox.com/s/ow63i45t14lu7u4/2014%20-%20TASK%20ORDER%203028%20FINAL%20REPORT%20EXPLOSIVES%20IN%20MARINE%20TISSUE%20MULTI-LABORATORY%20STUDY.pdf?Dl=0⟩, 2014.

[36]. United States Environmental Protection Agency, Validation of Testing/Measurement Methods, 600/X-83–060, 1983.

[37]. United States Environmental Protection Agency, Nitroaromatics and Nitramines by HPLC, Second Update SW846 Method 8330. ⟨https://archive.epa.gov/epawaste/hazard/testmethods/web/pdf/method⟩ 8330, revision 1 – 1990.pdfs, 1994.

[38]. United States Environmental Protection Agency, Fact Sheet: Revision of Federal Human Health Criteria Applicable to Washington, U.S. EPA Office of Water, Washington, D.C., EPA 823-F-16–003, 11 ⟨https://www.epa.gov/sites/production/files/2016-11/documents/washington_hhc_final_rule_fact_sheet_508c.pdf⟩, 2016.

[39]. United States Environmental Protection Agency, Method 8000D, Determinative Chromatographic Separations, U.S. EPA Office of Solid Waste and Emergency Response, Washington, D.C., Revision 5, 3 ⟨https://www.epa.gov/sites/production/files/2015-12/documents/8000d.pdf⟩, 2018.

[40]. Hewitt AD, Jenkins TF, Walsh ME, Brochu S.. Validation of sampling protocol and promulgation of for method modifications for the characterization of energetic residues on military testing and training ranges. ERDC/CRREL TR 09–6, Hanover, NH (USA), 2009 ⟨http://www.dtic.mil/dtic/tr/fulltext/u2/a517341.pdf⟩.

[41]. Rosner B, Percentage points for a generalized ESD many outlier procedure, Technometrics 25 (1983) 165–172, 10.2307/1268549.

[42]. Horwitz W, Evaluation of analytical methods used for regulation of foods and drugs, Anal. Chem 54 (1982) 67A–76A, 10.1021/ac00238a002.

[43]. Boyer KW, Horwitz W, Albert R, Interlaboratory variability in trace element analysis, Anal. Chem 57 (1985) 454–459, 10.1021/ac50001a031. [PubMed: 3838422]

[44]. Bauer CF, Grant CL, Jenkins TF, Interlaboratory evaluation of high-performance liquid chromatographic determination of nitroorganics in munition plant wastewater, Anal. Chem 58 (1986) 176–182, 10.1021/ac00292a043.

[45]. Bauer CF, Koza SM, Jenkins TF, Liquid chromatographic method for the determination of explosives residues in soil: collaborative study, J. AOAC 73 (1990) 541–552.

[46]. Jenkins TFPG, Thorne EF, McCormick KF Myers. Preservation of Water Samples Containing Nitroaromatics and Nitramines. CRREL Special Report 95–16, Hanover, New Hampshire, 1995 ⟨http://www.dtic.mil/dtic/tr/fulltext/u2/a295723.pdf⟩.

[47]. Hoffsommer JC, Rosen JM, Hydrolysis of explosives in sea water, Bull. Environ. Contam. Toxicol 10 (1973) 78–79, 10.1007/BF01685876. [PubMed: 4749300]

[48]. McCormick NG, Feeherry FE, Levinson HS, Microbial transformation of 2,4,6trinitrotoluene and other nitroaromatic compounds, Appl. Environ. Microbiol 6 (1976) 949–958 ⟨http://aem.asm.org/content/31/6/949.full.pdf⟩.

