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DEVELOPMENT OF SAMPLING AND PRESERVATION TECHNIQUES TO RETARD CHEMICAL AND BIOLOGICAL CHANGES IN WATER SAMPLES

by

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Methods development	Cyclotrimethylenetrinitramine (RDX)
Preservation techniques	1,3,5-Trinitrobenzene (TNB)
High performance liquid chromatography	1,3-Dinitrobenzene (DNB)
Groundwater monitoring wells	2,4-Dinitrotoluene (2,4-DNT)
Dinitrophenol (DNP)	Trinitrotoluene (TNT)

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

A protocol was defined for the preservation of 12 munitions (Dinitrophenol DNP; Cyclotrimethylene trinitramine, RDX; 1,3,5-Trinitrobenzene, TNB; 1,3-Dinitrobenzene, DNB; 2,4-Dinitrotoluene, 2,4-DNT; Trinitrotoluene, TNT; 2,4,6-Trinitrophenylmethylnitramine, Tetryl; Diphenylamine, DPA; Nitrobenzene, NB; 2,6-Dinitrotoluene, 2,6-DNT; Nitroglycerin, NG; and Picric Acid, PA) in water samples. The samples were preserved by adding acetonitrile to achieve a 10% solution; the adjusting of the pH to 3.5 with glacial acetic acid; removing

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19. Key Words (concluded)

2,4,6-Trinitrophenylmethylnitramine (Tetryl)
Diphenylamine (DPA)
Nitrobenzene (NB)
2,6-Dinitrotoluene (2,6-DNT)
Nitroglycerin (NG)
Picric Acid (PA)

20. Abstract (concluded)

sediment by centrifugation; storing in amber glass bottles (cleaned according to specified instructions) sealed with Teflon cap liners; refrigerating at 4°C; and maintaining in the dark. The effectiveness of the protocol was demonstrated for munitions fortified in tap water and monitoring well water samples, and sediment-deionized water mixtures.

In order to carry out this study it was necessary to develop and validate methods for the analysis of the 12 munitions in water and sediment. Two high performance liquid chromatographic (HPLC-UV) systems were developed (System No. 1 for eight munitions; DNP, RDX, TNB, DNB, 2,4-DNT, TNT, Tetryl and DPA and System No. 2 for four munitions; NB, 2,6-DNT, NG and PA). Four separate sample preparation protocols were developed: 1 each for the 8-munition and 4-munition groups in water; and 1 each for the 8-munition and 4-munition groups in sediment.

The method for eight munitions (DNP, RDX, TNB, DNB, 2,4-DNT, TNT, tetryl and DPA) in water samples consists of adding 10 g sodium chloride, NaCl, to 100 ml water, adjusting the pH to 3.0 with acetic acid, and extracting with three 20-ml portions of methylene chloride, CH_2Cl_2. The extract is concentrated, exchanged into acetonitrile, CH_3CN, and analyzed by HPLC-UV (254 nm) using a Spherisorb ODS column and gradient elution with CH_3CN and 10% CH_3CN/0.08 M aqueous acetic acid.

The method for four munitions (NB, 2,6-DNT, NG and PA) in water samples consists of adding 10 g NaCl to 100 ml water, extracting with one 20-ml portion of CH_2Cl_2 followed by adding 1 ml 0.005 M t-butyl ammonium hydroxide (to ion-pair with PA) and extracting with three additional 20-ml portions of CH_2Cl_2. The extract is concentrated, exchanged into CH_3CN and analyzed by HPLC-UV (230 nm) using a Spherisorb ODS column and isocratic elution with 35/65 CH_3CN/0.005 M t-butyl ammonium hydroxide.

The methods for extraction of munitions from sediment are satisfactory for 10 of the 12 study compounds. DNP, RDX, TNB, DNB, 2,4-DNT, TNT and DPA are recovered using 95/5 CH_2Cl_2/methanol and hexane is used for the recovery of NB, NG, and 2,6-DNT (tetryl and picric acid are not successfully recovered from sediment with these solvents). Sample preparation consists of 1 hr of wrist-action shaking of a 10/1 (v/w) solvent sediment mixture. The mixture is centrifuged and supernatant is withdrawn, concentrated, and exchanged into CH_3CN. The HPLC conditions described for the water analyses are used for quantification of the munitions extracted from sediment.

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This work was conducted in the Analytical Chemistry Department, Dr. James L. Spigarelli, Director. The research effort was directed by Dr. Duane Lakings and Ms. Hope Miller and performed by Mr. Owen Gan, Mr. Randall Baker, Ms. Mary Valla Crook, Ms. Evelyn Conrad and Mr. Carlos Lizarraga. This report was prepared by Ms. Miller with assistance from Ms. Crook and Dr. Spigarelli.

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I. INTRODUCTION

The original objective of the work to be performed under Contract No. DAAK11-80-C-0007 was to develop sampling and preservation techniques that would retard chemically and biologically induced changes in selected munitions that might occur when water samples are removed from their parent source. The 12 munitions to be studied were nitroglycerin (NG), picric acid (PA), diphenylamine (DPA), trinitrotoluene (TNT), 2,4- and 2,6-dinitrotoluene (2,4-DNT, 2,6-DNT), cyclotrimethylene trinitramine (RDX), tetryl, 2,4-dinitrophenol (DNP), nitrobenzene (NB), 1,3-dinitrobenzene (DNB) and 1,3,5-trinitrobenzene (TNB). At the direction of the U.S. Army Toxic and Hazardous Materials Agency, the scope was ultimately changed to eliminate the development and evaluation sampling techniques.

After performance of an extensive literature survey, it was determined that none of the published methods were for the determination of more than 3 of the 12 study compounds and, in general, the sensitivities of the techniques were poor ($\geq$ 1 µg/ml). The scope of work was, therefore, expanded to include the development of the required analytical procedures.

Preliminary storage experiments indicated the presence of sediment could affect munition recovery from water. After USTHAMA indicated that a large number of monitoring wells would contain sediment, it was agreed that a contract modification was needed to address the effects of sediment. This modification included development of an analytical method for munitions on sediment and a series of storage stability studies.

The specific tasks discussed in this report are as follows: literature survey for preservation and analysis techniques; development and validation of analytical procedures; experiments conducted to define the proposed preservation techniques; validation of preservation protocol during long-term storage; and field test of the preservation protocol.

II. LITERATURE SURVEY

The literature survey was made of research on the analysis of and preservation techniques for munitions or related compounds in water. The sources searched included Chemical Abstracts (1972-1980) and Environline (1971-1980).

Although the computer search using the keywords "munitions" and "analysis" yielded approximately 150 citations, none of the procedures allowed for the determination of more than three of the study compounds and, in general, their sensitivity was poor (1 mg/liter). Since MRI had developed a reverse phase HPLC system for RDX, 2,4-DNT, TNT and tetryl with 100 µg/liter sensitivities, it seemed expedient to modify this method to accommodate the requirements for the determination of the remaining eight munitions.

Two of the munitions compounds (PA and DNP) are highly ionized at normal water pH ranges, which could prevent their analysis by the reverse phase HPLC technique. However, a column chromatographic technique was defined for the separation of these compounds [J. Indian Chem. Soc., 56(7): 737-738 (1979)]. Thus, a modification (use of an amine to ion pair with the compounds) of the reverse phase HPLC method promised to provide the necessary separation for the quantitative determination of PA and DNP.

Although 45 references were obtained from a search based on the keywords "sampling," "preservation" and "munitions," none were applicable to this study. However, two references, "Monitoring Well Sampling and Preservation Techniques," by J. P. Gibbs ["Disposal of Hazardous Waste," Proceedings of the Annual Research Symposium (6th), held at Chicago, Illinois, March 17 to 20, 1980] and J. Environ. Qual., 5:42-46 (1976), provided information on preservation and sampling techniques of inorganic ions. Both reports indicated that important aspects of sample preservation include pH adjustment, filtration to remove particulate matter, addition of antimicrobial agents and storage at 4°C. No data were presented on the application of these techniques to the preservation of organic compounds in water samples.

III. DEVELOPMENT AND VALIDATION OF ANALYTICAL PROCEDURES

This section contains a discussion of (1) the development of and validation of two HPLC systems for the determination of the 12 munitions and (2) the development and validation of the analytical methods to recover the 12 munitions from water.

A. Chromatography Development and Validation

It was first necessary to develop a chromatographic system capable of resolution and detection of the 12 munitions to be studied. Information from the literature indicated that picric acid would require ion-paired HPLC; and preliminary experiments indicated that nitroglycerin would require detection at 230-nm wavelength.

The remaining 10 munitions gave excellent sensitivity and linearity at 254 nm using reverse phase HPLC. However, 1,3-dinitrobenzene and nitrobenzene were only partially resolved and the 2,4- and 2,6-isomers of dinitrotoluene coeluted. Since a second system would already be required for PA and NG, it was decided to include the determination of nitrobenzene and 2,6-dinitrotoluene in the development of the second system. The development and use of two HPLC systems was considered the expedient approach in accomplishing the original scope of work (development of sampling and preservation techniques).

System No. 1 is described in Table 1. Quadruplicate SARM reference solutions of approximately 0, 50, 125, 250, 500, 1,250, and 2,500 µg/liter were analyzed. Assuming a water sample of 100 ml and a final volume of 2 ml,

the series corresponded to 0, 1, 2.5, 5, 10, 25 and 50 µg/liter, equivalent to parts per billion, ppb. A linear response for each munition was obtained over the concentration range evaluated. The results obtained for this system are given in Table 2.

TABLE 1

HPLC SYSTEM NO. 1 FOR EIGHT MUNITION GROUPS

System No. 1
HPLC-UV 254 nm
Column: Spherisorb ODS, 5 µ, 250 x 4.6 mm ID
Pre-Column: CO:PELL ODS, 25 to 35 µ, 50 x 2 mm ID
Solvent: Gradient Elution 22/78 (v/v) to 44/56 (v/v) in 35 min. 100% Acetonitrile/10% acetonitrile, 0.08 M aqueous acetic acid (pH adjusted to 3.1 with ammonium hydroxide)[a]
Flow Rate: 1.0 ml/min
Injection Volume: 40-100 µl

a Composition of eluant changed from that reported in Technical Report Nos. 1-4 when bacterial growth was observed. No change was noted in compound's retention volumes.

TABLE 2

RETENTION VOLUMES, CORRELATION COEFFICIENTS AND DETECTION LIMITS FOR EIGHT MUNITIONS USING SYSTEM NO. 1

Compounds	Retention Volume (Time)	Correlation Coefficients	Detection Limits[a] µg/liter
DNP	12.5	0.9993	62
RDX	13.5	0.9990	89
TNB	17.0	0.9992	68
DNB	18.0	0.9996	50
Propiophenone (internal standard)	20.0		
2,4-DNT	24.0	0.9996	50
TNT	25.0	0.9997	50
Tetryl	26.0	0.9995	52
DPA	38.0	0.9986	88

a Detection limits determined by Hubaux and Vos statistical evaluation of data.

System No. 2 is described in Table 3.

TABLE 3

HPLC SYSTEM NO. 2 FOR FOUR MUNITION GROUPS

System No. 2
HPLC-UV 230 nm
Column: Spherisorb ODS, 5 μ, 250 x 4.6 mm ID
Pre-Column: CO:PELL ODS, 25 to 35 μ, 50 x 2 mm ID
Solvent: Isocratic 35/65 (v/v) acetonitrile/0.005 M t-butyl ammonium hydroxide (pH adjusted to 6.5 with phosphoric acid)
Flow Rate: 1.0 ml/min
Injection Volume: 40-100 μl

Quadruplicate SARM reference solutions were analyzed for the four munitions at the following concentration ranges:

(1) NB, 2,6-DNT and PA	0, 50, 125, 250, 500, 1,250, and 2,500 μg/liter
(2) NG	0, 1,500, 3,750, 7,500, 15,000, 375,000, and 750,000 μg/liter

Assuming a 100-ml water sample and a final volume of 2 ml, the series analyzed corresponds to the following water concentrations:

(1) NB, 2,6-DNT and PA	0, 1, 2.5, 5, 10, 25, and 50 μg/liter
(2) NG	0, 30, 75, 150, 300, 750, and 1,500 μg/liter

A linear response for each munition was obtained on the concentration range evaluated.

The results for the analysis of four munitions using this system are given in Table 4.

TABLE 4

RETENTION VOLUMES, CORRELATION COEFFICIENT AND DETECTION LIMITS FOR FOUR MUNITIONS USING SYSTEM NO. 2

Compounds	Retention Volume (Time)	Correlation Coefficients	Detection Limits[a] µg/liter
NB	14	0.9997	50
IS Propiophenone	16		
2,6-DNT	20	0.9997	50
NG	23	0.9995	1,600
PA	24.5	0.9988	89

a Detection limits determined by Hubaux and Vos statistical evaluation of data.

Appendices A and B are appended to provide the raw data used in the precision and accuracy assessments of the two HPLC analytical systems.

B. Sample Preparation and Overall Method Validation

Following the validation of the HPLC separation and detection techniques for the 12 munitions, two sample preparation procedures were developed and coupled with the HPLC techniques to define the overall analytical methods for the quantitative determination of the 12 munitions.

The sample preparation procedure for eight munitions (DNP, RDX, TNB, DNB, 2,4-DNT, TNT, Tetryl and DPA) consists of adding 10-g sodium chloride to a 100-ml water sample and adjusting the pH to 3.0 with acetic acid. The munitions are extracted with three 20-ml portions of methylene chloride. The extract is concentrated to approximately 2 ml and solvent exchanged with acetonitrile. The acetonitrile solution is concentrated to 200 µl, mixed with 1 ml of internal standard solution and 800 µl of 45/55 (v/v) acetonitrile/0.08 M acetic acid solution giving a final volume of approximately 2 ml. The extract is passed through a 0.45 µl filter and analyzed by HPLC-UV (254 nm).

The analytical method for the eight munition compounds in water was validated for precision and accuracy by preparing and analyzing water samples spiked with the munitions at 0, 0.2X, 0.5X, X, 2X, 5X, and 10X, where X is equal to 5 µg/liter for each munition on four separate days. A linear response for each munition compound was obtained over the water concentration range evaluated. The results are given in Table 5.

TABLE 5

LINEAR REGRESSION EQUATIONS, CORRELATION COEFFICIENTS AND DETECTION LIMITS FOR ANALYTICAL METHOD FOR EIGHT MUNITIONS

Compound	Equation	Correlation Coefficient	Detection Limit[a] (µg/liter)
DNP	y = 0.931X + 0.107	0.9981	2.2
RDX	y = 1.004X + 0.047	0.9972	3.0
TNB	y = 0.954X + 0.097	0.9988	1.6
DNB	y = 0.911X + 0.066	0.9996	1.0
2,4-DNT	y = 0.872X + 0.296	0.9987	1.7
TNT	y = 0.918X + 0.257	0.9986	1.8
Tetryl	y = 0.943X - 0.089	0.9954	3.2
DPA	y = 0.829X + 0.045	0.9957	3.1

a A statistical evaluation of the data by the Hubaux and Vos program was used to determine the method detection limits.

The sample preparation procedure for four munitions (NB, 2,6-DNT, NB, and PA) consists of adding 10-g sodium chloride to a 100-ml water sample. The munition compounds are extracted with 20-ml methylene chloride; the remaining aqueous phase is adjusted with 1.0 ml 0.005 M t-butyl ammonium hydroxide, pH 6.5 (to ion-pair with the PA); and the water sample is extracted with 3 x 20 ml methylene chloride. The four extracts are combined and concentrated to about 2 ml. The solvent is exchanged to acetonitrile, and the sample is transferred to a culture tube. After concentrating the sample to about 200 µl, 1,000 µl of an internal standard stock solution and 800 µl of 35/65 (v/v) acetonitrile/water, 0.005 M t-butyl ammonium hydroxide, pH 6.5, are added to make the final sample volume approximately 2 ml. The prepared sample is filtered through a 0.45 µ filter, and an aliquot is analyzed by the HPLC-UV (230 nm) analytical technique. The analytical method for the four munition compounds in water was validated for precision and accuracy by preparing and analyzing water samples spiked with the munitions at 0, 0.2X, 0.5X, X, 2X, 5X, and 10X, where X is equal to 5 µg/liter for NB, 2,6-DNT, and PA and 150 µg/liter for NG, on four separate days. A linear response for each munition compound was obtained over the water concentration range evaluated. The results are given in Table 6.

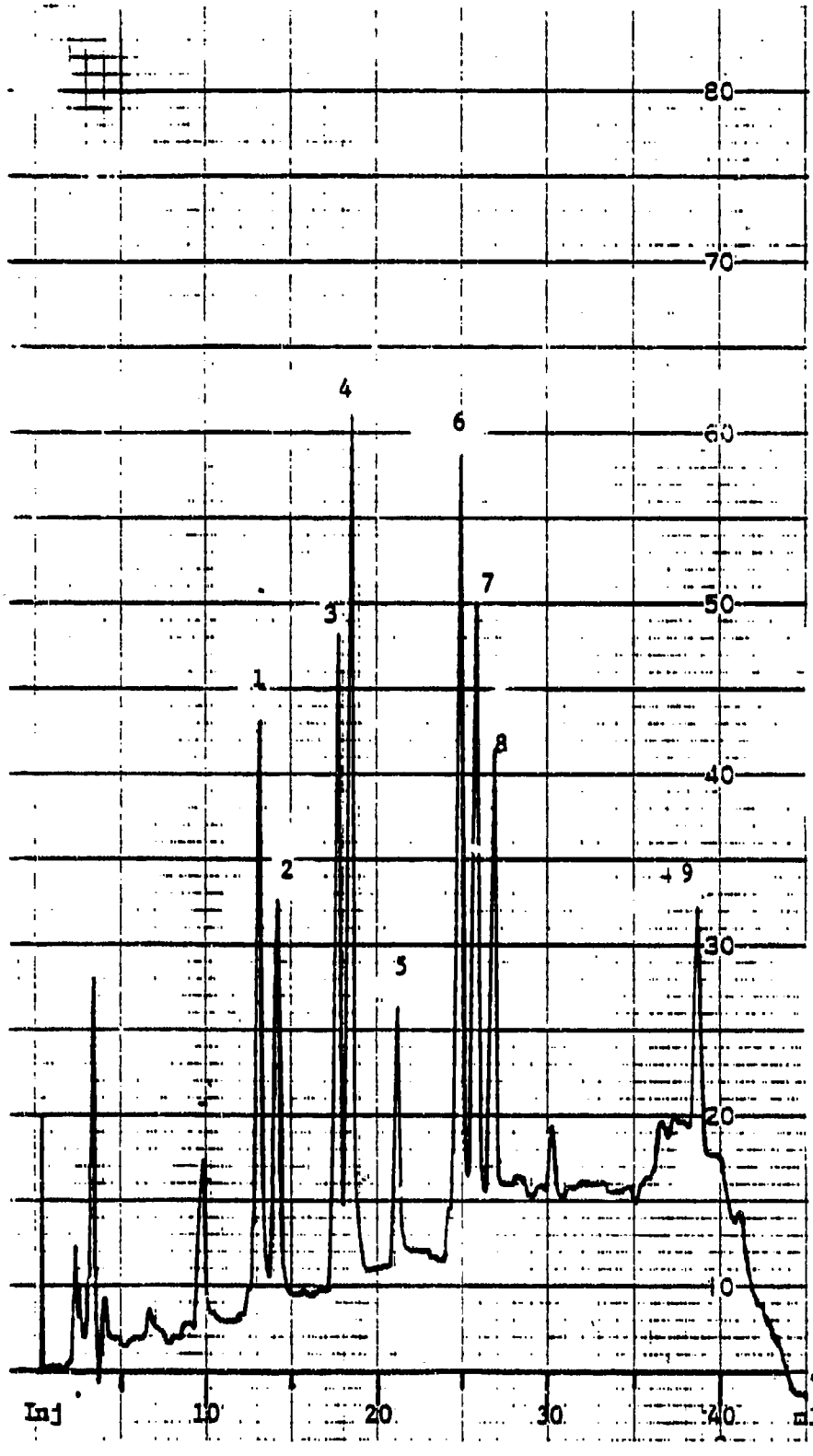
TABLE 6

LINEAR REGRESSION EQUATIONS, CORRELATION COEFFICIENTS AND DETECTION LIMITS FOR ANALYTICAL METHOD FOR FOUR MUNITIONS

Compound	Equation	Correlation Coefficient	Detection Limit[a] (µg/liter)
NB	y = 0.676X + 0.22	0.9800	6.9
2,6-DNT	y = 0.886X - 0.06	0.9986	1.9
NG	y = 0.945X + 4.4	0.9988	48.5
PA	y = 1.019X + 0.013	0.9996	1.0

a Method detection limits determined by the Hubaux and Vos program.

Appendices C and D are appended to provide the raw data used in the method validation. Figures No. 1 and 2 are copies of chromatograms of munitions recovered from a 100-ml water sample.



HPLC Parameters

Column: Spherisorb ODS, 5 µ, 250 x 4.6 mm ID
Precolumn: CO:PELL ODS, 25 to 35 µ, 50 x 2 mm ID
Eluent: Linear Gradient
Initial: 22/78 (v/v) CH_3CN/10% CH_3CN 0.08 M acetic acid adjusted to pH 3.1 with NH_4OH.
Final: 44/56 (v/v) CH_3CN/10% CH_3CN 0.08 M acetic acid adjusted to pH 3.1 with NH_4OH.
Time: 35 min
Flow Rate: 1.0 ml/min
Chart Speed: 0.1 in./min
Detector: UV, 254 nm
Injection Volume: 100 µl
Attenuation: 0.01 A.U.F.S.

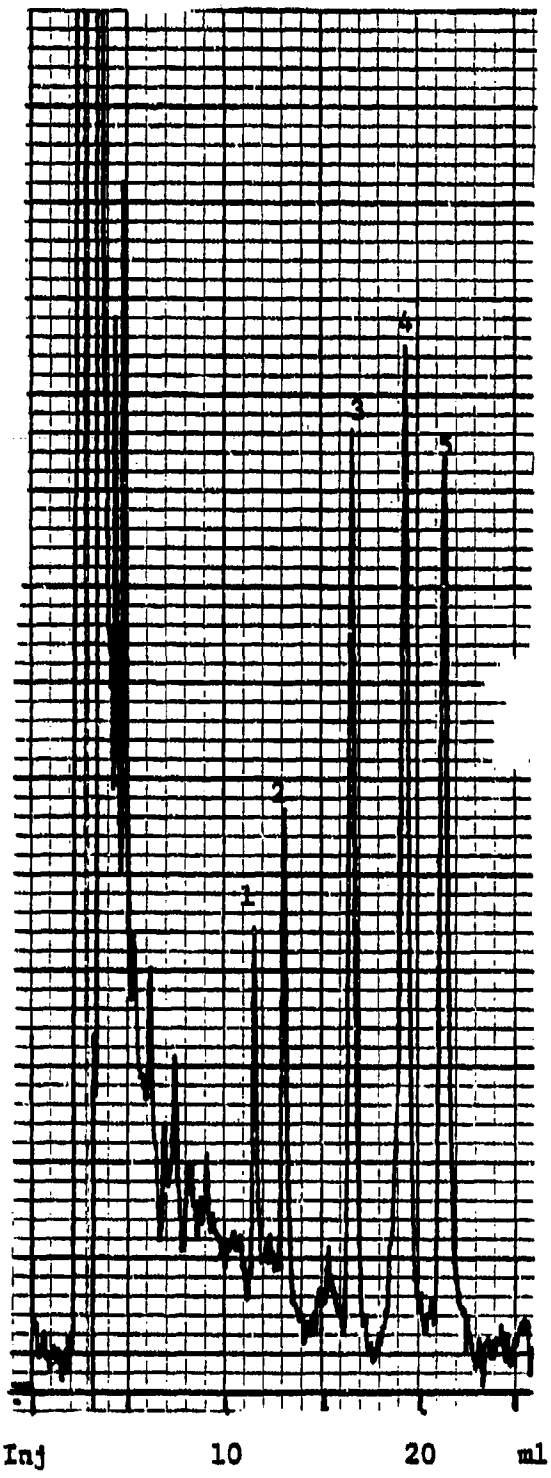
Sample Characteristics

No.	Compound	Added (ppb)	Recovered (ppb)
1	DNP	5.20	4.58
2	RDX	6.00	5.63
3	TNB	5.14	4.73
4	DNB	5.01	4.33
5	IS*	-	-
6	2,4-DNT	5.05	4.43
7	TNT	4.97	4.65
8	Tetryl	5.09	4.32
9	DPA	5.00	4.26

* IS - 0.222 µg Propiophenone (IS Stock No. 2) added during final sample preparation. Final sample volume is ∿ 2 ml.

Figure 1 - HPLC-UV (254 nm) Separation of DNP, RDX, TNB, DNB, 2,4-DNT, TNT, Tetryl, and DPA Recovered from a 100-ml Water Sample

Sample preparation procedure listed in text.



HPLC Parameters

Column: Spherisorb ODS, 5 µ, 250 x 4.6 mm ID
Precolumn: Co:Pell ODS, 25-35 µ, 50 x 2 mm ID
Eluent: 35/65 (v/v) CH_3CN/0.005 t-butyl ammonium hydroxide, pH 6.5 (H_3PO_4 adjusted)
Flow Rate: 1.0 ml/min
Chart Speed: 0.1 in/min
Detector: UV, 230 nm
Injection Volume: 100 µl
Attenuation: 0.005 X

Sample Characteristics

No.	Compound	Added (ppb)	Recovered (ppb)
1	NB	5.25	3.11
2	IS*	-	-
3	2,6-DNT	5.62	4.67
4	NG	152	154
5	PA	5.44	5.52

* IS - 0.222 µg propiophenone (IS Stock No. 2) added during final sample preparation. Final sample volume is ∿ 2 ml.

Figure 2 - HPLC-UV (230 nm) Separation of NB, 2,6-DNT, NG, and PA Recovered From a 100-ml Water Sample. Sample preparation procedure listed in text.

IV. PRESERVATION AND STORAGE STUDIES

A. Preliminary Studies

Based on information obtained from the literature, a set of storage parameters were selected for short-term studies of munition stability. Parameters evaluated included pH, temperature, light, containers, and the presence of sediment, salts and acetonitrile (antibacterial agent) and their effect on munition stability. The bulk of the experiments were carried out on the set of eight munitions. The results given in Table 7 indicate that the parameter with the greatest effect on stability was pH.

On the assumption that the remaining four munitions would exhibit similar behavior, an abbreviated short-term storage study, focusing on the effects of pH, was performed for NB, 2,6-DNT, NG, and PA. The results of this study are summarized in Table 8.

The data from these two studies show that basic pH (11) solutions caused reduced recovery of tetryl, diphenylamine, and nitroglycerin. At neutral pH (6.5), DPA showed a slight reduction in recovery after 7 days and at this pH, in the presence of 2% salts, losses were seen for nitrobenzene and diphenylamine.

B. Effect of Anions and Cations

Since an effect of salts was demonstrated at pH 6.5 and the data suggested that several compounds would require reduced pH (3.5) for storage stability, an additional study was conducted to determine ionic effects on munition stability at pH 3.5. In addition, after discussion with the project officer, it was decided that 100 mg/liter, equivalent to parts per million, ppm, salt concentrations would be more reflective of conditions in samples obtained from groundwater monitoring wells.

Two solutions were prepared containing 100 ppm concentrations of: (a) (anions) $NaNO_3$, $NaCl$, Na_2CO_3, Na_3PO_4, Na_2SO_4, and $NaBr$; and (b) (cations) $NaCl$, $CaCl_2$, $FeCl_3$, and $CuCl_2$. Aliquots of these solutions were fortified with munition at 0.5X, X and 5X levels (X = detection limit) stored, and analyzed after 0, 24, and 168 hr. Duplicate analyses were performed at 24 hr. Table 9 shows the results of this study. The mean of the four replicates analyzed during the method precision and accuracy study is also provided. A comparison of this value with anion/cation 0 hr values gives an indication of the effect of ionic content on the method's accuracy. The data demonstrate that any reduction in the recovery of the munitions occurs immediately but remains relatively unchanged over a 7-day period.

TABLE 7

PERCENT RECOVERY OF EIGHT MUNITIONS AFTER STORAGE FOR 0, 24, AND 168 HR

Compound (µg added to 100 ml)		DNP 10.4	RDX 12	TNB 10.3	DNB 10	2,4-DNT 10.1	TNT 9.9	Tetryl 10.2	DPA 10
Storage Parameters Compared	Hr	% Recovery							
1. Clear and amber bottles									
Clear bottle, 25°C	0	89	97	95	86	88	95	100	68
Room light	24	89	96	94	93	97	96	102	72
Teflon cap liner	168	86	94	89	84	86	94	79	74
Amber bottle, 25°C	0	89	96	94	83	86	95	101	65
Room light	24	80	85	95	98	100	94	103	93
Teflon cap liner	168	91	100	92	82	82	94	90	76
2. Teflon and wax paper cap liners									
Amber bottle, 25°C	0	89	96	94	83	86	95	101	65
Room light	24	80	85	95	98	100	94	103	93
Teflon cap liner	168	91	100	93	82	82	94	90	76
Amber bottle, 25°C	0	90	99	96	86	92	102	103	79
Room light	24	85	98	94	92	96	96	101	87
Wax paper cap liner	168	92	97	91	89	88	96	87	84
3. Ambient and refrigerated									
Amber bottle, 25°C	0	89	96	94	83	86	95	101	65
Room light	24	80	85	95	98	100	94	103	93
Teflon cap liner	168	91	100	93	82	82	94	90	76
pH 6.5									
Amber bottle, 4°C	0	91	98	97	84	91	98	100	76
Dark, Teflon cap liner	24	81	95	92	87	91	97	106	65
pH 6.5	168	87	98	92	87	88	94	92	65

TABLE 7 (continued)

Compound (µg added to 100 ml)		DNP 10.4	RDX 12	TNB 10.3	DNB 10	2,4-DNT 10.1	TNT 9.9	Tetryl 10.2	DPA 10
Storage Parameters Compared	Hr	% Recovery							
4. With and without acetonitrile									
a. At pH 3.5, adjust with HOAc									
Amber bottle, 4°C	0	84	98	95	86	87	94	97	83
Dark, Teflon cap liner	24	82	99	92	83	85	95	101	72
10% CH_3CN, pH 3.5 (HOAc)	168	88	99	98	92	100	101	99	82
Amber bottle, 4°C	0	88	97	96	88	93	100	105	92
Dark, Teflon cap liner	24	85	93	93	90	93	96	102	76
pH 3.5 (HOAc) % CH_3CN	168	91	100	96	91	97	100	96	81
b. At pH 6.5									
Amber bottle, 4°C	0	92	99	90	66	72	91	92	68
Dark, Teflon cap liner,	24	93	100	100	94	95	103	103	85
10% CH_3CN	168	90	97	97	94	97	103	101	82
Amber bottle, 4°C	0	91	98	97	84	91	98	100	76
Dark, Teflon cap liner	24	81	95	92	87	91	97	106	65
pH 6.5 0% CH_3CN	168	87	98	92	87	88	94	92	65
5. pH 3.5, 6.5, 11[a]									
a. With CH_3CN									
Amber bottle, 4°C	0	84	98	95	86	87	94	97	83
Dark, Teflon cap liner	24	82	99	92	83	85	95	101	72
10% CH_3CN, pH 3.5 (HOAc)	168	88	99	98	92	100	101	99	82
Amber bottle, 4°C	0	92	99	90	66	72	91	92	68
Dark, Teflon cap liner	24	93	100	100	94	95	103	103	85
10% CH_3CN, pH 6.5	168	90	97	97	94	97	103	101	82

TABLE 7 (continued)

Compound (µg added to 100 ml)		DMP 10.4	RDX 12	TNB 10.3	DNB 10	2,4-DNT 10.1	TNT 9.9	Tetryl 10.2	DPA 10
Storage Parameters Compared	Hr	% Recovery							
b. Without CH_3CN									
Amber bottle, 4°C	0	88	97	96	88	93	100	105	92
Dark, Teflon cap liner	24	85	93	93	90	93	96	102	76
pH 3.5 (HOAc)	168	91	100	96	91	97	100	96	81
Amber bottle, 4°C	0	91	98	97	84	91	98	100	76
Dark, Teflon cap liner	24	81	95	92	87	91	97	106	65
pH 6.5	168	87	98	92	87	88	94	92	65
Amber bottle, 4°C	0	84	95	89	85	91	89	62	56
Dark, Teflon cap liner	24	87	95	90	73	79	88	24	60
pH 11.0 (NaOH)	168	80	83	85	89	90	81	ND	27
6. pH adjust to 3.5 with HOAc or H_2SO_4									
Amber bottle, 4°C	0	88	97	96	88	93	100	105	92
Dark, Teflon cap liner	24	85	93	93	90	93	96	102	76
pH 3.5 (HOAc)	168	91	100	96	91	97	100	96	81
Amber bottle, 4°C	0	82	97	90	85	85	94	99	73
Dark, Teflon cap liner	24	84	96	90	85	90	92	97	78
pH 3.0 (H_2SO_4)	168	77	93	90	79	80	86	85	46
7. Deionized and ionic solutions at pH 6.5									
Amber bottle, 4°C	0	91	98	97	84	91	98	100	76
Dark, Teflon cap liner	24	81	95	92	87	91	97	106	65
pH 6.5	168	87	98	92	87	88	94	92	65

TABLE 7 (concluded)

Compound (µg added to 100 ml)		DNP 10.4	RDX 12	TNB 10.3	DNB 10	2,4-DNT 10.1	TNT 9.9	Tetryl 10.2	DPA 10
Storage Parameters Compared	Hr	% Recovery							
Amber bottle, 4°C	0	84	94	89	81	85	93	99	53
Dark, Teflon cap liner	24	55	86	74	56	56	71	84	26
NaCl, $CaCl_2$, $FeCl_3$, $CuCl_1$ pH 6.5	168	80	96	94	84	89	101	99	44
Amber bottle, 4°C	0	81	98	91	85	87	94	98	81
Dark Teflon cap liner	24	81	95	93	86	86	95	98	75
$NaNO_3$, NaCl, Na_2CO_3, Na_3PO_4 Na_2SO_4, NaBr, pH 6.5	168	70	99	90	78	85	92	97	55
Amber bottle, 4°C	0	NA	NA	NA	NA	NA	NA	NA	NA
Dark, Teflon cap liner	24	NA	NA	NA	NA	NA	NA	NA	NA
1/1 (v/v) cation and anion mixtures, pH 6.5	168	80	99	95	76	85	92	98	52
8. Presence or absence of soap film									
Amber bottle, 4°C	0	91	98	97	84	91	98	100	76
Dark, Teflon cap liner	24	81	95	92	87	91	97	106	65
pH 6.5	168	87	98	92	87	88	94	92	65
Amber bottle, 4°C, pH 6.5	0	94	97	97	84	94	102	103	91
Dark, Teflon cap liner	24	87	94	95	90	96	100	101	83
Cleaned by standard method then made slightly soapy	168	87	94	91	91	97	96	95	84

[2] No sample at pH 11 with CH_3CN.

TABLE 8

PERCENT RECOVERY OF FOUR MUNITIONS AFTER SHORT-TERM STORAGE

Compound µg/100 ml			NB 10.5	2,6-DNT 11.2	NG 303	PA 10.8
Storage	Parameter	Hr				
pH 3	(H_2SO_4)	117	65	82	94	100
		228	68	85	96	97
pH 3.5	(HOAc),	117	57	87	111	101
	10% CH_3CN	228	68	85	98	105
pH 6	2% Salts	117	66	83	90	98
		228	43	75	121	101
pH 6.5		0[a]	76	86	96	102
		117	66	86	95	97
		228	66	91	92	98
pH 6.5	10% CH_3CN	117	63	83	88	98
		228	60	79	94	104
pH 6.5	Oil film	117	66	116	91	Interferences
		228	62	114	82	Interferences
pH 11		117	68	85	18	93
		228	59	84	14	98

a Average recovery from precision and accuracy study, four replicates.

TABLE 9

EFFECT OF ANIONS AND CATIONS ON PERCENT RECOVERY OF MUNITIONS FROM WATER AT pH 3.5

Compound	Hr	% Recovery — Concentration of Munitions								
		Deionized Water[a]			100 ppm Anions			100 ppm Cations		
		0.5X	1.0X	5X	0.5X	1.0X	5X	0.5X	1.0X	5X
Dinitrophenol X = 5.2 µg/ℓ	0	100	90	95	82	82, 92	93	79	85, 75	90
	24				87	88, 90	91	84	82, 91	85
	168				88	88, 92	91	103	84, 88	85
RDX X = 6.0 µg/ℓ	0	107	92	107	94	83, 88	89	93	87, 89	87
	24				88	94, 98	94	104	94, 96	98
	168				82	87, 95	94	105	90, 94	95
TNB X = 5.1 µg/ℓ	0	101	87	98	79	73, 87	89	92	85, 82	91
	24				89	85, 98	92	88	88, 93	86
	168				73	78, 80	82	86	73, 77	79
DNB X = 5.0 µg/ℓ	0	96	88	93	81	76, 83	91	125	82, 73	89
	24				103	90, 92	90	88	82, 82	84
	168				91	78, 80	75	90	78, 82	76
2,4-DNT X = 5.1 µg/ℓ	0	99	91	80	71	66, 75	87	93	87, 74	88
	24				80	80, 90	88	97	89, 80	78
	168				77	81, 85	77	85	78, 84	76
TNT X = 5.0 µg/ℓ	0	101	93	95	74	70, 81	85	117	80, 74	82
	24				81	80, 93	88	82	88, 86	79
	168				86	92, 94	83	93	77, 80	83
Tetryl X = 5.1 µg/ℓ	0	83	84	96	77	69, 76	81	96	86, 86	84
	24				66	74, 79	81	82	84, 90	83
	168				69	73, 77	79	106	92, 78	90

TABLE 9 (concluded)

Compound	Hr	% Recovery — Concentration of Munitions: Deionized Water[a] 0.5X	Deionized Water[a] 1.0X	Deionized Water[a] 5X	100 ppm Anions 0.5X	100 ppm Anions 1.0X	100 ppm Anions 5X	100 ppm Cations 0.5X	100 ppm Cations 1.0X	100 ppm Cations 5X
DPA	0	88	84	81	67	63, 72	84	77	60, 52	29
X = 5.0 µg/ℓ	24				86	36, 72	Interferences	50	53, 75	34
	168				64	87, 77	73	113	72, 71	70
Nitrobenzene	0	73	69	80	86	68, 78	78	69	85, 77	75
X = 5.2 µg/ℓ	24				79	65, 68	64	71	89, 75	80
	168				65	68, 73	67	67	64, 67	67
2,6-Dinitro-	0	89	85	89	82	79, 84	78	76	127, 89	90
toluene	24				18	14, 15	15	84	105, 87	89
X = 5.6 µg/ρ	168				91	71, 77	83	84	78, 84	82
Nitroglycerin	0	97	96	98	76	71, 85	80	80	193, 90	87
X = 152 µg/ℓ	24				15	14, 12	16	87	96, 85	91
	168				77	69, 76	87	71	81, 71	89
Picric acid	0	101	99	104	86	91, 106	72	96	129, 105	67
X = 5.4 µg/ℓ	24				35	29, ND	ND	112	122, 108	72
	168				98	88, 102	65	142	98, 143	66

a Mean of four replicates - precision and accuracy study.

C. Effect of Sediment

Preliminary studies on the effect of sediment on the recovery of munitions gave some indication that diphenylamine was lost when the 2% added sediment was separated by filtering through glass wool. These experiments included only single 24 hr determinations and since USTHAMA suggested that a large number of monitoring well samples would contain sediment, a more detailed effort was undertaken.

This effort included: development of an analytical method for determination of munitions in sediment including an abbreviated precision and accuracy study; a 7-day storage stability study of munitions in sediment; and a 7-day storage stability study of munitions in sedimented water.

1. Method development: Acetone, acetonitrile, methylene chloride/methanol (95/5) and hexane were evaluated as extraction solvents for fortified sediment. Although tetryl exhibited unsatisfactory recoveries, the best solvent for recovery of the majority of the eight compound set was CH_2Cl_2/CH_3OH. All the polar solvents led to serious background interferences for NB, NG, and PA. Therefore, hexane was used for analysis of the four remaining munitions although picric acid could not be recovered.

The method consisted of 1 hr of wrist-action shaking of a 10/1, solvent/sediment mixture. The mixture was centrifuged and the supernatant liquid was carefully withdrawn in a volume equal to half of the original added volume. This aliquot was concentrated and exchanged into the solvent appropriate for HPLC analyses described earlier in this report. Tables 10 and 11 show the results of the abbreviated precision and accuracy evaluation done for the sediment methods.

2. Storage studies: The presence of sediment in a water sample might effect the recovery of munitions in the following ways: (1) adsorption of munitions on surface; (2) degradation of munitions by sediment leachates; and (3) chromatographic interferences due to sediment leachates. In order to determine if a reduction of munition is due to adsorption or degradation by leachate, it would be necessary to analyze both phases of sedimented water after storage. Since the munitions might become irreversibly bound to sediment, a storage study was conducted on wetted sediment fortified with munitions. The results of storage on sediment (Table 12) will allow a more accurate determination of munition material balance in sedimented water stored over time.

DNP, DNB, 2,4-DNT and DPA all showed appreciable losses after 7 days storage on sediment.

Munitions and sediment were added to deionized water at levels of 1 µg/20 ml and 2 g/20 ml, respectively. Nitroglycerin was added at a level of 5 µg/20 ml because of its higher detection limit. Samples were analyzed immediately (0 day), and after 1 and 7 days storage. Both water and sediment were analyzed separately. In addition, fortified water samples, without added sediment, were analyzed concurrently. The results are given in Table 13.

TABLE 10

PERCENT RECOVERY OF EIGHT MUNITIONS FROM SEDIMENT (2 g)

Compound Spiking Level	DNP	RDX	TNB	DNB	2,4-DNT	TNT	Tetryl	DPA
0.5X[a]	63	92	70	90	89	92	ND[b]	53
0.5X	86	111	89	91	94	99	ND	57
0.5X	86	102	52	85	85	89	ND	56
0.5X	68	86	63	83	84	84	ND	40
2X	83	101	72	85	89	94	ND	48
2X	85	104	89	84	88	104	ND	53
2X	83	105	78	81	84	94	ND	42
2X	89	116	65	84	88	95	ND	57
10X	82	99	77	89	92	91	7.5	58
10X	98	96	107	80	96	89	14	54
10X	98	98	106	83	92	87	24	43
10X	96	95	107	84	94	83	19	48

a X ranged from 0.5 to 0.6 µg.
b ND = Not detected.

TABLE 11

PERCENT RECOVERY OF FOUR MUNITIONS FROM SEDIMENT (2 g)

Compound Spiking Level	NB (x = 0.52 µg)	2,6-DNT (x = 0.56 µg)	NG (x = 2.5 µg)	PA (x = 0.5 µg)
0.5X	108	80	ND[a]	ND
	95	70	138	ND
	94	96	ND	ND
	98	76	135	ND
2X	77	79	84	ND
	78	86	84	ND
	70	80	89	ND
	88	91	73	ND
10X	78	80	77	ND
	77	76	63	ND
	78	83	85	ND
	80	82	74	ND

a ND = Not detected.

TABLE 12

PERCENT RECOVERY OF MUNITIONS FROM 2 g OF WETTED SEDIMENT AFTER 0, 1 AND 7 DAYS

Compound	Concentration	% Recovery 0 Day	1 Day	7 Days
DNP	2X	74, 83	74, 83	11, 39
x = 0.5 µg	10X	67, 74	77, 80	19, 44
RDX	2X	95, 105	93, 95	96, 98
x = 0.6 µg	10X	91, 95	96, 97	101, 102
TNB	2X	65, 36	54, 79	65, 79
x = 0.5 µg	10X	81, 86	92, 88	79, 89
DNB	2X	70, 71	70, 78	7.8, 33
x = 0.5 µg	10X	69, 79	80, 80	16, 39
2,4-DNT	2X	73, 72	74, 80	11, 41
x = 0.5 µg	10X	72, 79	80, 79	18, 43
TNT	2X	76, 72	76, 80	57, 72
x = 0.5 µg	10X	73, 81	86, 83	61, 67
Tetryl	2X	0, 0	0, 0	89, 78
x = 0.5 µg	10X	0, 55	84, 72	83, 83
DPA	2X	54, 64	41, 42	7.1, 14
x = 0.5 µg	10X	78, 88	68, 58	6.3, 11
NB	2X	73, 81	145, 149	88, 69
x = 0.5 µg	10X	95, 86	81, 93	86, 82
2,6-DNT	2X	79, 88	93, 101	81, 81
x = 0.6 µg	10X	95, 89	87, 98	91, 88
NG	2X	41, 56	70, 78	58, 90
x = 2.5 µg	10X	62, 46	52, 51	51, 51
PA	2X	ND, ND[a]	ND, ND	ND, ND
x = 0.5 µg	10X	ND, ND	ND, ND	ND, ND

a ND = Not detected.

TABLE 13

PERCENT RECOVERY OF MUNITIONS FROM WATER WITH AND WITHOUT SEDIMENT AFTER 0, 1 AND 7 DAYS

Compound	Sediment Added (g/20 ml)	0 Days	1 Day	7 Days
DNP	0	104, 94	95, 93	93, 89
	2	90, 92	85, 92	85, 83
RDX	0	94, 99	97, 96	96, 91
	2	86, 88	84, 84	79, 79
TNB	0	Not resolved	104, 95	117, 110
	2	Not resolved	98, 89	97, 90
DNB	0	87, 81	87, 85	88, 82
	2	74, 72	81, 84	75, 72
2,4-DNT	0	89, 93	90, 88	90, 82
	2	74, 71	78, 82	75, 71
TNT	0	66, 78	75, 72	84, 83
	2	65, 46	79, 71	76, 77
Tetryl	0	1.8, 22	ND, ND[a]	39, 75
	2	4, ND	68, 9.8	50, 57
DPA	0	86, ND	81, 81	82, 80
	2	98, 54	115, 57	27, 26
NB	0	92, 76	71, 71	76, 68
	2	79, 72	79, 58	62, 66
2,6-DNT	0	92, 80	83, 83	95, 84
	2	90, 82	90, 63	55, 61
NG	0	53, 75	51, 88	44, 59
	2	69, 55	57, 43	40, 48
PA	0	81, 77	116, 56	34, 30
	2	25, 41	140, 153	61, 82

a ND = Not detected.

In general, there was a slightly lower recovery of munitions from sedimented water. In part this may be due to that water which remained with the sediment after withdrawal of the supernatant liquid. Over a period of 7 days the amount of munitions recovered from the 2 g of sediment increased by not more than 50%. The average amount of munitions associated with the sediment after 7 days was less than 15% of the added amount as shown in Table 14.

The results of this experiment suggest that separation of sediment would be advisable for long-term storage but that it would not be a field requirement.

D. Long-Term Storage Study

1. Procedures: The protocol for preservation of 12 munitions in water was proposed on the basis of (1) information obtained from the literature review, (2) the results of short-term storage studies, and (3) assumptions on optimal storage conditions.

- Container - Amber glass with Teflon cap liner.
- Cleaning
  - Rinse with hot water.
  - Wash with hot water/soap solution.
  - Rinse with tap water followed by deionized water.
  - Soak for 1 hr in 0.1 N HCl.
  - Air dry using precautions to prevent contamination.
- Preservation - Add acetonitrile to achieve a 10% solution and mix thoroughly. Add glacial acetic acid dropwise until a pH of 3.5 is obtained and mix thoroughly. Removal of sediment by centrifugation and decantation of supernatant liquid.
- Storage - Refrigerate (4°C) and maintain in dark.

2. Validation studies with tap water: Tap water was fortified with munitions (in all cases, DNP, RDX, DNB, 2,4-DNT, TNT, tetryl, DPA, TNB, NB, 2,6-DNT, NG, and picric acid were fortified into water as compound sets of 8 and 4 as described earlier in Section III.B) at concentration levels of 0, 0.2X, 0.5X, X, 2X, 5X, 10X; X equals 5 µg/liter for all compounds except NG, where X equals 150 µg/liter. The samples were treated according to the protocol and stored for 3 weeks. At this time 110-ml aliquots were taken for analysis.

The analysis of the first sample set containing dinitrophenol and diphenylamine indicated a total loss of these compounds at all concentration levels. The remaining 10 munitions were satisfactorily recovered and the results of the short-term storage did not predict losses of this magnitude. Therefore, the remaining replicate analyses of the 8-compound tap water samples were postponed while an attempt was made to account for the reduced recovery. The analyses of the 4-compound group continued on schedule and the results are given in Appendix C.

TABLE 14

RECOVERY OF MUNITIONS FROM SEDIMENTED WATER AFTER 0, 1 AND 7 DAYS

µg Added	Days	µg Recovered: Water (20 ml) a	Water (20 ml) b	Sediment (2 µg) a	Sediment (2 µg) b	Total a	Total b	% Recovery Total a	% Recovery Total b
DNP	0	0.94	0.92	0.06	0.06	1.00	0.98	96	94
1.04	1	0.87	0.96	0.06	0.12	0.93	1.08	89	104
	7	0.88	0.87	0.10	0.08	0.98	0.95	94	91
RDX	0	1.04	1.05	0.12	0.12	1.16	1.17	97	98
1.2	1	1.01	1.01	0.18	0.16	1.19	1.17	99	98
	7	0.95	0.95	0.18	0.18	1.13	1.13	94	94
TNB	0	Not resolved		0.14	0.12	-	-	-	-
1.03	1	1.01	0.92	0.12	0.14	1.03	1.06	100	103
	7	1.00	0.93	0.20	0.18	1.20	1.11	117	108
DNB	0	0.74	0.72	0.06	0.06	0.80	0.78	80	78
1.0	1	0.81	0.84	0.06	0.06	0.87	0.90	87	90
	7	0.75	0.72	0.10	0.08	0.85	0.80	85	80
2,4-DNT	0	0.74	0.72	0.06	0.06	0.80	0.78	79	77
1.01	1	0.79	0.82	0.06	0.08	0.85	0.90	84	89
	7	0.76	0.72	0.12	0.10	0.88	0.82	87	81
TNT	0	0.65	0.45	0.06	0.06	0.71	0.51	72	51
0.99	1	0.79	0.71	0.06	0.08	0.85	0.79	86	80
	7	0.75	0.77	0.10	0.06	0.85	0.83	86	84
Tetryl	0	0.04	ND[a]	ND	ND	-	-	-	-
1.02	1	0.70	0.10	ND	ND	-	-	-	-
	7	0.51	0.58	ND	ND	-	-	-	-
DPA	0	0.98	0.54	ND	0.06	-	0.60	-	60
1.0	1	1.14	0.56	0.03	0.05	1.17	0.61	117	61
	7	0.27	0.26	ND	ND	-	-	-	-
NB	0	0.83	0.76	ND	ND	-	-	-	-
1.05	1	0.83	0.61	0.28	ND	111	-	106	-
	7	0.66	0.69	ND	ND	0.66	0.69	63	66
2,6-DNT	0	1.02	0.92	ND	ND	-	-	-	-
1.13	1	1.02	0.71	0.16	ND	1.18	-	104	-
	7	0.62	0.69	ND	ND	-	-	-	-
NG	0	3.5	2.8	ND	ND	-	-	-	-
5.06	1	2.9	2.2	ND	ND	-	-	-	-
	7	2.0	2.4	ND	ND	-	-	-	-
PA	0	0.27	0.45	ND	ND	-	-	-	-
1.09	1	1.5	1.7	ND	ND	-	-	-	-
	7	0.67	0.90	ND	ND	-	-	-	-

ND = Not detected.

The major differences between the analytical method validation and the preservation protocol validation were time, presence of chlorine in tap water (~ 3 ppm) and ionic concentration. Tap water was fortified with DNP and DPA individually and together with the other six munitions. Half the samples were treated with sodium thiosulfate to eliminate the effect of chlorination. The samples were stored for 7 days with aliquots being analyzed after 0, 1, and 7 days. The results shown in Tables 15 and 16 indicate that the presence of residual chlorine was the cause of the loss of DNP and DPA, and the addition of sodium thiosulfate eliminated the problem. However, after 7 days the recoveries of tetryl were 23 and 12% with thiosulfate while recoveries of tetryl from untreated tap water were 77 and 90%.

TABLE 15

PERCENT RECOVERY OF DINITROPHENOL FROM CHLORINATED TAP WATER (5 µg/100 ml)

Additives	Recovery (%) Storage Time (Days) 0	1	7
None	30, 27	33, 29	36, 30
RDX, DNB, 2,4-DNT, TNT, tetryl, DPA, and TNB	67, 0[a]	17, 0[a]	20, 0[a]
Sodium thiosulfate	102, 92	99, 93	87, 92
Sodium thiosulfate and RDX, DNB, 2,4-DNT, TNT, tetryl, DPA, and TNB	78, 77	86, 85	81, 86

a Fortification of this replicate with DNP is in doubt.

TABLE 16

PERCENT RECOVERY OF DIPHENYLAMINE FROM CHLORINATED TAP WATER (5 µg/100 ml)

Additives	Recovery (%) Storage Time (Days) 0	1	7
None	I,[a] I	I, I	0, 45
RDX, DNB, 2,4-DNT, TNT, tetryl, DPA, and TNB	11, I	I, I	0, I
Sodium thiosulfate	I, 127	I, 108	88, 105
Sodium thiosulfate and RDX, DNB, 2,4-DNT, TNT, tetryl, DPA, and TNB	I, 92	I, I	81, 88

a I = Interferences

Since chlorine is not likely to be found in groundwater and sodium thiosulfate had an adverse effect on the recovery of tetryl, the addition of sodium thiosulfate as a preservative for munitions is not recommended.

Following the experiments to determine the cause of the loss of DNP and DPA, four replicate analyses at seven concentration levels were performed on the remaining six munitions which had been stored in tap water for 10 weeks. The precision and accuracy analyses of these results are provided in Appendix D.

3. Field testing the protocol: The final test of the proposed preservation protocol involved the collection, fortification, preservation, storage and analysis of groundwater samples collected from monitoring well No. 17 at Sunflower Arsenal in DeSoto, Kansas. Ten liters of sediment-free water was obtained.

From the 10 liters the following samples were prepared:

1) One liter fortified with 25 μg of each compound in the eight munition group.

2) One liter designated as the blank for the eight munition group.

3) One liter fortified with 25 μg of NB, 2,6-DNT and PA and 725 μg of NG.

4) One liter designated as the blank for the four munition group.

The pH of each of these samples were adjusted to 3.5 by dropwise addition of glacial acetic acid. Acetonitrile was then added to prepare a 10% CH_3CN solution. Two aliquots of each prepared sample (1-4) were removed and analyzed immediately (day 0). The remaining sample was stored in amber bottles at 4°C in the dark for 3 weeks and reanalyzed. The results shown in Table 17 indicate only minor reductions in the recovery of two munitions (NB, NG) over time.

4. Comparison of long-term storage of munitions in various water types: After 3-week storage munitions stored in tap water generally showed the same % inaccuracy values obtained during the method precision and accuracy assessment conducted in deionized water as shown in Table 18. The exceptions were DNP and DPA whose losses were shown to be directly attributable to the presence of chlorine in tap water. DNB was the only other munition to show a slight reduction in recovery. An additional experiment was run in which munitions were added to the well water and these samples were stored in the dark at 4°C with no chemical additives for 1 week. These results are also included in Table 18.

The preserved well water samples also showed excellent agreement between 0 and 21 day % inaccuracy values. Results for the untreated (pH 7, no CH_3CN) fortified well water samples stored for 7 days in the dark at 4°C showed that DNP, RDX, DPA, NB, and NG could be stored in this matrix without chemical treatment for up to 7 days. However TNB, DNB, 2,4-DNT, tetryl, 2,6-DNT and picric acid showed significant losses.

TABLE 17

FIELD TEST[a] OF PRESERVATION PROTOCOL

Compound	µg Added	µg Found Day 0	µg Found Day 21
DNP	0	ND[b]	ND
		ND	ND
	2.45	1.6	1.5
		1.3	1.3[c]
RDX	0	ND	ND
		ND	ND
	2.76	2.1	2.1
		2.1	2.1[c]
TNB	0	ND	ND
		ND	ND
	2.56	1.9	2.0
		1.9	1.3[c]
DNB	0	ND	ND
		ND	ND
	2.45	1.5	1.4
		1.6	0.16[c]
2,4-DNT	0	ND	ND
		ND	ND
	2.54	1.6	1.6
		1.7	0.40[c]
TNT	0	ND	ND
		ND	ND
	2.46	1.8	2.0
		1.8	1.3[c]
Tetryl	0	ND	ND
		ND	NS
	2.50	2.2	2.3
		2.2	0.5[c]
DPA	0	ND	ND
		ND	ND
	2.35	0.90	1.0
		1.0	0.48[c]
NB	0	ND	ND
		ND	ND
	2.42	1.1	0.93
		1.1	0.78

TABLE 17 (concluded)

Compound	µg Added	µg Found Day 0	µg Found Day 21
2,6-DNT	0	ND	ND
		ND	ND
	2.63	1.8	1.9
		1.8	1.7
NG	0	ND	ND
		ND	ND
	72.6	17	13
		17	14
PA	0	ND	ND
		ND	ND
	2.56	1.8	2.1
		2.1	2.3

a 100 ml Sunflower Arsenal Monitoring Well No. 17 fortified with munitions followed by pH adjustment to 3.5 with glacial acetic acid and addition of 10% CH_3CN.
b ND = not detected.
c The response for the majority of compounds in this extract were low compared to the duplicate.

