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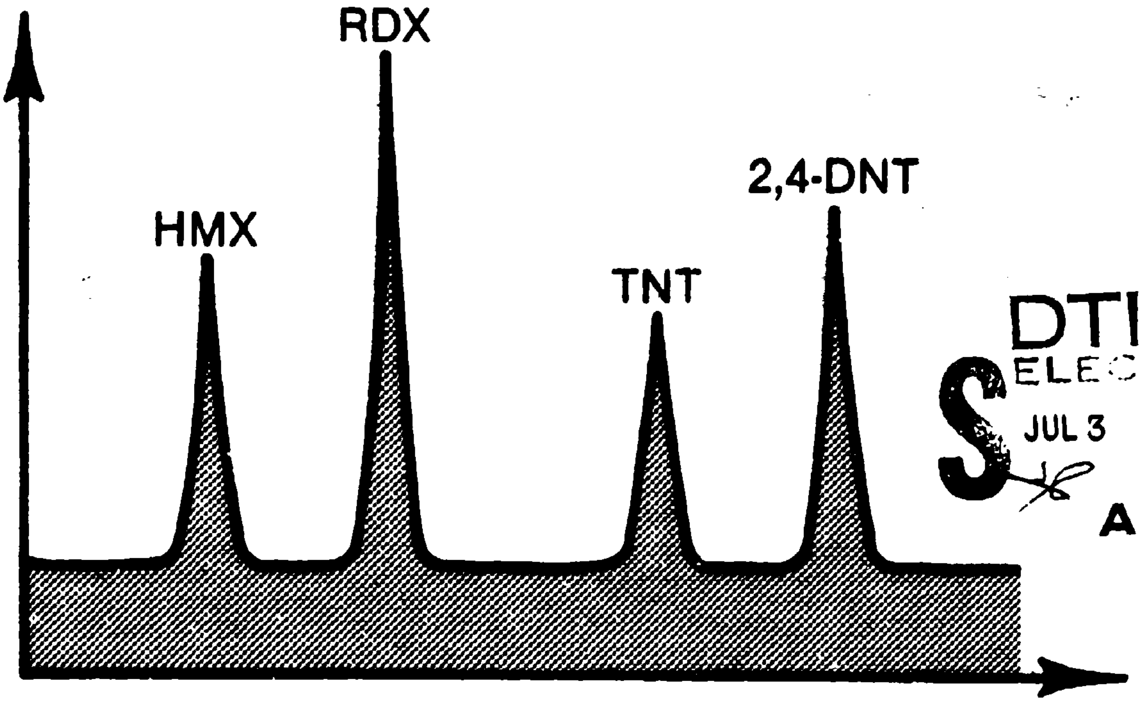
Reverse phase HPLC method for analysis of TNT, RDX, HMX and 2,4-DNT in munitions wastewater



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For conversion of SI metric units to U.S./British customary units of measurement consult ASTM Standard E380, Metric Practice Guide, *published by the American Society for Testing and Materials, 1916 Race St., Philadelphia, Pa. 19103.*

Cover: Liquid chromatogram of a typical explosive mixture.

CRREL Report 84-29

December 1984

Reverse phase HPLC method for analysis of TNT, RDX, HMX and 2,4-DNT in munitions wastewater

T.F. Jenkins, C.F. Bauer, D.C. Leggett and C.L. Grant

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An analytical method was developed to determine the concentrations of HMX, RDX, TNT and 2,4 DNT in munitions wastewater. The method involves dilution of an aqueous sample with an equal volume of methanol-acetonitrile solvent mixture, filtration through a 0.4-µm polycarbonate membrane and analysis of a 100-µL subsample by Reverse-phase, high-performance liquid chromatography using an LC-8 column. Retention times of these four analytes, their degradation products, and impurities expected in wastewater matrices were determined for two eluent compositions. An eluent of 50% water, 38% methanol and 12% acetonitrile successfully separated HMX, RDX and TNT from each other and the potential interferents. The method provided linear calibration curves over a wide range of concentrations. Detection limits were conservatively estimated to be 26, 22, 14 and 10 µg/L for HMX, RDX, TNT

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2,4-DNT respectively. Analytical precision was estimated at ± 3.4, 3.3, 4.4 and 4.6 μg/L. A ruggedness test involving the major manipulative steps in the procedure indicated that use of glass sampling containers, the portion of filtrate chosen for analysis and a carefully measured sample-to-organic-solvent ratio was necessary to obtain consistent analytical results. The method was tested with munition wastewater from several Army ammunition plants and found to perform adequately for load and pack wastewaters, wastewater from HMX/RDX manufacture and contaminated groundwater. An interlaboratory test of the method was conducted with nine participating organizations, including laboratories at four Army ammunition plants, the EPA's Environmental Monitoring and Support Laboratory, three Army research organizations and a university. For the four analytes collectively, the analytical accuracy was within 5%, the intralaboratory precision (repeatability) was 5 to 9% based on the average of duplicate injections, and the interlaboratory precision (reproducibility) was 7 to 10%. This evaluation excluded about 10% of the data, which were identified as outliers.

PREFACE

This report was prepared by Thomas F. Jenkins and Daniel C. Leggett, Research Chemists, Earth Sciences Branch, Research Division, U.S. Army Cold Regions Research and Engineering Laboratory and Dr. Christopher F. Bauer, Associate Professor, and Dr. Clarence L. Grant, Professor, Chemistry Department, University of New Hampshire (UNH), Durham, New Hampshire.

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1. Iowa Army Ammunition Plant, Mason Hangar Corporation: L. Shahan, Analyst; E. Pollpeter, Supervisor.
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4. Radford Army Ammunition Plant: F. Heim and W.C. Jackson, Analysts; R. Mundy, supervisor.
5. U.S. Army Large Caliber Weapons Systems Laboratory (LCWSL), Energetic Materials Division: D. Robertson, analyst; Dr. T.H. Chen, section chief.
6. U.S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory; Dr. D. Forest, analyst/supervisor.
7. U.S. Army Environmental Hygiene Agency (AEHA), Dr. R. Bishop, analyst/supervisor.
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P. Butler obtained the data for suspended solids, pH and total organic carbon used in the filtration-recovery study.

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ABBREVIATIONS

AAP	Army Ammunition Plant
AEHA	Army Environmental Hygiene Agency
ANOVA	Analysis of Variance
GC-ECD	Gas Chromatographic-Electron Capture Detector
GC-FID	Gas Chromatographic-Flame Ionization Detector
GOCO	Government-Owned, Contractor-Operated
LCWSL	Large Caliber Weapons Systems Laboratory (U.S. Army)
MS	Mass spectroscopy
NBS	National Bureau of Standards
RP-HPLC	Reverse Phase, High Performance Liquid Chromatography
RSD	Relative Standard Deviation
SARM	Standard Analytical Reference Materials
TLC	Thin-Layer Chromatography
USEPA, EMSL	U.S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory
UV	Ultraviolet

SUMMARY

This report documents the experiments conducted during the development and collaborative testing of a Reverse-Phase, High-Performance Liquid Chromatography (RP-HPLC) method for HMX, RDX, TNT and 2,4-DNT in water. This method utilizes an LC-8 column with an eluent of 50% water, 38% methanol and 12% acetonitrile. Retention times for HMX, RDX, TNT and 2,4-DNT were 3.2, 4.1, 7.0 and 7.8 minutes, respectively, at an eluent flow rate of 1.5 mL/minute. Measurement of the retention times of expected matrix contaminants and degradation products indicated that none would interfere with the determination of HMX, RDX or TNT. The presence of 4-amino-2,6-dinitrotoluene (a microbial degradation product of TNT) 2,4,5-TNT and 2,6-DNT could interfere with 2,4-DNT determination since they all elute within 0.2 minutes of 2,4-DNT.

Water samples were analyzed as follows. A 10-mL aqueous sample was diluted with 10 mL of a mixed solvent composed of 76% methanol 24% acetonitrile (V/V) in a scintillation vial. The sample was capped, shaken and allowed to stand for 15 minutes. The sample was then filtered through a 0.4-µm Nuclepore polycarbonate membrane into a second scintillation vial. A 100-µL subsample of this solution was then injected into an LC-8 column and eluted with 1.5 mL/minute of 50/38/12% water/methanol/acetonitrile (V/V/V). The column effluent was directed to a fixed wavelength, 254-nm UV detector and the response measured with a digital integrator.

Detector response was linear from the detection limits to 5580 µg/L for HMX, 6200 µg/L for RDX, 4200 µg/L for TNT and 1600 µg/L for DNT. The linear range can be extended by use of smaller injection volumes. Using peak height measurements and linear regression analysis, analytical sensitivity was established for HMX, RDX, TNT and 2,4-DNT at 5.0×10^{-3}, 6.8×10^{-3}, 1.1×10^{-2} and 1.4×10^{-2} absorbance units per mg/L, respectively, for 100-µL injection volumes.

Since removal of suspended solids is necessary to protect expensive HPLC columns, experiments were conducted to assess the degree of loss during filtration by adsorption on various types of filters. Nuclepore 0.4-µm polycarbonate membranes were found to be well suited for this application. Dilution of sample with an equal volume of methanol-acetonitrile solution prior to filtration was found to result in quantitative recovery of spikes of 2,4-DNT, TNT and RDX. There were small losses of HMX, which appeared to be proportional to the concentration of suspended material present in the sample. Even in the worst case tested, over 92% of the spiked HMX was recovered.

Detection limits of this method were obtained by the method of Hubaux and Vos (1970) using data from peak area measurements from a digital integrator. The values of 26 µL for HMX, 22 µg/L for RDX, 14 µg/L for TNT and 10 µg/L for 2,4-DNT are considered to be conservative, and are sufficient to meet current and projected discharge limits. Analytical precision was estimated at ±3.4 µg/L for HMX, ±3.3 µg/L for RDX, ±4.4 µg/L for TNT and ±4.6 µg/L for DNT at concentrations below 245 µg/L, 136 µg/L, 77 µg/L and 64 µg/L respectively.

A ruggedness test was conducted to determine the sensitivity of the method to small deviations in the analytical protocol. The results indicated that use of glass containers rather than polyethylene was desirable, particularly for 2,4-DNT. Accu-

rate 2,4-DNT analysis also required consistency in the filtration procedure, washing the filter with the first 10-mL portion of sample–organic solvent solution and using the second 10-mL portion for analysis. Care was also found to be very important in the volumetric measurements used to dilute the sample with the organic solvent. The solvent strength affected the measured HMX and RDX peak areas over and above the effect expected because of the resulting differences in analyte concentration.

Munitions wastewaters were collected at four Army ammunition plants. These included wastewater from a load and pack facility and an RDX-HMX manufacturing line and also an RDX contaminated groundwater. The method appeared to be adequate for analysis of all three types of matrices.

Results of a collaborative study, where nine laboratories each analyzed four aqueous matrices spiked with the analytes, showed that the overall performance of the RP-HPLC method is very good for the concentration ranges studied. The evidence supporting this evaluation is summarized below:

1. For DNT, RDX and HMX the median "found" concentrations are within 3% of the true values. For TNT the difference is within 5%. Considering that the "true" values themselves are necessarily somewhat uncertain, the overall accuracy is very good.

2. The repeatability, based on duplicate injections of each of two aliquots, is about 7, 9, 15 and 10 μg/L for DNT, TNT, RDX and HMX respectively. These values represent percent relative deviations on the order of 5 to 9%. If single injections were used the repeatabilities would be inflated by a factor of 1.414 (square root of 2).

3. Reproducibilities for each analyte are about 6, 21, 40 and 44% greater than repeatabilities for DNT, TNT, RDX and HMX respectively. This gives percent interlaboratory deviations, based on average concentration examined, of about 7% for DNT, RDX and HMX and 10% for TNT. The most likely source of these differences between laboratories is the calibration of the instrumental response.

4. Recoveries of a given analyte were similar regardless of matrix. Overall, DNT and RDX were recovered quantitatively, and TNT and HMX showed small losses of about 5%.

The standard deviation of replication was independent of concentration in the concentration ranges examined in this collaborative study. Because of this, the relative standard deviations for RDX and HMX are better than those of DNT and TNT when in fact RDX and HMX have poorer absolute precisions.

Valid statistical analysis required rejection of about 10% of the individual data values. Even where a substantial number of outliers was identified, the repeatabilities for those analytes most effected (RDX and HMX) grew from 5% relative to only 12% relative when no values were eliminated. This larger relative deviation is still quite acceptable for analysis at the microgram-per-litre level.

At this point we are confident in recommending that this HPLC method be implemented as a means of monitoring munitions plant wastewaters and natural waters for DNT, TNT, RDX and HMX at the submilligram-per-litre level. The accuracy and reproducibility in the analysis of real environmental samples have proven to be adequate for this task.

REVERSE PHASE HPLC METHOD FOR ANALYSIS OF TNT, RDX, HMX AND 2,4-DNT IN MUNITIONS WASTEWATER

T.F. Jenkins, C.F. Bauer, D.C. Leggett and C.L. Grant

PART 1. METHOD DEVELOPMENT

INTRODUCTION

Monitoring requirements

One of the Army's most serious water pollution problems is the disposal of wash waters used to clean equipment and interior surfaces at TNT and RDX manufacturing and demilitarization facilities. It has been estimated that up to a half million gallons (190,000,000 L) of this type of wastewater is generated from a single production line each day (Walsh et al. 1973). Since this washdown process is necessary for safe operation, it is unlikely that this waste stream will be eliminated in the near future.

Current practice is to collect wash water from these processing operations in a holding tank and pump the wastewater through a carbon adsorption column. This procedure is capable of reducing TNT and RDX levels to the low parts-per-billion range. The treated wastewater is then typically discharged to a nearby surface stream. These point discharges are subject to state and federal National Pollution Discharge Elimination System (NPDES) permits, which generally limit the acceptable concentrations of TNT and RDX (2,4,6-trinitrotolune and hexahydro-1,3,5-trinitro-1,3,5-triazine [see Appendix B]). Carbon adsorption technology can at present meet discharge limitations, but these carbon columns have finite lifetimes. Eventually, breakthrough occurs and regeneration or replacement is necessary.

To satisfy permit requirements and to check on system performance, daily monitoring of wastewater from the carbon adsorption columns is generally necessary during manufacturing. Current monitoring requires separate determinations for TNT and RDX, the two most common explosives used by the U.S. Army.

Additionally, monitoring for HMX (octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazine [see Appendix B]) also an Army explosive and a common impurity in RDX and 2,4-DNT (2,4-dinitrotoluene [see Appendix B])—a low-level impurity in TNT—may also be required in the near future. At present no standard analytical method is available for TNT, RDX or HMX. Hence, individual Army installations have developed their own procedures, which differ widely in their detection limits, specificity and precision. Since 2,4-DNT is one of EPA's priority pollutants, a standard method involving solvent extraction and gas chromatographic analysis has been developed for its determination (Federal Register 1979). No information is available on the suitability of this method for simultaneous determination of TNT, RDX and HMX.

Objectives

The first objective of this effort was to choose from among the various alternatives for measurement of TNT, RDX, HMX and 2,4-DNT the method best suited for compliance monitoring requirements at U.S. Army Ammunition Plants (AAP). The method of choice must satisfy the following requirements:

1. It must have detection limits sufficiently low to satisfy current and future monitoring requirements for point discharges.
2. It must be rapid to enable quick remedial ac-

Table 1. Survey of discharge limits and analytical methods at Government-owned, contractor-operated installations in 1981.

Installation	*Max. discharge concentrations (mg/L)**	*Monitoring requirement*	*Analytical method*
Lone Star AAP	TNT, 0.3 RDX, 15	1 grab/day	Solvent extraction, GC-FID
Louisiana AAP	TNT†, 2.0	1 composite/day	Silas Mason Colorimetric
Iowa AAP	TNT, 0.5 RDX, 15	2 composite/mo.	RD-HPLC
Holston AAP	**	—	RP-HPLC, or GC-FID
Radford AAP	**	—	RP-HPLC, or GC-ECD

* Maximum daily average as specified on NPDES permit.
† TNT and nitrobodies.
** None specified on permit.

tion if discharges are found to be in violation of discharge limits.

3. It should be precise and accurate so that the waste stream can be characterized using a minimum number of replicates.

4. It should be free of interferences from the common contaminants in AAP waste streams, including decomposition products and impurities commonly found in the explosives.

5. It should allow measurement of all four of the analytes in the same procedure since they will often occur together because of the types of formulations typically used in explosives production.

6. It should be as inexpensive as possible to implement, on both an initial capital cost and a per sample basis.

A second objective of this study was to conduct a collaborative test of the developed method to determine how well it works in a variety of laboratories. This was to include several laboratories that support munitions manufacturing operations, where monitoring of discharges for NPDES permit compliance is required.

Possible analytical approaches

Two parallel approaches were used to assess which analytical methods were best suited for the above analytes in a water matrix. First, a literature search was conducted which identified the methods that had been reported in the open literature as well as in published government reports. The second was by personal site visits to five AAPs and several government laboratories that had extensive experience with these types of analyses.

A summary of some of the most important information from the five AAPs, including their discharge limits, monitoring requirements and the analytical methods in use, is presented in Table 1. Clearly, the discharge limits vary somewhat from site to site primarily because of their location in different states and EPA regions and because of the lack of a nationwide discharge standard. At present both TNT and RDX are limited in most permits but HMX and DNT are not. Analytical approaches in use include a colorimetric method, Reverse-Phase, High-Performance Liquid Chromatography (RP-HPLC), and solvent extraction followed by Gas Chromatographic analysis using either a Flame Ionization Detector (GC-FID) or Electron Capture Detector (GC-ECD). Discussions with analytical chemists at each installation resulted in a consensus that for compliance monitoring, a direct approach such as RP-HPLC was the most desirable if sufficiently low detection limits could be obtained. While it is difficult to predict the discharge limits for these substances that may be set in the future, current research at the U.S. Army Medical Bioengineering Research and Development Laboratory indicates that limits as low as 300 μg/L, 920 μg/L, 120 μg/L and less than 40μg/L for RDX, HMX, DNT and TNT, respectively, are possible.*

* Personal communication with J. Barkley, U.S. Army Medical Bioengineering Research and Development Laboratory, Fort Detrick, Maryland.

Besides these five APPs, we also consulted Dr. John Walsh at U.S. Army Natick Laboratories and Dr. Richard Bishop from the U.S. Army Environmental Hygiene Agency. Both of these individuals had extensive analytical experience in the determination of the analytes from their research activities. Again the recommendation was RP-HPLC because of the difficulty associated with GC analysis of RDX and HMX, primarily attributable to thermal degradation at the temperatures required to volatilize these substances in the injector. Experience in our own laboratory also indicated that RP-HPLC could successfully be used to determine these four analytes in a single analysis (Leggett, in prep.).

Analytical methods for TNT, RDX, HMX and DNT in water are generally modifications of procedures developed for analysis of the explosives themselves. Yinon and Zitrin (1981) give an extensive review of these methods as they apply to the analysis of intact explosives and post explosion residues.

The approaches that have received attention for trace analysis of these substances in water are:

1. Direct colorimetric analysis
2. Thin-layer chromatography
3. Gas-liquid chromatography with a variety of detectors
4. High-performance liquid chromatography, normal and reverse-phase.

Colorimetric analysis

The production of characteristic colored products from alkaline hydrolysis of nitroaromatics has been known since the 19th century (Yinon and Zitrin 1981). Application of this concept for the analysis of trace levels of TNT in munitions wastewater was reported by Mudri (1968). In this method a sample of wastewater is diluted with an aqueous sodium sulfite-sodium hydroxide solution. Absorbance measurements at 500 nm are used to detect the extent of color development, which was found to be linearly related to TNT concentrations from less than 1 to 20 ppm. Recovery studies indicated that the procedure was accurate to ± 10% but possible interferences from other nitroaromatics or nitramines were not studied.

Jurinski et al. (1975) reported an automated colorimetric procedure for TNT analysis in wastewaters. In this method, the sample was diluted with 15% KOH and the transmittance at 440 nm measured. They found that the method obeyed Beer's law and was applicable in the 1-80 ppm range. No interference was found for mono- or dinitrotoluenes or RDX; however, other isomers of TNT, TNB (1,3,5-trinitrobenzene [see Appendix B]) and Tetryl (methyl-2,4,6-trinitrophenylnitramine [see Appendix B]) gave positive responses.

A modification of the colorimetric approach for TNT determination has been reported by Heller et al. (1977). A colored reaction product is produced by alkaline hydrolysis and immobilized on a quaternary ammonium ion-exchange resin that had been saturated with a fluorescent dye. The reduction in fluorescence when the immobilized resin is excited with UV radiation is proportional to concentration of TNT. Although it is not suited for precise laboratory determination of TNT, this method offers an approach to detecting breakthrough of TNT from activated carbon treatment columns. This concept has been extended to the development of portable detection tubes (Heller et al. 1982), which detect TNT at concentrations as low as 100 μg/L in fresh water. But these tubes are not suitable for RDX, HMX or DNT. None of the reported colorimetric procedures are capable of simultaneous measurement of these four analytes.

Thin-layer chromatography

Thin-layer chromatography (TLC) has been evaluated for use in determining some of these analytes in water and sediment. Hoffsommer et al. (1972) describe a method in which TNT and RDX are extracted from sediment with benzene. The solvent is removed by evaporation and the residue is dissolved in a small volume of benzene; this solution is spotted on a TLC plate and developed with a hexane-acetone solution. TNT and RDX appear as dark spots under 254-nm UV light. No data on detection limits, precision or accuracy are presented.

Glover and Hoffsommer (1973) report on the use of TLC to determine HMX and RDX in munitions wastewater. The water solution is extracted with benzene, the extract evaporated to dryness, taken up in acetone and spotted on a silica gel plate. The plates are developed with benzene-acetone and HMX and RDX are separated and detected as dark spots under a 254-nm UV light. Detection limits of 20 μg/L are estimated for HMX using this procedure, with analytical accuracy of about ± 10% in the 0.1- to 1.0-mg/L range.

Epstein et al. (1977) have used TLC to qualitatively characterize TNT wastewaters from several AAPs. TLC was very powerful in separating the many individual components, particularly in wastewater from the manufacture of TNT. Quantitation, however, was accomplished by other means. While solvent extraction followed by TLC analysis appears to be sufficiently sensitive, the semi-quantitative nature of TLC reduces its utility when precise and accurate quantitative analysis is needed.

A method for the determination of TNT in water by conversion to nitrate has been reported by Leggett (1977). Recovery was near 100% for TNT, but potential interference from other nitroaromatics or nitramines was not determined. Therefore, this method does not lend itself to determination of individual components. Since the various substances have different levels of toxicity and thus have different discharge limits, a total analysis is not sufficient.

A method for TNT analysis in water by differential pulse polarography has also been reported (Conley and Mikucki 1976). The potential was swept cathodically, reducing the three nitro groups sequentially at -0.28, -0.45 and -0.61 V versus the standard calomel electrode. Concentrations below 100 ppb can be analyzed directly. No information was presented, however, on whether the presence of RDX, HMX or DNT in the water would interfere with TNT analysis.

Gas-liquid chromatography

A number of researchers have reported gas chromatographic procedures for DNT, TNT and RDX. A method by Goerlitz and Law (1975) has been listed as the method of choice for TNT and RDX in water by the *National Handbook of Recommended Methods for Water-Data Acquisition* (U.S. Dept. of Interior 1977). This method involves four sequential extractions of water with benzene, combination of the extracts, volume reduction to 0.5 mL with a Kuderna-Danish evaporator, column chromatographic clean-up, and analysis of the column eluate using Gas Chromatography with an OV-17 column and an Electron Capture Detector (GC-ECD). The detection limits for this procedure are well below 1 μg/L for both TNT and RDX with recovery of 95 ±15% for TNT and 85 ±10% for RDX.

A similar method for DNT has been adopted by the EPA (Federal Register 1979). This procedure includes three sequential extractions with methylene chloride, solvent exchange with toluene, evaporative concentration, cleanup by column chromatography and analysis by GC-ECD using an OV-17 column. A detection limit of 0.06 μg/L for 2,4-DNT was reported with baseline separation between 2,4- and 2,6-DNT. Experience in our own laboratory and in others has indicated that the DNTs can be separated easily from TNT using GC (Murrmann et al. 1971).

Determination of HMX by GC has also been accomplished using a fused silica capillary column coated with OV-101. This is in contrast to earlier reports that RDX could be determined in an HMX matrix with no elution of an HMX peak (Rowe 1967). Personal discussions with a number of experienced analysts indicate that measurement of HMX by GC methods was difficult because of significant and nonreproducible decomposition at the temperatures required to volatilize the compound. Douse (1981) reported that the peak shape and response for HMX was improved using temperature programming. The lower analytical precision (10%), compared to other analytes, indicates that HMX was probably thermally degrading even in this work. Similar problems were encountered with RDX, which would apparently chromatograph acceptably for long periods and then, for no explainable reason, start erratically decomposing during analysis.

A number of other papers have also presented GC-ECD methods for analysis of DNT, TNT and its microbial metabolites, the aminodinitrotoluenes and diaminonitrotoluenes (Hoffsommer and Rosen 1972, Glover et al. 1977, Hashimoto et al. 1980). These methods differ primarily in the choice of extraction solvent and the specific column used for analysis. Jurinski et al. (1975) and Spanggord et al. (1982) present similar methods using an FID detector rather than ECD.

Krull et al. (1983) address the problem of potential interferences in measurement of various dinitrotoluenes by using ECD and photoionization detectors, and in documenting response ratios. This technique is very valuable for trace analysis of groundwater or surface waters but is probably unnecessary in analysis of the wastewater matrix, particularly following carbon column cleanup. GC/MS methods for unequivocal identification of DNT and TNT and their metabolites have also been reported (Pereira et al. 1979, Weinberg and Hsu 1983).

High-performance liquid chromatography

The use of Reverse-Phase, High-Performance Liquid Chromatography (RP-HPLC) for analysis of TNT wastewaters was first reported by Walsh et al. (1973). Walsh was able to separate TNT from 2,4-DNT using a C-18 column with 10:90 V/V acetonitrile/water under isocratic conditions. Direct injection of 10 μL of wastewater permitted concentration estimates in the low micrograms-per-litre range. No figures of merit with respect to precision, percent recovery or detection limits were provided.

Doali and Juhasz (1974) reported on the use of normal phase HPLC for the analysis of several explosive formulations. Conditions were provided for the separation of TNT and DNT and also for RDX and HMX, but not for all four in one matrix. Because this paper describes methods suitable for analysis of solid explosive, rather than trace levels in water, no information on detection limits was provided. Since normal phase HPLC typically uses non-polar elution solvents, an extraction step would be required, unlike reverse phase where aqueous solutions can be injected directly.

Stanford (1977) reported a RP-HPLC method that separated 2,4,6-TNT from the various isomers of DNT, and TNT from RDX in water. This method uses a C-18 column and various elution solvents of ethanol/water, acetonitrile/water and methanol/acetonitrile/water. For injections of 100 μL, detection limits of 50–250 μg/L were reported using a UV detector at 230 nm. Stanford observed a reversal in elution order for TNT and DNT between methanol/water and acetonitrile/water which he attributed to a specific interaction between acetonitrile and nitroaromatics.

Stidham (1979) described a RP-HPLC method for the determination of nitramines and TNT from a RDX-HMX manufacturing operation. Using a gradient elution technique and a ternary solvent mixture of methanol/acetonitrile/water, he achieved detection limits of less than 65 μg/L with direct injection of 700 μL of aqueous sample. A C-8 column was used with UV detection at 230 nm. This method achieves good separation for HMX, RDX and TNT as well as SEX (octahydro-1-(N)-acetyl-3,5,7-trinitro-1,3,5,7-tetrazine [see Appendix B]) and TAX (hexahydro-1-(N)-acetyl-3,5-dinitro-1,3,5-triazine [see Appendix B]), two major impurities in RDX-HMX manufacture. Stidham measured UV spectra of TNT, RDX, HMX, TAX and SEX to choose the best wavelength for detection, which generally was in the 240- to 245-nm region for these five compounds. Detailed assessments of analytical precision were presented at concentration ranges from 50 to 10 mg/L. Precision was generally better than 10%. Recovery of spiked samples indicated that the inaccuracy was generally better than ±10%. Stidham reported that direct injection with RP-HPLC gave superior performance with respect to accuracy and precision compared to methods that required sample extraction or preconcentration by "...avoiding tedious analytical steps and minimizing potential degradation or sample loss."

Bratin et al. (1981) compared the limits of detection obtainable using UV detection at 254 nm versus electrochemical detection with RP-HPLC for HMX, RDX, TNT and DNT. A gold-mercury amperometric detector improved detection limits by factors of 3.5–5.1 at equivalent signal-to-noise ratios. If very low detection limits are required this detector shows great promise; however, it is not currently in common use.

Hoffsommer et al. (1981) compared UV detection at 200 nm versus 254 nm for TNT, RDX, HMX and DNT and found an improvement in detection limits of only about a factor of two. Detection limits of about 200 g/L were found for injection volumes of 30 μL. Lakings et al. (1981) reported detection limits of 89 μg/L for RDX and 50 μg/L for TNT and DNT for a similar RP-HPLC method using UV detection at 254 nm and 100-μL injection volumes.

An innovative use of electron capture detection with HPLC was reported by Krull et al. (1981). Detection limits are expected to be very low because of the extreme sensitivity of the ECD for nitro-containing aromatics (e.g. TNT, DNT); but because it requires the analyte to be volatilized, it suffers the same problems as GC analysis, i.e., the very low vapor pressures and thermal instability of HMX and RDX. Use of ECD with HPLC is still in the research stage and is not currently in common use.

West* has also reported a RP-HPLC method for RDX, TNT and DNT in munitions wastewaters: 500 μL of filtered wastewater is injected into an ODS column eluted with 30/70 (V/V) methanol/water, and the column effluent is analyzed by UV at 254 nm. West obtained detection limits of 3 μg/L for RDX, 5 μg/L for TNT and 7 μg/L for DNT. Subsequent discussions with him indicated that for routine analysis, however, injection volumes should probably be reduced somewhat.

In some subsequent studies within our own laboratory, RP-HPLC has been successfully used to determine TNT, RDX, HMX and DNT in leachate from PVC pipes (Parker et al., in prep.), in soil and sediment extracts (Cragin et al., in prep.), in plant tissue digests† and in sorption isotherm experiments with bentonite drilling muds (Leggett and Foley, in prep.)

Assessment of alternatives

Of the alternative methods, clearly the two best suited for compliance monitoring are GC-ECD

* Personal communication with Dr. J. West, Louisiana AAP, 1982.

† Personal communication with D. Leggett and B. Foley, CRREL, 1984.

and RP-HPLC. GC-ECD is particularly attractive because of its sensitivity and selectivity for nitroaromatics and nitramines. Detection limits of better than 1 µg/L for all four analytes are achievable using this approach, which will certainly meet all current and projected needs. GC-ECD instrumentation is currently available in most Army and GOCO (government-owned, contractor-operated) installations so little capital cost would be required to implement this method.

GC methods, however, require extraction from the water matrix into a nonpolar organic solvent. Partition coefficients between nonpolar solvents and water for these substances are not very favorable, particularly for RDX and HMX (Leggett and Foley, in prep.), and hence, a number of sequential extractions would be required to approach complete recovery. Following extraction, the solvent must be concentrated by evaporation prior to analysis. This entire procedure is very time consuming, resulting in a turn-around time of at least several hours between delivery of sample to the laboratory and availability of the data. This is clearly not desirable if the values are not within compliance limits and discharge has continued while the analyses are underway. In addition the large number of exacting steps will reduce analytical precision unless very highly trained technicians are available, a situation only rarely true for compliance monitoring activities. GC analysis also requires that the analytes be thermally stable within the injector and analytical column. This is a problem for HMX, which has a very low vapor pressure and is thermally labile, and to a lesser extent for RDX and TNT, which have shown unpredictable thermal instability problems.

RP-HPLC is attractive because aqueous solutions can be analyzed directly, without the necessity of solvent extraction. Good detection limits can be obtained without sample preconcentration, because, relative to GC, large volumes can be injected. The ability to analyze aqueous solutions directly allows a turn-around time of 30 minutes or less, which is very desirable for discharge monitoring. HPLC instrumentation is currently available at most AAPs.

Several detection concepts have been reported for these analytes in RP-HPLC. Electro-chemical detectors and ECDs are both very sensitive, but are not readily available or routinely used. Detection by UV is somewhat less sensitive for these analytes, but is available on most HPLC equipment. The most common UV detector is a single wavelength 254-nm detector, although variable wavelength systems are becoming more common. For reliability, however, the 254-nm UV detector is excellent. All four analytes absorb strongly at 254 nm, although their absorptivity is somewhat higher at slightly shorter wavelengths. Most HPLC systems at GOCO and Army installations are equipped with fixed wavelength 254-nm detectors, while only a few are equipped with variable wavelength systems. Detection limits of less than 100 µg/L have been reported by several investigators. These values are one-tenth the current discharge limits for TNT and less than one one-hundreth that currently set for RDX.

Several RP columns have been used to provide adequate separation for these four analytes, including C-8, C-18 and CN. The C-8 column can also separate TAX and SEX, the most significant impurities in HMX-RDX manufacture. Generally, the eluents for the analysis of these four compounds have been methanol/water, acetonitrile/water or a ternary system of methanol/acetonitrile/water. All seem to provide adequate separation, although the elution order of TNT and DNT is reversed in changing from water/methanol to water/acetonitrile. In addition, isocratic acetonitrile/water co-elutes HMX and RDX.

Most HPLC systems currently in use in the Army are not equipped to perform gradient elution. Isocratic conditions have most commonly been used in the past and adequate performance has been achieved. Therefore, it seems desirable to use an isocratic method, if possible, to minimize the necessity for capital expediture for new equipment for compliance monitoring. Isocratic analyses are also faster if a number of samples are to be analyzed.

In summary, we proposed RP-HPLC as the method most desirable for compliance monitoring for these four analytes in munitions wastewater. For initial testing we chose a C-8 column with a methanol/water eluent under isocratic conditions with UV detection at 254 nm.

EXPERIMENTAL

Instrumentation

All HPLC measurements at CRREL during method development were conducted on two instrumental set-ups. The first is a Perkin Elmer Series 3/LC-65T equipped with a variable wavelength UV detector set at 254 nm and a Rheodyne 7125 sample loop injector. The second utilizes the Perkin Elmer Series 3 pump with a Rheodyne 7125 loop injector and a Spectra-Physics SP8300 fixed 254-nm UV detector. Depending on the experi-

Table 2. Instrumentation used by various collaborative test participants.

