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TREASURE ISLAND
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Task Order 001

EXPLOSIVES IN SOIL BY HIGH PRESSURE LIQUID CHROMTOGRAPHY

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Prepared for:

U.S. ARMY TOXIC AND HAZARDOUS MATERIALS AGENCY
Aberdeen Proving Ground, MD. 21010-5401

ENCLOSURE (1) 46

USATHAMA METHOD NUMBER: LW12

DETERMINATION OF EXPLOSIVES IN SOIL BY HIGH PRESSURE LIQUID CHROMATOGRAPHY

I. SUMMARY

A. ANALYTES

This method is applicable to the Class 1 analysis of the following organic compounds in environmental soil samples.

Analytes

1,3-Dinitrobenzene
2,4-Dinitrotoluene
2,6-Dinitrotoluene
HMX (octahydro-1,3,5,7-tetranitro-s-tetrazocine)
Nitrobenzene
Nitroglycerin
PETN (pentaerythritol tetranitrate)
RDX (hexahydro-1,3,5-trinitro-s-triazine)
Tetryl (N-methyl-N,2,4,6-tetranitrobenzenamine)
1,3,5-Trinitrobenzene
2,4,6-Trinitrotoluene

B. MATRIX

This method is applicable to all relatively dry environmental soil matrices.

C. GENERAL METHOD

This method employs extraction of one gram of an environmental soil using two milliliters (2mL) of acetonitrile. Extraction is accomplished by vortexing followed by sonication of the

sample for two hours. The resulting extract is filtered and diluted 1 to 8 with water. The target analytes are separated on a high pressure liquid chromatography (HPLC) column using isocratic elution, and detected using ultraviolet absorbance (UV) at 230 nanometers (nm).

II. APPLICATION

A. TESTED CONCENTRATION RANGE.

The certification testing ranges in micrograms per gram (ug/g) are:

Analyte	Tested Range (ug/g)
1,3-Dinitrobenzene	0.496 - 24.8
2,4-Dinitrotoluene	0.424 - 21.2
2,6-Dinitrotoluene	0.524 - 26.2
HMX	0.666 - 33.3
Nitrobenzene	0.548 - 11.0
Nitroglycerin	4.00 - 200
PETN	4.00 - 80.0
RDX	0.438 - 21.9
Tetryl	0.404 - 20.2
1,3,5-Trinitrobenzene	0.488 - 24.4
2,4,6-Trinitrotoluene	0.456 - 22.8

C. SENSITIVITY

The instrumental responses for each compound, reported in peak area units at the certified reporting limit (CRL) are

Analyte	Certified Reporting Limit (ug/g)	Area Counts

1,3-Dinitrobenzene	0.496	40000
2,4-Dinitrotoluene	0.424	27500
2,6-Dinitrotoluene	0.524	20000
HMX	0.666	40000
Nitrobenzene	2.41	50000
Nitroglycerin	4.00	4000
PETN	4.00	4000
RDX	0.587	35000
Tetryl	0.731	40000
1,3,5-Trinitrobenzene	0.488	45000
2,4,6-Trinitrotoluene	0.456	30000

C. REPORTING LIMITS.

The certified reporting limits and upper certified limit for each analyte in environmental soil samples are:

Analyte	Certified Reporting Limit (ug/g)	Upper Certified Limit (ug/g)
1,3-Dinitrobenzene	0.496	24.8
2,4-Dinitrotoluene	0.424	21.2
2,6-Dinitrotoluene	0.524	26.2
HMX	0.666	33.3
Nitrobenzene	2.41	27.4
Nitroglycerin	4.00	200
PETN	4.00	80
RDX	0.587	21.9
Tetryl	0.731	20.2
1,3,5-Trinitrobenzene	0.488	24.4
2,4,6-Trinitrotoluene	0.456	22.8

D. INTERFERENCES.

Any materials which are extracted from soil with acetonitrile,

coelute with the explosives through the HPLC column, and which absorb ultraviolet radiation at 230 nm may cause interferences. Carryover from analysis of a highly contaminated sample can result in apparent contamination of the succeeding samples analyzed. Such contamination is often manifested by the presence of unusually broad chromatographic peaks nested among narrower peaks. This interference is minimized by reanalyzing heavily contaminated samples following dilution, running blanks after heavily contaminated samples until carry over is removed, and/or rinsing the system with a mobile phase containing a high proportion of organic modifier until the contamination is removed.

E. **ANALYSIS RATE.**

After instrument calibration, one analyst can analyze approximately 8 samples per 8-hour day.

F. **SAFETY INFORMATION.**

The target compounds in this method are toxic explosives and some are known carcinogens, e.g. 2,4-Dinitrotoluene. The preparation of all standards should be performed in a laboratory hood. Adequate dermal protection must be used when handling samples and standards.

Most of these compounds are either primary or secondary explosives and should be handled with care to avoid contact with electrostatic shocks or impacts. Tetryl, RDX, and PETN have intermediate sensitivity between initiating explosives and explosives used as bursting charges. However, PETN is used as an initiating agent and is extremely sensitive to initiation (more sensitive than either Tetryl or RDX). Tetryl is toxic when taken internally or by skin contact. RDX, HMX,and TNT are used as bursting charge explosives. Although TNT is less

sensitive to friction and impact than many other high explosives, it can be detonated with moderate force when confined between metal surfaces such as on the threads of bolts. TNT will form sensitive materials in the presence of alkalies.

III. APPARATUS AND CHEMICALS.

A. GLASSWARE/HARDWARE.

1. 4 mL vials with screw top cap and teflon septa (Waters Wisp autosampler vials).
2. Vortex mixer, Scientific Products Vortex-Genie, Model S8223.
3. Ultrasonic bath, Bransson 3200.
4. Filter assembly, Gelman Acrodisc CR 0.45 um
5. 5 mL syringe, Hamilton 10005TLL
6. 250 uL syringe, Hamilton 7255NR
7. 1000 uL syringe, Hamilton 1001LTN
8. Class A Volumetric flasks -100, 25 and 50mL
9. Class A Volumetric pipets-0.5, 1.0, 2.0, 5.0, and 10.0mL
10. Micro pipets 50, 100, and 200 uL
11. Balance, capable of weighing to 0.0001 gram

B. INSTRUMENTATION

1. HPLC: Shimadzu model LC-6A high-pressure liquid chromatograph with Shimadzu model SIL-6A autosampler (or, equivalent).
2. Detector: Perkin-Elmer LC-75 variable wavelength ultraviolet absorbance detector (UV) set at 230 nanometers.
3. Column: Supelcosil LC-18 octadecyldimethylsilyl, reverse-phase column, 25 centimeters (cm) length x 4.6 millimeters

(mm) I.D., 5 micrometers (um) particle size (Supelco, Inc., Bellefonte, Pa.).

4. Mobile phase: 50 percent methanol: 50 percent water.
5. Flow rate: 1.0 milliliters per minute (mL/min).
6. Sample Volume: 250 microLiters (uL).