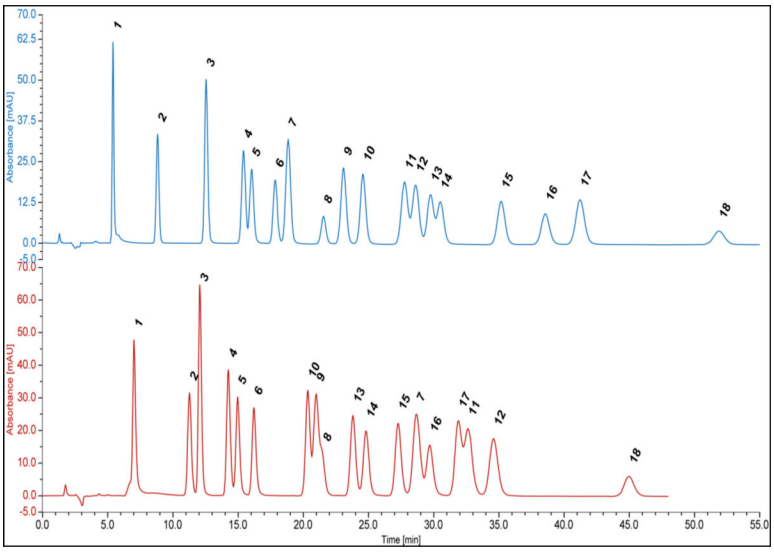

Fig. 1.

2500 μg/L standard (on-column) UV @ 210nm Peaks: 1) HMX, 2) RDX, 3) 1,3,5-TNB, 4) 1,2-DNB, 5) 1,3-DNB, 6) NB, 7) 3,5-DNA, 8) NG, 9) Tetryl, 10) TNT, 11) 4-Am-DNT, 12) 2-Am-DNT, 13) 2,6-DNT, 14) 2,4-DNT, 15) 2-NT, 16) 4-NT, 17) 3-NT, 18) PETN.