Participant	*System description*
AEHA	Waters Model 6000A pump Waters U6K Universal Injector (100-μL loop) Waters Model 440 absorbance, fixed wavelength 254-nm detector Integrator—HP 3390A
USEPA, EMSL	Waters Model 6000A pump Waters Model M710 WISP autosampler Waters Model 440 UV-254-nm detector Waters Model 721 microprocessor
Univ. of New Hampshire	Waters Model 6000 pump Waters Model U6K injector Waters Model 1205 UV-254-nm detector Integrator—HP 3390A
CRREL	Perkin Elmer Series 3 pump Rheodyne 7125 sample loop injector Spectra-Physics Model SP8300 UV-254-nm detector Integrator—HP 3390A
Louisiana AAP	Perkin Elmer Series 3 pump Perkin Elmer ISS-100 autosampler, sample loop 150 μL Perkin Elmer UV-VIS variable wavelength detector set at 254 nm Perkin Elmer Sigma 15 Chromatography data station
Iowa AAP	Waters Model ALC-204 pump Waters Model M710B WISP Autosampler Waters Model 440, UV-254-nm detector Manual peak height determination
Holston AAP	Spectra Physics Model 8700 pump Valco injection valve Perkin Elmer LC75, variable wavelength UV set at 254 nm Spectra Physics SP4000 Integrator
Radford AAP	Dupont 870 pump Rheodyne Model 7120 injection valve LCD UV-3, UV-254-nm detector Spectra Physics SP4000 Integrator
LCWSL	Spectra Physics 8100 pump Valco injection valve Perkin Elmer Model 250, fixed wavelength 254-nm detector Spectra Physics Model 4100 integrator

ment, peak heights were measured manually or peak areas were obtained using HP3390A Integrators. In all cases, 100 μL of sample was injected via a 100-μL sample loop. A collaborative test was conducted following method development to assess overall performance of the method. The instruments used by the participants are summarized in Table 2.

All analyses for both method development and the collaborative test were conducted on Supelco 25 cm by 4.6 mm LC-8 columns (5 μ). The number of theoretical plates for these columns averaged about 5000. A 2-cm precolumn of LC-8 was frequently used.

Chemicals

All analytical standards for TNT, RDX, HMX, 2,4-DNT, 2,6-DNT, Tetryl and TNB were prepared from Standard Analytical Reference Materials (SARM) obtained from the Armament Research and Development Center, Large Caliber Weapon Systems Laboratory (LCWSL), Energetic Materials Division. SARM quality material from the same batch was supplied to each collaborative test participant for each of the four analytes determined. Standards were dried to constant weight in a vacuum desiccator over dry calcium chloride in the dark.

Standards for the aminodinitrotoluenes, the diaminonitrotoluenes, SEX and TAX, used for retention time confirmation, were obtained from Dr. David Kaplan at the U.S. Army Natick Laboratories and used without further purification.

Methanol, acetonitrile and water used to prepare the mobile phases for various experiments were Baker HPLC grade solvents. They were combined in the proper proportions and vacuum filtered through a solvent-washed 0.4-μm Nuclepore filter to remove particulate matter and to degas the solvent. Fresh mobile phase was prepared daily.

RESULTS AND DISCUSSION

Retention times of major analytes and common impurities

In addition to separating the analytes of interest from one another, the RP-HPLC method must be able to distinguish these analytes from other common components of munitions waste matrices, including impurities in the explosive formulations and decomposition products. It is impossible to test all the wastewaters from the many manufacturing operations over the range of conditions expected. However, several studies have documented the major impurities in these types of wastes. A study conducted by Stidham (1979), for example, identified the types of impurities common to RDX-HMX manufacturing and processing. Stidham found that in addition to RDX and HMX, TAX and SEX were present at concentrations as high as 5.2 and 2.0 mg/L, respectively. No other nitramines were detected. Since wastewater from this process is ultimately disposed of in surface waters, compliance monitoring of this type of wastewater will be required.

Four other compounds, unrelated to nitramines, were also detected by Stidham in cyclohexanone wastes. These substances contain only carbonyl- and hydroxyl-functionality and would therefore have very low UV absorptivity. Consequently, they would only be detectable at very high concentrations, well above expected levels. Cyclohexanone has a very low UV absorptivity, but because it is used as a recrystallizing solvent for purification of RDX, it could be present in rather large concentration. Thus, it is important for the method to be capable of separating cyclohexanone from the four analytes of interest.

For wastewater from load and pack operations, the waste is primarily generated from washdown of equipment used to melt solid explosives and pour them into shell casings. Thus, any explosive that becomes associated with this waste stream is completely dissolved. The explosive itself and its major impurities and their decomposition products will become important components of the wastewater.

The major impurities in production grade TNT have been identified by a number of investigators as TNB, DNTs and several of the unsymmetrical isomers of TNT. Of the DNTs, the 2,4-isomer is present in the greatest concentration, ranging from 0.06% (Leggett et al. 1977) to 0.72% (Gehring and Shirk 1967). The sum of the other isomers is, at most, present at only about one-third of the concentration of the 2,4-isomer (Leggett et al. 1977).

Munitions wastewaters are generally held in collection tanks prior to carbon treatment and ultimate disposal. During the holding period, these wastes are subject to microbial transformation. TNT degrades metabolically under both aerobic and anaerobic conditions (McCormick et al. 1976) by a stepwise reduction of the nitro groups initially forming 4-amino-2,6-dinitrotoluene (4-Am-DNT) and 2-amino-4,6-dinitrotoluene (2-Am-DNT) (see Appendix B). These substances have been detected along with TNT in contaminated groundwater at the Hawthorne Naval Ammunition Depot, Nevada (Pereira et al. 1979). Further reduction of these components results in 2,4-diamino-6-nitrotoluene (2,4 DAm-NT) and 2,6-diamino-4-nitrotoluene (2,6 DAm-NT) (see Appendix B), which are apparently stable to further reduction (Kaplan and Kaplan 1982). Thus, the periodic presence of these amino-containing decomposition products is expected in wastewater from load and pack operations. Any analytical method used for these wastewaters must be able to separate these substances from the major components and also allow quantitation if sufficient concentrations are present.

Spanggord et al. (1982), using capillary GC-MS, have recently reported the identification of 32 different substances in the condensate wastewater from manufacture of TNT using capillary GC-MS. These include mono-, di-, and tri-nitrotoluenes and mono-, di- and tri-nitrobenzenes, several nitro-containing phenols, two nitro-containing benzonitriles, toluene, several aminonitrotoluenes and a couple of more exotic substances. This type of wastewater amounts to a very small portion of the munitions related wastewater and is far too complex for analysis by RP-HPLC using standard columns. No attempt was made to study the elution behavior of these substances.

Experiments were conducted to determine the retention times of major impurities and decompo-

Table 3. Retention times of primary analytes, impurities and decomposition products in two eluents (flow rate of 1.5 mL/minute at 25°C).

Substance	Retention times (min) Eluent A*	Eluent B†
HMX	2.69	3.15
RDX	3.94	4.15
TNT	7.15	7.45
2-4-DNT	8.67	8.24
SEX	2.43	2.58
TAX	2.86	2.84
TNB	4.61	4.86
Tetryl	6.49	7.18
2-Am-DNT	8.44	7.86
4-Am-DNT	8.63	8.03
2,4-DAm-NT	2.73	2.78
2,6-DAm-NT	2.57	2.63
2,6-DNT	8.97	8.41
2,4,5-TNT	7.50	8.11
Cyclohexanone	3.93	3.60
Diethylphthalate	15.26	12.61

* Eluent A—50% water, 50% methanol.
† Eluent B—50% water, 38% methanol, 12% acetonitrile.

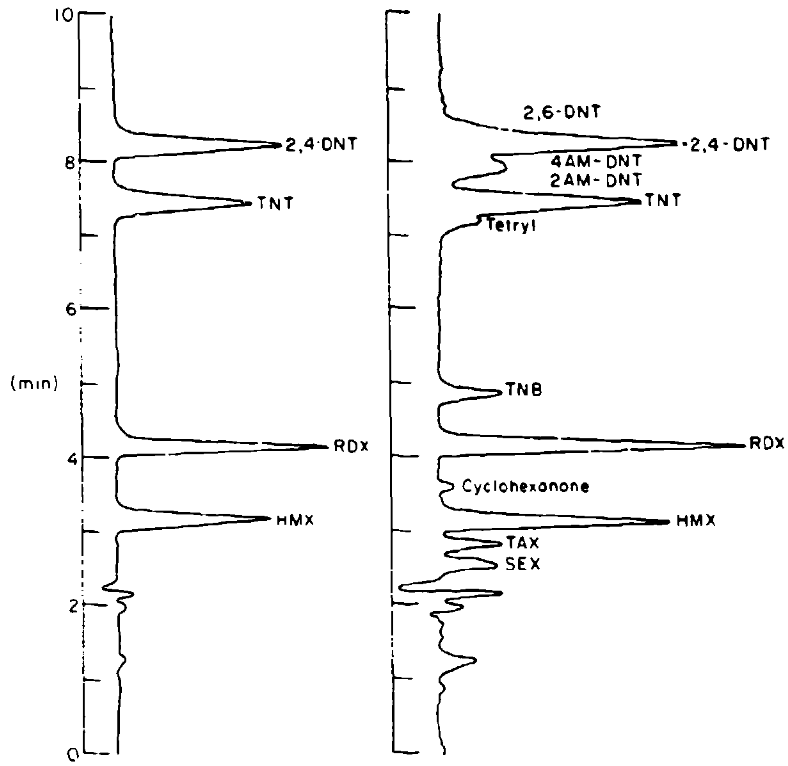


Figure 1. Chromatogram of HMX, RDX, TNT and 2,4-DNT with and without major contaminants using eluent B.

sition products using the LC-8 column with two different eluents: A—50% methanol and 50% water (V/V) and B—38% methanol, 12% acetonitrile and 50% water (V/V/V). The retention times for these substances and the four primary analytes are presented in Table 3.

The elution order for the two eluents is quite similar for the four primary analytes and the group of possible interfering substances. Eluent A separates TNT from 2,4-DNT by about 1.5 minutes compared to 0.8 minutes for eluent B. Eluent A also separates TNT from tetryl more completely, in about 0.7 minutes compared to 0.27 minutes for eluent B. However, eluent A does not separate HMX from SEX or TAX, both significant contaminants in HMX-RDX wastes. In addition, cyclohexanone co-elutes with RDX. Eluent B separates RDX and cyclohexanone by about 0.6 minutes and separates HMX from SEX and TAX by 0.6 and 0.3 minutes, respectively (Fig. 1). In addition, some recent experiments by Gleichauf* indicate that components of natural humic material interfere with HMX when eluent A is used, but are sufficiently separated for quantitation when eluent B is used.

Unfortunately, neither eluent separates 2,4-DNT efficiently from the aminodinitrotoluenes or 2,6-DNT. Of the four primary analytes, however, 2,4-DNT is certainly the least important. It is significant only as an impurity in TNT, where it is present at concentrations less than 0.5% of that for TNT.

An eluent of acetonitrile/water was not studied because it is known that HMX and RDX co-elute using this eluent.

Clearly for HMX, RDX and TNT determination, B (38% methanol, 12% acetonitrile and 50% water) is the eluent of choice. Its only drawback is the poor separation of TNT and tetryl; however, tetryl is rarely used at present and its occurrence is only likely in the analysis of wastewater in old disposal lagoons. Since these are being phased out, tetryl should not pose an analytical problem for current or future analyses.

*Personal communication with G. Gleichauf, University of New Hampshire, 1984.

Linearity tests

To test response linearity, standard solutions of HMX, RDX, TNT and DNT were prepared at eight concentrations ranging from 11-5580, 12-6200, 9-4300 and 6-3200 µg/L, respectively. These solutions were diluted with an equal volume of methanol/acetonitrile solution (76/24% V/V) and 100 µL was injected into an LC-8 column and eluted with 50/38/12% water/methanol/acetonitrile at 1.5 mL/min. Quantitation was achieved using a fixed wavelength 254-nm detector coupled to both a strip chart recorder and an integrator (Table A1). The retention times were as follows: HMX—3.16 minutes, RDX—4.18 minutes, TNT—7.53 minutes and DNT—8.36 minutes.

Random error variances of the three replicates at each concentration were obtained (Table A1) and tested for homogeneity using Bartlett's test. For HMX, a χ^2 value of 2.04 was calculated, less than a critical value of 12.59 for $\alpha = 0.05$ with six degrees of freedom. Thus for HMX, the variances are considered homogeneous over the entire range tested. A similar result was obtained for TNT. The χ^2 value was calculated to be 10.43, again less than a critical value of 12.59 and the variances are considered homogeneous for TNT as well.

For DNT, the χ^2 value for the entire range was 15.17, indicating nonhomogeneous variance. Looking at individual variances, however, indicated that the variance for the highest concentration sample was greater than the sum of the variances for the other six concentrations. When Bartlett's test was repeated for the lowest six concentrations, the χ^2 value dropped to 5.04 and the variances were considered homogeneous within the reduced range.

Use of Bartlett's test for RDX also indicated that the variances were nonhomogeneous. Inspection of individual variance values (Table A1) indicates that this is attributable to two abnormally low variances at intermediate concentrations. Dropping the highest concentration data as was done for DNT will not improve this situation. Since the other five variances appear to be quite similar, we assumed the variances to be sufficiently homogeneous for regression analysis.

Within the regions of homogeneity, peak areas were individually regressed against known concentrations to obtain the best fit linear calibration curves (Table 4). Regression analysis tables were obtained to test whether the linear model adequately describes the data, or more simply, whether the responses were linear with concentration

Table 4. Regression analysis for linearity tests.

Variable	*Sum of squares*	*Degrees of freedom*	*Mean squares*	F*
HMX (area) b_0 = 2631.8, b_1 = 337.53				
Total	0.12934E+14	21		
b_0†	0.46238E+13	1		
b_1**	0.83112E+13	1		
L of F††	0.57030E+08	5	1.1406E+7	2.36
Error	0.67761E+08	14	4.8401E+6	
HMX (peak height) b_0 = 3.987E−04, b_1 = 4.995E−06				
Total	0.29517E−02	21		
b_0	0.11202E−02	1		
b_1	0.18204E−02	1		
L of F	0.25666E−05	5	5.1332E−07	0.84
Error	0.85984E−05	14	6.1417E−07	
RDX (area) b_0 = 6807.0, b_1 = 459.62				
Total	0.29694E+14	21		
b_0	0.10667E+14	1		
b_1	0.19026E+14	1		
L of F	0.21270E+09	5	4.2540E+7	0.10
Error	0.59884E+09	24	4.2774E+8	
RDX (peak height) b_0 = 3.822E−04, b_1 = 6.789E−06				
Total	0.66013E−02	21		
b_0	0.24471E−02	1		
b_1	0.41398E−02	1		
L of F	0.32312E−05	5	6.4624E−07	0.81
Error	0.11160E−04	14	7.9714E−07	
TNT (area) b_0 = 3580.8, b_1 = 959.64				
Total	0.61991E+14	21		
b_0	0.22096E+14	1		
b_1	0.39895E+14	1		
L of F	0.14631E+09	5	2.9262E+7	1.18
Error	0.34575E+09	14	2.4696E+7	
TNT (peak height) b_0 = 2.285E−04, b_1 = 1.060E−05				
Total	0.76724E−02	21		
b_0	0.27888E−02	1		
b_1	0.48715E−02	1		
L of F	0.25564E−05	5	5.113E−07	0.75
Error	0.95943E−05	14	6.853E−07	
DNT (area) b_0 = 2835.5, b_1 = 1266.7				
Total	0.59790E+14	21		
b_0	0.21293E+14	1		
b_1	0.38497E+14	1		
L of F	0.24198E+09	5	4.8396E+7	0.78
Error	0.52928E+09	14	3.7806E+7	
DNT (peak height) b_0 = 1.836E−04, b_1 = 1.358E−05				
Total	0.69466E−02	21		
b_0	0.25152E−02	1		
b_1	0.44210E−02	1		
L of F	0.22113E−05	5	4.423E−07	0.76
Error	0.81805E−05	14	5.843E−07	

* Variance ratio, critical value is 2.99 for 5 and 14 degrees of freedom.

† Intercept.

** Slope.

†† Lack of fit.

over the concentration ranges examined (Table 4). In all cases F values comparing lack of fit (L of F) to random error were much lower than 2.99, the critical value for 5 and 14 degrees of freedom at a 95% confidence level. Since L of F is not significant, the responses are adequately described by linear models over the ranges tested.

Variances for manually measured peak height data were not homogeneous over the concentration range tested because of, in part, quantitation error. Quantitation error results from the limited number of significant figures obtainable in manual peak height estimation. Even so, the regression lines obtained for peak heights can be used to describe the sensitivity of this method. For HMX, RDX, TNT and DNT, the sensitivities were 5.0×10^{-3}, 6.8×10^{-3}, 1.1×10^{-2} and 1.4×10^{-2} absorbance units/ppm, respectively, for 100-μL injection volumes. The noise level, peak to peak, was about 4.1×10^{-5} absorbance units. Using a signal-to-noise ratio criterion of 3 to 1, we estimated detection limits of 25, 18, 11 and 9 μg/L for HMX, RDX, TNT and 2,4-DNT respectively.

Linearity tests were also conducted using a variable wavelength detector set at 254 nm. Noise levels on the variable wavelength system were much higher. This was reflected in poorer precision and higher detection limits. Thus, where a choice is available, use of a fixed wavelength detector will probably result in better performance, particularly at low concentration.

Filtration tests

Typical aqueous environmental samples will contain particulates in amounts that are unacceptable for direct injection into an HPLC column. For this reason, we decided that a filtration step was necessary to protect expensive HPLC columns.

An experiment was conducted to assess which types of filters could be used to remove particulates without adversely affecting the ability to analyze for trace levels of the four analytes by RP-HPLC. The following types of filter materials were tested with pink water and lagoon water from Louisiana AAP: glass fibre, polyvinyl chloride, polycarbonate and cellulose acetate-nitrate. The results of this study are presented in detail elsewhere (Leggett, in prep.). In general, use of cellulose-ester membranes was not recommended because of loss of the analytes, presumably by sorption on the membrane surface. The extent of loss was inversely related to the rate of filtration. No losses were encountered using polycarbonate filter materials nor with plastic syringes or polycarbonate filter holders, nor was anything leached at concentrations that interferred with the HPLC analysis. Subsequent work has indicated that disposable PTFE filter membranes are also acceptable for this application.

Another concern was whether absorption of analytes on natural particulates could bind significant amounts, with losses as the particulates were removed by filtration. It has been argued on the basis of octanol-water partition coefficients (see Table 6) that significant loss by this mechanism was unlikely, but it was not possible to be certain without experimental evidence (Leggett, in prep.). In addition, because of the slow rate of dissolution in water, small particles of the solid explosives could be removed by filtration. Since it was our goal to develop a method that would determine the total amount of material in the discharge, both in solution and associated with the particulate phase, we decided to dilute the aqueous samples one to one with methanol or methanol-acetonitrile prior to filtration. We feel that this procedure is desirable because 1) it further lessens the possible loss of analyte by adsorption on filters, particularly if filter media other than polycarbonate are used; 2) it would enhance desorption from natural particulates prior to their removal by filtration; and 3) it would increase the rate of dissolution of small particles of solid explosive.

To test the adequacy of this procedure, an experiment was conducted to see whether measurable amounts of the four analytes would be sorbed by particulates in various types of waters. We used five different types of water in the study: 1) Connecticut River water collected at Hanover, New Hampshire; 2) Hanover, New Hampshire, tapwater; 3) groundwater from a deep well in Canaan, New Hampshire; 4) water from a stagnant pond in Lebanon, New Hampshire; and 5) Milli-Q water. Total suspended solids (TSS), pH and total organic carbon (TOC) were determined on aliquots of each water (Table 5). Three replicate samples of

Table 5. Total suspended solids, pH and total organic carbon in waters used for recovery study.

Sample	*TSS (mg/L)*	*pH*	*TOC (mg/L)*
Milli-Q	< 0.1	4.4	< 0.1
Groundwater	0.2	7.1	0.7
Tapwater	1.1	5.8	3.2
Connecticut River	1.7	7.8	4.3
Pond water	4.2	7.9	10.2

Table 6. Physical constants for TNT, RDX, HMX and DNT.

Substance	*Melting point (°C)*		*Solubility (mg/L at 24°C)*		K_{ow}*		*Vapor pressure at 20°C* *torr*		*Pascal*
TNT	80.1	a	136	d	45.0	d	1.3×10^{-6}	e	1.7×10^{-4}
RDX	203.5	b	43	d	7.55	d	1.0×10^{-9}	f	1.3×10^{-7}
HMX	280.0	b	5.0	-	1.38	d	—		—
DNT	70.0	h	180	d	75.2	d	2×10^{-4}	g	3×10^{-2}

*Octanol-water partition coefficient.
a—Jenkins et al. (1973).
b—Stidham (1979).
c—Glover and Hoffsommer (1973).
d—Leggett (unpubl.)
e—Leggett (1977).
f—Coates et al. (1970).
g—Leggett et al. (1977).
h—Dean (1979).

500 mL of each type of water were autoclaved for 1 hour at 121 °C, cooled to room temperature and spiked with stock solutions of HMX, RDX, TNT and DNT in methanol.

Each sample of spiked water was stirred for 1 hour and allowed to stand overnight in the dark. A 10-mL subsample was removed with a volumetric pipet and placed in a 20-mL scintillation vial, 10 mL of methanol was added, and the samples were shaken and allowed to stand at least 15 minutes. Each sample was then filtered through a 0.4-μm Nuclepore polycarbonate membrane into a clean scintillation vial. Processed samples were analyzed in duplicate by injection of 100 μl into a LC-8 HPLC column followed by elution with 1.5 mL/minute of 50% water, 38% methanol and 12% acetonitrile. The Milli-Q water was also analyzed in duplicate without filtration. The results of these analyses are presented in Table A2.

An analysis of variance test of these data was done, considering the duplicate analyses of three replicate samples as six total replicates since the variance for analytical replicates was about the same magnitude as that for replicate samples. The results indicated that there was no significant difference in analyte concentrations (at the 95% confidence level) between any of the five types of filtered water or the unfiltered Milli-Q water for TNT, RDX or DNT. This indicates that for these analytes, the addition of methanol prior to filtration eliminates any sorption on particulates or filter membranes. For HMX, however, a significant difference at the 95% confidence level was found, with an F ratio of 5.92 compared to a table value of 2.53. A Duncan's Multiple Range test revealed that the Connecticut River water and the pond water were the two samples that differed significantly from the unfiltered Milli-Q water. These were also the two samples that had the highest total suspended solids and total organic carbon (Table 5). The mean values of the river and pond for HMX were 60.8 and 58.4 μg/L, respectively, compared to a mean value of 63.2 μg/L, for the unfiltered Milli-Q water. It seems likely that adsorption of HMX on particulates and removal by filtration is not completely eliminated by dilution with methanol or that some irreversible chemisorption has occurred. Octanol-water partition coefficients have been used to simulate the nonspecific partitioning of hydrophobic organics between water and soil or sediments (Karickhoff et al. 1979). Since HMX has an octanol-water partition coefficient of 1.38, lower than those for the other three analytes (Table 6), if physical adsorption is responsible for loss, it must be due to some specific adsorption sites that are active for it because of its particular size or shape. Even so, only about 7.5% and 3.8% of the HMX was lost for the pond water and river water, compared to the unfiltered Milli-Q water. Thus dilution of the sample with an equal volume of methanol seems desirable to minimize sorptive losses on filters and particulates.

In some recent experiments, Gleichauf* compared the recovery of TNT spiked into solutions containing soluble and particulate humic acid at concentrations up to 20 mg/L. When these solutions were equilibrated with an equal volume of

* Personal communication with G. Gleichauf, University of New Hampshire, 1984.

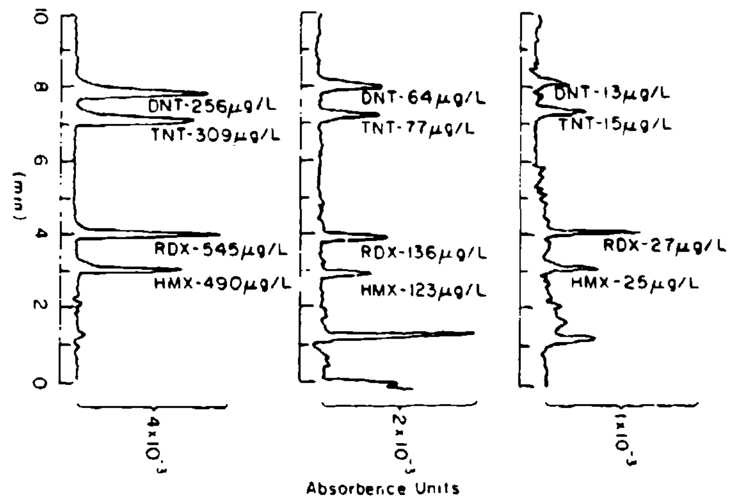


Figure 2. Examples of chromatograms for HMX, RDX, TNT and 2,4-DNT at several analyte concentrations.

methanol prior to filtration, complete recovery of TNT was observed using this HPLC method with equilibration times as short as 15 minutes. If the solutions were filtered without the addition of the organic modifier, only about 80% of the spiked TNT was recovered. Thus, addition of the organic modifier can reduce analyte loss during filtration, presumably by removal of TNT sorbed on the surface of particulate humic acid prior to removal of the particulate on the filter.

Detection limit determination

A study was conducted to establish the detection limits of this RP-HPLC method for the four analytes in a common distilled water matrix. The study was configured as specified in the U.S. Army Toxic and Hazardous Materials Agency Quality Assurance Program (USATHAMA 1982), which is based on a method by Hubaux and Vos (1970). In this approach, detection limits were assumed for each analyte and standards were prepared at 0.5, 1, 1.5, 2, 5, 10 and 20* times these values. The estimated detection limits were 25, 27, 15 and 13 µg/L, for HMX, RDX, TNT and 2,4-DNT, respectively, close to the detection limits estimated from signal-to-noise ratio measurements in the linearity tests described earlier. Aqueous standards at each of these seven levels were prepared in quadruplicate on each of four days and analyzed in random order as described below. The results are shown in Table A3. Figure 2 shows three example chromatograms.

Analyses were conducted by diluting 10 mL of each sample with 10 mL of a solution that was 76% methanol and 24% acetonitrile (V/V), allowing the solution to stand at least 15 minutes and filtering it through a 0.4-µm Nuclepore polycarbonate filter. The first 10 mL portion of filtrate was discarded and the second 10 mL portion was saved for analysis. Samples were analyzed by filling a 100-µL sample loop to capacity, injecting into an LC-8 column maintained at 25 ±1°C and eluting with a mobile phase of 50% water, 38% methanol and 12% acetonitrile (V/V/V) at 1.5 mL/minute. Retention times were 3.1, 4.1, 7.3 and 8.1 minutes for HMX, RDX, TNT and DNT, respectively. A fixed wavelength 254-nm detector was used, with the output attached to a digital integrator.

To determine detection limits for each analyte, first the mean and variance were obtained for the integrator readings at each concentration (Table 7). Bartlett's test was used to determine over what concentration ranges the variances were homogeneous. For HMX, Bartlett's test gave a χ^2 value of 18.23 when all the data were used, relative to a critical value of 12.59. When the data for the highest concentration were eliminated, the χ^2 value dropped to 6.23 compared to a critical value of 11.07. Thus in this range the variance was accepted as homogeneous at the 95% confidence level. For DNT, an analogous situation was found. Inclusion of all the data resulted in a significant χ^2

* These two concentration levels are not specified in the USATHAMA Quality Assurance Program, but were included in this analysis.

value while eliminating the two highest concentrations resulted in a χ^2 value of 0.99 compared to a critical value of 9.49. Thus within the range of the five lowest standards, we considered the variance homogeneous.

For RDX and TNT, on the other hand, Bartlett's tests, using the data for all seven and the lowest six standards, all resulted in significant χ^2 values at the 95% confidence level. The χ^2 values for the lowest five standards were also barely significant in both cases, but there seemed to be no direct relationship between variance and concentration in this range. Therefore, for the purposes of the estimation of detection limits, the variances were considered homogeneous in this range. For HMX, variances were considered homogeneous over the concentration range 12.55-245.1 µg/L. For RDX, TNT and 2,4-DNT, the homogeneous ranges were 13.63-136.31, 7.72-77.2 and 6.4-64.0 µg/L respectively.

Using the data within these ranges, we regressed the known concentrations against the 16 individual integrator readings for each analyte at each concentration; the best fit linear equations obtained are presented in Table 8. Regression analysis tables were obtained for these data to test whether the assumption of a linear relationship between concentration and response was justified. In all cases the linear model adequately described the data at the 95% confidence level.

Confidence limits about the regression lines were determined at the 90% confidence level. The detection limit was obtained from the value of X (the target concentration) corresponding to the point on the lower confidence limit curve where the value of Y (integrator units) equals the value of Y on the upper confidence limit curve at $X = 0$ (Hubaux and Vos 1970). This is shown graphically for HMX in Figure 3. The detection limits for HMX, RDX, TNT and DNT obtained in this manner were 26, 22, 14 and 10 respectively.

The random error variances obtained at each concentration can also be used to define analytical precision. For HMX, within the region of homogeneous variance, the average variance was about 7.3×10^6 integrator units and hence the standard deviation was about 2.7×10^3. When this was converted to concentration units using the regression line, the analytical precision in the concentration range 12-245 µg/L was estimated at ±3.4 µg/L. Above 245 µg/L, the relative standard deviation is probably constant at about ±2%. In a similar manner, the analytical precision for RDX was estimated at ±3.3 µg/L in the 13-136 µg/L concentration range, for TNT at 4.4 µg/L in the 7-77

Table 7. Variance analysis at measured concentrations for detection limit test.

Analyte	Concentration (µg/L)	Integrator units Mean	Variance	Bartlett's test (χ^2)*
HMX	12.55	5,111	4.85×10^6	
	24.51	10,690	5.18×10^6	
	36.76	13,881	4.20×10^6	
	49.02	20,220	8.67×10^6	
	122.05	44,168	1.11×10^7	
	245.1	88,135	1.00×10^7	6.23
	490.2	179,720	2.47×10^7	18.23†
RDX	13.63	7,265	4.24×10^6	
	27.26	14,707	8.87×10^6	
	40.89	21,084	6.20×10^6	
	54.52	27,990	2.09×10^7	
	136.3	65,707	9.57×10^6	10.82†
	272.6	130,669	2.08×10^7	14.69†
	545.2	263,501	4.76×10^7	31.26†
TNT	7.72	6,718	1.24×10^7	
	15.44	15,987	1.09×10^7	
	23.16	23,612	4.25×10^7	
	30.88	31,315	1.17×10^7	
	77.2	77,309	1.82×10^7	10.84†
	154.4	155,133	6.63×10^7	22.87†
	308.8	308,734	1.64×10^8	52.32†
DNT	6.40	6,071	1.93×10^7	
	12.80	15,9[illegible]	1.84×10^7	
	19.20	24,854	2.80×10^7	
	25.60	34,192	2.25×10^7	
	64.0	85,156	1.84×10^7	0.99
	128.0	171,249	8.86×10^7	—
	256.0	336,551	3.96×10^7	17.50†

* Critical χ^2 values ($\alpha = 0.05$) are 12.59 when data for all seven concentrations are used, 11.07 when the highest concentration is dropped and 9.49 when the data for the two highest concentrations are dropped.

† Variances are significant at the 95% confidence level.

Table 8. Regression equations for detection limit tests (in the form: Peak area = $b_0 + b_1$ [concentration]).

Analyte	Concentration range (µg/L)	b_0	b_1
HMX	12.55-245.1	2631.8	337.53
RDX	13.63-272.6	6807.0	459.62
TNT	7.72- 77.2	3580.8	959.64
2,4-DNT	6.40- 64.0	2835.5	1266.7

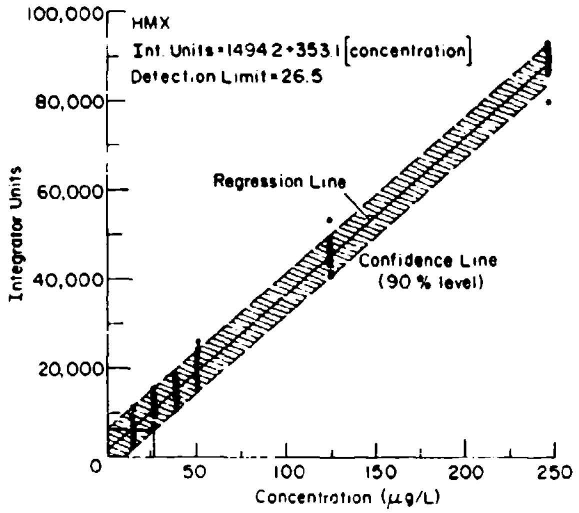


Figure 3. Detection limit determination for HMX.

μg/L range and for DNT at 4.6 μg/L in the 6-64 μg/L range. At higher concentrations, relative standard deviation is estimated at ±2% for RDX and ±4% for TNT and DNT.

Ruggedness test

When a published analytical method is selected for use, it is often difficult to reproduce the levels of performance obtained by the original investigator. The originator of the method was meticulous in reproducing the many individual steps required. While some of these steps are called out in detail in the published method, many others may not be. Although these steps may be common practice in the originator's laboratory, they may not be elsewhere, and strict adherence to procedural details may be critical in the outcome of analytical determinations.

A method that has been used to help assess the sensitivity of analytical methods to small deviations in test protocols is called a "ruggedness test" (Youden and Steiner 1975). To conduct such a test, the originator of a method carefully scrutinizes every step involved in the procedure to identify variables, such as composition of containers, types of filters and holders, storage conditions, temperatures, holding times, etc. Consideration is given, using experience and chemical intuition, as to whether deviations in a specific step could modify the analytical result. An experiment is then designed to test small variations in those steps that seem likely to result in analytical deviations.

For the RP-HPLC method under investigation, the method was studied carefully and four specific aspects were selected as being the most likely areas where small deviations in procedure could produce significant changes in results. These variables are designated X_1-X_4: X_1, use of plastic rather than glass vials for sample storage; X_2, during filtration, use of the first 10-mL portion of filtrate rather than the second 10 mL as specified; X_3, determination of whether the 15-minute holding time after dilution with methanol was more or less effective than a much longer (4-hour) holding time; and X_4, how critical the volume ratio of sample to methanol was in determining peak areas for the four analytes.