C. **ANALYTES**

Analyte	USATHAMA Abbrev.	CAS Number
1,3-Dinitrobenzene	DNB	99-65-01
2,4-Dinitrotoluene	24DNT	121-14-2
2,6-Dinitrotoluene	26DNT	606-20-2
HMX	HMX	2691-41-0
Nitrobenzene	NB	98-95-3
Nitroglycerin	NG	53-63-0
PETN	PETN	75-11-5
RDX	RDX	121-84-4
Tetryl	TETRYL	479-45-8
1,3,5-Trinitrobenzene	TNB	25377-32-6
2,4,6-Trinitrotoluene	TNT	118-96-7

D. **REAGENTS AND SARMS**

1. The standards used for target compound certification and calibration are USATHAMA supplied standard analytical refernce materials (SARMS). Equivalent standards may be used as long as they have been characterized according to Section 6.5.3 of the USATHAMA Quality Assurance Plan (2nd Edition, March, 1987). USATHAMA SARMS were used in this certification, and their lot numbers are listed below:

Analyte	SARM LOT NUMBER
1,3-Dinitrobenzene	2250
2,4-Dinitrotoluene	1147

2,6-Dinitrotoluene	1148
HMX	1217
Nitrobenzene	2177
Nitroglycerin	1150
PETN	1151
RDX	1130
Tetryl	1149
1,3,5-Trinitrobenzene	1153
2,4,6-Trinitrotoluene	1129

2. Methanol (HPLC grade - American Burdick & Jackson, McGaw Park, Illinois).
3. Water (HPLC grade - American Burdick & Jackson, McGaw Park, Illinois).
4. Acetonitrile (HPLC grade - American Burdick & Jackson, McGaw Park, Illinois).

IV. CALIBRATION

A. INITIAL CALIBRATION.

1. Preparation of Standards.

Precertification Calibration. Separate primary stock standards (SPSS) for each target analyte are prepared according to the dilution scheme presented in Table 4-1. The SPSS solutions should be prepared fresh every 2 months. Tetryl needs to be made fresh every 2 weeks. Each separate stock solution is made to volume with acetonitrile. Two SARMS (nitroglycerin and PETN) are provided by USATHAMA as solutions containing a total of 200 milligrams. The entire contents of the vial were used to prepare the respective primary stock standards. Aliquots of the separate primary stock standards (SPSS) are used to prepare the combined stock standard (CSS) by dilution to a final volume of 50 mL using acetonitrile as described in Table 4-2. The CSS solutions should be prepared fresh every week. All primary stocks and combined stocks should be stored in a freezer.

For precertification calibration, duplicate composite calibration standards (CCS-1 through CCS-7) are prepared from the combined stock standard (CSS) as given in Tables 4-3 and 4-4. HPLC-grade water is used for dilution to final volumes for the composite calibration standards. Standards are prepared fresh daily.

Initial Calibration. Standards CCS-1, CCS-3, CCS-5, CCS-6 CCS-7, and a blank described in Tables 4-3 and 4-4 are prepared. These solutions are prepared fresh for every run.

2. Instrument Calibration.

Table 4-1. Preparation of Separate Primary Stock Standards (SPSS).

Analyte	13DNB	24DNT	26DNT	HMX	NB	NG	PETN	RDX	TETRYL	135TNB	246TNT
mg SARM added	12.4	10.6	13.1	41.6	13.7	200	200	109.5	10.1	12.2	11.4
Final Volume (mL)	10.0	10.0	10.0	25.0	10.0	50.0	50.0	100.0	10.0	10.0	10.0
Conc. of SPSS (ug/mL)	1240	1060	1310	1664	1370	4000	4000	1095	1010	1220	1140

Note: Each SARM diluted to the volume indicated with acetonitrile.

Source: ESE, 1988.

Table 4-2. Preparation of Composite Stock Standard (CSS).

Analyte	13DNB	24DNT	26DNT	HMX	NB	NG	PETN	RDX	TETRYL	135TNB	246TNT
mL SPSS added	1.0	1.0	1.0	1.0	1.0	2.5	2.5	1.0	1.0	1.0	1.0
Final Volume (mL)	50	50	50	50	50	50	50	50	50	50	50
Conc. of CSS (ug/mL)	24.8	21.2	26.2	33.28	27.4	200	200	21.9	20.2	24.4	22.9

Note: Acetonitrile used for dilution to the 50 mL final volume for the Composite Stock Standard (CSS).

Source: ESE, 1988.

Table 4-3. Preparation of Composite Calibration Standards 1 Through 4 (CCS-1 through CCS-4).

Analyte	13DNB	24DNT	26DNT	HMX	NB	NG	PETN	RDX	TETRYL	135TNB	246TNT
Conc. in CCS-1 (ug/mL)	2.480	2.120	2.620	3.330	2.740	20.0	20.0	2.19	2.02	2.44	2.28
Conc. in CCS-2 (ug/mL)	0.992	0.848	1.048	1.330	1.096	8.00	8.00	0.876	0.808	0.976	0.912
Conc. in CCS-3 (ug/mL)	0.496	0.424	0.524	0.666	0.548	4.00	4.00	0.438	0.404	0.488	0.456
Conc. in CCS-4 (ug/mL)	0.248	0.212	0.262	0.333	0.274	2.00	2.00	0.219	0.202	0.244	0.228

Note: CCS-1 prepared by diluting 5.0 mL of CSS to 50 mL with HPLC-grade water.
CCS-2 prepared by diluting 2.0 mL of CSS to 50 mL with HPLC-grade water.
CCS-3 prepared by diluting 1.0 mL of CSS to 50 mL with HPLC-grade water.
CCS-4 prepared by diluting 0.5 mL of CSS to 50 mL with HPLC-grade water.

Source: ESE, 1988.

Table 4-4. Preparation of Composite Calibration Standards 1 Through 4 (CCS-5 through CCS-7).

Analyte	13DNB	24DNT	26DNT	HMX	NB	NG	PETN	RDX	TETRYL	135TNB	246TNT
Conc. in CCS-5 (ug/mL)	0.0992	0.0848	0.1048	0.133	0.1096	0.800	0.800	0.0876	0.0876	0.0976	0.0912
Conc. in CCS-6 (ug/mL)	0.0496	0.0424	0.052	0.0666	0.0548	0.400	0.400	0.0438	0.0404	0.0488	0.0456
Conc. in CCS-7 (ug/mL)	0.0248	0.0212	0.0262	0.0333	0.0274	0.200	0.200	0.0129	0.0202	0.0244	0.0228

Note: CCS-5 prepared by diluting 0.2 mL of CSS to 50 mL with HPLC-grade water.
CCS-6 prepared by diluting 0.1 mL of CSS to 50 mL with HPLC-grade water.
CCS-7 prepared by diluting 0.05 mL of CSS to 50 mL with HPLC-grade water.

Source: ESE, 1988.

To calibrate the instrument, 250 uL of each standard in Tables 4-3 and 4-4 is injected into the instrument in the same manner as a sample extract. Each duplicate composite calibration standard is analyzed during precertification calibration, and the single dilutions of the composite standards are analyzed during initial calibration.

