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TECHNICAL REPORT QAL-89-1

HEXACHLOROETHANE PURITY AND ASSAY IN SMOKE MIX
BY HIGH PRESSURE LIQUID CHROMATOGRAPHY

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MTT TASK # 1705

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HEXACHLOROETHANE PURITY AND ASSAY IN SMOKE MIX
BY HIGH PRESSURE LIQUID CHROMATOGRAPHY
MTT TASK # 1705

1. INTRODUCTION

Hexachloroethane is a component of white smoke mixtures used by the Department of Defense. Pure hexachloroethane is currently purchased using specifications set forth in MIL-H-235C dtd 23 Feb 1984. This specification requires the material to be a minimum 98.00 percent hexachloroethane by weight. The assessment of purity (para 4.2.4.2) requires the use of procedures in both ASTM E256 and ASTM D2989. These procedures require ignition in a sodium peroxide bomb which is hazardous and labor intensive. For smoke mixtures, the percent by weight of hexachloroethane is currently determined by heating the mixture in a vented oven for two hours at 200°C. In order for the procedure to be valid, water content must also be determined using ASTM E203 with Karl Fischer Reagent and hexachloroethane must be the only volatile component besides water. Thus, determining the percent by weight of hexachloroethane in smoke mixes is currently a multistep process of questionable reliability which generates hazardous waste and fumes. A faster, more efficient and safer method of assessing hexachloroethane purity and percent by weight in smoke mixes was investigated using high pressure liquid chromatography (HPLC).

2. EXPERIMENTAL PROCEDURES

2.1 MATERIALS

Hexachloroethane was provided by the Pine Bluff Arsenal. Standard material was prepared and purity assessed as indicated in the results section. All solvents were purchased as HPLC grade. Smoke mix components Zinc Oxide and Aluminum powder were obtained from the stocks actually being used on the production lines.

2.2 GAS CHROMATOGRAPHY/MASS SPECTROMETRY

Hexachloroethane standard was analyzed on a Finnigan Model 5100 GC/MS using electron impact ionization. The oven fitted with a 30m DB1701 capillary column was programmed from 30°C to 90°C at 10°C/min, held at 90°C for 5 min, then increased to 230°C at 20°C/min. Injection sample size was 1 ul, injector temperature was 200°C, interface temperature was 230°C, and the ion source temperature was 100°C. The mass range was scanned from 30-300 amu at two scans per second. Calibration and tuning was with FC-43, perfluorotributylamine.

2.3 HIGH PRESSURE LIQUID CHROMATOGRAPHY (HPLC)

HPLC analysis utilized a mobile phase consisting of acetonitrile/water (75/25) flowing through a Brownlee Spheri-5 reverse phase 5 um, 250 x 4.6 mm column at 1 ml/min. The detector was an LKB 2151 set at 215 nm. Injections were made with a Rheodyne 7125 valve fitted with a 50 ul loop. The loop was loaded using 100 ul of sample. Results of each injection were recorded on an LKB 2220 recording integrator. Areas under each peak were obtained using an attenuation of 2×10^6 , chart speed 1 cm/min and a threshold of 2×10^4 .

2.4 SMOKE MIX CONTROLS FOR SUITABILITY AND PRECISION AND ACCURACY STUDIES

Smoke mixes of known composition were difficult to prepare because of the volatility of hexachloroethane and difficulty getting a uniform blend of the components. Suitable mixtures were finally achieved by mixing the solid using a 3/8 inch stir bar in a 4 ml closed container filled to 80% capacity and placed over a magnetic stir plate. All weighing of solid samples was achieved as quickly as possible. QP's for the P&A study were prepared by weighing the smoke mix components directly into acetonitrile.

2.5 PRECISION AND ACCURACY STUDY DESIGN

A set of calibration standards was prepared at concentrations from 17.5 to 32.5 ug hexachloroethane per 50 ul acetonitrile in 100 ml volumetric flasks. A set of artificial smoke mixes (QP's) was prepared at 3 concentrations in quadruplicate representing smoke mix compositions of 40, 50 and 60% hexachloroethane.

A 25 ug per 50 ul standard was used as a QL to make sure the instrument was not drifting out of calibration. All samples were injected in the order presented in Table 2 on two consecutive days. Peak response is peak area of a single injection as recorded by the LKB integrator.

2.6 DATA ANALYSIS

Statistical analysis of calibration data was by a least mean square fit to a linear equation as described in Appendix A. Statistical treatment of the data from the Precision and Accuracy Study of smoke mix analysis is described in Appendix B. The detection limit and confidence limits are determined.