TABLE 18

COMPARISON OF PERCENT INACCURACY VALUES FOR ANALYSES OF MUNITIONS STORED FOR 0, 7, 21 AND 70 DAYS IN VARIOUS WATER TYPES
(µg/liter fortification level)

Compound	0 Days		7 Days		21 Days		70 Days
	Deionized Water	Well Water w/ CH_3CN at pH 3.5	Deionized w/ CH_3CN at pH 3.5	Well Water[a] (Not Preserved)	Well Water w/ CH_3CN at pH 3.5	Tap Water w/ CH_3CN at pH 3.5	Tap Water w/ CH_3CN at pH 3.5
DNP 10X[b]	-7	-	-12	-	-	-	-
5X	-5	-40	-	-45	-42	ND	ND
2X	-4	-	-	-	-	ND	ND
1X	-10	-	-	-	-	ND	ND
0.5X	-2	-	-	-	-	ND	ND
0.2X	+3	-	-	-	-	ND	ND
RDX 10X	-1	-	-1	-	-	19	2
5X	+4	-23	-	-23	-23	6	1
2X	-1	-	-	-	-	7	-8
1X	-8	-	-	-	-	4	2
0.5X	+5	-	-	-	-	-2	-6
0.2X	+6	-	-	-	-	4	36
TNB 10X	-5	-	-2	-	-	-3	-15
5X	-2	-27	-	-54	-35	-10	-13
2X	-2	-	-	-	-	-13	-16
1X	-9	-	-	-	-	-4	-13
0.5X	-1	-	-	-	-	-7	-13
0.2X	+4	-	-	-	-	-13	+34
DNB 10X	-9	-	-8	-	-	-17	-27
5X	-7	-39	-	-60	-42	-17	-26
2X	-6	-	-	-	-	-24	-29
1X	-13	-	-	-	-	-17	-26
0.5X	-6	-	-	-	-	-15	-32
0.2X	-10	-	-	-	-	-28	-31

TABLE 18 (continued)

Compound	0 Days		7 Days		21 Days		70 Days
	Deionized Water	Well Water w/ CH_3CN at pH 3.5	Deionized w/ CH_3CN at pH 3.5	Well Water[a] (Not Preserved)	Well Water w/ CH_3CN at pH 3.5	Tap Water w/ CH_3CN at pH 3.5	Tap Water w/ CH_3CN at pH 3.5
2,4-DNT 10X	-13	-	-13	-	-	-17	-28
5X	-11	-36	-	-53	-37	-14	-30
2X	-4	-	-	-	-	-17	-25
1X	-10	-	-	-	-	-11	-16
0.5X	-2	-	-	-	-	-10	-30
0.2X	-1	-	-	-	-	-13	-3
TNT 10X	-8	-	+1	-	-	-8	-21
5X	-5	-27	-	-36	-34	-10	-23
2X	-1	-	-	-	-	-14	-19
1X	-7	-	-	-	-	-4	-13
0.5X	-1	-	-	-	-	-11	-21
0.2X	+1	-	-	-	-	-12	-9
Tetryl 10X	-7	-	-1	-	-	+5	-12
5X	-4	-12	-	-54	-8	-4	-13
2X	-4	-	-	-	-	-8	-11
1X	-16	-	-	-	-	-1	-5
0.5X	-18	-	-	-	-	-8	-5
0.2X	-17	-	-	-	-	-6	-2
DPA 10X	-17	-	-28	-	-	ND	ND
5X	-19	-60	-	-60	-59	ND	ND
2X	-8	-	-	-	-	ND	ND
1X	-17	-	-	-	-	ND	ND
0.5X	-26	-	-	-	-	ND	ND
0.2X	-20	-	-	-	-	ND	ND

TABLE 18 (concluded)

Compound	0 Days		7 Days		21 Days		70 Days
	Deionized Water	Well Water w/ CH_3CN at pH 3.5	Deionized w/ CH_3CN at pH 3.5	Well Water[a] (Not Preserved)	Well Water w/ CH_3CN at pH 3.5	Tap Water w/ CH_3CN at pH 3.5	Tap Water w/ CH_3CN at pH 3.5
NB 10X	-32	-	NA	-	-	-35	NA
5X	-31	-54	-	-56	-64	-27	NA
2X	-24	-	-	-	-	-34	NA
1X	-31	-	-	-	-	-31	NA
0.5X	-27	-	-	-	-	-22	NA
0.2X	-24	-	-	-	-	-26	NA
2,6-DNT 10X	-12	-	NA	-	-	-17	NA
5X	-11	-31	-	-40	-32	-13	NA
2X	-14	-	-	-	-	-13	NA
1X	-15	-	-	-	-	-19	NA
0.5X	-11	-	-	-	-	-8	NA
0.2X	-10	-	-	-	-	-7	NA
NG 10X	-6	-	NA	-	-	-12	NA
5X	-2	-77	-	-79	-81	-11	NA
2X	-4	-	-	-	-	-9	NA
1X	-4	-	-	-	-	-4	NA
0.5X	-3	-	-	-	-	-3	NA
0.2X	-2	-	-	-	-	-2	NA
PA 10X	+2	-	NA	-	-	-5	NA
5X	+3	-23	-	-69	-15	-4	NA
2X	+2	-	-	-	-	-8	NA
1X	-1	-	-	-	-	-3	NA
0.5X	+2	-	-	-	-	+2	NA
0.2X	-2	-	-	-	-	+7	NA

ND - Not detected.
NA - Not analyzed.
a CH_3CN added and pH adjusted to 3.5 just prior to analysis after 7 days storage without treatment.
b X = 5 µg/liter except for NG where X = 150 µg/liter.

Equally significant is the matrix effect seen for 0 day analyses. The % recovery from well water was significantly lower than in deionized water for all munitions with the exception of tetryl. Although the cause of this reduction was not investigated, the preservation protocol was effective in maintaining these levels after 3 weeks.

V. CONCLUSIONS AND RECOMMENDATIONS

The results of two 3-week storage studies conducted in tap water and well water demonstrate the adequacy of the proposed preservation techniques. Although the control of temperature, exposure to light and sample container are recommended, short-term storage studies summarized in Table 19 indicate that maintenance of samples at 4°C, in dark, and in amber glass bottles is not critical for periods of 7 days or less. Also, the need for the addition of acetonitrile as an antibacterial agent was not demonstrated. However, its efficacy in this capacity has been proven in previous work at MRI.

Waters Associates recommends that HPLC columns used with aqueous eluants be stored with 50% organic solvent to prevent microbial growth. On the basis of this recommendation, samples of apple juice and Milli-Q water, which had previously exhibited microbial growth during storage, were treated with CH_3CN (10:1, v/v). Ten percent CH_3CN was selected as a possible treatment because of its transparency in the UV, its miscibility with water, and its compatibility with the analytical method in use at the time. Fresh samples of both Milli-Q water and apple juice which were treated with 10% CH_3CN showed no growth even after 3 weeks storage. The use of CH_3CN during this project did not decrease munition stability. The parameter with the greatest effect on munition stability was pH, and adjustment to 3.5 was selected on the basis of (1) lower recoveries obtained at pH 11 and 6.5, and (2) its compatibility with the analytical method developed and used during this study.

It should be noted that although the preservation techniques maintained stability in tap water and well water, the percent inaccuracy of the analytical method at zero time was substantially different in the two water types. However, these studies were not conducted at the same time or for the purposes of comparing the effect of water type on the zero time recovery values. It is therefore recommended that an experiment be designed to compare zero time recoveries from a variety of water types. Aliquots of each water sample should be fortified with the same standard munition solution and analyzed immediately (zero time) and after 21 days with preservation. If the results of this experiment confirm that zero time recoveries do vary with water type but remain unchanged after 21 days of storage with preservation, then the following conclusions can be drawn:

1. Preserved water samples can be stored for up to 21 days before analysis.

2. Munition concentrations in different water types can be compared only after a recovery correction is made for each water type.

TABLE 19

SUMMARY OF EFFECTS OF STORAGE PARAMETERS ON REDUCTION OF MUNITION RECOVERY[a]

(7-Day[b] Storage)

	Parameters								
								Sediment	
		Ambient	Absence of CH_3CN		pH			Analysis of	
Munition	Light	Temperature (25°C)	at pH 3.5	at pH 6.5	6.5	11	Presence of Anions/Cations	H_2O Only	H_2O and Sediment
DNP	None	None	None	None	None	Slight[c]	Slight	Slight	None
RDX	None	None	None	None	None	Slight	None	Slight	None
TNB	None	None	None	None	None	Slight	None	Slight	None
DNB	None	None	None	None	None	None	None	Slight	None
2,4-DNT	None	None	None	Slight	Slight	None	None	Slight	None
TNT	None	None	None	None	None	Slight	None	None	None
Tetryl	Slight	None	None	None	None	Major[c]	None	None	None
DPA	None	Slight	None	Slight	Slight	Major	Slight	Major	Major
NB	NA[d]	NA	NA	NA	None	Slight	None	None	None
2,6-DNT	NA	NA	NA	NA	None	None	None	Slight	Slight
NG	NA	NA	NA	NA	None	Major	None	None	None
PA	NA	NA	NA	NA	None	None	None	None	None

a Recoveries compared to protocol which specifies (1) amber bottles, (2) 4°C, (3) 10% CH_3CN, and (4) pH 3.5.
b pH studies for NB, 2,6-DNT, NG, and PA were 10-day storage.
c Slight > 10% < 50% reduction; major > 50% reduction.
d NA = not analyzed.

APPENDIX A

PRECISION AND ACCURACY ASSESSMENT OF THE HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC ANALYTICAL TECHNIQUE FOR THE DETERMINATION OF DINITROPHENOL (DNP); CYCLOTRIMETHYLENETRINITRAMINE (RDX); 1,3-DINITROBENZENE (DNB); 1,3,5-TRINITROBENZENE (TNB); 2,4-DINITROTOLUENE (2,4-DNT); TRINITROTOLUENE (TNT); 2,4,6-TRINITROPHENYLMETHYLNITRAMINE (TETRYL); AND DIPHENYLAMINE (DPA)

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1. Application

The developed analytical technique is for the quantitative determination of DNP, RDX, TNB, DNB, 2,4-DNT, TNT, tetryl, and DPA using high performance liquid chromatography (HPLC) with ultraviolet (UV) detection at 254 nm. The technique can be employed to analyze samples, i.e., water, that have been properly prepared.

a. Evaluated Concentration Range: The concentration ranges of DNP, RDX, TNB, DNB, 2,4-DNT, TNT, tetryl, and DPA studied in the reference solutions were 100, 250, 500, 1,000, 2,500, and 5,000 ng/2.0 ml. This concentration range corresponds to a series of 0.2X, 0.5X, X, 2X, 5X, and 10X, where X is 5 ng/ml (parts per billion, ppb) of a munition in water and a 100-ml water sample is utilized.

b. Sensitivity: A signal-to-noise ratio of 20 to 1 for DNP (peak height (PH) = 23 mm), 15 to 1 for RDX (PH = 16 mm), 20 to 1 for TNB (PH = 25 mm), 30 to 1 for DNB (PH = 35 mm), 30 to 1 for 2,4-DNT (PH = 31 mm), 20 to 1 for TNT (PH = 23 mm), 20 to 1 for tetryl (PH = 19 mm), and 10 to 1 for DPA (PH = 10 mm) was obtained with an injection of 100 µl of a 100 ng/2.0 ml solution of each compound (ca. 5 ng each compound on column).

c. Detection Limits: The detection limits of the analytical technique for reference solutions using the Hubaux and Vos detection limit program were 125 ng/2.0 ml for DNP, 178 ng/2.0 ml for RDX, 136 ng/2.0 ml for TNB, 100 ng/2.0 ml for DNB, 100 ng/2.0 ml for 2,4-DNT, 100 ng/2.0 ml for TNT, 103 ng/2.0 ml for tetryl, and 176 ng/2.0 ml for DPA.

d. Interferences: No interfering peaks were observed on the chromatograms of reference solutions.

2. Chemistry

DNP, RDX, TNB, DNB, 2,4-DNT, TNT, tetryl, and DPA are munition-related compounds manufactured at various installations. The assessment of potential environmental contamination by these compounds in water requires knowledge that the level of the compounds present at the time of sampling does not change prior to analysis and that the sampling technique provides a representative sample. The evaluation of the preservation and sampling parameters to be employed requires an analytical technique capable of assaying the compounds with sufficient precision, accuracy, and sensitivity to provide quantitative data.

3. Apparatus

a. Instrumentation: A Waters programmable liquid chromatographic system consisting of two Model 6000A pumps, Waters Model 720 system programmer, a Rheodyne Model 7125 100-µl fixed loop injector, a Model 440 UV detector with a 254 nm filter and a single pen Model SR-204 Heath-Schlumberger recorder were used. (Note: Equivalent instrumentation will provide similar results.)

b. HPLC Parameters:

1. Column: Spherisorb ODS, 5 µ, 250 x 4.6 mm ID.

2. Precolumn: Co:Pell ODS, 25 µ to 35 µ, 50 x 2 mm ID.

3. Eluent:
   Initial: 30/70 (v/v) acetonitrile/water 0.08 M in acetic acid, adjusted to pH 3.1 with ammonium hydroxyide
   Final: 50/50 (v/v) acetonitrile/water 0.08 M in acetic acid, adjusted to pH 3.1 with ammonium hydroxide

4. Program: Linear gradient from initial eluent to final eluent over a 35-min period.

5. Flow Rate: 1.0 ml/min.

6. Chart Speed: 0.1 in/min.

7. Detector: UV, 254 nm.

8. Internal Standard: Propiophenone.

9. Injection Volume: 50 to 100 µl.

10. Retention Volumes:

Compound	Milliliters
DNP	12.5
RDX	13.5
TNB	17.0
DNB	18.0
2,4-DNT	24.0
TNT	25.0
Tetryl	26.0
DPA	38.0
IS	20.0

Note: Slight changes in the retention indices may occur with fresh eluent or a change in precolumn or analytical column.

A representative HPLC-UV (254 nm) chromatogram for DNP, RDX, TNB, DNB, 2,4-DNT, TNT, tetryl, DPA, and the IS is shown in Figure 1.

c. Laboratory Glassware and Equipment:

1. Culture tubes (Pyrex) with Teflon-lined screw caps.

2. Volumetric flasks (100 ml).

3. Volumetric syringes (0-100, 0-500, and 0-1,000 µl).

d. Chemicals:

1. DNP, RDX, TNB, DNB, 2,4-DNT, TNT, tetryl, and DPA SARMs, obtained from the U.S. Army Toxic and Hazardous Materials Agency.

2. Propiophenone, analytical grade.

3. Acetonitrile, "Distilled in Glass" grade; acetic acid and ammonium hydroxide, ACS grade.

4. High purity water from a Milli-Q water purification system.

4. Standards

a. Stock: Weigh approximately 20 mg of DNP, RDX, TNB, DNB, 2,4-DNT, TNT, tetryl, and DPA SARM or interim SARM into separate 100-ml volumetric flasks and dissolve in acetonitrile (concentration of each compound, 200 µg/ml). Quantitatively pipette 2.5 ml from each stock above into a 100-ml volumetric flask and dilute to volume with a 45/55 (v/v) acetonitrile and water solution 0.08 M in acetic acid (concentration each compound, 5 µg/ml): working stock No. 1. Quantitatively pipette 20 ml of working stock No. 1 into a 100-ml volumetric flask and dilute to volume with a 45/55 (v/v)

acetonitrile and water solution 0.08 M in acetic acid: working stock No. 2 (concentration each compound, 1 µg/ml).

b. Internal Standard Stock: Weigh 20 mg propiophenone into a 100-ml volumetric flask and dilute to volume with acetonitrile (concentration, 200 µg/ml). Quantitatively pipette 1.0 ml of the stock above into a 100-ml volumetric flask and dilute to volume with a 45/55 (v/v) acetonitrile and water solution 0.08 M in acetic acid (concentration, 2 µg/ml).

c. Reference Solution Preparation: The working stocks No. 1 and No. 2 and the IS stock were employed to prepare the reference solutions for precision and accuracy testing of the analytical technique as follows:

Working Stock No.	µl Working Stock	µl IS Stock	µl HPLC Eluent	Concentration Each Compound ng/2.0 ml	
				Compound	IS
1	1,000	1,000	-	5,000	2,000
1	500	1,000	500	2,500	2,000
2	1,000	500	500	1,000	1,000
2	500	250	1,250	500	500
2	250	100	1,650	250	200
2	100	50	1,850	100	100
-	-	50	1,950	-	100

Each reference solution was prepared and analyzed on four separate days to define the precision and accuracy of the analytical technique.

5. Calculations

The reference solutions described in Section 4.c. were prepared and analyzed in quadruplicate. The relative weight response (RWR) (Equation 1) of each compound to the IS was calculated and the average RWR for each compound utilized to calculate the nanograms of that compound in every reference solution (Equation 2). The nanograms found were plotted against the nanograms added and a linear regression analysis of the data performed. The slope, intercept, and correlation coefficient of each compound were then determined. The data are summarized in Table 1 and include the average value at each level (Equation 3), the standard deviation (Equation 4), coefficient of variation (Equation 5), and percent inaccuracy (Equation 6). The raw data and calculations are given in Tables 10 through 17.

$$RWR = \frac{\text{Peak Height Cpd}}{\text{Peak Height IS}} \times \frac{\text{ng/2.0 ml IS}}{\text{ng/2.0 ml Cpd}} \qquad \text{(Eq. 1)}$$

$$\text{ng/2.0 ml cpd} = \frac{\text{Peak Height Cpd}}{\text{Peak Height IS}} \times \frac{\text{ng/2.0 ml IS}}{\text{Avg. RWR Cpd}} \qquad \text{(Eq. 2)}$$

$$\text{Average value} = \bar{x} = \Sigma x/n \qquad \text{(Eq. 3)}$$

$$\text{Standard deviation} = \sigma = \left(\frac{n\Sigma x^2 - (\Sigma x)^2}{n(n-1)}\right)^{\frac{1}{2}} \qquad \text{(Eq. 4)}$$

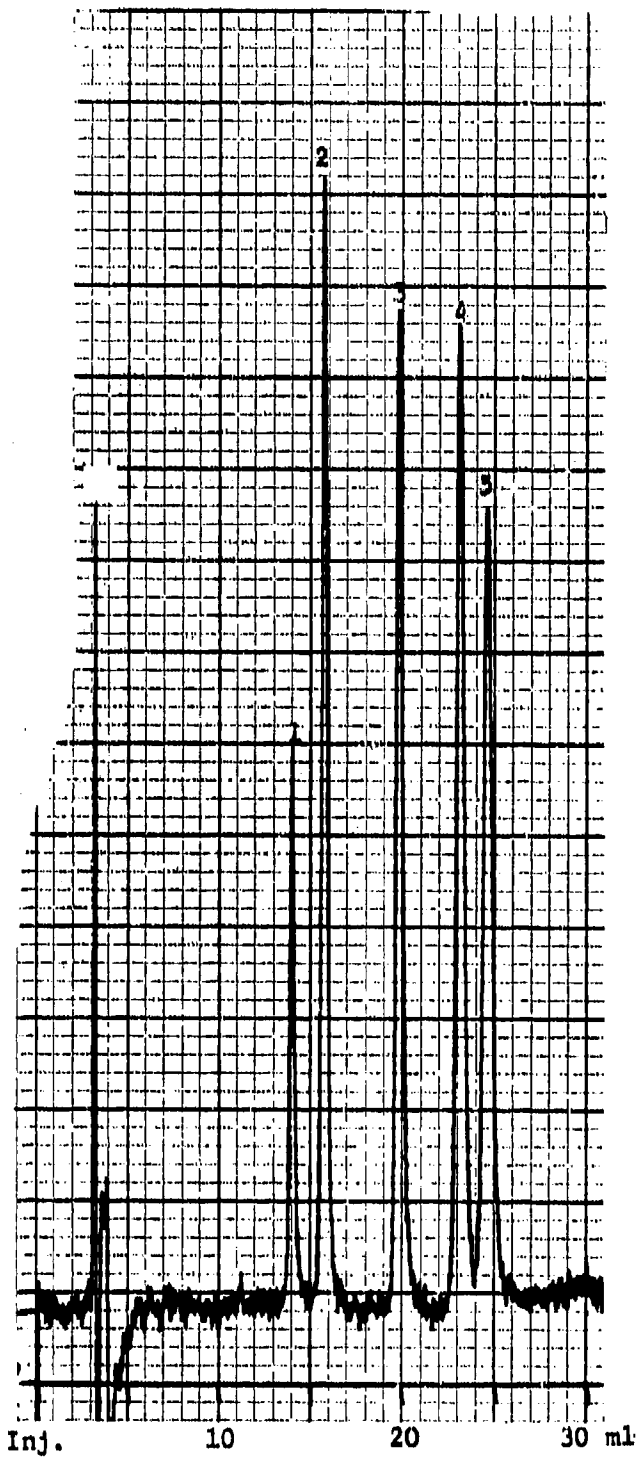
$$\text{Coefficient of variation} = \sigma/\bar{x} \times 100 \qquad \text{(Eq. 5)}$$

$$\text{Percent inaccuracy} = \frac{\bar{x} - \text{ng added}}{\text{ng added}} \times 100 \qquad \text{(Eq. 6)}$$

Graphic presentations of the standard deviation, coefficient of variation, and percent inaccuracy are given in Figures 2 through 9.

6. Statistical Evaluation of Data

A statistical evaluation of the data obtained for the precision and accuracy evaluation of the analytical technique for the determination of DNP, RDX, TNB, DNB, 2,4-DNT, TNT, tetryl, and DPA was made using the Hubaux and Vos detection limit program provided by the U.S. Army Toxic and Hazardous Materials Agency. The results of these evaluations are given in Tables 2 through 9. Detection limits for each compound were as follows: DNP, 125; RDX, 178; TNB, 136; DNB, 100; 2,4-DNT, 100; TNT, 100; tetryl, 103; and DPA, 176, using all the data points. The average nanograms per 2.0 ml value found at each level for each compound were determined from the linear regression equation for the 28 data points and the nanograms per 2.0 ml added at that level. The standard deviation and percent imprecision at each level were calculated based on this average, and thus do not agree with the values given in Table 1.



HPLC Parameters

Column: Spherisorb ODS, 5 µ
250 x 4.6 mm ID
Pre-column: CO:PELL ODS, 35 µ
50 x 2 mm ID
Eluent: 35/65 (v/v) CH_3CN/0.005M
t-Butyl Ammonium Hydroxide, pH 6.5
with 1N H_8PO_4
Flow Rate: 1.0 ml/min
Chart Speed: 0.1 in/min
Detector: UV, 230 nm
Attenuation: 0.01X
Injection Volume: 50 µl

Sample Characteristics

No.	Name	µg/2.0 ml
1	NB	1.0
2	IS	1.0
3	2,6-DNT	1.0
4	NG	30
5	PA	1.0

Figure 1 - HPLC-UV (230 nm) Separation of NB, 2,6-DNT, NG, and PA in a 2.0 ml Reference Solution

TABLE 1

LINEARITY AND PRECISION OF THE HPLC-UV (254 nm) DETERMINATION OF SARM REFERENCE SOLUTIONS OF DNP, RDX, TNB, DNB, 2,4-DNT, TNT, TETRYL, AND DPA

Compound	ng 2.0 ml Added	ng/2.0 ml Detected A	B	C	D	Average[a]	Standard[b] Deviation	Coefficient[c] of Variation	Percent[d] Inaccuracy
DNP	5,200	5,160	5,030	5,070	5,070	5,080	± 55	1	-2
	2,600	2,640	2,630	2,680	2,760	2,680	± 59	2	+3
	1,040	930	970	970	970	960	± 20	2	-8
	520	460	480	480	470	470	± 10	2	-10
	260	310	300	290	280	290	± 13	4	+12
	104	110	110	100	100	105	± 6	6	+1
	0	ND[e]	ND	ND	ND	-	-	-	-
Linear Regression DNP, y = 0.985x + 2.0; Correlation Coefficient, 0.9993									
RDX	6,000	6,170	5,960	5,960	6,060	6,040	± 100	2	+1
	3,000	3,220	3,140	3,200	3,300	3,210	± 66	2	+7
	1,200	1,060	1,120	1,160	1,140	1,120	± 43	4	-7
	600	530	530	540	520	530	± 8	2	-12
	300	340	330	320	320	330	± 10	3	+10
	120	120	110	120	120	120	± 5	4	0
	0	ND	ND	ND	ND	-	-	-	-
Linear Regression RDX, y = 1.019x - 11.5; Correlation Coefficient, 0.9990									
TNB	5,140	5,390	5,290	5,170	5,000	5,210	± 168	3	+1
	2,570	2,720	2,610	2,670	2,630	2,660	± 49	2	+4
	1,030	930	970	980	950	960	± 22	2	-7
	514	460	480	480	460	470	± 12	2	-9
	257	290	290	270	270	280	± 12	4	+9
	103	110	100	100	100	100	± 5	5	-3
	0	ND	ND	ND	ND	-	-	-	-
Linear Regression TNB; y = 1.020x - 17.5; Correlation Coefficient, 0.9992									

TABLE 1 (continued)

Compound	ng 2.0 ml Added	ng/2.0 ml Detected A	B	C	D	Average[a]	Standard[b] Deviation	Coefficient[c] of Variation	Percent[d] Inaccuracy
DNB	5,010	5,110	5,030	5,160	5,090	5,100	± 54	1	+2
	2,500	2,610	2,550	2,640	2,610	2,600	± 38	1	+4
	1,000	920	930	950	940	940	± 13	1	-6
	500	450	460	460	450	460	± 6	1	-8
	250	300	290	280	270	280	± 13	5	+12
	100	100	100	90	100	100	± 5	5	0
	0	ND	ND	ND	ND	-	-	-	-
Linear Regression DNB, y = 1.023x - 14.8; Correlation Coefficient, 0.9996									
2,4-DNT	5,050	5,060	5,170	5,050	5,030	5,080	± 63	1	+1
	2,520	2,630	2,570	2,640	2,650	2,620	± 36	1	+5
	1,010	960	970	970	940	960	± 14	1	-5
	505	460	470	470	450	460	± 10	2	-9
	252	300	290	280	270	280	± 13	5	+11
	101	100	100	100	90	100	± 5	5	-1
	0	ND	ND	ND	ND	-	-	-	-
Linear Regression 2,4-DNT, y = 1.012x - 6.4; Correlation Coefficient, 0.9996									
TNT	4,970	5,050	5,030	5,040	4,980	5,020	± 31	1	+1
	2,480	2,510	2,510	2,530	2,570	2,530	± 28	1	+2
	994	920	930	890	920	920	± 17	2	-7
	497	440	440	460	450	450	± 10	2	-9
	248	280	270	270	280	270	± 6	2	+9
	99	100	90	110	100	100	± 8	8	+1
	0	ND	ND	ND	ND	-	-	-	-
Linear Regression TNT, y = 1.015x - 19.1; Correlation Coefficient, 0.9997									

TABLE 1 (concluded)

Compound	ng / 2.0 ml Added	ng/2.0 ml Detected A	B	C	D	Average[a]	Standard Deviation[b]	Coefficient of Variation[c]	Percent Inaccuracy[d]
Tetryl	5,090	5,090	5,160	5,120	5,020	5,100	± 59	1	+1
	2,540	2,620	2,660	2,690	2,550	2,630	± 61	2	+4
	1,020	960	970	960	940	960	± 13	1	-6
	509	450	470	480	450	460	± 15	3	-10
	254	300	290	280	270	280	± 13	5	+10
	102	100	100	100	100	100	0	0	-2
	0	0	0	0	0	0	-	-	-
Linear Regression Tetryl, y = 1.008x - 8.2; Correlation Coefficient, 0.9995									
DPA	5,000	4,660	5,070	5,170	5,140	5,010	± 237	5	+1
	2,500	2,540	2,530	2,580	2,520	2,540	± 26	1	+2
	1,000	930	910	900	890	910	± 17	2	-9
	500	420	470	480	450	460	± 26	6	-8
	250	300	310	270	290	290	± 17	6	+16
	100	90	100	100	100	100	± 5	5	0
	0	ND	ND	ND	ND	-	-	-	-
Linear Regression DPA; y = 1.006x - 13.9; Correlation Coefficient, 0.9986									

a Average = $\Sigma x/n = \bar{x}$

b Standard deviation = $\left(n\Sigma x^2 - (\Sigma x)^2/n(n-1)\right)^{\frac{1}{2}} = \sigma$

c Coefficient of Variation = $\sigma/\bar{x} \times 100$

d Percent Inaccuracy = $\frac{\bar{x} - \text{ng added}}{\text{ng added}} \times 100$

e ND - Not detectable, less than 20 ng/2.0 ml.

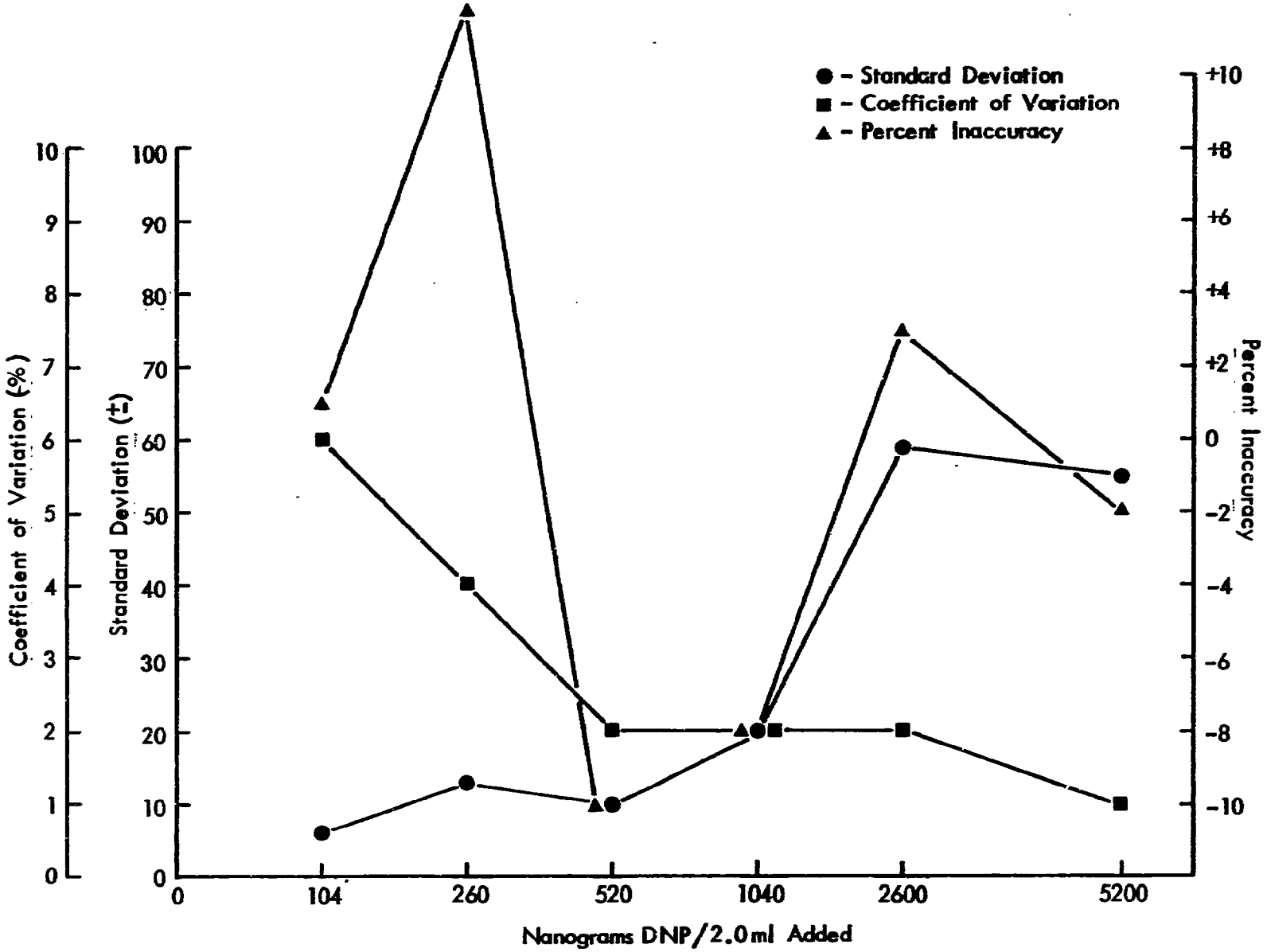


Figure 2 - Standard Deviation, Coefficient of Variation, and Percent Inaccuracy for DNP in Reference Solutions

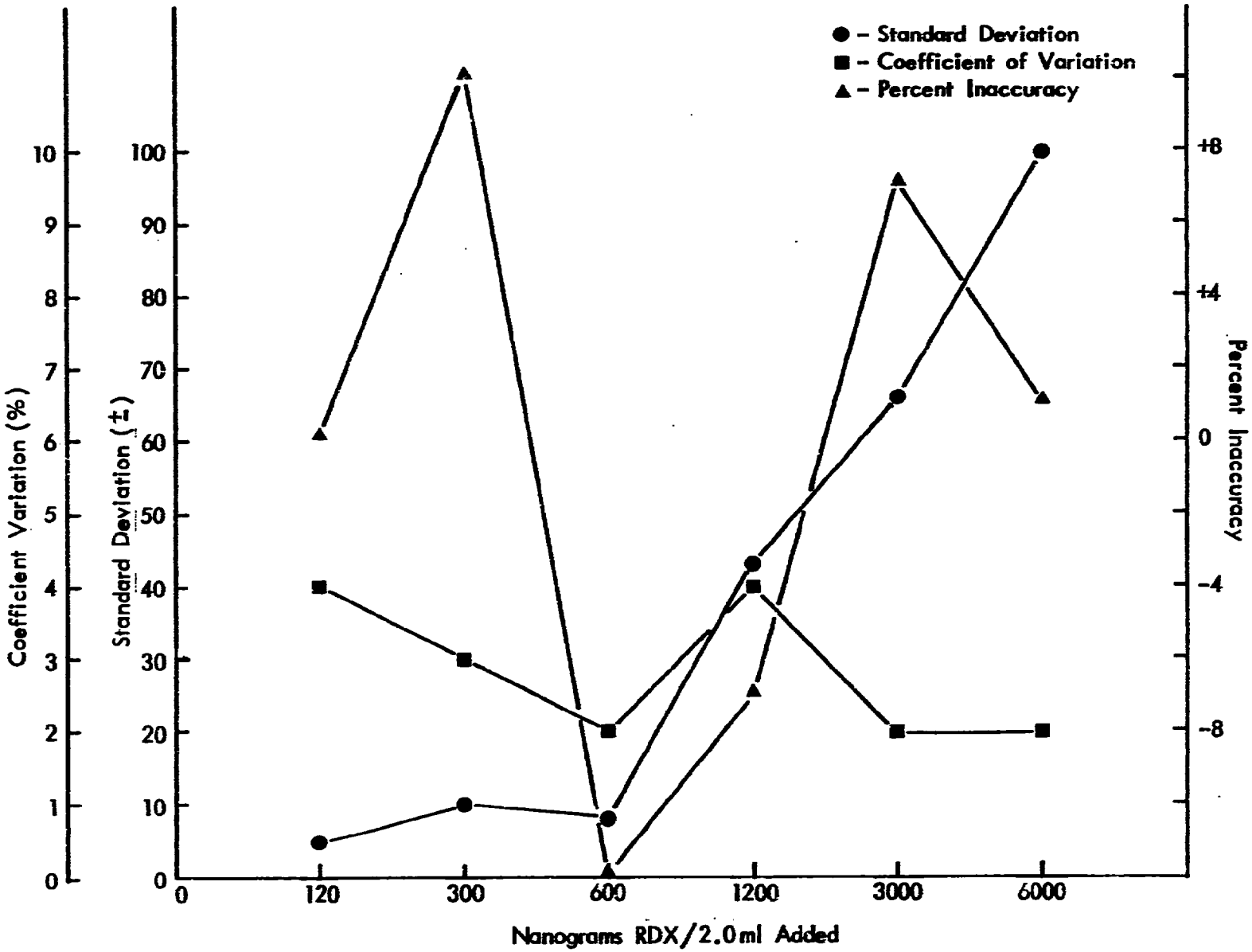


Figure 3 - Standard Deviation, Coefficient of Variation, and Percent Inaccuracy for RDX in Reference Solutions

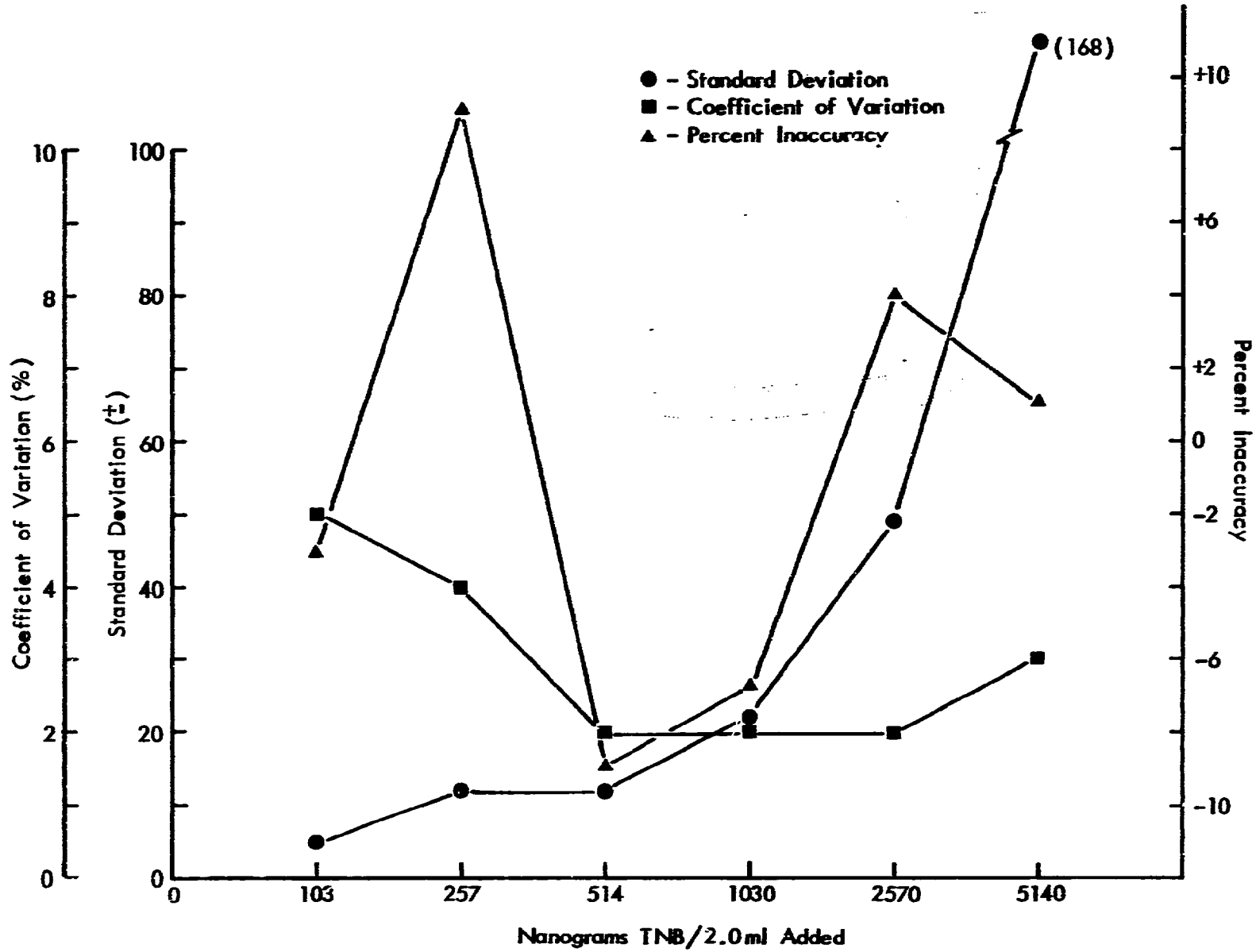


Figure 4 - Standard Deviation, Coefficient of Variation, and Percent Inaccuracy for TNB in Reference Solutions

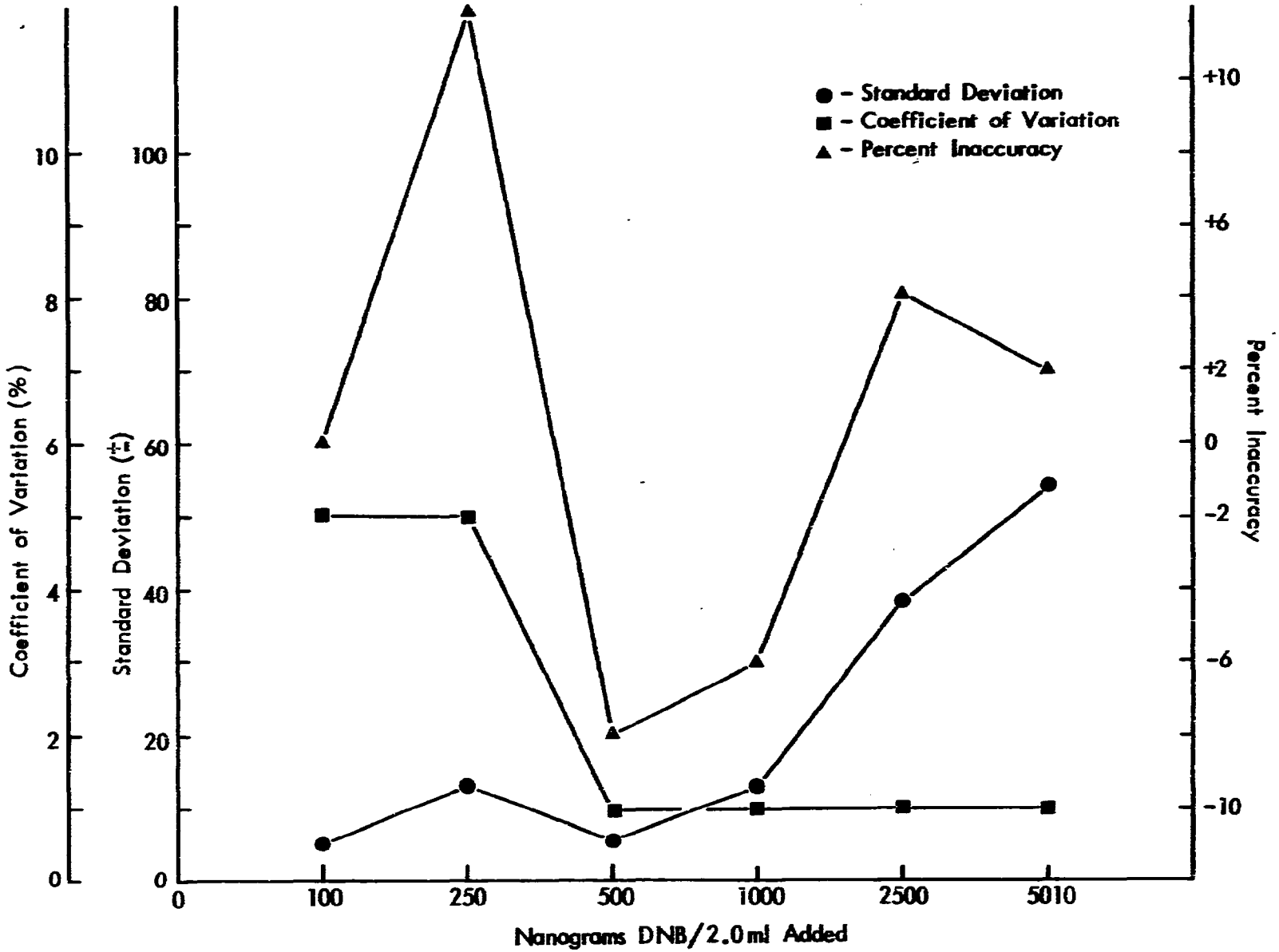


Figure 5 - Standard Deviation, Coefficient of Variation, and Percent Inaccuracy for DNB in Reference Solutions

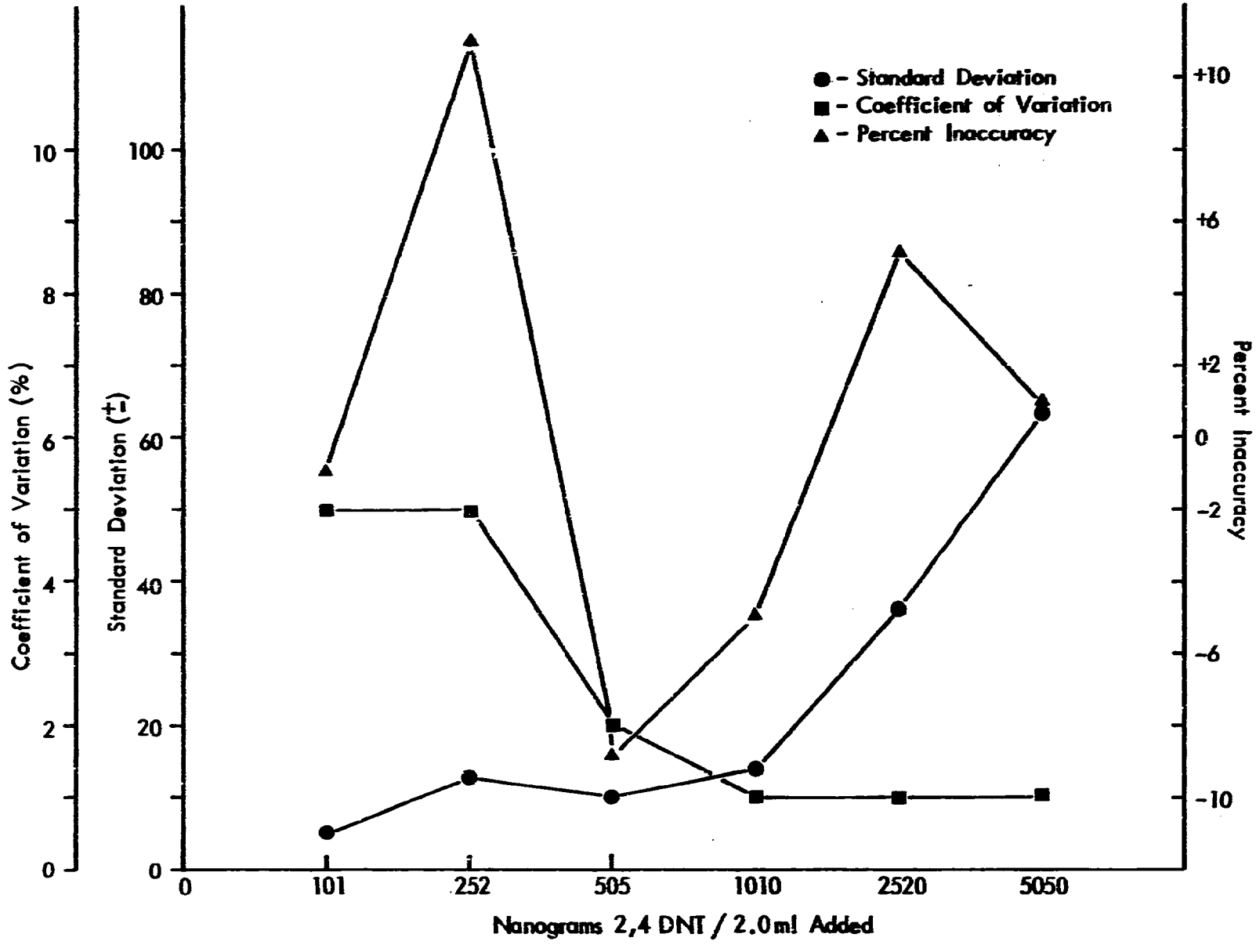


Figure 6 - Standard Deviation, Coefficient of Variation, and Percent Inaccuracy for 2,4-DNT in Reference Solutions

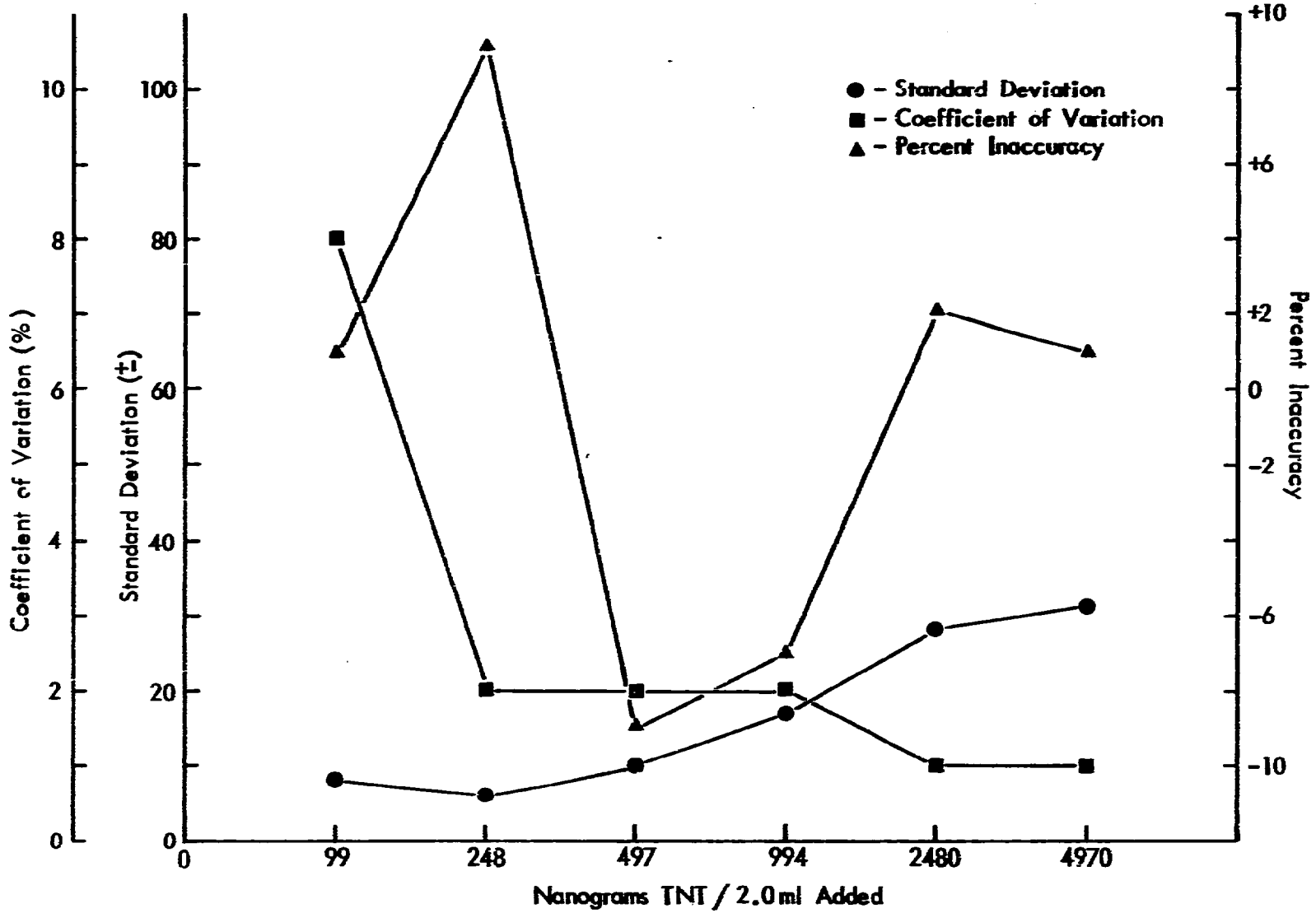


Figure 7 – Standard Deviation, Coefficient of Variation, and Percent Inaccuracy for TNT in Reference Solutions

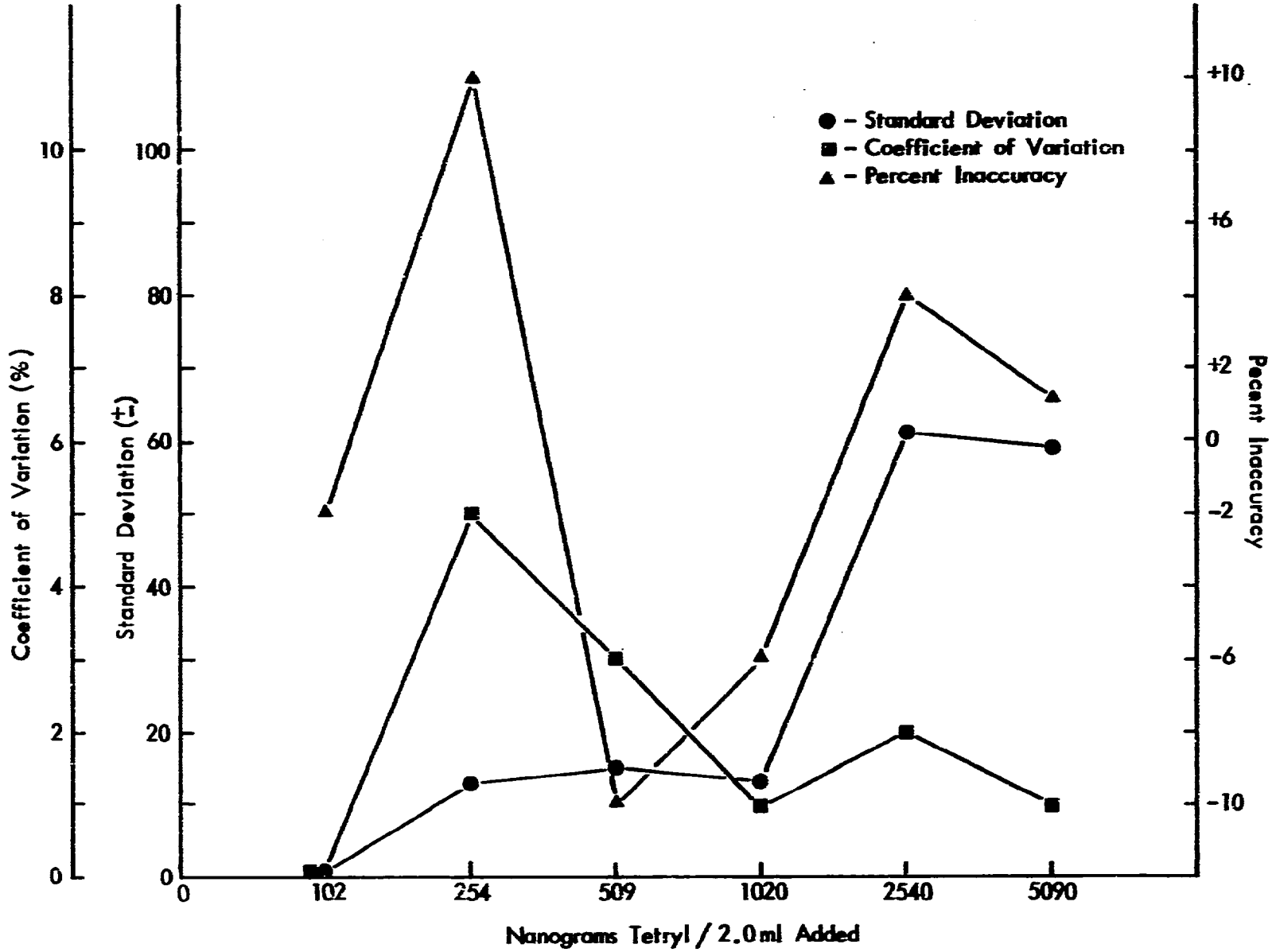


Figure 8 - Standard Deviation, Coefficient of Variation, and Percent Inaccuracy for Tetryl in Reference Solutions

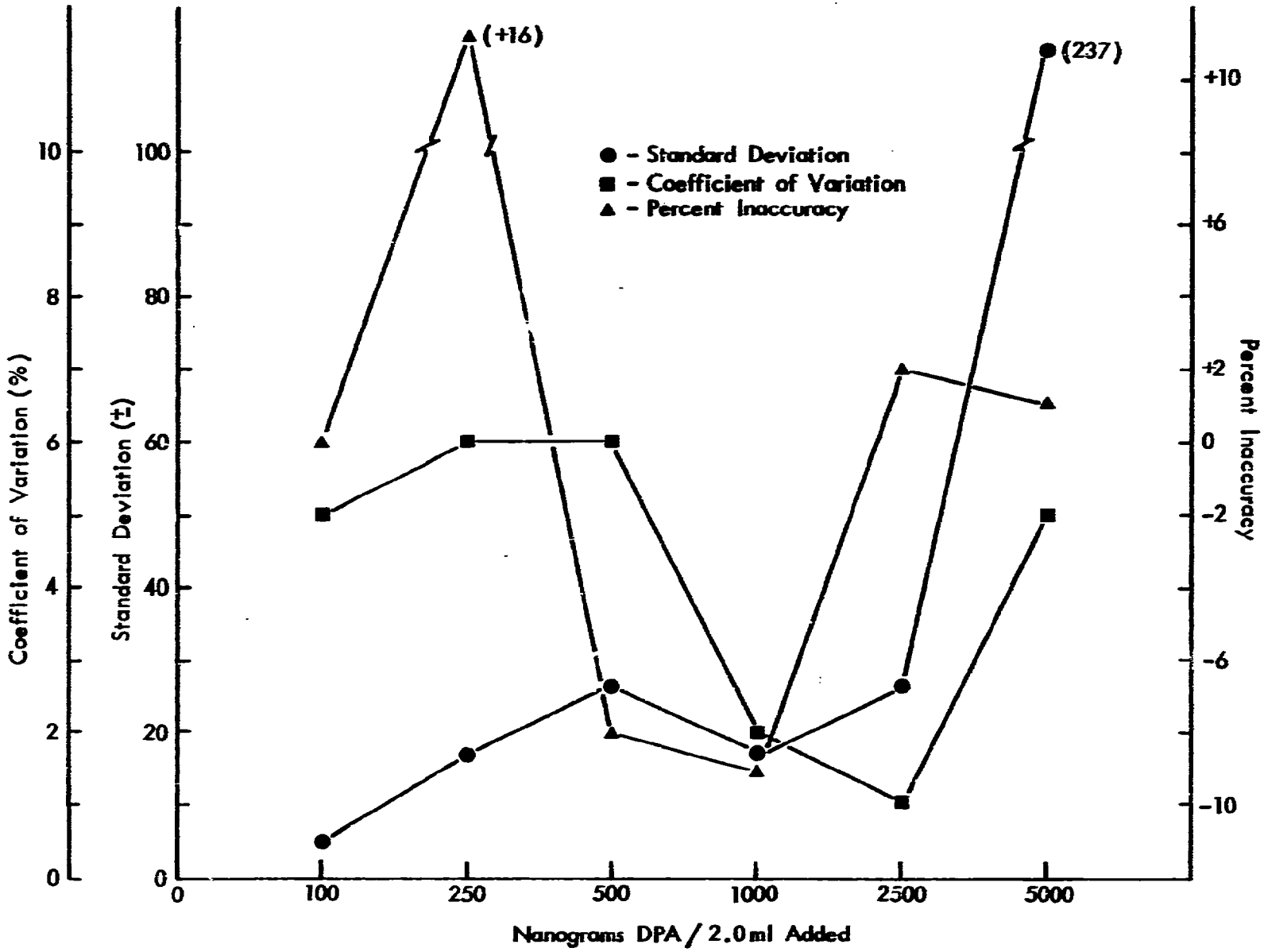


Figure 9 - Standard Deviation, Coefficient of Variation, and Percent Inaccuracy for DPA in Reference Solutions

TABLE 2

STATISTICAL EVALUATION OF DNP IN ANALYTICAL TECHNIQUE SARM REFERENCE SOLUTIONS BY THE HUBAUX AND VOS DETECTION LIMIT PROGRAM

Number of Data Points[a]	Linear Regression	Correlation Coefficient	Degrees of Freedom	t[b]	y-Intercept[c]	Detection Limit[d]
28	y = 0.985x + 2.0	0.9993	26	1.706	64	125

ng/2.0 ml DNP Added	Average[e] ng/2.0 ml Found	Standard[f] Deviation	Percent[g] Imprecision	Percent[h] Inaccuracy
0	ND[i]	-	-	-
104	100	± 3.3	3.2	+1.0
260	290	± 7.5	2.5	+13.5
520	470	± 5.5	1.2	-9.1
1,040	950	± 11.5	1.2	-7.7
2,600	2,640	± 34.1	1.3	+3.0
5,200	5,010	± 31.7	0.6	-2.3

a Number of data points - data points utilized in calculation of the linear regresion equation and detection limits = 28, all data.

b t - 2-tail p level (usually 0.1, each confidence band is 0.05 so total p = 0.1).

c y-intercept - intercept on y-axis of upper confidence limit line.

d Detection limit - x-intercept of y-intercept and lower confidence limit line.

e Average ng/ml found - average at each of the seven levels determined from linear regression equation for each of the four found concentrations within each level.

f Standard deviation - determined from average value (e above) and observed values.

g Percent imprecision - standard deviation divided by average value times 100%.

h Percent inaccuracy - determined from the average values of the seven observed values at each level.

$$\% \text{ Inaccuracy} = \frac{\text{Average observed values - level added}}{\text{level added}} \times 100$$

i ND - not detectable, less than 20 ng/2.0 ml.