3. Independent Reference Standard.

An independent stock will be prepared to serve as a reference standard for explosives in soil. The independent reference standard must be analyzed along with the initial and precertification calibration standards, and the results must be within ± 25% of the expected value, for the calibration standards to be considered valid. If the analysis of the independent reference standard fails, the source of the problem must be identified and corrected. The results of the second analysis of the independent reference standard must be within the acceptable limits before the analysis of samples may proceed. Since a new initial calibration is performed daily, a reference, if available, is required at least weekly.

4. Analysis of Calibration Data.

After analyzing the standards (i.e., one blank and ten standards), the data are tabulated and graphed. For precertification calibration, the duplicate calibration data are analyzed using the lack-of-fit (LOF) and zero intercept (ZI) tests (USATHAMA QA Plan, 2nd Edition, March, 1987).

B. DAILY CALIBRATION.

1. **PREPARATION OF STANDARDS.**

The daily calibration standards are CCS-1, CCS-3, CCS-5, CCS-6, CCS-7, and a blank as presented in Tables 4-3 and 4-4. These standards must be prepared fresh daily. The daily calibration curve used by ESE for this method is actually the same as an initial calibration curve as defined by the USATHAMA QA Plan, March 1987.

2. **INSTRUMENT CALIBRATION.**

At the beginning of each analytical run, inject 250 uL of each standard presented in Sec. IV.B.1, above. At the end of the analytical run, one standard CCS-1 will be analyzed.

3. **ANALYSIS OF CALIBRATION DATA.**

The response for the target compounds does not have to be less than 25 percent different from the response obtained during the previous initial calibration, because each run is an initial calibration. Since reference solutions are not readily available responses could be monitored to evaluate trends changes in stocks. It is advised that stocks for standards and spike solutions be staggered to ensure that degradation of the solutions is dissimilar. The response of the target compounds in the end run standard must be less than 25 percent different from response factors obtained from the standard analyzed at the beginning of the day. If the response is greater than 25 percent different, the standard will be reanalyzed. If reanalysis still fails the 25-percent criterion, a new initial calibration must be performed and all analyses since the last acceptable calibration must be repeated. After seven calibrations have been completed, the end of

run response must agree to within two times the standard deviation of the mean response rather than a percentage. Failure of the tighter criteria will not be an automatic requirement for reanalyses if documentation exists to ensure that data quality of the samples is not affected by instrument drift (i.e increase in sensitivity and all samples less than the CRL). In addition, drifts outside criteria and within 25 percent should be evaluated in the light of expected method performance.

V. CERTIFICATION TESTING.

Spiked samples for certification testing are prepared in USATHAMA standard soil (USATHAMA Standard Soil). A combined stock spiking solution is made up as shown in Section IV, then is used as spiking solution A. A 1 to 5 dilution of the combined stock is made into acetonitrile to obtain spiking solution B as shown in Table 4.5. Spiking is performed for certification testing on four separate days. The spiking scheme is given in Table 4.6. Analysis of the spiked samples follows the procedure outlined in Section VII. The target versus found data are analyzed using the lack of fit (LOF) and zero intercept (ZI) tests (USATHAMA QA Plan, March, 1987). The result of these tests and the certification data are presented in Section XI.D (see Attachment 3) for each target analyte.

VI. SAMPLING HANDLING AND STORAGE

A. SAMPLING PROCEDURE

Samples will be collected using adequate dermal and inhalation protection and must follow Sections 5.6 and 5.7 of the USATHAMA Quality Assurance Plan (March 1987).

Table 4.5 Spiking Solutions for Soil Validation Concentrations (ug/mL).

Spiking Solution	HMX	RDX	TNB	DNB	TetryL	NB	NC	TNT	26DNT	24DNT	PETN
A	33.3	21.9	24.4	24.8	20.2	27.4	200	22.8	26.2	21.2	200
B	6.66	4.38	4.88	4.96	4.04	5.48	40	4.56	5.24	4.24	40

Table 4.6 Spiking Scheme for Soil Validation (ug/g).

:vel	Spiking Volume	Spiking Solution	Weight Soil	HMX	RDX	TNB	DNB	TetryL	NB	NG	TNT	26DNT	24DNT	PETN
'2X	0.05 mL	B	1 g	0.333	0.219	0.244	0.248	0.202	0.274	2.00	0.228	0.262	0.212	2.00
ı X	0.10 mL	B	1 g	0.666	0.438	0.488	0.496	0.404	0.548	4.00	0.456	0.524	0.424	4.00
2 X	0.20 mL	B	1 g	1.33	0.876	0.976	0.992	0.808	1.10	8.00	0.912	1.05	0.848	8.00
5 X	0.50 mL	B	1 g	3.33	2.19	2.44	2.48	2.02	2.74	20.0	2.28	2.62	2.12	20.0
0 X	0.20 mL	A	1 g	6.66	4.38	4.88	4.96	4.04	5.48	40.0	4.56	5.24	4.24	40.0
0 X	0.40 mL	A	1 g	13.3	8.76	9.76	9.92	8.08	11.0	80.0	9.12	10.5	8.48	80.8
0 X	1.0 mL	A	1 g	33.3	21.9	24.4	24.8	20.2	27.4	200	22.8	26.2	21.2	200

B. **CONTAINERS**

Amber colored glass jars with Teflon-lined lids are required.

C. **HOLDING TIME LIMITS**

Samples must be extracted within 7 days of sampling date, and the extract must be analyzed within 40 days of extraction date. Samples should be stored at 4 C until extraction.

D. **SOLUTION VERIFICATION**

Verification of the calibration standards is based on the analyses of daily QC spikes and analysis of independent reference standards (if available). Since stable reference solutions are not readily available, staggered preparation of stock solutions for control spikes and standards needs to be implemented ensure acceptable solution verification. An unextracted control spike solution could be analyzed weekly as a check to avoid extraction and storage affects. The response of this check must be within 25 percent of the true value or ± 2 standard deviations for recent performance (last 7 runs). If criteria cannot be met for the target compounds new stock solutions must be perpared.

VII. PROCEDURE

A. **SAMPLE PREPARATION**

A portion of the sample is spread out on a sheet of aluminum foil and allowed to air dry overnight under a hood. The dried soil needs to be submitted for moisture determination.

B. **SAMPLE EXTRACTION**

One gram of dried soil is weighed into a 4 mL vial and with a pipet 2.0 ml of acetonitrile is added. The sample is

vortexed for 30 seconds and then placed in a sonicator for 2 hours. Following settling, a portion of the extract is removed with a dispo pipet and placed into a 5 mL syringe equipped with a 0.45 um acrodisc filter. The extract is filtered and collected into a 1 mL sample vial. The extract is stored in the dark at 4 C until analysis. Prior to analysis, 100 uL of the extract is mixed with 700 uL of water directly in the autosampler vial.

C. CHEMICAL REACTIONS.

This method does not involve any chemical reactions.