To conduct this experiment, a 2^4 factorial design was used. The factors, levels tested, and design and interaction matrices in coded units are presented in Tables A4 and A5. The 16 individual trials and analytical standards were analyzed in random order in duplicate. The test solution contained the following approximate concentrations: HMX—202 μg/L, RDX—131 μg/L, TNT—177 μg/L and DNT—96 μg/L.

Analysis was conducted in a 25 cm by 4 mm LC-8 column using a mobile phase of 50% methanol and 50% water at 1.5 mL/minute. Injections were made by filling a 100-μL sample loop to capacity with the filtered sample or standard. Quantitation of each peak was made using a variable wavelength UV detector set at 254 nm, and peak areas were measured using a digital integrator. Retention times for HMX, RDX, TNT and DNT were 2.6, 3.8, 6.7 and 8.2 minutes respectively.

We constructed calibration curves for the four standards run in duplicate using least squares linear regression analysis with and without an intercept. An F-ratio test was conducted to determine whether the true intercepts were equal to zero for each analyte. In all cases, the hypothesis of zero intercept could not be rejected at the 95% confidence level and the model with a zero intercept was accepted as the proper calibration curve. Using these models, we converted peak areas for individual trials to concentrations (Table 9). The following estimates of relative standard deviation were obtained from these 16 sets of duplicate analyses: 10.7% for HMX, 6.5% for RDX, 6.2% for TNT and 9.5% for 2,4-DNT.

The data in Table 9 were evaluated by analysis of variance techniques (Table A6). A summary of the effects for all four analytes is presented in Table 10.

Variable X_1 is significant only for 2,4-DNT where the samples stored in glass gave a higher average concentration than the samples stored in polyethylene. Of the four analytes, DNT has the highest octanol-water partition coefficient and,

Table 9. Duplicate concentration values (μg/L)* for trials in the ruggedness test.

Trial	*HMX*		*RDX*		*TNT*		*2,4-DNT*	
1	145.168	150.439	116.680	104.814	158.849	153.894	89.413	87.093
2	229.971	210.516	158.197	147.957	199.183	203.885	131.903	130.816
3	315.186	333.983	126.885	131.402	190.567	204.919	103.302	86.663
4	129.717	146.785	122.668	119.178	120.180	150.541	89.934	66.533
5	243.024	233.021	139.573	126.348	201.818	192.011	110.314	125.308
6	141.500	178.869	128.817	111.820	169.579	165.918	87.286	87.800
7	183.772	170.126	145.881	113.694	158.928	155.164	90.615	86.573
8	211.514	246.641	141.376	142.570	213.203	188.184	105.600	114.588
9	195.495	256.575	137.557	143.220	205.274	207.088	98.225	113.778
10	161.946	152.550	117.080	114.011	124.220	161.263	87.864	69.457
11	146.774	189.100	122.646	121.794	162.092	157.153	67.021	91.245
12	237.584	185.207	144.085	137.380	199.822	177.911	114.730	103.719
13	208.300	173.637	127.027	120.125	166.040	167.102	85.647	78.719
14	238.640	243.794	153.350	140.139	200.200	196.895	108.958	98.000
15	241.290	216.503	156.969	146.139	202.212	203.167	98.700	96.700
16	166.529	193.062	127.124	116.924	161.855	162.131	81.500	78.687

* Concentration are not really known to six significant figures but the values were retained since the statistical analysis was performed without rounding off the values to three significant figures.

Table 10. Effects of variations in sample handling on results for HMX, RDX, TNT and 2,4-DNT in water by HPLC.

Effects were estimated in a 2^4 factorial experiment—bold face values are significant at the 95% confidence level.

	Effects: Concentration differences (μg/L)†			
*Variable**	*HMX*	*RDX*	*TNT*	*2,4-DNT*
X_1	+ 3.95	− 2.98	− 1.35	+ 7.66
X_2	− 9.40	− 1.88	+ 3.70	+ 7.27
X_3	− 6.45	− 4.52	− 7.60	− 0.07
X_4**	**+75.0**	**−21.4**	**− 43.6**	**−25.9**
X_1X_2	**−16.3**	+ 0.69	+ 4.23	+ 6.00
X_1X_3	+13.1	+ 1.73	− 0.25	− 2.73
X_1X_4	**−22.1**	+ 2.59	− 1.50	− 2.02
X_2X_3	−13.3	+ 3.56	+ 2.61	+ 3.38
X_2X_4	+ 7.71	− 4.32	+ 0.40	− 4.62
X_3X_4	**−16.7**	− 2.05	− 6.47	− 3.46
$X_1X_2X_3$	− 8.43	+ 4.50	+ 1.86	+ 6.55
$X_1X_2X_4$	**+14.3**	− 4.33	+ 7.52	− 4.16
$X_1X_3X_4$	**−14.5**	− 4.38	− 2.22	+ 1.44
$X_2X_3X_4$	**+14.4**	− 5.79	− 4.65	− 1.26
$X_1X_2X_3X_4$	**+20.5**	− 2.62	+ 5.33	− 3.14

* X_1 = Two-day sample storage in glass (+1 level) and in polyethylene bottles (−1 level).
X_2 = First 10-mL filtrate from 0.4-μm Nuclepore (+1 level) and second 10-mL portion (−1 level).
X_3 = Standing 15 minutes before filtering MeOH/H_2O solution (+1 level) and 4 hours standing (−1 level), both in glass vials.
X_4 = 8/10 sample-to-MeOH volume ratio (+1 level) and 10/8 sample-to-MeOH ratio (−1 level).

† Average concentration for each compound was: HMX = 202 μg/L, RDX = 131 μg/L, TNT = 177 μg/L, and 2,4-DNT = 95.8 μg/L.

** See text for explanation of large effect of variable X_4.

hence, is the substance most prone to adsorbing or partitioning into an organic surface such as polyethylene by non-specific or hydrophobic mechanisms.

Variable X_2 is also significant only for 2,4-DNT where the first 10 mL of filtrate appears to have a higher concentration than the second 10 mL. The reason for this effect is unclear and seems to be in the opposite direction one might expect if there was adsorption in the filter membrane.

Variable X_3 was not found to be significant for any of the four analytes. Thus a 15-minute standing time seems sufficient after dilution with methanol, at least when the analyte was present as a dissolved species.

Variable X_4, the volume ratio of sample to methanol, was found to be significant for all four analytes. This was expected since the actual concentration of the +1 coded trials was 25% lower than that for the −1 coded trials. The raw data were not adjusted for this effect because of concerns about adverse effects on error estimates. Consequently, it is necessary to examine the size of the effects in comparison to the expected values. For this purpose, the average concentrations were calculated for both coded levels and the +1 coded levels were multiplied by 1.25 to account for the expected 25% differences. An adjusted sum of squares was also calculated using the corrected concentrations and new F ratios were estimated based on the original error mean squares. The results of these calculations are displayed in Table 11. Clearly, the sample-to-methanol ratio exhibits a substantial effect on the results for HMX even

Table 11. Further analysis of the effect of sample/methanol ratio by adjusting for volume differences.

	HMX	*RDX*	*TNT*	*2,4-DNT*
Average concentration (μg/L) for (−1) coded level	239.9	142.1	199.1	108.7
Average concentration (μg/L) for (+1) coded level	164.9	120.6	155.6	82.8
Decrease (%) for (+1) coded level compared to (−1) level	−45.5	−17.8	−28.0	−31.2
Average concentration for (+1) multiplied by 1.25 to account for volume	206.1	150.8	194.4	103.5
Difference (%) in adjusted means: (+1) coded level compared to (−1) coded level	−16.4	+5.8	−2.4	−5.0
F values for adjusted data:	19.7*	8.45*	1.46	2.62

* Significant at 95% level.

Table 12. Results of methanol/water ratio test, concentration in μg/L.

Sample	*HMX*	*RDX*	*TNT*	*DNT*
1 (1)	522.0	289.0	94.8	129.8
(2)	495.9	294.5	94.5	124.2
2 (1)	496.9	292.3	97.3	95.7
(2)	506.8	292.5	95.0	127.6
3 (1)	495.8	285.5	94.3	128.6
(2)	493.0	291.7	92.6	124.9
4 (1)	488.0	286.2	92.6	128.0
(2)	503.0	297.4	89.5	126.2
5 (1)	490.8	291.6	94.0	127.5
(2)	507.5	290.7	88.4	125.1
6 (1)	472.4	271.8	95.9	125.3
(2)	480.7	284.5	97.9	123.8
7 (1)	473.1	276.2	94.9	123.3
(2)	441.3	265.3	94.5	118.1
8 (1)	455.9	280.8	97.4	100.0
(2)	478.8	273.1	82.9	120.1
9 (1)	478.5	276.2	94.5	123.2
(2)	475.3	276.1	93.6	120.0
10 (1)	466.4	277.7	101.2	94.5
(2)	471.1	277.2	91.4	122.6

after volume corrections are applied. A smaller but significant effect is also in evidence for RDX, which elutes second, but no significant effect is noted for TNT or 2,4-DNT. Further testing of this effect is desirable when the actual analyte concentrations are equivalent in two cases. However, it appears that proper maintenance of this sample-to-methanol ratio is essential for reliable results.

The results of the ruggedness test indicate that it is important to be very specific with regard to the types of containers and the portion of filtrate chosen for analysis. It also appears that sample-to-methanol ratio is important over and above the obvious effect on the resulting concentration in the final solution.

Solvent strength test

To test further the effect of various sample-to-methanol ratios in the solution injected into the HPLC, two standards were prepared, one in water and one in methanol, but both with equal concentrations of HMX, RDX, TNT and DNT. Five replicate 10-mL portions of the methanol standard were each diluted with 8 mL of water (samples 1-5). Five replicates of the water were diluted in a like manner with methanol (samples 6-10). These 10 samples thus had equivalent concentrations of the four analytes, but five had a 10/8 methanol-to-water ratio and five had an 8/10 methanol-to-water ratio.

The 10 samples were analyzed randomly in duplicate (Table 12). A variance ratio (*F*) test on the two solvent types indicated no significant difference in random error for any of the four analytes at the 95% confidence level. Therefore, the variances were pooled for both types of samples for a given analyte and a *t*-test was run to compare treatment means. The calculated *t* values were 32.9, 7.3, 0.7 and 1.4 for HMX, RDX, TNT and DNT, respectively, while the *t*-table value is 2.101 for the 95% confidence level. Thus, there again is a significant difference in the peak areas for HMX and to a lesser degree for RDX but not for TNT and DNT. This result confirms that found earlier for the effect of variable X_4 in the ruggedness test for all four analytes after adjustment was made for actual concentration differences. Therefore, it is important to ensure that the solvent strengths of samples and standards are carefully matched or inaccurate results for HMX and RDX will result. The reason for this is uncertain but may be due to differences in absorptivities of these substances in solvents of varying composition, which would be the most significant for substances eluting early in the chromatogram, like HMX.

Methanol-water equilibrium times with river water

In the ruggedness test, two contact times (15 minutes and 4 hours) between methanol addition

Table 13. Results of equilibration time study with Connecticut River water, concentrations in µg/L.

Sample	TNT 15 min	TNT 4 hr	TNT Difference	RDX 15 min	RDX 4 hr	RDX Difference
1	106.4	105.8	+0.6	75.6	75.9	−0.3
2	99.2	101.3	−2.1	78.8	81.0	−2.2
3	100.0	102.2	−2.2	74.7	75.5	−0.8
4	99.6	96.3	+3.3	75.0	80.3	−5.3
5	105.7	107.9	−2.2	76.6	78.4	−1.8
6	97.8	94.1	+3.7	76.1	72.3	+3.8
7	102.5	106.2	−3.7	80.5	78.6	+1.9
8	52.2	55.8	−3.6	74.5	74.3	+0.2
			$\bar{X}$ = −0.78			$\bar{X}$ = −0.56
			S = 2.72			S = 2.74
	t value = 0.81			t value = 0.58		
	t.95 (df = 7) = 2.365			t.95 (df = 7) = 2.365		

and filtration were tested; no significant differences were found in analyte concentration for a distilled water matrix. It is possible, however, that in a natural water sample, a significant amount of these analytes might be adsorbed to natural particulate matter. If so, it seems that addition of methanol should desorb at least a portion of this material, but the process could be rate limited and a longer contact time could be useful.

To test the role of adsorption, a sample of Connecticut River water was collected, spiked with TNT and RDX and divided into eight subsamples. Typical levels of suspended solids for this water are 2–3 mg/L. Four subsamples were stored in glass and four in plastic for 9 days. Then, two 10-mL portions of the water in each bottle were withdrawn. One was mixed with 10 mL of methanol, allowed to stand 15 minutes and filtered through a 0.4-µm Nucleopore polycarbonate filter. The second aliquot was mixed with methanol and allowed to stand for 4 hours prior to filtering.

A 100-µL portion of each filtered sample was injected into a LC-8 column and eluted with a mobile phase of 38% methanol, 12% acetonitrile and 50% water. Peak areas were obtained for RDX (4.2 minutes) and TNT (7.3 minutes) (Table 13).

A paired t-test was conducted on these data. The results indicated that there was no significant difference at the 95% confidence level for either TNT or RDX. This agrees with the result obtained in the ruggedness test for this variable for these two substances and indicates that a 15-minute equilibration time is sufficient for natural waters containing low levels of natural particulate matter.

Analysis of real munitions wastes

In our discussion of the RP-HPLC method thus far, we have considered only synthetic samples; aqueous samples prepared by spiking distilled water or several natural water matrices with low levels of TNT, RDX, HMX and DNT. To test the method with a variety of real munitions wastes, samples were collected at Louisiana AAP, Iowa AAP, Holston AAP and Milan AAP.

The initial samples were collected at the Louisiana AAP from two different sources. The first was wastewater from a load and pack operation following activated carbon treatment. The second was from an old lagoon, once used for disposal of load and pack wastewater prior to use of the carbon treatment process. Figure 4 is an example of the chromatograms obtained. These samples were analyzed in the usual manner using a mobile phase of 50/50% methanol/water. The concentrations of TNT, RDX and HMX found in these samples are presented in Table 14.

Clearly, the wastewater samples following the carbon treatment represent the simpler matrix with well-defined peaks only for TNT, RDX and HMX. No significant interferences are apparent in this matrix. The lagoon water sample, on the other hand, has several other peaks in addition to those for TNT, RDX and HMX. For the most part, they are well separated from the analytes of interest.

At Milan AAP, groundwater from a contaminated water supply well was sampled and analyzed as described above (Fig. 5). A peak for RDX was observed at a retention time of 3.97 minutes, with a peak area corresponding to an aqueous concentration of 70 µg/L. No analytical problems were

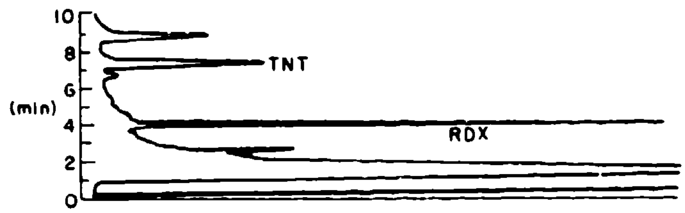


Figure 4. Chromatogram for disposal pond at Louisiana Army Ammunition Plant.

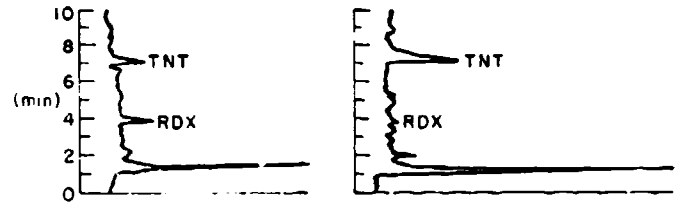


Figure 5. Examples of chromatograms for two samples of contaminated groundwater at Milan Army Ammunition Plant.

Table 14. Analysis of munition wastes from Louisiana, Milan and Iowa AAPs, concentrations in μg/L.

Sample	*HMX*	*RDX*	*TNT*	*DNT*
Louisiana				
Load and pack wastewater	289	2,430	19	< d
Lagoon water	1,652	6,280	1,314	915
Milan				
Water supply well	< d	70	< d	< d
Iowa wastewater				
Before carbon column	4,600	19,700	51,300	< d
After one carbon column	606	586	128	< d
After two carbon columns	< d	< d	< d	< d

d—detection limits are estimated at about 26 μg/L for HMX, 22 μg/L for RDX, 14 μg/L for TNT, and 10 μg/L for DNT.

encountered and the method appeared to function well for this type of water matrix.

At Iowa AAP, samples were collected from a waste stream produced from melt and pour operations for loading of artillery shells. Three types of samples were collected: wastewater prior to carbon treatment, wastewater following treatment with one carbon column and wastewater following treatment with two carbon columns. Analyses of these samples were conducted as usual, using a mobile phase of water/methanol/acetonitrile in a ratio of 50:38:12. Retention times for HMX, RDX, TNT and DNT standards were 3.4, 4.4, 7.4 and 8.3 minutes, respectively. Chromatograms obtained are shown in Figure 6 and quantitative results results are presented in Table 14. The method seemed to work very well for these samples. Concentration of these analytes in the wastewater prior to carbon treatment was quite high (≈60 mg/L for TNT) and injection volumes between 2 μL and 100 μL were tried. The results demonstrated that the concentrations obtained were independent of sample volume when the volumes were properly considered in calculations.

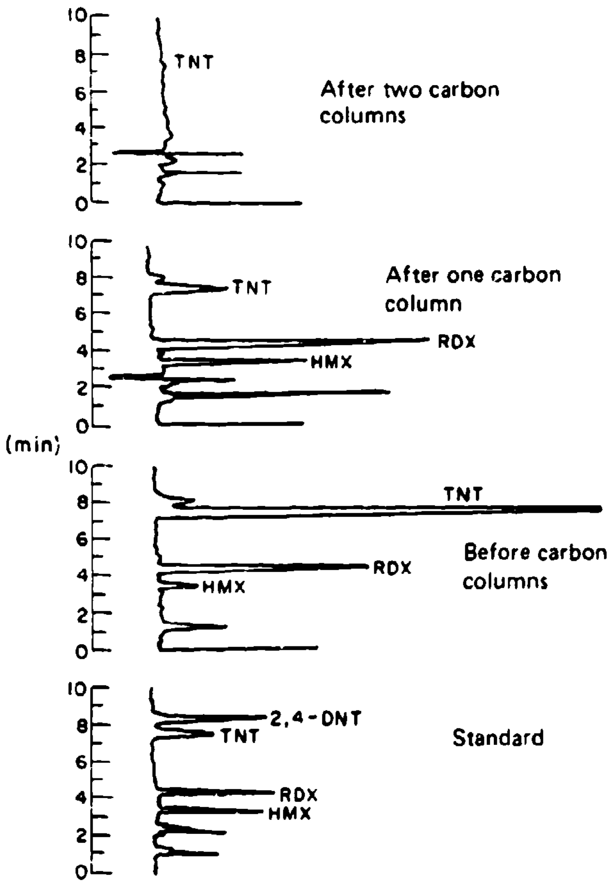


Figure 6. Chromatograms for treatment sequence at Iowa Army Ammunition Plant.

The Iowa sample before carbon treatment and after the first carbon column had an additional peak just after TNT but too early for DNT. A peak in this region had been observed in samples containing TNT that were held at room temperature for several days. This peak is probably a microbial degradation product of TNT, perhaps one of the isomers of aminodinitrotoluene.

A sample of wastewater from an RDX-HMX manufacturing operation at Holston AAP was also subjected to this analytical procedure. Large peaks for HMX and RDX were found along with several other very early eluting peaks. The concentrations of HMX and RDX were found to be about 3.0 and 27.4 mg/L respectively. Two of the early eluting peaks are thought to be SEX and TAX, two known impurities in RDX-HMX manufacturing.

Preparations for collaborative test

It appears that the RP-HPLC method is suitable for use in compliance monitoring at Army and GOCO installations, based on CRREL research. In order to determine how well this method works in a variety of other laboratories, a collaborative test of the method was conducted. A number of laboratories were contacted and nine agreed to participate. Seven of these were within the Army system including four ammunition plants and three research laboratories. The other two were a university and the USEPA Environmental Monitoring and Support Laboratory. Thus the nine laboratories included some rather diverse participants in both background and experience with this type of analysis.

The broad strategy for conducting the collaborative test was as follows: a set of water matrices was to be chosen and sent to each participant along with a set of solutions containing the four analytes with which the matrices were to be spiked. The matrices were to represent some of the types of water that might be analyzed by this method and were to be stabilized as much as possible to retard chemical or microbiological modifications during storage. The spiking solutions were to be made up in methanol and were to represent various concentration ranges. Preparation of the samples and the beginning of the test were to be coordinated to minimize the storage times and, hence, the chances for deterioration.

Since the bulk of the study was to be based on the spiking of aqueous matrices with a methanol-based stock solution, it was necessary to ensure that evaporation of this volatile solvent during shipment and storage was eliminated. To do so we planned to store the methanol solutions in flame sealed glass ampules. A study was conducted to determine whether this procedure resulted in any measurable change in concentration of the four analytes in a methanol stock solution.

A stock solution of HMX, RDX, TNT and DNT in methanol was prepared at about 330, 250, 100 and 125 μg/L respectively. Approximately 10 mL of this solution was poured into four glass ampules. (The glass ampules were 1.8 cm in diameter with a capacity of about 10 mL, obtained from OIC, College Station, Texas.) One ampule was sealed immediately using the methane-oxygen flame from an OIC Purging and Sealing Module of an organic carbon analyzer. The neck of the other three ampules were covered with aluminum foil and they were placed in a freezer for 30 minutes. They were then removed and quickly sealed while cold.

The methanol solutions in the four ampules were analyzed as follows. A volume of solution was withdrawn from the ampule and mixed with

Table 15. Results of flame sealing test, mean concentrations in $\mu g/L$.*

Sample	HMX	RDX	TNT	DNT
Ampule 1†	339.4 a††	250.7 b	104.6	129.6 ab
Ampule 2	341.4 a	252.2 b	107.7	125.5 c
Ampule 3	341.2 a	258.8 a	105.9	132.5 ab
Ampule 4	340.2 a	259.6 a	109.0	133.7 a
Stock**	329.5 b	247.5 b	100.8	127.5 bc

* Mean of three replicates.

† Ampule 1 was sealed immediately; the other three ampules were cooled in a freezer for 30 minutes prior to sealing.

** Same solution used in ampules but not flame sealed.

†† Values with different letters are considered significantly different from each other at the 95% confidence level using Duncan's multiple range test.

an equal volume of HPLC grade water. A 100-μL volume of each was injected in triplicate into a LC-8 column and eluted with 1.5 mL/minute of 50:38:12% water/methanol/acetonitrile. Quantitation was obtained by comparison of the peak areas to that obtained when the same methanol stock solution was analyzed in a like manner but without flame sealing. The results are presented in Table 15.

For HMX, the four sealed ampules were significantly higher in concentration at the 95% confidence level than the unsealed stock solution by about 3%. For RDX, two of the sealed ampules were significantly higher than the other two and the unsealed stock. The average ampule concentration was again about 3% higher than the unsealed stock solution. For TNT, there was more variability in the replicates, which resulted in no significant difference between any of the ampules and the unsealed stock. For DNT, there were significant differences among the ampules, with ampule 2 being lower then the other three. This result is caused by one very low value in the three replicates. Even so, the average concentration of the ampules is again about 3% higher than the unsealed stock.

If the result for DNT in ampule 2 is ignored, the mean values for the stock solution are lower than the mean for any of the ampules for all analytes. The value for ampule 1 is next lowest in all cases. Recall that ampule 1 was sealed immediately and not allowed to stand in the freezer for 30 minutes prior to sealing as were the others. It appears that a small amount of methanol evaporated during the 30 minutes the ampules were cooled in the freezer, resulting in a slight but measurable increase in concentration for all four analytes. Since the ampule that was sealed immediately was only significantly different from the unsealed stock for one analyte, it appears that the major portion of the evaporation did not occur during the sealing process itself, but rather in the standing. Since the vapor pressure of methanol is reduced from about 112 torr (14.9 kPa) at 25° C to only 28 torr (3.7 kPa) at 0°C, the majority of the evaporation probably occurred while the solution was cooling rather than after it reached the final temperature. Thus ampules 2, 3 and 4 were not significantly different from each other even though they were sealed several minutes apart, resulting in slightly different standing periods.

In conclusion, it appeared that sealing the methanol solutions in glass ampules could be used to ensure that evaporation did not cause the collaborative test participants to receive spiking solutions that differed in analyte concentrations. In addition, if the solutions were cooled in the freezer before the ampules were filled, the small amount of evaporation that was observed could be reduced to insignificant levels.

A test of the long-term stability of methanol solutions of these analytes was also conducted. A solution was prepared with HMX, RDX, TNT and DNT concentrations of 279, 310, 215 and 160 $\mu g/L$. It was sealed as described above, except that a capped flask of the solution was cooled in the refrigerator prior to being placed in a glass ampule and flame-sealed. This solution was allowed to stand in the refrigerator in the dark for 5 months. It was then opened and analyzed using RP-HPLC with a LC-8 column and the ternary eluent described earlier. The mean determined concentrations of three replicates for HMX, RDX, TNT and DNT were 273, 316, 212 and 163 $\mu g/L$, respectively. Clearly these analytes were stable in methanol solution over this 5-month period.

PART 2. COLLABORATIVE TEST

PROTOCOL STRATEGY

A number of important decisions were made in setting up the analytical protocol for the collaborative test. Since the rationale behind these decisions is not obvious in many cases, a discussion was considered helpful. The following comments are presented in roughly the same sequence as the procedural steps in the protocol (Appendix C).

The style of the protocol is that of a very detailed recipe. In fact, it is so detailed as to be insulting to any competent analyst because it ignores his or her judgment in even the most trivial matters. There is an excellent reason for this approach, namely the need to focus attention on the performance of the test method alone. Consequently, it was necessary to eliminate or control unknown sources of experimental error by requiring strict adherence to the protocol. The protocol itself explains to the analyst the reasons for such rigidness. All deviations from the procedure had to be cleared with Tom Jenkins at CRREL. For similar reasons, the collaborative test was to be handled by a single analyst in each laboratory. Different analysts certainly perform with different levels of skill; having multiple analysts would only reduce our ability to derive useful information from statistical evaluation of the data.

The standards and water matrices shipped from CRREL and the standards prepared within each laboratory had to be stored in the dark and at a temperature of around 4°C. These measures were necessary to prevent photochemical or biological degradation of the analyte species in these materials, and to reduce solvent evaporation from standards that were used throughout the study. Some of these solutions were more susceptible to changes than others. For example, biological activity is completely inhibited in methanol solutions but not in aqueous ones. The *Preparation of Aqueous Matrices* section below discusses other precautions taken to minimize this problem.

The instrumentation required for the HPLC method and also the collaborative study were not the ultimate in state of the art quality. Rather, the method was constructed around routine instruments that most laboratories were likely to have already, especially the AAPs, which will be using this method routinely to test their wastewater purification systems. We chose isocratic liquid chromatography using a single-wavelength UV absorption detector and a digital electronic integrator. Quantitation using electronically integrated peak areas was required because other approaches to area measurement are much more labor intensive. It was unlikely that the HPLC method would be accepted as a standard method if an integrator were not used.

The reagent solutions were prepared as follows:

1. The sample modifier was 76% methanol and 24% acetronitrile (V/V). An equal volume of this solvent was added to each aqueous sample. The mixture was prepared using volumetric pipettes rather than volumetric flasks to minimize systematic differences with the mobile phase because of volume contraction. Dilution of the samples with this solvent, rather than with methanol alone, eliminates a negative peak that elutes just before HMX and results in unpredictable integration.

2. The HPLC mobile phase was 50% water, 38% methanol and 12% acetonitrile (V/V/V). Graduated cylinders were used to prepare this solvent. It also had to be prepared daily because bacterial growth was not insignificant even with so much methanol present. A substantial bacterial population clogged the inlet filters of the HPLC. An additional reason for daily preparation was that selective evaporation of one of the solvent components was possible. This would lead to a systematic change in the retention volumes of the analytes as the solvent composition changed. For storing the column after use, pure methanol was used. This fully inhibits bacterial activity.

3. Individual-analyte stock, combined-analyte working stock and working standards were prepared as follows. First, working standard concentrations were selected in the range of concentrations of interest for the collaborative study. Then,

stock standard concentrations and dilution factors were chosen to minimize the number of transfers necessary to prepare the working standards and to minimize the errors introduced by volumetric tolerances. To dissolve the SARM solids, methanol was adequate for DNT and TNT, but not for RDX and HMX, which needed 40% acetonitrile. This difference in solvent composition had a negligible effect on the working standards. Creation of the combined-analyte stock entailed a 40-fold dilution with methanol of the RDX and HMX stock solutions. Thus, this combined stock contained only 1% acetonitrile. The next dilution down to the working standards further reduced this level.

Each aqueous matrix was spiked at low, medium and high levels. The particular levels were selected to cover the range of concentations likely to be found in treated munitions wastewaters and in contaminated natural waters: about 30 to 500 μg/L. This range extends roughly from 20 to 30 times the detection limits estimated, in the method development phase, by the Hubaux and Vos (1970) method. Having analytical results from three concentration levels permits evaluation of accuracy and precision near to and far from the detection limits. Actually, spikes of four different concentrations were used. Two of these spike levels, either the highest two or the middle two, were close in value—no more different from each other than a factor of 1.15. These two spikes together represented the high or medium concentration level respectively. The other two spikes were set off by factors of at least 0.3 and as much as 2 or more. For example, for matrix B the DNT analyte spike levels were 61.4, 76.8, 115 and 128 μg/L; for RDX the levels were 74.3, 248, 273 and 372 μg/L. For DNT, the two high values are together the high range; for RDX, the two middle values are the medium range.

The purpose of having two closely spaced concentrations is so that our chosen statistical evaluation method, called the Youden two-sample chart, can be applied to the data. This method displays graphically the relative magnitudes of systematic and random errors that exist in the method.

The analytical work was divided into two segments: establishment of statistical control of the procedure and analysis of the spiked water matrices. During the first segment the analyst certified that the HPLC column was performing within its specifications, established working curves for each of the four analytes, and analyzed a test sample whose composition was specified by CRREL. If the results were acceptable, the analyst could proceed with the analysis of the spiked water matrices. Since these working curves would be the basis for all other measurements, the characteristics of these curves had to be well established using linear regression statistics. Theoretically, the UV detector response should be linear in concentration and have a zero intercept. Previous experience at CRREL in developing the HPLC method had shown that in practice this behavior did take place. Consequently, the laboratories participating in this study were expected to be able to achieve the same results. The advantages of linearity and a zero intercept are simplified daily calibration and significant time savings.

To construct the working curves, chromatograms of the four working standards and the blank were obtained in duplicate. The 10 injections were sequenced randomly, a necessary prerequisite for valid statistical evaluation. Unweighted linear regression analysis was applied to the data, using models with and without a zero intercept. Since many analytical chemists are not familiar with proper curve fitting procedures, step-by-step instructions were provided in the protocol. First, the model with an intercept was tested to determine whether it was adequately fit by a straight line. Existence of significant lack of fit required taking steps to ascertain the source of the nonlinearity and to correct it. After successful completion of this task, the regression line was tested to determine whether it passed through the origin. Again, action might have had to be taken to achieve this. The responses of the two blank samples were not included in the regression analysis because "zero" values force the fitted curve toward the origin. Omission of these data represent taking a conservative approach toward fitting the lines. The blanks were analyzed only to see whether significant contamination existed in the reagents.

An unweighted least squares approach was used instead of a weighted approach, which may be considered more generally appropriate, because the former was easier to carry out. Experiments at CRREL found that in the lower concentration ranges of the HPLC method the variances are homogeneous. This means that the weighted and unweighted approaches are equivalent for the concentration levels of interest.

The water matrices chosen for study were representative of waters for which the HPLC method was devised. The matrices were:

A. Final effluent from an AAP pink water treatment facility. It contained no detectable analytes and was spiked with TNT and RDX.

B. Distilled or deionized water from each participating laboratory's supply. Exact methods of preparation for these matrices are discussed in the *Preparation of Aqueous Matrices* section.

C. Uncontaminated well water from Canaan, New Hampshire. No detectable analytes were present and it was spiked with DNT and HMX.

D. Contaminated well water from an AAP site that contained RDX.

The ampule solutions used for spiking the water matrices were prepared and labeled in a manner that avoided creating a predictable pattern that an analyst might discern. The concentrations were not identical for every matrix nor were they sequenced to match their labels for each matrix—if such a precaution were not observed and an analyst discovered the pattern, subsequent samples could no longer be considered independent because the analyst's subjective judgment might influence how these later samples were handled.

The sequence in which the matrices were analyzed was randomized. All of the analyses on a given matrix had to be done in a single working day. This avoided the problem of day-to-day variability associated with remaking standards and recalibration. Four aliquots of the matrix were taken. Each was spiked using a different ampule spike solution. Another aliquot of the matrix was taken as the unspiked sample. Each of these solutions was processed in duplicate following the procedure described in the protocol. Then each of the processed samples was to be injected onto the HPLC column in duplicate. This meant a total of 20 chromatograms had to be obtained in addition to those necessary for establishing the working curve for that day (at least eight). More than an 8-hour day would have been necessary for completing all these tasks. To alleviate the time crunch, daily calibration was performed by preparation and analysis of only the highest concentration standard instead of all of the standards.

Relying on the response of a single standard seems somewhat risky. In this particular case the decision can be justified because the fundamental relationship between the response of the UV absorption detector and concentration of the analytes is well understood and is well controlled by the instrumentation. Furthermore, each participating laboratory would have already established that their instrument's response was linear and through the origin. Extensive experience at CRREL with the HPLC method has indicated that a linear response is to be expected. Hence, there was no need to verify this condition every day of the interlaboratory study. Instead, a single-standard calibration approach was taken.

Triplicates of newly made working standard of high concentration were obtained. The mean peak areas for the four analytes were compared with the confidence intervals around the working curves previously established. Detailed instructions for carrying out this comparison were given. If no differences were found, the analyses could begin. A significant difference would indicate either systematic error in preparation of the standard or instrumental response drift. To distinguish between these two possibilities, a second set of triplicates then were run using another newly made high standard. The mean of this set was tested against the working curve. If a difference still existed, another statistical test was performed—a t-test for equivalence between the two sets of triplicates. If this last test indicated no difference, then nothing was wrong with the way the standard had been prepared and instrumental response drift was suspected. The analyst could then proceed with analysis of the spiked water samples. If the t-test indicated a difference, then either the instrument was subject to strong short-term drift or noise or there was insufficient reproducibility in the analyst's technique of solution preparation. At this point, CRREL would have had to be consulted.