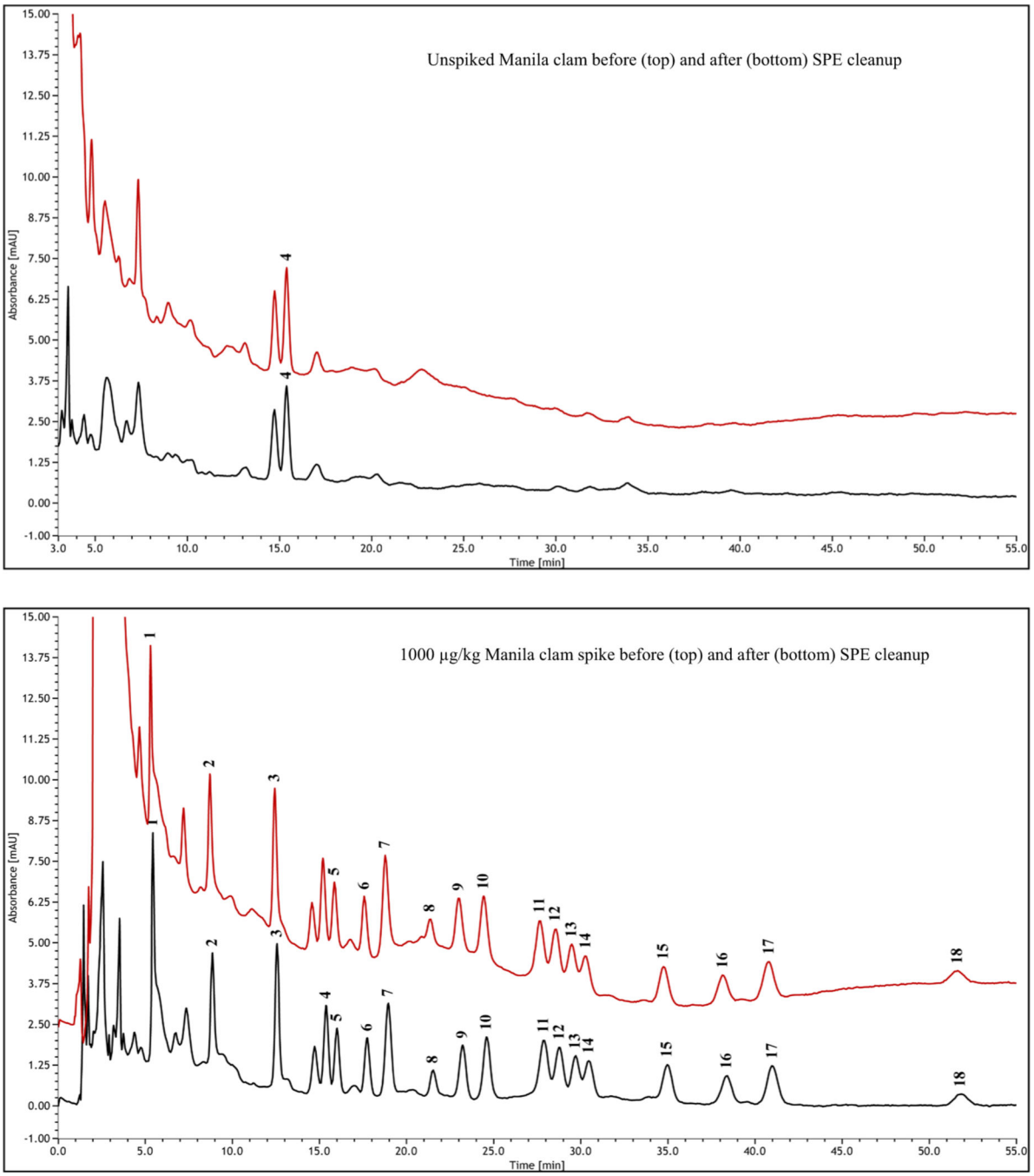

Fig. 2.

Primary Column (E1), UV @210 nm Peaks: 1) HMX, 2) RDX, 3) 1,3,5-TNB, 4) 1,2-DNB, 5) 1,3-DNB, 6) NB, 7) 3,5-DNA, 8) NG, 9) Tetryl, 10) TNT, 11) 4-Am-DNT, 12) 2-Am-DNT, 13) 2,6-DNT, 14) 2,4-DNT, 15) 2-NT, 16) 4-NT, 17) 3-NT, 18) PETN.