D. INSTRUMENTAL ANALYSIS

Instrumental analysis involves injection of 250 uL of the diluted extract onto the analytical column described in Section III.B. The instrumental conditions are specified in Sec. III.B.3, and the integrated output of the UV detector is used in the calculations of Section VIII.

VIII. CALCULATIONS

A linear regression equation is calculated from calibration data by using the response versus the concentration for each compounds. The concentration of a target compound in the soil extract is calculated by substituting the response into the calibration curve equation. The following formula is then used to calculate the concentration (ug/g) of each compound in the soil.

$$C = \frac{EC \times EV \times (DV + EAV/EAV)}{SW}$$

Where: EC is the extract concentration from the standard curve in ug/mL.

EV is the extraction volume (2 mL).

DV is the dilution volume (700 uL).
EAV is the extract aliquot volume (100 uL), and
SW is the soil sample weight in gm (1 gm).

IX. DAILY QUALITY CONTROL

A. CONTROL SAMPLES.

Daily quality control samples consist of a standard matrix method blank (USATHAMA Standard soil), duplicate spikes at the upper concentration of the certified range, and a single spike at approximately twice the certified reporting limit. These quality control samples should be carried throughout the entire method at the same time samples are run. Separate stock solutions for the daily control spike samples are prepared in the same manner as for the calibration stock solutions (see Section IV.A.1 and Tables 4-1). The spiking combined stock solution made up as described in Table 4.7 and is used as spiking solution A (see Table 4.8).

Daily Spiking Scheme

Daily spiking should be performed into USATHAMA standard soil using spiking solution A (Table 4-7). The spiking scheme presented in Table 4.8 should be used. Control analytes for this method are RDX, NB, TNB, 24DNT, NG and PETN.

B. CONTROL CHARTS.

Control charts are prepared for all of the control analytes being analyzed for using the percent recovery data from both the duplicate high level spikes and the low level spikes calculated according to the following equation:

$$\% \text{ Recovery} = \frac{\text{Found Conc.}}{\text{Spiked Concentration}} \times 100 \text{ percent}$$

Table 4-7. Preparation of Combined Spike Composite Stock Standard (SCSS).

Analyte	13DNB	24DNT	26DNT	HMX	NB	NG	PETN	RDX	TETRYL	135TNB	246TNT
mL SPSS added to Final Volume (mL)	1.0 50	1.0 50	1.0 50	1.0 50	2.0 50	2.5 50	2.5 50	1.0 50	1.0 50	1.0 50	1.0 50
Conc. of SCSS (ug/mL)	24.8	21.2	26.2	33.28	27.4	200	200	21.9	20.2	24.4	22.9

Note: Acetonitrile used for dilution to the 50 mL final volume for the Spike Composite Stock Standard (SCSS).

Source: ESE, 1988.

Table 4.8 Daily Spiking Scheme

mL of Spiking Solution A used	gm of USATHAMA Std. Soil	HMX	RDX	TNB	DNB	TetryL	NB	NG	TNT	26DMT	24DNT	PETN
Blank 0	1	0	0	0	0	0	0	0	0	0	0	0
Low Level 0.05	1	1.64	1.10	1.22	1.24	1.01	2.74	10.0	1.14	1.31	1.06	10.0
High Level 0.4	1	13.3	8.76	9.76	9.92	8.08	22.0	80.0	9.12	10.5	8.48	80.0

The found response is corrected for method blank response prior to calculation of the found concentration. Method blank correction may be in terms of instrument response or in terms of the concentration in the blank.

Preparation of control charts requires the following data:

1. Average percent recovery (X) of the two high concentration spiked QC samples in each lot,
2. Difference (R) between the two high concentration spiked QC samples in each lot,
3. Three-point moving average (X) percent recovery control chart for the low level spike in each lot, and
4. Three-point moving average difference (R) control chart for the high concentration spike.

For values that fall outside the control limits and data points that are deemed as outliers, the data will be evaluated and corrective action will be taken.

X. REFERENCES.

A. U.S. Army Toxic and Hazardous Materials Agency, 1987, USATHAMA QA Program (December 1985, 2nd Ed., March 1987).

XI. DATA

A. OFF-THE-SHELF ANALYTICAL REFERENCE MATERIALS CHARACTERIZATION

Only SARMS were used in this certification.

B. INITIAL/PRECERTIFICATION CALIBRATION - see ATTACHMENT 1.

Response of each target analyte is tabulated at each calibration target concentration. The results from the lack of fit (LOF) and zero intercept (ZI) tests are presented.

C. DAILY CALIBRATION DURING CERTIFICATION - see ATTACHMENT 2.

Calibration responses and required percentage on the end run standard.

D. **STANDARD CERTIFICATION SAMPLES - see ATTACHMENT 3.**

E. **CHROMATOGRAM**

ATTACHMENT 1 – INITIAL/PRECERTIFICATION CALIBRATION

ATTACHMENT 2 – DAILY CALIBRATION DURING CERTIFICATION

ATTACHMENT 3 – STANDARD CERTIFICATION SAMPLES

THESE ATTACHMENTS ARE NOT AVAILABLE.

EXTENSIVE RESEARCH WAS PERFORMED BY NAVFAC SOUTHWEST RECORDS OFFICE TO LOCATE THE MISSING ATTACHMENTS. THIS PAGE HAS BEEN INSERTED AS A PLACEHOLDER AND WILL BE REPLACED SHOULD THE MISSING ITEMS BE LOCATED.