Once the analysis had been shown to be under statistical control on that day, the spiked solutions could be prepared and analyzed. As stated above there were 20 separate aqueous samples to be analyzed. Five replicates of the high concentration working standard prepared that day were also analyzed. These 25 analyses were done in random sequence. The day's working curve for each analyte was based on the mean response of the five replicates of the standard and assuming a zero intercept.

PREPARATION OF METHANOL SOLUTIONS

Standard Analytical Reference Materials (from LCWSL) of TNT, RDX, HMX and DNT were dried in a vacuum dessicator until successive weights did not differ by greater than 0.2 mg (approximately 24 hours). A sample of each solid (about 100 mg) was carefully weighed out on weighing paper to the nearest 0.01 mg, transferred to individual volumetric flasks and diluted to volume with a solution of 90% methanol/10% aceto-

Table 16. Concentrations (mg/L) of HMX, RDX, TNT and DNT in ampules supplied to each participant.

Solution	*HMX*	*RDX*	*TNT*	*DNT*
Test	27.85	30.98	21.46	16.01
A1 and D4	4.46	9.91	3.43	5.12
A2 and D3	13.37	19.82	10.30	8.96
A3 and D2	14.70	22.30	11.67	10.24
A4 and D1	22.28	49.56	17.16	12.80
B1 and C4	6.68	7.43	5.15	6.14
B2 and C3	22.28	24.78	13.73	11.52
B3 and C2	24.51	27.26	15.44	12.80
B4 and C1	33.42	37.17	8.58	7.68

nitrile. The concentrations of HMX, RDX, TNT and DNT in these stock standards were 111.40, 123.92, 85.83 and 64.04 mg/L respectively.

The spiking and test solutions for the collaborative study were prepared from these four stock standards by combining various volumes of each using volumetric pipettes and diluting to volume with methanol in ground-glass-stoppered volumetric flasks. To further prevent loss of methanol by evaporation, the tops of the stoppers were carefully wrapped with Parafilm. For the test solution, 25 mL of each stock standard was used with no additional dilution. For the eight spiking solutions, the volumes of individual stock solutions used varied from 10 to 100 mL. The concentrations of the four analytes in the resulting solutions are presented in Table 16.

These solutions were cooled overnight in a refrigerator and then approximately 5 mL was dispensed into individual ampules using an automatic pipet that was cleaned carefully with methanol before use and between individual solutions. These ampules were labeled as shown in Table 16. It should be noted that two types of ampules with different labels were filled from the same solution. For example, the contents of ampules labeled A1 and D4 were identical, although the participants in the collaborative test were not informed of this.

A set of sealed ampules consisting of one test solution, 16 ampules labeled A1-A4, B1-B4, C1-C4 and D1-D4, and an empty ampule were placed in a square plastic container. The outside of the ampules were packed with paper towels so they wouldn't break during shipment. The ampule sets were stored in a refrigerator in the dark overnight.

Table 17. Concentrations ($\mu g/L$) of analytes in aqueous matrices.

Matrix	*HMX*	*RDX*	*TNT*	*DNT*
A	—	55.6	38.1	—
B*	—	—	—	—
C	124	—	—	71.7
D	—	112	—	—

* Distilled or deionized water from each location.

PREPARATION OF AQUEOUS MATRICES

Matrix A was prepared on 2 September from water collected earlier at the Iowa AAP. This water was collected from the effluent of the second carbon column from a pink water treatment line. This carbon column had just been placed in operation and analysis of the water indicated that the concentrations of HMX, RDX, TNT and DNT were below detection limits.

This water and sufficient well water to bring the volume to 18 L were combined and sterilized by autoclaving in a 23-L (5-gal.) glass jug for 2½ hours at 127°C. The jug was cooled and spiked with 8 mL of the TNT stock solution and 20 mL of a 50-mg/L RDX solution to recreate concentrations of these analytes found at Iowa AAP in effluent from the first carbon column. The pH of the solution was reduced to approximately 5.5 with 1 N HCl to inhibit hydrolysis reactions. The concentration of the analytes in solution are shown in Table 17.

We autoclaved 15 1-L glass bottles as described above, cooled and filled them with the above solution and labeled them "Matrix A." These bottles were immediately placed in a refrigerator in the dark until shipment. Sample bottles for the other two matrices were prepared and stored the same way.

Matrix C was prepared on 2 September from well water collected in Canaan, New Hampshire. We autoclaved 18 L of this water for 2½ hours at 127°C, cooled it and spiked it with 20 mL of the DNT and HMX stock solutions. The resulting concentrations of these analytes are presented in Table 17. The pH of this solution was also adjusted to 5.5 with 1 N HCl.

Table 18. Timetable for receipt of samples and analysis of aqueous matrices.

Laboratory	Samples received	Matrix analyzed A	B	C	D
USEPA, EMSL	9 Sep	16 Sep	22 Sep	21 Sep	20 Sep
AEHA	7 Sep	15 Sep	16 Sep	14 Sep	13 Sep
CRREL	6 Sep	27 Sep	21 Sep	26 Sep	22 Sep
UNH	6 Sep	26 Sep	28 Sep	21 Sep	27 Sep
LCWSL	6 Sep	18 Oct	14 Oct	17 Oct	19 Oct
Iowa AAP	10 Sep	18 Oct	14 Oct	11 Oct	16 Oct
Louisiana AAP	7 Sep	29 Sep	27 Sep	28 Sep	30 Sep
Holston AAP	8 Sep	22 Nov	30 Nov	1 Dec	29 Nov
Radford AAP	7 Sep	12 Oct	14,18 Oct	12 Oct	14 Oct

Matrix D was prepared on 1 September from contaminated well water from the Milan AAP. Upon analysis, this water had an RDX concentration of about 72 µg/L, while the concentration of the other analytes was below detection limits. Since our experience indicated that RDX is destroyed when the water is autoclaved, this solution was not subjected to this procedure but was mixed with autoclaved well water to obtain a sufficient volume for the test. A small amount of the RDX stock solution was added to increase the concentration of RDX above 100 µg/L (Table 17). Since the solution was not sterile, it was reduced to pH 3.5 with 1 N HCl to prolong its stability.

SHIPMENT OF SAMPLES

Samples were shipped to the various participants on 6 September 1983. All samples were kept on ice in the dark during shipment. The samples that went to Louisiana AAP were shipped by air freight and were received the following day (Table 18). Samples to all other locations were delivered by car and care was taken to keep the samples cold during transit. Samples arrived at the various locations between 6 and 10 September (Table 18).

SUMMARY OF PROTOCOL FOR COLLABORATIVE STUDY

The protocol (Appendix C) consisted of a detailed procedure that the participating laboratories were required to follow explicitly. Strict adherence was essential in order for the statistical analysis of results to provide unbiased estimates of method performance. The reasoning behind this is that unknown sources of random or systematic error had to be eliminated insofar as possible. Any deviations from the protocol had to be cleared by CRREL.

A single analyst in each laboratory was responsible for all aspects of this study, from receipt of materials through data analysis. All efforts were to be documented in duplicate in a project notebook. Detailed instructions concerning the following items were given:

1. Inspection of materials received from CRREL.
2. Storage of these materials.
3. Required instrumentation and settings.
4. Hardware and glassware—types and cleaning.
5. Chemical reagents.
6. Preparation and storage of HPLC mobile phase and calibration standards.
7. Conditioning of HPLC column and test of its performance.
8. Practice run through analytical procedure using a test sample.
9. Spiking and analysis of water matrices (four matrices at four spike concentration levels).
10. Data calculations and reporting.

The analytical work was done in two steps. The analyst first spent some time becoming familiar with the procedures. During this period working curves for each of the four analytes were prepared and steps taken to establish that they were linear and passed through the origin. A test sample whose composition was specified by CRREL was analyzed. If the results were acceptable, the analyst could procede with the analysis of the collaborative test samples. These statistical procedures and their rationale were described thoroughly in the protocol.

The second portion of the work consisted of analysis of the four water matrices; three of these were provided by CRREL and the fourth was the laboratory's own reagent-grade water. These matrices were analyzed directly and after spiking with standard analyte solutions. Four different spiking solutions were provided. Each contained all four analytes. All of the work associated with a given water matrix could be performed in a single workday; the chronological order for matrix analysis was random. The daily procedure consisted of the following:

1. The most concentrated standard was analyzed and its response was statistically compared with the previously established working curves.

2. Barring unresolved discrepancies in the first step, the spiked matrix samples were prepared: four separate spiked samples and one unspiked sample.

3. Each of these solutions was processed in duplicate and each of these twin processed samples was injected in duplicate onto the HPLC column (20 total injections) along with five replicates of the highest standard; the injection sequence was random.

4. The day's working curve for each analyte was based on the mean response of the five replicates of the highest standard, assuming a zero intercept.

5. The concentrations for the 20 injections of spiked and unspiked water samples were calculated.

STATISTICAL ANALYSIS

Rationale

A primary goal of this collaborative study was to assess the capability of the HPLC method to determine DNT, TNT, RDX and HMX concentrations under typical environmental conditions. The performance characteristics evaluated were accuracy, repeatability (precision within individual laboratories), and reproducibility (precision between laboratories) (Youden and Steiner 1975). A number of standard statistical tests were applied to the data to extract these summary characteristics (Youden and Steiner 1975). It must be emphasized that, although these calculations may seem straightforward, it is often necessary to apply chemical intuition to assist in making reasonable decisions.

The sequence of tasks was roughly: inspection of raw data and construction of Youden two-sample plots to obtain a "feel" for overall performance, rejection of extreme values (outliers), analysis of variance to extract estimates of precision, and regression analysis to evaluate overall accuracy. Most calculations were made using the computer program MINITAB, which is available on one of the mainframe computers at the University of New Hampshire.

The nine participating laboratories reported data in a uniform format using a form provided with the collaborative test protocol. For each laboratory there were 320 individual concentration values in micrograms per litre (four analytes × four aqueous matrices × five analyte concentration levels per matrix × two aliquots processed per spiked solution × two injections per aliquot). These data sets are collected in Table A7. After loading this information into a computer file, it was proofread scrupulously to correct transcription errors. Individual laboratories were identified only by number to avoid potential bias where value judgment was required. All laboratories followed the required analytical protocol except for laboratory 7; consequently, this one was rejected. By requiring adherence to the protocol we assured that every laboratory would have the same general sources of variation. Since laboratory 7 followed its own protocol, its results were subject to different sources of error; therefore, laboratory 7's data set and that of the other laboratories are not comparable.

An initial impression of analytical performance can be gleaned from inspection of the results of the test sample analysis, which had been a prerequisite for carrying out the water sample analyses. Table 19 lists individual results, means, standard deviations and actual concentrations. The differences between the mean determined concentrations and the actual values is quite small: less than

Table 19. Determination of test sample composition, concentrations in μg/L.

Laboratory	*DNT*	*TNT*	*RDX*	*HMX*
1	157.0	210.7	304.7	268.4
2	162.0	217.0	315.0	275.0
3	156.6	212.9	336.1	237.8
4	160.0	220.7	306.6	270.3
5	152.1	220.6	315.5	249.2
6	148.0	193.0	265.0	256.0
7	190.3	279.5	336.0	288.9
8	164.0	187.0	321.0	259.0
9	155.9	208.6	311.4	278.1
Mean	160.7	216.7	312.4	264.7
Actual value	160	215	310	279
Standard deviation	12.1	26.3	21.1	15.8
% RSD	7.8	7.0	4.1	3.2

1% for all analytes but HMX, for which the difference is still less than 5%. The relative standard deviations are not unreasonably large. It must be recognized that these values represent the relative performance across the laboratories; consequently, we may expect large scatter.

Detailed inspection for gross errors was the next step. This was aided by calculation of the mean concentration for each set of four replicate analyses on each sample. (Henceforth, the word "sample" will refer specifically to the solutions from which the two aliquots were withdrawn for processing. Thus, the "four replicates" represent the two aliquots, each of which was injected into the HPLC in duplicate.) These means were compared with the concentrations that should have been found (henceforth called the "true" values), given the concentrations of the added spiking solutions and the amount of analyte already present in each matrix. This comparison was made by looking simultaneously at the results of all the participants for a given sample and analyte (see Table A8). In addition, each set of four replicates was inspected for internal consistency. Any datum that seemed to be disparate from its group was checked against the original lab notebook. Only two out of about 30 suspect values were resolved in this manner. Transcription errors were the cause. In one case we found that two data columns in a laboratory's report had been mislabeled. Although this inspection approach did find some errors, it is clearly very inefficient.

A comment regarding the definition of "true value" is in order. Spiking solutions were prepared using SARM solids. The quality of these standards is not as good as NBS primary reference materials, but the assays are certified to be within 98 mole %. As far as the collaborative test is concerned, the assay of these standards should not affect the evaluation of interlaboratory precision because the SARM sent to each collaborating laboratory was prepared from the same batch. There could be a small effect on accuracy because the SARM used for the spiking solutions was from a different batch than the SARM distributed to the collaborators.

For 11 of the 16 analyte-matrix combinations, diluted solutions of SARM were added to a material in which no analyte was already present. In this case the accuracy can be affected only by the propagation of error through the SARM assays and volumetric measurement tolerances (assuming no errors in manipulation). For the remaining five analyte-matrix combinations (DNT matrix C, TNT matrix A, RDX matrices A and D, HMX matrix C), analyte was already present. These existing levels were determined at CRREL by the HPLC method that is under scrutiny in this collaborative study. Because of the added errors inherent in analytical measurement, the "true values" derived in these five cases are subject to a larger degree of uncertainty.

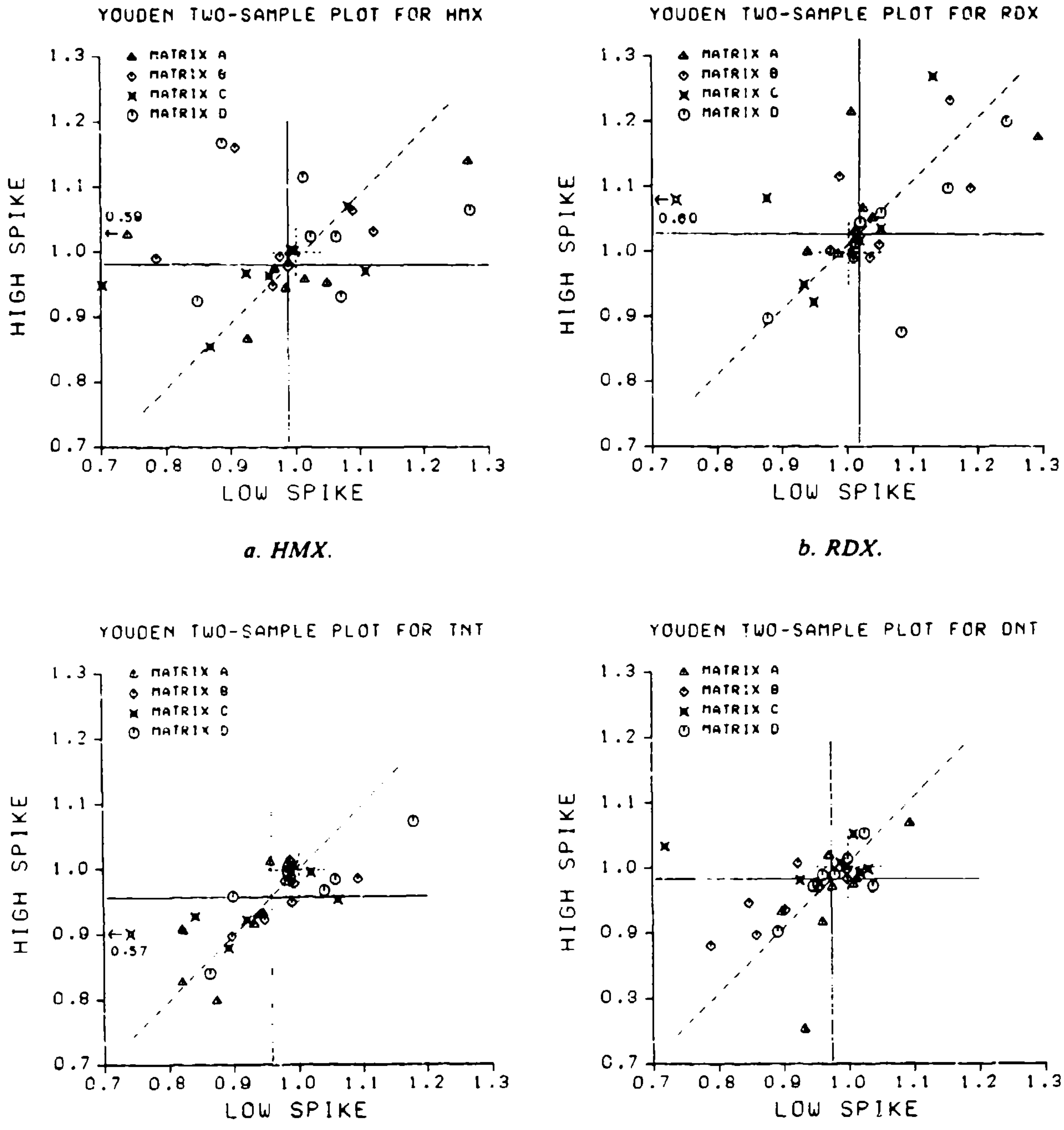
Youden two-sample plots

To aid further with inspection of the results and to begin to consider the problem of outliers, Youden two-sample plots were constructed for each analyte (Fig. 7). A Youden plot (Youden and Steiner 1975) concisely summarizes the relative amount of systematic error between laboratories in comparison to the amount of random error in the method and also indicates the relative accuracy of the results. In these diagrams, the reported concentrations for two of the spiked solutions are plotted against each other. The two solutions involved were those two that were purposely made similar to each other in concentration. (See *Protocol Strategy* section for details.) In order to display these plots in an easily digestible fashion, the data were contracted to permit display of all matrices on a single set of axes: the values plotted were the means of the four replicate determinations, normalized to the true values (Table A9). Eight points were excluded because they were so far off from the expected value and from the other measured values in that data set.

Each plot contains a large amount of information. The higher concentration spike was plotted versus the lower concentration in all cases. The origin of the solid axes locates the medians for the entire data set for that analyte. The shortened dashed axis locates the true values, which after normalization equal (1.0, 1.0). The medians have been used here instead of means because the former are not affected greatly by the few outlying points. Table 20 lists the median values. Both the tabulated values and the Youden plots show that the overall accuracy is quite good, the disparity being 3% or less for DNT, RDX and HMX, and less than 5% for TNT. The shapes of the Youden plot for DNT and TNT hug the 45° line. This indi-

Table 20. Grand medians for each analyte.

Analyte	*Low spike (range)*	*High spike (range)*
DNT	0.970 (0.719-1.094)	0.982 (0.753-1.068)
TNT	0.957 (0.567-1.180)	0.955 (0.798-1.072)
RDX	1.017 (0.601-1.293)	1.030 (0.875-1.268)
HMX	0.990 (0.593-1.272)	0.983 (0.854-1.167)



a. HMX.

b. RDX.

c. TNT.

d. DNT.

Figure 7. Youden two-sample plots.

cates that systematic error between laboratories is larger than the method's random error. For RDX and HMX, the pattern is more circular, indicating that random and systematic errors are more nearly equivalent. Note that the relative amount of systematic error, as shown by the spread of points along the 45° line, is roughly the same for all analytes but that the random error, as shown by the straight-line distances to the 45° line, is larger for RDX and HMX than for DNT and TNT. The most likely source of the systematic error is the calibration procedure.

Table 21. Number of laboratories that fall into each quadrant of the Youden plots for individual matrices.

Matrix	*Analyte*	*Quadrant count (relative to medians)* (+ +)	(- -)	(+ -)	(- +)	*Bias?*
A	DNT	3	3	2	0	
	TNT	0	7	0	1	Low
	RDX	3	3	0	2	
	HMX	1	3	2	2	High
B	DNT	2	5	0	1	Low
	TNT	5	2	1	0	High*
	RDX	2	2	2	1	
	HMX	2	2	0	3	
C	DNT	4	2	0	1	High*
	TNT	4	4	0	0	
	RDX	2	3	1	2	
	HMX	3	4	1	0	
D	DNT	3	2	1	1	
	TNT	5	1	0	1	High*
	RDX	4	2	1	0	High*
	HMX	4	1	1	1	High

* Biased with respect to data medians but not to "true" values.

Method performance for each matrix may be estimated by counting the number of laboratories that fall into each quadrant of the Youden plots (Table 21). By comparing the relative number of points in quadrant I (+ +) vs quadrant III (- -), one can identify where bias exists for particular analytes and particular matrices. The only clear cases of low bias are for TNT and HMX in matrix A (treated pink water) and DNT in matrix B (laboratory water) and of high bias for HMX in matrix D (RDX-contaminated groundwater). Several other combinations are biased with respect to the grand medians, but are instead clustered around the true values. Hence, we did not consider these data to be outliers. The total number of points in the (+ +) and (- -) quadrants compared to the (+ -) and (- +) clearly supports the statement that the major errors are systematic rather than random. Of course, we must remember that only the most extreme outliers have been eliminated from the data at this point.

Rejection of outliers

More sophisticated statistical methods had to be applied at this point to help us decide whether outliers existed and whether or not to reject them. There is a need to be cautious about wielding these methods, as Youden suggests (Youden and Steiner 1975):

> Our task is that of presenting a realistic picture of the population of laboratories. This last objective has to be balanced against the distortion of the picture that would occur from keeping a result so out of line that the estimate of error does not mirror the real merit of the analytical method.... The inclusion in the statistical analysis of even one or two points emphatically apart from the main pattern considerably increases the estimates of standard deviation...obtained. The danger is that a really promising analytical method may fail to receive a positive recommendation for adoption because of a lapse by one or two collaborators.

The particular reasons for excluding outliers are 1) that the HPLC method is being tested here, not the individual laboratories, 2) there is no other way to find mistakes that are not obvious by inspection, and 3) analysis of variance assumes homogeneity in the data set variance.

Lastly, it is inevitable that a data set this large will contain some outliers. Inspection found many instances of suspect values that could not be rejected or corrected by reference to the laboratory notebooks. The collaborators should be commended for their honesty in reporting data that they could have censored had they observed apparently errant values. Rejection of outliers is more safely done with reference to the entire population of analytical results rather than with reference to the results within a single laboratory.

The particular statistical tests applied are described in detail in Youden and Steiner (1975). The tests were applied in the following sequence: ranking test on laboratories, Dixon's range test on individual data values, range test for homogeneity of variance among laboratories, and Cochran's test for homogeneity of variance between replicates. For these calculations, a single analytical datum is defined as the average result on duplicate injections from a given vial. Only the results of these tests in terms of outliers rejected will be discussed here.

Ranking test

The ranking test for laboratories was applied to the collection of means of the four replicated determinations on each spiked sample (Table A8). For each different sample, laboratories were ranked according to their reported concentrations. These rankings were then summed across all the samples. The distribution of total scores was compared to limiting scores expected for the case of completely random errors. Any laboratory having a score outside these limits indicates systematic error. Only one data set could be eliminated without ambiguity: RDX for laboratory 5. In several other instances, the ranking test indicated systematic error, but inspection of the data led us to decide not to reject them because only a few of the concentration values were extreme. These could be eliminated on an individual basis instead of by eliminating the entire laboratory, which would be throwing away many valid data points.

Dixon's test

Next, Dixon's test was used to uncover individual stray data. To apply this test and in preparation for the analysis of variance, we decided to define one "analytical value" as the average of the responses for the two duplicate injections from the same aliquot sample. This reduced the number of apparent replicates per sample to two instead of four. Although averaging eliminates information on the variability between duplicate injections of the same sample, it mimics the probable approach that most analysts would take in practice, namely to base their quantitative result on the average of (at least) two injections instead of just one injection.

Dixon's test is sensitive to values that lie outside the range expected in the case of randomly distributed results. When an individual datum was flagged for rejection, its duplicate was also rejected. (Recall that each datum here represents an analytical measurement of one of two duplicate aliquots removed from each sample.) In order to maintain balance in the data sets, we chose this procedure rather than the alternative of filling in for the rejected datum by calculating an expected value. Table 22 lists the numbers of pairs of values that

Table 22. Catalogue of aliquot pairs rejected on basis of Dixon's test.

	Number rejected			
Laboratory	*DNT*	*TNT*	*TDX*	*HMX*
1	3	1	1	1
2	0	0	1	2
3	2	1	1	1
4	0	0	0	0
5	1	2	18*	2
6	0	1	6	17†
8	5	5	6	17†
9	0	0	0	0
Total rejected	11	10	33	40
Number per laboratory before rejection	17	17	18	17
Total pairs before rejection	136	136	144	136
Percent rejected	8	7	23	29
Percent rejected disregarding rejected laboratories	8	7	12	6

* Entire laboratory rejected via laboratory rank test.

† Entire laboratory rejected because at least half of individuals were outliers.

were rejected. The relative number of outliers for each analyte is between 6 and 12% when excluded laboratories are not considered part of the total. This does not represent a significant loss to the data set and seems to be typical for collaborative studies (Horwitz 1982).

In some of these cases we observed that the results for all four analytes in a given sample were identified as outliers and that the amount of deviation was similar in magnitude and direction. This is a clear indication of mishandling of the sample during processing, such as erroneous use of volumetric glassware.

Note that only those samples with non-zero concentration levels were considered. Specifically, 3 out of the 20 samples (four matrices × five spike levels) contained no DNT, TNT or HMX, and two contained no RDX. These samples were not included because in most cases laboratories reported duplicate "0.0" concentrations. Since the variance here is zero, the within laboratory variance would be decreased in the analysis of variance tests because of addition of degrees of freedom without concommitant increase in the sum of squares. Hence, inclusion of these data falsely sensitizes the tests for homogeneity of variance and the analysis of variance.

Range test

We found the variance among laboratories to be homogeneous by using a range test based on the sums of the data for the duplicate aliquots. A range was calculated for laboratories within each sample. The maximum range was compared with the sum of all the ranges. Only RDX failed this comparison. Although dropping one sample did result in passing the test, we decided that this was undesirable since a large number of values had already been eliminated by previous tests. Furthermore, analysis of variance is a robust test—it can handle a small amount of heterogeneity without risk.

Cochran's test

Finally, Cochran's test compares the maximum variance between the duplicate aliquots with respect to the total sum of squares of duplicates. The results were that DNT and TNT were homogeneous after rejection of the pairs of outliers identified by Dixon's test and that RDX and HMX were slightly heterogeneous. No data, however, were excluded from the latter two analytes because too many values had to be dropped to pass the test. This artificially contracts the variance to levels that are not realistic. Relative to DNT and TNT, RDX and HMX should have larger random error components as indicated by the Youden plots.

Table A10 lists the entire data set, showing which values were rejected using the statistical evaluations described above.

Analysis of variance

Since outliers had been rejected and the data sets were now adequately homogeneous, analysis of variance (ANOVA) was carried out to separate individual contributions to the overall variance (Table 23). Several items in Table 23 must be explained. The degrees of freedom change for each analyte for two reasons: different numbers of rejected outliers and different numbers of samples (17 or 18). Note that for all analytes the laboratories are significantly different from each other. Frankly, we expected this result since the vast majority of collaborative studies show this trend (Youden and Steiner 1975). Furthermore, it is reasonable to expect more variability among several laboratories than within any given laboratory. For RDX and HMX, the laboratory-sample interaction is also significant, indicating an inconsistent bias among laboratories. The size of this effect is much smaller than the consistent laboratory bias, however.

This table also shows the grand average of the measured concentrations, the standard deviation of replication (the within-laboratory standard deviation or the repeatability), and the percent Relative Standard Deviation (RSD). These last values are all between 5 and 9%. The reason that the percent RSDs for RDX and HMX are lower than those of the other analytes is that the average concentrations measured for RDX and HMX were two to three times greater than those for TNT and DNT. Since previous studies at CRREL, as well as this study, have demonstrated that the variance in this concentration range is independent of concentration, the RSD must decrease with increasing concentration.

To demonstrate the effect on ANOVA of not rejecting outliers, the uncensored data set for RDX and HMX was subjected to ANOVA. This is shown in Table 24. The tangible result of ignoring outliers is that all mean square values are larger. Specifically, the interaction between laboratories and samples becomes much stronger, and the standard deviation of replication and the % RSD increase by factors of about 2.5. These values seem uncharacteristically large and give the impression that the HPLC method cannot be expected to achieve precisions better than 12%. The uncen-

Table 23. Analysis of variance.

	SS	*DF*	*MS*	F	*SS*	*DF*	*MS*	F
	DNT				**TNT**			
Total	3,300,508	250			3,474,037	252		
CF	2,848,709	1			2,869,867	1		
Labs	1,385.69	7	197.96	3.76*	6,279.94	7	897.13	10.76*
Samples	438,125	16			575,532	16		
Replicates	6,574.84	125	52.60		10,500.64	126	83.33	
Interaction Lab × sample	5,713.47	101	56.57	1.08†	11,857.42	102	116.25	1.39†

DNT: Grand Average = 106.75; Std. dev. of replication = $\sqrt{\text{Replicate MS}}$ = 7.25; % RSD = 6.79

TNT: Grand Average = 106.72; Std. dev. of replication = $\sqrt{\text{Replicate MS}}$ = 9.13; % RSD = 8.55

	SS	*DF*	*MS*	F	*SS*	*DF*	*MS*	F
	RDX				**HMX**			
Total	21,591,195	222			10,253,207	192		
CF	16,815,188	1			7,883,430	1		
Labs	30,045	6	5,007.5	22.8*	7,525.31	5	1,505.06	14.34*
Samples	4,688,011	17			2,333,177	16		
Replicates	24,326.27	111	219.2		10,075.24	96	104.95	
Interaction Lab × sample	33,624.73	86	391.0	1.78*	18,999.45	74	256.75	2.45*

RDX: Grand Average = 275.22; Std. dev. of replication = $\sqrt{\text{Replicate MS}}$ = 14.8; % RSD = 5.38

HMX: Grand Average = 202.63; Std. dev. of replication = $\sqrt{\text{Replicate MS}}$ = 10.25; % RSD = 5.06

*Significant at 0.99 probability.
† Not significant.

Table 24. Analysis of variance for uncensored RDX and HMX data.

	SS	*DF*	*MS*	F	*SS*	*DF*	*MS*	F
	RDX				**HMX**			
Total	30,478,680	288			15,911,994	272		
CF	23,686,095	1			12,012,847	1		
Labs	143,535	7	20,503.57	17.7*	88,077.3	7	12,582.46	20.8*
Samples	6,097,818	17			3,303,801	16		
Replicates	167,219	144	1,161.24		82,211.3	136	604.5	
Interaction Lab × sample	384,033	119	3,227.17	2.78*	425,057.4	112	3,795.16	6.28*

RDX: Grand Average = 286.78; Std. dev. of replication = $\sqrt{\text{Replicate MS}}$ = 34.1; % RSD = 11.9

HMX: Grand Average = 210.15; Std. dev. of replication = $\sqrt{\text{Replicate MS}}$ = 24.59; % RSD = 11.7

*Significant at 0.99 probability.

Table 25. Repeatability and reproducibility of HPLC method (μg/L).

Analyte	Repeatability	% RSD	Reproducibility	% RSD
DNT	7.25	6.8	7.66	7.2
TNT	9.13	8.6	11.08	10.4
RDX	14.80	5.4	20.80	7.6
HMX	10.25	5.1	14.75	7.3

sored data sets for DNT and TNT were not subjected to ANOVA because there were so few outliers that the changes would have been minimal.

Next, the variance was segregated according to its sources, in particular, so we could calculate the reproducibility (between laboratory variance). This is accomplished easily using the mean square values from the ANOVA tables. The results are listed in Table 25.

The repeatability values in Table 25 represent the standard deviation to be expected for a single determination (based on duplicate injections) by the HPLC method when compared with all other results within one laboratory. The reproducibility values represent the standard deviation to be expected for a single determination by the HPLC method when compared with all other results from many laboratories. As expected, the reproducibility is the larger of the two values, although the magnitude of this difference is not unusually large. It should be recognized that the inclusion of values considered to be outliers would produce a greater increase in the reproducibility estimate than in the repeatability estimate. The reason for this expectation is that most outliers were identified according to their magnitude with respect to the rest of the data set (which contributes to reproducibility) and not according to the amount of variation between duplicates (which contributes to repeatability).

Regression analysis

The last task was to evaluate accuracy by linear least squares regression analysis of "found" concentrations (y) plotted versus "true concentrations" (x). A perfectly accurate method should have an intercept of 0 and a slope of 1.00. Regression equations were determined for each of the four analytes in each of the four matrices using the data after rejection of outliers. The 16 equations are given in Table 26. Clearly, all slopes are quite close to the theoretically expected value of 1.00. Intercepts will be considered below.

For each analyte, an analysis of variance was conducted according to the procedure described in Volk (1958) to test the hypothesis that the slopes for the four matrices were homogeneous. Another way of saying this is accepting the hypothesis means that the amount of deviation removed by fitting individual least squares lines for each matrix, over that removed by using a pooled slope for all four matrices together, is not statistically significant. As shown in Table 27, the hypothesis of homogeneity could not be rejected at the 95% confidence level for any of the analytes. In fact, the largest F ratio found was 1.74 for DNT, with a value of 2.65 required for rejection of the hypothesis. Based on these analysis, we concluded that each analyte could be represented by a single fitted curve regardless of sample matrix. These pooled equations are in Table 28.

Table 28 also shows the least squares equations for the model through the origin, i.e., the model in which the intercept is required to be zero. An F test as described by Youden (1951) was employed to test the hypothesis that the intercepts were equal to zero. For DNT and TNT, it was not possible to reject the hypothesis so we concluded that the model through the origin was the best one to describe the data. For RDX and HMX, the zero intercept hypothesis was rejected and equations with both intercept and slope were deemed best. Appropriate confidence intervals were calculated for the slopes, as shown in Table 29.

To interpret these results for the pooled data, we must remember that the regressions were of "found" concentration versus "true" concentration. Thus, a perfectly accurate method should have an intercept of 0.00 and a slope of 1.00. The intercepts for DNT and TNT have been shown above to be equivalent to 0. In the case of DNT, the slope of the fitted model, 0.986, is extremely close to the theoretically expected value. The small difference may arise from the fact that the SARM used to prepare the spiking solutions at CRREL was from a different batch than the SARM dis-

Table 26. Linear least squares regression equations for each matrix and each analyte (x and y are in μg/L).