TABLE 3

STATISTICAL EVALUATION OF RDX IN ANALYTICAL TECHNIQUE SARM REFERENCE SOLUTIONS BY THE HUBAUX AND VOS DETECTION LIMIT PROGRAM

Number of[a] Data Points	Linear Regression	Correlation Coefficient	Degrees of Freedom	t[b]	y-Intercept[c]	Detection[d] Limit
28	y = 1.019x - 11.5	0.9990	26	1.706	79	178

ng/2.0 ml RDX Added	Average[e] ng/2.0 ml Found	Standard[f] Deviation	Percent[g] Imprecision	Percent[h] Inaccuracy
0	ND[i]	-	-	-
120	110	± 2.9	2.5	-2.1
300	320	± 5.5	1.7	+9.2
600	530	± 4.7	0.9	-11.7
1,200	1,130	± 24.9	2.2	-6.7
3,000	3,260	± 38.2	1.2	+7.2
6,000	6,140	± 57.8	1.0	+0.6

a Number of data points - data points utilized in calculation of the linear regresion equation and detection limits = 28, all data.

b t - 2-tail p level (usually 0.1, each confidence band is 0.05 so total p = 0.1).

c y-intercept - intercept on y-axis of upper confidence limit line.

d Detection limit - x-intercept of y-intercept and lower confidence limit line.

e Average ng/ml found - average at each of the seven levels determined from linear regression equation for each of the four found concentrations within each level.

f Standard deviation - determined from average value (e above) and observed values.

g Percent imprecision - standard deviation divided by average value times 100%.

h Percent inaccuracy - determined from the average values of the seven observed values at each level.

$$\% \text{ Inaccuracy} = \frac{\text{Average observed values - level added}}{\text{level added}} \times 100$$

i ND - not detectable, less than 20 ng/2.0 ml.

TABLE 4

STATISTICAL EVALUATION OF TNB IN ANALYTICAL TECHNIQUE SARM REFERENCE SOLUTIONS BY THE HUBAUX AND VOS DETECTION LIMIT PROGRAM

Number of Data Points[a]	Linear Regression	Correlation Coefficient	Degrees of Freedom	t[b]	y-Intercept[c]	Detection Limit[d]
28	y = 1.020x - 17.5	0.9992	26	1.706	52	136

ng/2.0 ml TNB Added	Average[e] ng/2.0 ml Found	Standard[f] Deviation	Percent[g] Imprecision	Percent[h] Inaccuracy
0	ND[i]	-	-	-
103	90	± 2.9	2.8	-0.5
257	270	± 6.7	2.4	+8.9
514	460	± 6.7	1.4	-8.6
1,030	960	± 12.8	1.3	-7.0
2,570	2,690	± 28.0	1.1	+3.4
5,140	5,300	± 96.9	1.9	+1.4

a Number of data points - data points utilized in calculation of the linear regresion equation and detection limits = 28, all data.
b t - 2-tail p level (usually 0.1, each confidence band is 0.05 so total p = 0.1).
c y-intercept - intercept on y-axis of upper confidence limit line.
d Detection limit - x-intercept of y-intercept and lower confidence limit line.
e Average ng/ml found - average at each of the seven levels determined from linear regression equation for each of the four found concentrations within each level.
f Standard deviation - determined from average value (e above) and observed values.
g Percent imprecision - standard deviation divided by average value times 100%.
h Percent inaccuracy - determined from the average values of the seven observed values at each level.

$$\% \text{ Inaccuracy} = \frac{\text{Average observed values - level added}}{\text{level added}} \times 100$$

i ND - not detectable, less than 20 ng/2.0 ml.

TABLE 5

STATISTICAL EVALUATION OF DNB IN ANALYTICAL TECHNIQUE SARM REFERENCE SOLUTIONS BY THE HUBAUX AND VOS DETECTION LIMIT PROGRAM

Number of Data Points[a]	Linear Regression	Correlation Coefficient	Degrees of Freedom	t[b]	y-Intercept[c]	Detection Limit[d]
28	y = 1.023x - 14.8	0.9996	26	1.706	33	93

ng/2.0 ml DNB Added	Average[e] ng/2.0 ml Found	Standard[f] Deviation	Percent[g] Imprecision	Percent[h] Inaccuracy
0	ND[i]	-	-	-
100	80	± 2.9	3.0	-2.5
250	280	± 7.5	2.6	+14.0
500	450	± 3.3	0.7	-9.0
1,000	940	± 7.5	0.8	-6.5
2,500	2,650	± 21.8	0.8	+4.1
5,010	5,200	± 31.0	0.6	+1.7

a Number of data points - data points utilized in calculation of the linear regresion equation and detection limits = 28, all data.

b t - 2-tail p level (usually 0.1, each confidence band is 0.05 so total p = 0.1).

c y-intercept - intercept on y-axis of upper confidence limit line.

d Detection limit - x-intercept of y-intercept and lower confidence limit line.

e Average ng/ml found - average at each of the seven levels determined from linear regression equation for each of the four found concentrations within each level.

f Standard deviation - determined from average value (e above) and observed values.

g Percent imprecision - standard deviation divided by average value times 100%.

h Percent inaccuracy - determined from the average values of the seven observed values at each level.

$$\% \text{ Inaccuracy} = \frac{\text{Average observed values - level added}}{\text{level added}} \times 100$$

i ND - not detectable, less than 20 ng/2.0 ml.

TABLE 6

STATISTICAL EVALUATION OF 2,4-DNT IN ANALYTICAL TECHNIQUE SARM REFERENCE SOLUTIONS BY THE HUBAUX AND VOS DETECTION LIMIT PROGRAM

Number of Data Points[a]	Linear Regression	Correlation Coefficient	Degrees of Freedom	t[b]	y-Intercept[c]	Detection Limit[d]
28	y = 1.012x - 6.4	0.9996	26	1.706	43	97

ng/2.0 ml 2,4-DNT Added	Average[e] ng/2.0 ml Found	Standard[f] Deviation	Percent[g] Imprecision	Percent[h] Inaccuracy
0	ND[i]	-	-	-
101	90	± 2.9	3.0	-3.5
252	280	± 7.5	2.6	+13.1
505	460	± 5.5	1.2	-8.4
1,010	960	± 8.2	0.9	-5.0
2,520	2,650	± 20.7	0.8	+4.1
5,050	5,130	± 36.3	0.7	+0.5

a Number of data points - data points utilized in calculation of the linear regresion equation and detection limits = 28, all data.
b t - 2-tail p level (usually 0.1, each confidence band is 0.05 so total p = 0.1).
c y-intercept - intercept on y-axis of upper confidence limit line.
d Detection limit - x-intercept of y-intercept and lower confidence limit line.
e Average ng/ml found - average at each of the seven levels determined from linear regression equation for each of the four found concentrations within each level.
f Standard deviation - determined from average value (e above) and observed values.
g Percent imprecision - standard deviation divided by average value times 100%.
h Percent inaccuracy - determined from the average values of the seven observed values at each level.

$$\% \text{ Inaccuracy} = \frac{\text{Average observed values - level added}}{\text{level added}} \times 100$$

i ND - not detectable, less than 20 ng/2.0 ml.

TABLE 7

STATISTICAL EVALUATION OF TNT IN ANALYTICAL TECHNIQUE SARM REFERENCE SOLUTIONS BY THE HUBAUX AND VOS DETECTION LIMIT PROGRAM

Number of[a] Data Points	Linear Regression	Correlation Coefficient	Degrees of Freedom	t[b]	y-Intercept[c]	Detection[d] Limit
28	y = 1.015x - 19.1	0.9997	26	1.706	22	81

ng/2.0 ml TNT Added	Average[e] ng/2.0 ml Found	Standard[f] Deviation	Percent[g] Imprecision	Percent[h] Inaccuracy
0	ND[i]	-	-	-
99	80	± 4.7	4.7	+1.0
248	260	± 3.3	1.2	+10.9
497	440	± 5.5	1.2	-10.0
994	910	± 10.0	1.1	-7.9
2,480	2,550	± 16.3	0.6	+2.0
4,970	5,080	± 18.0	0.4	+1.1

a Number of data points - data points utilized in calculation of the linear regresion equation and detection limits = 28, all data.

b t - 2-tail p level (usually 0.1, each confidence band is 0.05 so total p = 0.1).

c y-intercept - intercept on y-axis of upper confidence limit line.

d Detection limit - x-intercept of y-intercept and lower confidence limit line.

e Average ng/ml found - average at each of the seven levels determined from linear regression equation for each of the four found concentrations within each level.

f Standard deviation - determined from average value (e above) and observed values.

g Percent imprecision - standard deviation divided by average value times 100%.

h Percent inaccuracy - determined from the average values of the seven observed values at each level.

$$\% \text{ Inaccuracy} = \frac{\text{Average observed values - level added}}{\text{level added}} \times 100$$

i ND - not detectable, less than 20 ng/2.0 ml.

TABLE 8

STATISTICAL EVALUATION OF TETRYL IN ANALYTICAL TECHNIQUE SARM REFERENCE SOLUTIONS BY THE HUBAUX AND VOS DETECTION LIMIT PROGRAM

Number of Data Points[a]	Linear Regression	Correlation Coefficient	Degrees of Freedom	t[b]	y-Intercept[c]	Detection Limit[d]
28	y = 1.008x - 8.2	0.9995	26	1.706	44	103

ng/2.0 ml Tetryl Added	Average[e] ng/2.0 ml Found	Standard[f] Deviation	Percent[g] Imprecision	Percent[h] Inaccuracy
0	ND[i]	-	-	-
102	90	0	0	-2.0
254	280	± 7.5	2.6	+12.2
509	460	± 8.7	1.9	-9.1
1,020	960	± 7.3	0.8	-6.1
2,540	2,640	± 35.0	1.3	+3.5
5,090	5,130	± 34.1	0.7	+0.1

a Number of data points - data points utilized in calculation of the linear regresion equation and detection limits = 28, all data.
b t - 2-tail p level (usually 0.1, each confidence band is 0.05 so total p = 0.1).
c y-intercept - intercept on y-axis of upper confidence limit line.
d Detection limit - x-intercept of y-intercept and lower confidence limit line.
e Average ng/ml found - average at each of the seven levels determined from linear regression equation for each of the four found concentrations within each level.
f Standard deviation - determined from average value (e above) and observed values.
g Percent imprecision - standard deviation divided by average value times 100%.
h Percent inaccuracy - determined from the average values of the seven observed values at each level.

$$\% \text{ Inaccuracy} = \frac{\text{Average observed values - level added}}{\text{level added}} \times 100$$

i ND - not detectable, less than 20 ng/2.0 ml.

TABLE 9

STATISTICAL EVALUATION OF DPA IN ANALYTICAL TECHNIQUE SARM REFERENCE SOLUTIONS
BY THE HUBAUX AND VOS DETECTION LIMIT PROGRAM

Number of[a] Data Points	Linear Regression	Correlation Coefficient	Degrees of Freedom	t[b]	y-Intercept[c]	Detection[d] Limit
28	y = 1.006x - 13.9	0.9986	26	1.706	75	176

ng/2.0 ml DPA Added	Average[e] ng/2.0 ml Found	Standard[f] Deviation	Percent[g] Imprecision	Percent[h] Inaccuracy
0	ND[i]	-	-	-
100	80	± 2.9	3.0	-2.5
250	280	± 9.9	3.4	+17.0
500	440	± 15.3	3.4	-9.0
1,000	900	± 9.9	1.1	-9.2
2,500	2,540	± 15.2	0.6	+1.7
5,000	5,030	± 136.9	2.7	+0.2

a Number of data points - data points utilized calculation of the linear regresion equation and detection limits = 28, all data.
b t - 2-tail p level (usually 0.1, each confidence band is 0.05 so total p = 0.1).
c y-intercept - intercept on y-axis of upper confidence limit line.
d Detection limit - x-intercept of y-intercept and lower confidence limit line.
e Average ng/ml found - average at each of the seven levels determined from linear regression equation for each of the four found concentrations within each level.
f Standard deviation - determined from average value (e above) and observed values.
g Percent imprecision - standard deviation divided by average value times 100%.
h Percent inaccuracy - determined from the average values of the seven observed values at each level.

$$\% \text{ Inaccuracy} = \frac{\text{Average observed values - level added}}{\text{level added}} \times 100$$

i ND - not detectable, less than 20 ng/2.0 ml.

TABLE 10

LINEARITY AND PRECISION OF SARM DNP BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

Reference Solution No.	ng/2.0 ml[a] DNP Added	Peak Height (mm)[b] DNP	Peak Height (mm)[b] IS	ng/2.0 ml[c] IS	RWR[d]	Calculated ng[e] 2.0 ml
A-1	5,200	104.2	39.0	2,220	1.14	5,160
A-2	2,600	130.1	95.1	2,220	1.17	2,640
A-3	1,040	99.7	103.4	1,110	1.03	930
A-4	520	106.0	110.9	555	1.02	460
A-5	260	65.5	41.0	222	1.36	310
A-6	104	53.8	46.9	111	1.22	110
A-7	0	< 2	46.5	111	-	< 20
B-1	5,200	122.8	47.1	2,220	1.11	5,030
B-2	2,600	128.3	94.0	2,220	1.17	2,630
B-3	1,040	102.0	101.3	1,110	1.07	970
B-4	520	115.4	116.5	555	1.06	480
B-5	260	62.9	40.6	222	1.32	300
B-6	104	57.0	47.9	111	1.27	110
B-7	0	< 2	44.2	111	-	< 20
C-1	5,200	125.0	47.6	2,220	1.12	5,070
C-2	2,600	148.5	107.0	2,220	1.19	2,680
C-3	1,040	134.8	134.0	1,110	1.07	970
C-4	520	123.2	124.5	555	1.06	480
C-5	260	70.3	46.9	222	1.28	290
C-6	104	46.0	46.1	111	1.07	100
C-7	0	< 2	47.0	111	-	< 20
D-1	5,200	121.0	46.1	2,220	1.12	5,070
D-2	2,600	143.0	100.0	2,220	1.22	2,760
D-3	1,040	152.3	151.9	1,110	1.07	970
D-4	520	114.0	118.2	555	1.03	470
D-5	260	57.3	39.0	222	1.25	280
D-6	104	43.1	39.5	111	1.16	100
D-7	0	< 2	46.0	111	-	< 20
				Average	1.15	
				Standard Deviation	± 0.10	
				Relative Standard Deviation	8.6%	

a ng/2.0 ml Added - nanograms of DNP added to reference standard having a total volume of 2.0 ml.

b Peak Height - measured height of DNP and IS in millimeters.

c ng/2.0 ml IS - nanograms of IS present in the 2.0 ml reference solution.

d RWR - relative weight response.

$$RWR = \frac{\text{Peak Height DNP}}{\text{Peak Height IS}} \times \frac{\text{ng/2.0 ml IS}}{\text{ng/2.0 ml DNP}}$$

e Calculated ng/2.0 ml - level of DNP calculated to be in the reference solution using the average RWR of all solutions.

$$\text{Calculated ng/2.0 ml} = \frac{\text{Peak Height DNP}}{\text{Peak Height IS}} \times \frac{\text{ng/2.0 ml IS}}{\text{Avg. RWR}}$$

TABLE 11

LINEARITY AND PRECISION OF SARM RDX BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

Reference Solution No.	ng/2.0 ml[a] RDX Added	Peak Height (mm)[b] RDX	Peak Height (mm)[b] IS	ng/2.0 ml[c] IS	RWR[d]	Calculated ng[e] 2.0 ml
A-1	6,000	66.1	39.0	2,220	0.63	6,170
A-2	3,000	84.2	95.1	2,220	0.66	3,220
A-3	1,200	60.0	103.4	1,110	0.54	1,060
A-4	600	64.2	110.9	555	0.54	530
A-5	300	38.2	41.0	222	0.69	340
A-6	120	30.0	46.9	111	0.59	120
A-7	0	< 2	46.5	111	-	< 20
B-1	6,000	77.1	47.1	2,220	0.61	5,960
B-2	3,000	81.2	94.0	2,220	0.64	3,140
B-3	1,200	62.2	101.3	1,110	0.57	1,120
B-4	600	67.7	116.5	555	0.54	530
B-5	300	36.5	40.6	222	0.67	330
B-6	120	30.0	47.9	111	0.58	110
B-7	0	< 2	44.2	111	-	< 20
C-1	6,000	78.0	47.6	2,220	0.61	5,960
C-2	3,000	94.0	107.0	2,220	0.65	3,200
C-3	1,200	85.3	134.0	1,110	0.59	1,160
C-4	600	74.0	124.5	555	0.55	540
C-5	300	41.7	46.9	222	0.66	320
C-6	120	31.2	46.1	111	0.63	120
C-7	0	< 2	47.0	111	-	< 20
D-1	6,000	76.8	46.1	2,220	0.62	6,060
D-2	3,000	90.8	100.0	2,220	0.67	3,300
D-3	1,200	95.0	151.9	1,110	0.59	1,140
D-4	600	67.8	118.2	555	0.53	520
D-5	300	34.2	39.0	222	0.65	320
D-6	120	26.4	39.5	111	0.62	120
D-7	0	< 2	46.0	111	-	< 20
				Average	0.61	
				Standard Deviation	± 0.05	
				Relative Standard Deviation	7.8%	

a ng/2.0 ml Added - nanograms of RDX added to reference standard having a total volume of 2.0 ml.

b Peak Height - measured height of RDX and IS in millimeters.

c ng/2.0 ml IS - nanograms of IS present in the 2.0 ml reference solution.

d RWR - relative weight response.

$$RWR = \frac{\text{Peak Height RDX}}{\text{Peak Height IS}} \times \frac{\text{ng/2.0 ml IS}}{\text{ng/2.0 ml RDX}}$$

e Calculated ng/2.0 ml - level of RDX calculated to be in the reference solution using the average RWR of all solutions.

$$\text{Calculated ng/2.0 ml} = \frac{\text{Peak Height RDX}}{\text{Peak Height IS}} \times \frac{\text{ng/2.0 ml IS}}{\text{Avg. RWR}}$$

TABLE 12

LINEARITY AND PRECISION OF SARM TNB BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

Reference Solution No.	ng/2.0 ml[a] TNB Added	Peak Height (mm)[b] TNB	Peak Height (mm)[b] IS	ng/2.0 ml[c] IS	RWR[d]	Calculated ng[e] 2.0 ml
A-1	5,140	113.7	39.0	2,220	1.26	5,390
A-2	2,570	140.0	95.1	2,220	1.27	2,720
A-3	1,030	104.5	103.4	1,110	1.09	930
A-4	514	111.0	110.9	555	1.08	460
A-5	257	64.4	41.0	222	1.36	290
A-6	103	54.0	46.9	111	1.24	110
A-7	0	< 2	46.5	111	-	< 20
B-1	5,140	134.7	47.1	2,220	1.24	5,290
B-2	2,570	132.5	94.0	2,220	1.22	2,610
B-3	1,030	105.8	101.3	1,110	1.13	970
B-4	514	121.0	116.5	555	1.12	480
B-5	257	64.9	40.6	222	1.38	290
B-6	103	50.0	47.9	111	1.12	100
B-7	0	< 2	44.2	111	-	< 20
C-1	5,140	133.0	47.6	2,220	1.21	5,170
C-2	2,570	154.3	107.0	2,220	1.25	2,670
C-3	1,030	142.0	134.0	1,110	1.14	980
C-4	514	128.0	124.5	555	1.11	480
C-5	257	69.0	46.9	222	1.27	270
C-6	103	49.2	46.1	111	1.15	100
C-7	0	< 2	47.0	111	-	< 20
D-1	5,140	124.5	46.1	2,220	1.17	5,000
D-2	2,570	142.2	100.0	2,220	1.23	2,630
D-3	1,030	156.7	151.9	1,110	1.11	950
D-4	514	118.2	118.2	555	1.08	460
D-5	257	58.1	39.0	222	1.29	270
D-6	103	44.1	39.5	111	1.20	100
D-7	0	< 2	46.0	111	-	< 20
				Average	1.20	
				Standard Deviation	± 0.08	
				Relative Standard Deviation	7.0%	

a ng/2.0 ml Added - nanograms of TNB added to reference standard having a total volume of 2.0 ml.

b Peak Height - measured height of TNB and IS in millimeters.

c ng/2.0 ml IS - nanograms of IS present in the 2.0 ml reference solution.

d RWR - relative weight response.

$$RWR = \frac{\text{Peak Height TNB}}{\text{Peak Height IS}} \times \frac{\text{ng/2.0 ml IS}}{\text{ng/2.0 ml TNB}}$$

e Calculated ng/2.0 ml - level of TNB calculated to be in the reference solution using the average RWR of all solutions.

$$\text{Calculated ng/2.0 ml} = \frac{\text{Peak Height TNB}}{\text{Peak Height IS}} \times \frac{\text{ng/2.0 ml IS}}{\text{Avg. RWR}}$$

TABLE 13

LINEARITY AND PRECISION OF SARM DNB BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

Reference Solution No.	ng/2.0 ml[a] DNB Added	Peak Height (mm)[b] DNB	Peak Height (mm)[b] IS	ng/2.0 ml[c] IS	RWR[d]	Calculated ng[e] 2.0 ml
A-1	5,010	159.0	39.0	2,220	1.81	5,110
A-2	2,500	198.0	95.1	2,220	1.85	2,610
A-3	1,000	152.0	103.4	1,110	1.63	920
A-4	500	159.7	110.9	555	1.60	450
A-5	250	98.8	41.0	222	2.14	300
A-6	100	77.0	46.9	111	1.82	100
A-7	0	< 2	46.5	111	-	< 20
B-1	5,010	189.0	47.1	2,220	1.78	5,030
B-2	2,500	191.4	94.0	2,220	1.81	2,550
B-3	1,000	151.0	101.3	1,110	1.65	930
B-4	500	170.5	116.5	555	1.62	460
B-5	250	93.3	40.6	222	2.04	290
B-6	100	76.5	47.9	111	1.77	100
B-7	0	< 2	44.2	111	-	< 20
C-1	5,010	195.8	47.6	2,220	1.82	5,160
C-2	2,500	224.9	107.0	2,220	1.87	2,640
C-3	1,000	204.0	134.0	1,110	1.69	950
C-4	500	182.0	124.5	555	1.62	460
C-5	250	104.2	46.9	222	1.97	280
C-6	100	66.1	46.1	111	1.59	90
C-7	0	< 2	47.0	111	-	< 20
D-1	5,010	187.2	46.1	2,220	1.80	5,090
D-2	2,500	208.0	100.0	2,220	1.85	2,610
D-3	1,000	227.3	151.9	1,110	1.66	940
D-4	500	169.7	118.2	555	1.59	450
D-5	250	82.9	39.0	222	1.89	270
D-6	100	60.0	39.5	111	1.69	100
D-7	0	< 2	46.0	111	-	< 20
				Average	1.77	
				Standard Deviation	± 0.15	
				Relative Standard Deviation	8.3%	

a ng/2.0 ml Added - nanograms of DNB added to reference standard having a total volume of 2.0 ml.

b Peak Height - measured height of DNB and IS in millimeters.

c ng/2.0 ml IS - nanograms of IS present in the 2.0 ml reference solution.

d RWR - relative weight response.

$$RWR = \frac{\text{Peak Height DNB}}{\text{Peak Height IS}} \times \frac{\text{ng/2.0 ml IS}}{\text{ng/2.0 ml DNB}}$$

e Calculated ng/2.0 ml - level of DNB calculated to be in the reference solution using the average RWR of all solutions.

$$\text{Calculated ng/2.0 ml} = \frac{\text{Peak Height DNB}}{\text{Peak Height IS}} \times \frac{\text{ng/2.0 ml IS}}{\text{Avg. RWR}}$$

TABLE 14

LINEARITY AND PRECISION OF SARM 2,4-DNT BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

Reference Solution No.	ng/2.0 ml[a] 2,4-DNT Added	Peak Height (mm)[b] 2,4-DNT	Peak Height (mm)[b] IS	ng/2.0 ml[c] IS	RWR[d]	Calculated ng[e] 2.0 ml
A-1	5,050	119.2	39.0	2,220	1.34	5,060
A-2	2,520	151.0	95.1	2,220	1.40	2,630
A-3	1,010	120.4	103.4	1,110	1.28	960
A-4	505	123.1	110.9	555	1.22	460
A-5	252	73.3	41.0	222	1.57	300
A-6	101	54.9	46.9	111	1.29	100
A-7	0	< 2	46.5	111	-	< 20
B-1	5,050	147.0	47.1	2,220	1.37	5,170
B-2	2,520	145.9	94.0	2,220	1.37	2,570
B-3	1,010	118.9	101.3	1,110	1.29	970
B-4	505	130.9	116.5	555	1.23	470
B-5	252	72.2	40.6	222	1.57	290
B-6	101	60.3	47.9	111	1.38	100
B-7	0	< 2	44.2	111	-	< 20
C-1	5,050	145.0	47.6	2,220	1.34	5,050
C-2	2,520	170.3	107.0	2,220	1.40	2,640
C-3	1,010	157.6	134.0	1,110	1.29	970
C-4	505	140.9	124.5	555	1.24	470
C-5	252	79.0	46.9	222	1.48	280
C-6	101	57.0	46.1	111	1.36	100
C-7	0	< 2	47.0	111	-	< 20
D-1	5,050	140.0	46.1	2,220	1.34	5,030
D-2	2,520	160.3	100.0	2,220	1.41	2,650
D-3	1,010	172.0	151.9	1,110	1.24	940
D-4	505	127.8	118.2	555	1.19	450
D-5	252	63.5	39.0	222	1.43	270
D-6	101	43.2	39.5	111	1.20	90
D-7	0	< 2	46.0	111	-	< 20
				Average	1.34	
				Standard Deviation	± 0.10	
				Relative Standard Deviation	7.8%	

a ng/2.0 ml Added - nanograms of 2,4-DNT added to reference standard having a total volume of 2.0 ml.

b Peak Height - measured height of 2,4-DNT and IS in millimeters.

c ng/2.0 ml IS - nanograms of IS present in the 2.0 ml reference solution.

d RWR - relative weight response.

$$RWR = \frac{\text{Peak Height 2,4-DNT}}{\text{Peak Height IS}} \times \frac{\text{ng/2.0 ml IS}}{\text{ng/2.0 ml 2,4-DNT}}$$

e Calculated ng/2.0 ml - level of 2,4-DNT calculated to be in the reference solution using the average RWR of all solutions.

$$\text{Calculated ng/2.0 ml} = \frac{\text{Peak Height 2,4-DNT}}{\text{Peak Height IS}} \times \frac{\text{ng/2.0 ml IS}}{\text{Avg. RWR}}$$

TABLE 15

LINEARITY AND PRECISION OF SARM TNT BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

Reference Solution No.	ng/2.0 ml[a] TNT Added	Peak Height (mm)[b] TNT	Peak Height (mm)[b] IS	ng/2.0 ml[c] IS	RWR[d]	Calculated ng[e] 2.0 ml
A-1	4,970	92.3	39.0	2,220	1.06	5,050
A-2	2,480	112.0	95.1	2,220	1.05	2,510
A-3	994	89.0	103.4	1,110	0.96	920
A-4	497	91.1	110.9	555	0.92	440
A-5	248	54.2	41.0	222	1.18	280
A-6	99	44.9	46.9	111	1.07	100
A-7	0	< 2	46.5	111	-	< 20
B-1	4,970	110.9	47.1	2,220	1.05	5,030
B-2	2,480	110.4	94.0	2,220	1.05	2,510
B-3	994	88.0	101.3	1,110	0.97	930
B-4	497	96.7	116.5	555	0.93	440
B-5	248	51.9	40.6	222	1.14	270
B-6	99	42.0	47.9	111	0.98	90
B-7	0	< 2	44.2	111	-	< 20
C-1	4,970	112.4	47.6	2,220	1.05	5,040
C-2	2,480	126.8	107.0	2,220	1.06	2,530
C-3	994	111.9	134.0	1,110	0.93	890
C-4	497	108.0	124.5	555	0.97	460
C-5	248	59.2	46.9	222	1.13	270
C-6	99	45.8	46.1	111	1.11	110
C-7	0	< 2	47.0	111	-	< 20
D-1	4,970	107.5	46.1	2,220	1.04	4,980
D-2	2,480	120.3	100.0	2,220	1.08	2,570
D-3	994	131.0	151.9	1,110	0.96	920
D-4	497	100.2	118.2	555	0.95	450
D-5	248	51.0	39.0	222	1.17	280
D-6	99	35.6	39.5	111	1.01	100
D-7	0	< 2	46.0	111	-	< 20
				Average	1.04	
				Standard Deviation	± 0.08	
				Relative Standard Deviation	7.5%	

a ng/2.0 ml Added - nanograms of TNT added to reference standard having a total volume of 2.0 ml.

b Peak Height - measured height of TNT and IS in millimeters.

c ng/2.0 ml IS - nanograms of IS present in the 2.0 ml reference solution.

d RWR - relative weight response.

$$RWR = \frac{\text{Peak Height TNT}}{\text{Peak Height IS}} \times \frac{\text{ng/2.0 ml IS}}{\text{ng/2.0 ml TNT}}$$

e Calculated ng/2.0 ml - level of TNT calculated to be in the reference solution using the average RWR of all solutions.

$$\text{Calculated ng/2.0 ml} = \frac{\text{Peak Height TNT}}{\text{Peak Height IS}} \times \frac{\text{ng/2.0 ml IS}}{\text{Avg. RWR}}$$

TABLE 16

LINEARITY AND PRECISION OF SARM TETRYL BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

Reference Solution No.	ng/2.0 ml[a] Tetryl Added	Peak Height (mm)[b] Tetryl	Peak Height (mm)[b] IS	ng/2.0 ml[c] IS	RWR[d]	Calculated ng[e] 2.0 ml
A-1	5,090	77.8	39.0	2,220	0.87	5,090
A-2	2,540	97.7	95.1	2,220	0.90	2,620
A-3	1,020	77.9	103.4	1,110	0.82	960
A-4	509	78.3	110.9	555	0.77	450
A-5	254	49.0	41.0	222	1.04	300
A-6	102	38.6	46.9	111	0.90	100
A-7	0	< 2	46.5	111	-	< 20
B-1	5,090	95.2	47.1	2,220	0.88	5,160
B-2	2,540	98.0	94.0	2,220	0.91	2,660
B-3	1,020	77.2	101.3	1,110	0.83	970
B-4	509	85.3	116.5	555	0.80	470
B-5	254	46.0	40.6	222	0.99	290
B-6	102	37.0	47.9	111	0.84	100
B-7	0	< 2	44.2	111	-	< 20
C-1	5,090	95.5	47.6	2,220	0.88	5,120
C-2	2,540	112.9	107.0	2,220	0.92	2,690
C-3	1,020	101.2	134.0	1,110	0.82	960
C-4	509	93.0	124.5	555	0.81	480
C-5	254	52.3	46.9	222	0.97	280
C-6	102	37.0	46.1	111	0.87	100
C-7	0	< 2	47.0	111	-	< 20
D-1	5,090	90.7	46.1	2,220	0.86	5,020
D-2	2,540	100.0	100.0	2,220	0.87	2,550
D-3	1,020	111.9	151.9	1,110	0.80	940
D-4	509	83.2	118.2	555	0.77	450
D-5	254	41.2	39.0	222	0.92	270
D-6	102	30.5	39.5	111	0.84	100
D-7	0	< 2	46.0	111	-	< 20
				Average	0.87	
				Standard Deviation	± 0.07	
				Relative Standard Deviation	7.8%	

a ng/2.0 ml Added - nanograms of tetryl added to reference standard having a total volume of 2.0 ml.

b Peak Height - measured height of tetryl and IS in millimeters.

c ng/2.0 ml IS - nanograms of IS present in the 2.0 ml reference solution.

d RWR - relative weight response.

$$RWR = \frac{\text{Peak Height tetryl}}{\text{Peak Height IS}} \times \frac{\text{ng/2.0 ml IS}}{\text{ng/2.0 ml tetryl}}$$

e Calculated ng/2.0 ml - level of tetryl calculated to be in the reference solution using the average RWR of all solutions.

$$\text{Calculated ng/2.0 ml} = \frac{\text{Peak Height tetryl}}{\text{Peak Height IS}} \times \frac{\text{ng/2.0 ml IS}}{\text{Avg. RWR}}$$

TABLE 17

LINEARITY AND PRECISION OF SARM DPA BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

Reference Solution No.	ng/2.0 ml[a] DPA Added	Peak Height (mm)[b] DPA	Peak Height (mm)[b] IS	ng/2.0 ml[c] IS	RWR[d]	Calculated ng[e] 2.0 ml
A-1	5,000	36.8	39.0	2,220	0.42	4,660
A-2	2,500	49.0	95.1	2,220	0.46	2,540
A-3	1,000	39.0	103.4	1,110	0.42	930
A-4	500	38.2	110.9	555	0.38	420
A-5	250	24.7	41.0	222	0.53	300
A-6	100	18.0	46.9	111	0.43	90
A-7	0	< 2	46.5	111	-	< 20
B-1	5,000	48.4	47.1	2,220	0.46	5,070
B-2	2,500	48.2	94.0	2,220	0.46	2,530
B-3	1,000	37.5	101.3	1,110	0.41	910
B-4	500	44.6	116.5	555	0.42	470
B-5	250	25.2	40.6	222	0.55	310
B-6	100	19.0	47.9	111	0.44	100
B-7	0	< 2	44.2	111	-	< 20
C-1	5,000	49.9	47.6	2,220	0.47	5,170
C-2	2,500	55.9	107.0	2,220	0.46	2,580
C-3	1,000	49.0	134.0	1,110	0.41	900
C-4	500	48.0	124.5	555	0.43	480
C-5	250	25.8	46.9	222	0.49	270
C-6	100	19.5	46.1	111	0.47	100
C-7	0	< 2	47.0	111	-	< 20
D-1	5,000	48.0	46.1	2,220	0.46	5,140
D-2	2,500	51.0	100.0	2,220	0.45	2,520
D-3	1,000	54.8	151.9	1,110	0.40	890
D-4	500	43.2	118.2	555	0.41	450
D-5	250	23.0	39.0	222	0.52	290
D-6	100	16.5	39.5	111	0.46	100
D-7	0	< 2	46.0	111	-	< 20
				Average	0.45	
				Standard Deviation	± 0.04	
				Relative Standard Deviation	9.4%	

a ng/2.0 ml Added - nanograms of DPA added to reference standard having a total volume of 2.0 ml.

b Peak Height - measured height of DPA and IS in millimeters.

c ng/2.0 ml IS - nanograms of IS present in the 2.0 ml reference solution.

d RWR - relative weight response.

$$RWR = \frac{\text{Peak Height DPA}}{\text{Peak Height IS}} \times \frac{\text{ng/2.0 ml IS}}{\text{ng/2.0 ml DPA}}$$

e Calculated ng/2.0 ml - level of DPA calculated to be in the reference solution using the average RWR of all solutions.

$$\text{Calculated ng/2.0 ml} = \frac{\text{Peak Height DPA}}{\text{Peak Height IS}} \times \frac{\text{ng/2.0 ml IS}}{\text{Avg. RWR}}$$

APPENDIX B

PRECISION AND ACCURACY ASSESSMENT OF THE HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC ANALYTICAL TECHNIQUE FOR THE DETERMINATION OF NITROBENZENE (NB), 2,6-DINITROTOLUENE (2,6-DNT), NITROGLYCERIN (NG), AND PICRIC ACID (PA)

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1. Application

The developed analytical technique is for the quantitative determination of NB, 2,6-DNT, NG, and PA using high performance liquid chromatography (HPLC) with ultraviolet (UV) detection at 230 nm. The technique can be employed to analyze samples, i.e., water, that have been properly prepared.

a. Evaluated Concentration Range: The concentration range of NB, 2,6-DNT, and PA studied in reference solutions was 0, 100, 250, 500, 1,000, 2,500, and 5,000 ng/2.0 ml, and of NG, 0, 3, 7.5, 15, 30, 75, and 150 µg/2.0 ml. The higher levels evaluated for NG were required since this compound has a UV molar absorptivity approximately 30 times less than the other munition compounds. The concentration ranges correspond to a series of 0, 0.2X, 0.5X, X, 2X, 5X, and 10X, where X is 5 µg/liter (parts per billion, ppb) for NB, 2,6-DNT, and PA, and 150 µg/liter for NG and a 100 ml water sample is utilized.

b. Sensitivity: A signal-to-noise ratio of 3 to 1 for NB (peak height (PH) = 17 mm), 5 to 1 for 2,6-DNT (PH = 27 mm), 5 to 1 for NG (PH = 27 mm), and 4 to 1 for PA (PH = 22 mm) was obtained with an injection of 100 µl of a 100 ng NB, 2,6-DNT, and PA and 3 µg NG per 2.0 ml reference solution (ca. 5 ng NB, 2,6-DNT, and PA, and 150 ng NG on column.

c. Detection Limits: The detection limits of the analytical technique for reference solutions using the Hubaux and Vos detection limit program were 100 ng/2.0 ml for NB, 100 ng/2.0 ml for 2,6-DNT, 3,200 ng/2.0 ml for NG, and 178 ng/2.0 ml for PA. These detection limits for reference solutions correspond to the expected detection limits for a 100-ml water sample and a final extract volume of 2.0 ml. The corresponding detection limits for a 100-ml water sample expressed in µg/liter (ppb) would be 2 µg/liter for NB, 2 µg/liter for 2,6-DNT, 64 µg/liter for NG, and 3.56 µg/liter for PA.

d. Interferences: No interfering chromatographic peaks were observed on the HPLC chromatograms of reference solutions.

2. Chemistry

NB, 2,6-DNT, NG, and PA are munition-related compounds manufactured at various installations. The assessment of potential environmental contamination by these compounds in water requires knowledge that the level of the compounds present at the time of sampling does not change prior to analysis and that the sampling technique provides a representative sample. The evaluation of the preservation and sampling parameters to be employed requires an analytical technique capable of assaying the compounds with sufficient precision, accuracy, and sensitivity to provide quantitative data. NG has an ultraviolet (UV) wavelength maxima (λ max) at 230 nm and a molar absorptivity (ε max) substantially less than the other compounds. Thus, to obtain the highest possible sensitivity for NG and still have sufficient sensitivity to detect and quantitate NB, 2,6-DNT, and PA, a 230 nm UV detector is required. PA is a strong acid (pKa 0.38) and exists in an anionic form in aqueous media. Reverse phase HPLC cannot resolve ionic species, and to obtain a good chromatographic peak for PA requires coupling the anion with a cation (ion-pairing chromatography). Quaternary butyl ammonium hydroxide is the cation utilized for many ion-paired compounds.

3. Apparatus

a. Instrumentation: A Chem Research Series 2000 HPLC unit with a Rheodyne 7120 variable loop injector, a Tracor Model 970A variable wavelength UV-VIS detector, and a single pen Model SR-204 Heath-Schlumberger recorder were used. (Note: Equivalent instrumentation will provide similar results.)

b. HPLC Parameters:

1. Column: Spherisorb ODS, 5 μ, 250 x 4.6 mm ID.

2. Precolumn: Co:Pell ODS, 25 μ - 35 μ, 50 x 2 mm ID.

3. Eluent: 35/65 (V/V) acetonitrile/0.005M t-Butyl Ammonium Hydroxide, pH 6.5 (pH adjusted with 1N phosphoric acid).

4. Flow Rate: 1.0 ml/min.

5. Chart Speed: 0.1 in/min.

7. Internal Standard: Propiophenone.

8. Injection Volume: 40 to 100 µl of a 2.0 ml reference solution.

9. Retention Volumes:

Compound	Milliliters
NB	14.0
2,6-DNT	20.0
NG	23.0
PA	24.5
IS	16.0

Note: Slight changes in the retention indices may occur with fresh eluent or a change in precolumn or analytical column.

A representative HPLC-UV (230 nm) chromatogram for NB, 2,6-DNT, NG, PA, and the IS is shown in Figure 1.

c. Laboratory Glassware and Equipment:

1. Culture tubes (Pyrex) with Teflon-lined screw caps.

2. Volumetric flasks (100 ml).

3. Volumetric syringes (0-100, 0-250, 0-500, and 0-1,000 µl).

d. Chemicals:

1. NB, 2,6-DNT, NG, and PA SARMs, obtained from the U.S. Army Toxic and Hazardous Materials Agency.

2. Propiophenone, analytical grade.

3. t-Butyl ammonium hydroxide, HPLC grade.

4. Acetonitrile, "Distilled in Glass" grade: phosphoric acid, analytical grade.

5. High purity water from a Milli-Q water purification system.

4. Standards

a. Stock: Weigh approximately 20 mg of NB, 2,6-DNT, NG, and PA SARM or interim SARM into separate 100 ml volumetric flasks and dissolve in acetonitrile (concentration of each compound, 200 µg/ml). Quantitatively pipette 2.5 ml of the NB, 2,6-DNT, and PA stocks and 75 ml of the NG stock into a 100 ml volumetric flask and dilute to volume with acetonitrile (concentration of NB, 2,6-DNT, and PA, 5 µg/ml and of NG, 150 µg/ml): working stock No. 1. Quantitatively pipette 20 ml of working stock No. 1 into a 100 ml volumetric flask and dilute to volume with 15 ml acetonitrile and 65 ml high purity water (concentration of NB, 2,6-DNT, and PA, 1 µg/ml and of NG, 30 µg/ml): working stock No. 2.

b. Internal Standard Stock: Weigh approximately 20 mg propiophenone into a 100 ml volumetric flask and dilute to volume with acetonitrile (concentration, 200 µg/ml). Quantitatively pipette 10 ml of the stock to a 100 ml volumetric flask and dilute to volume with 35/65 (v/v) acetonitrile in water (concentration 20 µg/ml): working IS stock No. 1. Quantitatively pipette 10 ml working stock No. 1 to a 100 ml volumetric flask and dilute to volume with 35/65 (v/v) acetonitrile in water (concentration, 2 µg/ml).

c. Reference Solution Preparation: The working stock Nos. 1 and 2 and the working IS stock Nos. 1 and 2 were employed to prepare the reference solutions for precision and accuracy testing of the analytical technique as follows:

Working Stock No.	µl Working Stock	Working IS Stock No.	µl Working IS Stock	µl HPLC Eluent	Each Compound X ng/2.0 ml Compound	IS
1	1,000	1	100	900	10X	2,000
1	500	1	100	1,400	5X	2,000
2	1,000	1	100	900	2X	2,000
2	500	2	100	1,400	1X	200
2	250	2	100	1,650	0.5X	200
2	100	2	100	1,800	0.2X	200
-	0	2	100	1,900	0	200

The term "X" refers to 500 ng for NB, 2,6-DNT and PA, and 15,000 ng for NG. Each reference solution was prepared and analyzed on four separate days to define the linearity of the analytical technique.

5. Calculations

The reference solutions described in Section 4.c. were prepared and analyzed in quadruplicate. The relative weight response (RWR) (Equation 1) of each compound to the IS was calculated and average RWR for each compound utilized to calculate the nanograms of that compound in every reference solution (Equation 2). The nanograms found were plotted against the nanograms added and a linear regression analysis of the data performed. The slope, intercept, and correlation coefficient of each compound were determined. The data is summarized in Table 1 and include the average value at each level (Equation 3), the standard deviation (Equation 4), coefficient of variation (Equation 5), and percent inaccuracy (Equation 6). The raw data and calculations are given in Tables 6 through 9.

$$\text{RWR} = \frac{\text{Peak Height Cpd}}{\text{Peak Height IS}} \times \frac{\text{ng/2.0 ml IS}}{\text{ng/2.0 ml Cpd}} \qquad \text{(Eq. 1)}$$

$$\text{ng/2.0 ml cpd} = \frac{\text{Peak Height Cpd}}{\text{Peak Height IS}} \times \frac{\text{ng/2.0 ml IS}}{\text{Avg. RWR Cpd}} \qquad \text{(Eq. 2)}$$

$$\text{Average value} = \bar{x} = \Sigma x/n \qquad \text{(Eq. 3)}$$

$$\text{Standard deviation} = \sigma = \left(\frac{n\Sigma x^2 - (\Sigma x)^2}{n(n-1)}\right)^{\frac{1}{2}} \qquad \text{(Eq. 4)}$$

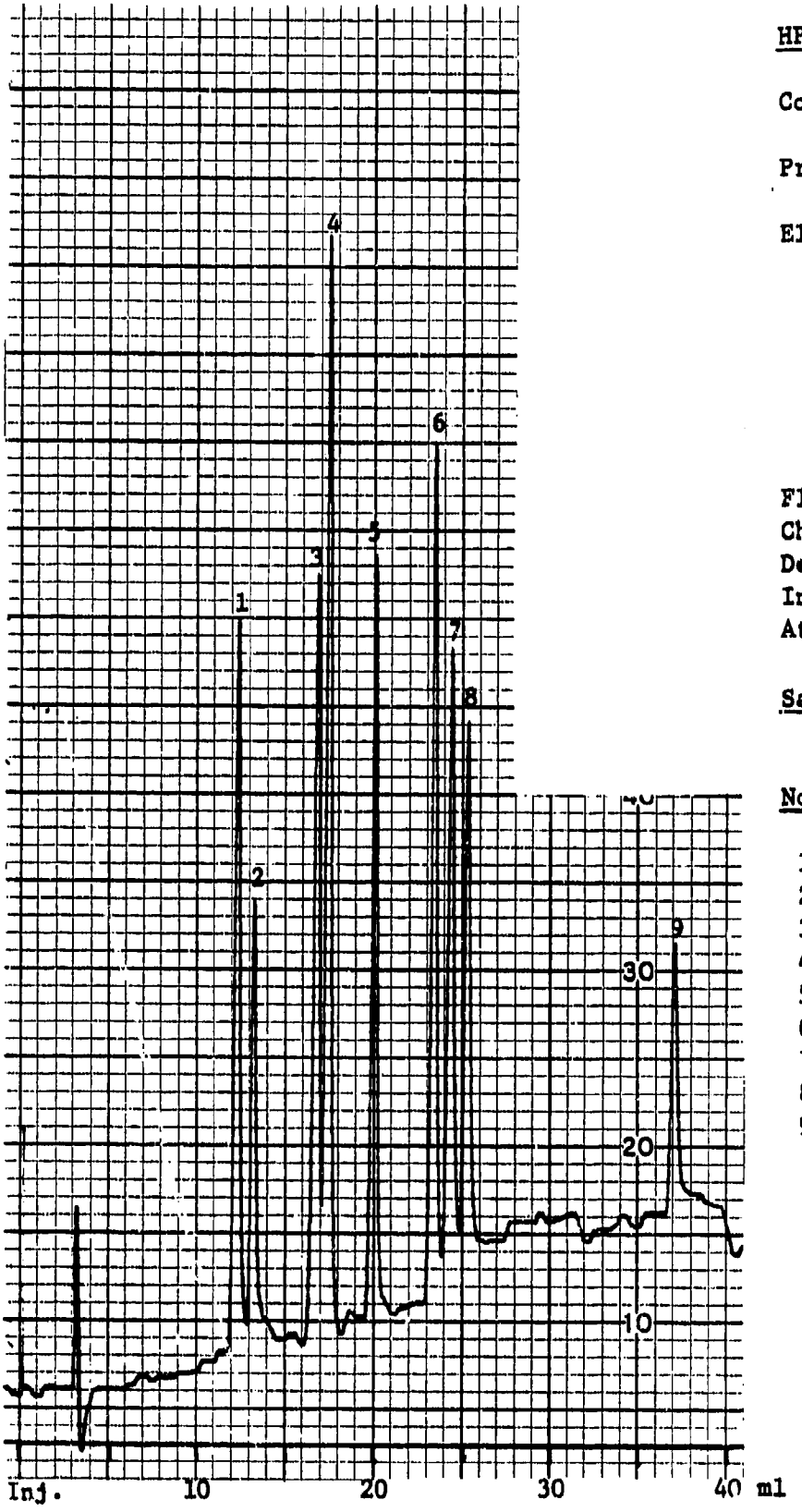
$$\text{Coefficient of variation} = \sigma/\bar{x} \times 100 \qquad \text{(Eq. 5)}$$

$$\text{Percent inaccuracy} = \frac{\bar{x} - \text{ng added}}{\text{ng added}} \times 100 \qquad \text{(Eq. 6)}$$

Graphic presentations of the standard deviation, coefficient of variation, and percent inaccuracy are given in Figures 2 through 5.

6. Statistical Evaluation Of Data

A statistical evaluation of the data obtained for the precision and accuracy evaluation of the analytical technique for the determination of NB, 2,6-DNT, NG, and PA was made using the Hubaux and Vos detection limit program provided by the U.S. Army Toxic and Hazardous Materials Agency. The results of these evaluations are given in Tables 2 through 5. Detection limits for each compound were as follows: NB, 100; 2,6-DNT, 100; NG, 3,200; and PA, 178 ng/2.0 ml. The average nanograms per 2.0 ml values found at each level for each compound (Tables 2-5) were determined from the linear regression equation for the 28 data points. The standard deviation and percent imprecision (coefficient of variation) at each level were calculated based on this average, and thus do not agree with the values given in Table 1.