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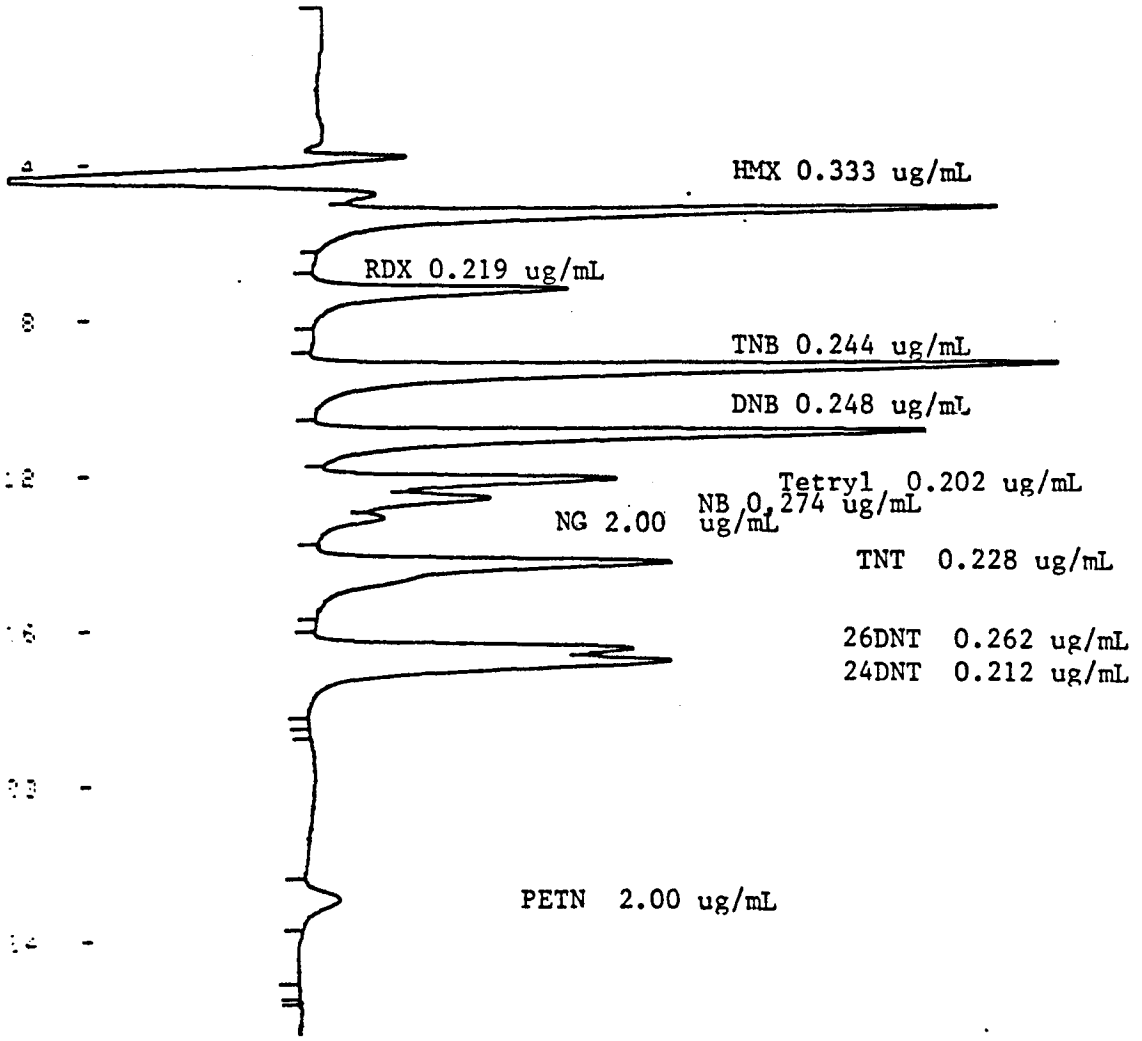
CHROMATOGRAM OF EXPLOSIVES IN SOIL



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Task Order 00002AN

DEVELOPMENT OF STANDARD ANALYTICAL METHODS EXPLOSIVES IN WATER BY HIGH PRESSURE LIQUID CHROMTOGRAPHY CERTIFICATION CALIBRATION REPORT

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Prepared for:

U.S. ARMY TOXIC AND HAZARDOUS MATERIALS AGENCY
Aberdeen Proving Ground, MD. 21010-5401

USATHAMA METHOD NUMBER: UW14

DETERMINATION OF EXPLOSIVES IN WATER BY HIGH PRESSURE LIQUID CHROMATOGRAPHY

I. SUMMARY

A. ANALYTES

This method is applicable to the Class 1 analysis of the following organic compounds in environmental water samples.

Analytes

1,3-Dinitrobenzene
2,4-Dinitrotoluene
2,6-Dinitrotoluene
HMX (octahydro-1,3,5,7-tetranitro-s-tetrazocine)
Nitrobenzene
RDX (hexahydro-1,3,5-trinitro-s-triazine)
Tetryl (N-methyl-N,2,4,6-tetranitrobenzenamine)
1,3,5-Trinitrobenzene
2,4,6-Trinitrotoluene

B. MATRIX

This method is applicable to all environmental water matrices.

C. GENERAL METHOD

This method employs solid phase extraction of 500 milliliters of an environmental water using a tube packed with Porapak R. The target analytes are desorbed with 3 mls of acetonitrile and the extract is diluted to a final volume of 10 ml with water. The analytes are separated by high pressure liquid

chromatography (HPLC) using isocratic elution and detected using ultraviolet absorbance (UV) at 250 nanometers (nm).

II. APPLICATION

A. TESTED CONCENTRATION RANGE.

The certification testing ranges in micrograms per liter (ug/L) are:

Analyte	Tested Range (ug/L)
1,3-Dinitrobenzene	0.501 - 40.1
2,4-Dinitrotoluene	0.503 - 40.2
2,6-Dinitrotoluene	0.655 - 52.4
HMX	0.361 - 28.9
Nitrobenzene	0.686 - 54.9
RDX	0.549 - 43.9
Tetryl	0.556 - 44.5
1,3,5-Trinitrobenzene	0.526 - 42.1
2,4,6-Trinitrotoluene	0.502 - 40.2

C. SENSITIVITY

The instrumental responses for each compound, reported in peak area units at the certified reporting limit (CRL) are

Analyte	Certified Reporting Limit (ug/L)	Area Counts
1,3-Dinitrobenzene	0.519	75000
2,4-Dinitrotoluene	0.612	80300
2,6-Dinitrotoluene	1.15	83200
HMX	1.65	43700
Nitrobenzene	1.07	104000

RDX	2.11	85100
Tetryl	0.556	36300
1,3,5-Trinitrobenzene	0.626	63100
2,4,6-Trinitrotoluene	0.588	58700

C. REPORTING LIMITS.

The certified reporting limits and upper certified limit for each analyte in environmental water samples are:

Analyte	Certified Reporting Limit (ug/L)	Upper Certified Limit (ug/L)
1,3-Dinitrobenzene	0.519	40.1
2,4-Dinitrotoluene	0.612	40.2
2,6-Dinitrotoluene	1.15	52.4
HMX	1.65	28.9
Nitrobenzene	1.07	54.9
RDX	2.11	43.9
Tetryl	0.556	44.5
1,3,5-Trinitrobenzene	0.626	42.1
2,4,6-Trinitrotoluene	0.588	40.2

D. INTERFERENCES.

Any materials which are adsorbed from water on the cartridge, coelute with the explosives through the HPLC column, and which absorb ultraviolet radiation at 250 nm may cause interferences.

The Porapak R material must be thoroughly cleaned to minimize interference. A late eluting component arising from the Porapak R required a 6 minute delay in sample injection following analysis of an extract. Carryover from analysis of a highly contaminated sample can result in apparent contamination of the succeeding samples analyzed. Such contamination is

often manifest by the presence of unusually broad chromatographic peaks nested among narrower peaks. This interference is minimized by reanalyzing heavily contaminated samples following dilution, running blanks after heavily contaminated samples until carry over is removed, and/or rinsing the system with a mobile phase containing a high proportion of organic modifier until the contamination is removed.

E. **ANALYSIS RATE.**

After instrument calibration, one analyst can analyze approximately 8 samples per 8-hour day.

F. **SAFETY INFORMATION.**

The target compounds in this method are toxic explosives and some are known carcinogens, e.g. 2,4-Dinitrotoluene. The preparation of all standards should be performed in a laboratory hood. Adequate dermal protection must be used when handling samples and standards.