	DNT	*TNT*
Matrix A	$y = 1.28 + 0.979x$	$y = -8.26 + 0.964x$
B	$y = 3.20 + 0.909x$	$y = -0.729 + 0.973x$
C	$y = 1.14 + 0.998x$	$y = -3.18 + 0.970x$
D	$y = 3.12 + 0.942x$	$y = 3.14 + 0.960x$
	RDX	*HMX*
Matrix A	$y = 8.75 + 0.997x$	$y = 1.93 + 0.959x$
B	$y = 4.03 + 1.01x$	$y = 8.69 + 0.978x$
C	$y = -0.803 + 1.00x$	$y = 6.88 + 0.951x$
D	$y = 8.49 + 0.984x$	$y = 15.3 + 0.933x$

Table 27. Analysis of variance test for homogeneity of slopes.

Source of variation	*SS*	*df*	*MS*	F
DNT: Between slopes	321.5	3	107.17	1.74*
Error	14,915.5	242	61.63	
TNT: Between slopes	15.3	3	5.1	0.04†
Error	29,266.7	244	119.9	
RDX: Between slopes	471	3	157	0.37
Error	91,054	214	425.5	
HMX: Between slopes	311	3	103.7	0.51
Error:	37,773	184	205.3	

* $F_{0.95}(3,242) = 2.65$.
† Any F value below 1 is not significant.

tributed to the collaborators. Since SARM assays are certified to be at least 98 mole %, this 2% uncertainty could account for the observed slope being slightly less than 1.00. The largest deviation expected from SARM assay inaccuracy is 2.8% as calculated by propagation of errors test (mean square of the 2% inaccuracy for each SARM batch).

For TNT, the slope of 0.944 cannot be attributed to SARM assay differences alone. This means that as a whole the collaborators recovered only 94.4% of the TNT. The reason for this low recovery is probably related to the fact that TNT is susceptible to decomposition by chemical, photochemical and microbial action. This happened despite steps taken during the preparation of matrices and spiking solutions and in the storage of these materials to try to minimize such losses.

Table 28. Linear least squares regression equations for each analyte over all matrices.

	Model with intercept	*Model through origin*
DNT	$y = -2.15 + 1.00x$	*$y = -0.986x$
TNT	$y = -0.798 + 0.950x$	*$y = 0.944x$
RDX	*$y = 5.82 + 0.996x$	$y = 0.996x$
HMX	*$y = 8.36 + 0.955x$	$y = 0.987x$

* These models are the accepted ones.

Table 29. Confidence intervals (95%) for intercepts of accepted models (μg/L).

DNT ±0.0088	TNT ±0.0116
RDX ±0.0186	HMX ± 0.0183

In the case of RDX, the slope of 0.996 indicates nearly quantitative recovery, but a small positive intercept of 5.8 μg/L is found. This intercept could arise either because negative curvature exists in the plot of "found" versus "true," which would tend to cause the fitted linear model to have a smaller slope and larger intercept, or because a small positive bias exists. An inspection of the residuals from the regression analysis showed no indication of curvature; therefore, the bias appears to be real.

Finally for HMX, both the intercept and slope depart from theory but not by a large amount. The small positive intercept represents a real bias since inspection of the regression residuals indicates no curvature in the relationship. The slope value indicates a small loss of HMX, more than can be accounted for by SARM assay errors.

Clearly, the results indicate very good accuracy considering that eight laboratories were represented and all concentrations were below 1 mg/L.

CONCLUSIONS

Given the inevitable errors associated with the quantitative determination of trace level organic compounds in natural waters, the overall performance of the HPLC method for DNT, TNT, RDX and HMX is very good for the concentration ranges studied. The evidence supporting this evaluation is summarized below:

1. For DNT, RDX and HMX the median

"found" concentrations are within 3% of the "true" values. For TNT the difference is within 5%. Considering that the true values themselves are somewhat uncertain, the overall accuracy is very good.

2. The repeatability, based on duplicate injections of each of two aliquots, is about ±7, 9, 15 and 10 μg/L for DNT, TNT, RDX and HMX respectively. These values represent percent relative deviations on the order of 5 to 9%. If single injections were used, the repeatabilities would be inflated by a factor of 1.414 (square root of 2).

3. Reproducibilities for each analyte are about ±6, 21, 40 and 44% greater than repeatabilities for DNT, TNT, RDX and HMX respectively. This gives percent interlaboratory deviations, based on average concentration examined, of about 7% for DNT, RDX and HMX, and 10% for TNT. The most likely source of these differences between laboratories is the calibration of the instrumental response.

4. Recoveries of a given analyte were similar regardless of matrix. Overall, DNT and RDX were recovered quantitatively, and TNT and HMX showed small losses of about 5%.

We found that the standard deviation of replication was independent of concentration in the concentration ranges examined in this collaborative study. This observation confirms a similar finding from Part 1 of this report. Thus the relative standard deviations (RSD) for RDX and HMX are better than those of DNT and TNT when in fact RDX and HMX have poorer absolute precisions. This is clearly shown by the Youden plots, in which RDX and HMX have a much larger degree of scatter than DNT and TNT.

Valid statistical analysis required rejection of about 10% of the individual data values. This was not done blindly but with the aid of chemical intuition and with the view that the method, and not the individual laboratories, was being evaluated. Even where substantial number of outliers were identified, the repeatabilities for those analytes most affected (RDX and HMX) grew from 5% relative to only 12% relative when no values were eliminated. This larger RSD is still quite acceptable for analysis at the microgram-per-litre level.

In order to put the performance of this method in perspective, it is instructive to compare the results of this collaborative study with others dealing with measurement of trace level constituents. Horwitz (1982) has discussed the interrelationship between interlaboratory reproducibility and concentration of analyte. Using data from over 150 independent collaborative studies, he found a clear logarithmic relationship between percent relative standard deviation of reproducibility and concentration of analyte. The reproducibility roughly doubles for each decrease of concentration of two orders of magnitude. Furthermore, this trend is independent of analyte or of analytical method. For the concentration levels measured by the HPLC method in this study, the expected reproducibility according to Horwitz is about 20%. Reproducibilities of 7 to 10% were actually found. The difference is most likely attributable to the fact that the samples distributed to the collaborating laboratories were homogeneous whereas many of the studies cited by Horwitz involved heterogeneous materials

At this point we are confident in recommending that this HPLC method be implemented for monitoring munitions plant wastewaters and natural waters for DNT, TNT, RDX and HMX at the submilligram-per-litre level. The accuracy and reproducibility in the analysis of real environmental samples have proven to be adequate for this task. The instrumental response was calibrated daily by using a single high standard in order to make as much time available for analysis of real samples as possible. This single-standard approach can be implemented efficiently by means of quality control charts.

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APPENDIX A: DATA

Method development

Table A1. Analytical results of linearity tests.

HMX Concentration (µg/L)	HMX Peak Area (integrator units)	HMX Peak height (absorbance units)	RDX Concentration (µg/L)	RDX Peak area (integrator units)	RDX Peak height (absorbance units)
22,3	9707	$1,46\times10^{-4}$	24,8	17222	$2,17\times10^{-4}$
	12872	$1,50\times10^{-4}$		23376	$3,15\times10^{-4}$
	9463	$1,48\times10^{-4}$		14638	$2,19\times10^{-4}$
	$\bar{Y}$=10681			$\bar{Y}$=18412	
	σ^2=3,62E6			σ^2=2,02E7	
55,8	21720	$3,05\times10^{-4}$	62,0	30051	$4,06\times10^{-4}$
	28074	$3,01\times10^{-4}$		31804	$4,13\times10^{-4}$
	21370	$3,13\times10^{-4}$		30837	$4,10\times10^{-4}$
	$\bar{Y}$=23721			$\bar{Y}$=30897	
	σ^2=1,42E7			σ^2=7,71E5	
111,6	39035	$6,00\times10^{-4}$	124,0	61334	$8,52\times10^{-4}$
	35474	$5,91\times10^{-4}$		58965	$8,22\times10^{-4}$
	41373	$6,00\times10^{-4}$		60696	$8,48\times10^{-4}$
	$\bar{Y}$=38627			$\bar{Y}$=60332	
	σ^2=8,82E6			σ^2=1,50E6	
558	187470	$3,29\times10^{-3}$	620	288580	$4,74\times10^{-3}$
	191470	$3,23\times10^{-3}$		289080	$4,65\times10^{-3}$
	192390	$3,18\times10^{-3}$		305970	$4,65\times10^{-3}$
	$\bar{Y}$=190443			$\bar{Y}$=294543	
	σ^2=6,84E6			σ^2=9,80E7	
1116	379810	$6,30\times10^{-3}$	1240	591220	$9,29\times10^{-3}$
	378210	$6,46\times10^{-3}$		574470	$9,22\times10^{-3}$
	379800	$6,46\times10^{-3}$		584170	$9,34\times10^{-3}$
	$\bar{Y}$=379273			$\bar{Y}$=583287	
	σ^2=8,48E5			σ^2=7,07E7	
2232	752980	$1,26\times10^{-2}$	2480	1146000	$1,81\times10^{-2}$
	755900	$1,27\times10^{-2}$		1144400	$1,81\times10^{-2}$
	756130	$1,28\times10^{-2}$		1148700	$1,83\times10^{-2}$
	$\bar{Y}$=755003			$\bar{Y}$=1146367	
	σ^2=3,08E6			σ^2=7,07E7	
5580	1883800	$2,60\times10^{-2}$	6200	2846200	$3,95\times10^{-2}$
	1885700	$2,86\times10^{-2}$		2854600	$4,32\times10^{-2}$
	1890100	$2,86\times10^{-2}$		2864500	$4,31\times10^{-2}$
	$\bar{Y}$=1886533			$\bar{Y}$=2855100	
	σ^2=1,04E7			σ^2=8,39E7	
17,2	22386	$2,00\times10^{-4}$	12,8	18044	$1,80\times10^{-4}$
	18883	$1,79\times10^{-4}$		16891	$1,75\times10^{-4}$
	20296	$1,97\times10^{-4}$		25403	$2,17\times10^{-4}$
	$\bar{Y}$=20522			$\bar{Y}$=20113	
	σ^2=3,11E6			σ^2=2,13E7	
43,0	38434	$4,28\times10^{-4}$	32,0	44425	$4,01\times10^{-4}$
	42977	$4,23\times10^{-4}$		38845	$3,98\times10^{-4}$
	46234	$4,05\times10^{-4}$		34601	$3,89\times10^{-4}$
	$\bar{Y}$=42548			$\bar{Y}$=39290	
	σ^2=1,53E7			σ^2=2,43E7	
86,0	84853	$8,85\times10^{-4}$	64,0	84288	$8,26\times10^{-4}$
	98201	$8,66\times10^{-4}$		85186	$8,07\times10^{-4}$
	86431	$8,78\times10^{-4}$		92984	$8,23\times10^{-4}$
	$\bar{Y}$=89828			$\bar{Y}$=87486	
	σ^2=5,32E7			σ^2=2,29E7	

Table A1 (cont'd). Analytical results of linearity tests.

TNT Concentration (µg/L)	TNT Peak area (integrator units)	TNT Peak height (absorbance units)	2,4-DNT Concentration (µg/L)	2,4-DNT Peak area (integrator units)	2,4-DNT Peak height (absorbance units)
430	411360	$4,93 \times 10^{-3}$	320	403880	$4,57 \times 10^{-3}$
	413900	$4,94 \times 10^{-3}$		405520	$4,61 \times 10^{-3}$
	414770	$4,67 \times 10^{-3}$		405930	$4,41 \times 10^{-3}$
	$\bar{Y}$=413343			$\bar{Y}$=405110	
	σ^2=3,14E6			σ^2=1,18E6	
860	826690	$9,60 \times 10^{-3}$	640	815160	$9,06 \times 10^{-3}$
	825590	$9,68 \times 10^{-3}$		808080	$9,17 \times 10^{-3}$
	834460	$9,62 \times 10^{-3}$		815120	$9,09 \times 10^{-3}$
	$\bar{Y}$=828910			$\bar{Y}$=812787	
	σ^2=2,34E7			σ^2=1,66E7	
1720	1650600	$1,92 \times 10^{-2}$	1280	1625500	$1,81 \times 10^{-2}$
	1659900	$1,92 \times 10^{-2}$		1628200	$1,83 \times 10^{-2}$
	1655800	$1,93 \times 10^{-2}$		1633200	$1,84 \times 10^{-2}$
	$\bar{Y}$=1655433			$\bar{Y}$=1628967	
	σ^2=2,17E7			σ^2=1,55E7	
4300	4119300	$4,30 \times 10^{-2}$	3200	4041100	$4,10 \times 10^{-2}$
	4134800	$4,67 \times 10^{-2}$		4055100	$4,44 \times 10^{-2}$
	4135200	$4,67 \times 10^{-2}$		4068400	$4,45 \times 10^{-2}$
	$\bar{Y}$=4129767			$\bar{Y}$=4054867	
	σ^2=8,22E7			σ^2=1,86E8	

Table A2. Results of recovery study for analytes in filtered waters, concentration in µg/L.

Sample	Replicate 1 A	Replicate 1 B	Replicate 2 A	Replicate 2 B	Replicate 3 A	Replicate 3 B	Mean
				HMX			
Milli-Q (unfiltered)	61,7	65,3	61,7	65,3	65,3	63,3	63,2
Milli-Q	61,1	63,3	64,2	65,9	68,1	63,9	64,1
Groundwater	62,5	64,4	52,5	64,4	66,9	61,1	63,6
Tapwater	60,6	64,4	61,1	62,5	58,9	63,6	61,9
Conn. River	61,1	59,7	61,7	61,1	64,4	56,9	60,8
Pond water	58,1	55,6	57,8	60,6	56,1	61,1	58,4
				RDX			
Milli-Q (unfiltered)	47,5	49,3	47,5	50,0	49,5	45,8	48,3
Milli-Q	49,2	49,2	47,3	47,6	52,4	48,8	49,1
Groundwater	47,8	46,6	47,8	49,2	45,6	46,1	47,2
Tapwater	44,9	50,8	46,1	49,5	47,5	49,0	48,0
Conn. River	48,8	47,1	45,8	47,8	49,0	43,9	47,1
Pond water	43,9	49,2	48,3	47,1	47,1	46,1	47,0
				TNT			
Milli-Q (unfiltered)	28,9	30,7	30,6	28,4	30,7	28,1	29,6
Milli-Q	28,6	29,9	29,9	27,4	29,9	27,0	28,8
Groundwater	30,7	29,7	29,0	29,5	32,1	28,0	29,8
Tapwater	27,9	28,4	29,8	28,5	28,5	29,0	28,7
Conn. River	27,3	29,0	27,2	30,7	27,3	27,3	28,1
Pond water	28,4	28,1	28,1	29,6	30,7	27,0	28,7
				DNT			
Milli-Q (unfiltered)	36,9	36,4	37,3	37,3	38,2	35,3	36,9
Milli-Q	35,0	36,3	37,3	36,0	36,5	35,7	36,1
Groundwater	37,2	37,6	38,3	36,4	36,2	36,0	37,0
Tapwater	35,7	37,9	35,7	36,0	37,3	38,1	36,8
Conn. River	35,7	36,4	36,4	36,3	36,6	33,6	35,8
Pond water	36,4	36,6	36,4	37,9	38,8	36,5	37,1

Table A3. Detection limit test.

Sample	Concentration (µg/L)	Integrator Units Day 1	Day 2	Day 3	Day 4
			HMX		
0.5 x	12.55	5470	8226	4944	4122
		5161	6058	1813	2717
		6189	7961	4080	4808
		10021	4073	3557	2583
1 x	24.51	9799	12199	9832	11280
		14258	8571	10183	9320
		13953	13774	13393	6363
		8696	10334	10390	8516
1.5 x	36.76	15296	12994	15573	12387
		17054	14227	10658	4442
		16339	12928	14201	15416
		9807	13714	15450	11514
2 x	49.02	19857	17974	15054	[illegible]
		22153	17988	15671	[illegible]
		21407	16601	19435	[illegible]
		19578	22953	24936	23106
5 x	122.55	39900	46777	46132	44799
		40092	44212	48049	40623
		39996	44855	52438	43555
		43064	44413	42379	45404
10 x	245.1	85817	86432	92414	85057
		89839	88957	91065	91337
		86705	88328	87972	86766
		78953	89659	89836	90021
20 x	490.2	191920	174210	174200	180610
		178900	178450	176330	171750
		173000	171170	176480	177980
		179720	172130	175120	176620
			RDX		
0.5 x	13.63	10827	9102	4283	9332
		7998	4725	6632	5926
		8052	8229	6022	8313
		10021	4005	7169	5609
1 x	27.26	13539	16224	11374	12974
		13811	16999	16627	14896
		16864	21338	15723	11115
		8696	15639	16487	13013
1.5 x	40.89	23896	18606	20011	18122
		27645	20954	20042	21994
		22255	23802	20306	18335
		22042	19091	20540	19704
2 x	54.52	23414	25833	22073	28472
		24933	27443	22480	39795
		31127	27310	35456	27864
		29914	27636	27861	26234
5 x	136.3	65160	64177	66898	63175
		65584	70117	61318	69453
		62131	70842	65691	62227
		64665	68595	62503	68770
10 x	272.6	130950	132570	130050	130990
		131500	132880	139980	129980
		129140	130580	133520	127210
		116950	130380	131960	132070
20 x	545.2	283470	257350	260890	268830
		268770	260470	264420	254050
		260400	263800	266370	267950
		262480	261340	259250	256170

Sample	Concentration (µg/L)	Integrator Units Day 1	Day 2	Day 3	Day 4
			TNT		
0.5 x	7.72	8886	12670	15149	5511
		5427	7777	9332	8706
		4447	4687	3968	2565
		6869	3116	4217	4157
1 x	15.44	8884	13679	16627	16046
		13899	15892	10961	16144
		14584	20084	14178	20390
		17651	20083	13784	20226
1.5 x	23.16	29895	25004	10295	20357
		26544	19894	31070	24452
		21521	23618	26327	31302
		33471	12561	24289	17398
2 x	30.88	29535	28553	28822	32068
		29180	35802	25531	31266
		30844	30918	26888	32269
		30389	37674	35908	35399
5 x	77.2	80861	72409	84584	71905
		77016	75697	77560	78429
		82260	73255	80177	73795
		74898	84961	75760	72374
10 x	154.4	169020	154840	144850	149290
		172710	156130	151150	147460
		155010	153930	154660	157100
		140380	155490	161600	158510
20 x	308.3	301420	304960	309680	306450
		352540	313790	307150	296040
		304590	311760	304800	301190
		305020	298090	306580	315900
			DNT		
0.5 x	6.4	2356	4361	3544	1815
		2072	2098	8431	4325
		13502	5590	6398	4538
		15304	2081	7941	12777
1 x	12.8	15255	11652	17582	7395
		19140	9834	18771	15072
		18950	10123	16074	18421
		20986	16286	15826	23091
1.5 x	19.2	29875	26739	24828	29636
		24341	26862	26147	26479
		27416	29507	27307	24133
		10636	13846	25633	24281
2 x	25.6	29654	39336	32735	31287
		30818	34449	27855	34230
		38308	39463	25039	33136
		35157	38000	43303	34307
5 x	64.0	87897	81195	85665	88945
		83164	82577	85145	84416
		91657	83519	87978	89198
		85272	87709	85275	72881
10 x	128.0	167080	169940	171370	164410
		196730	173250	169360	167960
		169810	172310	167050	180110
		149740	172880	176820	171140
20 x	256.0	340580	329940	334050	333320
		352540	333430	342800	325880
		336620	337820	337970	334710
		344340	334220	336770	331450

Table A4. Factors employed in 2^4 factorial experiment to test the ruggedness of HPLC determination of HMX, RDX, TNT and 2, 4-DNT in aqueous samples.

Factors	Coded levels (+)	Coded levels (-)
X_1 = Sample storage (2 days)	Glass vials	Polyethylene vials
X_2 = Portion of filtrate from 0.4 μm nuclepore filter	First 10 mL	Second 10 mL
X_3 = Equilibration time with methanol before filtration	15 minutes	4 hours
X_4 = Volume ratio of sample-to-methanol	8/10	10/8

Table A5. Design and interaction matrices in coded units.

Trial	X_1	X_2	X_3	X_4	X_1X_2	X_1X_3	X_1X_4	X_2X_3	X_2X_4	X_3X_4	$X_1X_2X_3$	$X_1X_2X_4$	$X_1X_3X_4$	$X_2X_3X_4$	$X_1X_2X_3X_4$
1	+1	+1	+1	+1	+1	+1	+1	+1	+1	+1	+1	+1	+1	+1	+1
2	+1	+1	+1	-1	+1	+1	-1	+1	-1	-1	+1	-1	-1	-1	-1
3	[illegible]	-1	+1	-1	-1	+1	-1	-1	+1	-1	-1	+1	-1	+1	+1
4	+1	-1	+1	+1	-1	+1	+1	-1	-1	+1	-1	-1	+1	-1	-1
5	+1	+1	-1	-1	+1	-1	-1	-1	-1	+1	-1	-1	+1	+1	+1
6	+1	+1	-1	+1	+1	-1	+1	-1	+1	-1	-1	+1	-1	-1	-1
7	+1	-1	-1	+1	-1	-1	+1	+1	-1	-1	+1	-1	-1	+1	+1
8	+1	-1	-1	-1	-1	-1	-1	+1	+1	+1	+1	+1	+1	-1	-1
9	-1	+1	+1	-1	-1	-1	+1	+1	-1	-1	-1	+1	+1	-1	+1
10	-1	+1	+1	+1	-1	-1	-1	+1	+1	+1	-1	-1	-1	+1	-1
11	-1	-1	+1	+1	+1	-1	-1	-1	-1	+1	+1	+1	-1	-1	+1
12	-1	-1	+1	-1	+1	-1	+1	-1	+1	-1	+1	-1	+1	+1	-1
13	-1	+1	-1	+1	-1	+1	-1	-1	+1	-1	+1	-1	+1	-1	+1
14	-1	+1	-1	-1	-1	+1	+1	-1	-1	+1	+1	+1	-1	+1	-1
15	-1	-1	-1	-1	+1	+1	+1	+1	+1	+1	-1	-1	-1	-1	+1
16	-1	-1	-1	+1	+1	+1	-1	+1	-1	-1	-1	+1	+1	+1	-1

Table A6. Analysis of variance for ruggedness test. The complete experimental design appears in Tables A4 and A5 and the results are in Table 9.

Variable	Effect (µg/L)	Sum of squares	Degrees of freedom	Mean squares	F*
			2,4-DNT		
Total		1385447,000	32		
C.F.**	202,413	1311072,000	1		
X_1	+3,955	124,998	1	Same as S.S.	0,27
X_2	-9,396	706,222	1	"	1,52
X_3	-6,451	332,949	1	"	0,72
X_4	-75,04	45052,515	1	"	97,0*
X_1X_2	-16,256	2114,125	1	"	4,55*
X_1X_3	+13,114	1375,764	1	"	2,96
X_1X_4	-22,143	3922,544	1	"	8,44*
X_2X_3	-13,314	1418,048	1	"	3,05
X_2X_4	+7,713	475,938	1	"	1,02
X_3X_4	-16,713	2510,038	1	"	5,40*
$X_1X_2X_3$	-8,428	568,266	1	"	1,22
$X_1X_2X_4$	+14,333	1643,364	1	"	3,54
$X_1X_3X_4$	-14,489	1679,391	1	"	3,61
$X_2X_3X_4$	+14,428	1665,222	1	"	3,58
$X_1X_2X_3X_4$	+20,473	3353,191	1	"	7,22*
Error		7434,02	16	464,626	

Standard Deviation (S_y) based on duplicates is equal to $\sqrt{\text{Error MS}}$ = <u>21,55</u>

For the entire experiment, the percent relative standard deviation (% RSD) is given by

$$\% \text{ RSD} = \frac{s_y (100)}{\text{grand mean}} = \frac{(21,55)(100)}{202,413} = 10,7\%$$

Variable	Effect (µg/L)	Sum of squares	Degrees of freedom	Mean squares	F*
			RDX		
Total		558395,000	32		
C.F.**	131,357	552150,000	1		
X_1	-2,982	71,156	1	Same as S.S.	0,99
X_2	-1,875	28,136	1	"	0,39
X_3	-4,521	163,479	1	"	2,26
X_4	-21,429	3673,531	1	"	50,9*
X_1X_2	+0,693	3,850	1	"	0,05
X_1X_3	+1,733	24,016	1	"	0,33
X_1X_4	+2,585	53,447	1	"	0,75
X_2X_3	+3,559	101,350	1	"	1,40
X_2X_4	-4,317	149,082	1	"	2,06
X_3X_4	-2,047	33,524	1	"	0,46
$X_1X_2X_3$	+4,500	161,978	1	"	2,24
$X_1X_2X_4$	-4,325	149,619	1	"	2,07
$X_1X_3X_4$	-4,383	153,672	1	"	2,13
$X_2X_3X_4$	-5,793	268,448	1	"	3,71
$X_1X_2X_3X_4$	-2,520	54,905	1	"	0,76
Error		1155,02	16	72,189	

Standard Deviation (S_y) based on duplicates is equal to $\sqrt{\text{Error MS}}$ = <u>8,50</u>

For the entire experiment, the % relative standard deviation (% RSD) is given by

$$\% \text{ RSD} = \frac{s_y (100)}{\text{grand mean}} = \frac{(8,50)(100)}{131,357} = 6,47\%$$

* $F_{.95}(1,16) = 4,49$; an F value in the table above which exceeds 4,49 is significant at the 95% probability level.

** C.F. = Correction factor $\left(\frac{(\Sigma y)^2}{n}\right)$. The difference between the total and C.F. is the total corrected sum of squares.

Table A6 (cont'd). Analysis of variance for ruggedness test.

Variable	Effect (µg/L)	Sum of squares	Degrees of Freedom	Mean squares	F*
			TNT		
Total		1025705.000	32		
C.F.**	177.352	1006514.000	1		
X_1	-1,350	14.585	1	Same as S.S.	0.12
X_2	+3.699	109.487	1	"	0.91
X_3	-7.598	461.852	1	"	3.82
X_4	-43.589	15200.269	1	"	125.*
X_1X_2	+4.232	143.279	1	"	1.19
X_1X_3	-0.251	0.502	1	"	<0.01
X_1X_4	-1.500	18.002	1	"	0.15
X_2X_3	+2.610	54.481	1	"	0.45
X_2X_4	+0.403	1.302	1	"	0.01
X_3X_4	-6.468	334.629	1	"	2.77
$X_1X_2X_3$	+1.860	27.679	1	"	0.23
$X_1X_2X_4$	+7.522	452.629	1	"	3.75
$X_1X_3X_4$	-2.215	39.254	1	"	0.32
$X_2X_3X_4$	-4.647	172.771	1	"	1.43
$X_1X_2X_3X_4$	+5.332	227.420	1	"	1.88
Error		1932.94	16	120.809	

Standard Deviation (S_y) based on duplicates is equal to $\sqrt{\text{Error MS}}$ = 11.0

For the entire experiment, the % relative standard deviation (% RSD) is given by

$$\% \text{ RSD} = \frac{s_y\,(100)}{\text{grand mean}} = \frac{(11.0)\,(100)}{177.352} = 6.20\%$$

Variable	Effect (µg/L)	Sum of squares	Degrees of Freedom	Mean squares	F*
			2,4-DNT		
Total		302434.000	32		
C.F.**	95.778	293548.000	1		
X_1	+7.663	469.711	1	Same as S.S.	5.75*
X_2	+7.268	0.038	1	"	5.17*
X_3	-0.069	0.038	1	"	<0.01
X_4	-25.882	5358.972	1	"	65.6*
X_1X_2	+5.998	287.838	1	"	3.92
X_1X_3	-2.734	59.817	1	"	0.73
X_1X_4	-2.024	32.775	1	"	0.40
X_2X_3	+3.384	91.498	1	"	1.12
X_2X_4	-4.621	170.825	1	"	2.09
X_3X_4	-3.464	95.998	1	"	1.18
$X_1X_2X_3$	+6.549	343.089	1	"	4.20
$X_1X_2X_4$	-4.160	138.474	1	"	1.70
$X_1X_3X_4$	+1.442	16.644	1	"	0.20
$X_2X_3X_4$	-1.257	12.632	1	"	0.15
$X_1X_2X_3X_4$	-3.141	78.914	1	"	0.97
Error		1306.89	16	81.681	

Standard Deviation (S_y) based on duplicates is equal to $\sqrt{\text{Error MS}}$ = 9.04

For the entire experiment, the % relative standard deviation (% RSD) is given by

$$\% \text{ RSD} = \frac{s_y\,(100)}{\text{grand mean}} = \frac{(9.04)\,(100)}{95.778} = 9.45\%$$

*$F_{.95}(1,16) = 4.49$; an F value in the table above which exceeds 4.49 is significant at the 95% probability level.

**C.F. = Correction factor $\left(\frac{(\Sigma y)^2}{n}\right)$. The difference between the total and C.F. is the total corrected sum of squares.

Table A7. Concentrations of DNT, TNT, RDX, and HMX ($\mu g/L$) reported by laboratories participating in collaborative test of HPLC method. (First set of four columns list identification indices for the other columns; remaining columns are also in sets of four—four analytes per laboratory.)

— PRINT C1-C4

COLUMN COUNT ROW	MATRIX 80	SPIKE 80	VIAL 80	INJECTN 80
1	1. = A	0. = unspiked	1.	1.
2	1.	0.	1.	2.
3	1.	0.	2.	1.
4	1.	0.	2.	2.
5	1.	1.	1.	1.
6	1.	1.	1.	2.
7	1.	1.	2.	1.
8	1.	1.	2.	2.
9	1.	2.	1.	1.
10	1.	2.	1.	2.
11	1.	2.	2.	1.
12	1.	2.	2.	2.
13	1.	3.	1.	1.
14	1.	3.	1.	2.
15	1.	3.	2.	1.
16	1.	3.	2.	2.
17	1.	4.	1.	1.
18	1.	4.	1.	2.
19	1.	4.	2.	1.
20	1.	4.	2.	2.
21	2. = B	0.	1.	1.
22	2.	0.	1.	2.
23	2.	0.	2.	1.
24	2.	0.	2.	2.
25	2.	1.	1.	1.
26	2.	1.	1.	2.
27	2.	1.	2.	1.
28	2.	1.	2.	2.
29	2.	2.	1.	1.
30	2.	2.	1.	2.
31	2.	2.	2.	1.
32	2.	2.	2.	2.
33	2.	3.	1.	1.
34	2.	3.	1.	2.
35	2.	3.	2.	1.
36	2.	3.	2.	2.
37	2.	4.	1.	1.
38	2.	4.	1.	2.
39	2.	4.	2.	1.
40	2.	4.	2.	2.
41	3. = C	0.	1.	1.
42	3.	0.	1.	2.
43	3.	0.	2.	1.
44	3.	0.	2.	2.
45	3.	1.	1.	1.
46	3.	1.	1.	2.
47	3.	1.	2.	1.
48	3.	1.	2.	2.
49	3.	2.	1.	1.
50	3.	2.	1.	2.

Table A7 (cont'd) Concentrations of DNT, TNT, RDX, and HMX (μg/L) reported by laboratories participating in collaborative test of HPLC method.

51	3.		2.	2.	1.
52	3.		2.	2.	2.
53	3.		3.	1.	1.
54	3.		3.	1.	2.
55	3.		3.	2.	1.
56	3.		3.	2.	2.
57	3.		4.	1.	1.
58	3.		4.	1.	2.
59	3.		4.	2.	1.
60	3.		4.	2.	2.
61	4.	– D	0.	1.	1.
62	4.		0.	1.	2.
63	4.		0.	2.	1.
64	4.		0.	2.	2.
65	4.		1.	1.	1.
66	4.		1.	1.	2.
67	4.		1.	2.	1.
68	4.		1.	2.	2.
69	4.		2.	1.	1.
70	4.		2.	1.	2.
71	4.		2.	2.	1.
72	4.		2.	2.	2.
73	4.		3.	1.	1.
74	4.		3.	1.	2.
75	4.		3.	2.	1.
76	4.		3.	2.	2.
77	4.		4.	1.	1.
78	4.		4.	1.	2.
79	4.		4.	2.	1.
80	4.		4.	2.	2.

-- PRINT C10-C13

COLU#N	ONT LAB1	TNT LAB1	RCX LAB1	HMX LAB1
COUNT	80	80	80	80
ROW				
1	0.000	29.800	54.900	0.000
2	0.000	27.200	50.500	0.000
3	0.000	27.400	58.100	0.000
4	0.000	32.300	58.400	0.000
5	48.500	63.900	149.900	35.800
6	48.300	60.900	151.600	36.800
7	50.000	62.800	145.500	32.300
8	46.900	60.300	148.400	37.700
9	84.900	133.300	247.200	116.100
10	87.900	131.100	257.600	131.300
11	83.500	129.400	244.300	119.700
12	87.800	135.800	252.700	129.000
13	92.100	142.000	273.300	131.700
14	90.500	143.500	260.200	120.700
15	96.400	143.600	273.300	127.200
16	95.300	147.600	304.100	129.600
17	124.300	203.900	555.500	202.800
18	125.300	197.600	539.500	209.200
19	126.600	205.500	544.600	208.300
20	125.700	202.300	558.600	203.800
21	0.000	0.000	0.000	0.000
22	0.000	0.000	0.000	0.000
23	0.000	0.000	0.000	0.000
24	0.000	0.000	0.000	0.000
25	48.900	47.300	61.200	61.000

Table A7 (cont'd).