3. RESULTS AND DISCUSSION

3.1 MOBILE PHASE CONDITIONS

HPLC conditions were chosen which gave a retention time of about 8.2 minutes. The standard hexachloroethane sample was found to have an impurity that was not adequately resolved at shorter retention times and completely hidden at the 3.6 minutes retention time which resulted from using a 100% acetonitrile mobile phase. An acetonitrile/water (75:25) mobile phase flowing at 1 ml per minute resulted in a symmetrical peak with no apparent hidden impurities (Figure 1). The lack of unresolved impurities was indicated by the unchanging ratio of peak areas obtained at three different wavelengths and two different mobile phases (Table 1). The 13.6 minute retention time peaks were obtained using an acetonitrile/water (65:35) mobile phase flowing at 1 ml per minute.

3.2 WAVELENGTH SELECTION

Hexachloroethane did not exhibit a characteristic lambda max in the range 190 to 600 nm range. A wavelength was chosen, therefore, which maximized the signal to noise and reproducibility. As the wavelength is decreased, sample absorbance increases as well as background noise. As the wavelength is increased, sample absorbance decreased and electronic amplification noise necessarily increases. The best wavelength may have to be chosen for each detector depending on its level of sophis-

tication. The longer wavelengths are less likely to have interference from contaminants. Figure 2 shows the results of injecting the same amount of hexachloroethane in acetonitrile with the detector set to various wavelengths.

3.3 HEXACHLOROETHANE STANDARD PURITY

The purity of the standard provided by the Pine Bluff Arsenal was determined to be 89.3% by weight. Purity was determined by comparison of HPLC peak heights with a sample prepared by sublimation on a cold finger. Less than 1% contamination of the sublimed material could be detected by HPLC or GC/MS. The melting point of sublimed material was 186.0°C while that of the PBA standard was 185.5°C. By comparison, a certified 98.7% purity sample (Ref. 7) had a melting point of 185.6°C. The volatility of the sublimed material at 200°C for one hour was 100% while that of the PBA standard was 99.3%. The identity of the major contaminant(s) is currently under investigation. At least one contaminant is readily trapped by the sublimed crystals and has very similar HPLC chromatographic characteristics as hexachloroethane. GC/MS analysis of the PBA standard identified a small amount of tetrachloroethane eluting before hexachloroethane.

3.4 IDENTITY OF STANDARD AND HEXACHLOROETHANE IN AUTHENTIC SMOKE MIXES

The mass spectrum shown in figure 3 indicates the presence of a hydrocarbon containing six chlorine atoms, a molecular weight of 234 and matches the spectral library for this compound. The material assessed in smoke mix as hexachloroethane had the same retention times on HPLC and GC and the same mass spectral pattern as authentic hexachloroethane (Ref. 6).

3.5 SUITABILITY OF HPLC METHOD FOR DETERMINATION OF HEXACHLOROETHANE PURITY

Eighteen calibration standards were prepared at concentrations from 5 to 50 ug per 50 ul acetonitrile and injected into the HPLC in triplicate. Using the average peak response for all 36 injections as a calibration factor, the ug content of each sample was calculated and plotted versus its actual concentration

(Figure 4). A Hubaux and Vos analysis of this data indicates a lower detection limit for 2 replications to be 2.87 ug with a variance of 0.2741903. The correlation coefficient for this data was 0.9994348. Additional evaluation of the method was performed by preparing standard hexachloroethane at 4 different concentrations and injecting each 4 times. A least squares fit was performed on the data generated. The correlation coefficient of the relationship between concentration and peak area was found to be 0.9999107 (Figure 5).

The following peak areas were obtained for 4 injections of each of the following standards:

	<u>10.02ug/100ul</u>	<u>20.13ug/100ul</u>	<u>50.55ug/100ul</u>	<u>103.55ug/100ul</u>
$\bar{X}$	1,640,825	3,191,450	7,957,875	15,899,250
S.D.	±0.588%	±1.34%	±0.565%	±0.529%

Analyzing the response factors (peak area/ug injected) for each injection of the 4 different standards yields:

$$\text{Response Factor } \bar{x} = 316,632 \text{ (n=16)}$$

$$\text{S.D.} = \pm 2.49\%$$

Using this Response Factor and the average peak areas for the different standards, a simple back calculation yields the ug injected, the concentration of standard solution and hence, the percent purity:

	<u>10.02ug/100ul</u>	<u>20.13ug/100ul</u>	<u>50.55ug/100ul</u>	<u>103.55ug/100ul</u>
ug inj.	5.1821	10.0794	25.1329	50.2137
conc.	10.36	20.16	50.27	100.43
% pur.	103.4	100.15	99.45	96.99

3.6 SUITABILITY FOR DETERMINATION OF HEXACHLOROETHANE IN SMOKE MIXES

A set of calibration standards was prepared at concentrations of 17.5 to 32.5 ug per 50 ul acetonitrile. A set of artificial smoke mixes (QP's) was prepared at 3 concentrations in quadruplicate representing smoke mix compositions of 40, 50 and 60% hexachloroethane. A 25 ug/50 ul standard was used as a QL to make sure the instrument was not drifting out of calibration. All samples were injected once per day for two days. Peak areas and retention times are shown in Table 2. A least squares fit program was used to determine a linear relationship

between the micrograms of hexachloroethane in the standards to their respective peak areas for each of the two day's trials (Figure 6 and 7). From this relationship, the instrumental found micrograms of hexachloroethane present in the QP samples and QL samples analyzed on each day were calculated (Table 3).