Most of these compounds are either primary or secondary explosives and should be handled with care to avoid contact with electrostatic shocks or impacts. Tetryl and RDX have intermediate sensitivity between initiating explosives and explosives used as bursting charges. Tetryl is toxic when taken internally or by skin contact. RDX, HMX,and TNT are used as bursting charge explosives. Although TNT is less sensitive to friction and impact than many other high explosives, it can be detonated with moderate force when confined between metal surfaces such as on the threads of bolts. TNT will form sensitive materials in the presence of alkalies.

III. APPARATUS AND CHEMICALS.

A. GLASSWARE/HARDWARE.

1. Sorbent Cartridge - 6 mL OctyL Disposable Extraction Columns (J.T. Baker, Phillipsburg, N.J.) were used. After removing the top plug and the packing material, 0.5 gm of cleaned Porapak R was added to each tube.
2. Baker 10 Solid Phase Extraction System, (J.T. Baker, Phillipsburg, NJ) including manifold, 75 mL reservoirs and adapters.
3. Class A Volumetric flasks - 10, 100 and 500 mL.
4. Class A Volumetric pipets - 0.5, 1.0, 2.0 mL.
5. Aspirator
6. Disposable micro pipets - 25, 50, 100 and 200 uL.

B. INSTRUMENTATION

1. HPLC: Shimadzu model LC-6A high-pressure liquid chromatograph (or, equivalent).
2. Detector: Perkin-Elmer LC-75 variable wavelength ultraviolet absorbance detector (UV) set at 250 nanometers.
3. Column: Zorbax ODS (octadecylsilane), reverse-phase column, 25 centimeters (cm) length x 4.6 millimeters (mm) I.D., 5 micrometers (um) particle size (Mac-Mod Analytical Inc., Chadds Ford, PA).
4. Altex 210A Injection Valve, (Beckman Instr. Inc., Berkeley, CA).
5. Mobile phase: Isocratic, 25% methanol/17% acetonitrile/48% water.
6. Flow rate: 1.0 milliliters per minute (mL/min).
7. Sample Volume: 500 microliters (uL).

C. ANALYTES

Analyte	USATHAMA Abbrev.	CAS Number
1,3-Dinitrobenzene	DNB	99-65-01
2,4-Dinitrotoluene	24DNT	121-14-2
2,6-Dinitrotoluene	26DNT	606-20-2
HMX	HMX	2691-41-0
Nitrobenzene	NB	98-95-3
RDX	RDX	121-84-4
Tetryl	TETRYL	479-45-8
1,3,5-Trinitrobenzene	TNB	25377-32-6
2,4,6-Trinitrotoluene	TNT	118-96-7

D. REAGENTS AND SARMS

1. The standards used for target compound certification and calibration are USATHAMA supplied standard analytical reference materials (SARMS). Equivalent standards may be used as long as they have been characterized according to Section 6.5.3 of the USATHAMA Quality Assurance Plan (2nd Edition, March, 1987). USATHAMA SARMS were used in this certification, and their lot numbers are listed below:

Analyte	SARM LOT NUMBER
1,3-Dinitrobenzene	2250
2,4-Dinitrotoluene	1147
2,6-Dinitrotoluene	1148
HMX	1217
Nitrobenzene	2177
RDX	1130
Tetryl	1149
1,3,5-Trinitrobenzene	1154
2,4,6-Trinitrotoluene	1129

2. Methanol (HPLC grade - American Burdick & Jackson, McGaw Park, Illinois).
3. Water (ASTM Type II/HPLC grade - American Burdick & Jackson, McGaw Park, Illinois).
4. Acetonitrile (HPLC grade - American Burdick & Jackson, McGaw Park, Illinois).
5. Acetone (HPLC grade - American Burdick & Jackson, McGaw Park, IL).
6. Porapak R, 80-100 Mesh - (Supleco, Inc., Bellefont, PA), cleaned by six acetone extractions, six acetonitrile extractions and six methanol extractions followed by air drying. (75 cc of the resin was contacted with 200 mL of solvent for 10 minutes in a sonicator at each extraction step.

IV. CALIBRATION

A. INITIAL CALIBRATION.

1. Preparation of Standards.

Precertification Calibration. Separate primary stock standards (SPSS) for each target analyte are prepared according to the dilution scheme presented in Table 4-1. The SPSS solutions should be prepared fresh every 2 months. Tetryl needs to be made fresh every 2 weeks. Each separate stock solution is made to volume with acetonitrile.

Aliquots of the separate primary stock standards (SPSS) are used to prepare the combined stock standard (CSS) by dilution to a final volume of 10 mL using acetonitrile as described in Table 4-2. The CSS solution should be prepared fresh every day.

For precertification calibration, duplicate composite

calibration standards (CCS-1 through CCS-7) are prepared from the combined stock standard (CSS) as given in Tables 4-3 and 4-4. HPLC-grade water is used for dilution to final volumes for the composite calibration standards. Standards are prepared fresh daily.

Initial Calibration. Standards CCS-1, CCS-3, CCS-5, CCS-6 CCS-7, and a blank described in Tables 4-3 and 4-4 are prepared. These solutions are prepared fresh for every run. Reference materials are not available for verification of the calibration curve, therefore staggering of spikes will be important.

2. Instrument Calibration.

To calibrate the instrument, 500 uL of each standard in Tables 4-3 and 4-4 is injected into the instrument in the same manner as a sample extract. Each duplicate composite calibration standard is analyzed during precertification calibration, and the single dilutions of the composite standards are analyzed during initial calibration.

3. Independent Reference Standard.

An independent stock will be prepared to serve as a reference standard for explosives in water. The independent reference standard must be analyzed along with the initial and precertification calibration standards, and the results must be within ± 25% of the expected value, for the calibration to be considered valid. If the analysis of the independent reference standard fails, the source of the problem must be identified and corrected. The results of the second analysis of the independent reference standard must be within the acceptable limits before the analysis of samples may proceed. Since a new initial calibration is

Table 4-1. Preparation of Separate Primary Stock Standards (SPSS).

Analyte	13DNB	24DNT	26DNT	HMX	NB	RDX	TETRYL	135TNB	246TNT
mg SARM added	100.2	100.5	13.1	97.6	13.7	109.8	27.8	105.2	100.4
Final Volume (mL)	100	100	10.0	100	10.0	100.0	25	100	100
Conc. of SPSS (ug/mL)	1002	1005	1310	976	1370	1098	1112	1052	1004

Note: Each SARM diluted to the volume indicated with acetonitrile.

Source: ESE, 1988.

Table 4-2. Preparation of Composite Stock Standard (CSS).

Analyte	13DNB	24DNT	26DNT	HMX	NB	RDX	TETRYL	135TNB	246TNT
mL SPSS added	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Final Volume (mL)	10	10	10	10	10	10	10	10	10
Conc. of CSS (ug/mL)	10.02	10.05	13.1	9.76	13.7	10.98	11.12	10.52	10.04

Note: Acetonitrile used for dilution to the 10 mL final volume for the Composite Stock Standard (CSS).

Source: ESE, 1988.

Table 4-3. Preparation of Composite Calibration Standards 1 Through 4 (CCS-1 through CCS-4).