HPLC Parameters

Column: Spherisorb ODS, 5 µ, 250 x 4.6 mm ID
Precolumn: CO:PELL ODS, 25 to 35 µ, 50 x 2 mm ID
Eluent: Gradient linear
Initial: 30/70 (v/v) CH_3CN/0.08 M acetic acid adjusted to pH 3.1 with NH_4OH
Final: 50/50 (v/v) CH_3CN/0.08 M acetic acid adjusted to pH 3.1 with NH_4OH
Time: 35 min
Flow Rate: 1.0 ml/min
Chart Speed: 0.1 in/min
Detector: UV, 254 nm
Injection Volume: 100 µl
Attenuation: 0.01X

Sample Characteristics

No.	Name	Level ng/2 ml
1	DNP	520
2	RDX	600
3	1,3,5-TNB	514
4	1,3-DNB	501
5	IS	555
6	2,4-DNT	505
7	TNT	497
8	tetryl	509
9	DPA	500

Figure 1 - HPLC-UV (254 nm) Separation of DNP, RDX, TNB, DNB, DNT, TNT, tetryl, and DPA at the 500 ng/2.0 ml Level

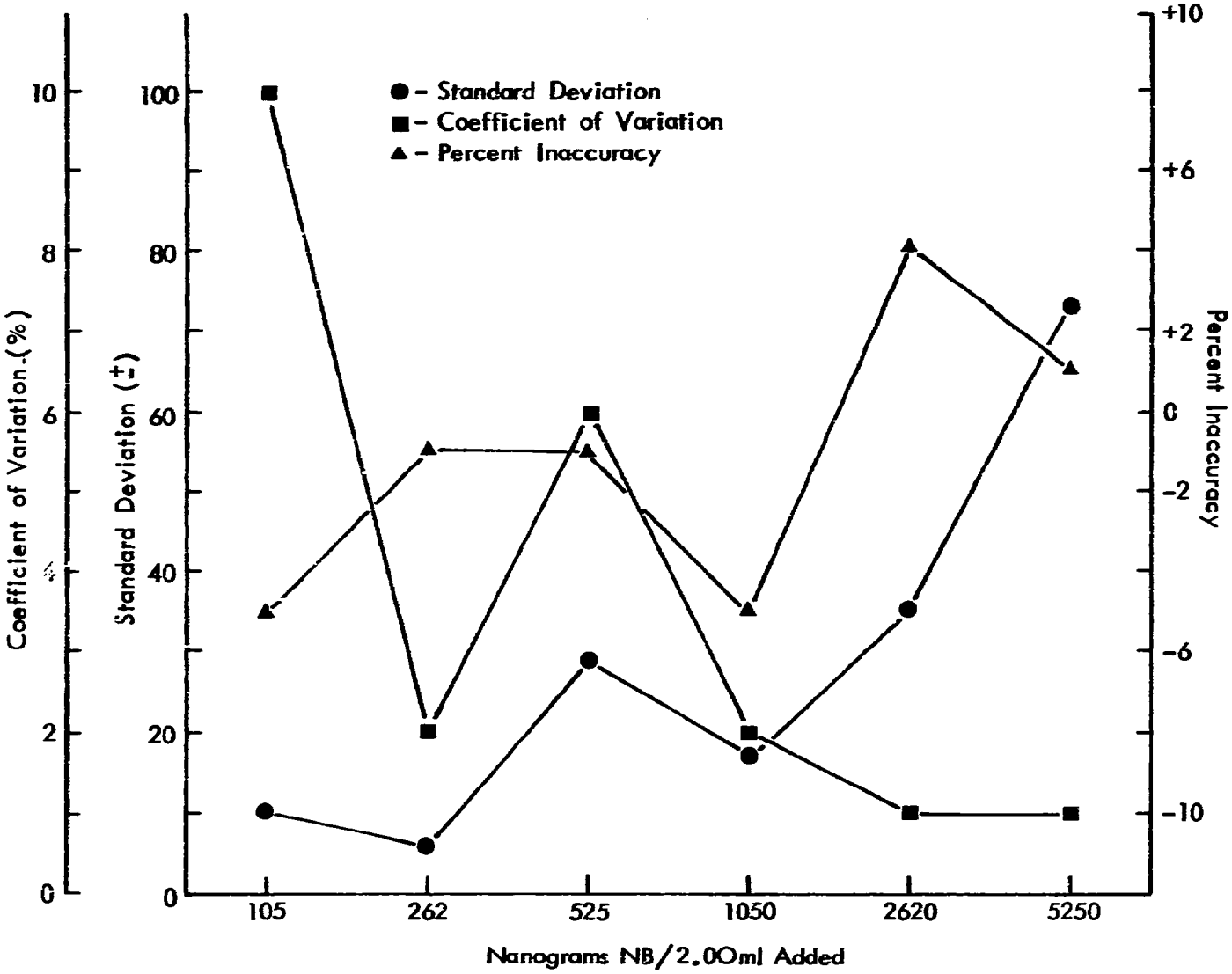


Figure 2 - Standard Deviation, Coefficient of Variation, and Percent Inaccuracy for NB in Reference Solutions

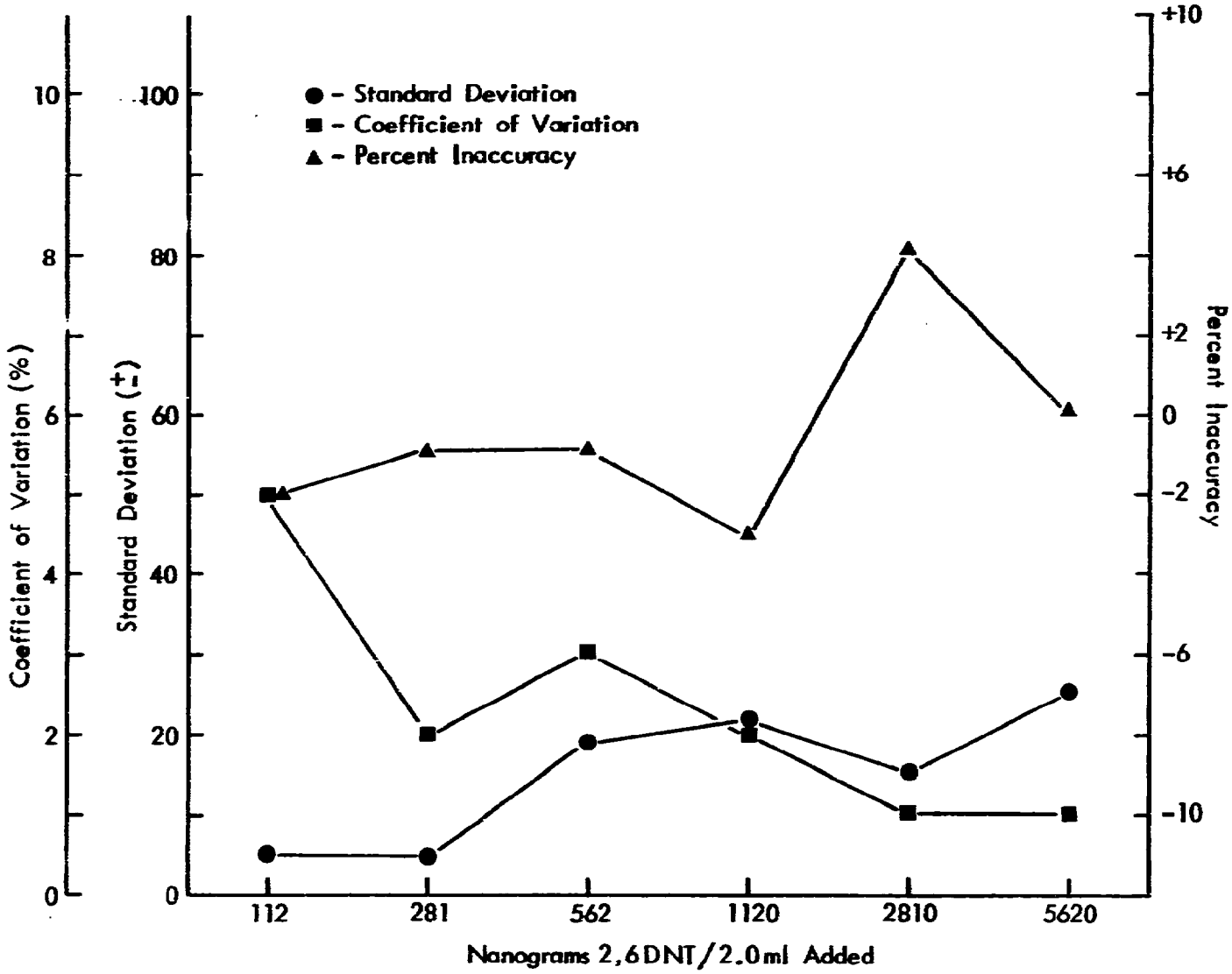


Figure 3 - Standard Deviation, Coefficient of Variation, and Percent Inaccuracy for 2,6-DNT in Reference Solutions

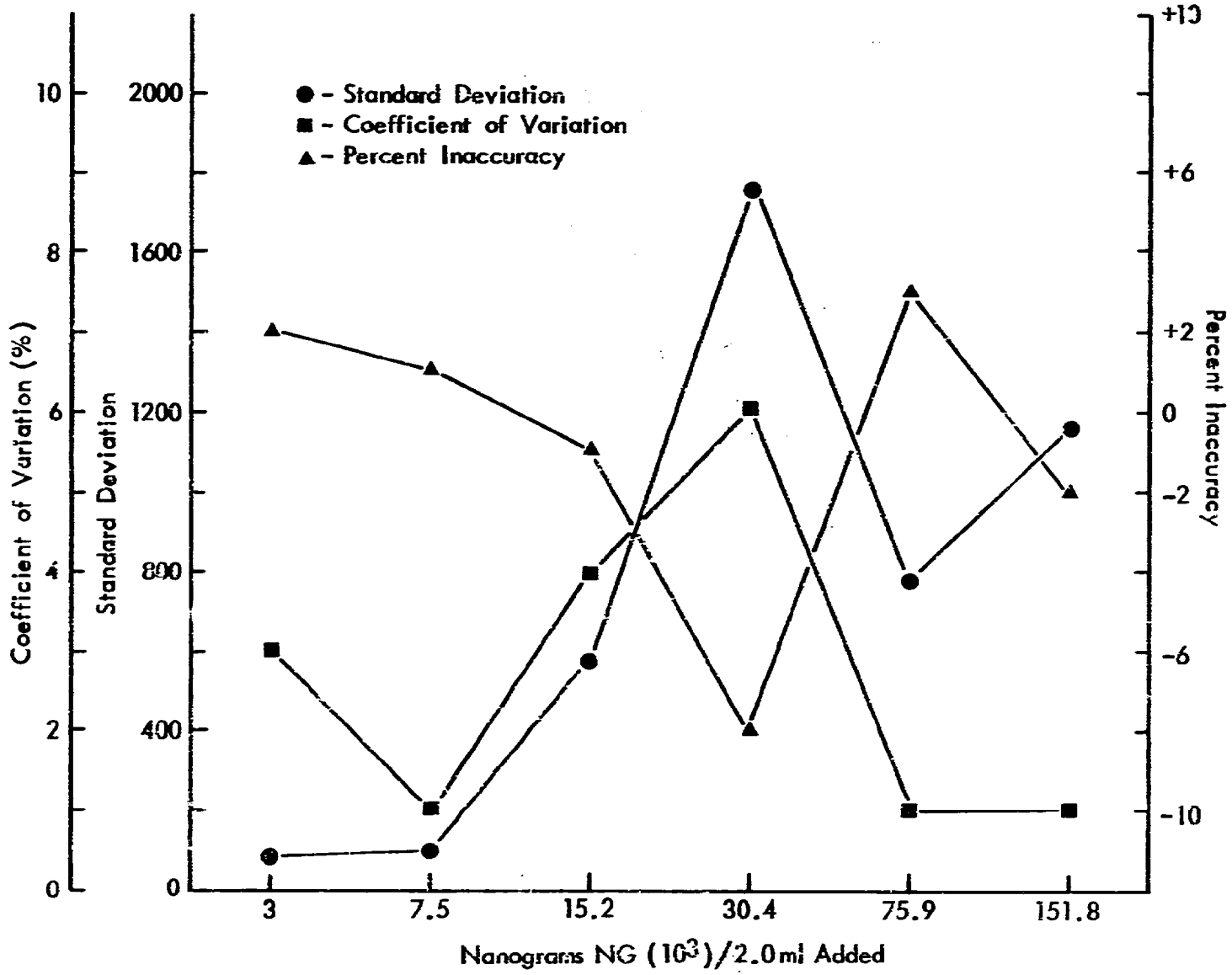


Figure 4 - Standard Deviation, Coefficient of Variation, and Percent Inaccuracy for NG in Reference Solutions

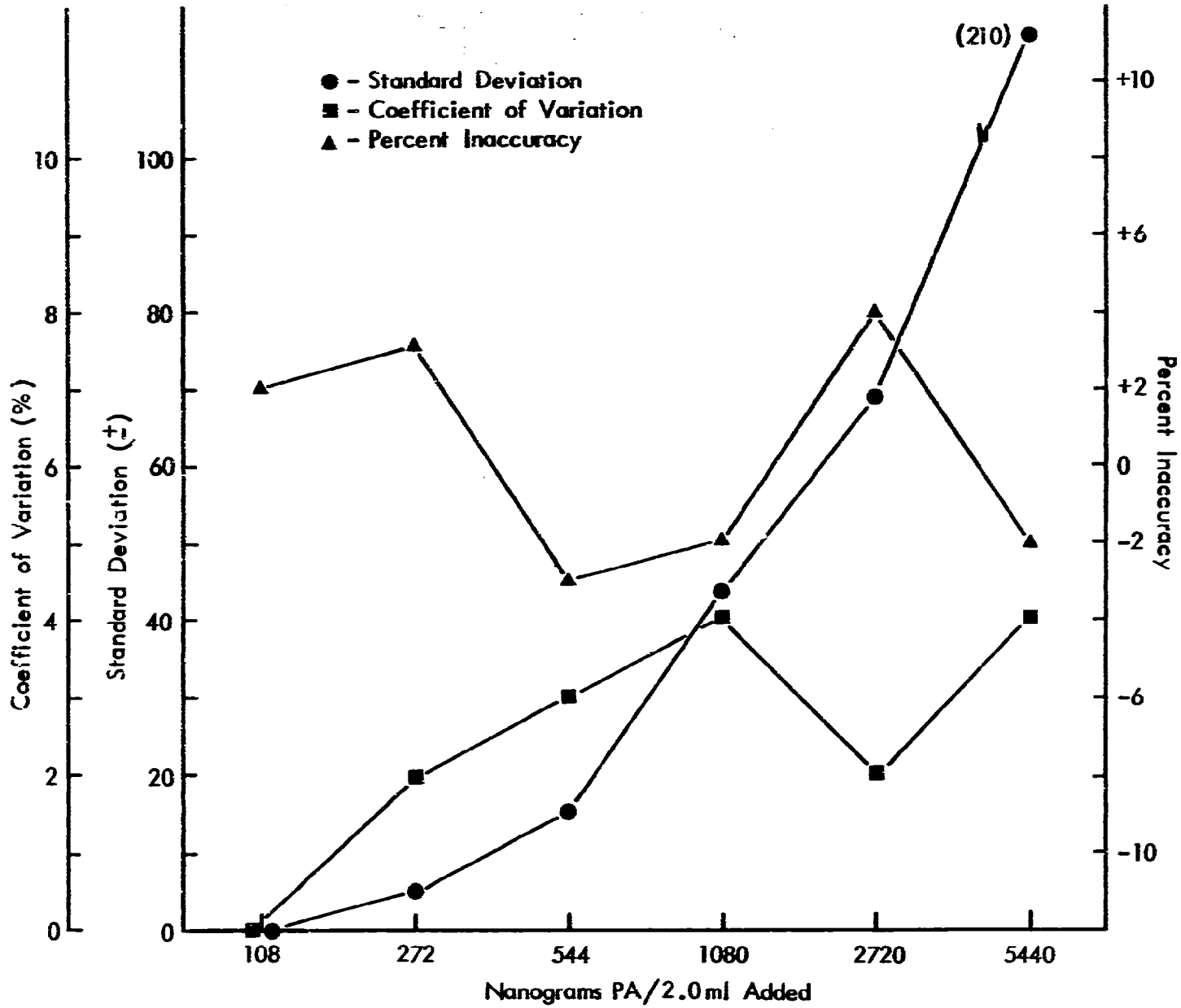


Figure 5 - Standard Deviation, Coefficient of Variation, and Percent Inaccuracy for PA in Reference Solutions

TABLE 1

LINEARITY AND PRECISION OF THE HPLC-UV (230 nm) DETERMINATION OF SARM REFERENCE SOLUTIONS OF NB, 2,6-DNT, NG, PA

Compound	ng 2.0 ml Added	ng/2.0 ml Detected A	B	C	D	Average[a]	Standard[b] Deviation	Coefficient[c] of Variation	Percent[d] Inaccuracy
Nitro-	5,250	5,350	5,400	5,240	5,270	5,320	± 73	1	+1
benzene	2,620	2,740	2,750	2,690	2,680	2,720	± 35	1	+4
	1,050	1,020	980	990	1,000	1,000	± 17	2	-5
	525	530	560	500	500	520	± 29	6	-1
	262	270	260	260	270	260	± 6	2	-1
	105	120	100	100	100	100	± 10	10	-5
	0	ND[e]	ND	ND	ND	-	-	-	-
		Linear Regression, y = 1.017x - 8.8; Correlation Coefficient, 0.9997							
2,6-Dini-	5,620	5,610	5,650	5,620	5,590	5,620	± 25	1	0
trotoluene	2,810	2,940	2,940	2,940	2,910	2,930	± 15	1	+4
	1,120	1,100	1,090	1,060	1,110	1,090	± 22	2	-3
	562	570	570	530	560	560	± 19	3	-1
	281	290	280	280	280	280	± 5	2	-1
	112	110	110	100	110	110	± 5	5	-2
	0	ND	ND	ND	ND	-	-	-	-
		Linear Regression, y = 1.007x + 1.7; Correlation Coefficient, 0.9997							
Trinitro-	151,830	147,700	150,000	148,500	150,100	149,100	± 1,170	1	-2
glycerin	75,900	77,700	77,500	79.200	77,800	78,000	± 780	1	+3
	30,370	26,700	29,500	29,600	26,300	28,000	± 1,770	6	-8
	15,180	15,600	15,400	14,500	14,500	15,000	± 580	4	-1
	7,590	7,600	7,700	7,500	7,700	7,600	± 100	1	+1
	3,040	3,100	3,200	3,000	3,100	3,100	± 80	3	+2
	0	ND	ND	ND	ND	-	-	-	-
		Linear Regression, y = 0.989x + 16.5; Correlation Coefficient, 0.9995							

TABLE 1 (concluded)

Compound	ng / 2.0 ml Added	ng/2.0 ml Detected A	B	C	D	Average[a]	Standard[b] Deviation	Coefficient[c] of Variation	Percent[d] Inaccuracy
Picric	5,440	5,030	5,470	5,480	5,390	5,340	± 210	4	-2
Acid	2,720	2,720	2,850	2,850	2,870	2,820	± 69	2	+4
	1,080	1,030	1,090	1,020	1,110	1,060	± 44	4	-2
	544	540	540	510	540	530	± 15	3	-3
	272	280	280	280	270	280	± 5	2	+3
	108	110	110	110	110	110	0	0	+2
	0	ND	ND	ND	ND	-	-	-	-

Linear Regression, $y = 0.989x + 13.0$; Correlation Coefficient, 0.9988

a Average = $\Sigma x/n = \bar{x}$

b Standard deviation = $\left(n\Sigma x^2 - (\Sigma x)^2/n(n-1)\right)^{\frac{1}{2}} = \sigma$

c Coefficient of Variation = $\sigma/\bar{x} \times 100$

d Percent Inaccuracy = $\frac{\bar{x} - \text{ng added}}{\text{ng added}} \times 100$

e ND - Not detectable, less than 20 ng/2.0 ml for NB, 2,6-DNT, PA; 300 ng/2.0 ml for NG.

TABLE 2

STATISTICAL EVALUATION OF NB IN ANALYTICAL TECHNIQUE SARM REFERENCE SOLUTIONS BY THE HUBAUX AND VOS DETECTION LIMIT

Number of[a] Data Points	Linear Regression	Correlation Coefficient	Degrees of Freedom	t[b]	y[c] Intercept	Detection[d] Limit
28	y = 1.017 x -8.8	0.9997	26	1.706	34	85

ng/2.0 ml NB Added	Average[e] ng/2.0 ml Found	Standard[f] Deviation	Percent[g] Imprecision	Percent[h] Inaccuracy
0	ND[i]	-	-	-
105	98	± 5.8	5.5	0
262	261	± 3.3	1.3	+ 1.1
525	523	± 16.6	3.2	- 0.5
1,050	1,010	± 9.9	1.0	- 5.0
2,620	2,750	± 20.3	0.7	+ 3.6
5,250	5,400	± 40.3	0.8	+ 1.2

[a] Number of data points - data points utilized in calculation of the linear regression equation and detection limits - 28, all data.

[b] t - 2 tail p level (usually 0.1, each confidence band is 0.05 so total p = 0.1).

[c] y intercept - intercept on y-axis of upper confidence limit line

[d] Detection limit - x-intercept of y-intercept and lower confidence limit line.

[e] Average ng/2.0 ml found - average at each level determined from linear regression equation for 28 points.

[f] Standard deviation - determined from average value (e above) and observed values.

[g] Percent imprecision - standard deviaion divided by average value times 100%.

[h] Percent inacurracy - determined from the average values of the four observed values at each level

$$\% \text{ Inaccuracy} = \frac{\text{Average observed values - level added}}{\text{level added}} \times 100$$

[i] ND - not detectable, less than 20 ng/2.0 ml.

TABLE 3

STATISTICAL EVALUATION OF 2,6-DNT IN ANALYTICAL TECHNIQUE SARM REFERENCE SOLUTIONS BY THE HUBAUX AND VOS DETECTION LIMIT

Number of[a] Data Points	Linear Regression	Correlation Coefficient	Degrees of Freedom	t[b]	y[c] Intercept	Detection[d] Limit
28	y = 1.007X + 1.7	0.9997	26	1.706	47	91

ng/2.0 ml 2,6-DNT Added	Average[e] ng/2.0 ml Found	Standard[f] Deviation	Percent[g] Imprecision	Percent[h] Inaccuracy
0	ND[i]	-	-	-
112	110	± 2.9	2.7	- 4.0
281	286	± 2.9	1.0	+ 0.5
562	563	± 10.9	2.0	- 0.8
1,120	1,100	± 12.5	1.1	- 2.7
2,810	2,950	± 8.7	0.3	+ 4.4
5,620	5,660	± 14.4	0.3	- 0.1

[a] Number of data points - data points utilized in calculation of the linear regression equation and detection limits - 28, all data.

[b] t - 2 tail p level (usually 0.1, each confidence band is 0.05 so total p = 0.1).

[c] y intercept - intercept on y-axis of upper confidence limit line

[d] Detection limit - x-intercept of y-intercept and lower confidence limit line.

[e] Average ng/2.0 ml found - average at each level determined from linear regression equation for 28 points.

[f] Standard deviation - determined from average value (e above) and observed values.

[g] Percent imprecision - standard deviaion divided by average value times 100%.

[h] Percent inacurracy - determined from the average values of the four observed values at each level

$$\% \text{ Inaccuracy} = \frac{\text{Average observed values - level added}}{\text{level added}} \times 100$$

[i] ND - not detectable, less than 20 ng/2.0 ml.

TABLE 4

STATISTICAL EVALUATION OF NG IN ANALYTICAL TECHNIQUE SARM REFERENCE SOLUTIONS BY THE HUBAUX AND VOS DETECTION LIMIT

Number of[a] Data Points	Linear Regression	Correlation Coefficient	Degrees of Freedom	t[b]	y[c] Intercept	Detection[d] Limit
28	y = 0.989X + 16.5	0.9995	26	1.706	1,600	3,200

ng/2.0 ml NG Added	Average[e] ng/2.0 ml Found	Standard[f] Deviation	Percent[g] Imprecision	Percent[h] Inaccuracy
0	ND[i]	-	-	-
3,040	3,080	± 47	1.5	+ 2.0
7,590	7,560	± 55	0.7	+ 0.5
15,180	14,900	± 337	2.2	- 1.2
30,370	27,700	± 1,021	3.6	- 7.7
75,900	77,200	± 448	0.6	+ 2.8
151,830	147,500	± 677	0.5	- 1.8

[a] Number of data points - data points utilized in calculation of the linear regression equation and detection limits - 28, all data.

[b] t - 2 tail p level (usually 0.1, each confidence band is 0.05 so total p = 0.1).

[c] y intercept - intercept on y-axis of upper confidence limit line

[d] Detection limit - x-intercept of y-intercept and lower confidence limit line.

[e] Average ng/2.0 ml found - average at each level determined from linear regression equation for 28 points.

[f] Standard deviation - determined from average value (e above) and observed values.

[g] Percent imprecision - standard deviation divided by average value times 100%.

[h] Percent inacurracy - determined from the average values of the four observed values at each level

$$\% \text{ Inaccuracy} = \frac{\text{Average observed values - level added}}{\text{level added}} \times 100$$

[i] ND - not detectable, less than 300 ng/2.0 ml.

TABLE 5

STATISTICAL EVALUATION OF PA IN ANALYTICAL TECHNIQUE SARM REFERENCE SOLUTIONS BY THE HUBAUX AND VOS DETECTION LIMIT

Number of[a] Data Points	Linear Regression	Correlation Coefficient	Degrees of Freedom	t[b]	y[c] Intercept	Detection[d] Limit
28	y = 0.989X + 13.0	0.9988	26	1.706	101	178

ng/2.0 ml PA Added	Average[e] ng/2.0 ml Found	Standard[f] Deviation	Percent[g] Imprecision	Percent[h] Inaccuracy
0	ND[i]	-	-	-
108	122	0	0	+ 1.9
272	287	± 2.9	1.0	+ 2.0
544	540	± 8.7	1.6	- 2.1
1,080	1,060	± 25.5	2.4	- 1.6
2,720	2,800	± 39.8	1.4	+ 3.8
5,440	5,300	± 122.5	2.3	- 1.8

[a] Number of data points - data points utilized in calculation of the linear regression equation and detection limits - 28, all data.

[b] t - 2 tail p level (usually 0.1, each confidence band is 0.05 so total p = 0.1).

[c] y intercept - intercept on y-axis of upper confidence limit line

[d] Detection limit - x-intercept of y-intercept and lower confidence limit line.

[e] Average ng/2.0 ml found - average at each level determined from linear regression equation for 28 points.

[f] Standard deviation - determined from average value (e above) and observed values.

[g] Percent imprecision - standard deviaion divided by average value times 100%.

[h] Percent inacurracy - determined from the average values of the four observed values at each level

$$\% \text{ Inaccuracy} = \frac{\text{Average observed values - level added}}{\text{level added}} \times 100$$

[i] ND - not detectable, less than 20 ng/2.0 ml.

TABLE 6

LINEARITY AND PRECISION OF SARM NB BY HPLC-UV (230 nm)

Reference Solution No.	ng/2.0 ml Added[a]	Peak Heights[b] NB	Peak Heights[b] IS	ng IS/2.0 ml[c] Reference Solution	RWR[d]	Calculated ng[e] 2.0 ml
A-1	5,250	115.2	90.2	2,220	0.54	5,350
A-2	2,620	109.8	167.9	2,220	0.55	2,740
A-3	1,050	52.5	215.5	2,220	0.52	1,020
A-4	525	56.9	45.0	222	0.54	530
A-5	262	36.8	57.7	222	0.54	270
A-6	105	16.8	60.2	222	0.59	120
A-7	0	< 2	28.0	222	-	-
B-1	5,250	118.0	91.5	2,220	0.54	5,400
B-2	2,620	112.2	171.0	2,220	0.56	2,750
B-3	1,050	50.0	212.5	2,220	0.50	980
B-4	525	63.8	48.0	222	0.56	560
B-5	262	39.0	61.5	222	0.54	260
B-6	105	15.0	60.8	222	0.52	100
B-7	0	< 2	59.2	222	-	-
C-1	5,250	119.0	95.2	2,220	0.53	5,240
C-2	2,620	111.2	173.0	2,220	0.54	2,690
C-3	1,050	51.2	216.0	2,220	0.50	990
C-4	525	59.2	49.2	222	0.50	500
C-5	262	38.5	62.2	222	0.52	260
C-6	105	15.2	63.2	222	0.51	100
C-7	0	< 2	61.5	222	-	-
D-1	5,250	117.0	93.0	2,220	0.53	5,270
D-2	2,620	111.8	174.5	2,220	0.54	2,680
D-3	1,050	56.6	237.1	2,220	0.50	1,000
D-4	525	58.2	48.8	222	0.50	500
D-5	262	40.2	63.0	222	0.54	270
D-6	105	14.5	60.9	222	0.50	100
D-7	0	< 2	59.5	222	-	-

Average RWR	0.53
Standard Deviation	± 0.02
Relative Standard Deviation	4.4%

a ng/2.0 ml Reference Solution: ng NB added to 2.0 ml reference solution.

b Peak Heights: peak heights of NB and IS measures in millimeters.

c ng IS/2.0 ml Reference Solution: ng IS added to 2.0 ml reference solution.

d RWR: Relative Weight Response = $\frac{\text{Peak height NB}}{\text{Peak height IS}} \times \frac{\text{ng IS/2.0 ml}}{\text{ng NB/2.0 ml}}$

e Calculated ng/2.0 ml: ng of NB calculated to be in the 2.0 ml reference solution using the average RWR

$$\frac{\text{ng calculated}}{\text{2.0 ml}} = \frac{\text{Peak height NB}}{\text{Peak height IS}} \times \frac{\text{ng/2.0 ml IS}}{\text{Avg. RWR}}$$

TABLE 7

LINEARITY AND PRECISION OF SARM 2,6-DNT BY HPLC-UV (230 nm)

Reference Solution No.	ng/2.0 ml Added[a]	Peak Heights[b] 2,6-DNT	Peak Heights[b] IS	ng IS/2.0 ml[c] Reference Solution	RWR[d]	Calculated ng[e] 2.0 ml
A-1	5,620	207.3	90.2	2,220	0.91	5,610
A-2	2,810	202.1	167.9	2,220	0.95	2,940
A-3	1,120	97.0	215.5	2,220	0.89	1,100
A-4	562	105.7	45.0	222	0.93	570
A-5	281	67.9	57.7	222	0.93	290
A-6	112	27.0	60.2	222	0.89	110
A-7	0	< 2	56.0	222	-	-
B-1	5,620	211.8	91.5	2,220	0.91	5,650
B-2	2,810	206.0	171.0	2,220	0.95	2,940
B-3	1,120	95.0	212.5	2,220	0.89	1,090
B-4	562	112.0	48.0	222	0.92	570
B-5	281	72.0	61.5	222	0.92	280
B-6	112	28.5	60.8	222	0.93	110
B-7	0	< 2	59.2	222	-	-
C-1	5,620	219.2	95.2	2,220	0.91	5,620
C-2	2,810	208.2	173.0	2,220	0.95	2,940
C-3	1,120	93.8	216.0	2,220	0.86	1,060
C-4	562	107.5	49.5	222	0.86	530
C-5	281	70.8	62.2	222	0.90	280
C-6	112	27.2	63.2	222	0.85	100
C-7	0	< 2	59.5	222	-	-
D-1	5,620	213.2	93.0	2,220	0.90	5,590
D-2	2,810	208.5	174.5	2,220	0.94	2,910
D-3	1,120	107.8	237.1	2,220	0.90	1,110
D-4	562	112.5	48.8	222	0.91	560
D-5	281	72.2	63.0	222	0.90	280
D-6	112	27.9	60.9	222	0.91	110
D-7	0	< 2	59.5	222	-	-

Average RWR	0.91
Standard Deviation	± 0.03
Relative Standard Deviation	3.0%

a ng/2.0 ml Reference Sclution: ng 2,6-DNT added to 2.0 ml reference solution.

b Peak Heights: peak heights of 2,6-DNT and IS measures in millimeters.

c ng IS/2.0 ml Reference Solution: ng IS added to 2.0 ml reference solution.

d RWR: Relative Weight Response = $\frac{\text{Peak height 2,6-DNT}}{\text{Peak height IS}} \times \frac{\text{ng IS/2.0 ml}}{\text{ng 2,6-DNT/2.0 ml}}$

e Calculated ng/2.0 ml: ng of 2,6-DNT calculated to be in the 2.0 ml reference solution using the average RWR

$$\frac{\text{ng calculated}}{\text{2.0 ml}} = \frac{\text{Peak height 2,6-DNT}}{\text{Peak height IS}} \times \frac{\text{ng/2.0 ml IS}}{\text{Avg RWR}}$$

TABLE 8

LINEARITY AND PRECISION OF SARM NG BY HPLC-UV (230 nm)

Reference Solution No.	ng/2.0 ml Added[a]	Peak Heights[b] NG	Peak Heights[b] IS	ng IS/2.0 ml[c] Reference Solution	RWR[d]	Calculated ng[e] 2.0 ml
A-1	151,830	192.0	90.2	2,220	0.031	147,670
A-2	75,900	188.0	167.9	2,220	0.033	77,680
A-3	30,370	83.0	215.5	2,220	0.028	26,720
A-4	15,180	101.0	45.0	222	0.033	15,570
A-5	7,590	63.0	57.7	222	0.032	7,570
A-6	3,040	27.0	60.2	222	0.032	3,110
A-7	0	< 2	28.0	222	-	-
B-1	151,830	197.8	91.5	2,220	0.032	149,970
B-2	75,900	191.0	171.0	2,220	0.033	77,490
B-3	30,370	90.5	212.5	2,220	0.031	29,540
B-4	15,180	106.8	48.0	222	0.032	15,440
B-5	7,590	68.5	61.5	222	0.033	7,730
B-6	3,040	28.5	60.8	222	0.034	3,250
B-7	0	< 2	59.2	222	-	-
C-1	151,830	206.0	95.2	2,220	0.032	150,120
C-2	75,900	194.0	173.0	2,220	0.033	77,800
C-3	30,370	82.0	216.0	2,220	0.028	26,340
C-4	15,180	103.2	49.5	222	0.030	14,460
C-5	7,590	69.0	62.2	222	0.032	7,700
C-6	3,040	28.0	63.2	222	0.032	3,070
C-7	0	< 2	61.5	222	-	-
D-1	151,830	199.0	93.0	2,220	0.031	148,450
D-2	75,900	199.2	174.5	2,220	0.033	79,190
D-3	30,370	101.0	237.1	2,220	0.031	29,550
D-4	15,180	102.2	48.8	222	0.031	14,530
D-5	7,590	68.5	63.0	222	0.032	7,540
D-6	3,040	25.9	60.9	222	0.031	2,950
D-7	0	< 2	59.5	222	-	-

Average RWR	0.032
Standard Deviation	± 0.001
Relative Standard Deviation	4.6%

a ng/2.0 ml Reference Solution: ng NG added to 2.0 ml reference solution.

b Peak Heights: peak heights of NG and IS measures in millimeters.

c ng IS/2.0 ml Reference Solution: ng IS added to 2.0 ml reference solution.

d RWR: Relative Weight Response = $\frac{\text{Peak height NG}}{\text{Peak height IS}} \times \frac{\text{ng IS/2.0 ml}}{\text{ng NG/2.0 ml}}$

e Calculated ng/2.0 ml: ng of NG calculated to be in the 2.0 ml reference solution using the average RWR

$$\frac{\text{ng calculated}}{\text{2.0 ml}} = \frac{\text{Peak height NG}}{\text{Peak height IS}} \times \frac{\text{ng/2.0 ml IS}}{\text{Avg. RWR}}$$

TABLE 9

LINEARITY AND PRECISION OF SARM PA BY HPLC-UV (230 nm)

Reference Solution No.	ng/2.0 ml Added[a]	Peak Heights[b] PA	Peak Heights[b] IS	ng IS/2.0 ml[c] Reference Solution	RWR[d]	Calculated ng[e] 2.0 ml
A-1	5,440	145.0	90.2	2,220	0.66	5,030
A-2	2,720	146.3	167.9	2,220	0.71	2,720
A-3	1,080	71.2	215.5	2,220	0.68	1,030
A-4	544	78.2	45.0	222	0.71	540
A-5	272	50.9	57.7	222	0.72	280
A-6	108	22.0	60.2	222	0.75	110
A-7	0	< 2	28.0	222	-	-
B-1	5,440	160.2	91.5	2,220	0.71	5,470
B-2	2,720	155.8	171.0	2,220	0.74	2,850
B-3	1,080	74.2	212.5	2,220	0.72	1,090
B-4	544	83.8	48.0	222	0.71	540
B-5	272	54.2	61.5	222	0.72	280
B-6	108	22.0	60.8	222	0.74	110
B-7	0	< 2	59.2	222	-	-
C-1	5,440	166.8	95.2	2,220	0.72	5,480
C-2	2,720	157.5	173.0	2,220	0.74	2,850
C-3	1,080	70.2	216.0	2,220	0.67	1,020
C-4	544	81.0	49.5	222	0.67	510
C-5	272	54.8	62.2	222	0.72	280
C-6	108	22.2	63.2	222	0.72	110
C-7	0	< 2	61.5	222	-	-
D-1	5,440	160.2	93.0	2,220	0.70	5,390
D-2	2,720	160.2	174.5	2,220	0.75	2,870
D-3	1,080	84.4	237.1	2,220	0.73	1,110
D-4	544	85.0	48.8	222	0.71	540
D-5	272	54.2	63.0	222	0.70	270
D-6	108	22.0	60.9	222	0.74	110
D-7	0	< 2	59.5	222	-	-

Average RWR	0.71
Standard Deviation	± 0.02
Relative Standard Deviation	3.5%

a ng/2.0 ml Reference Solution: ng PA added to 2.0 ml reference solution.
b Peak Heights: peak heights of PA and IS measures in millimeters.
c ng IS/2.0 ml Reference Solution: ng IS added to 2.0 ml reference solution.

d RWR: Relative Weight Response = $\frac{\text{Peak height PA}}{\text{Peak height IS}} \times \frac{\text{ng IS/2.0 ml}}{\text{ng PA/2.0 ml}}$

e Calculated ng/2.0 ml: ng of PA calculated to be in the 2.0 ml reference solution using the average RWR

$$\frac{\text{ng calculated}}{\text{2.0 ml}} = \frac{\text{Peak height PA}}{\text{Peak height IS}} \times \frac{\text{ng/2.0 ml IS}}{\text{Avg. RWR}}$$

APPENDIX C

PRECISION AND ACCURACY ASSESSMENT OF THE ANALYTICAL METHOD FOR THE DETERMINATION OF DINITROPHENOL (DNP); CYCLOTRIMETHYLENE-TRINITRAMINE (RDX); 1,3,5-TRINITROBENZENE (TNB); 1,3-DINITROBENZENE (DNB); 2,4-DINITROTOLUENE (2,4-DNT); TRINITROTOLUENE (TNT); 2,4,6-TRINITROPHENYLMETHYL-NITRAMINE (TETRYL); AND DIPHENYLAMINE (DPA) IN WATER SAMPLES

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1. Application

The developed method for extraction and sample preparation of DNP, RDX, TNB, DNB, 2,4-DNT, TNT, tetryl, and DPA can be utilized for the quantitative recovery of the munitions from water samples that have been processed according to this methodology.

This method of extraction and sample preparation is designed to supplement "The High Performance Liquid Chromatographic Analytical Technique for the Determination of DNP, RDX, TNB, DNB, 2,4-DNT, TNT, Tetryl, and DPA," Technical Report No. 1 previously submitted in March 1981, under this same contract.

a. Evaluated concentration range: The concentration ranges of DNP, RDX, TNB, DNB, 2,4-DNT, TNT, tetryl, and DPA studied in the water samples were 0, 1.0, 2.5, 5.0, 10.0, 25.0, and 50.0 µg/liter (parts per billion, ppb). This corresponds to spiking levels of 0, 0.2X, 0.5X, X, 2X, 5X, and 10X, where X is 0.5 µg fortification and the water volume extracted is 0.1 liter.

b. Sensitivity: A signal-to-noise ratio of 22 to 1 for DNP (peak height (PH) = 22 mm), 14 to 1 for RDX (PH = 14 mm), 21 to 1 for TNB (PH = 21 mm), 32 to 1 for DNB (PH = 32 mm), 25 to 1 for 2,4-DNT (PH = 25 mm), 22 to 1 for TNT (PH = 22 mm), 15 to 1 for tetryl (PH = 15 mm), and 7 to 1 for DPA (PH = 7 mm) was obtained with a 100-µl injection of a 0.2X (1 µg/liter) level water sample prepared and analyzed by the procedure outlined below (ca. 2.5 ng of each munition on column).

c. Recovery detection limits: The recovery detection limits of the extraction and sample preparation methodology using the Hubaux and Vos recovery detection limit program were 2.12 µg/liter DNP, 2.96 µg/liter RDX, 1.64 µg/liter TNB, 1.00 µg/liter DNB, 1.70 µg/liter 2,4-DNT, 1.76 µg/liter TNT, 3.24 µg/liter tetryl, and 3.09 µg/liter for DPA.

d. Interferences: No interfering peaks were observed on the chromatographs of either the reference or the sample solutions.

e. Analysis rate: The chromatographic time per injection was 50 min. Two reference solutions were analyzed prior to injecting the prepared samples and one was analyzed during the day (150 min to total time). Thus, a total of seven prepared water samples (350 min total time) can be analyzed during an 8-hr working day, i.e., 8:00 a.m. to 5:00 p.m.

2. Chemistry

DNP, RDX, TNB, DNB, 2,4-DNT, TNT, tetryl, and DPA are munition related compounds manufactured at various installations. The assessment of potential environmental contamination by these compounds in water requires knowledge that the level of the compounds present at the time of sampling does not change prior to analysis and that the sampling technique provides a representative sample. The evaluation of the preservation and sampling parameters to be employed requires an extraction and sample preparation method capable of providing recovery of these compounds from water samples with sufficient precision and accuracy to provide quantitative data.

3. Apparatus

a. Instrumentation: A Waters programmable liquid chromatographic system consisting of two Model 6000A pumps, Waters Model 720 system programmer, a Rheodyne Model 7125 100-µl fixed loop injector, a Model 440 UV detector with a 254 nm filter and a single pen Model SR-204 Heath-Schlumberger recorder were used. (Note: Equivalent instrumentation will provide similar results.)

b. HPLC Parameters:

1. Column: Spherisorb ODS, 5 µ, 250 x 4.6 mm ID.

2. Precolumn: Co:Pell ODS, 25 µ to 35 µ, 50 x 2 mm ID.

3. Eluent:
   Initial: 30/70 (v/v) acetonitrile/water 0.08 M in acetic acid, adjusted to pH 3.1 with ammonium hydroxyide
   Final: 50/50 (v/v) acetonitrile/water 0.08 M in acetic acid, adjusted to pH 3.1 with ammonium hydroxide

4. Program: Linear gradient from initial eluent to final eluent over a 35-min period.

5. Flow Rate: 1.0 ml/min.

6. Chart Speed: 0.1 in/min.

7. Detector: UV, 254 nm.

8. Internal Standard: Propiophenone.

9. Injection Volume: 50 to 100 µl.

10. Retention Volumes:

Compound	Milliliters
DNP	12.5
RDX	13.5
TNB	17.0
DNB	18.0
2,4-DNT	24.0
TNT	25.0
Tetryl	26.0
DPA	38.0
IS	20.0

Note: Slight changes in the retention indices may occur with fresh eluent or a change in precolumn or analytical column.

c. Laboratory Glassware and Equipment:

1. Pasteur pipettes
2. Beakers (100 ml)
3. Separatory funnels (125 ml) with Teflon stopcock
4. Culture tubes (12 ml) with Teflon-lined screw caps
5. Graduated cylinders (250, 100, and 10 ml)
6. pH meter
7. Nitrogen gas stream drying system (set up in safety ventilation hood)
8. Hot plate (variable temperature)
9. Vortex mixer
10. Filters 0.45 µm (organic solvent compatability)
11. Disposable 5 cc syringes (compatible with filter fitting)
12. Volumetric syringes (0-100, 0-500, 0-1,000 µl)

d. Chemicals:

1. DNP, RDX, TNB, DNB, 2,4-DNT, TNT, tetryl, and DPA SARMs, obtained from the U.S. Army Toxic and Hazardous Materials Agency
2. Propiophenone, analytical grade
3. Acetic acid ACS grade
4. Methylene chloride "distilled in glass" grade
5. Acetonitrile "distilled in glass" grade
6. Sodium chloride ACS grade
7. pH 4.0 calibration buffer
8. High purity water from a Milli-Q water purification system

4. Standards

a. Stock: Weigh accurately ~ 20 mg of DNP, RDX, TNB, DNB, 2,4-DNT, TNT, tetryl, and DPA SARM or interim SARM into separate 100-ml volumetric flasks and dissolve in acetonitrile (concentration of each compound, 200 µg/ml). Working Stock No. 1 is prepared by quantitatively pipetting 2.5 ml from each stock above into a 100-ml volumetric flask and diluting to volume with a 45/55 (v/v) acetonitrile and water solution, 0.08 M in acetic acid (concentration each compound, 5 µg/ ml). Working Stock No. 2 is prepared by quantitatively pipetting 10 ml of working stock No. 1 into a 100-ml volumetric flask and diluting to volume with a 45/55 (v/v) acetonitrile and water solution, 0.08 M in acetic acid (concentration each compound, 0.5 µg/ml).

b. Internal Standard Stock: Weigh accurately ~ 20 mg propiophenone into a 100-ml volumetric flask and dilute to volume with acetonitrile (concentration, 200 µg/ml). Internal standard (IS) stock solution No. 1 is prepared by quantitatively pipetting 1.0 ml of the stock above into a 100-ml volumetric flask and diluting to volume with a 45/55 (v/v) acetonitrile and water solution 0.08 M in acetic acid (concentration, 2 µg/ml). IS Stock No. 2 is prepared by quantitatively pipetting 10-ml IS Stock No. 1 into a 100-ml volumetric flask and diluting to volume with a 45/55 (v/v) acetonitrile and water solution 0.08 M in acetic acid (concentration, 0.2 µg/ml).

c. Reference Standards: Working stocks No. 1 and No. 2 and the IS stocks No. 1 and No. 2 were employed to prepare the reference standards for the precision and accuracy testing of the extraction and sample preparation method as follows:

Working Stock No.	µl Working Stock	IS Stock No.	µl IS Stock	µl 45/55 (v/v) CH_3CN/Water 0.08 M Acetic Acid	Concentration Each Compound ng/2.0 ml	
					Compounds	IS
1	500	1	1,000	500	2,500	2,000
2	1,000	2	1,000	-	500	200
2	200	2	1,000	800	100	200

Each reference standard solution was prepared fresh on four separate days correlating with each separate extraction series performed to define the precision and accuracy assessment of the extraction and sample preparation method.

d. Sample Fortification: Working stocks No. 1 and No. 2 were employed to spike the 100-ml water samples to be extracted as follows:

Working Stock No.	µl Working Stock	Concentration Each Compound µg/ℓ (ppb)	µg Fortification Each Compound when X equals 0.5 µg
1	1,000	50	10X
1	500	25	5X
1	200	10	2X
2	1,000	5	1X
2	500	2.5	0.5X
2	200	1	0.2X
-	-	0	0

Each of these 100-ml spiked water samples and the blank were prepared and extracted on four separate days to define the precision and accuracy of the extraction and sample preparation method.

e. Internal Standard Sample Addition: To each sample extracted and concentrated according to the protocol outlined below, IS stocks No. 1 and No. 2 were employed to add the appropriate level of IS as follows:

Munitions Sample According to X equals 0.5 µg Fortification	IS Stock No.	µl IS Stock Added	µg IS Stock in Sample
10X	1	1,000	2
5X	1	1,000	2
2X	1	1,000	2
1X	2	1,000	0.2
0.5X	2	1,000	0.2
0.2X	2	1,000	0.2
0	2	1,000	0.2

Each sample had an additional 800 µl of 45/55 (v/v) acetonitrile and water solution, 0.08 M in acetic acid added to aid in the dissolution of the munitions and to make the final sample volume approximately 2 ml.

5. Sample Preparation Procedure

The procedure outlined below was defined for the quantitative extraction of DNP, RDX, TNB, 2,4-DNT, TNT, tetryl, and DPA from a water sample.

1. Place 100 ml of the water sample into a 125-ml separatory funnel.

2. Add the appropriate level of the munition compounds as given in Section 4.d.

3. Add 8.5 ml sodium chloride crystals, i.e., approximately 10 g. The water sample is 10% w/v in sodium chloride.

4. Adjust the pH of the sample to pH 3 by adding dropwise glacial acetic acid.

5. Mix thoroughly.

6. Add 20 ml methylene chloride and hand shake for 20 sec. Note: During the extraction, vent the separatory funnel through the stopper not the stopcock to prevent pressure buildup.

7. Allow the phases to completely separate.

8. Drain the methylene chloride layer into a 100-ml beaker. Note: Be careful not to drain any of the aqueous layer into the beaker.

9. Repeat steps 6 through 8 twice more combining the extracts in the beaker.

10. Concentrate the extract to approximately 2 ml on a 40°C hot plate under a stream of nitrogen. Note: A higher temperature than 40°C may cause the extract to boil. The evaporation process is to be accomplished in a hood.

11. Add approximately 10 ml acetonitrile to the beaker to solvent exchange from methylene chloride to acetonitrile. Note: Add the acetonitrile to wash the sides of beaker to insure that the munitions are completely dissolved.

12. Mix by swirling and observe the sample to determine if the two solvents are completely miscible. If necessary, add additional acetonitrile until one phase is obtained.

13. Concentrate the sample to approximately 2 ml on a 40°C hot plate under a stream of nitrogen.

14. Transfer the sample to a culture tube with a Teflon-lined screw cap.

15. Wash the beaker with 3 x 1 ml acetonitrile and add the washes to the culture tube.

16. Concentrate the sample to approximately 200 µl on a 40°C hot plate under a stream of nitrogen.

17. Add 800 µl of a 45/55 (v/v) acetonitrile/water, 0.08 M acetic acid solution.

18. Add 1,000 µl of the appropriate IS stock solution (see Section 4.e.).

19. Mix thoroughly.

20. Filter through a 0.45 µ filter into a clean culture tube.

21. Cap tightly and store at 4°C in the dark until analysis by HPLC-UV (254 nm) using the parameters outlined in Section 3.

A representative HPLC-UV (254 nm) chromatogram of a 100-ml water extract of DNP, RDX, TNB, DNB, 2,4-DNT, TNT, tetryl, and DPA at the 5 µg/liter level is shown in Figure 1.

6. Calculations

The reference standards described in Section 4.c. were prepared fresh at the time of each series extraction. The relative weight response (RWR) (Equation 1) of each compound to the IS was calculated for each reference solution and the average RWR for each compound was utilized to calculate the level of that compound in each of the seven 100-ml water extracted samples (Equation 2). The micrograms per liter found were plotted against the micrograms per liter added and a linear regression analysis of the data was performed. The slope, intercept, and correlation coefficient of each compound were then determined. The data is summarized in Table 1 and include the average value found at each level (Equation 3), the standard deviation (Equation 4), coefficient of variation (Equation 5), and percent inaccuracy (Equation 6). The raw data and calculations for the reference standard solutions and extraction samples are given in Tables 10 through 17.

$$\text{RWR} = \frac{\text{Peak Height Cpd}}{\text{Peak Height IS}} \times \frac{\text{µg IS/reference solution}}{\text{µg Cpd/reference solution}} \qquad \text{(Eq. 1)}$$

$$\frac{\text{µg Cpd Found}}{\text{100 ml Water Extract Sample}} = \frac{\text{Peak Height Cpd}}{\text{Peak Height IS}} \times \frac{\text{µg IS}}{\text{Avg. RWR Cpd}} \qquad \text{(Eq. 2)}$$

$$\text{Average } \frac{\mu g}{\ell} \text{ Found} = \bar{x} = \Sigma x/n \qquad \text{(Eq. 3)}$$

$$\text{Standard deviation} = \sigma = \left(\frac{n\Sigma x^2 - (\Sigma x)^2}{n(n-1)}\right)^{\frac{1}{2}} \qquad \text{(Eq. 4)}$$

$$\text{Coefficient of variation} = (\sigma/\bar{x}) \times 100 \qquad \text{(Eq. 5)}$$

$$\text{Percent inaccuracy} = \left(\frac{\bar{x} - \mu g/\ell \text{ added}}{\mu g/\ell \text{ added}} \right) \times 100 \qquad \text{(Eq. 6)}$$

Graphic presentations of the data points and linear regression line along with graphic representations of the standard deviation, coefficient of variation, and percent inaccuracy are given in Figures 2 through 17.

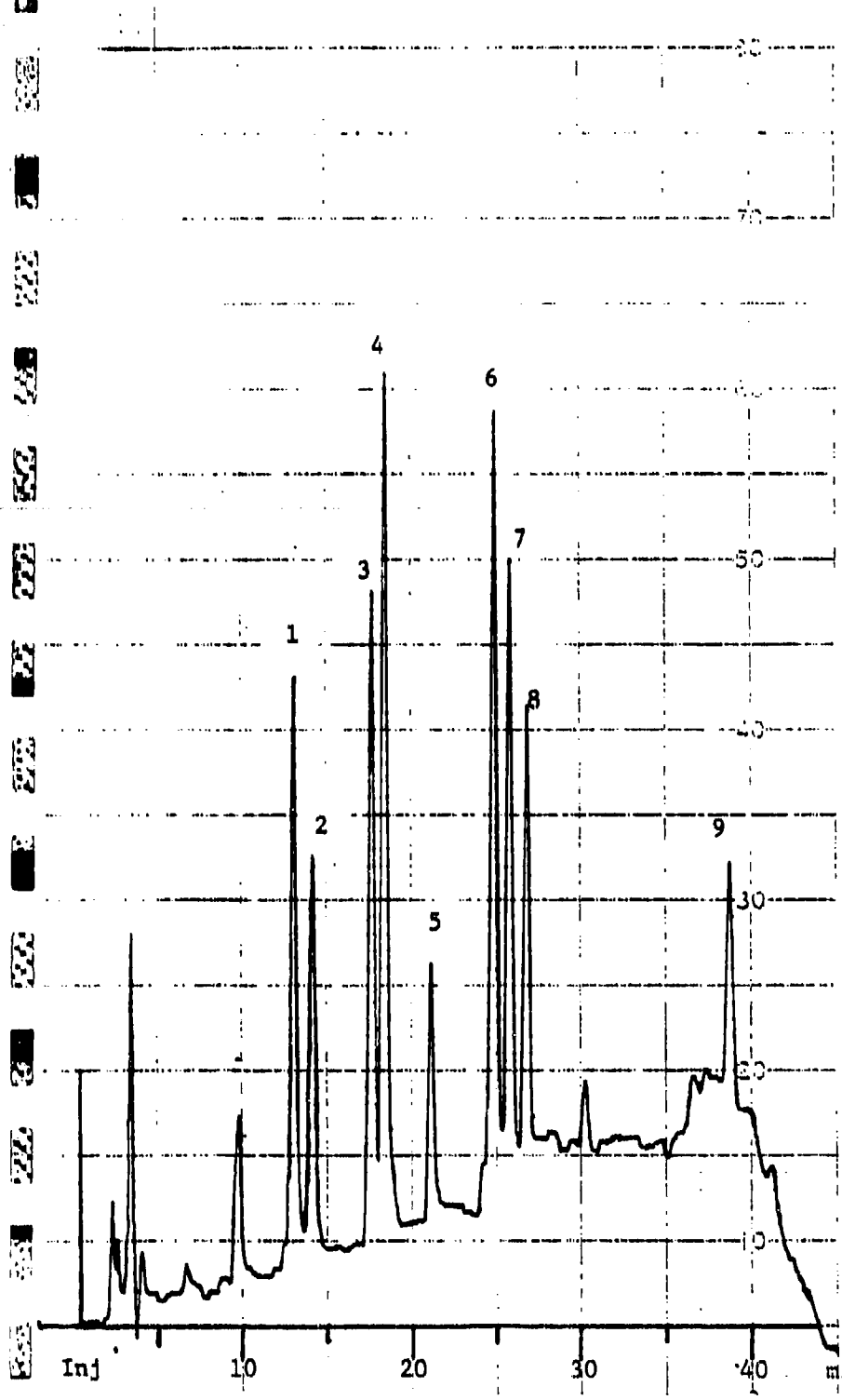
7. Statistical Evaluation of Data

A statistical evaluation of the data obtained for the "Precision and Accuracy Determination of the Extraction from Water and Sample Preparation Method" for DNP, RDX, TNB, DNB, 2,4-DNT, TNT, tetryl, and DPA was performed utilizing the Hubaux and Vos detection limit program provided by the U.S. Army Toxic and Hazardous Materials Agency.

Detection limits for each compound were as follows: DNP, 2.12 µg/ℓ; RDX, 2.96 µg/ℓ; TNB, 1.64 µg/ℓ; DNB, 1.00 µg/ℓ; 2,4-DNT, 1.70 µg/ℓ; TNT, 1.76 µg/ℓ; tetryl, 3.24 µg/ℓ; and DPA, 3.09 µg/ℓ using all the data points. The average micrograms found at each level for each compound were determined from the linear regression equation for the 28 data points and the four found concentrations within each level (Equation 7). The standard deviation and percent imprecision at each level were calculated based on this average, and thus do not agree with the values given in Table 1.

$$\text{Avg. } \mu g/\ell = \frac{\Sigma \left(\frac{\mu g/\ell \text{ found} - \text{intercept}}{\text{slope}} \right)}{n} \qquad \text{(Eq. 7)}$$

The results of the Hubaux and Vos evaluations are given in Tables 2 through 9.



HPLC Parameters

Column: Spherisorb ODS, 5 µ, 250 x 4.6 mm ID
Precolumn: CO:PELL ODS, 25 to 35 µ, 50 x 2 mm ID
Eluent: Linear Gradient
Initial: 30/70 (v/v) CH_3CN/water, solution 0.08 M acetic acid adjusted to pH 3.1 with NH_4OH.
Final: 50/50 (v/v) CH_3CN/water, solution 0.08 M acetic acid adjusted to pH 3.1 with NH_4OH.
Time: 35 min
Flow Rate: 1.0 ml/min
Chart Speed: 0.1 in./min
Detector: UV, 254 nm
Injection Volume: 100 µl
Attenuation: 0.01 A.U.F.S.

Sample Characteristics

No.	Compound	Added (ppb)	Recovered (ppb)
1	DNP	5.20	4.58
2	RDX	6.00	5.63
3	TNB	5.14	4.73
4	DNB	5.01	4.33
5	IS*	-	-
6	2,4-DNT	5.05	4.43
7	TNT	4.97	4.65
8	Tetryl	5.09	4.32
9	DPA	5.00	4.26

* IS - 0.222 µg Propiophenone (IS Stock No. 2) added during final sample preparation. Final sample volume is ∿ 2 ml.

Figure 1 - HPLC-UV (254 nm) Separation of DNP, RDX, TNB, DNB, 2,4-DNT, TNT, Tetryl, and DPA Recovered from a 100-ml Water Sample

Sample preparation procedure listed in text.