26	52.700	43.900	62.300	64.100
27	48.300	47.100	72.000	60.900
28	52.000	39.600	68.800	57.700
29	83.100	121.700	274.900	222.600
30	87.800	121.600	265.100	212.000
31	138.300	177.100	248.800	211.900
32	85.300	121.200	251.900	214.200
33	100.200	133.900	287.800	232.500
34	97.800	137.800	271.800	234.900
35	162.400	197.300	280.100	232.100
36	98.900	135.300	261.800	229.200
37	63.000	88.000	376.700	312.400
38	58.400	77.200	375.100	313.300
39	61.100	77.000	374.600	320.700
40	64.300	76.700	382.800	315.800
41	56.300	0.000	0.000	113.400
42	58.700	0.000	0.000	118.800
43	52.700	0.000	0.000	116.400
44	54.900	0.000	0.000	116.500
45	140.700	63.900	379.400	446.200
46	134.500	67.500	417.200	440.700
47	147.400	85.200	377.000	430.500
48	141.400	62.200	383.100	436.600
49	204.200	157.300	279.600	347.900
50	235.700	191.300	238.000	362.200
51	206.100	152.600	234.500	357.300
52	191.000	111.500	284.100	353.500
53	185.700	132.900	243.900	337.700
54	183.800	132.600	212.900	330.000
55	196.100	142.300	257.600	333.300
56	184.700	134.900	212.000	330.000
57	127.700	37.300	74.700	180.300
58	131.000	35.400	67.200	182.700
59	129.600	40.800	54.000	173.000
60	120.400	35.900	70.700	180.900
61	0.000	0.000	95.700	0.000
62	0.000	0.000	109.400	0.000
63	0.000	0.000	90.800	0.000
64	0.000	0.000	113.300	0.000
65	123.400	173.500	570.600	205.100
66	121.300	170.000	595.400	204.900
67	139.000	154.300	557.000	203.100
68	154.500	135.800	569.300	206.600
69	63.900	105.500	325.300	136.500
70	107.400	126.000	324.700	136.800
71	64.000	99.500	336.300	137.700
72	104.900	126.400	322.800	131.300
73	178.000	206.300	463.100	260.900
74	174.600	206.600	481.300	246.700
75	180.700	205.800	490.700	251.800
76	173.100	198.900	466.400	233.300
77	40.900	22.400	197.400	39.700
78	35.500	24.600	208.500	38.100
79	45.600	31.100	199.300	47.100
80	35.300	18.900	177.900	38.800

— PRINT C20-C23

COLUMN	DNT LAB2	TNT LAB2	RDX LAB2	HMX LAB2
COUNT	80	80	80	80
ROW				
1	0.000	37.000	198.900	0.000
2	0.000	30.900	202.100	0.000

Table A7 (cont'd). Concentrations of DNT, TNT, RDX, and HMX (μg/L) reported by laboratories participating in collaborative test of HPLC method.

3	0.000	26.200	76.400	0.000
4	0.000	33.000	113.800	0.000
5	46.900	64.400	159.100	46.300
6	48.000	67.300	163.600	33.400
7	52.400	73.200	163.800	46.000
8	46.300	67.600	158.100	48.900
9	86.900	129.100	267.900	129.100
10	86.200	134.300	266.900	126.100
11	85.500	138.300	264.200	128.100
12	89.100	138.200	256.700	135.400
13	106.400	157.800	292.900	147.200
14	108.900	165.000	283.400	145.400
15	102.900	151.700	301.500	143.100
16	97.400	152.400	295.400	137.000
17	121.300	207.100	574.700	225.300
18	125.200	208.000	570.900	237.400
19	131.100	207.700	553.000	222.300
20	124.100	207.400	545.400	222.800
21	0.000	0.000	0.000	0.000
22	0.000	0.000	0.000	0.000
23	0.000	0.000	0.000	0.000
24	0.000	0.000	0.000	0.000
25	64.100	53.900	71.100	73.800
26	62.900	54.100	69.000	77.700
27	62.600	54.700	76.400	123.700
28	62.100	49.600	79.100	186.700
29	116.000	142.200	262.500	286.000
30	116.300	138.000	264.900	229.100
31	112.400	135.300	254.100	223.000
32	114.100	125.900	245.100	233.200
33	127.200	157.100	280.500	243.400
34	126.100	153.900	259.300	230.600
35	128.900	156.700	272.200	331.300
36	128.400	156.500	268.700	236.100
37	79.200	90.700	374.500	423.600
38	78.600	90.000	367.800	328.600
39	75.500	84.700	373.100	316.500
40	76.000	85.300	364.100	343.400
41	72.000	0.000	0.000	128.200
42	72.400	0.000	0.000	114.700
43	71.200	0.000	0.000	125.000
44	77.000	0.000	0.000	128.100
45	153.400	90.800	380.500	446.500
46	150.000	86.800	379.300	452.200
47	147.200	82.500	374.100	461.000
48	151.200	88.800	399.600	453.300
49	198.800	155.000	274.600	359.900
50	199.700	149.900	291.900	368.800
51	202.000	157.700	288.000	365.900
52	189.900	150.100	275.000	337.700
53	194.100	146.700	259.300	350.400
54	183.200	136.200	257.100	342.900
55	193.500	136.200	264.400	491.700
56	186.300	140.000	262.700	354.800
57	133.500	51.400	76.100	193.400
58	135.400	50.000	74.200	198.300
59	139.000	54.600	78.800	201.800
60	136.300	53.800	76.600	189.700
61	0.000	0.000	174.900	0.000
62	0.000	0.000	122.500	0.000
63	0.000	0.000	117.700	0.000
64	0.000	0.000	130.200	0.000
65	123.400	177.900	624.200	229.900
66	121.300	165.800	614.200	223.500

Table A7 (cont'd).

67	118.300	162.400	593.500	200.100
68	128.600	174.400	634.400	222.600
69	99.500	120.800	344.900	166.100
70	101.000	113.900	366.300	164.300
71	99.600	113.900	349.100	164.200
72	103.400	121.100	357.800	160.400
73	86.400	102.000	326.800	134.800
74	86.400	99.200	314.400	140.600
75	84.700	102.800	337.200	131.900
76	86.900	101.700	324.900	135.200
77	53.100	39.200	219.300	46.300
78	48.600	31.800	312.800	73.500
79	46.900	34.400	228.500	64.800
80	48.500	32.700	227.200	50.700

-- PRINT C30-C33

COLUMN	DNT LAB3	TNT LAB3	RDX LAB3	HMX LAB3
COUNT	80	80	80	80
ROW				
1	0.000	29.600	76.600	0.000
2	0.000	19.200	90.300	0.000
3	0.000	28.000	74.200	0.000
4	0.000	18.200	58.100	0.000
5	56.000	41.900	181.500	53.100
6	48.400	49.100	183.600	53.200
7	50.300	81.300	160.900	58.200
8	52.700	67.300	169.900	51.200
9	107.600	124.700	278.400	128.600
10	97.800	131.400	247.200	140.400
11	81.500	120.600	278.000	154.200
12	74.500	87.600	237.900	104.600
13	93.500	123.700	310.000	153.200
14	102.800	146.000	301.400	120.700
15	93.500	138.300	285.700	144.600
16	107.900	152.900	292.900	136.700
17	139.600	187.200	539.900	210.600
18	127.900	166.800	609.700	221.900
19	172.400	196.800	566.400	252.100
20	180.300	223.800	586.200	205.400
21	0.000	0.000	0.000	0.000
22	0.000	0.000	0.000	0.000
23	0.000	0.000	0.000	0.000
24	0.000	0.000	0.000	0.000
25	52.200	71.600	106.800	87.600
26	60.100	51.400	98.600	87.300
27	53.900	38.100	87.800	56.500
28	62.500	31.100	84.000	87.500
29	98.200	103.900	230.300	191.600
30	107.300	117.200	260.700	200.700
31	88.400	117.300	276.100	221.500
32	68.400	153.300	213.900	196.300
33	105.100	153.100	318.200	275.500
34	106.400	124.700	277.100	300.700
35	122.200	139.200	314.600	228.900
36	117.600	135.400	307.100	331.300
37	71.800	95.100	384.800	328.300
38	56.900	67.000	460.400	298.900
39	72.700	76.700	391.900	323.100
40	59.600	90.800	355.900	341.800
41	69.200	0.000	0.000	86.400
42	82.300	0.000	0.000	152.000
43	70.400	0.000	0.000	107.300
44	68.900	0.000	0.000	111.200
45	165.900	74.100	377.900	434.900
46	137.900	72.900	363.000	381.500

Table A7 (cont'd). Concentrations of DNT, TNT, RDX, and HMX (μg/L) reported by laboratories participating in collaborative test of HPLC method.

47	154.600	82.600	400.400	406.700
48	148.200	85.900	363.500	402.300
49	198.000	124.400	297.200	333.600
50	203.400	142.700	297.800	335.600
51	207.700	130.500	285.300	334.300
52	213.400	156.600	297.000	395.300
53	132.600	85.600	145.500	245.500
54	137.900	80.700	168.400	243.300
55	129.600	70.900	141.400	243.300
56	135.100	73.300	141.400	242.200
57	134.400	59.700	76.700	173.700
58	132.500	57.700	81.400	191.600
59	137.100	71.900	100.500	210.500
60	138.400	52.200	70.700	196.900
61	0.000	0.000	137.400	0.000
62	0.000	0.000	129.700	0.000
63	0.000	0.000	120.100	0.000
64	0.000	0.000	151.900	0.000
65	126.600	175.500	676.000	224.800
66	140.900	184.900	709.100	247.900
67	114.800	167.400	647.000	232.400
68	133.200	184.800	674.200	235.100
69	115.500	116.300	374.300	157.800
70	101.000	116.100	339.300	134.800
71	108.100	120.800	378.200	166.900
72	104.900	107.400	376.700	166.200
73	94.400	87.400	371.100	159.200
74	94.800	115.200	356.900	155.900
75	79.000	121.200	336.100	213.600
76	99.000	112.100	367.500	153.300
77	55.100	33.000	223.100	46.000
78	47.600	35.500	250.700	50.000
79	47.300	34.200	233.500	61.700
80	63.200	25.800	260.300	40.700

-- PRINT C40-C43

COLUMN	DNT LAB4	TNT LAB4	RDX LAB4	HMX LAB4
COUNT	80	80	80	80
ROW				
1	0.000	30.700	56.900	0.000
2	0.000	35.100	55.900	0.000
3	0.000	34.800	63.900	0.000
4	0.000	29.600	61.000	0.000
5	56.000	69.300	163.000	48.900
6	39.200	70.100	159.300	53.000
7	55.100	70.500	165.700	34.000
8	50.700	71.700	160.900	51.800
9	97.200	140.400	270.800	140.600
10	88.500	129.600	249.100	131.100
11	88.200	120.000	255.500	147.800
12	89.200	135.300	252.300	124.100
13	99.700	141.000	282.300	136.200
14	100.400	141.300	296.600	142.900
15	102.300	144.400	287.000	139.900
16	98.600	141.200	283.500	143.600
17	122.400	197.600	550.600	228.000
18	128.200	209.400	559.300	227.100
19	127.700	201.700	544.000	220.100
20	122.400	206.600	542.200	226.600
21	0.000	0.000	0.000	0.000
22	0.000	0.000	0.000	0.000
23	0.000	0.000	0.000	0.000
24	0.000	0.000	0.000	0.000

Table A7 (cont'd).

25	63.500	49.100	77.300	69.700
26	64.900	52.400	71.000	63.400
27	56.500	48.600	72.200	61.900
28	57.200	51.100	77.100	71.100
29	110.000	136.800	242.700	214.800
30	110.900	133.100	245.000	226.600
31	112.100	133.500	246.400	211.100
32	91.200	139.700	232.300	217.800
33	130.600	147.200	283.500	248.900
34	125.700	136.800	263.000	247.700
35	132.200	150.100	274.300	244.300
36	127.300	150.700	272.100	232.400
37	84.800	81.900	375.600	325.200
38	76.800	90.200	396.100	344.300
39	76.200	90.100	391.300	323.200
40	60.800	87.200	370.700	324.000
41	80.000	0.000	0.000	125.900
42	72.900	0.000	0.000	128.900
43	70.600	0.000	0.000	127.700
44	74.600	0.000	0.000	127.700
45	148.800	88.400	378.100	460.400
46	150.500	85.300	368.400	460.900
47	151.800	87.400	374.700	459.500
48	149.900	73.400	373.100	469.300
49	201.600	152.400	277.500	367.900
50	199.800	153.800	275.900	375.900
51	197.800	154.500	275.000	367.900
52	203.200	159.200	285.800	368.200
53	180.200	135.900	253.000	347.600
54	187.300	137.300	250.900	347.000
55	181.400	135.000	252.000	343.900
56	186.400	135.800	251.300	339.600
57	134.400	52.600	81.400	199.200
58	134.200	45.700	83.000	189.500
59	130.200	43.600	91.200	197.000
60	136.500	49.200	88.100	191.400
61	0.000	0.000	112.600	0.000
62	0.000	0.000	121.000	0.000
63	0.000	0.000	125.000	0.000
64	0.000	0.000	120.600	0.000
65	136.100	178.700	611.000	225.300
66	123.300	167.100	608.900	221.600
67	94.100	166.700	612.800	218.900
68	125.500	165.400	605.000	220.800
69	105.700	103.900	347.200	151.800
70	106.800	108.400	348.200	154.300
71	103.300	114.400	346.800	146.100
72	97.900	126.000	357.200	151.300
73	88.700	107.500	315.700	145.700
74	88.900	104.900	319.200	147.300
75	86.600	109.600	314.900	145.000
76	94.000	106.800	313.800	132.000
77	47.000	38.400	223.200	52.500
78	51.500	38.500	226.100	71.400
79	58.300	39.000	227.400	39.200
80	49.500	34.000	224.600	56.400

-- PRINT C50-C53

COLUMN	DNT LABS	TNT LABS	RDX LABS	HMX LABS
COUNT	80	80	80	80
ROW				
1	0.000	30.700	111.800	0.000
2	0.000	80.400	89.800	0.000
3	0.000	40.300	226.900	0.000
4	0.000	35.900	93.800	0.000

Table A7 (cont'd) Concentrations of DNT, TNT, RDX, and HMX (µg/L) reported by laboratories participating in collaborative test of HPLC method.

5	66.800	64.800	331.100	36.300
6	71.800	84.200	412.600	86.300
7	42.900	89.900	288.200	49.200
8	66.500	83.400	278.500	17.800
9	82.200	135.700	196.100	144.000
10	65.500	114.000	247.000	143.300
11	76.300	153.300	240.500	147.200
12	97.600	129.500	339.200	127.800
13	110.300	118.400	343.500	95.700
14	95.600	164.700	402.800	132.800
15	82.500	162.100	318.400	164.500
16	92.000	133.100	289.800	167.000
17	127.700	212.100	692.800	219.900
18	110.400	186.000	647.600	238.100
19	139.700	186.400	683.200	131.200
20	143.400	230.200	572.800	209.200
21	0.000	0.000	0.000	0.000
22	0.000	0.000	0.000	0.000
23	0.000	0.000	0.000	0.000
24	[illegible]	0.000	0.000	0.000
25	[illegible]	46.900	160.100	112.000
26	[illegible]	83.600	200.100	79.700
27	[illegible]	72.800	125.100	89.700
28	75.200	83.300	197.100	98.700
29	65.200	144.500	258.100	267.700
30	126.600	165.900	268.800	220.500
31	122.700	149.600	304.600	222.600
32	100.700	139.100	317.200	290.300
33	113.800	137.800	262.700	244.500
34	115.700	155.200	311.900	284.900
35	128.000	150.900	433.300	228.300
36	121.500	163.000	336.700	253.100
37	87.400	93.800	458.900	340.000
38	71.200	86.400	387.900	401.700
39	75.600	101.900	448.100	338.000
40	74.500	[illegible]	424.900	352.800
41	93.500	0.000	0.000	144.000
42	75.300	0.000	0.000	250.500
43	77.500	0.000	0.000	188.400
44	78.900	0.000	0.000	63.100
45	167.100	136.400	389.000	404.100
46	174.400	68.600	399.500	440.200
47	167.100	79.600	433.000	419.900
48	134.900	109.800	651.600	441.200
49	197.400	145.700	222.000	382.700
50	189.800	[illegible]	427.900	333.500
51	198.500	142.000	378.900	326.500
52	208.400	145.200	356.000	385.200
53	184.400	152.800	270.400	288.900
54	185.900	136.500	318.500	339.200
55	212.000	[illegible]	273.900	297.300
56	185.000	[illegible]	260.900	357.400
57	119.100	[illegible]	228.900	226.200
58	141.900	61.500	97.000	220.700
59	126.300	39.500	118.800	349.800
60	139.700	45.900	109.200	203.100
61	0.000	0.000	219.400	0.000
62	0.000	0.000	175.800	0.000
63	0.000	0.000	297.700	0.000
64	0.000	0.000	147.400	0.000
65	107.900	172.000	581.000	194.400
66	107.900	164.100	704.300	275.800

67	140.400	167.800	681.700	257.700
68	119.100	155.700	607.600	209.800
69	97.100	130.800	393.000	181.900
70	93.000	116.700	432.800	109.300
71	101.300	126.000	350.400	142.400
72	105.300	128.400	429.700	114.100
73	79.400	109.400	287.100	138.400
74	78.600	114.700	438.400	174.900
75	88.400	127.200	444.700	120.000
76	125.500	134.700	373.100	140.800
77	55.700	48.700	270.400	75.100
78	73.200	45.300	263.800	78.900
79	38.200	39.900	413.100	77.800
80	44.800	35.300	206.900	94.400

-- PRINT C60-C63

COLUMN	DNT LAB6	TNT LAB6	RDX LAB6	HMX LAB6
COUNT	80	80	80	80
ROW				
1	0.000	0.000	92.200	0.000
2	0.000	0.000	143.100	0.000
3	0.000	0.000	79.000	0.000
4	0.000	0.000	126.400	0.000
5	45.200	29.700	211.200	79.200
6	48.900	39.600	225.500	68.600
7	38.500	30.200	161.800	62.200
8	52.500	37.500	188.400	83.200
9	92.100	117.800	340.400	174.000
10	99.600	110.000	333.500	166.800
11	108.000	159.300	339.300	181.500
12	92.500	105.200	300.700	158.400
13	101.800	119.300	301.700	163.600
14	102.200	122.200	337.700	157.200
15	117.200	134.300	330.200	170.600
16	114.400	118.800	340.500	178.400
17	136.200	185.000	584.800	219.600
18	132.500	179.400	533.300	217.000
19	136.600	181.200	614.400	226.600
20	127.700	172.200	537.200	203.700
21	0.000	0.000	0.000	0.000
22	0.000	0.000	0.000	0.000
23	0.000	0.000	0.000	0.000
24	0.000	0.000	67.200	0.000
25	55.100	46.500	271.500	182.800
26	66.800	44.900	269.100	192.800
27	54.000	52.600	81.400	135.700
28	58.000	54.100	95.200	136.700
29	114.000	133.000	441.900	410.600
30	105.300	142.400	453.300	602.200
31	112.000	139.700	276.300	543.200
32	107.900	122.100	272.500	592.700
33	121.800	151.200	691.900	569.200
34	121.600	158.200	659.100	556.300
35	122.000	148.700	350.400	493.600
36	130.900	146.700	340.800	492.300
37	68.400	85.300	538.900	478.400
38	69.300	75.900	527.600	474.400
39	66.600	77.200	632.900	512.900
40	75.800	66.600	651.600	502.600
41	62.100	0.000	0.000	148.400

Table A7 (cont'd). Concentrations of DNT, TNT, RDX, and HMX (µg/L) reported by laboratories participating in collaborative test of HPLC method.

42	73.700	0.000	0.000	107.400
43	72.400	0.000	0.000	107.400
44	62.100	0.000	0.000	90.100
45	135.600	57.600	317.100	445.100
46	143.500	66.700	345.900	403.700
47	146.800	77.200	342.700	470.400
48	148.100	68.200	342.000	523.500
49	200.900	137.000	242.700	408.200
50	194.000	126.100	249.600	334.600
51	193.300	147.900	259.200	405.700
52	189.100	130.400	253.900	430.900
53	180.000	117.200	240.600	325.000
54	171.900	114.800	225.400	388.600
55	184.300	123.200	236.900	335.100
56	175.200	133.400	238.500	453.100
57	122.500	39.100	76.200	273.200
58	136.600	42.500	59.000	232.700
59	123.700	42.500	68.900	166.000
60	126.400	54.400	73.900	179.200
61	0.000	0.000	100.700	0.000
62	0.000	0.000	95.700	0.000
63	0.000	0.000	97.400	0.000
64	0.000	0.000	92.000	0.000
65	120.400	160.600	553.900	194.300
66	121.600	167.000	543.100	186.900
67	122.100	160.500	574.400	201.000
68	119.100	156.300	544.200	185.200
69	101.700	106.700	320.600	130.400
70	105.200	106.200	287.700	128.[illegible]
71	102.100	116.200	297.600	140.5[illegible]
72	87.500	119.100	295.100	144.[illegible]
73	83.400	86.000	253.600	92[illegible]
74	85.200	97.400	278.600	[illegible]
75	85.900	93.900	273.400	[illegible]
76	84.500	91.500	282.200	[illegible]
77	46.200	22.900	183.000	[illegible]00
78	48.800	26.600	196.600	0.000
79	46.700	31.800	187.600	0.000
80	52.800	27.600	188.100	0.000

-- PRINT C70-C73

COLUMN	DNT LAB7	TNT LAB7	RDX LAB7	HMX LAB7
COUNT	80	80	80	80
ROW				
1	0.000	53.900	55.200	0.000
2	0.000	40.400	61.500	0.000
3	0.000	32.400	69.200	0.000
4	0.000	49.200	67.500	0.000
5	66.800	93.800	288.900	0.000
6	84.000	102.200	463.300	0.000
7	39.200	80.700	154.000	137.700
8	72.800	91.600	155.800	0.000
9	99.100	167.600	248.100	370.300
10	************	************	************	************
11	104.000	164.900	262.700	392.800
12	111.600	176.100	498.700	272.900
13	126.900	187.500	267.900	419.200
14	128.500	179.800	280.000	371.100
15	123.500	186.300	704.700	325.700
16	119.400	189.600	278.700	388.400
17	153.200	252.100	635.400	336.400
18	157.900	283.200	554.800	452.100
19	149.400	261.600	554.700	453.900

Table A7 (cont'd).

20	158.300	254.700	551.300	434.000
21	0.000	0.000	0.000	0.000
22	0.000	0.000	0.000	0.000
23	0.000	0.000	0.000	0.000
24	0.000	0.000	0.000	0.000
25	73.100	63.100	88.700	80.100
26	72.300	61.900	94.100	96.800
27	66.500	61.500	102.000	94.700
28	74.600	63.700	83.200	85.400
29	137.800	171.900	237.100	198.500
30	138.800	168.100	263.500	201.700
31	132.000	165.300	279.000	245.200
32	139.200	172.400	267.500	248.000
33	141.000	172.700	271.500	229.300
34	160.200	190.900	294.100	265.900
35	159.900	196.000	297.700	255.200
36	154.000	189.400	292.500	282.300
37	104.800	111.600	428.100	382.300
38	97.400	112.900	415.700	401.300
39	88.600	100.100	396.900	374.500
40	94.900	113.100	378.700	352.300
41	77.100	0.000	0.000	230.900
42	73.500	0.000	0.000	307.200
43	71.200	0.000	0.000	304.100
44	76.400	0.000	0.000	309.200
45	167.500	108.400	360.200	661.700
46	170.700	102.400	379.700	664.400
47	169.800	129.100	376.700	645.300
48	160.800	101.900	373.100	654.000
49	231.300	200.000	258.500	596.200
50	235.500	196.300	281.600	590.300
51	231.400	190.900	275.900	577.600
52	228.100	190.600	282.000	572.900
53	215.900	171.900	258.600	555.900
54	216.600	172.300	258.300	588.000
55	224.800	174.100	236.100	547.300
56	214.200	176.700	242.100	536.300
57	157.100	64.400	76.600	386.400
58	161.200	67.200	74.900	373.100
59	115.300	70.200	67.700	379.100
60	162.900	64.500	71.900	370.100
61	0.000	0.000	126.500	0.000
62	0.000	0.000	129.300	0.000
63	0.000	0.000	120.400	0.000
64	0.000	0.000	114.400	0.000
65	212.400	251.100	611.500	192.300
66	172.400	246.300	856.900	451.800
67	156.100	219.200	720.800	432.300
68	160.000	225.500	623.400	241.500
69	123.800	148.300	329.400	145.700
70	134.500	152.500	590.100	400.000
71	126.900	144.000	332.600	157.000
72	122.800	146.000	333.700	154.000
73	114.000	133.600	574.900	381.700
74	106.800	135.500	328.400	133.000
75	100.200	136.700	330.900	135.200
76	97.500	121.600	320.800	145.300
77	69.700	46.400	216.600	53.300
78	64.100	48.900	216.000	54.700
79	36.400	43.200	225.600	56.200
80	56.100	44.900	208.100	110.900

Table A7 (cont'd). Concentrations of DNT, TNT, RDX, and HMX (μg/L) reported by laboratories participating in collaborative test of HPLC method.

-- PRINT C80-C83

COLUMN	DNT LAB8	TNT LAB8	RDX LAB8	HMX LAB8
COUNT	80	80	80	80
ROW				
1	77.100	17.700	72.800	0.000
2	0.000	157.100	0.000	0.000
3	0.000	56.200	0.000	0.000
4	0.000	0.000	0.000	0.000
5	98.300	39.300	83.800	0.000
6	49.000	57.200	140.400	33.800
7	65.300	91.400	140.600	205.800
8	31.100	44.100	115.800	0.000
9	113.900	185.500	286.300	146.100
10	55.800	73.800	270.100	55.300
11	59.200	90.400	206.000	75.300
12	65.000	113.500	191.700	41.300
13	61.200	152.400	263.800	110.600
14	84.300	137.700	270.600	152.500
15	77.400	184.900	258.900	81.700
16	64.500	88.100	321.100	258.400
17	165.800	158.500	604.200	517.800
18	113.100	222.100	549.900	176.800
19	141.800	202.700	640.100	251.500
20	103.100	167.600	491.400	178.600
21	0.000	0.000	0.000	0.000
22	0.000	0.000	0.000	0.000
23	0.000	0.000	0.000	0.000
24	0.000	0.000	0.000	82.700
25	61.600	47.100	49.300	0.000
26	87.700	20.700	204.400	96.200
27	53.500	49.700	50.400	123.700
28	59.200	34.300	81.600	153.800
29	82.700	139.100	351.900	181.200
30	94.800	156.900	243.800	153.800
31	87.600	113.800	364.200	241.500
32	124.400	109.000	221.000	123.700
33	138.400	197.800	307.200	229.800
34	99.800	110.300	346.000	168.700
35	106.400	146.600	253.000	330.500
36	139.700	113.300	290.900	241.100
37	49.900	64.900	404.600	421.700
38	111.100	104.000	319.100	320.800
39	46.100	63.300	358.700	279.700
40	89.600	203.700	368.300	276.700
41	89.100	0.000	0.000	174.400
42	57.100	12.600	0.000	98.400
43	34.000	0.000	0.000	101.100
44	58.800	0.000	0.000	81.700
45	169.300	91.300	353.200	475.900
46	147.900	32.600	336.100	377.900
47	127.900	66.100	386.500	444.600
48	114.200	20.900	554.500	365.700
49	181.700	113.000	337.600	302.400
50	183.600	130.400	233.700	304.300
51	192.700	152.600	310.000	350.100
52	222.500	175.000	298.700	303.400
53	158.400	125.500	230.300	306.900
54	164.600	96.400	256.400	252.100
55	192.700	122.300	199.100	369.200
56	173.500	116.500	183.700	276.600
57	142.200	63.800	148.300	195.600
58	131.700	63.200	36.700	118.400
59	85.300	51.000	57.100	109.600
60	80.100	132.200	35.600	174.200

Table A7 (cont'd).

61	0.000	0.000	118.500	0.000
62	0.000	0.000	127.300	0.000
63	0.000	0.000	238.000	0.000
64	0.000	0.000	148.300	0.000
65	152.400	125.200	720.300	318.700
66	105.100	97.500	710.900	268.400
67	89.600	101.500	611.600	204.500
68	129.100	113.900	581.300	313.600
69	81.500	80.600	287.400	122.800
70	84.200	106.400	297.600	176.200
71	116.800	106.100	310.200	154.700
72	85.500	99.800	277.500	232.500
73	71.300	84.900	349.000	82.800
74	48.500	62.300	319.800	171.600
75	143.800	119.400	391.800	131.800
76	55.800	89.000	281.900	89.300
77	53.300	37.800	216.900	0.000
78	0.000	22.900	167.800	0.000
79	19.900	36.700	244.100	222.900
80	17.700	98.800	181.500	134.800

-- PRINT C90-C93

COLUMN	DNT LAB9	TNT LAB9	RDX LAB9	HMX LAB9
COUNT	80	80	80	80
ROW				
1	0.000	16.400	57.400	0.000
2	0.000	16.400	58.900	0.000
3	0.000	17.300	58.900	0.000
4	0.000	16.400	57.400	0.000
5	49.800	49.100	157.400	41.500
6	51.300	49.100	154.500	45.100
7	49.000	49.100	156.000	45.100
8	49.800	50.000	154.500	45.100
9	86.600	114.500	254.600	135.400
10	87.300	115.500	256.000	131.800
11	88.100	117.400	254.600	133.600
12	87.300	115.500	257.500	130.000
13	98.800	127.000	278.100	148.100
14	98.100	128.000	279.600	142.700
15	100.400	129.000	278.100	142.700
16	98.800	129.000	278.100	144.500
17	124.100	181.900	548.900	222.100
18	124.100	180.900	550.300	220.300
19	124.900	182.900	550.300	220.300
20	125.600	181.900	548.900	222.100
21	0.000	0.000	0.000	0.000
22	0.000	0.000	0.000	0.000
23	0.000	0.000	0.000	0.000
24	0.000	0.000	0.000	0.000
25	60.400	49.600	75.600	66.200
26	61.200	50.600	75.600	66.200
27	61.200	52.500	74.100	64.300
28	61.900	51.600	75.600	66.200
29	114.600	136.200	250.400	220.500
30	111.500	133.300	246.000	216.800
31	117.700	139.100	256.400	226.000
32	115.300	136.200	249.000	218.700
33	125.400	150.800	271.200	238.900
34	126.200	149.800	266.700	238.900
35	124.600	149.800	268.200	238.900
36	127.000	151.800	272.700	240.700
37	75.900	84.600	370.500	325.200
38	75.100	82.700	367.500	323.400
39	77.400	84.600	379.400	332.600
40	75.900	83.700	369.000	323.400

Table A7 (cont'd) Concentrations of DNT, TNT, RDX, and HMX (µg/L) reported by laboratories participating in collaborative test of HPLC method.

41	71.600	0.000	0.000	125.500
42	72.300	0.000	0.000	130.800
43	72.300	0.000	0.000	132.600
44	70.800	0.000	0.000	125.500
45	147.000	78.400	374.600	454.400
46	150.900	80.300	386.300	470.300
47	147.800	79.300	380.500	456.200
48	146.200	76.400	376.100	456.200
49	198.600	140.300	276.600	369.500
50	201.700	142.200	281.000	373.100
51	199.400	143.200	278.000	366.000
52	198.600	142.200	273.600	369.500
53	186.300	126.800	253.100	346.500
54	186.300	126.800	254.600	350.100
55	185.500	125.800	253.100	343.000
56	183.200	124.800	250.200	344.800
57	133.900	48.400	76.100	194.500
58	134.700	48.400	76.100	198.000
59	131.600	46.400	74.600	194.500
60	130.800	45.500	74.600	192.700
61	0.000	0.000	111.000	0.000
62	0.000	0.000	114.000	0.000
63	0.000	0.000	114.000	0.000
64	0.000	0.000	114.000	0.000
65	125.700	170.400	613.000	225.500
66	124.900	167.500	615.900	225.500
67	121.800	164.500	584.800	220.000
68	126.400	169.400	610.000	223.700
69	99.300	116.500	336.100	148.500
70	101.600	116.500	342.000	150.300
71	101.600	116.500	339.100	150.300
72	101.600	116.500	340.500	152.200
73	87.700	100.900	313.900	135.700
74	87.700	100.900	315.400	137.500
75	89.200	102.800	315.400	139.300
76	86.100	100.900	310.900	135.700
77	50.400	36.200	214.700	51.300
78	50.400	35.300	217.600	51.300
79	50.400	34.300	213.200	51.300
80	50.400	35.300	216.200	49.500

Table A8. Mean concentrations (µg/L) for each set of four replicate determinations on each sample.

— PRINT C3-C8

COLUMN			LAB	SPIKE 0	SPIKE 1	SPIKE 2	SPIKE 3	SPIKE 4
COUNT			160	160	160	160	160	160
ROW								
1	A	DNT	1.	0.000	48.425	36.000	93.575	125.630
2			2.	0.000	48.400	86.925	103.900	125.430
3			3.	0.000	51.850	90.350	99.425	155.050
4			4.	0.000	50.250	90.775	100.250	125.180
5			5.	0.000	62.000	80.400	95.100	130.300
6			6.	0.000	46.275	98.050	108.900	133.250
7			7.	0.000	65.700	104.900	124.570	154.700
8			8.	19.275	60.925	83.475	76.850	130.950
9			9.	0.000	49.975	87.325	99.025	124.680
10			10. mean=	0.000	51.200	89.600	102.000	128.000
11		TNT	1.	29.175	61.975	132.400	144.180	202.330
12			2.	31.775	68.125	134.980	156.730	207.550
13			3.	23.750	59.900	116.070	140.230	193.650
14			4.	32.550	70.400	131.330	141.980	203.830
15			5.	46.825	80.575	133.130	144.580	203.680

Table A8 (cont'd).