Analyte	13DNB	24DNT	26DNT	HMX	NB	RDX	TETRYL	135TNB	246TNT
Conc. in CCS-1 (ug/L)	2000	2010	2620	1950	2740	2200	2220	2100	2010
Conc. in CCS-2 (ug/L)	1000	1010	1310	976	1370	1100	1110	1050	1000
Conc. in CCS-3 (ug/L)	501	503	655	488	686	549	556	526	502
Conc. in CCS-4 (ug/L)	200	201	262	195	274	220	222	210	201

Note: CCS-1 prepared by diluting 2.0 mL of CSS to 10 mL with ASTM Type II/HPLC-grade water.
CCS-2 prepared by diluting 1.0 mL of CSS to 10 mL with ASTM Type II/HPLC-grade water.
CCS-3 prepared by diluting 0.5 mL of CSS to 10 mL with ASTM Type II/HPLC-grade water.
CCS-4 prepared by diluting 0.2 mL of CSS to 10 mL with ASTM Type II/HPLC-grade water.

Source: ESE, 1988.

Table 4-4. Preparation of Composite Calibration Standards 1 Through 4 (CCS-5 through CCS-7).

Analyte	13DNB	24DNT	26DNT	HMX	NB	RDX	TETRYL	135TNB	246TNT
Conc. in CCS-5 (ug/L)	100	101	131	97.6	137	110	111	105	100
Conc. in CCS-6 (ug/L)	50.1	50.3	65.5	48.8	68.6	54.9	55.6	52.6	50.2
Conc. in CCS-7 (ug/L)	25.1	25.1	32.8	24.4	34.3	27.5	27.8	26.3	25.1

Note: CCS-5 prepared by diluting 0.1 mL of CSS to 10 mL with ASTM Type II/HPLC-grade water.
CCS-6 prepared by diluting 0.05 mL of CSS to 10 mL with ASTM Type II/HPLC-grade water.
CCS-7 prepared by diluting 0.025 mL of CSS to 10 mL with ASTM Type II/HPLC-grade water.

Source: ESE, 1988.

performed daily, a reference is required at least weekly.

4. Analysis of Calibration Data.

After analyzing the standards (i.e., one blank and seven standards), the data are tabulated and graphed. For precertification calibration, the duplicate calibration data are analyzed using the lack of fit (LOF) and zero intercept (ZI) tests (USATHAMA QA Plan, 2nd Edition, March, 1987).

B. DAILY CALIBRATION.

1. Preparation of Standards

The daily calibration standards are CCS-1, CCS-3, CCS-5, CCS-6, CCS-7, and a blank as presented in Tables 4-3 and 4-4. These standards must be prepared fresh daily. The daily calibration curve used by ESE for this method is actually the same as an initial calibration curve defined by the USATHAMA QA Plan, March 1987.

2. Instrument Calibration

At the beginning of each analytical run, inject 500 uL of each standard presented in Section IV.B.1, above. At the end of the analytical run, the CCS-1 standard will be analyzed.

3. Analysis of Calibration Data

The response for the target compounds does not have to be less than 25 percent different from the response obtained during the previous initial calibration, because each run is an initial calibration. Since reference solutions are not readily available, responses should be monitored to evaluate trends changes in stocks. It is advised that stocks for standards and spike solutions be staggered to monitor for degradation of the solutions.

The response of the target compounds in the end run

standard (CSS-1) must be less than 25 percent different from response factors obtained from the CSS-1 standard analyzed at the beginning of the day. If the response is greater than 25 percent different, the standard will be reanalyzed. If reanalysis still fails the 25-percent criterion, a new initial calibration must be performed and all analyses since the last acceptable calibration must be repeated. After seven calibrations have been completed, the end of run response must agree to within two times the standard deviation of the mean response rather than a percentage. Failure of the tighter criteria will not be an automatic requirement for reanalyses if documentation exists to ensure that data quality of the samples is not affected by instrument drift (i.e increase in sensitivity and all samples less than the CRL). In addition, drifts outside criteria and within 25 percent should be evaluated in the light of expected method performance.

V. CERTIFICATION TESTING.

Spiked samples for certification testing are prepared in standard water (ASTM Type II grade water containing 100 mg/L of sulfate and chloride, see Section 4.5.1 of the USATHAMA QA Plan, March, 1987 Edition) as outlined in Tables 4-5 and 4-6. A composite stock standard (CSS) (see Table 4-2) is prepared for use as a spiking solution. Spiking is performed for certification testing on four separate days. Analysis of the spiked samples follows the procedure outlined in Section VII.

The target versus found data are analyzed using the lack of fit (LOF) and zero intercept (ZI) tests (USATHAMA QA Plan, March, 1987). The result of these tests and the certification data are presented in Section XI.D (see Attachment 3) for each target analyte.

Table 4-5 Spiking Scheme for Certification Testing (0X through 2X levels).

Analyte	HMX	RDX	TNB	DNB	NB	TetryL	TNT	26DNT	24DNT
Conc. in 0X (ug/L) Level	0	0	0	0	0	0	0	0	0
Conc. in 0.5X (ug/L) Level	0.361	0.549	0.526	0.501	0.686	0.556	0.502	0.655	0.503
Conc. in 1X (ug/L) Level	0.722	1.10	1.05	1.00	1.37	1.11	1.00	1.31	1.01
Conc. in 2X (ug/L) Level	1.44	2.20	2.10	2.00	2.74	2.22	2.01	2.62	2.01

Note: 0X Level was 500 mL of unspiked standard water.
0.5X Level prepared by spiking 25 uL of CSS into 500 mL of standard water.
1X Level prepared by spiking 50 uL of CSS into 500 mL of standard water.
2X Level prepared by spiking 100 uL of CSS into 500 mL of standard water.

Source: ESE, 1988

Table 4-6 Spiking Scheme for Certification Testing (5X through 40X levels).

Analyte	HMX	RDX	TNB	DNB	NB	TetryL	TNT	26DNT	24DNT
Conc. in 5X (ug/L) Level	3.61	5.49	5.26	5.01	6.86	5.56	5.02	6.55	5.03
Conc. in 10X (ug/L) Level	7.04	11.0	10.5	10.0	13.7	11.1	10.0	13.1	10.1
Conc. in 20X(ug/L) Level	14.4	22.0	21.0	20.0	27.4	22.2	20.1	26.2	20.1
Conc. in 40X(ug/L) Level	28.9	43.9	42.1	40.1	54.9	44.5	40.2	52.4	40.2

Notes: 5X Level prepared by spiking 250 uL of CSS into 500 mL of standard water.
10X Level parepared by spiking 0.50 mL of CSS into 500 mL of standard water.
20X Level prepared by spiking 1.0 mL of CSS into 500 mL of standard water.
40X Level prepared by spiking 2.0 mL of CSS into 500 mL of standard water.

Source: ESE, 1988.

Table 4-7 Preparation of Daily Control Spiking Solution (DCSS).