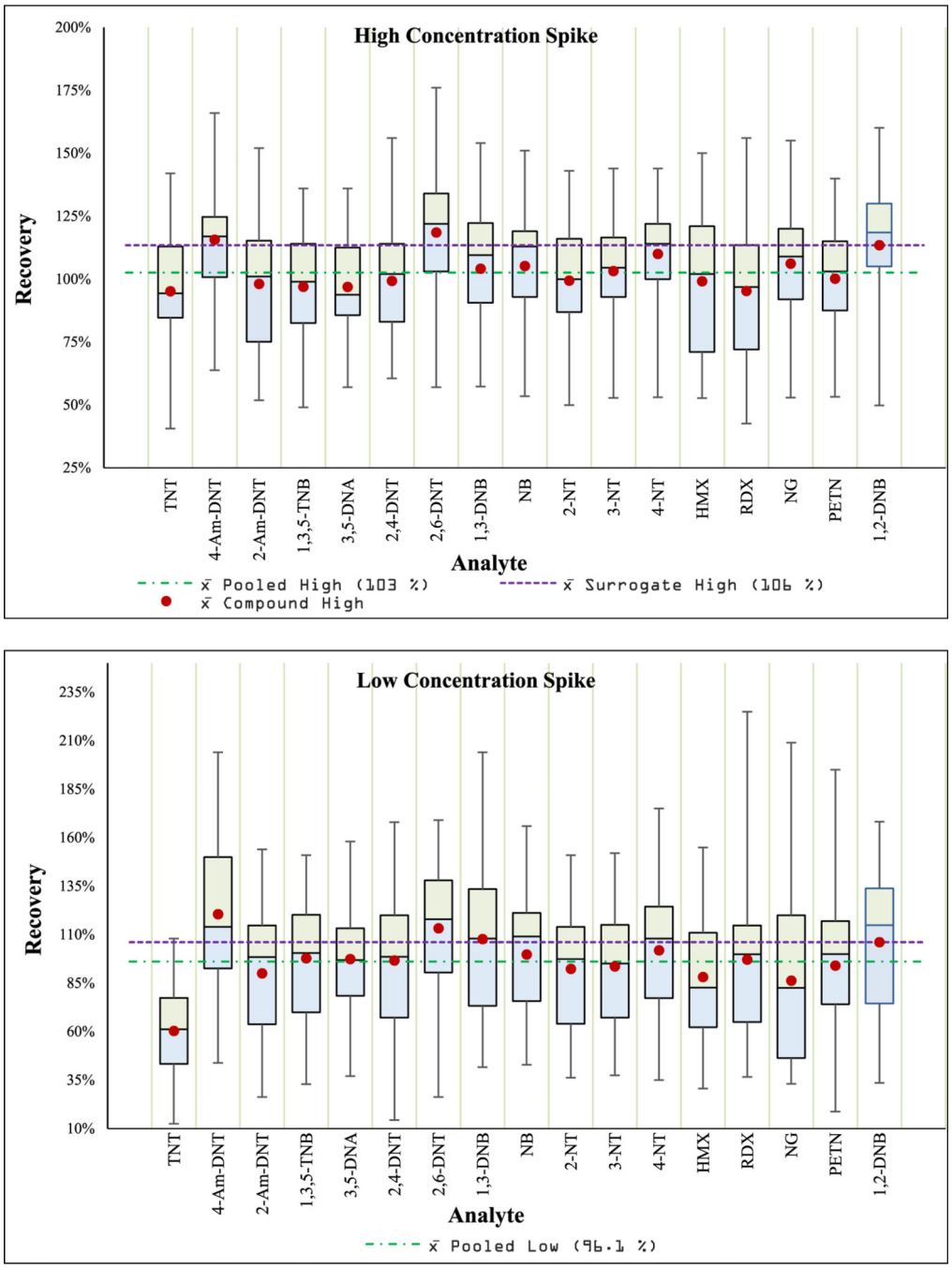

Fig. 3.

Box-plots from laboratory intercomparison study (pooled tissue types).

Table 1

Target analytes,their abbreviations,CAS numbers,and military relevance.

Compound	Abbreviation	CAS#	Military Use/Source in residues
Octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine	HMX	2691-41-0	Military explosive
Hexahydro-1,3,5-trinitro-1,3,5-triazine	RDX	121-82-4	Military explosive
1,3,5-trinitrobenzene	1,3,5-TNB	99-35-4	Photodegradation product of TNT
1,3-dinitrobenzene	1,3-DNB	99-65-0	Impurity in military grade TNT
Methyl-2,4,6-trinitrophenylnitramine	Tetryl	479-45-8	Former military explosive
Nitrobenzene	NB	98-95-3	Impurity in military grade DNT
2,4,6-trinitrotoluene	TNT	118-96-7	Military explosive
4-Amino-2,6-dinitrotoluene	4-Am-DNT	19406-51-0	Transformation product of TNT
2-Amino-4,6-dinitrotoluene	2-Am-DNT	35572-78-2	Transformation product of TNT
2,4-dinitrotoluene	2,4-DNT	121-14-2	Impurity in military grade TNT, component of single base propellants
2,6-dinitrotoluene	2,6-DNT	606-20-2	Impurity in military grade TNT
2-nitrotoluene	2-NT	88-72-2	Impurity in military grade DNT
3-nitrotoluene	3-NT	99-08-1	Impurity in military grade DNT
4-nitrotoluene	4-NT	99-99-0	Impurity in military grade DNT
Nitroglycerin	NG	55-63-0	Component of double base propellants
Pentaerythritol tetranitrate	PETN	78-11-5	Military explosive
3,5-dinitroaniline	3,5-DNA	618-87-1	Transformation product of 1,3,5-TNB

Table 2

Spike levels for laboratory intercomparison test.

Analyte	Low-Level Spike (μg/kg)	High-Level Spike (μg/kg)	Stock Standard Concentration (μg/mL)	Vendor
HMX	700	2800	1000	Restek
RDX	800	3200	1000	Restek
1,3,5-TNB	700	2800	1000	Restek
1,3-DNB	600	2400	1000	Restek
Tetryl	700	2800	1000	Restek
NB	800	3200	1000	Restek
TNT	1000	4000	1000	Restek
4-Am-DNT	800	3200	1000	Restek
2-Am-DNT	700	2800	1000	Supelco
2,4-DNT	800	3200	1000	Restek
2,6-DNT	800	3200	1000	Restek
2-NT	800	3200	1000	Restek
3-NT	800	3200	1000	Restek
4-NT	800	3200	1000	Restek
NG	2000	8000	1000	Restek
PETN	2400	9600	1000	Restek
3,5-DNA	700	2800	1000	Restek

Table 3

Method detection limits for target analytes and risk criteria.