16			6.		0.000	34.250	123.070	123.650	179.450
17			7.		43.975	92.075	169.530	185.800	262.900
18			8.		67.750	68.000	115.800	140.770	197.730
19			9.		16.625	49.325	115.730	128.250	181.900
20			10.	mean=	38.100	72.400	141.000	155.000	210.000
21		RDX	1.		55.475	148.850	250.450	277.720	549.550
22			2.		147.800	161.150	263.930	293.300	561.000
23			3.		74.800	173.980	260.380	297.500	575.550
24			4.		59.425	62.230	256.920	287.350	549.030
25			5.		130.580	327.600	255.700	338.630	649.100
26			6.		110.180	196.730	328.480	327.530	567.430
27			7.		63.350	265.500	336.500	382.830	574.050
28			8.		18.200	120.150	238.520	276.600	571.400
29			9.		58.150	155.600	255.680	276.470	549.600
30			10.	mean=	55.600	155.000	254.000	275.000	551.000
31		HMX	1.		0.000	35.650	124.020	127.300	205.770
32			2.		0.000	43.650	129.680	143.180	226.950
33			3.		0.000	53.925	131.950	138.800	222.500
34			4.		0.000	46.925	135.900	140.650	225.450
35			5.		0.000	47.400	140.580	140.000	199.600
36			6.		0.000	73.300	170.170	167.450	216.730
37			7.		0.000	34.425	345.330	376.160	419.100
38			8.		0.000	59.900	79.500	150.800	281.170
39			9.		0.000	44.200	132.700	144.500	221.200
40			10.	mean=	0.000	44.600	134.000	147.000	223.000
41	B	DNT	1.		0.000	50.475	98.625	114.820	61.700
42			2.		0.000	62.925	114.700	127.650	77.325
43			3.		0.000	57.175	90.575	112.820	65.250
44			4.		0.000	60.525	106.050	128.950	74.650
45			5.		0.000	67.150	103.800	119.750	77.175
46			6.		0.000	58.475	109.800	124.070	70.025
47			7.		0.000	71.625	136.950	153.770	96.425
48			8.		0.000	65.500	97.375	121.070	74.175
49			9.		0.000	61.175	114.780	125.800	76.075
50			10.	mean=	0.000	61.400	115.000	126.000	76.800
51		TNT	1.		0.000	44.475	135.400	151.070	79.725
52			2.		0.000	53.075	135.350	156.050	87.675
53			3.		0.000	48.050	122.930	138.100	82.400
54			4.		0.000	50.300	135.770	146.200	87.350
55			5.		0.000	71.650	149.770	151.730	99.100
56			6.		0.000	49.525	134.300	151.200	76.250
57			7.		0.000	62.550	169.420	187.250	109.430
58			8.		0.000	37.950	129.700	142.000	108.980
59			9.		0.000	51.075	136.200	150.550	83.900
60			10.	mean=	0.000	51.500	137.000	154.000	85.800
61		RDX	1.		0.000	66.200	260.170	275.380	377.300
62			2.		0.000	73.900	256.650	270.170	369.880
63			3.		0.000	94.300	245.250	304.250	398.250
64			4.		0.000	74.400	241.600	273.220	383.420
65			5.		0.000	170.600	287.170	336.150	429.950
66			6.		16.800	179.300	361.000	510.550	587.750
67			7.		0.000	92.000	261.780	286.950	404.850
68			8.		0.000	96.425	295.230	299.280	362.670
69			9.		0.000	75.225	250.450	265.700	371.600
70			10.	mean=	0.000	74.300	248.000	273.000	372.000
71		HMX	1.		0.000	60.925	215.180	232.180	315.550
72			2.		0.000	115.480	242.830	260.350	353.030
73			3.		0.000	79.725	202.520	284.100	323.030
74			4.		0.000	66.525	217.580	243.330	329.170
75			5.		0.000	95.025	250.270	252.700	358.130
76			6.		0.000	162.000	537.170	527.850	492.080
77			7.		0.000	89.250	223.350	256.170	377.600
78			8.		20.675	93.425	175.050	242.520	324.730
79			9.		0.000	65.725	220.500	235.350	326.150
80			10.	mean=	0.000	66.800	223.000	245.000	334.000
81	C	DNT	1.		55.650	141.000	209.250	187.580	127.180
82			2.		73.150	150.450	197.600	189.270	136.050
83			3.		72.700	151.650	205.630	133.600	135.600
84			4.		74.525	150.250	200.600	183.830	133.830

Table A8 (cont'd). Mean concentrations ($\mu g/L$) for each set of four replicate determinations on each sample.

85			5.	81.300	160.880	198.520	191.830	131.750
86			6.	67.575	143.500	194.330	177.850	127.300
87			7.	74.550	167.200	231.580	217.880	149.130
88			8.	59.750	139.820	195.130	172.300	109.830
89			9.	71.750	147.980	199.580	185.330	132.750
90			10. mean=	71.100	148.000	199.000	186.000	125.000
91		TNT	1.	0.000	69.700	153.170	135.670	37.350
92			2.	0.000	87.225	153.180	139.780	52.450
93			3.	0.000	78.875	138.550	77.625	60.375
94			4.	0.000	83.625	154.980	136.000	47.775
95			5.	0.000	98.600	146.730	145.520	51.075
96			6.	0.000	67.425	135.350	122.150	44.625
97			7.	0.000	110.450	194.450	173.750	66.575
98			8.	3.150	52.725	142.750	115.180	77.550
99			9.	0.000	78.600	141.980	126.050	47.175
100			10. mean=	0.000	85.800	154.000	137.000	51.500
101		RDX	1.	0.000	389.170	259.050	231.600	66.650
102			2.	0.000	383.550	282.380	260.880	76.425
103			3.	0.000	376.200	294.330	145.170	82.325
104			4.	0.000	373.580	278.550	251.800	85.925
105			5.	0.000	468.280	346.200	280.530	138.480
106			6.	0.000	336.920	251.350	235.350	69.500
107			7.	0.000	372.420	274.500	248.770	72.775
108			8.	0.000	407.580	295.000	217.380	69.425
109			9.	0.000	379.380	277.300	252.750	75.350
110			10. mean=	0.000	371.000	273.000	248.000	74.300
111		HMX	1.	116.270	438.500	355.230	332.750	179.230
112			2.	124.000	453.250	358.080	384.950	195.800
113			3.	114.230	406.350	349.700	243.580	193.180
114			4.	127.550	462.530	369.980	344.530	194.270
115			5.	161.500	426.350	356.980	320.700	249.950
116			6.	113.320	460.670	394.850	375.450	212.780
117			7.	287.850	656.350	584.250	556.680	377.170
118			8.	113.900	416.030	315.050	301.200	149.450
119			9.	128.600	459.280	369.530	346.100	194.930
120			10. mean=	124.000	458.000	369.000	347.000	191.000
121	D	DNT	1.	0.000	134.550	85.050	176.600	39.325
122			2.	0.000	122.900	100.880	86.100	49.275
123			3.	0.000	128.880	107.380	91.800	53.300
124			4.	0.000	119.750	103.400	89.550	51.575
125			5.	0.000	118.820	99.175	92.975	52.975
126			6.	0.000	120.800	99.125	84.750	48.625
127			7.	0.000	175.230	127.000	104.630	56.575
128			8.	0.000	119.050	92.000	79.650	22.725
129			9.	0.000	124.700	101.030	87.675	50.400
130			10. mean=	0.000	128.000	102.000	89.600	51.200
131		TNT	1.	0.000	158.400	114.350	204.400	24.250
132			2.	0.000	170.130	117.430	101.430	34.525
133			3.	0.000	178.150	115.150	108.980	32.125
134			4.	0.000	169.480	113.180	107.200	37.475
135			5.	0.000	164.900	125.470	121.500	42.300
136			6.	0.000	161.100	112.050	92.200	27.225
137			7.	0.000	235.520	147.700	131.850	45.850
138			8.	0.000	109.520	98.225	88.900	49.050
139			9.	0.000	167.950	116.500	101.380	35.275
140			10. mean=	0.000	172.000	117.000	103.000	34.300
141		RDX	1.	102.300	573.080	327.280	475.380	195.770
142			2.	136.330	616.570	354.530	325.630	246.950
143			3.	134.770	676.570	367.130	357.900	241.900
144			4.	119.800	609.430	349.850	315.900	225.330
145			5.	210.080	643.650	401.480	385.830	288.550
146			6.	96.450	553.900	300.250	271.950	188.830
147			7.	122.650	703.150	396.450	388.750	216.530
148			8.	158.020	656.030	293.170	335.630	202.580
149			9.	113.250	605.930	339.420	313.900	215.430
150			10. mean=	111.600	607.000	335.000	309.800	211.000

151	HMX	1.	C.000	204.930	135.580	248.180	40.925
152		2.	0.000	219.020	163.750	135.630	58.825
153		3.	C.000	235.050	156.420	170.500	49.600
154		4.	0.000	221.650	150.880	142.500	54.875
155		5.	0.000	234.420	136.920	143.520	81.550
156		6.	0.000	191.850	135.800	113.730	0.000
157		7.	0.000	329.470	214.180	198.800	68.775
158		8.	0.000	276.300	171.550	118.680	89.425
159		9.	C.000	223.680	150.330	137.050	50.850
160		10. mean=	0.000	223.000	147.000	134.C00	44.600

Table A9. Spike 2 and spike 3 concentrations (from Table A8) normalized to means (column headings indicate matrix by first letter, analyte by next three, and spike number by last number; outliers are marked by asterisk*).

COLUMN	BADNT2	BADNT3	RBDNT2	BBDNT3
COUNT	8	8	8	8
ROW				
1	0.95982	0.91740	C.857609	0.89703
2	0.97015	1.01863	C.997391	0.99727
3	1.00837	0.97475	0.787609	0.88141
4	1.01311	C.98284	0.922174	1.00742
5	0.89732	0.93235	C.902609	0.93555
6	1.09431	1.C6765	0.954783	0.96330
7	0.93164	0.75343	0.846739	0.94586
8	0.97461	C.97083	0.998087	0.98281

— PRINT C21C22C31C32

COLUMN	RCDNT2	RCDNT3	RDCNT2	BDDNT3
COUNT	8	8	8	8
ROW				
1	1.05151	1.00849	0.83382	1.97098 *
2	0.99296	1.C1758	0.98902	0.96094
3	1.03332	C.71935	1.05275	1.02455
4	1.00804	0.98833	1.01373	0.99944
5	0.99759	1.03134	0.97230	1.03767
6	0.97653	0.95618	0.97181	0.94587
7	0.98055	C.92634	C.90196	0.89118
8	1.00291	0.99640	0.99049	0.97852

-- PRINT C3C4C13C14

COLUMN	RATNT2	BATNT3	RBTNT2	RBTNT3
COUNT	8	8	8	8
ROW				
1	0.939007	0.93019	0.98832	0.98097
2	0.957305	1.01116	0.98796	1.01331
3	0.823191	0.90471	0.89730	0.89675
4	0.931418	0.91600	0.99102	C.94935
5	0.944184	0.93277	1.09321	0.98526
6	0.872837	0.79774	0.98029	0.98182
7	0.821277	0.90819	0.94672	0.92208
8	0.820780	0.82742	0.99416	0.97760

Table A9 (cont'd). Spike 2 and spike 3 concentrations (from Table A8) normalized to means.

— PRINT C23C24C33C34

COLUMN	RCTNT2	RCTNT3	RDTNT2	RDTNT3
COUNT	8	8	8	8
ROW				
1	0.99461	0.99029	0.97735	1.98447 *
2	0.99468	1.02029	1.00368	0.98476
3	0.89968	0.56661	0.98419	1.05806
4	1.00636	0.99270	0.96735	1.04078
5	0.95279	1.06219	1.07239	1.17961
6	0.87890	0.89161	0.95769	0.89515
7	0.92695	0.84073	0.83953	0.86311
8	0.92195	0.92007	0.99573	0.98427

-- PRINT C5C6C15C16

COLUMN	RARDX2	RARDX3	RBRDX2	RBRDX3
COUNT	8	8	8	8
ROW				
1	0.98602	0.99541	1.04907	1.00872
2	1.03909	1.05125	1.03488	0.98963
3	1.02512	1.06631	0.98891	1.11447
4	1.01150	1.02993	0.97419	1.00081
5	1.00669	1.21373	1.15794	1.23132
6	1.29323	1.17394	1.45565 *	1.87015 *
7	0.93906	0.99857	1.19044	1.09626
8	1.00661	0.99810	1.00988	0.98791

— PRINT C25C26C35C36

COLUMN	RCRDX2	RCRDX3	RDRDX2	RDRDX3
COUNT	8	8	8	8
ROW				
1	0.94890	0.93387	0.97696	1.53447 *
2	1.03436	1.05194	1.05830	1.05174
3	1.07813	0.60149	1.09591	1.15526
4	1.02033	1.01532	1.04433	1.01969
5	1.26813	1.13278	1.19845	1.24542
6	0.92070	0.94899	0.89627	0.87782
7	1.08059	0.87653	0.87513	1.08338
8	1.01575	1.01915	1.01319	1.01323

-- PRINT C7C8C17C18

COLUMN	RAHMX2	RAHMX3	RBHMX2	RBHMX3
COUNT	8	8	8	8
ROW				
1	0.92552	0.86599	0.96493	0.94767
2	0.96776	0.97401	1.08892	1.06265
3	0.98470	0.94422	0.90816	1.15959
4	1.01418	0.95680	0.97570	0.99318
5	1.04910	0.95238	1.12229	1.03143
6	1.26993	1.13912	2.40883 *	2.15449 *
7	0.99328	1.02585	0.78498	0.98988
8	0.99030	0.98299	0.98879	0.97694

— PRINT C27C28C37C38

COLUMN	RCHMX2	RCHMX3	RDHMX2	RDHMX3
COUNT	8	8	8	8
ROW				
1	0.96268	0.95893	0.92231	1.85209 *
2	0.97041	1.10937	1.11395	1.01216
3	0.94770	0.70196	1.06408	1.27239
4	1.00266	0.99288	1.02639	1.06343
5	0.96743	0.92421	0.93143	1.07104
6	1.07005	1.08199	0.92381	0.84873
7	0.85379	0.86801	1.16701	0.88716
8	1.00144	0.99741	1.02265	1.02276

Table A10. Concentrations (µg/L) of aliquots taken from each sample (average of injection duplicate results). Outliers indicated by asterisk*; table is organized into subgroups by matrix; columns represent different spike levels and within each spike level are the two duplicate results for each aliquot from sample; rows are segregated by analyte and then by laboratory.

MATRIX A

-- PRINT C40-C45

	ROW		C40	C41	C42	C43	C44	C45
	COUNT		35	35	35	35	35	35
	1		1.000	1.000	1.000	1.000	1.000	1.000
	2	spike	0.000	0.000	1.000	1.000	2.000	2.000
	3		1.000	2.000	1.000	2.000	1.000	2.000
	4	lab 1	0.000	0.000	48.400	48.450	86.350	85.650
DNT	5	2	0.000	0.000	47.450	49.350	86.550	87.300
	6	3	0.000	0.000	52.200	51.500	102.700	78.000
	7	4	0.000	0.000	47.600	52.900	92.650	88.700
	8	5	0.000	0.000	69.300	54.700	73.850	96.950
	9	6	0.000	0.000	47.050	45.500	95.650	100.250
	10	8	38.550 *	0.000	73.650	48.200	84.850	82.100
	11	9	0.000	0.000	50.550	49.400	86.950	87.700
	12		28.500	29.850	62.400	61.550	132.200	132.600
TNT	13		33.950	29.600	65.850	70.400	131.700	138.250
	14		24.400	23.100	45.500	74.300	128.050	104.100
	15		32.900	32.200	69.700	71.100	135.000	127.650
	16		55.550	38.100	74.500	86.650	124.850	141.400
	17		0.000 *	0.000 *	34.650	33.850	113.900	132.250
	18		107.400 *	28.100 *	68.250	67.750	129.650	101.950
	19		16.400	16.850	49.100	49.550	115.000	116.450
	20		52.700	58.250	150.750	146.950	252.400	248.500
RDX	21		200.500 *	95.100 *	161.350	160.950	267.400	260.450
	22		83.450	66.150	182.550	165.400	262.800	257.950
	23		56.400	62.450	161.150	163.300	259.950	253.900
	24		100.800 *	160.350 *	371.850*	283.350 *	221.550 *	289.850 *
	25		117.650	102.700	218.350	175.100	336.950 *	320.000 *
	26		36.400 *	0.000 *	112.100	128.200	278.200 *	198.850 *
	27		58.150	58.150	155.950	155.250	255.300	256.050
	28		0.000	0.000	36.300	35.000	123.700	124.350
HMX	29		0.000	0.000	39.850	47.450	127.600	131.750
	30		0.000	0.000	53.150	54.700	134.500	129.400
	31		0.000	0.000	50.950	42.900	135.650	135.950
	32		0.000	0.000	61.300	33.500	143.650	137.500
	33		0.000	0.000	73.900 *	72.700 *	170.400 *	169.950 *
	34		0.000	0.000	16.900 *	102.900 *	100.700 *	58.300 *
	35		0.000	0.000	43.300	45.100	133.600	131.800

-- PRINT C46-C49

	ROW		C46	C47	C48	C49
	COUNT		35	35	35	35
	1		1.000	1.000	1.000	1.000
	2	spike	3.000	3.000	4.000	4.000
	3		1.000	2.000	1.000	2.000
	4		91.300	95.850	125.100	126.150
DNT	5		107.650	100.150	123.250	127.600
	6		98.150	100.700	133.750 *	176.350 *
	7		100.050	100.450	125.300	125.050
	8		102.950	87.250	119.050	141.550
	9		102.000	115.800	134.350	132.150
	10		72.750 *	80.950 *	139.450	122.450
	11		98.450	99.600	124.100	125.250
	12		142.750	145.600	200.750	203.900
TNT	13		161.400	152.050	207.550	207.550
	14		134.850	145.600	177.000	210.300
	15		141.150	142.800	203.500	204.150
	16		141.550	147.600	199.050	208.300
	17		120.750	126.550	182.200	176.700
	18		145.050	136.500	210.300	185.150
	19		127.500	129.000	181.400	182.400

Table A10 (cont'd). Concentrations (μg/L) of aliquots taken from each sample.

MATRIX A

	20	266.750	288.700	547.500	551.600
RDX	21	288.150	298.450	572.800	549.200
	22	305.700	289.300	574.800	576.300
	23	289.450	285.250	554.950	543.100
	24	373.150 *	304.100 *	670.200 *	628.000 *
	25	319.700 *	335.350 *	559.050	575.800
	26	267.200	290.000	577.050	565.750
	27	278.850	278.100	549.600	549.600
	28	126.200	128.400	205.500	206.050
HMX	29	146.300	140.050	231.350	222.550
	30	136.950	140.650	216.250	228.750
	31	139.550	141.750	227.550	223.350
	32	114.250	165.750	229.000	170.200
	33	160.400 *	174.500 *	218.300 *	215.150 *
	34	131.550 *	170.050 *	347.300 *	215.050 *
	35	145.400	143.600	221.200	221.200

MATRIX B

	COLUMN	C50	C51	C52	C53	C54	C55
	COUNT	35	35	35	35	35	35
	ROW						
	1	2.	2.0000	2.000	2.000	2.000	2.000
spike	2	0.	0.0000	1.000	1.000	2.000	2.000
	3	1.	2.0000	1.000	2.000	1.000	2.000
	4	0.	0.0000	50.800	50.150	85.450	111.800
DNT	5	0.	0.0000	63.500	62.350	116.150	113.250
	6	0.	0.0000	56.150	58.200	102.750	78.400
	7	0.	0.0000	64.200	56.850	110.450	101.650
	8	0.	0.0000	61.500	72.800	95.900	111.700
	9	0.	0.0000	60.950	56.000	109.650	109.950
	10	0.	0.0000	74.650	56.350	88.750	106.000
	11	0.	0.0000	60.800	61.550	113.050	116.500
	12	0.	0.0000	45.600	43.350	121.650	149.150
TNT	13	0.	0.0000	54.000	52.150	140.100	130.600
	14	0.	0.0000	61.500	34.600	110.550	135.300
	15	0.	0.0000	50.750	49.850	134.950	136.600
	16	0.	0.0000	65.250 *	78.050 *	155.200 *	144.350 *
	17	0.	0.0000	45.700	53.350	137.700	130.900
TNT	18	0.	0.0000	33.900	42.000	148.000	111.400
	19	0.	0.0000	50.100	52.050	134.750	137.650
	20	.	0.0000	62.000	70.400	270.000	250.350
RDX	21	0.	0.0000	70.050	77.750	263.700	249.600
	22	0.	0.0000	102.700	85.900	245.500	245.000
	23	0.	0.0000	74.150	74.650	243.850	239.350
	24	0.	0.0000	180.100 *	161.100 *	263.450 *	310.900 *
	25	0.	33.6000 *	270.300 *	88.300 *	447.600 *	274.400 *
	26	0.	0.0000	126.850 *	66.000 *	297.650	292.600
	27	0.	0.0000	75.600	74.850	248.200	252.700
	28	0.	0.0000	62.550	59.300	217.300	213.050
HMX	29	0.	0.0000	75.750 *	155.200 *	257.550	228.100
	30	0.	0.0000	87.450	72.000	196.150	208.900
	31	0.	0.0000	66.550	66.500	220.700	214.450
	32	0.	0.0000	95.850	94.200	244.100	256.450
	33	0.	0.0000	187.800 *	136.200 *	506.400 *	567.950 *
	34	0.	41.3500 *	48.100 *	138.750 *	167.500 *	182.600 *
	35	0.	0.0000	66.200	65.250	218.650	222.350

COLUMN	C56	C57	C58	C59
COUNT	35	35	35	35
ROW				
1	2.000	2.000	2.000	2.000
2	3.000	3.000	4.000	4.000
3	1.000	2.000	1.000	2.000

Table A10 (cont'd).

MATRIX B

	4	99.000 *	130.650 *	60.700	62.700
DNT	5	126.650	128.650	78.900	75.750
	6	105.750	119.900	64.350	66.150
	7	128.150	129.750	80.800	68.500
	8	114.750	124.750	79.300	75.050
	9	121.700	126.450	68.850	71.200
	10	119.100	123.050	80.500	67.850
	11	125.800	125.800	75.500	76.650
	12	135.850	166.300	82.600	76.850
TNT	13	155.500	156.600	90.350	85.000
	14	138.900	137.300	81.050	83.750
	15	142.000	150.400	86.050	88.650
	16	146.500	156.950	90.100	108.100
	17	154.700	147.700	80.600	71.900
	18	154.050	129.950	84.450 *	133.500 *
	19	150.300	150.800	83.650	84.150
	20	279.800	270.950	375.900	378.700
RDX	21	269.900	270.450	371.150	368.600
	22	297.650	310.850	422.600	373.900
	23	273.250	273.200	385.850	381.000
	24	287.300 *	385.000 *	423.400 *	436.500 *
	25	675.500 *	345.600 *	533.250 *	642.250 *
	26	326.600	271.950	361.850	363.500
	27	268.950	270.450	369.000	374.200
	28	233.700	230.650	312.850	318.250
HMX	29	237.000	283.700	376.100	329.950
	30	288.100	280.100	313.600	332.450
	31	248.300	238.350	334.750	323.600
	32	264.700	240.700	370.850	345.400
	33	562.750 *	492.950 *	476.400 *	507.750 *
	34	199.250 *	265.800 *	371.250 *	278.200 *
	35	238.900	239.800	324.300	328.000

MATRIX C

— PRINT C60-C65

	COLUMN	C60	C61	C62	C63	C64	C65
	COUNT	35	35	35	35	35	35
	ROW						
	1	3.000	3.000	3.000	3.000	3.000	3.000
spike	2	0.000	0.000	1.000	1.000	2.000	2.000
	3	1.000	2.000	1.000	2.000	1.000	2.000
	4	57.500	53.800	137.600	144.400	219.950	198.550
DNT	5	72.200	74.100	151.700	149.200	199.250	195.950
	6	75.750	69.650	151.900	151.400	200.700	210.550
	7	76.450	72.600	149.650	150.850	200.700	200.500
	8	84.400	78.200	170.750 *	151.000 *	193.600	203.450
	9	67.900	67.250	139.550	147.450	197.450	191.200
	10	73.100	46.400	158.600 *	121.050 *	182.650	207.600
	11	71.950	71.550	148.950	147.000	200.150	199.000
	12	0.000	0.000	65.700	73.700	174.300	132.050
TNT	13	0.000	0.000	88.800	85.650	152.450	153.900
	14	0.000	0.000	73.500	84.250	133.550	143.550
	15	0.000	0.000	86.850	80.400	153.100	156.850
	16	0.000	0.000	102.500	94.700	149.650	143.600
	17	0.000	0.000	62.150	72.700	131.550	139.150
	18	6.300 *	0.000	61.950	43.500	121.700	163.800
	19	0.000	0.000	79.350	77.850	141.250	142.700
	20	0.000	0.000	398.300	380.050	258.800	259.300
RDX	21	0.000	0.000	380.150	386.950	283.250	281.500
	22	0.000	0.000	370.450	381.950	297.500	291.150
	23	0.000	0.000	373.250	373.900	276.700	280.400
	24	0.000	0.000	394.250 *	542.300 *	324.950 *	367.450 *
	25	0.000	0.000	331.500	342.350	246.150	256.550
	26	0.000	0.000	304.650 *	470.500 *	285.650	304.350
	27	0.000	0.000	380.450	378.300	278.800	275.800

Table A10 (cont'd). Concentrations (μg/L) of aliquots taken from each sample.

MATRIX C

	ROW						
	28	116.100	116.450	443.450	433.550	355.050	355.400
HMX	29	121.450	126.550	449.350	457.150	364.350	351.800
	30	119.200	109.250	408.200	404.500	334.600	364.800
	31	127.400	127.700	460.650	464.400	371.900	368.050
	32	197.250 *	125.750 *	422.150	430.550	356.100	355.850
	33	127.900 *	98.750 *	424.400 *	496.950 *	371.400 *	419.300 *
	34	136.400 *	91.400 *	426.900 *	405.150 *	303.350 *	326.750 *
	35	128.150	129.050	462.350	456.200	371.300	367.750

— PRINT C66-C69

	COLUMN	C66	C67	C68	C69
	COUNT	35	35	35	35
	ROW				
	1	3.000	3.000	3.000	3.000
SPIKE	2	3.000	3.000	4.000	4.000
	3	1.000	2.000	1.000	2.000
	4	184.750	190.400	129.350	125.000
DNT	5	188.650	189.900	134.450	137.650
	6	135.250 *	132.350 *	133.450	137.750
	7	183.750	183.900	134.300	133.350
	8	185.150	198.500	130.500	133.000
	9	175.950	179.750	129.550	125.050
	10	161.500	183.100	136.950 *	82.700 *
	11	186.300	184.350	134.300	131.200
	12	132.750	138.600	36.350	38.350
TNT	13	141.450	138.100	50.700	54.200
	14	63.150 *	72.100 *	58.700	62.050
	15	136.600	135.400	49.150	46.400
	16	144.650	146.400	59.450	42.700
	17	116.000	128.300	40.800	48.450
	18	110.950	119.400	63.500 *	91.600 *
	19	126.800	125.300	48.400	45.950
	20	228.400	234.800	70.950	62.350
RDX	21	258.200	263.550	75.150	77.700
	22	156.950 *	141.400 *	79.050	85.600
	23	251.950	251.650	82.200	89.650
	24	294.450 *	267.400 *	162.950 *	114.000 *
	25	233.000	237.700	67.600	71.400
	26	243.350	191.400	92.500	46.350
	27	253.850	251.650	76.100	74.600
	28	333.850	331.650	181.500	176.950
HMX	29	346.650 *	423.250 *	195.850	195.750
	30	244.400 *	242.750 *	182.650	203.700
	31	347.300	341.750	194.350	194.200
	32	314.050	327.350	223.450 *	276.450 *
	33	356.800 *	394.100 *	252.950 *	172.600 *
	34	279.500 *	322.900 *	157.000 *	141.900 *
	35	348.300	343.900	196.250	193.600

MATRIX D

	COLUMN	C70	C71	C72	C73	C74	C75
	COUNT	35	35	35	35	35	35
	ROW						
	1	4.000	4.000	4.000	4.000	4.000	4.000
spike	2	0.000	0.000	1.000	1.000	2.000	2.000
	3	1.000	2.000	1.000	2.000	1.000	2.000
	4	0.000	0.000	122.350	146.750	85.650	84.450
DNT	5	0.000	0.000	122.350	123.450	100.250	101.500
	6	0.000	0.000	133.750	124.000	108.250	106.500
	7	0.000	0.000	129.700	109.800	106.250	100.550
	8	0.000	0.000	107.900	129.750	95.050	103.300
	9	0.000	0.000	121.000	120.600	103.450	94.800

Table A10 (cont'd).

MATRIX D

	10	0.000	0.000	128.750	109.350	82.650	101.150
	11	0.000	0.000	125.300	124.100	100.450	101.600
	12	0.000	0.000	171.750	145.050	115.750	112.950
TNT	13	0.000	0.000	171.850	168.400	117.350	117.500
	14	0.000	0.000	180.200	176.100	116.200	114.100
	15	0.000	0.000	172.900	166.050	106.150	120.200
	16	0.000	0.000	168.050	161.750	123.750	127.200
	17	0.000	0.000	163.800	158.400	106.450	117.650
TNT	18	0.000	0.000	111.350 *	107.700 *	93.500 *	102.950 *
	19	0.000	0.000	168.950	166.950	116.500	116.500
	20	102.550	102.050	583.000	563.150	325.000	329.550
RDX	21	148.700	123.950	619.200	613.950	355.600	353.450
	22	133.550	136.000	692.550	660.600	356.600	377.450
	23	116.800	122.800	609.950	608.900	347.700	352.000
	24	197.600 *	222.550 *	642.650 *	644.650 *	412.500 *	390.050 *
	25	98.200	94.700	548.500	559.300	304.150	296.350
	26	122.900 *	193.150 *	715.600 *	596.450 *	292.500	293.850
	27	112.500	114.000	614.450	597.400	339.050	339.800
	28	0.000	0.000	205.000	204.850	136.650	134.500
HMX	29	0.000	0.000	226.700	211.350	165.200	162.300
	30	0.000	0.000	236.350	233.750	146.300	166.550
	31	0.000	0.000	223.450	219.850	153.050	148.700
	32	0.000	0.000	235.100	233.750	145.600	128.250
	33	0.000	0.000	190.600 *	193.100 *	129.250 *	142.350 *
	34	0.000	0.000	293.550 *	259.050 *	149.500 *	193.600 *
	35	0.000	0.000	225.500	221.850	149.400	151.250

	COLUMN	C76	C77	C78	C79
	COUNT	35	35	35	35
	ROW				
	1	4.000	4.000	4.000	4.000
	2	spike 3.000	3.000	4.000	4.000
	3	1.000	2.000	1.000	2.000
	4	176.300 *	176.900 *	38.200 *	40.450 *
DNT	5	86.400	85.800	50.850	47.700
	6	94.600	89.000	51.350	55.250
	7	88.800	90.300	49.250	53.900
	8	79.000	106.950	64.450	41.500
	9	84.300	85.200	47.500	49.750
	10	59.900 *	99.800 *	26.650 *	18.800 *
	11	87.700	87.650	50.400	50.400
	12	206.450 *	202.350 *	23.500	25.000
TNT	13	100.600	102.250	35.500	33.550
	14	101.300	116.650	34.250	30.000
	15	106.200	108.200	38.450	36.500
	16	112.050	130.950	47.000	37.600
	17	91.700	92.700	24.750	29.700
	18	73.600	104.200	30.350	67.750
	19	100.900	101.850	35.750	34.800
	20	472.200 *	478.550 *	202.950	188.600
RDX	21	320.600	331.050	266.050	227.850
	22	364.000	351.800	236.900	246.900
	23	317.450	314.350	224.650	226.000
	24	362.750 *	408.900 *	267.100 *	310.000 *
	25	266.100	277.800	189.800	187.850
	26	334.400	336.850	192.350	212.800
	27	314.650	313.150	216.150	214.700
	28	253.800 *	242.550 *	38.900	42.950
HMX	29	137.700	133.550	59.900	57.750
	30	157.550	183.450	48.000	51.200
	31	146.500	138.500	61.950	47.800
	32	156.650	130.400	77.000	86.100
	33	107.400 *	120.050 *	0.000 *	0.000 *
	34	127.200 *	110.550 *	0.000 *	178.850 *
	35	136.600	137.500	51.300	50.400

APPENDIX B: CHEMICAL STRUCTURES

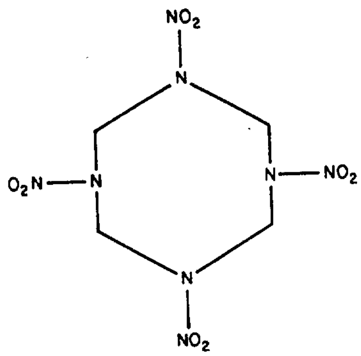

Figure B1. HMX

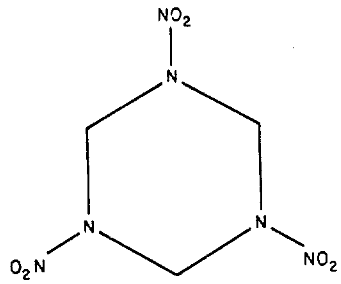

Figure B2. RDX

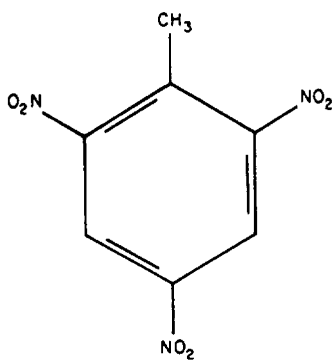

Figure B3. TNT

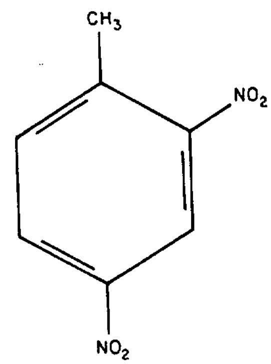

Figure B4. 2, 4-DNT

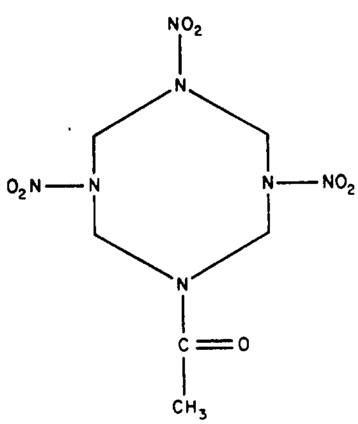

Figure B5. SEX

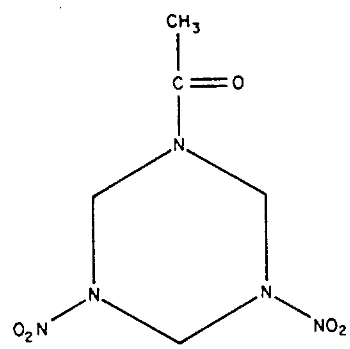

Figure B6. TAX

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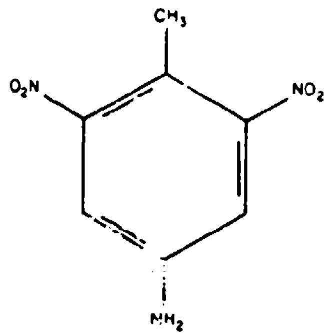

Figure B7. 2 Am-DNT.