Analyte	13DNB	24DNT	26DNT	HMX	NB	RDX	TETRYL	135TNB	246TNT
mL SPSS added	0.1	0.1	0.1	0.2	0.1	0.2	0.05	0.1	0.1
Final Volume (mL)	10	10	10	10	10	10	10	10	10
Conc. of CSS (ug/mL)	10.0	10.0	10.0	20.0	10.0	20.0	10.0	10.0	10.0

Note: Acetonitrile used for dilution to the 10 mL final volume for the Daily Control Spiking Solution (DCSS).

Source: ESE, 1988.

Table 4-8. Daily Control Spiking Scheme

	Spiking Volume of DCSS (mL)	Final Volume (mL)	13DNB	24DNT	26DNT	HMX	NB	RDX	TetryL	135TNB	246TNT
Low Level Spike	0.1	500	2.00	2.00	2.00	4.00	2.00	4.00	2.00	2.00	2.00
High Level Spike	1.0	500	20.0	20.0	20.0	40.0	20.0	40.0	20.0	20.0	20.0

Note: Standard Water (See Section V) used for dilution to the 500 mL final volume for the Spikes.

Source: ESE, 1988.

VI. SAMPLING HANDLING AND STORAGE

A. SAMPLING PROCEDURE

Samples will be collected using adequate dermal and inhalation protection and must follow Sections 5.6 and 5.7 of the USATHAMA Quality Assurance Plan (March 1987).

B. CONTAINERS

One Liter amber colored glass jars with Teflon-lined lids are required.

C. STORAGE CONDITIONS

Samples and extracts should be kept chilled to 4 C and in the dark.

D. HOLDING TIME LIMITS

Samples must be extracted within 7 days of sampling date, and the extract must be analyzed within 40 days of extraction date.

E. SOLUTION VERIFICATION

Verification of the calibration standards is based on the analyses of daily QC spikes and analysis of independent reference standards (if available). Since stable reference solutions are not readily available, staggered preparation of stock solutions for control spikes and standards needs to be implemented to ensure acceptable solution verification. An unextracted control spike solution should be analyzed weekly as a reference to check extraction and storage affects. The recovery of this reference must be within 25 percent of the true value or $\pm$ 2 standard deviations for recent performance (last 7 runs). If criteria cannot be met for the target compounds new stock solutions might need to be prepared.

VII. PROCEDURE

A. EXTRACTION

A 6 mL Baker Disposable Extraction Column in repacked with 0.5 grams of cleaned Porapak R. The column is rinsed with 15 mL of acetonitrile and 30 mL of water. 500 mL of the sample is measured out and passed through the column at a rate of 10 mL/minute. The column is then slowly eluted with 3 mL of acetonitrile which is collected in a 10 mL volumetric flask. The extract is diluted to volume with ASTM Type II/HPLC water.

B. CHEMICAL REACTIONS.

This method does not involve any chemical reactions.

C. INSTRUMENTAL ANALYSIS

Instrumental analysis involves injection of 500 mL of the extract onto the analytical column described in Section III.B. The instrumental conditions are specified in Section III.B.3, and the integrated output of the UV detector is used in the calculations of Section VIII. A six minute delay of injection is required following analysis of any extracts to allow a late eluting component (arising from the Porapak R) to elute.

VIII. CALCULATIONS

A linear regression equation is calculated from calibration data by regressing the response versus the concentration for each compound. The concentration of a target compound in the sample extract is calculated by substituting the response into the calibration curve equation. The same injection volume is used for standards and sample extracts. The following formula is used to calculate the analytical concentration in the

samples (SC).

$$SC\ (ug/L) = \frac{EC \times EV}{SV}$$

Where:

EC is the extract concentration determined from the calibration curve in ug/L.

EV is the extract volume (10 mL).

SV is the sample volume (500 mL).

Method blank correction may be necessary and can be in terms of instrument response or concentration of the blank.

IX. DAILY QUALITY CONTROL

A. CONTROL SAMPLES.

Daily quality control samples consist of a standard matrix method blank (ASTM Type I water), duplicate spikes at the upper concentrations of the certified range, and a single level spike at approximately twice the certified reporting limit. These quality control samples should be carried throughout the entire method at the same time samples are run. Primary Stock Standards (SPSS) at a concentration of 1000 mg/L are weighed up using 0.010 gm of each analyte diluted to 10 mL of acetonitrile. The SPSS are diluted to obtain a daily control spiking solution (DCSS) as presented in Table 4-7. Table 4-8 shows how the daily control spikes are prepared. Control analytes for this method are RDX, NB, TNB, TNT, and 24DNT.

B. CONTROL CHARTS.

Control charts are prepared for all of the target analytes

being analyzed for using the percent recovery data from both the duplicate high level spikes and the low level spikes calculated according to the following equation:

$$\% \text{ Recovery} = \frac{\text{Found Conc.}}{\text{Spiked Concentration}} \times 100 \text{ percent}$$

The found response is corrected for method blank response prior to calculation of the found concentration. Method blank correction may be in terms of instrument response or in terms of the concentration in the blank.

Preparation of control charts requires the following data:

1. Average percent recovery (X) of the two high concentration spiked QC samples in each lot,
2. Difference (R) between the two high concentration spiked QC samples in each lot,
3. Three-point moving average (X) percent recovery control chart for the low level spike in each lot, and
4. Three-point moving average difference (R) control chart for the high concentration spike.

For values that fall outside the control limits and data points that are deemed as outliers, the data will be evaluated and corrective action will be taken.

X. REFERENCES.

A. U.S. Army Toxic and Hazardous Materials Agency, 1987, USATHAMA QA Program (December 1985, 2nd Ed., March 1987).

XI. DATA

A. **OFF-THE-SHELF ANALYTICAL REFERENCE MATERIALS CHARACTERIZATION**
Only SARMS were used in this certification.

B. **INITIAL/PRECERTIFICATION CALIBRATION - see ATTACHMENT 1.**
Response of each target analyte is tabulated at each calibration target concentration. The results from the lack of fit (LOF) and zero intercept (ZI) tests are presented.

C. **DAILY CALIBRATION DURING CERTIFICATION - see ATTACHMENT 2.**
Calibration responses and required percentage on the end run standard.

D. **STANDARD CERTIFICATION SAMPLES - see ATTACHMENT 3.**

E. **INDEPENDENT REFERENCE STANDARDS DURING CERTIFICATION - see ATTACHMENT 4.**

ATTACHMENT 1 – INITIAL/PRECERTIFICATION CALIBRATION

ATTACHMENT 2 – DAILY CALIBRATION DURING CERTIFICATION

ATTACHMENT 3 – STANDARD CERTIFICATION SAMPLES

ATTACHMENT 4 – INDEPENDENT REFERENCE STANDARDS DURING CERTIFICATION

THESE ATTACHMENTS ARE NOT AVAILABLE.

EXTENSIVE RESEARCH WAS PERFORMED BY NAVFAC SOUTHWEST RECORDS OFFICE TO LOCATE THE MISSING ATTACHMENTS. THIS PAGE HAS BEEN INSERTED AS A PLACEHOLDER AND WILL BE REPLACED SHOULD THE MISSING ITEMS BE LOCATED.

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