TABLE 1

PRECISION AND ACCURACY ASSESSMENT OF THE METHOD FOR EXTRACTION FROM WATER AND SAMPLE PREPARATION OF DNP, RDX, TNB, DNB, 2,4-DNT, TNT, TETRYL, AND DPA

Compound	µg/ℓ Added	µg/ℓ Recovered A	B	C	D	Average[a]	Standard Deviation[b]	Coefficient of Variation[c]	Percent Inaccuracy[d]
DNP	52.0	46.6	46.6	48.7	51.4	48.3	± 2.28	5	-7
	26.0	22.2	24.3	25.8	26.4	24.7	± 1.87	8	-5
	10.4	10.6	10.4	9.30	9.60	10.0	± 0.62	6	-4
	5.20	4.30	4.60	4.80	5.00	4.70	± 0.30	6	-10
	2.60	2.50	2.50	2.50	2.70	2.60	± 0.10	4	0
	1.04	1.10	1.10	1.10	1.00	1.10	± 0.05	4	+6
	0	ND[e]	ND	ND	ND	-	-	-	-
Linear Regression DNP, y = 0.931x + 0.107; Correlation Coefficient, 0.9981									
RDX	60.0	54.9	58.9	64.4	61.1	59.8	± 3.99	7	-1
	30.0	29.3	30.9	33.2	31.9	31.3	± 1.65	5	+4
	12.0	12.2	12.7	11.5	11.3	11.9	± 0.64	6	-1
	6.00	5.10	5.70	5.80	5.50	5.50	± 0.31	6	-8
	3.00	3.00	3.10	3.30	3.20	3.20	± 0.13	4	+7
	1.20	1.30	1.40	1.20	1.20	1.30	± 0.10	7	+8
	0	ND	ND	ND	ND	-	-	-	-
Linear Regression RDX, y = 1.004x + 0.047; Correlation Coefficient, 0.9972									
TNB	51.4	46.5	48.1	49.7	51.2	48.9	± 2.03	4	-5
	25.7	24.5	24.6	25.4	26.2	25.2	± 0.79	3	-2
	10.3	10.2	11.0	9.40	9.70	10.1	± 0.70	7	-2
	5.14	4.30	4.70	4.80	4.90	4.70	± 0.26	6	-9
	2.57	2.50	2.40	2.70	2.60	2.60	± 0.13	5	+1
	1.03	1.10	1.10	1.00	1.10	1.10	± 0.05	5	+7
	0	ND	ND	ND	ND	-	-	-	-
Linear Regression TNB; y = 0.954x + 0.097; Correlation Coefficient, 0.9988									

TABLE 1 (continued)

Compound	µg/ℓ Added	µg/ℓ Recovered A	B	C	D	Average[a]	Standard Deviation[b]	Coefficient of Variation[c]	Percent Inaccuracy[d]
DNB	50.1	45.9	44.8	45.3	46.0	45.5	± 0.56	1	-9
	25.0	24.2	22.9	22.9	23.1	23.3	± 0.62	3	-7
	10.0	9.70	10.4	8.60	8.90	9.40	± 0.81	9	-6
	5.01	4.10	4.30	4.40	4.60	4.40	± 0.21	5	-12
	2.50	2.40	2.10	2.50	2.40	2.40	± 0.17	7	-4
	1.00	0.90	0.90	0.90	0.90	0.90	± 0	0	-10
	0	ND	ND	ND	ND	-	-	-	-
Linear Regression DNB, y = 0.911x + 0.066; Correlation Coefficient, 0.9996									
2,4-DNT	50.5	41.7	44.6	44.0	46.1	44.1	± 1.83	4	-13
	25.2	21.6	22.3	22.8	23.5	22.6	± 0.80	4	-10
	10.1	10.0	10.4	8.90	9.40	9.70	± 0.66	7	-4
	5.05	4.20	4.40	4.70	4.90	4.60	± 0.31	7	-9
	2.52	2.40	2.30	2.80	2.40	2.50	± 0.22	9	-1
	1.01	1.00	1.10	1.00	0.90	1.00	± 0.08	8	-1
	0	ND	ND	ND	ND	-	-	-	-
Linear Regression 2,4-DNT, y = 0.872x + 0.290; Correlation Coefficient, 0.9987									
TNT	49.7	44.1	44.0	47.4	46.6	45.5	± 1.73	4	-8
	24.8	22.8	22.5	25.4	23.8	23.6	± 1.31	6	-5
	9.94	10.2	10.6	9.40	9.20	9.80	± 0.66	7	-1
	4.97	4.20	4.60	4.80	4.80	4.60	± 0.28	6	-7
	2.48	2.50	2.30	2.60	2.50	2.50	± 0.13	5	+1
	0.99	1.00	1.10	0.90	1.00	1.00	± 0.08	8	+1
	0	ND	ND	ND	ND	-	-	-	-
Linear Regression TNT, y = 0.918x + 0.257; Correlation Coefficient, 0.9986									

TABLE 1 (concluded)

Compound	µg/ℓ Added	µg/ℓ Recovered A	B	C	D	Average[a]	Standard Deviation[b]	Coefficient of Variation[c]	Percent Inaccuracy[d]
Tetryl	50.9	43.5	44.4	50.4	52.0	47.6	± 4.25	9	-6
	25.4	23.2	23.3	25.3	26.2	24.5	± 1.49	6	-4
	10.2	8.40	10.9	9.80	10.1	9.80	± 1.04	11	-4
	5.09	3.80	4.30	4.70	4.30	4.30	± 0.37	9	-16
	2.54	2.30	1.80	2.80	1.40	2.10	± 0.61	29	-17
	1.02	1.00	0.70	1.00	0.70	0.90	± 0.17	19	-12
	0	ND	ND	ND	ND	-	-	-	-
Linear Regression Tetryl, y = 0.943x - 0.089; Correlation Coefficient, 0.9954									
DPA	50.0	38.2	42.4	45.8	40.0	41.6	± 3.29	8	-17
	25.0	18.2	20.8	21.7	20.2	20.2	± 1.48	7	-19
	10.0	9.00	10.5	9.10	8.10	9.20	± 0.99	11	-8
	5.00	3.10	4.30	4.50	4.70	4.20	± 0.72	17	-16
	2.50	1.60	2.00	1.60	2.20	1.90	± 0.30	16	-24
	1.00	1.00	0.80	0.70	0.70	0.80	± 0.14	18	-20
	0	ND	ND	ND	ND	-	-	-	-
Linear Regression DPA; y = 0.829x + 0.045; Correlation Coefficient, 0.9957									

a Average: $\Sigma x/n = \bar{x}$

b Standard deviation = $\left(\frac{n\Sigma x^2 - (\Sigma x)^2}{n(n-1)}\right)^{\frac{1}{2}} = \sigma$

c Coefficient of variation = $\sigma/\bar{x} \times 100$

d Percent inaccuracy = $\frac{\bar{x} - \mu g \text{ added}}{\mu g \text{ added}} \times 100$

e ND: Not detectable, less than 0.2 µg/ℓ.

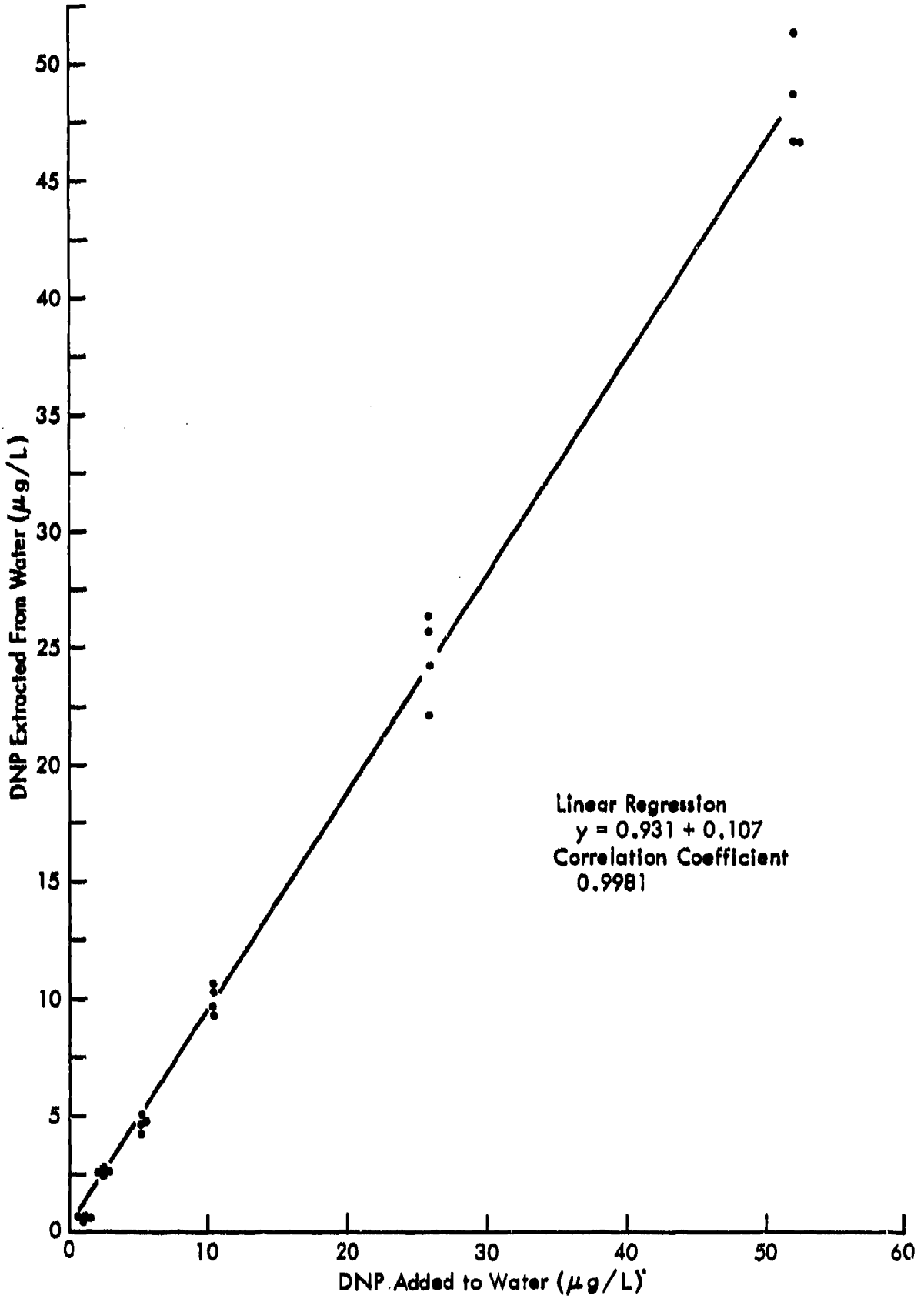


Figure 2 - Linearity of DNP Extracted From 100 ml Water

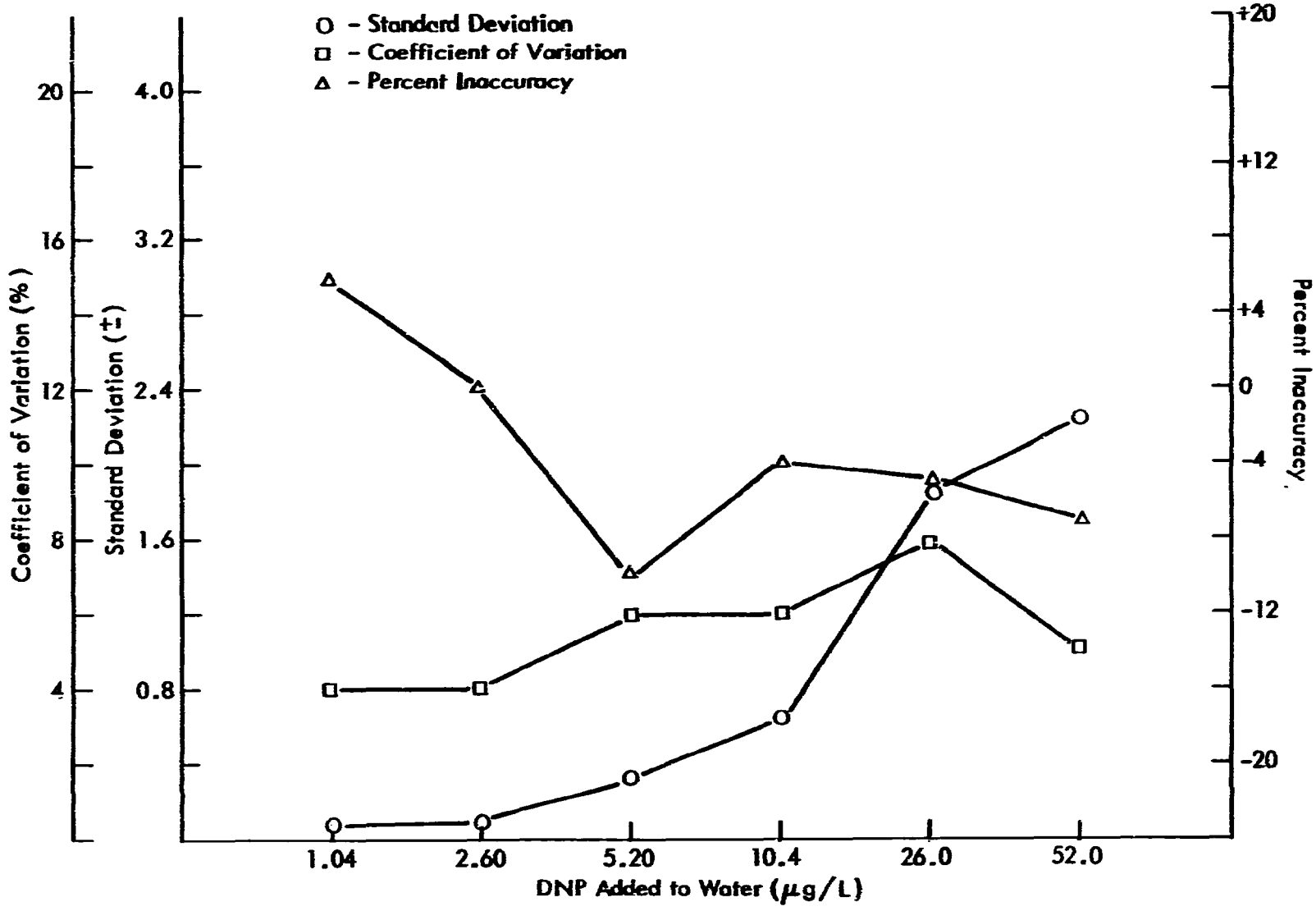


Figure 3 - Standard Deviation, Coefficient of Variation, and Percent Inaccuracy for DNP Extracted from 100 ml Water

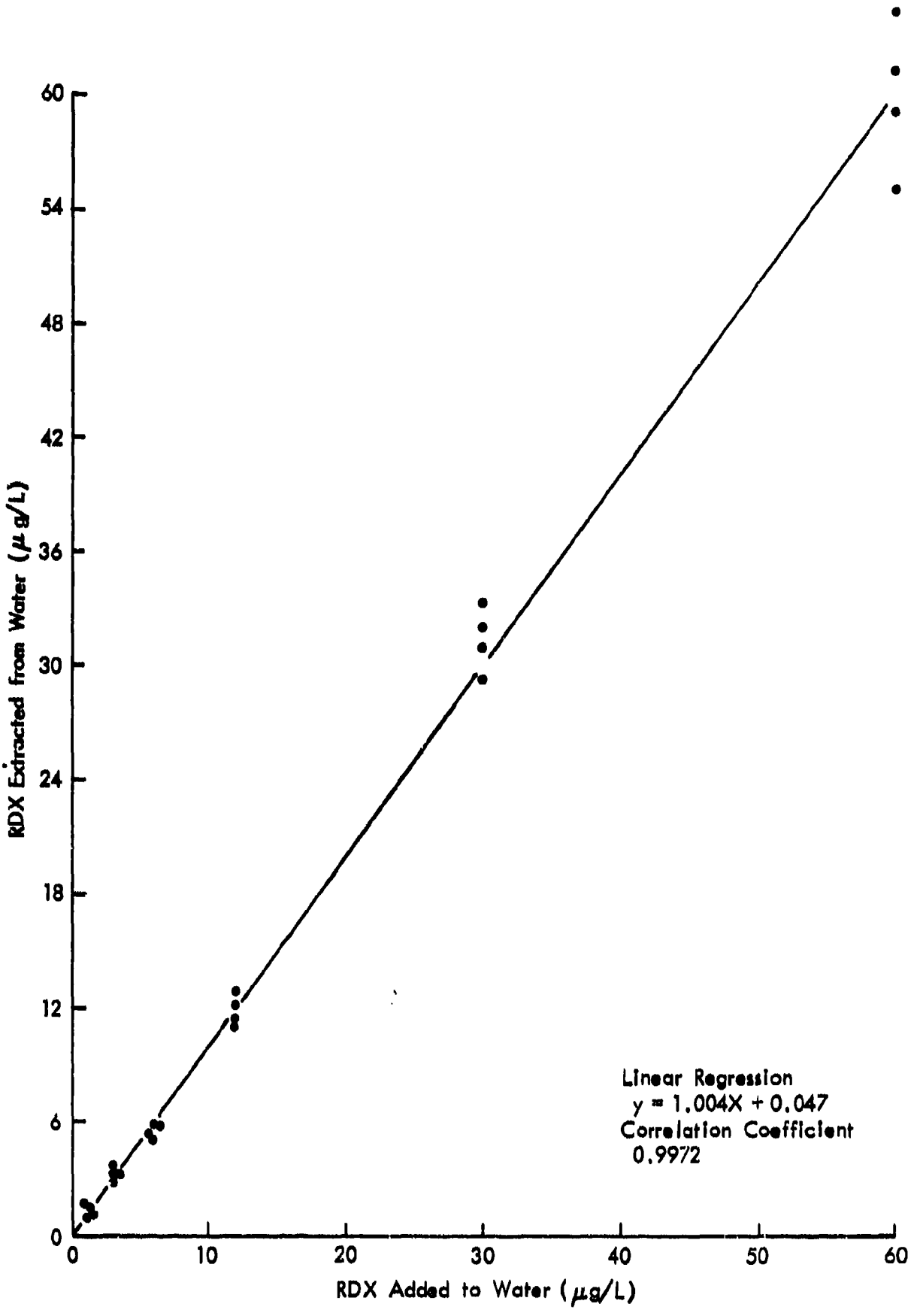


Figure 4 - Linearity of RDX Extracted from 100 ml Water

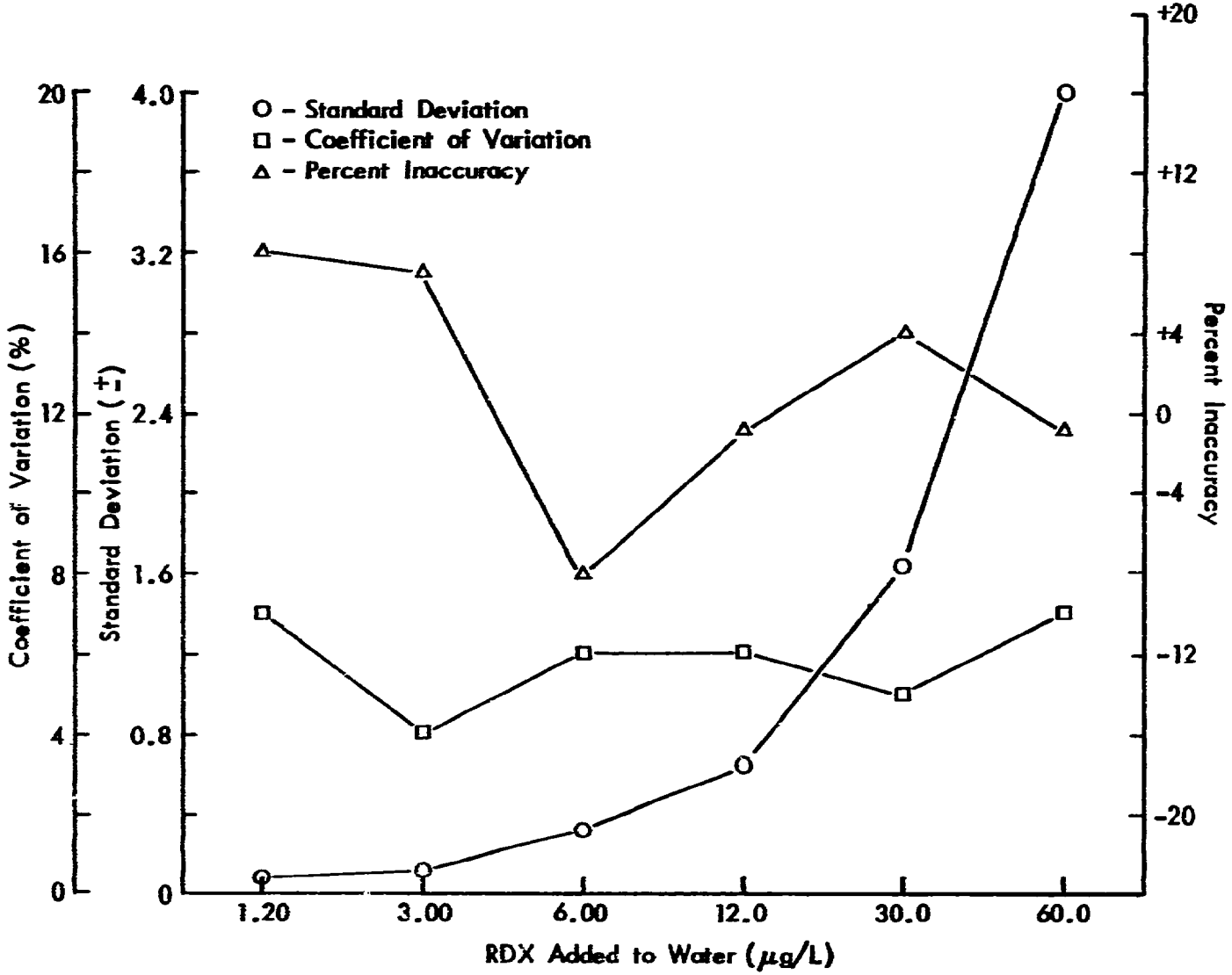


Figure 5 - Standard Deviation, Coefficient of Variation, and Percent Inaccuracy for RDX Extracted from 100 ml Water

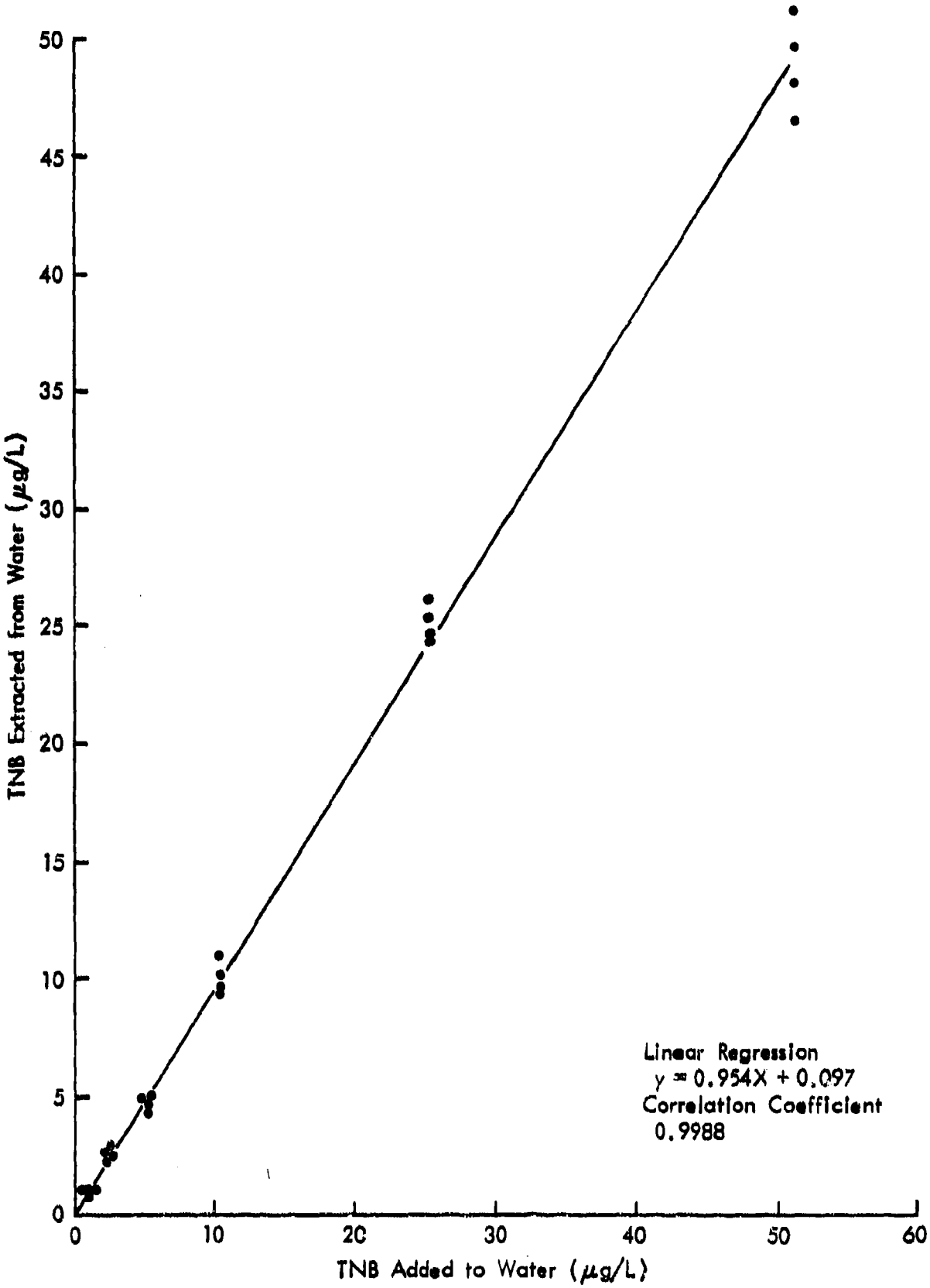


Figure 6 - Linearity of TNB Extracted from 100 ml Water

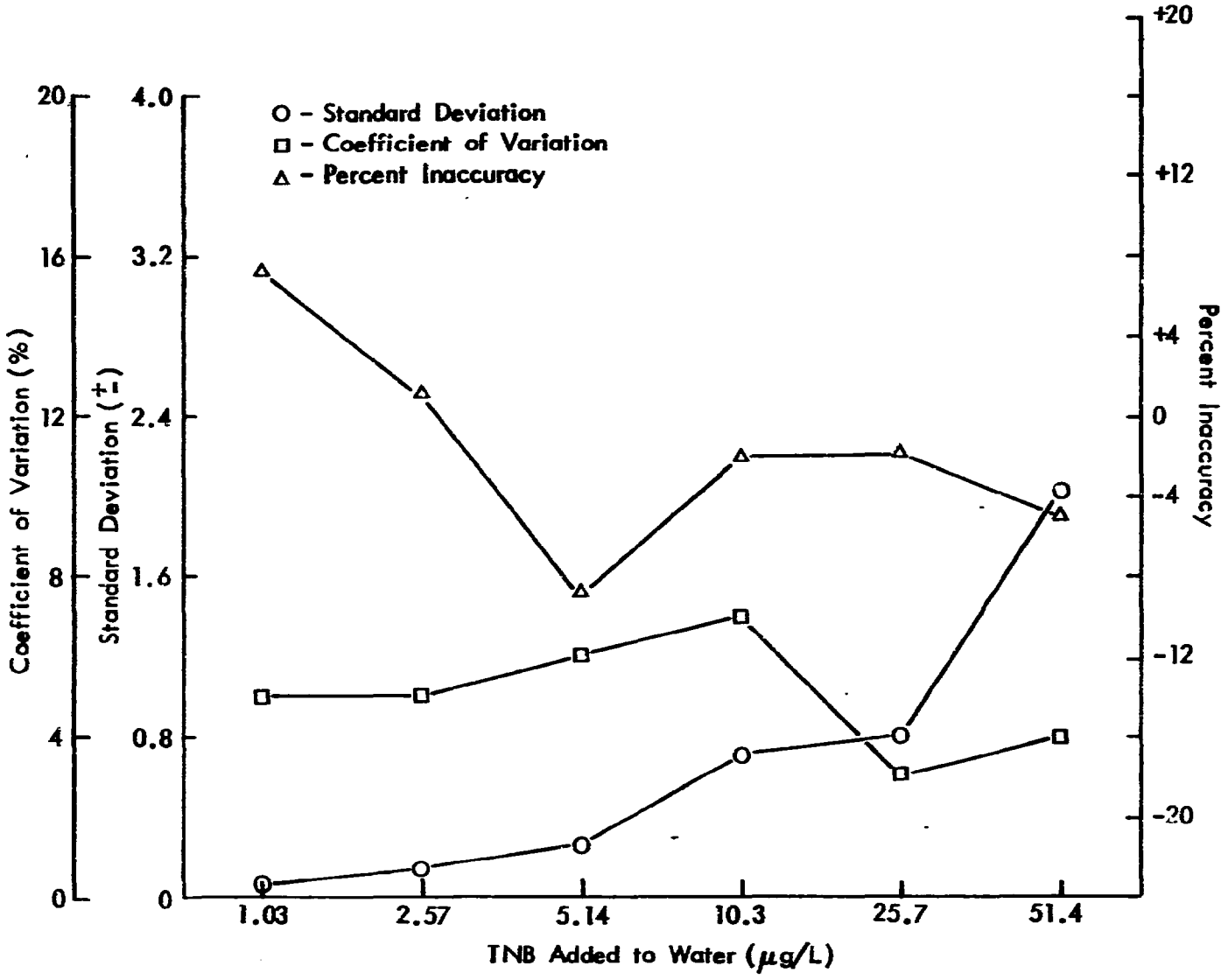


Figure 7 - Standard Deviation, Coefficinet of Variation, and Percent Inaccuracy for TNB Extracted from 100 ml Water

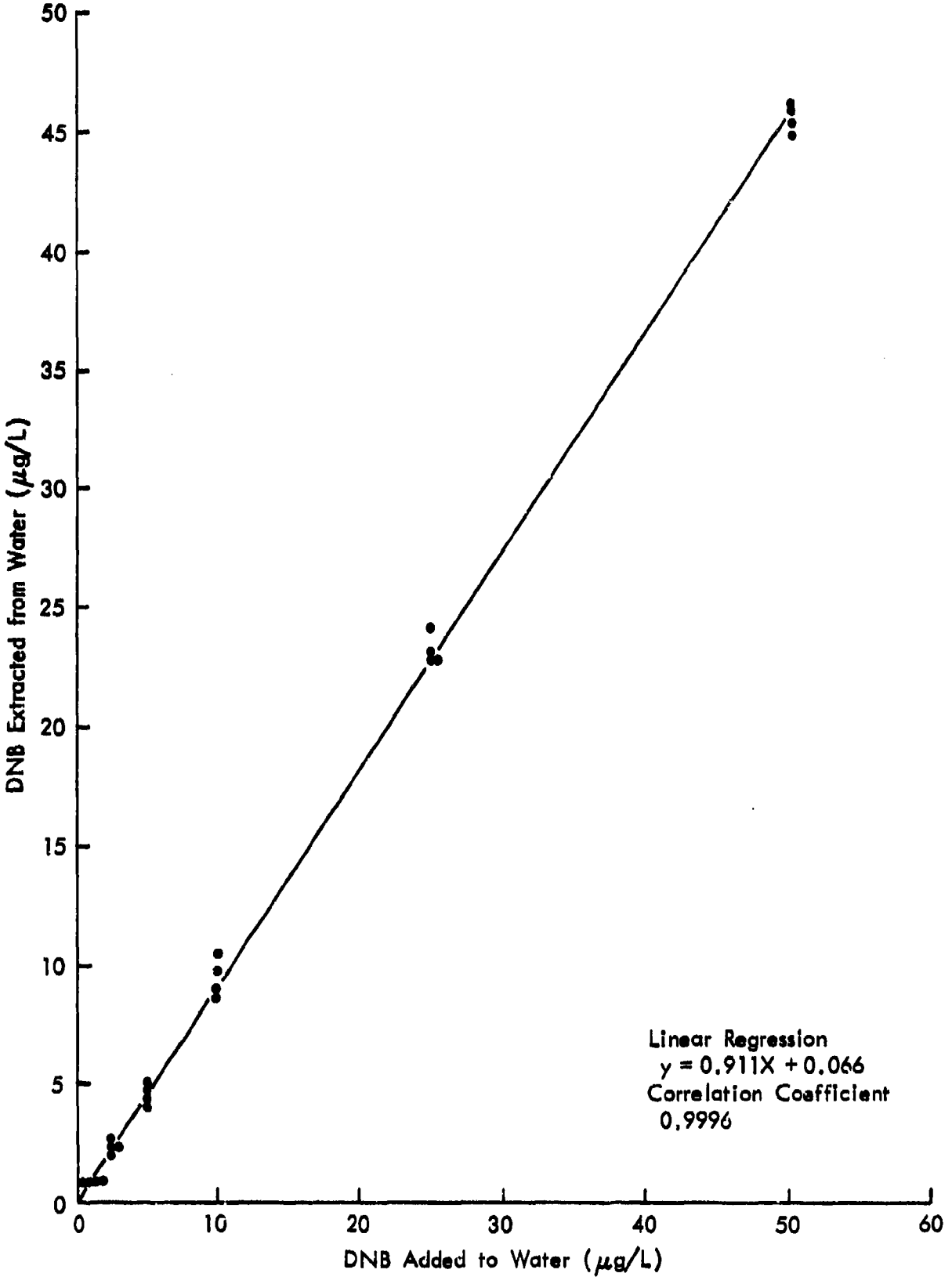


Figure 8 - Linearity of DNB Extracted from 100 ml Water

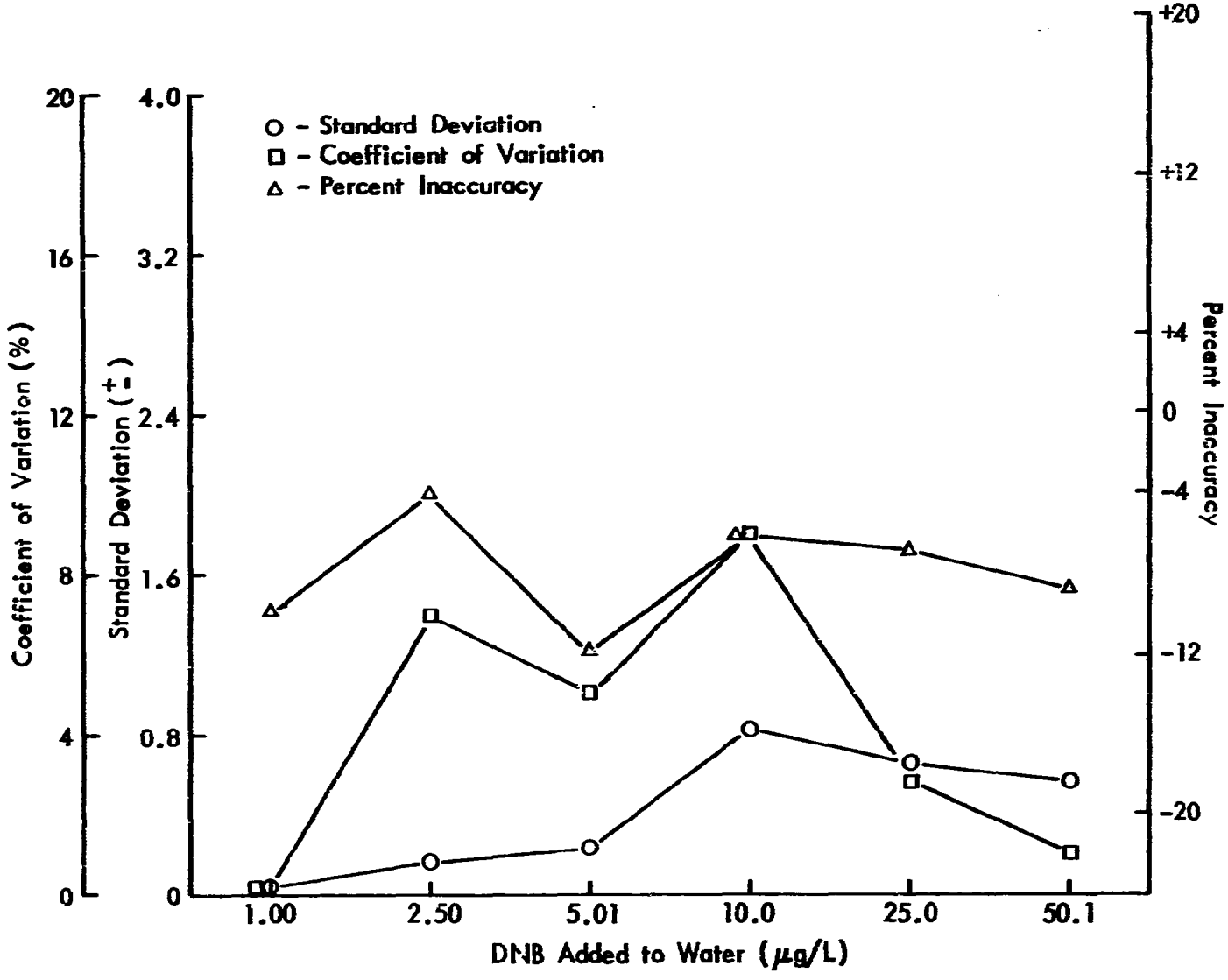


Figure 9 - Standard Deviation, Coefficient of Variation, and Percent Inaccuracy for DNB Extracted from 100 ml Water

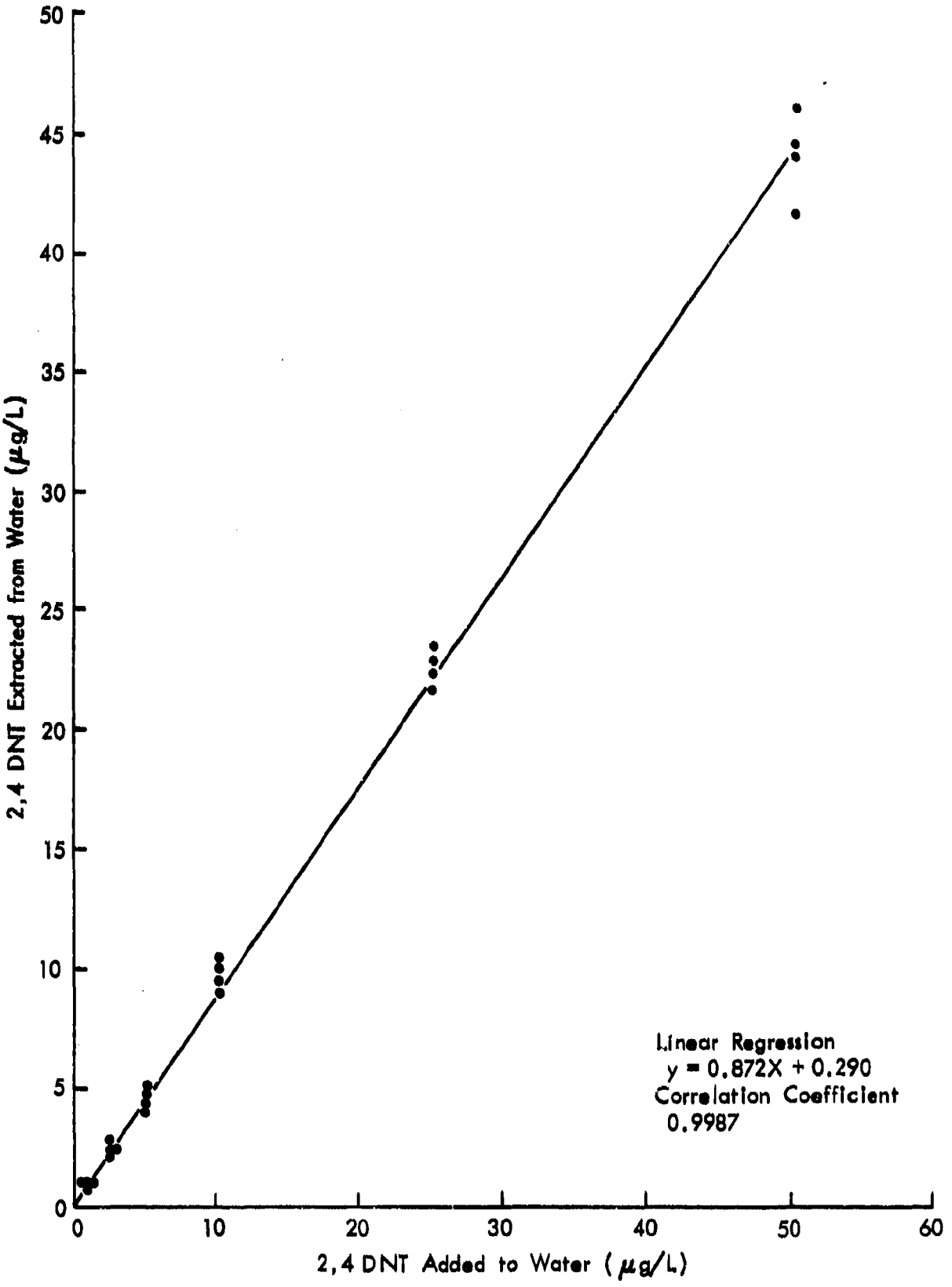


Figure 10 - Linearity of 2,4-DNT Extracted from 100 ml Water

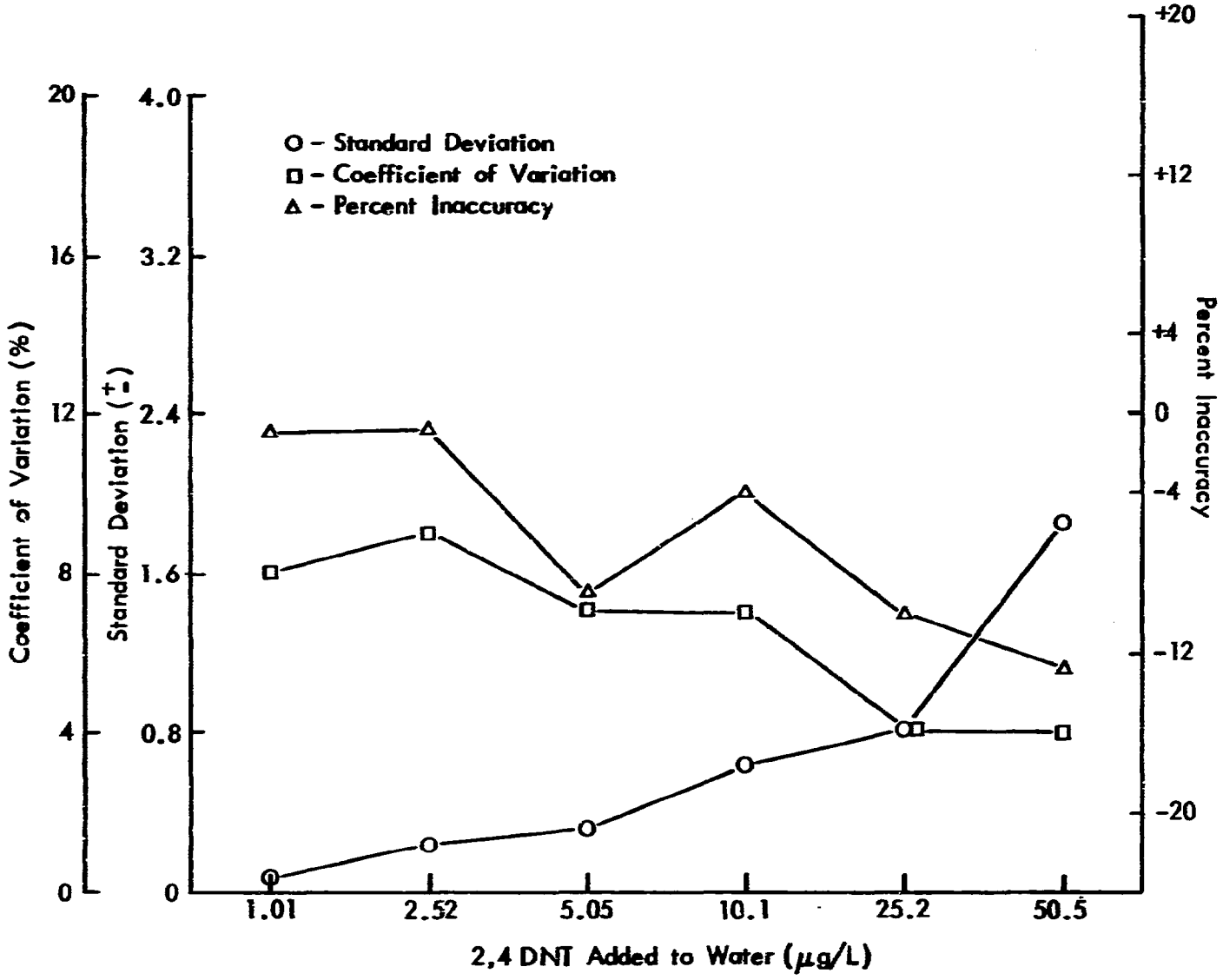


Figure 11 – Standard Deviation, Coefficient of Variation, and Percent Inaccuracy for 2,4-DNT Extracted from 100 ml Water

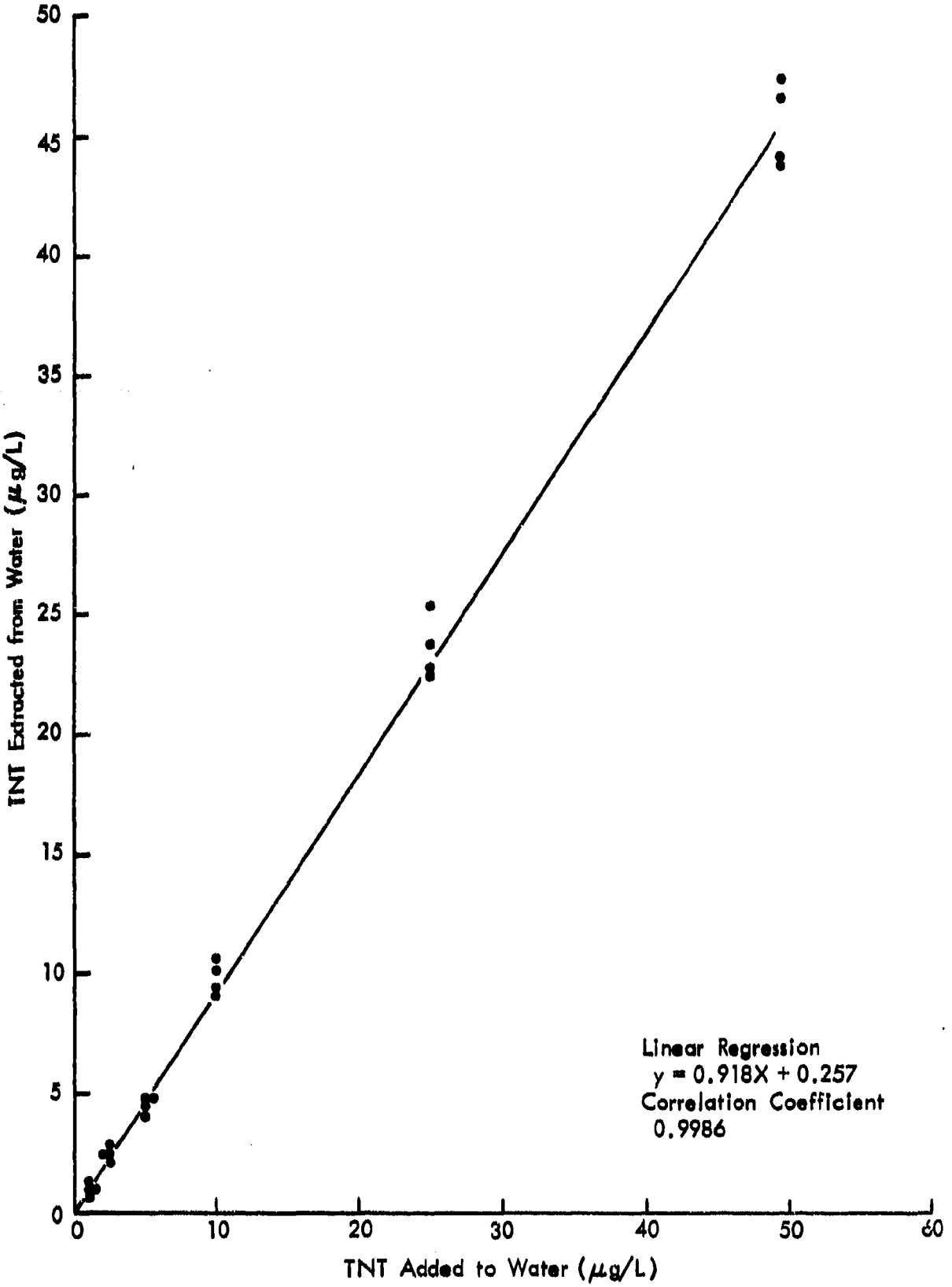


Figure 12 - Linearity of TNT Extracted from 100 ml Water

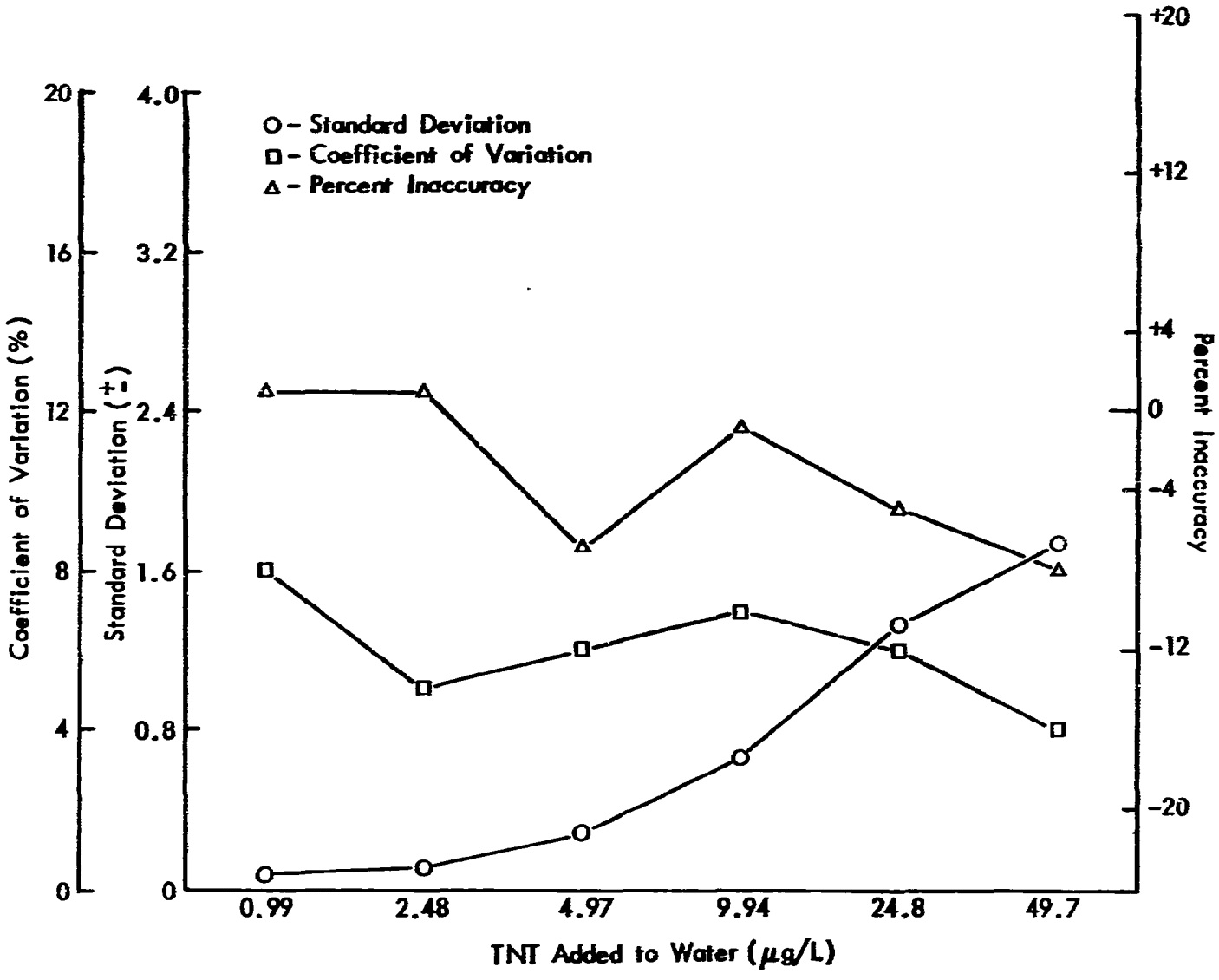


Figure 13 – Standard Deviation, Coefficient of Variation, and Percent Inaccuracy for TNT Extracted from 100 ml Water

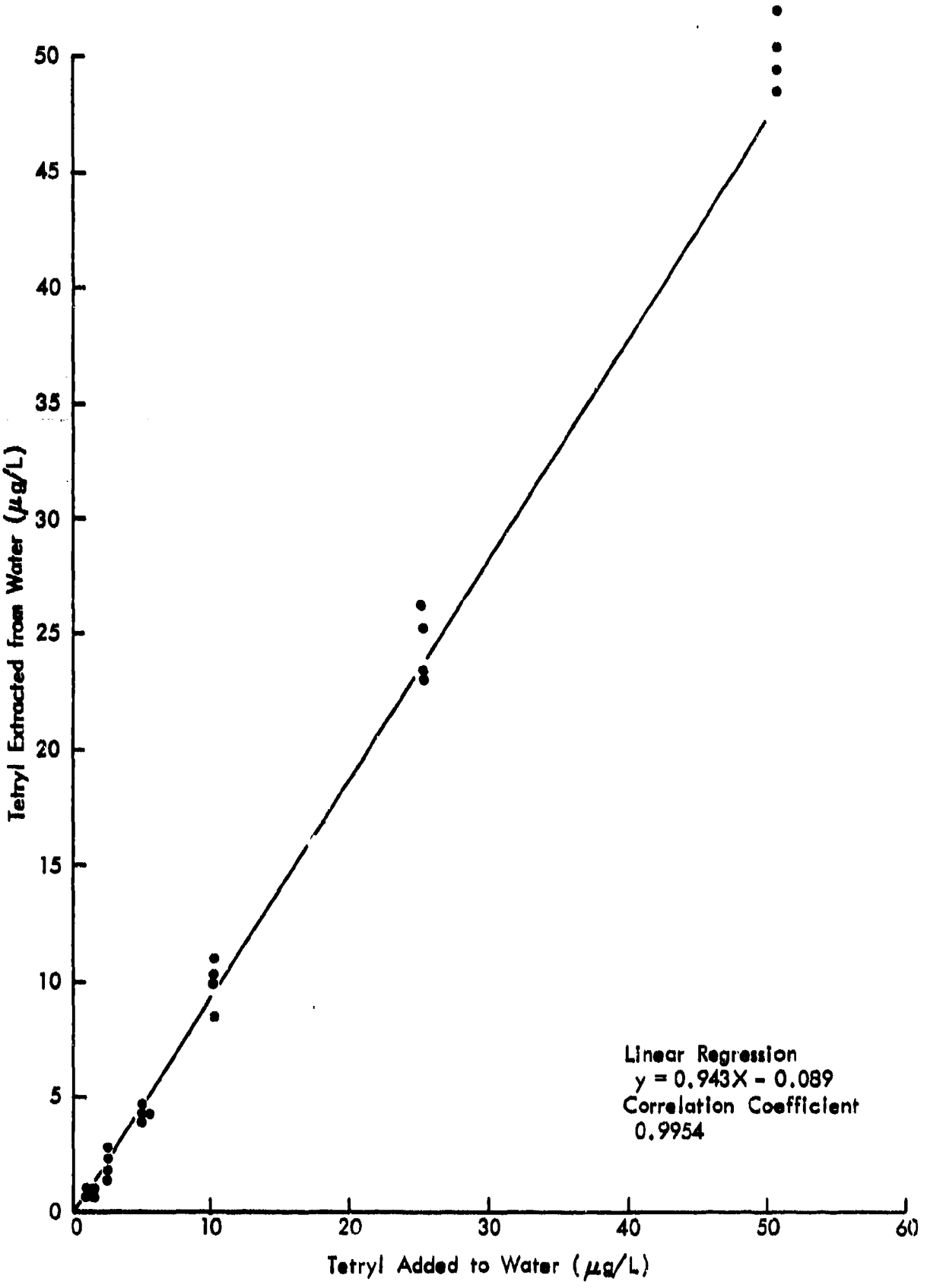


Figure 14 - Linearity of Tetryl Extracted from 100 ml Water

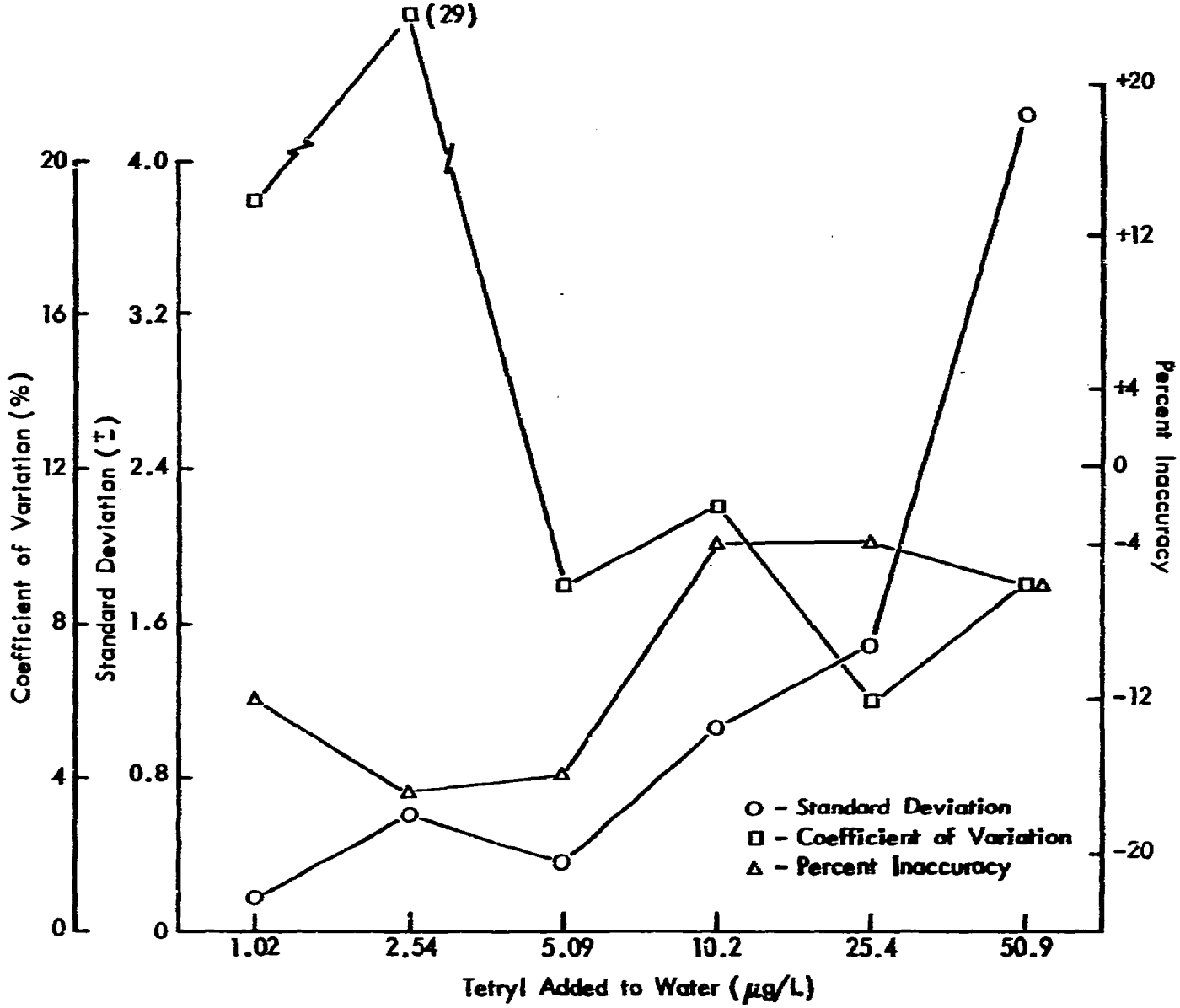


Figure 15 - Standard Deviation, Coefficient of Variation, and Percent Inaccuracy for Tetryl Extracted from 100 ml Water