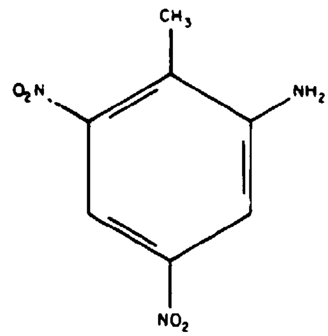

Figure B8. 4 Am-DNT

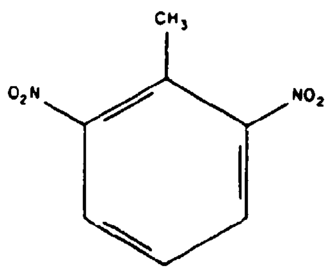

Figure B9. 2, 6 DNT

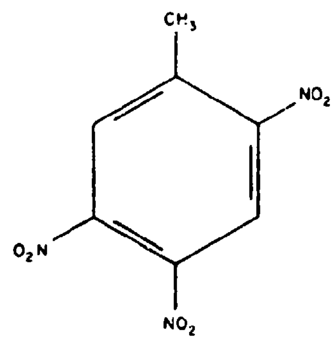

Figure B10. 2, 4, 5-TNT

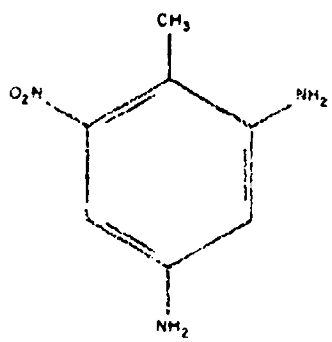

Figure B11. 2, 4-DAmNT

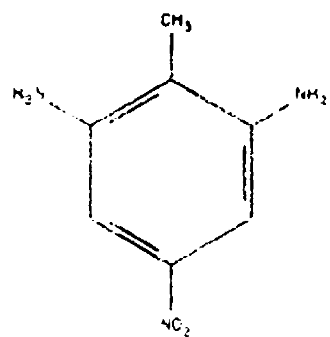

Figure B12. 2, 6-DAmNT

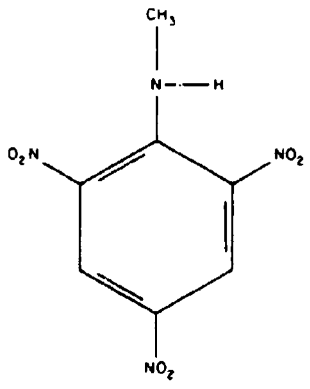

Figure B13. Tetryl

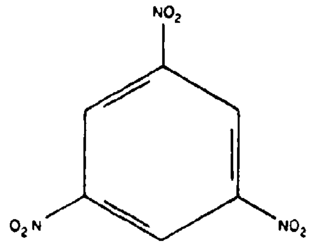

Figure B14. TNB

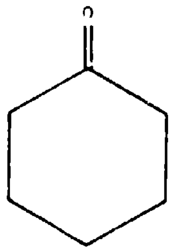

Figure B15. Cyclohexanone

APPENDIX C: PROTOCOL FOR INTERLABORATORY STUDY OF A REVERSE PHASE HPLC METHOD FOR THE DETERMINATION OF 2,4-DNT, TNT, RDX, AND HMX IN MUNITIONS WASTEWATER.

(This appendix exactly reproduces the protocol sent to the participating laboratories.)

I. INTRODUCTION

A. Objective

The goal of this study is to assess the capabilities of this HPLC method for the determination of 2,4-DNT, TNT, RDX and HMX* in the wastewater from munitions manufacturing and processing facilities and in groundwater.

B. Overview

Reverse phase HPLC will be used to determine the levels of the four analytes in four natural and waste water samples, and in these samples spiked with various amounts of standards. Strict adherence to the analytical protocol is essential in order for the statistical analysis of results to provide unbiased estimates of method performance. Bias in the intralaboratory precision can lead to the conclusion that laboratories differ systematically when they really do not. For instance, bias is introduced by discarding selected results and repeating analyses on an arbitrary basis.

Careful attention to detail is necessary to assure proper evaluation of the capabilities of the method for two reasons. Participation in this study represents the investment of a large amount of time and money by the organizers and the participating laboratories. Furthermore, if this method develops into a national regulatory method, a biased evaluation has much greater financial implications than just the cost of this interlaboratory study.

II. PREPARATIONS

A. Analyst

One analyst will be selected by the lab manager to be responsible for all aspects of this study, including receipt of materials through data

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* 2,4-DNT: 2,4-dinitrotoluene, henceforth referred to simply as DNT
TNT: 2,4,6-trinitrotoluene
RDX: 1,3,5-trinitro -1,3,5-triazacyclohexane
HMX: 1,3,5,7-tetranitro -1,3,5,7-tetraazacyclooctane

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analysis. There are several places where unsolved problems may call a halt to the protocol and require contacting Tom Jenkins* at CRREL. Further work cannot be performed until the problem is solved. The analyst, with the help of supervisors, should make reasonable attempts to resolve problems before calling.

B. Record Keeping

One notebook should be used exclusively for this study and should be labeled appropriately. Carbon or photo copies of notebook pages should be made. The original notebook must be submitted with the analytical report; the lab retains the copy. Complete documentation of experimental work and calculations is essential to help trace the sources of problems that may be discovered after data are returned to the coordinating laboratory.

C. Receipt of materials

The following materials will be shipped from CRREL:

Three 1-L bottles, each of which contains a different water sample typical of the sample type to which this method will be applied; shipped in ice water.

16 sealed glass ampules containing approximately 5 mL of mixed standards of the four analytes in methanol.

Two sealed glass ampules containing specified concentrations of the four analytes in methanol.

Two sealed glass ampules that are empty.

The following standard materials shipped from LCWSL:

Four vials of SARM**: two each of DNT and TNT, 200 mg neat, and two each of RDX and HMX, 200 mg under isopropanol.

Arrangements should be made to notify the analyst immediately when these materials arrive. Upon receipt, the analyst will log each container into the project notebook. Each entry should contain identification number, name, date of arrival, and description of condition. Inspect each

*(Commercial 603-646-4385, Autovon 684-4385, FTS 836-4385)
** Standard Analytical Reference Materials

container for damage. Broken, cracked, or leaking containers should be reported immediately to Tom Jenkins at CRREL, who will send replacements.

D. Storage of Materials

Water samples and ampules must be stored in the dark in a refrigerator or coldroom (temperature around 4°C, not below 0°C) immediately after receipt. The S/RMs should be stored in a freezer ($\leq$ 0°C).

III. ANALYSIS

A. Overview

The analytical work will be performed in two steps. The analyst first will spend some time becoming familiar with the test procedure. During this period working curves for each of the four analytes will be prepared and steps will be taken to establish that they are linear and pass through the origin. Then a sample whose composition is specified (provided by CRREL) will be analyzed. This experience should help to uncover potential systematic errors and allow the analyst to correct the causes. If uncorrected, these errors could cause a laboratory's results to be excluded from the statistical analysis at the end of the study.

The second portion of the work consists of analysis of four water samples; three of these will be provided by CRREL and the fourth is to be the laboratory's own reagent-grade water (distilled or deionized). These samples represent a range of matrices to which the HPLC method being tested should be applicable. Some amount of the four analytes (DNT, TNT, RDX, HMX) may be present. These matrices will be analyzed directly and after spiking with standard analyte solutions. Four separate spiking experiments will be performed for each matrix.

B. Experimental

1. Instrumentation

Chromatograph: The HPLC instrument should consist of a single high pressure pump and a 254-nm fixed wavelength ultraviolet absorption detector. If a fixed wavelength detector is not available, then a multiwavelength detector set to 254 nm may be used. A complete description of the instrument will be requested in the report.

Strip chart recorder: Full scale capacity should be compatible with the UV detector used. The trace is necessary to provide permanent record of experimental results. Computer storage of chromatograms is permissible if that is standard practice for the laboratory. These records should be retained by the participating laboratories unless requested by the coordinating laboratory.

Integrator: Calculates peak areas; may be a stand-alone digital integrator or computer-controlled integrator; mechanical or analog integrators may not be substituted without authorization.

Sample loop injector: Nominal 100-µL volume; syringe injection of 100 µL into a larger loop is not permissible without authorization.

2. Operating Parameters

Column: LC8 (Supelco) reverse phase, 25 cm × 4.6 mm; shipped from CRREL filled with methanol/water. Until this study has been completed, the column may not be used for any other purpose.

Column temperature: Room temperature; record hourly during analysis (± 1°C).

Solvent system: 50% water, 38% methanol, 12% acetonitrile by volume. Prepare using graduated cylinders, not volumetric flasks (because of solution contraction upon mixing). Prepare as a large batch (750 mL to 1000 mL), then vacuum filter through a solvent-washed 0.4-µm Nuclepore filter to remove particulate matter and to degas the solvent. Fresh solvent should be prepared daily.

Flow rate: 1.5 mL/min.

Detector: 254 nm

Integrator: Threshold set low enough to avoid negative intercept in working curve and high enough to avoid positive intercept (see section III. E).

Recorder: 0.2 in./min chart speed

3. Hardware/glassware

HPLC syringe: Any liquid-tight syringe of capacity 0.5 to 1.0 mL (e.g. Hamilton 750).

Filtration device: Nuclepore syringe filter, 25 mm diameter.

Filter: 0.4 µm Nuclepore polycarbonate, 25 mm diameter

Sample filtration syringe: 25 mL, glass or polyethylene (e.g. Plastipak; Becton, Dickinson and Co.; available through laboratory supply company; sterile -- no further cleaning necessary).

Volumetric flasks and pipets: Glass, class A or B; make sure condition is good (e.g. pipet tips not broken).

Scintillation vials: 20-mL glass with polyethylene cap insert (not aluminum); can be purchased from laboratory supply company (sterile; no further cleaning necessary).

Cleaning of volumetric glassware: Soak overnight in detergent, scrub briefly, rinse well with hot tap water, rinse with reagent-grade acetone, rinse with deionized water, oven dry at 105°C; rinse with appropriate solution before filling.

Reagents: Water, methanol, acetonitrile -- all HPLC grade.

Methanol-acetonitrile mixture: A solution consisting of 76% methanol and 24% acetonitrile is prepared and used throughout this method as a diluent for all water samples. This mixture is prepared using graduated cylinders rather than volumetric flasks to minimize systematic differences with the mobile phase because of volume contraction. Dilution with this mixture, rather than methanol alone, eliminates a negative peak which elutes just prior to HMX and results in unpredictable integration.

C. Calibration Standards

1. Individual Stock Standards for DNT, TNT, RDX, and HMX. These solutions must be used for the entire study.

For each material:

a. Vacuum dry SARMs at ambient temperature to constant weight (within 1 mg); a vacuum desiccator or vacuum oven attached to a water aspirator or vacuum pump will suffice. For RDX and HMX, remove most of the isopropanol by means of a Pasteur pipet, air dry for several hours, then vacuum dry. Store dried SARMs in a desiccator over dry calcium chloride or Drierite and place in the dark when not in use.

b. Accurately weigh about 0.1 g of each dried SARM onto weighing paper (e.g. VWR or Fisher-brand "Weighing Paper"); transfer carefully into separate 250-mL volumetric flasks. Reweigh weighing paper. Record mass to 0.1 mg.

c. For DNT and TNT dissolve and dilute to volume with methanol. For HMX and RDX, add 100 mL of acetonitrile to dissolve, then fill to volume with methanol.

d. Wrap the stoppered joint with Parafilm. This is an added protection against evaporation.

e. Calculate concentrations exactly in mg/L and label flasks.

f. Store in refrigerator at about 4°C (not below 0°C).

2. Combined-Analyte Working Stock Standard

a. Remove the stock standards from the refrigerator and allow to warm to room temperature (at least 30 min, but not overnight).

b. Invert flasks several times to mix.

c. Into a 1000-mL volumetric flask, pipet 10.0 mL each of DNT and TNT stock solutions and 25.0 mL each of RDX and HMX stock solutions. Dilute to volume with methanol. This standard will be about 4.0 mg/L in DNT and TNT and 10.0 mg/L in RDX and HMX.

d. Calculate the concentrations exactly in mg/L, label the flask, and date it.

e. Wrap the stoppered joint with Parafilm and store the flask in refrigerator when not in use. This standard may be used for one week from the date of preparation and then a fresh one must be prepared.

3. Working Standards

a. To be prepared fresh on each analysis day as instructed.

b. Remove the combined-analyte working stock standard from the refrigerator and allow to warm to room temperature (at least 30 min, but not overnight).

c. Invert flask several times to mix.

d. Transfer 2.00, 5.00, 10.00 and 20.0 mL by pipet into four 250-mL volumetric flasks, respectively.

e. Fill to mark with methanol/acetonitrile mixture. Stopper and invert ten times to mix.

f. Calculate the concentrations exactly in µg/L, label the flasks and date them.

4. Injected Standards

a. For each standard, pipet 10.0 mL into a scintillation vial.

b. Add 10.0 mL of HPLC grade water by means of a pipet.

c. Affix cap and shake to mix.

d. Prepare blank by combining 10.0 mL of methanol/acetonitrile mixture with 10.0 mL of water in vial. Affix cap and mix.

e. Label all vials appropriately.

The solutions that result represent the following concentrations in a 10.0-mL aqueous sample:

Aliquot volume of combined standard (mL)	Approximate concentrations (μg/L) For DNT and TNT	For RDX and HMX
2	32	80
5	80	200
10	160	400
20	320	800

Note that these values represent the concentrations before addition of the water. (The actual concentrations are half as large.) This can be done because the samples are treated similarly: a one-to-one dilution is made by adding 10.0 mL of methanol/acetonitrile mixture to 10.0 mL of aqueous sample. Thus, the analytical results derived from the working curve need not be corrected for this extra dilution.

The 10.0 mL methanol-acetonitrile/10.0 mL water mixtures are made in scintillation vials rather than in volumetric flasks because a slight volume contraction occurs. This might cause a systematic error because the standards would be diluted with water to volume and the samples diluted with the organic solvent to volume. Volume contraction therefore would lead to the samples being slightly richer in the organic solvent than the standards. Care must be taken in this step to pipet these 10-mL volumes accurately since experience has indicated that a significant error at this stage is compounded when peak areas are measured.

D. HPLC Procedure

1. Initial Conditioning

The HPLC column is new. Consequently, conditioning with the mobile phase and a test of performance are required before putting the column to work. This test may be performed the same day as the preliminary experiments (see Section III. E) but must be performed first.

a. Conditioning: Follow the procedure below (section III. D.2) for instrument warm-up, except pass at least 30 void volumes (about 60 mL) of mobile phase through the column. Continue until the UV detector baseline is level when set to its greatest sensitivity.

b. Performance test (calculation of plate number).

(1) Take a 1-mL aliquot from the combined-analyte working stock standard and dilute to 100 mL in a volumetric flask with methanol/acetonitrile.

(2) Use the proper sample injection procedure described in section III.D.3 below to obtain a chromatogram. All four analytes should elute within 10 minutes. Use the conditions described in section III.B.2 above, but select a chart speed that spreads the peaks out abnormally wide (such that widths at half height are at least 2.0 cm). Measure the peak width at half height to the closest millimetre.

(3) Calculate the number of plates (N) on the column from each peak using the equation

$$N = 5.54 \left(\frac{t_r}{t_{0.5}}\right)^2$$

where t_r is the retention time and $t_{0.5}$ is the width of the peak at half height, both in minutes.

(4) Average the results for all four analytes.

(5) If the average value is less than 3,000 plates, carefully recheck the calculation. If there is no error, allow another 30 void volumes of mobile phase to wash through the column and repeat the experiment. If the calculated value of N still does not exceed 3,000, the column is not performing up to its specification. If used it may invalidate results from this laboratory. Notify Tom Jenkins at CRREL immediately if this occurs.

2. Normal Warm-Up Procedure

a. Turn on all electronic equipment and allow to warm-up for at least 30 min.

b. Pass at least 15 void volumes of mobile phase through the column (20 min at 1.5 mL/min) and continue until the UV detector baseline is level when set to its greatest sensitivity.

c. Make certain the pumps are not experiencing vapor lock as indicated by large pressure fluctuations.

d. Check system thoroughly for leaks.

3. Sample Injection Procedure

a. Fill the analytical syringe with methanol/acetonitrile and discharge into a waste beaker.

b. Repeat twice more to remove traces of previous sample.

c. Rinse syringe three times with the sample.

d. Fill syringe with sample to at least 500 µL and inject most of this through sample loop, avoid introducing air bubbles. Overfilling the loop in this manner assures that the sample injected is not diluted by solvent in the loop.

E. Preliminary Experiments

Before beginning the analyses of the water samples, the analyst should become familiar with the analytical procedure. For this purpose an ampule containing the four analytes has been included as a test sample. This sample should be prepared by transferring a 1.00-mL aliquot of the ampule solution using a volumetric pipet into a 100-mL volumetric flask and diluting to volume with methanol/acetonitrile. This solution may be used up to three days after preparation. A 10.0-mL aliquot of this solution is transferred to a scintillation vial and 10.0 mL of HPLC-grade water is added. Cap tightly and shake.

Test HPLC column plate number specification, if not already done (see section III.D.1). Otherwise, follow the instrument warm-up and column conditioning instructions (see section II.D.2).

Prepare the working standards and blank as specified in sections III.C.3 and III.C.4. Using the procedure described in section III.D.3, inject each standard and blank into the HPLC at least once. Ascertain the detector range that provides sizeable but on-scale peaks so that a good chromatographic record results. Make certain that integration is occurring properly.

Next proceed as if the test material were a real sample to be analyzed.

1. Carefully prepare working curves for the four analytes (see section 4 below). <u>These curves will be the basis of all of the remaining quantitative work; consequently, it is essential that systematic errors be avoided</u>.

2. Carry the test sample through filtration (see section III.F.5 steps <u>c</u> to <u>e</u>) and analysis (see section III.D.3) at least three times and as many more times as is necessary to become accustomed to the procedure.

3. From the last two injections of test sample, determine the concentrations of all four analytes in the test sample using the working curves. Compare results with the specified values. The mean determined values should be within 15% of the specified values. If not, attempt to resolve discrepancies and then process another 10-mL aliquot of the solution in the 1-L flask. If all four analytes are within 15% of the specified values, proceed to section F; if not contact Tom Jenkins.

Construction of Working Curve

a. Obtain chromatograms of the four working standards and blank in duplicate (10 injections total). Sequence the injections randomly (see Appendix A).

b. Plot peak area versus concentration for each of the four analytes. Do not average the duplicates before plotting. Inspect the plot for gross deviations from linearity -- a set of duplicates wildly off line, or a large degree of curvature. Preliminary work has demonstrated that the analytical response is linear from 10 μg/L to 20 mg/L for DNT and TNT and 25 μg/L to 50 mg/L for RDX and HMX. Significant deviation from linearity is evidence for systematic bias. Whereas it is possible to make analytical determinations with a nonlinear working curve, it is preferable that the systematic error be found and corrected before beginning the interlaboratory test measurements. Once gross errors have been corrected and the plot looks reasonably linear, more rigorous statistical tests must be applied. (If obvious curvature still exists and you have the appropriate computational facilities, inspect the residuals as an aid in diagnosing the problem; otherwise, contact Tom Jenkins).

c. Calculate the regression analysis tables for each analyte using both the model through the origin and the model with an intercept (see Appendix B).

d. Test the model with an intercept for lack of fit for each analyte (see Appendix B). (Comparison of correlation coefficients alone is insufficient.)

- If a significant lack of fit exists for any of the analytes, plot the regression line on top of the data points. Inspect for wild points or curvature. (If you have the appropriate computational facilities, inspect the regression residuals.) Try to resolve the source of nonlinearity. If the problem cannot be resolved, contact Tom Jenkins.

e. Test the hypothesis that the intercept equals zero (see Appendix B).

- It is highly desirable to achieve a calibration that has a zero intercept because this simplifies the daily calibration routine. Thus for daily analysis instead of constructing a complete working curve, it is necessary only to run several replicates of the most concentrated standard.

- If it is found that an intercept is not zero, the most likely reason is that the integrator "zero" has been set too high (negative intercept) or too low (positive intercept). Adjust the integrator and repeat steps a through d. If this repetition fails to provide zero intercepts, search for other causes. If the problem cannot be resolved, contact Tom Jenkins.

F. Analysis of Water Samples

For each of the four water samples, all of the analyses must be performed on a single day. The sequence in which the matrices must be analyzed is given in Appendix C. Analysis of duplicates is an important part of this study. Sometimes duplicates will appear to be quite different in their response and there will be a strong inclination to discard a response and obtain a new one. Please do not do so, unless there is certainty that a systematic error has been made. Rejection of such data tends to make the within-lab reproducibility artificially good. This increases the sensitivity of statistical tests for differences between laboratories. Thus, significant differences may be found where no differences actually exist.

1. Remove matrix and its corresponding four ampules and the combined-analyte working stock standard from refrigerator and allow to warm to room temperature (at least 30 min., but not overnight). Note that the ampules are keyed to be used with a specific matrix (e.g., ampules A1 through A4 go with matrix A).

2. Warm up instrument and condition HPLC column (see section III.D.2).

3. Calibration

   If linear working curves with zero intercepts were obtained during the preliminary experiments, daily calibration only requires analysis of the most concentrated working standard. Proceed as follows:

   a. Prepare the most concentrated working standard from the combined-analyte stock standard (see section III.C.3).

   b. Prepare one vial of this standard for injection (see section III.C.4 a to c and e). Keep this vial tightly capped when not in use.

   c. Obtain chromatograms of this standard in triplicate (see section III.D.3).

   d. Calculate the mean and standard deviation of the peak areas for each of the four constituents.

   e. For each analyte, compare this mean with the response expected from the working curves already established (see Appendix D).

   f. If the test indicates no differences for any of the analytes, skip to instruction 4.

g. If the test is significant for any of the analytes, there may be a systematic preparation error, or instrumental response has drifted. To distinguish between these possibilities, carefully repeat steps a through e.

h. If the tests against the working curves (Appendix D) still indicate significant difference, test for equivalence between the two sets of triplicates run today (see Appendix E).

i. If the test in h indicates no difference, skip to instruction 4.

j. If the test in h indicates significant difference, either the instrument is subject to strong short term drift or noise or there is insufficient reproducibility in the analyst's technique of solution preparation. Call Tom Jenkins before proceeding further.

4. Proceeding one ampule at a time (to avoid solvent evaporation):

   a. Open ampule carefully by filing and breaking at neck.

   b. Transfer entire contents of ampule (about 5 mL) into a scintillation vial. Immediately pipet 1.00 mL of this solution into a 100-mL volumetric flask. Fill to volume with the water sample. Invert 10 times to mix. This solution will be referred to as the "spiked sample."

   c. Label this flask, indicating the ID number of the ampule from which the solution came.

   d. Repeat steps a through c for the other three ampules.

   e. Prepare the unspiked sample by repeating steps b and c, except begin with 1.00 mL of methanol instead of ampule solution.

5. Five solutions in 100-mL volumetrics are in hand. From each solution, two 10.0-mL aliquots will be taken, processed as below, and injected in duplicate into the HPLC instrument. In addition, the standard prepared in step 3b above will be injected five times. Consequently, a total of 25 injections will be made. The sequence of processing and injection must be randomized. Determine the order of injection of samples (see Appendix F). Then proceed through the following steps:

   a. When the injection sequence calls for injection of standard or for the second injection of a sample, skip to step f.

   b. Pipet 10.0 mL of the sample from its 100-mL volumetric flask into a scintillation vial. Add 10.0 mL of methanol/acetonitrile solution by pipet. Attach cap tightly. Shake vigorously. Let stand for at least 15 minutes before filtration (during this waiting period, the next samples in the sequence should be processed to avoid losing time later). The organic solvent is added for two reasons: (1) to help desorb analyte from the surfaces of particulates and dissolve small particles of analyte that could be present, and (2) to provide a sample compatible with the HPLC mobile phase.

c. Load new Nuclepore filter into filter holder.

d. Rinse 25-mL filtration syringe with methanol/acetonitrile solution then fill to about 10 mL with sample. Filter sample and discard this filtrate.

e. Fill syringe with remaining sample. Filter into a new scintillation vial. This solution will be analyzed. Label vial appropriately.

f. Using proper procedure (see section III.D.3), inject this solution into the HPLC.

g. Repeat steps a through f for each sample in the proper sequence.

G. Data Analysis

1. Determine working curves for each of the four analytes:
   a. Calculate the mean peak area ($\bar{y}$) for the five replicates of the standard.

   b. Solve the equation $\bar{y}/x_{HI} = b_1$ where x_{HI} is the known concentration of the highest standard and b_1 is the slope of the working curve.

2. Substitute the value for the slope into the working curve equation $y = b_1x$. Calculate the concentrations (x) for the 20 injections of spiked and unspiked water samples using individual peak areas (y).

H. Reporting of Results

An example of the format for reporting results is given in Appendix G.

APPENDIX A

Random Injection Sequence for Working Curve

The samples consist of a blank and four standards, each of which will be injected in duplicate (1 and 2). The sequence of injection of these 10 trials must be random. Use computer generated random numbers, random number tables, or pull slips of paper numbered 1 to 10 from a hat. Record the resulting sequence in the following table and in the notebook, then use this table to keep track of the order of injections.

Standard Concentration (μg/L nominal)			
DNT,TNT	RDX,HMX	Replicate	Sequence
0	0	1	
0	0	2	
32	80	1	
32	80	2	
80	200	1	
80	200	2	
160	400	1	
160	400	2	
320	800	1	
320	800	2	

APPENDIX B

Regression Analysis*

Previous testing has demonstrated (see section III.E for details) that chromatographic peak area (y) should be a linear function of analyte concentration (x). Two models may be tested, the model through the origin: $y = b_1x$, and the model with an intercept: $y = b_0 + b_1x$. The coefficients for these models can be calculated as follows:

For model through origin:

$$b_1 = \frac{\Sigma xy}{\Sigma x^2} \qquad\qquad b_0 = 0$$

For model with intercept:

$$b_1 = \frac{\Sigma xy - \frac{\Sigma x \Sigma y}{n}}{\Sigma x^2 - \frac{(\Sigma x)^2}{n}} \qquad\qquad b_0 = \bar{y} - b_1\bar{x}$$

where $\bar{y}$ and $\bar{x}$ represent respective mean values, n is the number of data points, and y is the value of y predicted by the regression equation.**

Regression analysis tables are used to determine whether the data fit the linear models well enough and which linear model is more applicable. The tables must be calculated as follows:

<u>Table for Model with Intercept</u>

	Sum of squares (SS)	Degrees of freedom (df)	Mean square (MS)	F-ratio (F)
Residual	$\Sigma y^2 - \frac{(\Sigma y)^2}{n} - b_1^2 \quad \Sigma x^2 - \frac{(\Sigma x)^2}{n}$	6	$\frac{\text{resid. SS}}{6}$	---
Error	$\frac{\Sigma d^2}{2}$	4	$\frac{\text{SS error}}{4}$	---
Lack of fit (LOF)	Residual SS - Error SS	2	$\frac{\text{SS LOF}}{2}$	$\frac{\text{MS LOF}}{\text{MS error}}$

*Do not round off intermediate numbers in calculations. Carry through at least six digits to avoid round off errors, even though in the final results less than six digits will be significant.

**The two replicate analyses of the blank (zero analyte) are <u>not</u> used to obtain regression equations.

where n is the number of data points and d is the difference between the peak areas of duplicates. For the model through the origin, the table is:

Table for Model through Origin

	Sum of squares (SS)	Degrees of freedom (df)	Mean square (MS)	F-ratio (F)
Residual	$\Sigma y^2 - \frac{(\Sigma xy)^2}{\Sigma x^2}$	7	$\frac{\text{resid. SS}}{7}$	---
Error	$\frac{\Sigma d^2}{2}$	4	$\frac{\text{SS error}}{4}$	---
Lack of fit (LOF)	Residual SS - Error SS	3	$\frac{\text{SS LOF}}{3}$	$\frac{\text{MS LOF}}{\text{MS error}}$

Test for lack of fit: For the model with an intercept, the critical value is $F_{.95}$ (2,4) = 6.94. If the F-ratio calculated in the right-hand column of the regression analysis table exceeds the critical value, there is a significant lack of fit; i.e., the working curve is not linear. Steps as suggested in the text must be taken to correct this problem. If the calculated value is less than 6.94, the linear model is satisfactory. It is not necessary to test the model through the origin.

After establishing linearity, the intercept must be tested to determine whether it is significantly different from zero. Calculate the F ratio:

$$F = \frac{\text{(resid. SS for model through origin)} - \text{(resid. SS of model with intercept)}}{\frac{\text{(residual SS of model with intercept)}}{6}}$$

where the 'residual SS" are in the tables. This can be done only after LOF has been shown to be insignificant. The critical value is $F_{.95}$ (1,6) = 5.99. If the calculated value exceeds the critical value, the intercept is significantly different from zero. Steps as suggested in the text must be taken to correct this problem. If the problem cannot be resolved, contact Tom Jenkins. If the calculated value is less than 5.99, the intercept is zero.

APPENDIX C

Determination of Chronological Order for Water Sample Analyses

Four water matrices are to be analyzed: three are provided by CRREL (A,B,C) and the fourth must be the participating laboratory's distilled or deionized water supply (D). Randomly select the sequence in which these four samples must be studied by means of computer-generated random numbers, random number tables, or pulling slips of paper numbers 1 to 4 out of a hat.

APPENDIX D

Daily check of instrument calibration is achieved by measuring the detector responses for the four analytes in the most concentrated standard. This is performed before beginning the analysis of a number of samples.

The statistical test is based on comparing the mean of triplicate peak area measurements of the standard with the confidence intervals around the working curve which was established during the preliminary experiments. The equations used to perform the comparison are as follows:

$$s_{yp} = \left[\frac{(n_{wc} - 1)\, s_{wc}^2 + (n - 1)\, s^2}{(n_{wc} - 1) + (n - 1)}\right]^{1/2} \quad (1)$$

$$PI = y_{HI} \pm t_{CRIT}\, s_{yp} \left(\frac{1}{n} + \frac{x_{HI}^2}{\Sigma x^2}\right)^{1/2} \quad (2)$$

$$t_{CRIT} = t_{.95}\ (df = 9) = 2.26 \quad (3)$$

where

n = 3, the number of data points in set to be compared with working curve

n_{wc} = 8, the number of measurements used to calculate working curve

s = standard deviation of triplicates

s_{wc} = square root of residual mean square from regression analysis table for model-through-origin,

s_{yp} = pooled standard deviation

PI = prediction interval

y_{HI} = peak area predicted for high standard by working curve

x_{HI} = known concentration of high standard

Σx^2 = summation over all of the standard concentrations squared (remember that each is used twice; value should be about 1,692,800 for HMX)

df = degrees of freedom, equals 9; 7 for working curve, 2 for

Notes on use:

a. Standard deviations of triplicates are most easily calculated by means of:

$$s = \left(\frac{\Sigma y^2 - \frac{(\Sigma y)^2}{n}}{n - 1} \right)^{1/2}$$

b. Example:

(1) Given: slope = 2.5 concentration = 800 μg/L HMX

$s_{wc} = 20$

$y_{HI} = 2.5 \times 800 = 2000.$

(2) At start of day, 3 replicates are run. Mean area is $\overline{y} = 1960$ with $s = 7$.

(3) Use equation 1:

$$s_{yp} = \left(\frac{7(20)^2 + 2(7)^2}{7 + 2} \right)^{1/2} = 17.944$$

(4) Use equation 2:

$$PI = 2000 \pm 2.26(17.944) \left(\frac{1}{3} + \frac{800^2}{1,692,800} \right)^{1/2}$$

Thus PI = 2000 ± 34.2 = [1965.8 - 2034.2]

(5) Is the mean of the triplicates within the PI? No, since prediction interval is [1965.8 - 2034.2] and $\overline{y} = 1960$ is outside interval.

APPENDIX E

Comparison of Two Sets of Triplicate Standards

Use the following equations:

$$S_{yp} = \sqrt{s_1^2 + s_2^2} \qquad (1)$$

$$t = \frac{(n)^{1/2} |\bar{y}_1 - \bar{y}_2|}{(2)^{1/2} s_{yp}} \qquad (2)$$

$$t_{.95} \text{ (df = 4)} = 2.78 \qquad (3)$$

where

$\bar{y}_1$ and $\bar{y}_2$ = means of the two sets of triplicates

s_1 and s_2 = corresponding standard deviations

$n = 3$

if $t < t_{.95}$, then the hypothesis that $\bar{y}_1 = \bar{y}_2$ is accepted.

APPENDIX F

Random Injection Sequence for Analyses of Spiked Water Matrices

The solutions to be analyzed on a given day consist of: a pair of unspiked samples, four pairs of spiked samples, each pair spiked from a different ampule, and a high-concentration standard. The water samples will be injected twice each into the HPLC; the standard, five times. The sequence of 20 sample injections for each water matrix must be random with the standards being injected at fixed points in this sequence (see table below). Use computer generated random numbers, random number tables, or pull slips of paper number 1 to 20 out of a hat. Record the results in table below, and also in the notebook. Repeat the random selection procedure for each matrix.

		Sequence			
Sample	Replicate	Matrix A	Matrix B	Martix C	Martix D
Standard	1	---------------First---------------			
Standard	2	----between 5th and 6th positions---			
Standard	3	----between 10th and 11th positions---			
Standard	4	----between 15th and 16th positions---			
Standard	5	---------------Last---------------			
1st vial, unspiked	1				
	2				
2nd vial, unspiked	1				
	2				
1st vial, spike 1	1				
	2				
2nd vial, spike 1	1				
	2				
1st vial, spike 2	1				
	2				
2nd vial, spike 2	1				
	2				
1st vial, spike 3	1				
	2				
2nd vial, spike 3	1				
	2				
1st vial, spike 4	1				
	2				
2nd vial, spike 4	1				
	2				

APPENDIX G
Format of Final Report

FINAL REPORT
on HPLC Determination of Ordinance Materials in Water

Sponsor Laboratory: USACRREL

Participating Laboratory:

Laboratory Manager:
Analyst:

Checklist of items to be included in report:

laboratory manager's profile of analyst ________

original project notebook ________

complete description of HPLC instrument and integrator ________

Preliminary Experiments

A. Plate count of HPLC column: ____________________

B. Masses of SARM solid taken for stock standards:

DNT __________ g
TNT __________ g
RDX __________ g
HMX __________ g

C. Working curves, in the form: (area) = b_1 (concentration)

DNT:
TNT:
RDX:
HMX:

D. Analysis of test sample composition; do not correct for 1000-fold dilution; report as µg/L

DNT:
TNT:
RDX:
HMX:

E. Retention times of analytes in test sample (min)
DNT:
TNT:
RDX:
HMX:

Analytical Results

Matrix Type: ____________ Date of Analysis: ____________________

		DNT	TNT	RDX	HMX
Working Curve Slopes					

Sample Analyses:	Replicate number	Determined Concentrations (μg/L)*
unspiked matrix, vial 1	1	
	2	
unspiked matrix, vial 2	1	
	2	
spike 1, vial 1	1	
	2	
spike 2, vial 2	1	
	2	
spike 2, vial 1	1	
	2	
spike 2, vial 2	1	
	2	
spike 3, vial 1	1	
	2	
spike 3, vial 2	1	
	2	
spike 4, vial 1	1	
	2	
spike 4, vial 2	1	
	2	

*Report values to the nearest tenth of a μg/L

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Jenkins, T.F.
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