Analyte	Min. MDL (µg/kg)	Max. MDL (µg/kg)	Mean MDL (µg/kg)	Fish Tissue Carcinogenic Target Risk (1E-06)[a] (µg/kg)	Fish Tissue Non-Carcinogenic Target Risk (HQ = 1)[a] (µg/kg)
HMX	25	67	52		77,000
RDX	21	80	56	38	4600
1,3,5-TNB	17	67	39		46,000
1,3-DNB	29	62	51		150
Tetryl	30	129	78		3100
NB	15	70	42		3100
TNT	26	84	55	140	770
4-Am-DNT	39	81	59		3100
2-Am-DNT	32	87	50		3100
2,4-DNT	21	77	45	13	3100
2,6-DNT	54	82	67	2.8	460
2-NT	36	80	65	19	1400
3-NT	24	75	54		150
4-NT	30	86	57	260	6200
NG	109	232	173	240	150
PETN	88	293	167	1000	3100
3,5-DNA	45	101	74		
1,2-DNB	29	77	64		

[a] https://www.epa.gov/risk/regional-fish-regional-screening-levels-rsls-june-2017.

Table 4

Recovery and reproducibility for various tissue types averaged across 16 target analytes (excluding tetryl).

Marine Species	Spike Level	% Recovery	RSD	N
Dungeness crab	High	114	15.9	237
Dungeness crab	Low	116	20.5	222
Manila clam	High	117	16.9	222
Manila clam	Low	117	26.2	226
Halibut	High	83	22.1	237
Halibut	Low	74	34.5	240
Sea cucumber	High	102	20.2	228
Sea cucumber	Low	89	29.4	225
Geoduck	High	97	20	223
Geoduck	Low	86	32.8	228

Table 5

Recovery, repeatability and reproducibility of selected analytes from laboratory intercomparison test.

Analyte	Concentration (μg/Kg)	Species	N	Mean Recovery (%)	Repeatability RSD_r (%)	Reproducibility RSD_R (%)
TNT High Level	3200	Dungeness crab	15	99	6.3	18.4
		Manila clam	14	116	3.0	12.9
		Halibut	15	73	6.4	28.3
		Sea cucumber	15	97	6.9	18.1
		Geoduck	15	93	2.6	19.2
TNT Low Level	800	Dungeness crab	14	71	8.7	27.4
		Manila clam	13	85	3.7	9.8
		Halibut	15	43	16.3	40.4
		Sea cucumber	15	57	8.1	35.7
		Geoduck	15	50	9.1	28.3
4-Am-DNT High Level	2800	Dungeness crab	15	131	3.4	14.6
		Manila clam	15	129	7.4	16.8
		Halibut	12	93	6.8	17.9
		Sea cucumber	12	111	7.6	16
		Geoduck	12	107	2.7	15.3
4-Am-DNT Low Level	700	Dungeness crab	14	162	10.6	15.7
		Manila clam	15	134	14.4	24.9
		Halibut	15	109	18.5	39.2
		Sea cucumber	12	98	23.2	28.8
		Geoduck	15	98	14.6	31.2
1,3,5-TNB High Level	2800	Dungeness crab	15	110	5.2	15.5
		Manila clam	15	108	12.0	21.5
		Halibut	15	75	5.8	18.8
		Sea cucumber	12	107	8.1	9.9
		Geoduck	15	86	2.5	21.5
1,3,5-TNB Low Level	700	Dungeness crab	14	114	8.4	17.9
		Manila clam	15	117	9.9	25.4
		Halibut	15	68	16.8	29.8

Analyte	Concentration (μg/Kg)	Species	N	Mean Recovery (%)	Repeatability RSD_r (%)	Reproducibility RSD_R (%)
		Sea cucumber	9	116	1.7	6.3
		Geoduck	15	82	8.9	29.4
2,4-DNT High Level	3200	Dungeness crab	15	110	4.9	22.9
		Manila clam	15	111	10.8	26.9
		Halibut	15	83	6.4	16
		Sea cucumber	15	97	7.9	17.8
		Geoduck	15	95	4.2	20.5
2,4-DNT Low Level	800	Dungeness crab	15	116	19.4	26.3
		Manila clam	15	126	7.3	31.8
		Halibut	15	67	23.6	41.4
		Sea cucumber	15	85	7.7	27.2
		Geoduck	15	89	8.9	32.1
RDX High Level	3200	Dungeness crab	15	101	5.4	19.7
		Manila clam	15	113	11.7	29.8
		Halibut	15	78	5.6	20.1
		Sea cucumber	15	94	7.5	23.1
		Geoduck	15	91	2.6	24.9
RDX Low Level	800	Dungeness crab	10	112	2.0	6.2
		Manila clam	15	130	17.7	38.6
		Halibut	15	70	14.1	32.2
		Sea cucumber	15	84	8.4	26.9
		Geoduck	15	94	7.4	33.4