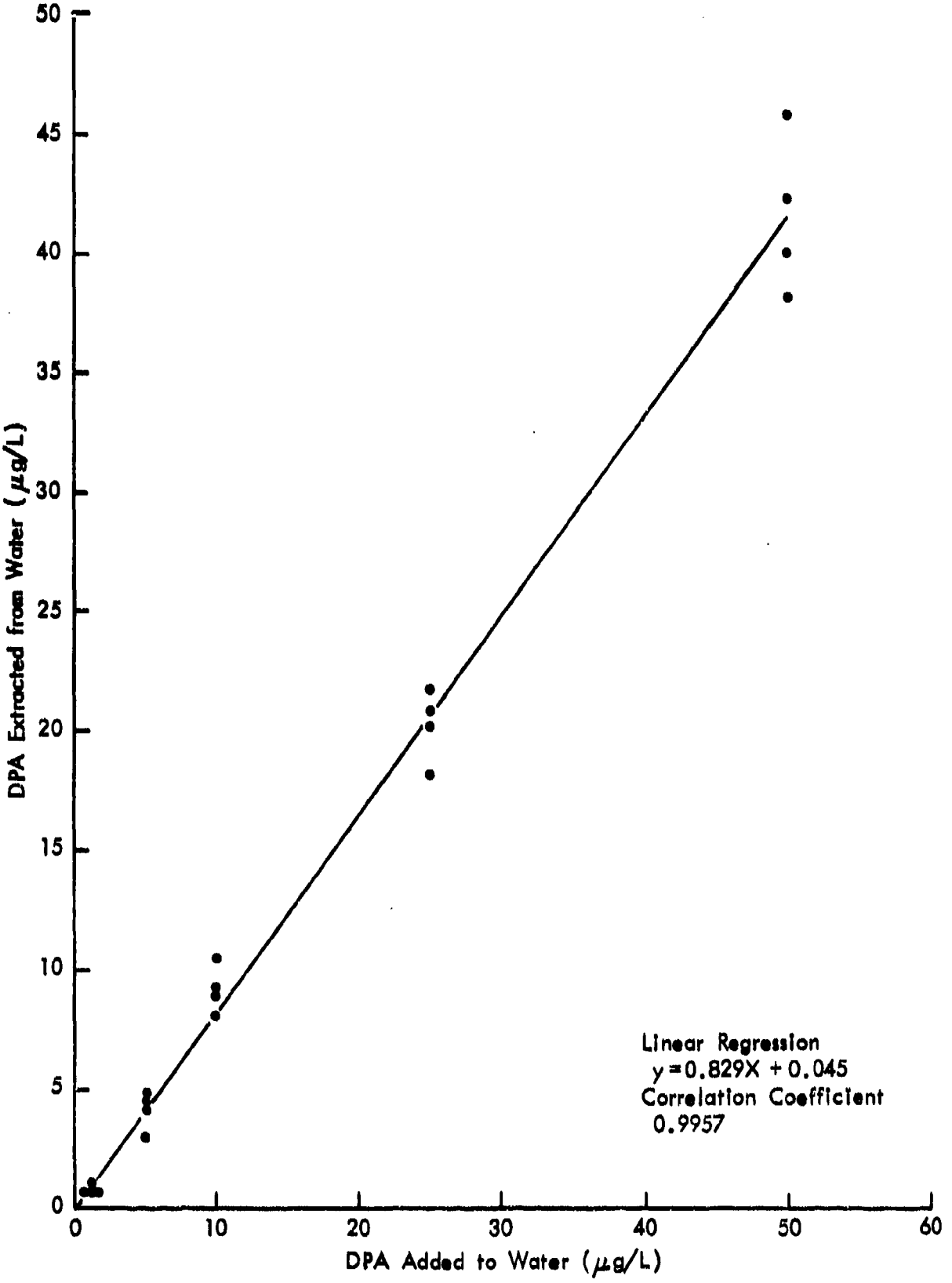


Figure 16 - Linearity of DPA Extracted from 100 ml Water

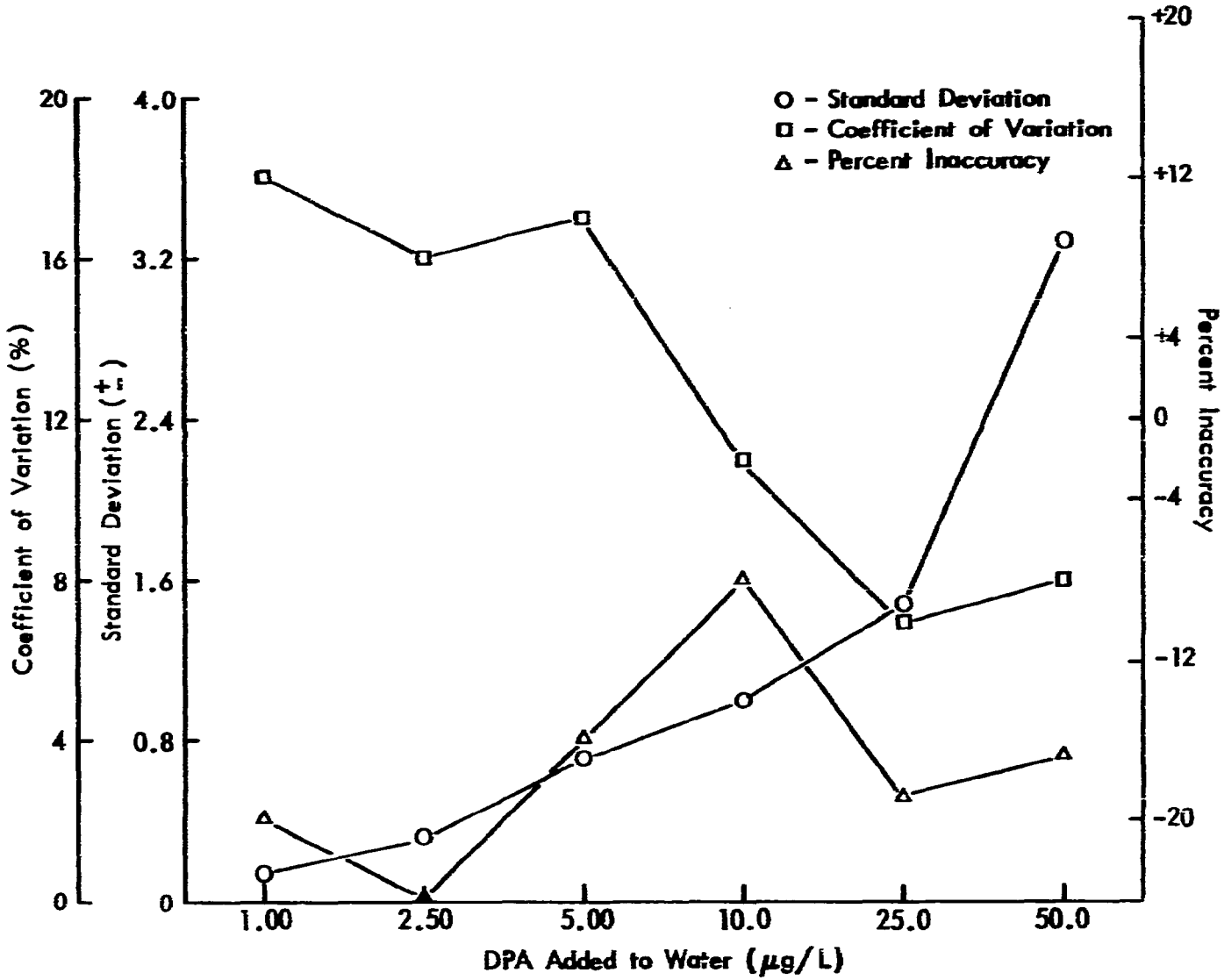


Figure 17 - Standard Deviation, Coefficinet of Variation, and Percent Inaccuracy for DPA Extracted from 100 ml Water

TABLE 2

STATISTICAL EVALUATION OF THE EXTRACTION FROM WATER AND SAMPLE PREPARATION OF DNP BY THE HUBAUX AND VOS RECOVERY DETECTION LIMIT PROGRAM

Number of[a] Data Points	Linear Regression	Correlation Coefficient	Degrees of Freedom	t[b]	y-Intercept[c]	Detection[d] Limit
28	y = 0.931x + 0.107	0.9981	26	1.706	1.10	2.12

µg/ℓ Water Added	Average[e] µg Found/Sample	Standard[f] Deviation	Percent[g] Imprecision	Percent[h] Inaccuracy
52.0	51.8	± 1.3	2.7	-7
26.0	26.4	± 1.1	4.4	-5
10.4	10.6	± 0.36	3.6	-4
5.20	4.91	± 0.17	3.7	-10
2.60	2.62	± 0.06	2.3	-2
1.04	1.04	± 0.03	2.7	+3
0	ND[i]	-	-	-

a Number of data points: data points utilized in calculation of the linear regresion equation and detection limits = 28, all data.
b t: 2-tail p level (usually 0.1, each confidence band is 0.05 so total p = 0.1).
c y-intercept: intercept on y-axis of upper confidence limit line.
d Detection limit: x-intercept of y-intercept and lower confidence limit line.
e Average µg/ℓ found: average at each of the seven levels determined from linear regression equation for each of the four found concentrations within each level.
f Standard deviation: determined from average value (e above) and observed values.
g Percent imprecision: standard deviation divided by average value times 100%.
h Percent inaccuracy: determined from the average values of the seven observed values at each level.

$$\% \text{ Inaccuracy} = \frac{\text{Average observed values - level added}}{\text{level added}} \times 100$$

i ND: not detectable, less than 0.2 µg/ℓ.

TABLE 3

STATISTICAL EVALUATION OF THE EXTRACTION FROM WATER AND SAMPLE PREPARATION OF RDX BY THE HUBAUX AND VOS RECOVERY DETECTION LIMIT PROGRAM

Number of[a] Data Points	Linear Regression	Correlation Coefficient	Degrees of Freedom	t[b]	y-Intercept[c]	Detection[d] Limit
28	y = 1.004x + 0.047	0.9972	26	1.706	1.54	2.96

µg/ℓ Water Added	Average[e] µg Found/Sample	Standard[f] Deviation	Percent[g] Imprecision	Percent[h] Inaccuracy
60.0	59.5	± 2.3	3.8	-1
30.0	31.1	± 0.95	3.0	+4
12.0	11.8	± 0.37	3.1	-1
6.00	5.45	± 0.18	3.2	-8
3.00	3.08	± 0.07	2.4	+5
1.20	1.22	± 0.06	4.3	+6
0	ND[i]	-	-	-

a Number of data points: data points utilized in calculation of the linear regresion equation and detection limits = 28, all data.

b t: 2-tail p level (usually 0.1, each confidence band is 0.05 so total p = 0.1).

c y-intercept: intercept on y-axis of upper confidence limit line.

d Detection limit: x-intercept of y-intercept and lower confidence limit line.

e Average µg/ℓ found: average at each of the seven levels determined from linear regression equation for each of the four found concentrations within each level.

f Standard deviation: determined from average value (e above) and observed values.

g Percent imprecision: standard deviation divided by average value times 100%.

h Percent inaccuracy: determined from the average values of the seven observed values at each level.

$$\% \text{ Inaccuracy} = \frac{\text{Average observed values - level added}}{\text{level added}} \times 100$$

i ND: not detectable, less than 0.2 µg/ℓ.

TABLE 4

STATISTICAL EVALUATION OF THE EXTRACTION FROM WATER AND SAMPLE PREPARATION OF TNB BY THE HUBAUX AND VOS RECOVERY DETECTION LIMIT PROGRAM

Number of[a] Data Points	Linear Regression	Correlation Coefficient	Degrees of Freedom	t[b]	y-Intercept[c]	Detection[d] Limit
28	y = 0.954x + 0.097	0.9988	26	1.706	0.88	1.64

µg/ℓ Water Added	Average[e] µg Found/Sample	Standard[f] Deviation	Percent[g] Imprecision	Percent[h] Inaccuracy
51.4	51.1	± 1.2	2.4	-5
25.7	26.3	± 0.46	1.8	-2
10.3	10.5	± 0.40	4.0	-2
5.14	4.80	± 0.15	3.2	-9
2.57	2.57	± 0.07	2.9	-1
1.03	1.02	± 0.03	2.7	+4
0	ND[i]	-	-	-

a Number of data points: data points utilized in calculation of the linear regresion equation and detection limits = 28, all data.

b t: 2-tail p level (usually 0.1, each confidence band is 0.05 so total p = 0.1).

c y-intercept: intercept on y-axis of upper confidence limit line.

d Detection limit: x-intercept of y-intercept and lower confidence limit line.

e Average µg/ℓ found: average at each of the seven levels determined from linear regression equation for each of the four found concentrations within each level.

f Standard deviation: determined from average value (e above) and observed values.

g Percent imprecision: standard deviation divided by average value times 100%.

h Percent inaccuracy: determined from the average values of the seven observed values at each level.

$$\% \text{ Inaccuracy} = \frac{\text{Average observed values - level added}}{\text{level added}} \times 100$$

i ND: not detectable, less than 0.2 µg/ℓ.

TABLE 5

STATISTICAL EVALUATION OF THE EXTRACTION FROM WATER AND SAMPLE PREPARATION OF DNB BY THE HUBAUX AND VOS RECOVERY DETECTION LIMIT PROGRAM

Number of[a] Data Points	Linear Regression	Correlation Coefficient	Degrees of Freedom	t[b]	y-Intercept[c]	Detection[d] Limit
28	y = 0.911x + 0.066	0.996	26	1.706	0.51	0.98

µg/ℓ Water Added	Average[e] µg Found/Sample	Standard[f] Deviation	Percent[g] Imprecision	Percent[h] Inaccuracy
50.1	49.9	± 0.32	0.7	-9
25.0	25.5	± 0.36	1.5	-7
10.0	10.2	± 0.47	5.0	-6
5.01	4.70	± 0.12	2.8	-13
2.50	2.51	± 0.10	4.3	-6
1.00	0.92	0	0	-10
0	ND[i]	-	-	-

a Number of data points: data points utilized in calculation of the linear regresion equation and detection limits = 28, all data.

b t: 2-tail p level (usually 0.1, each confidence band is 0.05 so total p = 0.1).

c y-intercept: intercept on y-axis of upper confidence limit line.

d Detection limit: x-intercept of y-intercept and lower confidence limit line.

e Average µg/ℓ found: average at each of the seven levels determined from linear regression equation for each of the four found concentrations within each level.

f Standard deviation: determined from average value (e above) and observed values.

g Percent imprecision: standard deviation divided by average value times 100%.

h Percent inaccuracy: determined from the average values of the seven observed values at each level.

$$\% \text{ Inaccuracy} = \frac{\text{Average observed values - level added}}{\text{level added}} \times 100$$

i ND: not detectable, less than 0.2 µg/ℓ.

TABLE 6

STATISTICAL EVALUATION OF THE EXTRACTION FROM WATER AND SAMPLE PREPARATION OF 2,4-DNT BY THE HUBAUX AND VOS RECOVERY DETECTION LIMIT PROGRAM

Number of[a] Data Points	Linear Regression	Correlation Coefficient	Degrees of Freedom	t[b]	y-Intercept[c]	Detection[d] Limit
28	y = 0.872x + 0.290	0.9987	26	1.706	1.04	1.70

µg/ℓ Water Added	Average[e] µg Found/Sample	Standard[f] Deviation	Percent[g] Imprecision	Percent[h] Inaccuracy
50.5	50.2	± 1.1	2.4	-13
25.2	25.2	± 0.46	2.1	-11
10.1	10.8	± 0.38	3.9	-4
5.05	4.88	± 0.18	3.9	-10
2.52	2.50	± 0.13	5.2	-2
1.01	0.81	± 0.05	4.7	-1
0	ND[i]	-	-	-

a Number of data points: data points utilized in calculation of the linear regresion equation and detection limits = 28, all data.
b t: 2-tail p level (usually 0.1, each confidence band is 0.05 so total p = 0.1).
c y-intercept: intercept on y-axis of upper confidence limit line.
d Detection limit: x-intercept of y-intercept and lower confidence limit line.
e Average µg/ℓ found: average at each of the seven levels determined from linear regression equation for each of the four found concentrations within each level.
f Standard deviation: determined from average value (e above) and observed values.
g Percent imprecision: standard deviation divided by average value times 100%.
h Percent inaccuracy: determined from the average values of the seven observed values at each level.

$$\% \text{ Inaccuracy} = \frac{\text{Average observed values - level added}}{\text{level added}} \times 100$$

i ND: not detectable, less than 0.2 µg/ℓ.

TABLE 7

STATISTICAL EVALUATION OF THE EXTRACTION FROM WATER AND SAMPLE PREPARATION OF TNT BY THE HUBAUX AND VOS RECOVERY DETECTION LIMIT PROGRAM

Number of[a] Data Points	Linear Regression	Correlation Coefficient	Degrees of Freedom	t[b]	y-Intercept[c]	Detection[d] Limit
28	y = 0.918x + 0.256	0.9986	26	1.706	1.07	1.76

µg/ℓ Water Added	Average[e] µg Found/Sample	Standard[f] Deviation	Percent[g] Imprecision	Percent[h] Inaccuracy
49.7	49.3	± 1.0	2.2	-8
24.8	25.5	± 0.75	3.2	-5
9.94	10.4	± 0.38	3.9	-1
4.97	4.73	± 0.16	3.5	-7
2.48	2.42	± 0.07	2.9	-1
0.99	0.81	± 0.05	4.7	+1
0	ND[i]	-	-	-

a Number of data points: data points utilized in calculation of the linear regresion equation and detection limits = 28, all data.
b t: 2-tail p level (usually 0.1, each confidence band is 0.05 so total p = 0.1).
c y-intercept: intercept on y-axis of upper confidence limit line.
d Detection limit: x-intercept of y-intercept and lower confidence limit line.
e Average µg/ℓ found: average at each of the seven levels determined from linear regression equation for each of the four found concentrations within each level.
f Standard deviation: determined from average value (e above) and observed values.
g Percent imprecision: standard deviation divided by average value times 100%.
h Percent inaccuracy: determined from the average values of the seven observed values at each level.

$$\% \text{ Inaccuracy} = \frac{\text{Average observed values - level added}}{\text{level added}} \times 100$$

i ND: not detectable, less than 0.2 µg/ℓ.

TABLE 8

STATISTICAL EVALUATION OF THE EXTRACTION FROM WATER AND SAMPLE PREPARATION OF TETRYL BY THE HUBAUX AND VOS RECOVERY DETECTION LIMIT PROGRAM

Number of[a] Data Points	Linear Regression	Correlation Coefficient	Degrees of Freedom	t[b]	y-Intercept[c]	Detection[d] Limit
28	y = 0.934x - 0.089	0.9953	26	1.706	1.45	3.24

µg/ℓ Water Added	Average[e] µg Found/Sample	Standard[f] Deviation	Percent[g] Imprecision	Percent[h] Inaccuracy
50.9	50.6	± 2.5	5.2	-7
25.4	26.1	± 0.86	3.5	-4
10.2	10.5	± 0.60	6.1	-4
5.09	4.63	± 0.21	4.9	-16
2.54	2.30	± 0.35	16.9	-18
1.02	1.00	± 0.10	11.7	-17
0	ND[i]	-	-	-

a Number of data points: data points utilized in calculation of the linear regresion equation and detection limits = 28, all data.
b t: 2-tail p level (usually 0.1, each confidence band is 0.05 so total p = 0.1).
c y-intercept: intercept on y-axis of upper confidence limit line.
d Detection limit: x-intercept of y-intercept and lower confidence limit line.
e Average µg/ℓ found: average at each of the seven levels determined from linear regression equation for each of the four found concentrations within each level.
f Standard deviation: determined from average value (e above) and observed values.
g Percent imprecision: standard deviation divided by average value times 100%.
h Percent inaccuracy: determined from the average values of the seven observed values at each level.

$$\% \text{ Inaccuracy} = \frac{\text{Average observed values - level added}}{\text{level added}} \times 100$$

i ND: not detectable, less than 0.2 µg/ℓ.

TABLE 9

STATISTICAL EVALUATION OF THE EXTRACTION FROM WATER AND SAMPLE PREPARATION OF DPA BY THE HUBAUX AND VOS RECOVERY DETECTION LIMIT PROGRAM

Number of[a] Data Points	Linear Regression	Correlation Coefficient	Degrees of Freedom	t[b]	y-Intercept[c]	Detection[d] Limit
28	y = 0.829x + 0.045	0.9956	26	1.706	1.33	3.09

µg/100 ml Water Added	Average[e] µg Found/Sample	Standard[f] Deviation	Percent[g] Imprecision	Percent[h] Inaccuracy
50.0	50.1	± 1.9	4.6	-17
25.0	24.4	± 0.86	4.2	-19
10.0	11.0	± 0.57	6.2	-8
5.00	4.95	± 0.41	10.0	-17
2.50	2.18	± 0.17	9.4	-26
1.00	0.91	± 0.08	10.2	-20
0	ND[i]	-	-	-

a Number of data points: data points utilized in calculation of the linear regresion equation and detection limits = 28, all data.

b t: 2-tail p level (usually 0.1, each confidence band is 0.05 so total p = 0.1).

c y-intercept: intercept on y-axis of upper confidence limit line.

d Detection limit: x-intercept of y-intercept and lower confidence limit line.

e Average µg/ℓ found: average at each of the seven levels determined from linear regression equation for each of the four found concentrations within each level.

f Standard deviation: determined from average value (e above) and observed values.

g Percent imprecision: standard deviation divided by average value times 100%.

h Percent inaccuracy: determined from the average values of the seven observed values at each level.

$$\% \text{ Inaccuracy} = \frac{\text{Average observed values - level added}}{\text{level added}} \times 100$$

i ND: not detectable, less than 0.2 µg/ℓ.

TABLE 10

EXTRACTION SAMPLE SOLUTIONS AND REFERENCE SOLUTIONS OF DNP FOR THE PRECISION AND ACCURACY DETERMINATION OF THE EXTRACTION FROM WATER AND SAMPLE PREPARATION METHOD

Extraction Sample Solutions

Water Extract Sample Number	µg/100 ml[a] Water Added	Peak Heights[b] DNP	Peak Heights[b] IS	µg IS[c]	µg Found/Sample[d]	µg/ℓ (ppb)[e]
A-10	5.20	95.9	40.1	2.22	4.66	46.6
A-5	2.60	110.4	97.0	2.22	2.22	22.2
A-2	1.04	105.0	193.8	2.22	1.06	10.6
A-1	0.520	83.1	38.0	0.222	0.430	4.30
A-0.5	0.260	45.1	35.7	0.222	0.250	2.50
A-0.2	0.104	21.0	37.6	0.222	0.110	1.10
A-0	-	< 2	32.0	0.222	< 0.02	< 0.2
B-10	5.20	91.2	38.8	2.22	4.66	46.6
B-5	2.60	136.2	111.0	2.22	2.43	24.3
B-2	1.04	98.0	186.0	2.22	1.04	10.4
B-1	0.520	88.0	38.0	0.222	0.460	4.60
B-0.5	0.260	50.0	40.0	0.222	0.250	2.50
B-0.2	0.104	20.5	37.8	0.222	0.110	1.10
B-0	-	< 2	34.5	0.222	< 0.02	< 0.2
C-10	5.20	98.6	42.0	2.22	4.87	48.7
C-5	2.60	123.5	99.0	2.22	2.58	25.8
C-2	1.04	81.7	183.1	2.22	0.930	9.30
C-0.5	0.520	87.2	38.0	0.222	0.480	4.80
C-0.5	0.260	41.0	34.0	0.222	0.250	2.50
C-0.2	0.104	24.5	46.0	0.222	0.110	1.10
C-0	-	< 2	38.0	0.222	< 0.02	< 0.2
D-10	5.20	114.0	46.0	2.22	5.14	51.4
D-5	2.60	122.3	96.0	2.22	2.64	26.4
D-2	1.04	92.7	200.9	2.22	0.960	9.60
D-1	0.520	80.2	33.2	0.222	0.500	5.00
D-0.5	0.260	49.0	37.7	0.222	0.270	2.70
D-0.2	0.104	20.5	42.0	0.222	0.100	1.00
D-0	-	< 2	37.0	0.222	< 0.02	< 0.2

TABLE 10 (continued)

Reference Solutions

Reference Solution Number	ng Added[f]	Peak Heights[b] DNP	IS	ng IS[c]	RWR[g]	Avg. RWR, S.D., R.S.D.[h]
A-5	2,600	141.0	107.8	2,220	1.12	
A'-5	2,600	142.1	110.3	2,220	1.10	A
A-1	520	77.9	30.2	222	1.10	RWR 1.14
A'-1	520	94.0	38.0	222	1.06	S.D. ± 0.07
A-0.2	104	23.0	40.0	222	1.23	R.S.D. 6.3%
A'-0.2	104	24.9	43.2	222	1.23	
B-5	2,600	138.2	106.5	2,220	1.11	
B'-5	2,600	143.5	106.8	2,220	1.15	B
B-1	520	96.8	39.8	222	1.04	RWR 1.12
B'-1	520	96.2	41.5	222	0.99	S.D. ± 0.10
B-0.2	104	21.8	39.0	222	1.19	R.S.D. 8.9%
B'-0.2	104	46.2	78.5	222	1.26	
C-5	2,600	131.2	103.0	2,220	1.09	
C'-5	2,600	128.8	100.0	2,220	1.10	C
C-1	520	96.2	41.0	222	1.00	$\bar{x}$ RWR 1.07
C'-1	520	95.8	38.8	222	1.05	S.D. ± 0.04
C-0.2	104	20.8	40.1	222	1.11	R.S.D. 3.8%
C'-0.2	104	21.0	42.2	222	1.06	
D-5	2,600	134.0	101.0	2,220	1.13	
D'-5	2,600	135.8	102.6	2,220	1.13	D
D-1	520	95.0	40.0	222	1.01	RWR 1.07
D'-1	104	91.2	39.0	222	1.00	S.D. ± 0.06
D-0.2	104	22.2	44.2	222	1.07	R.S.D. 5.4%
D'-0.2	104	22.0	42.5	222	1.10	

TABLE 10 (concluded)

a µg/100 ml Water added: µg of DNP added to 100 ml water.
b Peak heights: measured height of DNP and IS in millimeters.
c µg IS: µg IS present in the ~ 2 ml final sample.
d µg Found/sample: µg DNP recovered from 100 ml water.

$$\frac{\text{µg Found}}{\text{Sample}} = \frac{\text{Peak height DNP}}{\text{Peak height IS}} \times \frac{\text{µg IS/}\sim\text{ 2 ml sample}}{\text{Average RWR for DNP}}$$

e µg/ℓ (ppb): µg found in 100 ml sample extract equated to µg/ℓ.
f Added: nanograms of compound added to reference solution (~ 2 ml).

g RWR: relative weight response $= \frac{\text{Peak height DNP}}{\text{Peak height IS}} \times \frac{\text{µg IS/reference solution}}{\text{µg DNP/reference solution}}$

h Avg: average RWR $= \frac{\Sigma\text{RWR's}}{\text{Number of reference solutions (n)}}$

S.D.: standard deviation $= \left(\frac{n\Sigma RWR^2 - (\Sigma RWR)^2}{n(n-1)}\right)^{\frac{1}{2}}$

R.S.D.: relative standard deviation $= \frac{\text{S.D.}}{\text{Avg RWR}} \times 100$

TABLE 11

EXTRACTION SAMPLE SOLUTIONS AND REFERENCE SOLUTIONS OF RDX FOR THE PRECISION AND ACCURACY DETERMINATION OF THE EXTRACTION FROM WATER AND SAMPLE PREPARATION METHOD

Extraction Sample Solutions

Water Extract Sample Number	µg/100 ml[a] Water Added	Peak Heights[b] RDX	Peak Heights[b] IS	µg IS[c]	µg Found/Sample[d]	µg/ℓ (ppb)[e]
A-10	6.00	64.5	40.1	2.22	5.49	54.9
A-5	3.00	83.1	97.0	2.22	2.93	29.3
A-2	1.20	69.0	193.8	2.22	1.22	12.2
A-1	0.600	57.0	38.0	0.222	0.510	5.10
A-0.5	0.300	31.1	35.7	0.222	0.300	3.00
A-0.2	0.120	14.0	37.6	0.222	0.130	1.30
A-0	-	< 2	32.0	0.222	< 0.02	< 0.2
B-10	6.00	63.8	38.8	2.22	5.89	58.9
B-5	3.00	95.8	111.0	2.22	3.09	30.9
B-2	1.20	66.0	186.0	2.22	1.27	12.7
B-1	0.600	60.5	38.0	0.222	0.570	5.70
B-0.5	0.300	35.0	40.0	0.222	0.310	3.10
B-0.2	0.120	14.5	37.8	0.222	0.140	1.40
B-0	-	< 2	34.5	0.222	< 0.02	< 0.2
C-10	6.00	68.2	42.0	2.22	6.44	64.4
C-5	3.00	83.0	99.0	2.22	3.32	33.2
C-2	1.20	53.0	183.1	2.22	1.15	11.5
C-1	0.600	55.5	38.0	0.222	0.580	5.80
C-0.5	0.300	28.0	34.0	0.222	0.330	3.30
C-0.2	0.120	13.8	46.0	0.222	0.120	1.20
C-0	-	< 2	38.0	0.222	< 0.02	< 0.2
D-10	6.00	76.0	46.0	2.22	6.11	61.1
D-5	3.00	82.9	96.0	2.22	3.19	31.9
D-2	1.20	61.2	200.9	2.22	1.13	11.3
D-1	0.600	49.1	33.2	0.222	0.550	5.50
D-0.5	0.300	32.1	37.7	0.222	0.320	3.20
D-0.2	0.120	14.2	42.0	0.222	0.120	1.20
D-0	-	< 2	37.0	0.222	< 0.02	< 0.2

TABLE 11 (continued)

Reference Solutions

Reference Solution Number	ng Added[f]	Peak Heights[b] RDX	Peak Heights[b] IS	ng IS[c]	RWR[g]	Avg. RWR, S.D., R.S.D.[h]
A-5	3,000	93.8	107.8	2,220	0.64	
A'-5	3,000	93.5	110.3	2,220	0.63	A
A-1	600	60.5	38.0	222	0.59	RWR 0.65
A'-1	600	49.9	30.2	222	0.61	S.D. ± 0.06
A-0.2	120	15.9	40.0	222	0.74	R.S.D. 8.5%
A'-0.2	120	16.2	43.2	222	0.69	
B-5	3,000	93.8	106.5	2,220	0.65	
B'-5	3,000	94.5	106.8	2,220	0.65	B
B-1	600	62.5	39.8	222	0.58	RWR 0.62
B'-1	600	63.0	41.5	222	0.56	S.D. ± 0.04
B-0.2	120	27.2	78.5	222	0.64	R.S.D. 7.2%
B'-0.2	120	14.2	39.0	222	0.67	
C-5	3,000	85.5	103.0	2,220	0.61	
C'-5	3,000	83.0	100.0	2,220	0.61	C
C-1	600	56.5	41.0	222	0.51	RWR 0.56
C'-1	600	58.0	38.8	222	0.55	S.D. ± 0.04
C-0.2	120	12.0	40.1	222	0.55	R.S.D. 7.2%
C'-0.2	120	12.3	42.2	222	0.54	
D-5	3,000	86.8	101.0	2,220	0.64	
D'-5	3,000	87.8	102.6	2,220	0.63	D
D-1	600	58.9	40.0	222	0.54	RWR 0.60
D'-1	600	56.9	39.0	222	0.54	S.D. ± 0.05
D-0.2	120	15.0	44.2	222	0.63	R.S.D. 7.7%
D'-0.2	120	14.0	42.5	222	0.61	

TABLE 11 (concluded)

a µg/100 ml Water added: µg of RDX added to 100 ml water.
b Peak heights: measured height of RDX and IS in millimeters.
c µg IS: µg IS present in the ~ 2 ml final sample.
d µg Found/sample: µg RDX recovered from 100 ml water.

$$\frac{\text{µg Found}}{\text{Sample}} = \frac{\text{Peak height RDX}}{\text{Peak height IS}} \times \frac{\text{µg IS/}\sim\text{ 2 ml sample}}{\text{Average RWR for RDX}}$$

e µg/ℓ (ppb): µg found in 100 ml sample extract equated to µg/ℓ.
f Added: nanograms of compound added to reference solution (~ 2 ml).

g RWR: relative weight response $= \frac{\text{Peak height RDX}}{\text{Peak height IS}} \times \frac{\text{µg IS/reference solution}}{\text{µg RDX/reference solution}}$

h Avg: average RWR $= \frac{\Sigma \text{RWR's}}{\text{Number of reference solutions (n)}}$

S.D.: standard deviation $= \left(\frac{n\Sigma RWR^2 - (\Sigma RWR)^2}{n(n-1)}\right)^{\frac{1}{2}}$

R.S.D.: relative standard deviation $= \frac{\text{S.D.}}{\text{Avg RWR}} \times 100$

TABLE 12

EXTRACTION SAMPLE SOLUTIONS AND REFERENCE SOLUTIONS OF TNB FOR THE PRECISION AND ACCURACY DETERMINATION OF THE EXTRACTION FROM WATER AND SAMPLE PREPARATION METHOD

Extraction Sample Solutions

Water Extract Sample Number	µg/100 ml[a] Water Added	Peak Heights[b] TNB	Peak Heights[b] IS	µg IS[c]	µg Found/Sample[d]	µg/ℓ (ppb)[e]
A-10	5.14	105.9	40.1	2.22	4.65	46.5
A-5	2.57	135.1	97.0	2.22	2.45	24.5
A-2	1.03	112.1	193.8	2.22	1.02	10.2
A-1	0.514	93.4	38.0	0.222	0.430	4.30
A-0.5	0.257	50.8	35.7	0.222	0.250	2.50
A-0.2	0.103	23.0	37.6	0.222	0.110	1.10
A-0	-	< 2	32.0	0.222	< 0.02	< 0.2
B-10	5.14	100.8	38.8	2.22	4.81	48.1
B-5	2.57	147.8	111.0	2.22	2.46	24.6
B-2	1.03	111.0	186.0	2.22	1.10	11.0
B-1	0.514	97.0	38.0	0.222	0.470	4.70
B-0.5	0.257	51.2	40.0	0.222	0.240	2.40
B-0.2	0.103	22.0	37.8	0.222	0.110	1.10
B-0	-	< 2	34.5	0.222	< 0.02	< 0.2
C-10	5.14	112.0	42.0	2.22	4.97	49.7
C-5	2.57	135.1	99.0	2.22	2.54	25.4
C-2	1.03	92.3	183.1	2.22	0.940	9.40
C-1	0.514	98.0	38.0	0.222	0.480	4.80
C-0.5	0.257	49.8	34.0	0.222	0.270	2.70
C-0.2	0.103	25.0	46.0	0.222	0.100	1.00
C-0	-	< 2	38.0	0.222	< 0.02	< 0.2
D-10	5.14	121.0	46.0	2.22	5.12	51.2
D-5	2.57	129.3	96.0	2.22	2.62	26.2
D-2	1.03	100.4	200.9	2.22	0.970	9.70
D-1	0.514	83.3	33.2	0.222	0.490	4.90
D-0.5	0.257	50.1	37.7	0.222	0.260	2.60
D-0.2	0.103	23.0	42.0	0.222	0.110	1.10
D-0	-	< 2	37.0	0.222	< 0.02	< 0.2

TABLE 12 (continued)

Reference Solutions

Reference Solution Number	ng Added[f]	Peak Heights[b] TNB	IS	ng IS[c]	RWR[g]	Avg. RWR, S.D., R.S.D.[h]
A-5	2,570	156.2	107.8	2,220	1.25	
A'-5	2,570	160.0	110.3	2,220	1.25	A
A-1	514	101.0	38.0	222	1.15	RWR 1.26
A'-1	514	85.2	30.2	222	1.22	S.D. ± 0.08
A-0.2	103	25.0	40.0	222	1.35	R.S.D. 6.2%
A'-0.2	103	27.1	43.2	222	1.35	
B-5	2,570	153.0	106.5	2,220	1.24	
B'-5	2,570	146.0	106.8	2,220	1.18	B
B-1	514	107.2	39.8	222	1.16	RWR 1.20
B'-1	514	105.5	41.5	222	1.10	S.D. ± 0.06
B-0.2	103	23.2	39.0	222	1.28	R.S.D. 5.3%
B'-0.2	103	44.5	78.5	222	1.22	
C-5	2,570	147.1	103.0	2,220	1.23	
C'-5	2,570	139.1	100.0	2,220	1.20	C
C-1	514	108.0	41.0	222	1.14	RWR 1.19
C'-1	514	106.9	38.8	222	1.19	S.D. ± 0.04
C-0.2	103	22.3	42.2	222	1.14	R.S.D. 3.6%
C'-0.2	103	23.0	40.1	222	1.24	
D-5	2,570	142.0	101.0	2,220	1.21	
D'-5	2,570	144.2	102.6	2,220	1.21	D
D-1	514	104.9	40.0	222	1.13	RWR 1.14
D'-1	514	102.0	39.0	222	1.13	S.D. ± 0.06
D-0.2	103	22.4	44.2	222	1.09	R.S.D. 5.2%
D'-0.2	103	21.1	42.5	222	1.07	

TABLE 12 (concluded)

a µg/100 ml Water added: µg of TNB added to 100 ml water.
b Peak heights: measured height of TNB and IS in millimeters.
c µg IS: µg IS present in the ~ 2 ml final sample.
d µg Found/sample: µg TNB recovered from 100 ml water.

$$\frac{\text{µg Found}}{\text{Sample}} = \frac{\text{Peak height TNB}}{\text{Peak height IS}} \times \frac{\text{µg IS/}\sim\text{ 2 ml sample}}{\text{Average RWR for TNB}}$$

e µg/ℓ (ppb): µg found in 100 ml sample extract equated to µg/ℓ.
f Added: nanograms of compound added to reference solution (~ 2 ml).

g RWR: relative weight response $= \frac{\text{Peak height TNB}}{\text{Peak height IS}} \times \frac{\text{µg IS/reference solution}}{\text{µg TNB/reference solution}}$

h Avg: average RWR $= \frac{\Sigma\text{RWR's}}{\text{Number of reference solutions (n)}}$

S.D.: standard deviation $= \left(\frac{n\Sigma RWR^2 - (\Sigma RWR)^2}{n(n-1)}\right)^{\frac{1}{2}}$

R.S.D.: relative standard deviation $= \frac{\text{S.D.}}{\text{Avg RWR}} \times 100$

TABLE 13

EXTRACTION SAMPLE SOLUTIONS AND REFERENCE SOLUTIONS OF DNB FOR THE PRECISION AND ACCURACY DETERMINATION OF THE EXTRACTION FROM WATER AND SAMPLE PREPARATION METHOD

Extraction Sample Solutions

Water Extract Sample Number	µg/100 ml[a] Water Added	Peak Heights[b] DNB	Peak Heights[b] IS	µg IS[c]	µg Found/Sample[d]	µg/ℓ (ppb)[e]
A-10	5.01	146.9	40.1	2.22	4.59	45.9
A-5	2.50	187.0	97.0	2.22	2.42	24.2
A-2	1.00	149.3	193.8	2.22	0.970	9.70
A-1	0.501	123.0	38.0	0.222	0.410	4.10
A-0.5	0.250	66.9	35.7	0.222	0.240	2.40
A-0.2	0.100	28.0	37.6	0.222	0.090	0.900
A-0	-	< 2	32.0	0.222	< 0.02	< 0.2
B-10	5.01	136.2	38.8	2.22	4.48	44.8
B-5	2.50	199.0	111.0	2.22	2.29	22.9
B-2	1.00	151.0	186.0	2.22	1.04	10.4
B-1	0.501	129.0	38.0	0.222	0.430	4.30
B-0.5	0.250	66.0	40.0	0.222	0.210	2.10
B-0.2	0.100	26.0	37.8	0.222	0.090	0.900
B-0	-	< 2	34.5	0.222	< 0.02	< 0.2
C-10	5.01	151.0	42.0	2.22	4.53	45.3
C-5	2.50	180.0	99.0	2.22	2.29	22.9
C-2	1.00	124.2	183.1	2.22	0.860	8.60
C-1	0.501	131.0	38.0	0.222	0.440	4.40
C-0.5	0.250	67.2	34.0	0.222	0.250	2.50
C-0.2	0.100	34.0	46.0	0.222	0.090	0.900
C-0	-	< 2	38.0	0.222	< 0.02	< 0.2
D-10	5.01	161.1	46.0	2.22	4.60	46.0
D-5	2.50	169.0	96.0	2.22	2.31	23.1
D-2	1.00	136.3	200.9	2.22	0.890	8.90
D-1	0.501	116.3	33.2	0.222	0.460	4.60
D-0.5	0.250	69.3	37.7	0.222	0.240	2.40
D-0.2	0.100	27.8	42.0	0.222	0.090	0.900
D-0	-	< 2	37.0	0.222	< 0.02	< 0.2

TABLE 13 (continued)

Reference Solutions

Reference Solution Number	ng Added[f]	Peak Heights[b] DNB	IS	ng IS[c]	RWR[g]	Avg. RWR, S.D., R.S.D.[h]
A-5	2,500	207.0	107.8	2,220	1.70	
A'-5	2,500	211.9	110.3	2,220	1.70	A
A-1	501	144.0	38.0	222	1.68	RWR 1.77
A'-1	501	120.9	30.2	222	1.77	S.D. ± 0.10
A-0.2	100	35.3	40.0	222	1.96	R.S.D. 5.9%
A'-0.2	100	35.1	43.2	222	1.80	
B-5	2,500	212.0	106.5	2,220	1.77	
B'-5	2,500	212.2	106.8	2,220	1.76	B
B-1	501	144.2	39.8	222	1.60	RWR 1.74
B'-1	501	151.2	41.5	222	1.61	S.D. ± 0.11
B-0.2	100	32.2	39.0	222	1.83	R.S.D. 6.2%
B'-0.2	100	55.5	78.5	222	1.85	
C-5	2,500	204.5	103.0	2,220	1.76	
C'-5	2,500	203.3	100.0	2,220	1.80	C
C-1	501	157.3	41.0	222	1.70	RWR 1.76
C'-1	501	153.0	38.8	222	1.75	S.D. ± 0.04
C-0.2	100	32.0	40.1	222	1.77	R.S.D. 2.0%
C'-0.2	100	34.0	42.2	222	1.79	
D-5	2,500	202.3	101.0	2,220	1.78	
D'-5	2,500	205.0	102.6	2,220	1.77	D
D-1	501	146.7	40.0	222	1.62	RWR 1.69
D'-1	501	145.8	39.0	222	1.66	S.D. ± 0.06
D-0.2	100	33.0	44.2	222	1.66	R.S.D. 3.9%
D'-0.2	100	32.0	42.5	222	1.67	

TABLE 13 (concluded)

a µg/100 ml Water added: µg of DNB added to 100 ml water.
b Peak heights: measured height of DNB and IS in millimeters.
c µg IS: µg IS present in the ~ 2 ml final sample.
d µg Found/sample: µg DNB recovered from 100 ml water.

$$\frac{\text{µg Found}}{\text{Sample}} = \frac{\text{Peak height DNB}}{\text{Peak height IS}} \times \frac{\text{µg IS/}\sim\text{ 2 ml sample}}{\text{Average RWR for DNB}}$$

e µg/ℓ (ppb): µg found in 100 ml sample extract equated to µg/ℓ.
f Added: nanograms of compound added to reference solution (~ 2 ml).

g RWR: relative weight response $= \frac{\text{Peak height DNB}}{\text{Peak height IS}} \times \frac{\text{µg IS/reference solution}}{\text{µg DNB/reference solution}}$

h Avg: average RWR $= \frac{\Sigma\text{RWR's}}{\text{Number of reference solutions (n)}}$

S.D.: standard deviation $= \left(\frac{n\Sigma RWR^2 - (\Sigma RWR)^2}{n(n-1)}\right)^{\frac{1}{2}}$

R.S.D.: relative standard deviation $= \frac{\text{S.D.}}{\text{Avg RWR}} \times 100$

TABLE 14

EXTRACTION SAMPLE SOLUTIONS AND REFERENCE SOLUTIONS OF 2,4-DNT FOR THE PRECISION AND ACCURACY DETERMINATION OF THE EXTRACTION FROM WATER AND SAMPLE PREPARATION METHOD

Extraction Sample Solutions

Water Extract Sample Number	µg/100 ml[a] Water Added	Peak Heights[b] 2,4-DNT	Peak Heights[b] IS	µg IS[c]	µg Found/Sample[d]	µg/ℓ (ppb)[e]
A-10	5.05	115.3	40.1	2.22	4.17	41.7
A-5	2.52	144.8	97.0	2.22	2.16	21.6
A-2	1.01	133.0	193.8	2.22	1.00	10.0
A-1	0.505	109.0	38.0	0.222	0.420	4.20
A-0.5	0.252	59.8	35.7	0.222	0.240	2.40
A-0.2	0.101	27.0	37.6	0.222	0.100	1.00
A-0	-	< 2	32.0	0.222	< 0.02	< 0.2
B-10	5.05	120.8	38.8	2.22	4.46	44.6
B-5	2.52	173.0	111.0	2.22	2.23	22.3
B-2	1.01	135.0	186.0	2.22	1.04	10.4
B-1	0.505	118.0	38.0	0.222	0.440	4.40
B-0.5	0.252	64.9	40.0	0.222	0.230	2.30
B-0.2	0.101	28.5	37.8	0.222	0.110	1.10
B-0	-	< 2	34.5	0.222	< 0.02	< 0.2
C-10	5.05	118.3	42.0	2.22	4.40	44.0
C-5	2.52	144.2	99.0	2.22	2.28	22.8
C-2	1.01	104.2	183.1	2.22	0.890	8.90
C-1	0.505	113.4	38.0	0.222	0.470	4.70
C-0.5	0.252	59.9	34.0	0.222	0.280	2.80
C-0.2	0.101	28.5	46.0	0.222	0.100	1.00
C-0	-	< 2	38.0	0.222	< 0.02	< 0.2
D-10	5.05	130.0	46.0	2.22	4.61	46.1
D-5	2.52	138.1	96.0	2.22	2.35	23.5
D-2	1.01	115.3	200.9	2.22	0.940	9.40
D-1	0.505	99.2	33.2	0.222	0.490	4.90
D-0.5	0.252	56.0	37.7	0.222	0.240	2.40
D-0.2	0.101	22.3	42.0	0.222	0.090	0.90
D-0	-	< 2	37.0	0.222	< 0.02	< 0.2

TABLE 14 (continued)

Reference Solutions

Reference Solution Number	ng Added[f]	Peak Heights[b] 2,4-DNT	IS	ng IS[c]	RWR[g]	Avg. RWR, S.D., R.S.D.[h]
A-5	2,520	175.9	107.8	2,220	1.44	
A'-5	2,520	179.8	110.3	2,220	1.44	A
A-1	505	103.1	30.2	222	1.50	RWR 1.53
A'-1	505	127.0	38.0	222	1.47	S.D. ± 0.12
A-0.2	101	31.4	40.0	222	1.72	R.S.D. 7.6%
A'-0.2	101	32.1	43.2	222	1.63	
B-5	2,520	181.8	106.5	2,220	1.50	
B'-5	2,520	182.2	106.8	2,220	1.50	B
B-1	505	140.8	41.5	222	1.49	RWR 1.55
B'-1	505	131.5	39.8	222	1.45	S.D. ± 0.12
B-0.2	101	28.8	39.0	222	1.62	R.S.D. 7.5%
B'-0.2	101	62.8	78.5	222	1.76	
C-5	2,520	169.6	103.0	2,220	1.45	
C'-5	2,520	162.3	100.0	2,220	1.43	C
C-1	505	131.1	41.0	222	1.40	RWR 1.42
C'-1	505	127.3	38.8	222	1.44	S.D. ± 0.04
C-0.2	101	26.7	40.1	222	1.46	R.S.D. 2.9%
C'-0.2	101	26.0	42.2	222	1.35	
D-5	2,520	160.6	101.0	2,220	1.40	
D'-5	2,520	159.8	102.6	2,220	1.37	D
D-1	505	126.0	40.0	222	1.38	RWR 1.36
D'-1	505	116.0	39.0	222	1.31	S.D. ± 0.06
D-0.2	101	28.5	44.2	222	1.42	R.S.D. 4.5%
D'-0.2	101	24.6	42.5	222	1.27	

TABLE 14 (concluded)

a µg/100 ml Water added: µg of 2,4-DNT added to 100 ml water.
b Peak heights: measured height of 2,4-DNT and IS in millimeters.
c µg IS: µg IS present in the ~ 2 ml final sample.
d µg Found/sample: µg 2,4-DNT recovered from 100 ml water.

$$\frac{\mu g\ Found}{Sample} = \frac{Peak\ height\ 2,4\text{-}DNT}{Peak\ height\ IS} \times \frac{\mu g\ IS/\sim 2\ ml\ sample}{Average\ RWR\ for\ 2,4\text{-}DNT}$$

e µg/ℓ (ppb): µg found in 100 ml sample extract equated to µg/ℓ.
f Added: nanograms of compound added to reference solution (~ 2 ml).

g RWR: relative weight response $= \frac{Peak\ height\ 2,4\text{-}DNT}{Peak\ height\ IS} \times \frac{\mu g\ IS/reference\ solution}{\mu g\ 2,4\text{-}DNT/reference\ solution}$

h Avg: average RWR $= \frac{\Sigma RWR's}{Number\ of\ reference\ solutions\ (n)}$

S.D.: standard deviation $= \left(\frac{n\Sigma RWR^2 - (\Sigma RWR)^2}{n(n-1)}\right)^{\frac{1}{2}}$

R.S.D.: relative standard deviation $= \frac{S.D.}{Avg\ RWR} \times 100$

TABLE 15

EXTRACTION SAMPLE SOLUTIONS AND REFERENCE SOLUTIONS OF TNT FOR THE PRECISION AND ACCURACY DETERMINATION OF THE EXTRACTION FROM WATER AND SAMPLE PREPARATION METHOD

Extraction Sample Solutions

Water Extract Sample Number	µg/100 ml[a] Water Added	Peak Heights[b] TNT	Peak Heights[b] IS	µg IS[c]	µg Found/Sample[d]	µg/ℓ (ppb)[e]
A-10	4.97	94.9	40.1	2.22	4.41	44.1
A-5	2.48	118.5	97.0	2.22	2.28	22.8
A-2	0.994	106.0	193.8	2.22	1.02	10.2
A-1	0.497	86.3	38.0	0.222	0.420	4.20
A-0.5	0.248	47.2	35.7	0.222	0.250	2.50
A-0.2	0.099	21.0	37.6	0.222	0.100	1.00
A-0	-	< 2	32.0	0.222	< 0.02	< 0.2
B-10	4.97	90.8	38.8	2.22	4.40	44.0
B-5	2.48	133.0	111.0	2.22	2.25	22.5
B-2	0.994	105.0	186.0	2.22	1.06	10.6
B-1	0.497	93.8	38.0	0.222	0.460	4.60
B-0.5	0.248	49.5	40.0	0.222	0.230	2.30
B-0.2	0.099	21.5	37.8	0.222	0.110	1.10
B-0	-	< 2	34.5	0.222	< 0.02	< 0.2
C-10	4.97	92.3	42.0	2.22	4.74	47.4
C-5	2.48	110.0	99.0	2.22	2.54	25.4
C-2	0.994	79.7	183.1	2.22	0.940	9.40
C-1	0.497	85.0	38.0	0.222	0.480	4.80
C-0.5	0.248	41.0	34.0	0.222	0.260	2.60
C-0.2	0.099	19.5	46.0	0.222	0.090	0.900
C-0	-	< 2	38.0	0.222	< 0.02	< 0.2
D-10	4.97	105.3	46.0	2.22	4.66	46.6
D-5	2.48	112.4	96.0	2.22	2.38	23.8
D-2	0.994	90.9	200.9	2.22	0.920	9.20
D-1	0.497	78.3	33.2	0.222	0.480	4.80
D-0.5	0.248	46.1	37.7	0.222	0.250	2.50
D-0.2	0.099	21.0	42.0	0.222	0.100	1.00
D-0	-	< 2	37.0	0.222	< 0.02	< 0.2

TABLE 15 (continued)

Reference Solutions

Reference Solution Number	ng Added[f]	Peak Heights[b] TNT	IS	ng IS[c]	RWR[g]	Avg. RWR, S.D., R.S.D.[h]
A-5	2,480	135.1	107.8	2,220	1.12	
A'-5	2,480	136.9	110.3	2,220	1.11	A
A-1	497	79.5	30.2	222	1.18	RWR 1.19
A'-1	497	96.9	38.0	222	1.14	S.D. ± 0.08
A-0.2	99	23.0	40.0	222	1.29	R.S.D. 7.1%
A'-0.2	99	25.0	43.2	222	1.30	
B-5	2,480	132.2	106.5	2,220	1.11	
B'-5	2,480	136.0	106.8	2,220	1.14	B
B-1	497	103.2	39.8	222	1.16	$\bar{x}$ RWR 1.18
B'-1	497	105.2	41.5	222	1.13	S.D. ± 0.07
B-0.2	99	46.0	78.5	222	1.31	R.S.D. 6.3%
B'-0.2	99	21.2	39.0	222	1.22	
C-5	2,480	117.3	103.0	2,220	1.02	
C'-5	2,480	115.0	100.0	2,220	1.03	C
C-1	497	92.0	41.0	222	1.00	RWR 1.03
C'-1	497	93.7	38.8	222	1.08	S.D. ± 0.07
C-0.2	99	16.4	40.1	222	0.92	R.S.D. 6.7%
C'-0.2	99	21.0	42.2	222	1.12	
D-5	2,480	122.2	101.0	2,220	1.08	
D'-5	2,480	124.9	102.6	2,220	1.09	D
D-1	497	100.3	40.0	222	1.12	RWR 1.09
D'-1	497	90.2	39.0	222	1.03	S.D. ± 0.04
D-0.2	99	21.2	44.2	222	1.08	R.S.D. 4.0%
D'-0.2	99	22.0	42.5	222	1.16	

TABLE 15 (concluded)

a µg/100 ml Water added: µg of TNT added to 100 ml water.
b Peak heights: measured height of TNT and IS in millimeters.
c µg IS: µg IS present in the ~ 2 ml final sample.
d µg Found/sample: µg TNT recovered from 100 ml water.

$$\frac{\text{µg Found}}{\text{Sample}} = \frac{\text{Peak height TNT}}{\text{Peak height IS}} \times \frac{\text{µg IS/}\sim\text{ 2 ml sample}}{\text{Average RWR for TNT}}$$

e µg/ℓ (ppb): µg found in 100 ml sample extract equated to µg/ℓ.
f Added: nanograms of compound added to reference solution (~ 2 ml).

g RWR: relative weight response $= \frac{\text{Peak height TNT}}{\text{Peak height IS}} \times \frac{\text{µg IS/reference solution}}{\text{µg TNT/reference solution}}$

h Avg: average RWR $= \frac{\Sigma\text{RWR's}}{\text{Number of reference solutions (n)}}$

S.D.: standard deviation $= \left(\frac{n\Sigma RWR^2 - (\Sigma RWR)^2}{n(n-1)}\right)^{\frac{1}{2}}$

R.S.D.: relative standard deviation $= \frac{\text{S.D.}}{\text{Avg RWR}} \times 100$

TABLE 16

EXTRACTION SAMPLE SOLUTIONS AND REFERENCE SOLUTIONS OF TETRYL FOR THE PRECISION AND ACCURACY DETERMINATION OF THE EXTRACTION FROM WATER AND SAMPLE PREPARATION METHOD

Extraction Sample Solutions

Water Extract Sample Number	µg/100 ml[a] Water Added	Peak Heights[b] Tetryl	Peak Heights[b] IS	µg IS[c]	µg Found/Sample[d]	µg/ℓ (ppb)[e]
A-10	5.09	76.2	40.1	2.22	4.35	43.5
A-5	2.54	98.3	97.0	2.22	2.32	23.2
A-2	1.02	71.2	193.8	2.22	0.840	8.40
A-1	0.509	63.2	38.0	0.222	0.380	3.80
A-0.5	0.254	36.0	35.7	0.222	0.230	2.30
A-0.2	0.102	16.0	37.6	0.222	0.100	1.00
A-0	-	< 2	32.0	0.222	< 0.02	< 0.2
B-10	5.09	73.8	38.8	2.22	4.44	44.4
B-5	2.54	110.8	111.0	2.22	2.33	23.3
B-2	1.02	86.5	186.0	2.22	1.09	10.9
B-1	0.509	70.5	38.0	0.222	0.430	4.30
B-0.5	0.254	30.0	40.0	0.222	0.180	1.80
B-0.2	0.102	12.0	37.8	0.222	0.070	0.700
B-0	-	< 2	34.5	0.222	< 0.02	< 0.2
C-10	5.09	83.9	42.0	2.22	5.04	50.4
C-5	2.54	99.3	99.0	2.22	2.53	25.3
C-2	1.02	71.0	183.1	2.22	0.980	9.80
C-1	0.509	71.0	38.0	0.222	0.470	4.70
C-0.5	0.254	38.1	34.0	0.222	0.280	2.80
C-0.2	0.102	18.3	46.0	0.222	0.100	1.00
C-0	-	< 2	38.0	0.222	< 0.02	< 0.2
D-10	5.09	90.6	46.0	2.22	5.20	52.0
D-5	2.54	95.0	96.0	2.22	2.62	26.2
D-2	1.02	77.1	200.9	2.22	1.01	1.01
D-1	0.509	54.1	33.2	0.222	0.430	4.30
D-0.5	0.254	20.0	37.7	0.222	0.140	1.40
D-0.2	0.102	10.9	42.0	0.222	0.070	0.700
D-0	-	< 2	37.0	0.222	< 0.02	< 0.2

TABLE 16 (continued)

Reference Solutions

Reference Solution Number	ng Added[f]	Peak Heights[b] Tetryl	IS	ng IS[c]	RWR[g]	Avg. RWR, S.D., R.S.D.[h]
A-5	2,540	112.1	107.8	2,220	0.91	
A'-5	2,540	114.6	110.3	2,220	0.91	A
A-1	509	82.0	38.0	222	0.94	RWR 0.97
A'-1	509	63.9	30.2	222	0.92	S.D. ± 0.08
A-0.2	102	19.0	40.0	222	1.03	R.S.D. 7.8%
A'-0.2	102	21.7	43.2	222	1.09	
B-5	2,540	107.2	106.5	2,220	0.88	
B'-5	2,540	121.2	106.3	2,220	0.99	B
B-1	509	83.2	39.8	222	0.91	RWR 0.95
B'-1	509	87.8	41.5	222	0.92	S.D. ± 0.06
B-0.2	102	36.8	78.5	222	1.02	R.S.D. 5.9%
B'-0.2	102	17.8	39.0	222	0.99	
C-5	2,540	103.0	103.0	2,220	0.87	
C'-5	2,540	100.0	100.0	2,220	0.87	C
C-1	509	80.2	41.0	222	0.85	RWR 0.88
C'-1	509	82.0	38.8	222	0.92	S.D. ± 0.03
C-0.2	102	18.0	42.2	222	0.93	R.S.D. 3.8%
C'-0.2	102	15.8	40.1	222	0.86	
D-5	2,540	105.3	101.0	2,220	0.91	
D'-5	2,540	103.0	102.6	2,220	0.88	D
D-1	509	77.5	40.0	222	0.84	RWR 0.84
D'-1	509	76.8	39.0	222	0.86	S.D. ± 0.05
D-0.2	102	16.1	44.2	222	0.79	R.S.D. 6.0%
D'-0.2	102	15.2	42.5	222	0.78	

TABLE 16 (concluded)

a µg/100 ml Water added: µg of tetryl added to 100 ml water.
b Peak heights: measured height of tetryl and IS in millimeters.
c µg IS: µg IS present in the ~ 2 ml final sample.
d µg Found/sample: µg tetryl recovered from 100 ml water.

$$\frac{\text{µg Found}}{\text{Sample}} = \frac{\text{Peak height tetryl}}{\text{Peak height IS}} \times \frac{\text{µg IS/}\sim\text{ 2 ml sample}}{\text{Average RWR for tetryl}}$$

e µg/ℓ (ppb): µg found in 100 ml sample extract equated to µg/ℓ.
f Added: nanograms of compound added to reference solution (~ 2 ml).

g RWR: relative weight response $= \frac{\text{Peak height tetryl}}{\text{Peak height IS}} \times \frac{\text{µg IS/reference solution}}{\text{µg tetryl/reference solution}}$

h Avg: average RWR $= \frac{\Sigma\text{RWR's}}{\text{Number of reference solutions (n)}}$

S.D.: standard deviation $= \left(\frac{n\Sigma RWR^2 - (\Sigma RWR)^2}{n(n-1)}\right)^{\frac{1}{2}}$

R.S.D.: relative standard deviation $= \frac{\text{S.D.}}{\text{Avg RWR}} \times 100$

TABLE 17

EXTRACTION SAMPLE SOLUTIONS AND REFERENCE SOLUTIONS OF DPA FOR THE PRECISION AND ACCURACY DETERMINATION OF THE EXTRACTION FROM WATER AND SAMPLE PREPARATION METHOD

Extraction Sample Solutions

Water Extract Sample Number	µg/100 ml[a] Water Added	Peak Heights[b] DPA	Peak Heights[b] IS	µg IS[c]	µg Found/Sample[d]	µg/ℓ (ppb)[e]
A-10	5.00	35.2	40.1	2.22	3.82	38.2
A-5	2.50	40.5	97.0	2.22	1.82	18.2
A-2	1.00	39.9	193.8	2.22	0.900	9.00
A-1	0.500	27.1	38.0	0.222	0.310	3.10
A-0.5	0.250	13.2	35.7	0.222	0.160	1.60
A-0.2	0.100	8.0	37.6	0.222	0.100	1.00
A-0	-	< 2	32.0	0.222	< 0.02	< 0.2
B-10	5.00	34.8	38.8	2.22	4.24	42.4
B-5	2.50	49.0	111.0	2.22	2.08	20.8
B-2	1.00	41.5	186.0	2.22	1.05	10.5
B-1	0.500	34.5	38.0	0.222	0.430	4.30
B-0.5	0.250	17.0	40.0	0.222	0.200	2.00
B-0.2	0.100	6.5	37.8	0.222	0.080	0.800
B-0	-	< 2	34.5	0.222	< 0.02	< 0.2
C-10	5.00	38.1	42.0	2.22	4.58	45.8
C-5	2.50	42.6	99.0	2.22	2.17	21.7
C-2	1.00	33.0	183.1	2.22	0.910	9.10
C-1	0.500	33.8	38.0	0.222	0.450	4.50
C-0.5	0.250	11.0	34.0	0.222	0.160	1.60
C-0.2	0.100	6.0	46.0	0.222	0.070	0.700
C-0	-	< 2	38.0	0.222	< 0.02	< 0.2
D-10	5.00	34.0	46.0	2.22	4.00	40.0
D-5	2.50	35.9	96.0	2.22	2.02	20.2
D-2	1.00	30.2	200.9	2.22	0.810	8.10
D-1	0.500	28.9	33.2	0.222	0.470	4.70
D-0.5	0.250	15.0	37.7	0.222	0.220	2.20
D-0.2	0.100	5.5	42.0	0.222	0.070	0.700
D-0	-	< 2	37.0	0.222	< 0.02	< 0.2

TABLE 17 (continued)

Reference Solutions

Reference Solution Number	ng Added[f]	Peak Heights[b] DPA	IS	ng IS[c]	RWR[g]	Avg. RWR, S.D., R.S.D.[h]
A-5	2,500	55.6	107.8	2,220	0.46	
A'-5	2,500	58.3	110.3	2,220	0.47	A
A-1	500	34.0	30.2	222	0.50	RWR 0.51
A'-1	500	43.2	38.0	222	0.50	S.D. ± 0.04
A-0.2	100	10.0	40.0	222	0.56	R.S.D. 8.4%
A'-0.2	100	10.9	43.2	222	0.56	
B-5	2,500	53.0	106.5	2,220	0.44	
B'-5	2,500	49.8	106.8	2,220	0.41	B
B-1	500	43.2	39.8	222	0.48	RWR 0.47
B'-1	500	47.0	41.5	222	0.50	S.D. ± 0.04
B-0.2	100	17.5	78.5	222	0.49	R.S.D. 7.8%
B'-0.2	100	8.8	39.0	222	0.50	
C-5	2,500	52.1	103.0	2,220	0.45	
C'-5	2,500	47.1	100.0	2,220	0.41	C
C-1	500	43.4	41.0	222	0.47	RWR 0.44
C'-1	500	45.3	38.8	222	0.52	S.D. ± 0.05
C-0.2	100	8.1	42.2	222	0.43	R.S.D. 11.1%
C'-0.2	100	6.8	40.1	222	0.38	
D-5	2,500	51.4	101.0	2,220	0.45	
D'-5	2,500	45.7	102.6	2,220	0.40	D
D-1	500	40.5	40.6	222	0.45	RWR 0.41
D'-1	500	39.9	39.0	222	0.45	S.D. ± 0.05
D-0.2	100	7.0	44.2	222	0.35	R.S.D. 11.4%
D'-0.2	100	7.0	42.5	222	0.36	

TABLE 17 (concluded)

a µg/100 ml Water added: µg of DPA added to 100 ml water.
b Peak heights: measured height of DPA and IS in millimeters.
c µg IS: µg IS present in the ~ 2 ml final sample.
d µg Found/sample: µg DPA recovered from 100 ml water.

$$\frac{\text{µg Found}}{\text{Sample}} = \frac{\text{Peak height DPA}}{\text{Peak height IS}} \times \frac{\text{µg IS/}\sim\text{ 2 ml sample}}{\text{Average RWR for DPA}}$$

e µg/ℓ (ppb): µg found in 100 ml sample extract equated to µg/ℓ.
f Added: nanograms of compound added to reference solution (~ 2 ml).

g RWR: relative weight response $= \frac{\text{Peak height DPA}}{\text{Peak height IS}} \times \frac{\text{µg IS/reference solution}}{\text{µg DPA/reference solution}}$

h Avg: average RWR $= \frac{\Sigma\text{RWR's}}{\text{Number of reference solutions (n)}}$

S.D.: standard deviation $= \left(\frac{n\Sigma RWR^2 - (\Sigma RWR)^2}{n(n-1)}\right)^{\frac{1}{2}}$

R.S.D.: relative standard deviation $= \frac{\text{S.D.}}{\text{Avg RWR}} \times 100$

APPENDIX D

PRECISION AND ACCURACY ASSESSMENT OF THE ANALYTICAL METHOD FOR THE DETERMINATION OF NITROBENZENE (NB), 2,6-DINITROTOLUENE (2,6-DNT), NITROGLYCERIN (NG), AND PICRIC ACID (PA) IN WATER SAMPLES

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1. Application

The developed analytical method is for the quantitative determination of NB, 2,6-DNT, NG, and PA in water samples. The method uses liquid-liquid extraction to isolate the munition compound from the water and high performance liquid chromatography (HPLC) with ultraviolet (UV) detection at 230 nm for separation and quantification.

a. Evaluated concentration range: The concentration ranges of NB, 2,6-DNT, NG, and PA studied in water samples corresponded to a series of 0, 0.2X, 0.5X, X, 2X, 5X, and 10X where X was 5 µg/liter (parts per billion, ppb) for NB, 2,6-DNT, and PA and 150 µg/liter for NG.

b. Sensitivity: A signal-to-noise ratio of 5 to 1 for NB (peak height (PH) = 14 mm), 8 to 1 for 2,6-DNT (PH = 26 mm), 9 to 1 for NG (PH = 27 mm), and 7 to 1 for PA (PH = 22 mm) was obtained with a 100-µl injection of 0.2X (1 µg/liter for NB, 2,6-DNT, and PA and 30 µg/liter for NG) level water sample prepared and analyzed by the procedure outlined below (about 5 ng NB, 2,6-DNT, and PA and 150 ng NG injected on column).

c. Detection limits: The detection limits of the analytical method to be employed for water preservation evaluations using the Hubaux and Vos detection limit program were 6.92 µg/liter for NB, 1.93 µg/liter for 2,6-DNT, 48.5 µg/liter for NG, and 1.00 µg/liter for PA.

d. Interferences: Small interfering peaks were observed in some water samples near the elution positions of NB and 2,6-DNT. These peaks were attributed to the t-butyl ammonium hydroxide used as an ion-pairing agent and were eliminated by preparing fresh reagent.

e. Analysis rate: The chromatographic time per injection was 30 min. Two reference solutions were analyzed prior to injecting the prepared samples, and two were analyzed during the day (120 min total time). Thus, a total of 12 prepared water samples (360 min total time) can be analyzed during an 8-hr day.

2. Chemistry

NB, 2,6-DNT, NG, and PA are munition-related compounds manufactured at various installations. The assessment of potential environmental contamination by these compounds in water requires knowing that the level of the compounds present at the time of sampling does not change prior to analysis and that the sampling technique provides a representative sample. The evaluation of the preservation and sampling parameters to be employed requires an analytical method capable of assaying the compounds with sufficient precision, accuracy, and sensitivity to provide quantitative data. NG has an ultraviolet (UV) wavelength maxima (λ max) at 230 nm and a molar absorptivity (ε max) substantially less than the other compounds. Thus, to obtain the highest possible sensitivity for NG and still have sufficient sensitivity to detect and quantitate NB, 2,6-DNT, and PA, a 230 nm UV detector is required. PA is a strong acid (pKa 0.38) and exists in an anionic form in aqueous media. Reverse phase HPLC cannot resolve ionic species, and to obtain a good chromatographic peak for PA requires coupling the anion with a cation (ion-pairing chromatography). Quaternary butyl ammonium hydroxide is the cation utlized for many ion-paired compounds.

3. Apparatus

a. Instrumentation: A Chem Research Series 2000 HPLC unit with a Rheodyne 7120 variable loop injector, a Tracor Model 970A variable wavelength UV-VIS detector, and a single pen Model SR-204 Heath-Schlumberger recorder were used. (Note: Equivalent instrumentation will provide similar results.)

b. HPLC Parameters

1. Column: Spherisorb ODS, 5 µ, 250 x 4.6 mm ID.

2. Precolumn: Co:Pell ODS, 25 µ - 35 µ, 50 x 2 mm ID.

3. Eluent: 35/65 (V/V) acetonitrile/0.005M t-butyl ammonium hydroxide, pH 6.5 (pH adjusted with 1N phosphoric acid).

4. Flow Rate: 1.0 ml/min.

5. Chart Speed: 0.1 in/min.

6. Internal Standard: Propiophenone.

7. Injection Volume: 40 to 100 µl

8. Retention Volumes:

Compound	Milliliters
NB	14.0
2,6-DNT	20.0
NG	23.0
PA	24.5
IS	16.0

Note: Slight changes in the retention indices may occur with fresh eluent or a change in precolumn or analytical column.

c. Laboratory Glassware and Equipment:

1. Pasteur pipettes
2. Beakers (100 ml)
3. Separatory funnels (125 ml) with Teflon stopcock
4. Culture tubes (12 ml) with Teflon-lined screw caps
5. Graduated cylinders (250, 100, and 10 ml)
6. pH meter
7. Nitrogen gas stream baffle system (set up in safety ventilation hood)
8. Hot plate (variable temperature)
9. Vortex mixer
10. Filters 0.45 μm (organic solvent compatibility)
11. Disposable 5 cc syringes (compatible with filter fitting)
12. Volumetric syringes (0-100, 0-500, 0-1,000 μl)

d. Chemicals:

1. NB, 2,6-DNT, NG, and PA SARMS, obtained from the U.S. Army Toxic and Hazardous Materials Agency.

2. Propiophenone, analytical grade.

3. t-Butyl ammonium hydroxide, HPLC grade.

4. Acetonitrile, "Distilled in Glass" grade: phosphoric acid, analytical grade.

5. High purity water from a Milli-Q water purification system.

6. Methylene chloride "Distilled in Glass" grade.

7. Sodium chloride ACS grade.

8. pH 7.0 calibration buffer.

4. Standards

a. Stock: Weigh accurately 20 mg of NB, 2,6-DNT, NG, and PA SARM or interim SARM into separate 100 ml volumetric flasks, and dissolve in acetonitrile (concentration of each compound, 200 µg/ml). To prepare Working Stock No. 1, quantitatively pipette 2.5 ml of the NB, 2,6-DNT, and PA stocks and 75 ml of the NG stock into a 100 ml volumetric flask and dilute to volume with high purity water (concentration of NB, 2,6-DNT; and PA, 5 µg/ml; and of NG, 150 µg/ml). Working Stock No. 2 is prepared by quantitatively pipetting 10 ml of Working Stock No. 1 into a 100 ml volumetric flask and diluting to volume with 27 ml acetonitrile and high purity water (concentration of NB, 2,6-DNT; and PA, 0.5 µg/ml; and of NG, 15 µg/ml).

b. Internal Standard (IS) Stock: Weigh accurately 20 mg propiophenone into a 100 ml volumetric flask and dilute to volume with acetonitrile (concentration 200 µg/ml). IS Stock No. 1 is prepared by quantitatively pipetting 1.0 ml of the stock into a 100-ml volumetric flask and diluting to volume with 35/65 (v/v) acetonitrile in water (concentration 2 µg/ml). To prepare IS Stock No. 2, quantitatively pipette 10 ml Stock No. 1- to a 100-ml volumetric flask and dilute to volume with 35/65 (v/v) acetonitrile in water (concentration 0.2 µg/ml).

c. Reference Solution Preparation: Both Working Stocks 1 and 2 and IS Stocks 1 and 2 were employed to prepare the reference solutions for precision and accuracy testing of the extraction and sample preparation method as follows:

Working Stock No.	µl Working Stock	IS Stock No.	µl IS Stock	µl HPLC Eluent	Concentration Each Compound (ng/2.0 ml) NB; 2,6-DNT; PA	NG	IS
1	1,000	1	1,000	-	5,000	150,000	2,000
1	200	1	1,000	800	1,000	30,000	2,000
2	1,000	2	1,000	-	500	15,000	200
2	200	2	1,000	800	100	3,000	200

A minimum of three of the above reference solutions were prepared fresh on four separate days correlating with each separate extraction series performed to define the precision and accuracy of the total analytical method.

d. Sample Fortification: Working Stocks 1 and 2 were employed as follows to spike the 100-ml water samples to be extracted:

Working Stock No.	µl Working Stock	Concentration µg/ℓ (ppb) NB, 2,6-DNT, PA	Concentration µg/ℓ (ppb) NG	µg Fortification Each Compound when X Equals 0.5 µg or 150 µg (NG)
1	1,000	50	1,500	10X
1	500	25	750	5X
1	200	10	300	2X
2	1,000	5	150	1X
2	500	2.5	75	0.5X
2	200	1	30	0.2X
-	-	0	0	0

Each of these 100-ml spiked water samples and the blank were prepared and extracted on four separate days to define the precision and accuracy of the extraction and sample preparation method.

e. Internal Standard Sample Addition: To each sample extracted and concentrated according to the protocol outlined below, IS stocks 1 and 2 were employed to add the appropriate level of IS as follows:

Munitions Sample According to X Equals 0.5 or 150 µg Fortification	IS Stock No.	µl IS Stock Added	µg IS Stock in Sample
10X	1	1,000	2
5X	1	1,000	2
2X	1	1,000	2
1X	2	1,000	0.2
0.5X	2	1,000	0.2
0.2X	2	1,000	0.2
0	2	1,000	0.2

Each sample had an additional 800 µl of a 35/65 (v/v) CH_3CN/water, 0.005 M t-butyl ammonium hydroxide solution, pH 6.5 (H_3PO_4 adjusted), added to aid in the dissolution of the munitions, to ion-pair the PA, and to make the final sample volume approximately 2 ml.

5. Sample Preparation Procedure

The procedure outlined below was defined for the quantitative extraction of NB, 2,6-DNT, NG, and PA from water samples.

1. Place 100 ml of the water sample into a 125-ml separatory funnel.

2. Add the appropriate level of the munition compounds as given in Section 4.d.

3. Add 8.5 ml sodium chloride crystals, i.e., approximately 10 g. The water sample is 10% w/v in sodium chloride.

4. Mix thoroughly.

5. Add 20-ml methylene chloride and hand shake for 20 sec.

Note: During the extraction, vent the separatory funnel through stopper, not the stopcock, to prevent pressure buildup.

6. Allow the phases to completely separate.

7. Drain the methylene chloride layer into a 100-ml beaker.

Note: Be careful not to drain any of the aqueous layer into the beaker.

8. Add 1.0 ml 0.005 M t-butyl ammonium hydroxide, pH 6.5, to the aqueous phase.

Note: This step is necessary to provide a neutral (ion-paired) PA species for extraction.

9. Repeat steps 6 and 7 three more times combining the extracts in the beaker.

10. Concentrate the extract to approximately 2 ml on a 40°C hot plate under a stream of nitrogen.

Note: Higher temperature than 40°C may cause the extract to boil. The evaporation process is to be accomplished in a hood.

11. Add approximately 10 ml acetonitrile to the beaker to solvent exchange from methylene chloride to acetonitrile.

Note: Add the acetonitrile to wash the sides of beaker to ensure that the munitions are completely dissolved.

12. Mix by swirling and observe the sample to determine if the two solvents are completely miscible. If necessary, add additional acetonitrile until one phase is obtained.

13. Concentrate the sample to approximately 2 ml on a 40°C hot plate under a stream of nitrogen.

14. Transfer the sample to a culture tube with a Teflon-lined screw cap.

15. Wash the beaker with 3 x 1 ml acetonitrile, and add the washes to the culture tube.

16. Concentrate the sample to approximately 200 µl on a 40°C hot plate under a stream of nitrogen.

17. Add 800 µl of a 35/65 (v/v) acetonitrile/water, 0.005 M t-butyl ammonium hydroxide solution, pH 6.5.

18. Add 1,000 µl of the appropriate IS stock solution (see Section 4.e).

19. Mix thoroughly.

20. Filter through a 0.45-µ filter into a clean culture tube.

21. Cap tightly and store at 4°C in the dark until analysis by HPLC-UV (230 nm) using the parameters outlined in Section 3.

A representative HPLC-UV (230 nm) chromatogram of a 100-ml water extract of NB, 2,6-DNT, NG, and PA at the 1X level is shown in Figure 1.

6. Calculations

The reference standards described in Section 4.c were prepared fresh at the time of each series extraction. The relative weight response (RWR) (Equation 1) of each compound to the IS was calculated for each reference solution, and the average RWR for each compound was utilized to calculate the level of that compound in each of the seven 100-ml water extracted samples (Equation 2). The micrograms per liter found were plotted against the micrograms per liter added, and a linear regression analysis of the data was performed. The slope, intercept, and correlation coefficient of each compound were determined. The data are summarized in Table 1 and include the average value found at each level (Equation 3), standard deviation (Equation 4), coefficient of variation (Equation 5), and percent inaccuracy (Equation 6). The raw data and calculations for the reference standard solutions and extraction samples are given in Tables 6 through 9.

$$\text{RWR} = \frac{\text{Peak Height Cpd}}{\text{Peak Height IS}} \times \frac{\mu\text{g IS/reference solution}}{\mu\text{g Cpd/reference solution}} \qquad \text{(Eq. 1)}$$

$$\frac{\mu\text{g Cpd Found}}{\text{100 ml Water Extract Sample}} = \frac{\text{Peak Height Cpd}}{\text{Peak Height IS}} \times \frac{\mu\text{g IS}}{\text{Avg RWR Cpd}} \qquad \text{(Eq. 2)}$$

$$\text{Average } \frac{\mu g}{L} \text{ Found} = \bar{x} = \Sigma x/n \qquad \text{(Eq. 3)}$$

$$\text{Standard deviation} = \sigma = \left(\frac{n\Sigma x^2 - (\Sigma x)^2}{n(n-1)}\right)^{\frac{1}{2}} \qquad \text{(Eq. 4)}$$

$$\text{Coefficient of variation} = (\sigma/\bar{x}) \times 100 \qquad \text{(Eq. 5)}$$

$$\text{Percent inaccuracy} = \frac{\bar{x} - \mu g/L \text{ added}}{\mu g/L \text{ added}} \times 100 \qquad \text{(Eq. 6)}$$

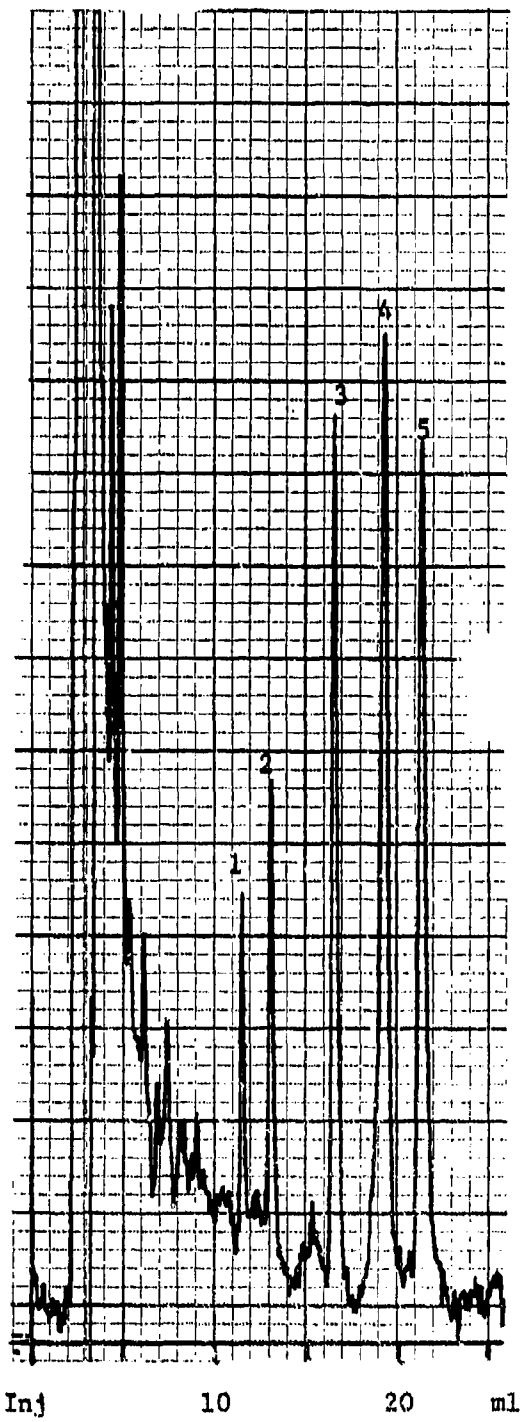
Graphic presentations of the data points and linear regression line along with graphic representations of the standard deviation, coefficient of variation, and percent inaccuracy are given in Figures 2 through 9.

7. Statistical Evaluation of Data

A statistical evaluation of the data obtained for the precision and accuracy determination of the extraction from water and sample preparation method for NB, 2,6-DNT, NG, and PA was performed utilizing the Hubaux and Vos detection limit program provided by the U.S. Army Toxic and Hazardous Materials Agency. Detection limits for each compound were as follows: NB, 6.92 µg/liter; 2,6-DNT, 1.93 µg/liter; NG 48.5 µg/ liter; and PA, 1.00 µg/liter using all the data points. The average micrograms found at each level for each compound were determined from the linear regression equation for the 28 data points and the micrograms added at that level (Equation 7). The standard deviation and percent imprecision at each level were calculated based on this average and thus do not agree with the values given in Table 1.

$$\text{Avg } \frac{\mu g}{L} = \frac{\Sigma\left(\frac{\text{found } \frac{\mu g}{L} - \text{intercept}}{\text{slope}}\right)}{n} \qquad \text{(Eq. 7)}$$

The results of the Hubaux and Vos evaluations are given in Tables 2 through 5.



HPLC Parameters

Column: Spherisorb ODS, 5 µ, 250 x 4.6 mm ID

Precolumn: Co:Pell ODS, 25-35 µ, 50 x 2 mm ID

Eluent: 35/65 (v/v) CH_3CN/0.005 t-butyl ammonium hydroxide, pH 6.5 (H_3PO_4 adjusted)

Flow Rate: 1.0 ml/min

Chart Speed: 0.1 in/min

Detector: UV, 230 nm

Injection Volume: 100 µl

Attenuation: 0.005 X

Sample Characteristics

No.	Compound	Added (ppb)	Recovered (ppb)
1	NB	5.25	3.11
2	IS*	-	-
3	2,6-DNT	5.62	4.67
4	NG	152	154
5	PA	5.44	5.52

* IS - 0.222 µg propiophenone (IS Stock No. 2) added during final sample preparation. Final sample volume is ∿ 2 ml.

Figure 1 - HPLC-UV (230 nm) Separation of NB, 2,6-DNT, NG, and PA Recovered From a 100-ml Water Sample. Sample preparation procedure listed in text.

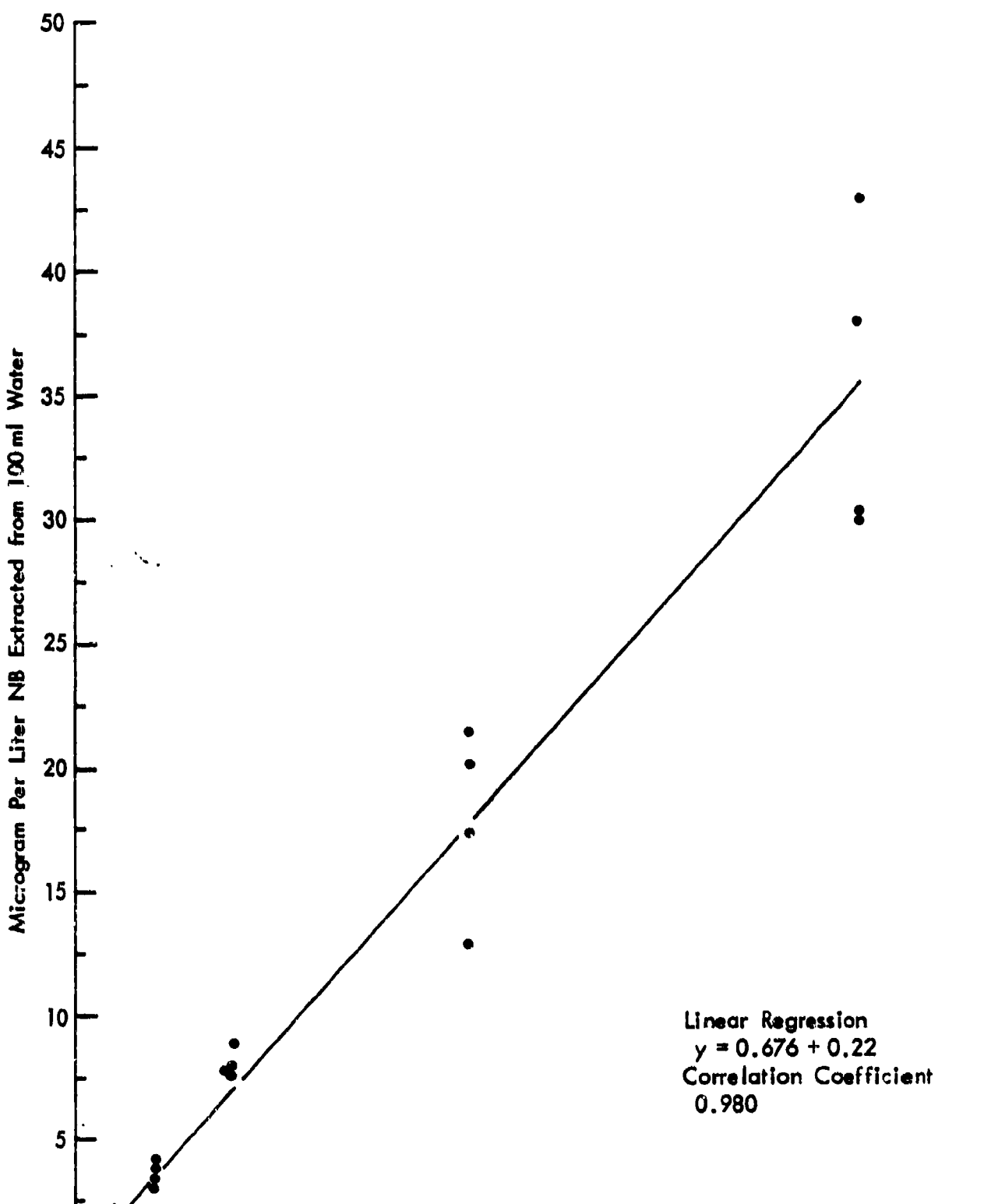


Figure 2 - Linearity of NB Extracted from 100 ml Water

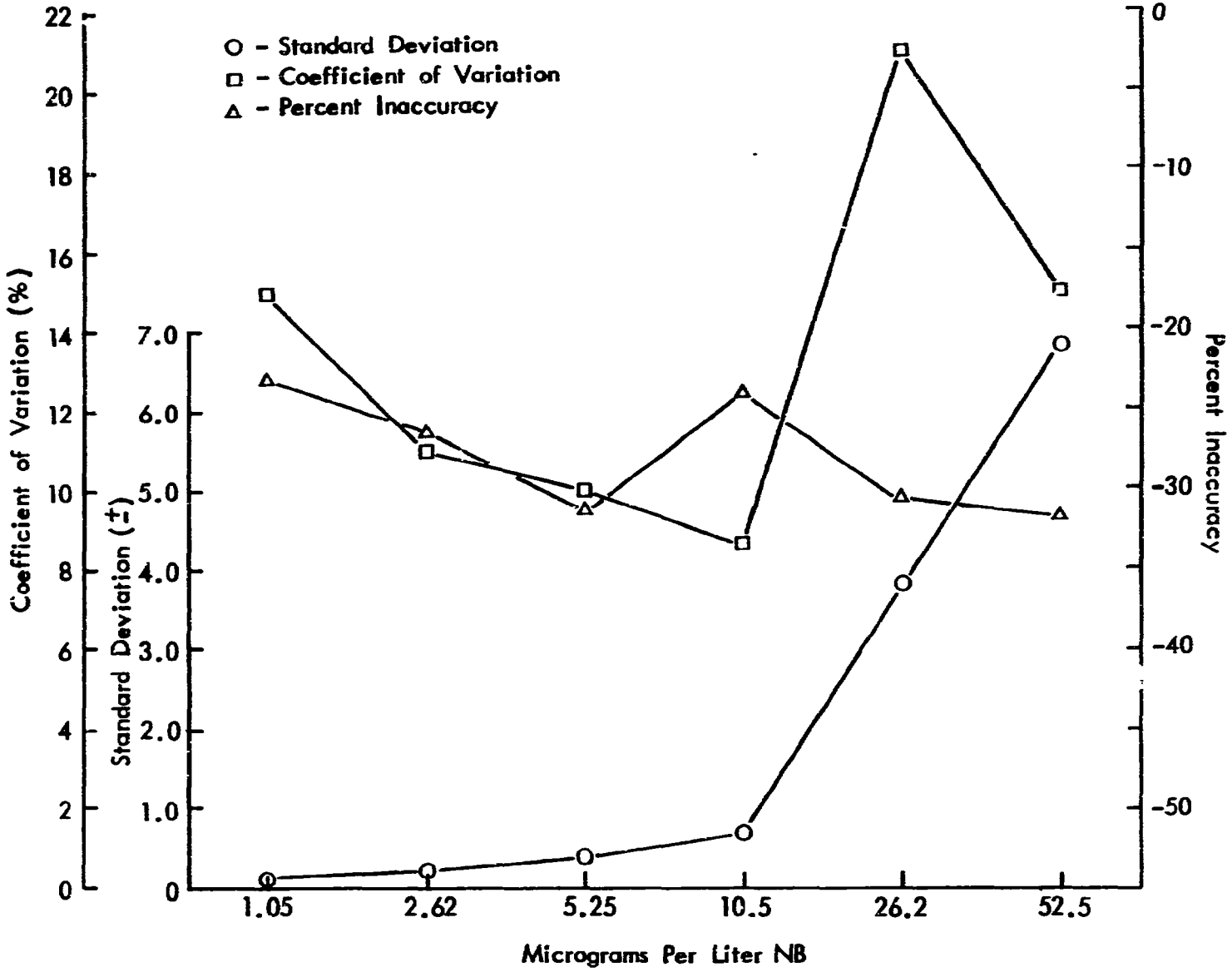


Figure 3 - Standard Deviation, Coefficient of Variation, and Percent Inaccuracy for NB

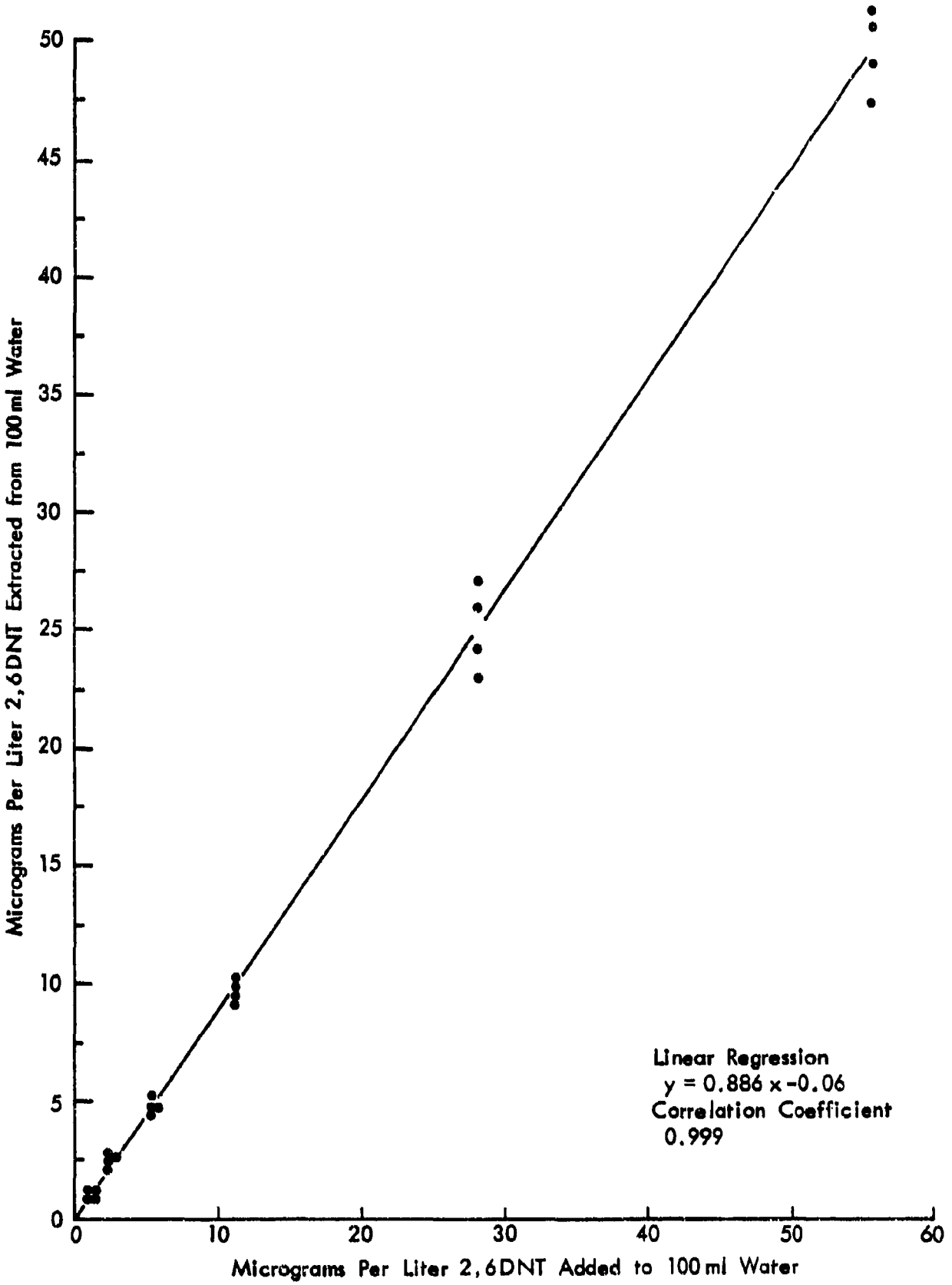


Figure 4 - Linearity of 2,6-DNT Extracted from 100 ml Water

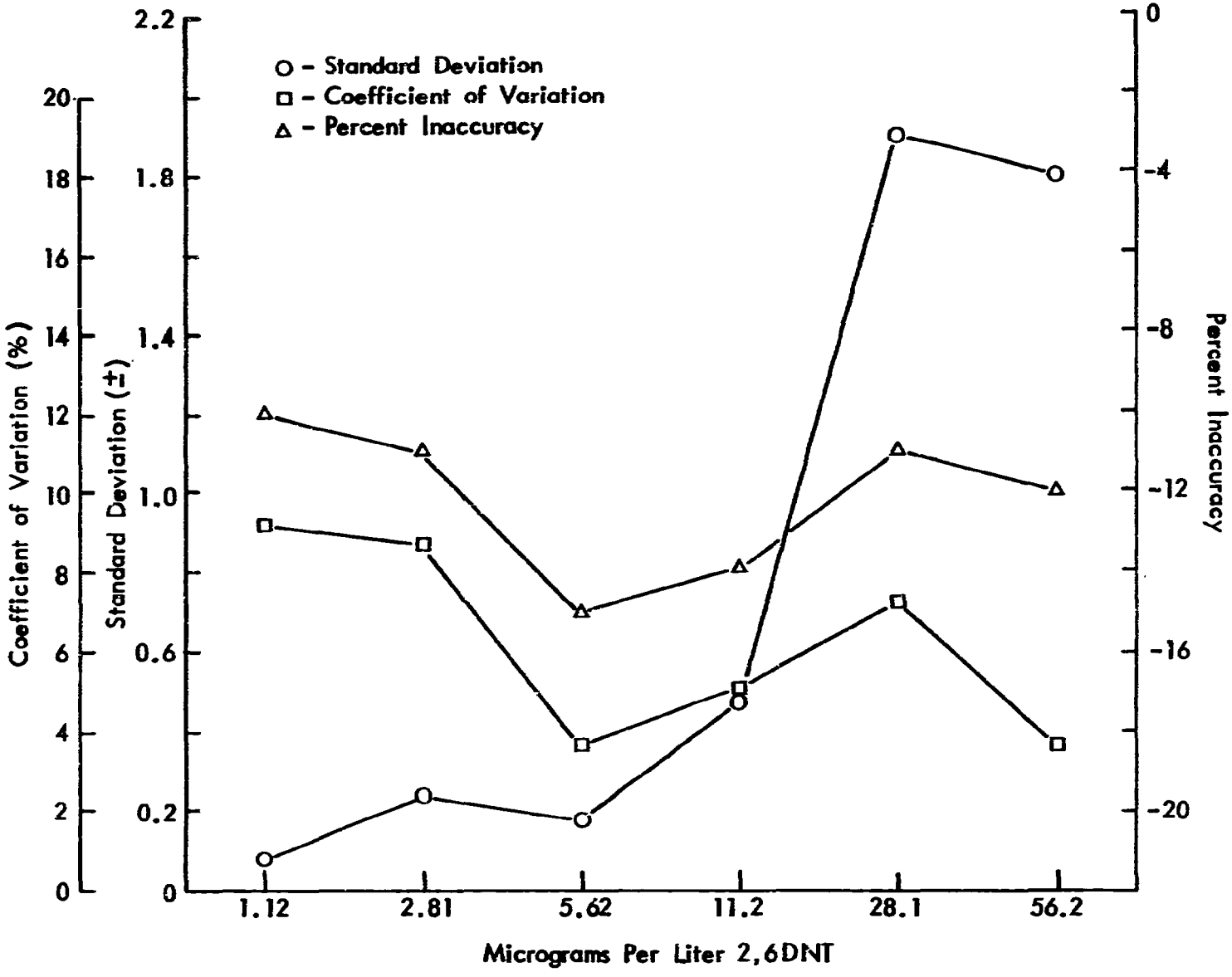


Figure 5 - Standard Deviation, Coefficient of Variation, and Percent Inaccuracy for 2,6-DNT

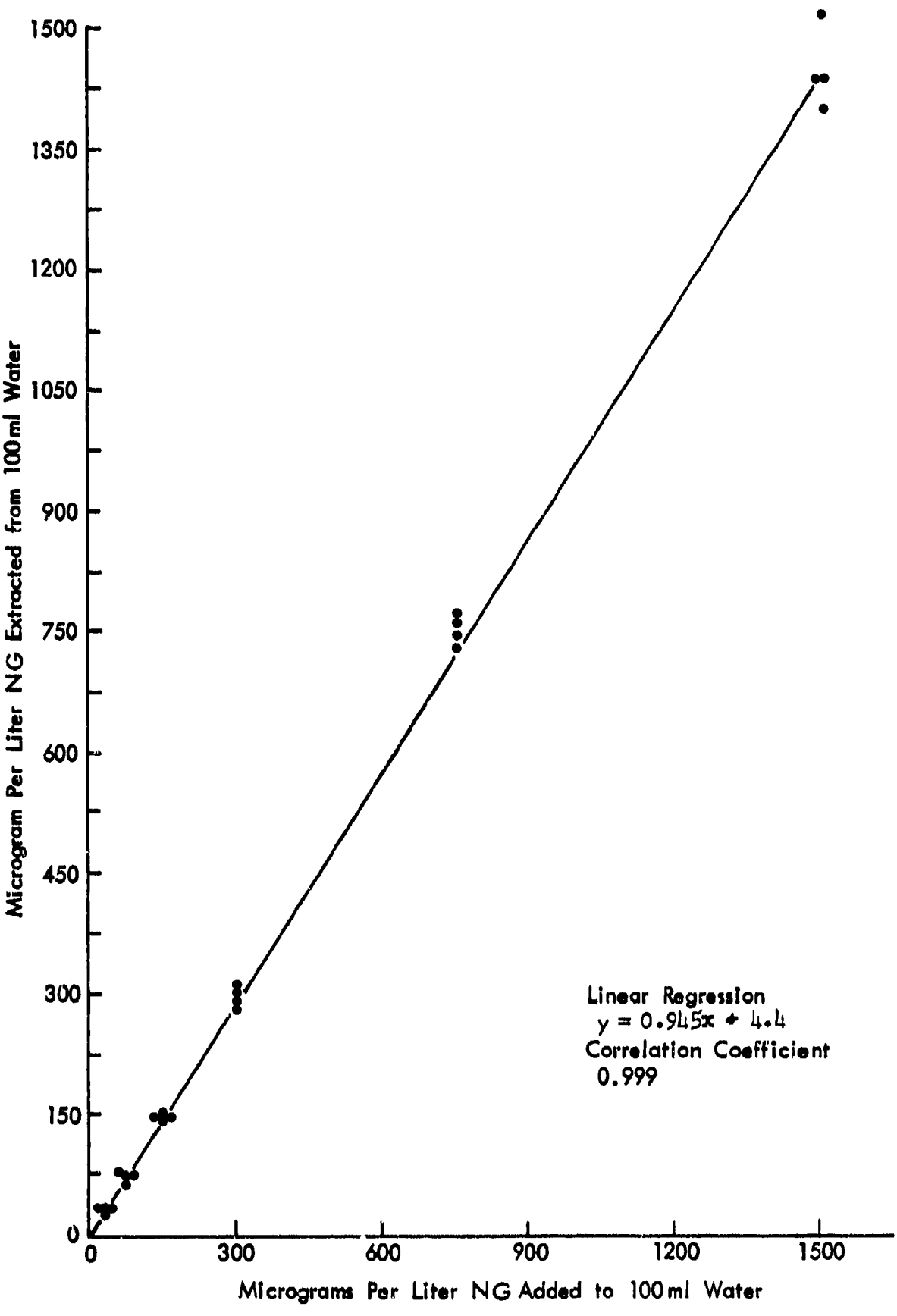


Figure 6 - Linearity of NG Extracted from 100 ml Water

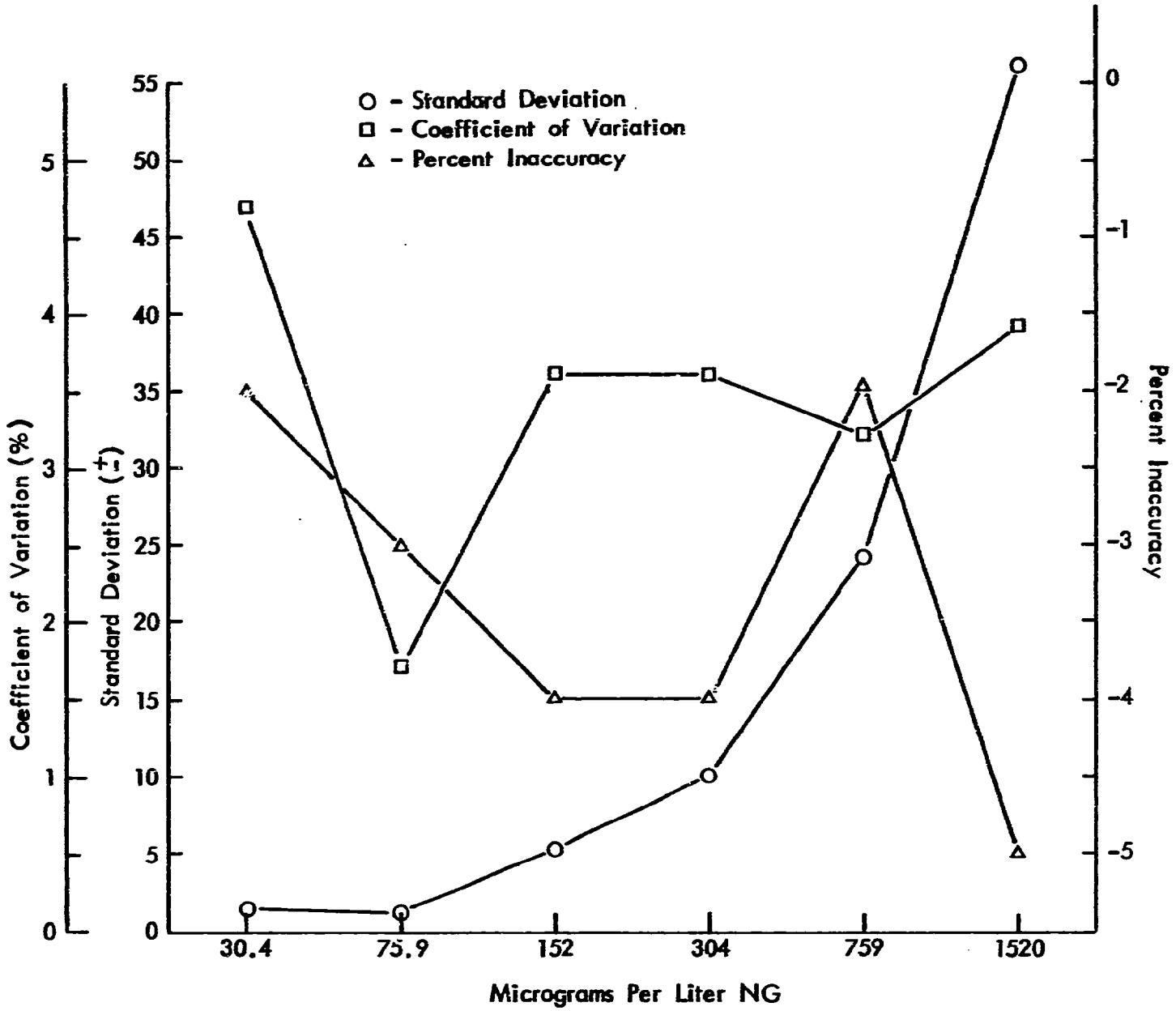


Figure 7 - Standard Deviation, Coefficient of Variation, and Percent Inaccuracy for NG

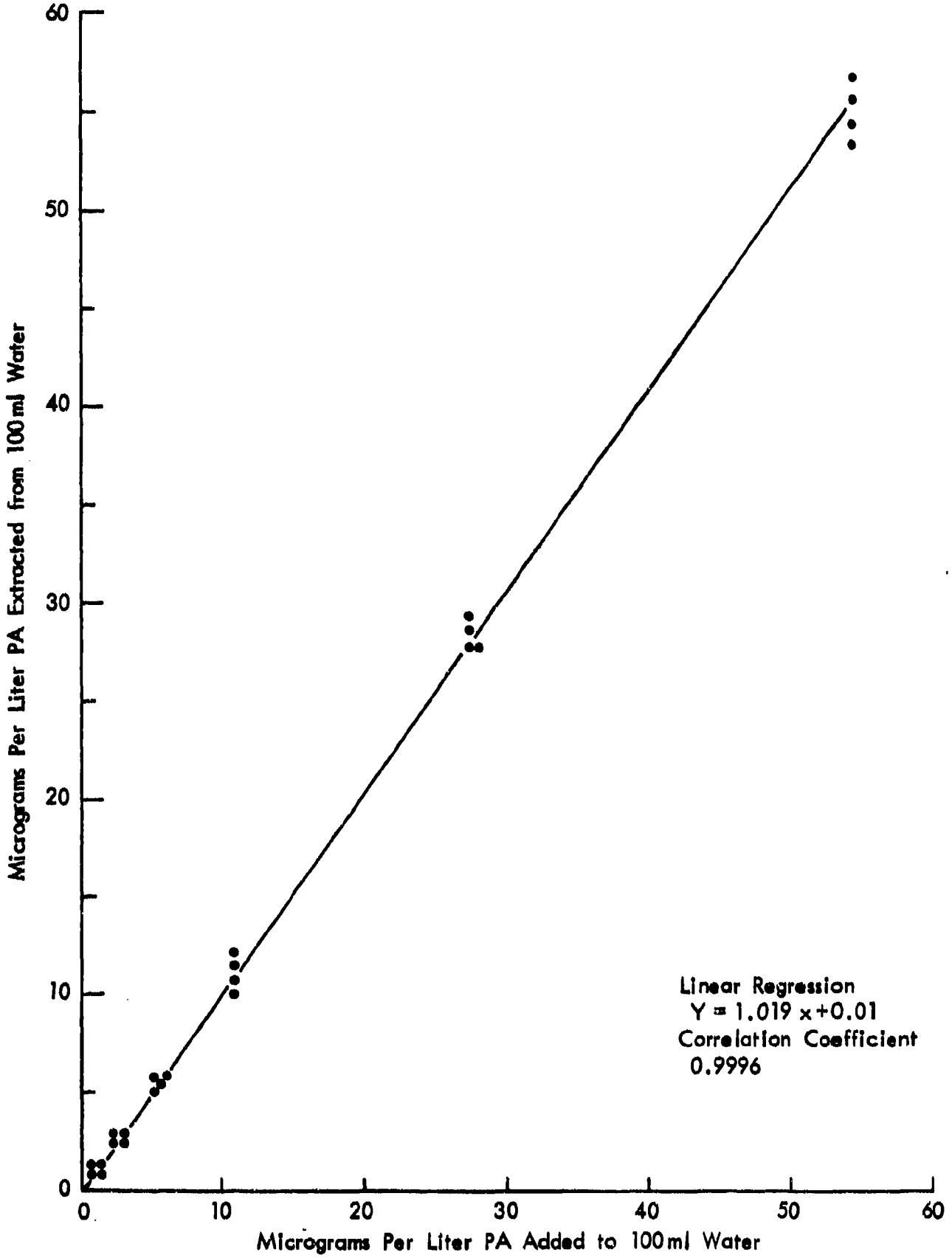


Figure 8 - Linearity of PA Extracted from 100 ml Water

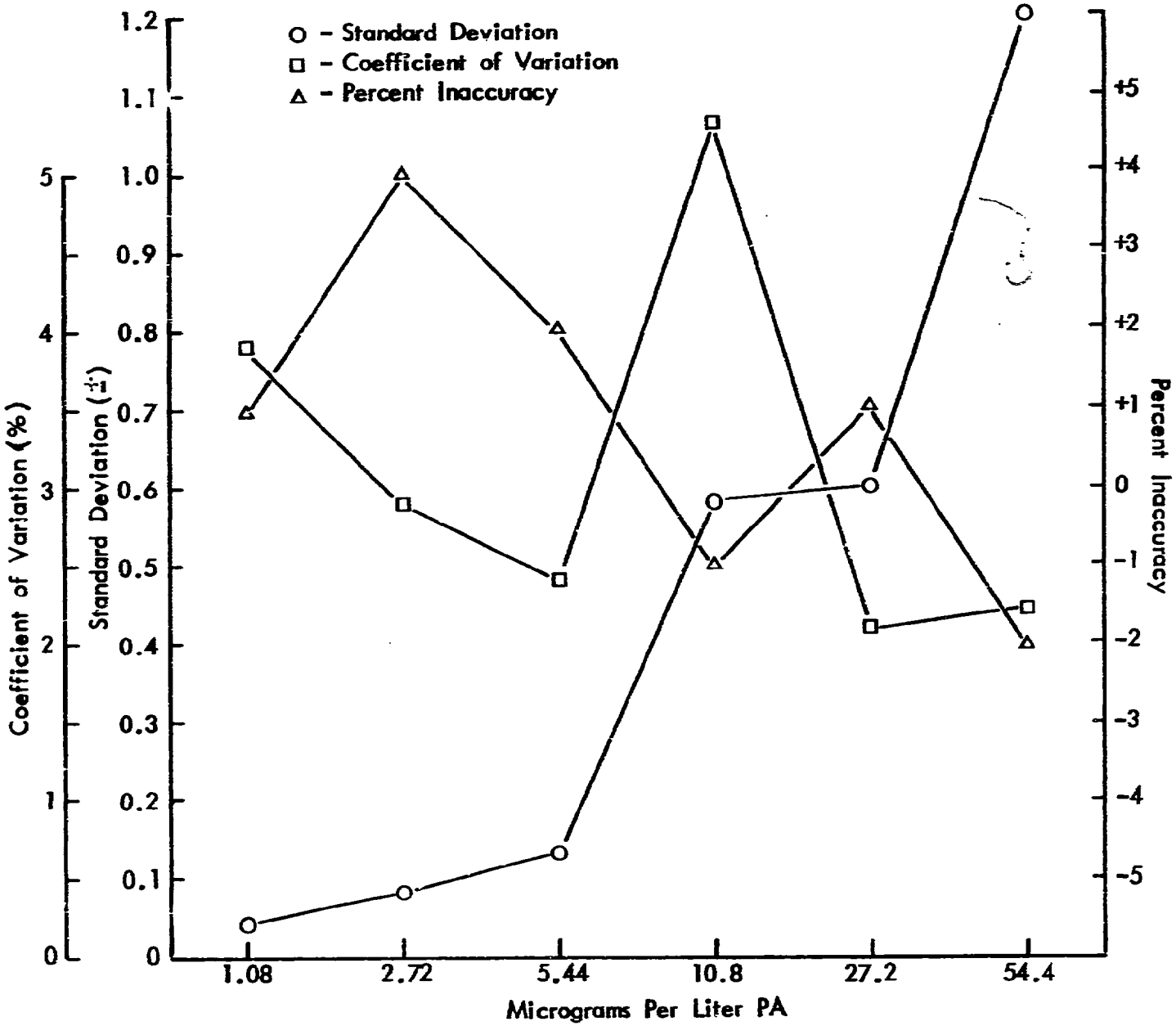


Figure 9 - Standard Deviation, Coefficient of Variation, and Percent Inaccuracy for PA

TABLE 1

PRECISION AND ACCURACY ASSESSMENT OF THE METHOD FOR EXTRACTION FROM WATER AND SAMPLE PREPARATION OF NB, 2,6-DNT, NG, AND PA

Compound	µg/ℓ Added	µg/ℓ Recovered A	B	C	D	Average	Standard Deviation	Coefficient of Variation	Percent Inaccuracy
NB	52.5	30.5	30.4	43.1	38.2	35.6	± 6.2	17	-32
	26.2	17.4	13.0	21.5	20.3	18.0	± 3.8	21	-31
	10.5	8.98	7.53	7.70	7.62	7.96	± 0.69	8.6	-24
	5.25	3.77	3.11	3.97	3.60	3.61	± 0.37	10	-31
	2.62	2.13	1.69	2.04	1.78	1.91	± 0.21	11	-27
	1.05	0.89	0.62	0.85	0.84	0.80	± 0.12	15	-23
	0	< 0.2	< 0.2	< 0.2	< 0.2	-	-	-	-
	Linear Regression y = 0.676x + 0.221; Correlation Coefficient, 0.980								
2,6-DNT	56.2	49.1	47.4	50.7	51.4	49.7	± 1.8	3.6	-12
	28.1	24.2	23.0	26.0	27.2	25.1	± 1.9	7.4	-11
	11.2	9.05	9.90	9.42	10.1	9.62	± 0.47	4.9	-14
	5.62	4.76	4.67	4.69	5.05	4.79	± 0.18	3.7	-15
	2.81	2.46	2.74	2.22	2.56	2.50	± 0.22	8.7	-11
	1.12	0.96	1.10	0.90	1.06	1.01	± 0.09	9.1	-10
	0	< 0.2	< 0.2	< 0.2	< 0.2		-	-	-
	Linear Regression y = 0.886x - 0.056; Correlation Coefficient, 0.9986								
NG	1,520	1,400	1,440	1,520	1,400	1,440	± 56	3.9	-5
	759	715	731	760	765	743	± 24	3.2	-2
	304	281	304	299	288	293	± 10	3.6	-4
	152	142	154	144	145	146	± 5.3	3.6	-4
	75.9	73.4	74.8	74.0	71.9	73.5	± 1.2	1.7	-3
	30.4	27.7	30.5	30.1	30.7	29.8	± 1.4	4.7	-2
	0	< 6	< 6	< 6	< 6	-	-	-	-
	Linear Regression y = 0.945x + 4.4; Correlation Coefficient, 0.9988								

TABLE 1 (continued)

Compound	µg/ℓ Added	µg/ℓ Recovered A	B	C	D	Average	Standard Deviation	Coefficient of Variation	Percent Inaccuracy
PA	54.4	54.7	53.9	55.7	56.7	55.2	± 1.2	2.2	+1
	27.2	27.9	27.6	28.1	29.0	28.2	± 0.60	2.1	+4
	10.8	10.9	11.6	10.3	11.4	11.0	± 0.58	5.3	+2
	5.44	5.38	5.52	5.45	5.22	5.39	± 0.13	2.4	-1
	2.72	2.79	2.86	2.74	2.67	2.76	± 0.08	2.9	+1
	1.08	1.02	1.02	1.10	1.08	1.06	± 0.04	3.9	-2
	0	< 0.2	< 0.2	< 0.2	< 0.2		-	-	-

Linear Regression y = 1.019x + 0.013; Correlation Coefficient, 0.9996

TABLE 2

STATISTICAL EVALUATION OF THE EXTRACTION FROM WATER AND SAMPLE PREPARATION OF NB, 2,6-DNT, NG, AND PA BY THE HUBAUX AND VOS RECOVERY DETECTION LIMIT PROGRAM

NB

Number of[a] Data Points	Linear Regression	Correlation Coefficient	Degrees of Freedom	t[b]	y-Intercept[c]	Detection[d] Limit
28	y = 0.676x + 0.221	0.980	26	1.706	2.59	6.92

µg/100 ℓ Water Added	Average[e] µg Found/Sample	Standard[f] Deviation	Percent[g] Imprecision	Percent[h] Inaccuracy
52.5	52.3	± 3.6	10.1	- 32
26.2	26.4	± 2.2	12.1	- 31
10.5	11.4	± 0.40	5.0	- 24
5.25	5.02	± 0.21	5.9	- 31
2.62	2.50	± 0.12	6.3	- 27
1.05	0.86	± 0.07	8.8	- 24
0	ND[i]	-	-	-

a Number of data points - data points utilized in calculation of the linear regression equation and detection limits = 28, all data.

b t - 2-tail p level (usually 0.1, each confidence band is 0.05 so total p = 0.1).

c y-intercept - intercept on y-axis of upper confidence limit line.

d Detection limit - x-intercept of y-intercept and lower confidence limit line.

e Average µg/liter found - average at each of the seven levels determined from linear regression equation for each of the four found concentrations within each level.

f Standard deviation - determined from average value (e above) and observed values.

g Percent imprecision - standard deviation divided by average value times 100%.

h Percent inaccuracy - determined from the average values of the seven observed values at each level.

$$\% \text{ Inaccuracy} = \frac{\text{Average observed values - level added}}{\text{level added}} \times 100$$

i ND - not detectable, less than 0.2 µg/liter.

TABLE 3

STATISTICAL EVALUATION OF THE EXTRACTION FROM WATER AND SAMPLE PREPARATION OF NB, 2,6-DNT, NG, AND PA BY THE HUBAUX AND VOS RECOVERY DETECTION LIMIT PROGRAM

2,6-DNT

Number of[a] Data Points	Linear Regression	Correlation Coefficient	Degrees of Freedom	t[b]	y-Intercept[c]	Detection[d] Limit
28	y = 0.886 - 0.056	0.9986	26	1.706	0.81	1.93

µg/100 £ Water Added	Average[e] µg Found/Sample	Standard[f] Deviation	Percent[g] Imprecision	Percent[h] Inaccuracy
56.2	56.1	± 1.0	2.1	- 12
28.1	28.4	± 1.1	4.3	- 11
11.2	10.9	± 0.27	2.8	- 14
5.62	5.47	± 0.10	2.1	- 15
2.81	2.88	± 0.13	5.0	- 11
1.12	1.20	± 0.05	5.3	- 10
0	ND[i]	-	-	-

a Number of data points - data points utilized in calculation of the linear regression equation and detection limits = 28, all data.

b t - 2-tail p level (usually 0.1, each confidence band is 0.05 so total p = 0.1).

c y-intercept - intercept on y-axis of upper confidence limit line.

d Detection limit - x-intercept of y-intercept and lower confidence limit line.

e Average µg/liter found - average at each of the seven levels determined from linear regression equation for each of the four found concentrations within each level.

f Standard deviation - determined from average value (e above) and observed values.

g Percent imprecision - standard deviation divided by average value times 100%.

h Percent inaccuracy - determined from the average values of the seven observed values at each level.

$$\% \text{ Inaccuracy} = \frac{\text{average observed values - level added}}{\text{level added}} \times 100$$

i ND - not detectable, less than 0.2 µg/liter.

TABLE 4

STATISTICAL EVALUATION OF THE EXTRACTION FROM WATER AND SAMPLE PREPARATION OF NB, 2,6-DNT, NG, AND PA BY THE HUBAUX AND VOS RECOVERY DETECTION LIMIT PROGRAM

NG

Number of Data Points[a]	Linear Regression	Correlation Coefficient	Degrees of Freedom	t[b]	y-Intercept[c]	Detection Limit[d]
28	y = 0.945x + 4.40	0.9988	26	1.706	27.4	48.5

µg/100 ℓ Water Added	Average[e] µg Found/Sample	Standard[f] Deviation	Percent[g] Imprecision	Percent[h] Inaccuracy
1,520	1,509	± 35	2.4	- 6
759	781	± 14	1.9	- 2
304	305	± 6.0	2.1	- 4
152	150	± 3.1	2.1	- 4
75.9	73.2	± 0.71	1.0	- 3
30.4	26.8	± 0.80	2.7	- 2
0	ND[i]	-	-	-

a Number of data points - data points utilized in calculation of the linear regression equation and detection limits = 28, all data.

b t - 2-tail p level (usually 0.1, each confidence band is 0.05 so total p = 0.1).

c y-intercept - intercept on y-axis of upper confidence limit line.

d Detection limit - x-intercept of y-intercept and lower confidence limit line.

e Average µg/liter found - average at each of the seven levels determined from linear regression equation for each of the four found concentrations within each level.

f Standard deviation - determined from average value (e above) and observed values.

g Percent imprecision - standard deviation divided by average value times 100%.

h Percent inaccuracy - determined from the average values of the seven observed values at each level.

$$\% \text{ Inaccuracy} = \frac{\text{Average observed values - level added}}{\text{level added}} \times 100$$

i ND - not detectable, less than 6 µg/liter.

TABLE 5

STATISTICAL EVALUATION OF THE EXTRACTION FROM WATER AND SAMPLE PREPARATION OF NB, 2,6-DNT, NG, AND PA BY THE HUBAUX AND VOS RECOVERY DETECTION LIMIT PROGRAM

PA

Number of[a] Data Points	Linear Regression	Correlation Coefficient	Degrees of Freedom	t[b]	y-Intercept[c]	Detection[d] Limit
28	y = 1.019x + 0.013	0.9996	26	1.706	0.52	1.00

µg/100 ℓ Water Added	Average[e] µg Found/Sample	Standard[f] Deviation	Percent[g] Imprecision	Percent[h] Inaccuracy
54.4	54.2	± 0.70	1.3	+ 2
27.2	27.6	± 0.35	1.2	+ 3
10.8	10.8	± 0.33	3.0	+ 2
5.44	5.28	± 0.07	1.4	- 1
2.72	2.70	± 0.05	1.7	+ 2
1.08	1.02	± 0.02	2.3	- 2
0	ND[i]	-	-	-

a Number of data points - data points utilized in calculation of the linear regression equation and detection limits = 28, all data.

b t - 2-tail p level (usually 0.1, each confidence band is 0.05 so total p = 0.1).

c y-intercept - intercept on y-axis of upper confidence limit line.

d Detection limit - x-intercept of y-intercept and lower confidence limit line.

e Average µg/liter found - average at each of the seven levels determined from linear regression equation for each of the four found concentrations within each level.

f Standard deviation - determined from average value (e above) and observed values.

g Percent imprecision - standard deviation divided by average value times 100%.

h Percent inaccuracy - determined from the average values of the seven observed values at each level.

$$\% \text{ Inaccuracy} = \frac{\text{Average observed values - level added}}{\text{level added}} \times 100$$

i ND - not detectable, less than 0.2 µg/liter.

TABLE 6

PRECISION AND ACCURACY DETERMINATION OF THE SAMPLE PREPARATION METHODOLOGY FOR NB

Water Extract Sample Number	µg/100 ml[a] Water Added	Peak Heights[b] NB	Peak Heights[b] IS	µg IS[c]	µg Found/Sample[d]	µg/ℓ (ppb)[e]
A-10	5.25	66.5	93.0	2.22	3.05	30.5
A-5	2.62	75.0	183.5	2.22	1.74	17.4
A-2	1.05	36.8	175.0	2.22	0.898	8.98
A-1	0.525	52.5	59.5	0.222	0.377	3.77
A-0.5	0.262	28.5	57.0	0.222	0.213	2.13
A-0.2	0.105	13.8	66.0	0.222	0.089	0.89
A-0	0	< 2	64.8	0.222	< 0.020	< 0.2
B-10	5.25	43.2	61.8	2.22	3.04	30.4
B-5	2.62	58.0	194.2	2.22	1.30	13.0
B-2	1.05	34.0	196.5	2.22	0.753	7.53
B-1	0.525	47.5	66.5	0.222	0.311	3.11
B-0.5	0.262	23.8	61.2	0.222	0.169	1.69
B-0.2	0.105	9.0	62.8	0.222	0.062	0.62
B-0	0	< 2	65.5	0.222	< 0.02	< 0.2
C-10	5.25	58.5	58.0	2.22	4.31	43.1
C-5	2.62	99.0	196.5	2.22	2.15	21.5
C-2	1.05	34.2	189.5	2.22	0.770	7.70
C-1	0.525	61.0	65.6	0.222	0.397	3.97
C-0.5	0.262	34.0	71.0	0.222	0.204	2.04
C-0.2	0.105	12.2	61.0	0.222	0.085	0.85
C-0	0	< 2	64.5	0.222	< 0.02	< 0.2
D-10	5.25	57.5	60.8	2.22	3.82	38.2
D-5	2.62	86.8	172.2	2.22	2.03	20.3
D-2	1.05	34.5	182.8	2.22	0.762	7.62
D-1	0.525	51.8	58.0	0.222	0.360	3.60
D-0.5	0.262	27.8	63.2	0.222	0.178	1.78
D-0.2	0.105	13.8	66.0	0.222	0.084	0.84
D-0	0	< 2	61.2	0.222	< 0.02	< 0.2

TABLE 6 (continued)

REFERENCE SOLUTIONS FOR NB FOR THE PRECISION AND ACCURACY DETERMINATION OF SAMPLE PREPARATION METHOD FOR NB

Reference Solution Number	ng Added[f]	Peak Heights[b] NB	Peak Heights[b] IS	ng IS[c]	RWR[g]	Avg RWR SD RSD[h]
A-10	5,250	116.0	90.2	2,220	0.54	A
A-2	1,050	47.0	199.2	2,220	0.50	Avg RWR 0.52
A-1	525	65.0	49.8	222	0.55	SD ± 0.03
A-0.2	105	14.5	62.0	222	0.49	RSD 5.8%
B-2	1,050	50.8	50.8	2,220	0.50	B
B-1	525	70.5	54.8	222	0.54	Avg RWR 0.51
B-0.2	105	15.2	15.2	222	0.50	SD ± 0.02
						RSD 3.9%
C-2	1,050	48.8	198.2	2,220	0.52	C
C'-2	1,050	52.8	210.0	2,220	0.53	Avg RWR 0.52
C-1	525	63.0	50.4	222	0.53	SD ± 0.02
C-0.2	105	16.0	65.0	222	0.52	RSD 3.8%
C'-0.2	105	16.0	64.0	222	0.53	
C"-0.2	105	15.0	66.2	222	0.48	
D-2	1,050	48.2	187.5	2,220	0.54	D
D-1	525	79.0	65.2	222	0.51	Avg RWR 0.55
D-0.2	105	16.8	60.2	222	0.59	SD ± 0.04
						RSD 7.3%

a µg/100 ml Water Added - µg of NB added to 100 ml water.
b Peak Heights - measured hieght of NB and IS in millimeters.
c µg IS - µg IS present in the ∿ 2 ml final sample.
d µg Found/Sample - µg NB recovered from 100 ml water.

$$\frac{\mu g \text{ Found}}{\text{Sample}} = \frac{\text{Peak Height NB}}{\text{Peak Height IS}} \times \frac{\mu g \text{ IS}/\sim 2 \text{ ml Sample}}{\text{Average RWR for NB}}$$

e µg/ℓ (ppb) - µg found in 100-ml sample extract equated to µg/ℓ.
f Added - nanograms compound added to reference solution (∿ 2 ml).

g RWR - Relative Weight Response = $\frac{\text{Peak Height NB}}{\text{Peak Height IS}} \times \frac{\mu g \text{ IS/reference solution}}{\mu g \text{ NB reference solution}}$

h Avg - Average RWR = $\frac{\Sigma \text{RWR's}}{\text{Number of Reference Solutions (N)}}$

SD - Standard Deviation = $\left(\frac{n \Sigma \text{RWR}^2 - (\Sigma \text{RWR})^2}{n(n-1)}\right)^{\frac{1}{2}}$

RSD - Relative Standard Deviation = $\frac{\text{SD}}{\text{Avg RWR}} \times 100$

TABLE 7

PRECISION AND ACCURACY DETERMINATION OF THE SAMPLE PREPARATION METHODOLOGY FOR 2,6-DNT

Water Extract Sample Number	µg/100 ml[a] Water Added	Peak Heights[b] 2,6-DNT	Peak Heights[b] IS	µg IS[c]	µg Found/Sample[d]	µg/ℓ (ppb)[e]
A-10	5.62	185.0	93.0	2.22	4.91	49.1
A-5	2.81	180.0	183.5	2.22	2.42	24.2
A-2	1.12	64.2	175.0	2.22	0.905	9.05
A-1	0.562	114.8	59.5	0.222	0.476	4.76
A-0.5	0.281	56.8	57.0	0.222	0.246	2.46
A-0.2	0.112	25.8	66.0	0.222	0.096	0.96
A-0	0	< 2	64.8	0.222	< 0.020	< 0.2
B-10	5.62	114.8	61.8	2.22	4.74	47.4
B-5	2.81	174.8	194.2	2.22	2.30	23.0
B-2	1.12	76.2	196.5	2.22	0.990	9.90
B-1	0.562	121.8	66.5	0.222	0.467	4.67
B-0.5	0.281	65.8	61.2	0.222	0.274	2.74
B-0.2	0.112	27.0	62.8	0.222	0.110	1.10
B-0	0	< 2	65.5	0.222	< 0.02	< 0.2
C-10	5.62	123.2	58.0	2.22	5.07	50.7
C-5	2.81	213.8	196.5	2.22	2.60	26.0
C-2	1.12	74.8	189.5	2.22	0.942	9.42
C-1	0.562	129.0	65.6	0.222	0.469	4.69
C-0.5	0.281	66.0	71.0	0.222	0.222	2.22
C-0.2	0.112	23.0	61.0	0.222	0.090	0.90
C-0	0	< 2	64.5	0.222	< 0.02	< 0.2
D-10	5.62	128.2	60.8	2.22	5.14	51.4
D-5	2.81	191.8	172.2	2.22	2.72	27.2
D-2	1.12	76.0	182.8	2.22	1.01	10.1
D-1	0.562	120.0	58.0	0.222	0.505	5.05
D-0.5	0.281	66.2	63.2	0.222	0.256	2.56
D-0.2	0.112	28.8	66.0	0.222	0.106	1.06
D-0	0	< 2	61.2	0.222	< 0.02	< 0.2

TABLE 7 (continued)

REFERENCE SOLUTIONS FOR 2,6-DNT FOR THE PRECISION AND ACCURACY DETERMINATION OF SAMPLE PREPARATION METHOD FOR 2,6-DNT

Reference Solution Number	ng Added[f]	Peak Heights[b] 2,6-DNT	Peak Heights[b] IS	ng IS[c]	RWR[g]	Avg RWR SD RSD[h]
A-10	5,620	211.8	90.2	2,220	0.93	A
A-2	1,120	92.0	199.2	2,220	0.92	Avg RWR 0.90
A-1	562	115.5	49.8	222	0.92	SD ± 0.04
A-0.2	112	26.2	62.0	222	0.84	RSD 4.4%
B-2	1,120	92.2	215.5	2,220	0.85	B
B-1	562	115.8	54.8	222	0.83	Avg RWR 0.87
B-0.2	112	30.0	64.2	222	0.93	SD ± 0.05
						RSD 5.7%
C-2	1,120	90.0	198.2	2,220	0.90	C
C'-2	1,120	99.2	210.0	2,220	0.94	Avg RWR 0.93
C-1	562	115.6	50.4	222	0.91	SD ± 0.04
C-0.2	112	32.0	65.0	222	0.98	RSD 4.3%
C'-0.2	112	31.2	64.0	222	0.97	
C'-0.2	112	28.8	66.2	222	0.86	
D-2	1,120	87.8	187.5	2,220	0.93	D
D-1	562	145.2	65.2	222	0.88	Avg RWR 0.91
D-0.2	112	28.0	60.2	222	0.92	SD ± 0.03
						RSD 3.3%

a µg/100 ml Water Added - µg of 2,6-DNT added to 100 ml water.
b Peak Heights - measured hieght of 2,6-DNT and IS in millimeters.
c µg IS - µg IS present in the ∿ 2 ml final sample.
d µg Found/Sample - µg 2,6-DNT recovered from 100 ml water.

$$\frac{\mu g\ Found}{Sample} = \frac{Peak\ Height\ 2,6\text{-}DNT}{Peak\ Height\ IS} \times \frac{\mu g\ IS/\sim 2\ ml\ Sample}{Average\ RWR\ for\ 2,6\text{-}DNT}$$

e µg/ℓ (ppb) - µg found in 100-ml sample extract equated to µg/ℓ.
f Added - nanograms compound added to reference solution (∿ 2 ml).

g RWR - Relative Weight Response = $\frac{Peak\ Height\ 2,6\text{-}DNT}{Peak\ Height\ IS} \times \frac{\mu g\ IS/reference\ solution}{\mu g\ 2,6\text{-}DNT\ reference\ solution}$

h Avg - Average RWR = $\frac{\Sigma RWR's}{Number\ of\ Reference\ Solutions\ (N)}$

SD - Standard Deviation = $\left(\frac{n\ \Sigma\ RWR^2 - (\Sigma\ RWR)^2}{n(n-1)}\right)^{\frac{1}{2}}$

RSD - Relative Standard Deviation = $\frac{SD}{Avg\ RWR} \times 100$

TABLE 8

PRECISION AND ACCURACY DETERMINATION OF THE SAMPLE PREPARATION METHODOLOGY FOR NG

Water Extract Sample Number	µg/100 ml[a] Water Added	Peak Heights[b] NG	Peak Heights[b] IS	µg IS[c]	µg Found/Sample[d]	µg/ℓ (ppb)[e]
A-10	152	193.2	93.0	2.22	140.0	1,400
A-5	75.9	195.0	183.5	2.22	71.5	715
A-2	30.4	73.0	175.0	2.22	28.1	281
A-1	15.2	126.0	59.5	0.222	14.2	142
A-0.5	7.59	62.2	57.0	0.222	7.34	73.4
A-0.2	3.04	27.2	66.0	0.222	2.77	27.7
A-0	0	< 2	64.8	0.222	< 0.6	< 6
B-10	152	116.2	61.8	2.22	144.0	1,440
B-5	75.9	185.5	194.2	2.22	73.1	731
B-2	30.4	78.0	196.5	2.22	30.4	304
B-1	15.2	133.5	66.5	0.222	15.4	154
B-0.5	7.59	59.8	61.2	0.222	7.48	74.8
B-0.2	3.04	25.0	62.8	0.222	3.05	30.5
B-0	0	< 2	65.5	0.222	< 0.6	< 6
C-10	152	119.0	58.0	2.22	152.0	1,520
C-5	75.9	201.8	196.5	2.22	76.0	760
C-2	30.4	76.5	189.5	2.22	29.9	299
C-1	15.2	127.6	65.6	0.222	14.4	144
C-0.5	7.59	71.0	71.0	0.222	7.40	74.0
C-0.2	3.04	24.8	61.0	0.222	3.01	30.1
C-0	0	< 2	64.5	0.222	< 0.6	< 6
D-10	152	122.8	60.8	2.22	140.0	1,400
D-5	75.9	189.8	172.2	2.22	76.5	765
D-2	30.4	76.0	182.8	2.22	28.8	288
D-1	15.2	121.0	58.0	0.222	14.5	145
D-0.5	7.59	65.5	63.2	0.222	7.19	71.9
D-0.2	3.04	29.2	66.0	0.222	3.07	30.7
D-0	0	< 2	61.2	0.222	< 0.6	< 6

TABLE 8 (continued)

REFERENCE SOLUTIONS FOR NG FOR THE PRECISION AND ACCURACY DETERMINATION OF SAMPLE PREPARATION METHOD FOR NG

Reference Solution Number	ng Added[f]	Peak Heights[b] NG	Peak Heights[b] IS	ng IS[c]	RWR[g]	Avg RWR SD RSD[h]
A-10	151,830	203.0	90.2	2,220	0.033	A
A-2	30,370	90.8	199.2	2,220	0.033	Avg RWR 0.033
A-1	15,180	111.2	49.8	222	0.033	SD ± 0.0005
A-0.2	3,040	29.2	62.0	222	0.034	RSD 1.5%
B-2	30,370	90.8	215.5	2,220	0.031	B
B-1	15,180	103.2	54.8	222	0.028	Avg RWR 0.029
B-0.2	3,040	24.8	64.2	222	0.028	SD ± 0.002
						RSD 6.9%
C-2	30,370	83.0	198.2	2,220	0.031	C
C'-2	30,370	88.2	210.0	2,220	0.031	Avg RWR 0.030
C-1	15,180	100.0	50.4	222	0.029	SD ± 0.002
C-0.2	3,040	24.0	65.0	222	0.027	RSD 6.7%
C'-0.2	3,040	30.0	64.0	222	0.034	
C"-0.2	3,040	27.0	66.2	222	0.029	
D-2	30,370	82.2	187.5	2,220	0.032	D
D-1	15,180	132.2	65.2	222	0.030	Avg RWR 0.032
D-0.2	3,040	27.2	60.2	222	0.033	SD ± 0.002
						RSD 6.2%

a µg/100 ml Water Added - µg of NG added to 100 ml water.
b Peak Heights - measured hieght of NG and IS in millimeters.
c µg IS - µg IS present in the ∿ 2 ml final sample.
d µg Found/Sample - µg NG recovered from 100 ml water.

$$\frac{\mu g \text{ Found}}{\text{Sample}} = \frac{\text{Peak Height NG}}{\text{Peak Height IS}} \times \frac{\mu g \text{ IS}/\sim 2 \text{ ml Sample}}{\text{Average RWR for NG}}$$

e µg/ℓ (ppb) - µg found in 100-ml sample extract equated to µg/ℓ.
f Added - nanograms compound added to reference solution (∿ 2 ml).

g RWR - Relative Weight Response = $\frac{\text{Peak Height NG}}{\text{Peak Height IS}} \times \frac{\mu g \text{ IS/reference solution}}{\mu g \text{ NG reference solution}}$

h Avg - Average RWR = $\frac{\Sigma \text{RWR's}}{\text{Number of Reference Solutions (N)}}$

SD - Standard Deviation = $\left(\frac{n \Sigma \text{RWR}^2 - (\Sigma \text{RWR})^2}{n(n-1)}\right)^{\frac{1}{2}}$

RSD - Relative Standard Deviation = $\frac{\text{SD}}{\text{Avg RWR}} \times 100$

TABLE 9

PRECISION AND ACCURACY DETERMINATION OF THE SAMPLE PREPARATION METHODOLOGY FOR PA

Water Extract Sample Number	µg/100 ml[a] Water Added	Peak Heights[b] PA	Peak Heights[b] IS	µg IS[c]	µg Found/Sample[d]	µg/ℓ (ppb)[e]
A-10	5.44	169.5	93.0	2.22	5.47	54.7
A-5	2.72	170.8	183.5	2.22	2.79	27.9
A-2	1.08	63.8	175.0	2.22	1.09	10.9
A-1	0.544	106.8	59.5	0.222	0.538	5.38
A-0.5	0.272	53.0	57.0	0.222	0.279	2.79
A-0.2	0.108	22.5	66.0	0.222	0.102	1.02
A-0	0	< 2	64.8	0.222	< 0.02	< 0.2
B-10	5.44	108.0	61.8	2.22	5.39	53.9
B-5	2.72	173.8	194.2	2.22	2.76	27.6
B-2	1.08	73.8	196.5	2.22	1.16	11.6
B-1	0.544	119.0	66.5	0.222	0.552	5.52
B-0.5	0.272	56.8	61.2	0.222	0.282	2.86
B-0.2	0.108	20.8	62.8	0.222	0.102	1.02
B-0	0	< 2	65.5	0.222	< 0.02	< 0.2
C-10	5.44	104.8	58.0	2.22	5.57	55.7
C-5	2.72	179.2	196.5	2.22	2.81	28.1
C-2	1.08	63.5	189.5	2.22	1.03	10.3
C-1	0.544	116.0	65.6	0.222	0.545	5.45
C-0.5	0.272	63.0	71.0	0.222	0.274	2.74
C-0.2	0.108	21.8	61.0	0.222	0.110	1.10
C-0	0	< 2	64.5	0.222	< 0.02	< 0.2
D-10	5.44	115.0	60.8	2.22	5.67	56.7
D-5	2.72	166.2	172.2	2.22	2.90	29.0
D-2	1.08	69.2	182.8	2.22	1.14	11.4
D-1	0.544	101.0	58.0	0.222	0.522	5.22
D-0.5	0.272	56.2	63.2	0.222	0.267	2.67
D-0.2	0.108	23.8	66.0	0.222	0.108	1.08
D-0	0	< 2	61.2	0.222	< 0.02	< 0.2

TABLE 9 (continued)

REFERENCE SOLUTIONS FOR PA FOR THE PRECISION AND ACCURACY DETERMINATION OF SAMPLE PREPARATION METHOD FOR PA

Reference Solution Number	ng Added[f]	Peak Heights[b] PA	Peak Heights[b] IS	ng IS[c]	RWR[g]	Avg RWR SD RSD[h]
A-10	5,440	164.2	90.2	2,220	0.74	A
A-2	1,080	72.0	199.2	2,220	0.74	Avg RWR 0.74
A-1	544	91.2	49.8	222	0.75	SD ± 0.005
A-0.2	108	22.2	62.0	222	0.74	RSD 0.7%
B-2	1,080	73.5	215.5	2,220	0.70	B
B-1	544	91.0	54.8	222	0.68	Avg RWR 0.72
B-0.2	108	24.8	64.2	222	0.79	SD ± 0.06 RSD 8.3%
C-2	1,080	71.0	198.2	2,220	0.74	C
C'-2	1,080	77.2	210.0	2,220	0.76	Avg RWR 0.72
C-1	544	88.0	50.4	222	0.71	SD ± 0.05
C-0.2	108	20.0	65.0	222	0.63	RSD 6.9%
C'-0.2	108	24.2	64.0	222	0.78	
C''-0.2	108	23.8	66.2	222	0.72	
D-2	1,080	74.2	187.5	2,220	0.81	D
D-1	544	118.8	65.2	222	0.74	Avg RWR 0.74
D-0.2	108	20.0	60.2	222	0.68	SD ± 0.06 RSD 8.3%

a µg/100 ml Water Added - µg of PA added to 100 ml water.
b Peak Heights - measured hieght of PA and IS in millimeters.
c µg IS - µg IS present in the ~ 2 ml final sample.
d µg Found/Sample - µg PA recovered from 100 ml water.

$$\frac{\mu g\ Found}{Sample} = \frac{Peak\ Height\ PA}{Peak\ Height\ IS} \times \frac{\mu g\ IS/\sim 2\ ml\ Sample}{Average\ RWR\ for\ PA}$$

e µg/ℓ (ppb) - µg found in 100-ml sample extract equated to µg/ℓ.
f Added - nanograms compound added to reference solution (~ 2 ml).

g RWR - Relative Weight Response = $\frac{Peak\ Height\ PA}{Peak\ Height\ IS} \times \frac{\mu g\ IS/reference\ solution}{\mu g\ PA\ reference\ solution}$

h Avg - Average RWR = $\frac{\Sigma RWR's}{Number\ of\ Reference\ Solutions\ (N)}$

SD - Standard Deviation = $\left(\frac{n\ \Sigma\ RWR^2 - (\Sigma\ RWR)^2}{n(n-1)}\right)^{\frac{1}{2}}$

RSD - Relative Standard Deviation = $\frac{SD}{Avg\ RWR} \times 100$

APPENDIX E

PRECISION AND ACCURACY RESULTS FOR MUNITIONS STORED FOR 3 WEEKS IN TAP WATER

TABLE 1

NB AFTER 3 WEEKS STORAGE IN TAP WATER

Target Conc. Versus Found Conc.

Target Conc. (µg/100 ml)	Found Conc. (µg/100 ml)
48.300	35.200
	39.600
	32.200
	19.100
24.200	17.500
	19.600
	15.300
	17.800
9.660	6.310
	7.830
	5.670
	6.200
4.830	3.280
	3.920
	2.660
	3.540
2.420	1.750
	2.170
	1.750
	1.890
0.966	0.849
	1.150
	0.669
	3.770
0.000	0.000
	0.000
	0.000
	0.000

TABLE 2

STATISTICAL DATA USED TO DETERMINE PERCENT INACCURACY AND IMPRECISION FOR NB

Mean Target Conc. (µg/100 ml)	Mean Found Conc. (µg/100 ml)	Standard Deviation	Mean % Inaccuracy	Imprecision
48.300	31.525	8.823	-34.731	27.988
24.200	17.550	1.764	-27.479	10.049
9.660	6.503	0.928	-32.686	14.272
4.830	3.350	0.530	-30.642	15.814
2.420	1.890	0.198	-21.901	10.476
0.966	1.610	1.454	66.615	90.335
0.000	0.000	0.000		
Means		1.957	-13.471	28.156

TABLE 3

ANALYSIS OF 28 TARGET CONC.-FOUND CONC. POINTS FOR NB

TARGET CONC.
MEAN= 12.9108571429 SD= 16.6672552379

FOUND CONC.
MEAN= 8.91814285714 SD= 11.3395637212

NO. RUNS 1 TOTAL X-Y ALL RUNS 28 NO. CONCENTR. 28
MEASURES (Y'S) EACH TARGET CONC. 1

INTERCEPT= 0.473285290478
SLOPE= 0.65408961413
  USE FOR ACCURACY
R= 0.961401938842
MEAN SQR DEV OF POINTS FROM REGRESSION= 10.1091629729
ST ERROR EST= 3.17949099274
  USE FOR PRECISION
T FOR CONFIDENCE BAND
D.F.= 26
TWO TAIL P LEVEL IS .1
t= 1.70561435167
X(D) FOR CALIBRATION CURVE OR UNKNOWN SAMPLE? C/U C
(EACH TARGET CONC. CONSIDERED INDEP SAMPLE
MEASURED 1 TIME(S))
y(c)= 6.05115756832
x(d)= 16.9750143671

TABLE 4

2,6-DNT AFTER 3 WEEKS STORAGE IN TAP WATER

Target Conc. Versus Found Conc.	
Target Conc. (µg/100 ml)	Found Conc. (µg/100 ml)
52.700	45.900
	45.900
	43.500
	39.000
26.300	24.200
	22.600
	21.400
	23.300
10.500	9.300
	9.500
	8.930
	8.700
5.270	4.640
	4.460
	4.030
	4.010
2.630	2.680
	2.520
	2.130
	2.380
1.050	0.984
	0.872
	0.933
	1.120
0.000	0.000
	0.000
	0.000
	0.000

TABLE 5

STATISTICAL DATA USED TO DETERMINE PERCENT INACCURACY AND IMPRECISION FOR 2,6-DNT

Mean Target Conc. (µg/100 ml)	Mean Found Conc. (µg/100 ml)	Standard Deviation	Mean % Inaccuracy	Imprecision
52.700	43.575	3.253	-17.315	7.465
26.300	22.875	1.181	-13.023	5.165
10.500	9.108	0.360	-13.262	3.952
5.270	4.285	0.315	-18.691	7.347
2.630	2.428	0.233	-7.700	9.604
1.050	0.977	0.106	-6.929	10.807
0.000	0.000	0.000		
Means		0.778	-12.820	7.390

TABLE 6

ANALYSIS OF 28 TARGET CONC.-FOUND CONC. POINTS FOR 2,6-DNT

TARGET CONC.
MEAN= 14.0642857143 SD= 18.1776964332

FOUND CONC.
MEAN= 11.8924642857 SD= 15.1505591377

NO. RUNS 1 TOTAL X-Y ALL RUNS 28 NO. CONCENTR. 28
MEASURES (Y'S) EACH TARGET CONC. 1

INTERCEPT= 0.209039834383
SLOPE= 0.830715806595
USE FOR ACCURACY
R= 0.996695872237
MEAN SQR DEV OF POINTS FROM REGRESSION= 1.5725935512
ST ERROR EST= 1.25403092115
USE FOR PRECISION
T FOR CONFIDENCE BAND
D.F.= 26
TWO TAIL P LEVEL IS .1
t= 1.70561435167
X(D) FOR CALIBRATION CURVE OR UNKNOWN SAMPLE? C/U C
(EACH TARGET CONC. CONSIDERED INDEP SAMPLE
MEASURED 1 TIME(S))
y(c)= 2.40896782509
x(d)= 5.27948509357

TABLE 7

NG AFTER 3 WEEKS STORAGE IN TAP WATER

Target Conc. Versus Found Conc.

Target Conc. (µg/100 ml)	Found Conc. (µg/100 ml)
1450.000	1090.000
	1200.000
	1480.000
	1340.000
726.000	582.000
	580.000
	739.000
	691.000
290.000	257.000
	250.000
	287.000
	265.000
145.000	129.000
	125.000
	155.000
	148.000
72.600	66.800
	66.400
	74.300
	74.000
29.000	27.700
	27.400
	32.100
	26.800
0.000	0.000
	0.000
	0.000
	0.000

TABLE 8

STATISTICAL DATA USED TO DETERMINE PERCENT INACCURACY AND IMPRECISION FOR NG

Mean Target Conc. (µg/100 ml)	Mean Found Conc. (µg/100 ml)	Standard Deviation	Mean % Inaccuracy	Imprecision
1450.000	1277.500	169.386	-11.897	13.259
726.000	648.000	79.812	-10.744	12.317
290.000	264.750	16.049	-8.707	6.062
145.000	139.250	14.523	-3.966	10.429
72.600	70.375	4.364	-3.065	6.201
29.000	28.500	2.429	-1.724	8.523
0.000	0.000	0.000		
Means		40.938	-6.684	9.465

TABLE 9

ANALYSIS OF 28 TARGET CONC.-FOUND CONC. POINTS FOR NG

TARGET CONC.
MEAN= 387.514285714 SD= 500.317159939

FOUND CONC.
MEAN= 346.910714286 SD= 444.121320044

NO. RUNS 1 TOTAL X-Y ALL RUNS 28 NO. CONCENTR. 28
MEASURES (Y'S) EACH TARGET CONC. 1

INTERCEPT= 6.39973771242
SLOPE= 0.878705609383
 USE FOR ACCURACY
R= 0.989890543568
MEAN SQR DEV OF POINTS FROM REGRESSION= 4120.50697154
ST ERROR EST= 64.1911751843
 USE FOR PRECISION
T FOR CONFIDENCE BAND
D.F.= 26
TWO TAIL P LEVEL IS .1
t= 1.70561435167
X(D) FOR CALIBRATION CURVE OR UNKNOWN SAMPLE? C/U C
(EACH TARGET CONC. CONSIDERED INDEP. SAMPLE
MEASURED 1 TIME(S))
y(c)= 119.011893096
x(d)= 255.119476406

TABLE 10

PA AFTER 3 WEEKS STORAGE IN TAP WATER

Target Conc. Versus Found Conc.	
Target Conc. (µg/100 ml)	Found Conc. (µg/100 ml)
51.300	52.500
	52.900
	46.400
	43.700
25.600	25.300
	23.000
	23.000
	26.500
10.300	10.600
	10.500
	7.480
	9.220
5.130	5.570
	5.400
	4.510
	4.510
2.560	2.980
	2.600
	2.530
	2.300
1.030	1.120
	1.300
	1.130
	0.859
0.000	0.000
	0.000
	0.000
	0.000

TABLE 11

STATISTICAL DATA USED TO DETERMINE PERCENT INACCURACY AND IMPRECISION FOR PA

Mean Target Conc. (µg/100 ml)	Mean Found Conc. (µg/100 ml)	Standard Deviation	Mean % Inaccuracy	Imprecision
51.300	48.875	4.555	-4.727	9.320
25.600	24.450	1.745	-4.492	7.135
10.300	9.450	1.456	-8.252	15.406
5.130	4.998	0.567	-2.583	11.349
2.560	2.603	0.282	1.660	10.852
1.030	1.102	0.182	7.015	16.511
0.000	0.000	0.000		
Means		1.255	-1.897	11.762

TABLE 12

ANALYSIS OF 28 TARGET CONC.-FOUND CONC. POINTS FOR PA

TARGET CONC.
MEAN= 13.7028571429 SD= 17.6915687928

FOUND CONC.
MEAN= 13.0681785714 SD= 16.9231489804

NO. RUNS 1 TOTAL X-Y ALL RUNS 28 NO. CONCENTR. 28
MEASURES (Y'S) EACH TARGET CONC. 1

INTERCEPT= 0.0282885850403
SLOPE= 0.95161832678
 USE FOR ACCURACY
R= 0.994827919569
MEAN SQR DEV OF POINTS FROM REGRESSION= 3.06848129019
ST ERROR EST= 1.75170810645
 USE FOR PRECISION
T FOR CONFIDENCE BAND
D.F.= 26
TWO TAIL P LEVEL IS .1
t= 1.70561435167
X(D) FOR CALIBRATION CURVE OR UNKNOWN SAMPLE? C/U C
(EACH TARGET CONC. CONSIDERED INDEP. SAMPLE
MEASURED 1 (TIME(S))
y(c)= 3.10135351066
x(d)= 6.43414617357

APPENDIX F

PRECISION AND ACCURACY RESULTS FOR MUNITIONS STORED FOR 10 WEEKS IN TAP WATER

TABLE 1

RDX AFTER 10 WEEKS STORAGE IN TAP WATER

Target Conc. Versus Found Conc.	
Target Conc. (µg/100 ml)	Found Conc. (µg/100 ml)
55.200	53.200
	57.000
	58.100
	56.200
27.600	27.500
	28.500
	27.900
	27.900
11.000	9.540
	10.500
	10.700
	9.610
5.520	5.670
	5.540
	5.960
	5.440
2.760	2.770
	2.900
	2.660
	2.030
1.100	1.580
	1.140
	1.620
	1.650
0.000	0.000
	0.000
	0.000
	0.000

TABLE 2

STATISTICAL DATA USED TO DETERMINE PERCENT INACCURACY AND IMPRECISION FOR RDX

Mean Target Conc. (µg/100 ml)	Mean Found Conc. (µg/100 ml)	Standard Deviation	Mean % Inaccuracy	Imprecision
55.200	56.150	2.053	1.721	3.657
27.600	27.950	0.412	1.268	1.475
11.000	10.088	0.598	-8.295	5.929
5.520	5.653	0.226	2.400	3.991
2.760	2.590	0.386	-6.159	14.904
1.100	1.498	0.240	36.136	16.030
0.000	0.000	0.000		
Means		0.559	4.512	7.664

TABLE 3

ANALYSIS OF 28 TARGET CONC.-FOUND CONC. POINTS FOR RDX

TARGET CONC.
MEAN= 14.74 SD= 19.0446835198

FOUND CONC.
MEAN= 14.8467857143 SD= 19.3886033829

NO. RUNS 1 TOTAL X-Y ALL RUNS 28 NO. CONCENTR. 28
MEASURES (Y'S) EACH TARGET CONC. 1

INTERCEPT= -0.144540721096
SLOPE= 1.01705064012
  USE FOR ACCURACY
R= 0.999009943226
MEAN SQR DEV OF POINTS FROM REGRESSION= 0.772606795027
ST ERROR EST= 0.878980543031
  USE FOR PRECISION
T FOR CONFIDENCE BAND
D.F.= 26
TWO TAIL P LEVEL IS .1
t= 1.70561435167
X(D) FOR CALIBRATION CURVE OR UNKNOWN SAMPLE? C/U C
(EACH TARGET CONC. CONSIDERED INDEP SAMPLE
MEASURED 1 TIME(S))
y(c)= 1.39745269575
x(d)= 3.026414863

TABLE 4

TNB AFTER 10 WEEKS STORAGE IN TAP WATER

Target Conc. Versus Found Conc.	
Target Conc. (µg/100 ml)	Found Conc. (µg/100 ml)
51.200	43.400
	44.500
	44.700
	41.200
25.600	21.300
	21.800
	22.500
	23.100
10.200	8.500
	8.720
	8.520
	8.360
5.120	4.330
	4.320
	4.770
	4.450
2.560	2.100
	2.450
	2.800
	1.510
1.020	0.999
	1.400
	2.000
	1.100
0.000	0.000
	0.000
	0.000
	0.000

TABLE 5

STATISTICAL DATA USED TO DETERMINE PERCENT INACCURACY AND IMPRECISION FOR TNB

Mean Target Conc. (µg/100 ml)	Mean Found Conc. (µg/100 ml)	Standard Deviation	Mean % Inaccuracy	Imprecision
51.200	43.450	1.605	-15.137	3.694
25.600	22.175	0.789	-13.379	3.558
10.200	8.545	0.149	-16.225	1.745
5.120	4.468	0.210	-12.744	4.704
2.560	2.215	0.550	-13.477	24.833
1.020	1.375	0.450	34.799	32.753
0.000	0.000	0.000		
Means		0.536	-6.030	11.881

TABLE 6

ANALYSIS OF 28 TARGET CONC.-FOUND CONC. POINTS FOR TNB

TARGET CONC.
MEAN= 13.6714285714 SD= 17.6647491023

FOUND CONC.
MEAN= 11.74675 SD= 14.9880449955

NO. RUNS 1 TOTAL X-Y ALL RUNS 28 NO. CONCENTR. 28
MEASURES (Y'S) EACH TARGET CONC. 1

INTERCEPT= 0.159057510879
SLOPE= 0.847584612579
  USE FOR ACCURACY
R= 0.998954135023
MEAN SQR DEV OF POINTS FROM REGRESSION= 0.487706834869
ST ERROR EST= 0.698360104007
  USE FOR PRECISION
T FOR CONFIDENCE BAND
D.F.= 26
TWO TAIL P LEVEL IS .1
t= 1.70561435167
X(D) FOR CALIBRATION CURVE OR UNKNOWN SAMPLE? C/U C
(EACH TARGET CONC. CONSIDERED INDEP SAMPLE
MEASURED 1 TIME(S))
y(c)= 1.38418790991
x(d)= 2.8851414553

TABLE 7

DNB AFTER 10 WEEKS STORAGE IN TAP WATER

Target Conc. Versus Found Conc.	
Target Conc. (µg/100 ml)	Found Conc. (µg/100 ml)
49.000	33.500
	37.300
	37.500
	34.000
24.500	17.600
	17.000
	20.000
	17.900
9.800	7.420
	7.080
	6.310
	7.030
4.900	3.270
	3.460
	4.130
	3.710
2.450	1.890
	1.780
	1.830
	1.130
0.980	0.526
	0.633
	0.806
	0.740
0.000	0.000
	0.000
	0.000
	0.000

TABLE 8

STATISTICAL DATA USED TO DETERMINE PERCENT INACCURACY AND IMPRECISION FOR DNB

Mean Target Conc. (µg/100 ml)	Mean Found Conc. (µg/100 ml)	Standard Deviation	Mean % Inaccuracy	Imprecision
49.000	35.575	2.119	-27.398	5.956
24.500	18.125	1.385	-26.020	7.199
9.800	6.960	0.467	-28.980	6.705
4.900	3.643	0.372	-25.663	10.202
2.450	1.658	0.355	-32.347	21.389
0.980	0.676	0.123	-30.995	18.180
0.000	0.000	0.000		
Means		0.677	-28.567	11.605

TABLE 9

ANALYSIS OF 28 TARGET CONC.-FOUND CONC. POINTS FOR DNB

TARGET CONC.
MEAN= 13.09 SD= 16.9042015378

FOUND CONC.
MEAN= 9.51946428571 SD= 12.3509492707

NO. RUNS 1 TOTAL X-Y ALL RUNS 28 NO. CONCENTR. 28
MEASURES (Y'S) EACH TARGET CONC. 1

INTERCEPT= -0.020665500409
SLOPE= 0.72881052606
  USE FOR ACCURACY
R= 0.997490941412
MEAN SQR DEV OF POINTS FROM REGRESSION= 0.793938226885
ST ERROR EST= 0.891032113274
  USE FOR PRECISION
T FOR CONFIDENCE BAND
D.F.= 26
TWO TAIL P LEVEL IS .1
t= 1.70561435167
X(D) FOR CALIBRATION CURVE OR UNKNOWN SAMPLE? C/U C
(EACH TARGET CONC. CONSIDERED INDEP SAMPLE
MEASURED 1 TIME(S))
y(c)= 1.5424866666
x(d)= 4.27725403816

TABLE 10

2,4-DNT AFTER 10 WEEKS STORAGE IN TAP WATER

Target Conc. Versus Found Conc.	
Target Conc. (µg/100 ml)	Found Conc. (µg/100 ml)
50.800	35.000
	36.700
	38.000
	35.900
25.400	13.400
	16.700
	20.400
	19.700
10.200	7.540
	7.420
	6.940
	8.670
5.080	3.630
	3.590
	4.160
	5.670
2.540	2.080
	1.870
	1.910
	1.190
1.020	0.810
	0.560
	1.050
	1.560
0.000	0.000
	0.000
	0.000
	0.000

TABLE 11

STATISTICAL DATA USED TO DETERMINE PERCENT INACCURACY AND IMPRECISION FOR 2,4-DNT

Mean Target Conc. (µg/100 ml)	Mean Found Conc. (µg/100 ml)	Standard Deviation	Mean % Inaccuracy	Imprecision
50.000	36.400	1.273	-28.346	3.497
25.400	17.550	3.198	-30.906	18.225
10.200	7.643	0.732	-25.074	9.583
5.080	4.263	0.974	-16.093	22.842
2.540	1.763	0.392	-30.610	22.262
1.020	0.995	0.426	-2.451	42.864
0.000	0.000	0.000		
Means		0.999	-22.247	19.879

TABLE 12

ANALYSIS OF 28 TARGET CONC.-FOUND CONC. POINTS FOR 2,4-DNT

TARGET CONC.
MEAN= 13.5771428571 SD= 17.5235999297

FOUND CONC.
MEAN= 9.80178571429 SD= 12.4802855065

NO. RUNS 1 TOTAL X-Y ALL RUNS 28 NO. CONCENTR. 28
MEASURES (Y'S) EACH TARGET CONC. 1

INTERCEPT= 0.183220407711
SLOPE= 0.708438101284
  USE FOR ACCURACY
R= 0.994719700559
MEAN SQR DEV OF POINTS FROM REGRESSION= 1.70364807185
ST ERROR EST= 1.30523870301
  USE FOR PRECISION
T FOR CONFIDENCE BAND
D.F.= 26
TWO TAIL P LEVEL IS .1
t= 1.70561435167
X(D) FOR CALIBRATION CURVE OR UNKNOWN SAMPLE? C/U C
(EACH TARGET CONC. CONSIDERED INDEP SAMPLE
MEASURED 1 TIME(S))
y(c)= 2.47304853674
x(d)= 6.43976922915

TABLE 13

TNT AFTER 10 WEEKS STORAGE IN TAP WATER

Target Conc. Versus Found Conc.	
Target Conc. (µg/100 ml)	Found Conc. (µg/100 ml)
49.200	39.800
	37.200
	41.100
	37.200
24.600	19.000
	17.900
	19.900
	19.300
9.840	7.740
	8.270
	7.840
	8.040
4.920	4.190
	3.990
	4.640
	4.250
2.460	2.070
	1.990
	2.170
	1.460
0.980	0.910
	0.750
	1.130
	0.790
0.000	0.000
	0.000
	0.000
	0.000

TABLE 14

STATISTICAL DATA USED TO DETERMINE PERCENT INACCURACY AND IMPRECISION FOR TNT

Mean Target Conc. (µg/100 ml)	Mean Found Conc. (µg/100 ml)	Standard Deviation	Mean % Inaccuracy	Imprecision
49.200	38.825	1.950	-21.087	5.023
24.600	19.025	0.838	-22.663	4.406
9.840	7.973	0.234	-18.979	2.939
4.920	4.268	0.272	-13.262	6.376
2.460	1.923	0.317	-21.850	16.489
0.980	0.895	0.171	-8.673	19.082
0.000	0.000	0.000		
Means		0.540	-17.752	9.052

TABLE 15

ANALYSIS OF 28 TARGET CONC.-FOUND CONC. POINTS FOR TNT

TARGET CONC.
MEAN= 13.1428571429 SD= 16.9736228607

FOUND CONC.
MEAN= 10.4153571429 SD= 13.3323089835

NO. RUNS 1 TOTAL X-Y ALL RUNS 28 NO. CONCENTR. 28
MEASURES (Y'S) EACH TARGET CONC. 1

INTERCEPT= 0.108664660777
SLOPE= 0.784204862767
  USE FOR ACCURACY
R= 0.99838652124
MEAN SQR DEV OF POINTS FROM REGRESSION= 0.595173931465
ST ERROR EST= 0.771475165812
  USE FOR PRECISION
T FOR CONFIDENCE BAND
D.F.= 26
TWO TAIL P LEVEL IS .1
t= 1.70561435167
X(D) FOR CALIBRATION CURVE OR UNKNOWN SAMPLE? C/U C
(EACH TARGET CONC. CONSIDERED INDEP SAMPLE
MEASURED 1 TIME(S))
y(c)= 1.4620741793
x(d)= 3.4434059094

TABLE 16

TETRYL AFTER 10 WEEKS STORAGE IN TAP WATER

Target Conc. Versus Found Conc.	
Target Conc. (µg/100 ml)	Found Conc. (µg/100 ml)
50.000	43.800
	43.400
	46.000
	43.800
25.000	22.000
	20.900
	22.000
	22.100
10.000	9.220
	9.460
	8.120
	8.840
5.000	4.980
	4.550
	4.900
	4.610
2.500	3.090
	2.390
	2.320
	1.730
1.000	1.010
	0.801
	1.260
	0.920
0.000	0.000
	0.000
	0.000
	0.000

TABLE 17

STATISTICAL DATA USED TO DETERMINE PERCENT INACCURACY AND IMPRECISION FOR TETRYL

Mean Target Conc. (µg/100 ml)	Mean Found Conc. (µg/100 ml)	Standard Deviation	Mean % Inaccuracy	Imprecision
50.000	44.250	1.182	-11.500	2.671
25.000	21.750	0.569	-13.000	2.614
10.000	8.910	0.585	-10.900	6.569
5.000	4.760	0.212	-4.800	4.450
2.500	2.383	0.557	-4.700	23.373
1.000	0.998	0.195	-0.225	19.510
0.000	0.000	0.000		
Means		0.471	-7.521	9.864

TABLE 18

ANALYSIS OF 28 TARGET CONC.-FOUND CONC. POINTS FOR TETRYL

TARGET CONC.
MEAN= 13.3571428571 SD= 17.2491852426

FOUND CONC.
MEAN= 11.8643214286 SD= 15.1845398449

NO. RUNS 1 TOTAL X-Y ALL RUNS 28 NO. CONCENTR. 28
MEASURES (Y'S) EACH TARGET CONC. 1

INTERCEPT= 0.113789011097
SLOPE= 0.879719004517
  USE FOR ACCURACY
R= 0.999334601206
MEAN SQR DEV OF POINTS FROM REGRESSION= 0.318537948378
ST ERROR EST= 0.564391662215
  USE FOR PRECISION
T FOR CONFIDENCE BAND
D.F.= 26
TWO TAIL P LEVEL IS .1
t= 1.70561435167
X(D) FOR CALIBRATION CURVE OR UNKNOWN SAMPLE? C/U C
(EACH TARGET CONC. CONSIDERED INDEP SAMPLE
MEASURED 1 TIME(S))
y(c)= 1.10391050806
x(d)= 2.24734809562

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