The QL samples were analyzed for percent imprecision and percent inaccuracy at the 95% confidence level for each day, and for the total analysis.

	<u>% inaccuracy</u>	<u>% imprecision</u>
Day #1 QL	0.43%	± 0.70%
Day #2 QL	- 0.77%	± 0.71%
Total QL	0.16%	± 0.52%

The QP samples were adjusted to conform to the analysis being carried out using three select quantities of hexachloroethane injected. This was accomplished by ratioing the select concentration to the actual quantity injected and by comparing directly this proportion to the ratio of the calculated recovered amount of hexachloroethane to the known amount of hexachloroethane recovered. i.e. for QPG day #1:

$$\frac{20 \text{ ug}}{19.95 \text{ ug}} = \frac{x}{19.68 \text{ ug}} ;$$

where x is the calculated recovered amount.

The select quantities of hexachloroethane injected and the calculated recovered amounts were determined to be as follows:

<u>SELECT QUANTITIES</u>			
	<u>20ug</u>	<u>25.05ug</u>	<u>29.7ug</u>
Day #1	19.73	25.24	29.87
	19.80	25.15	29.77
	19.95	25.14	29.57
	20.03	24.81	29.84
Day #2	19.74	24.99	29.69
	19.86	24.92	29.44
	19.83	24.82	29.23
	19.82	24.65	29.64

The calculated recovered amounts were analyzed using a basic program based on the methods of Hubaux and Vos to determine a lower detection limit (Figure 8).

This was found to be 1.03 ug of hexachloroethane. This data was also subjected to a statistical analysis to determine the percent imprecision, and percent inaccuracy at the 95% confidence level for each select quantity of hexachloroethane injected (Figure 9).

<u>select quantity injected</u>	<u>% inaccuracy</u>	<u>% imprecision</u>
20 ug	- 0.78%	± 0.67%
25.05 ug	- 0.34%	± 0.53%
29.7 ug	- 0.24%	± 0.45%

4. CONCLUSIONS

This HPLC method is a precise and accurate method for determination of hexachloroethane in pure sample purchases and in white smoke mixes. The use of this method should increase the level of confidence in these determinations over the old methods. The method is less susceptible to operator error and much safer than the sodium peroxide bomb method. Hazardous substances produced in this testing should be less hazardous to laboratory personnel and the environment.

ACKNOWLEDGEMENT

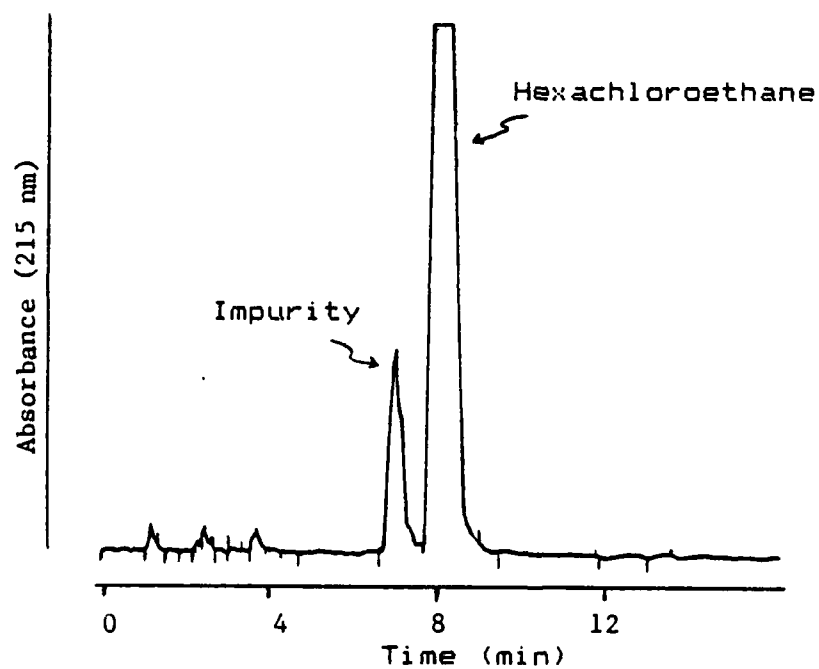
The authors wish to thank Joanie Payne for expert secretarial assistance.

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FIGURE 1

HPLC Chromatogram of Hexachloroethane Standard



Mobile phase was acetonitrile/water (3:1) flowing at 1 ml/min through a 25 cm C-18 RP column. LKB 2220 integrator was set with ATT2↑=6, PK WD = 0.04 and THRESH=4.

FIGURE 2

A plot of Hexachloroethane peak areas obtained at various wavelengths by injecting 19.6 ug standard using a mobile phase of acetonitrile/water (3:1).

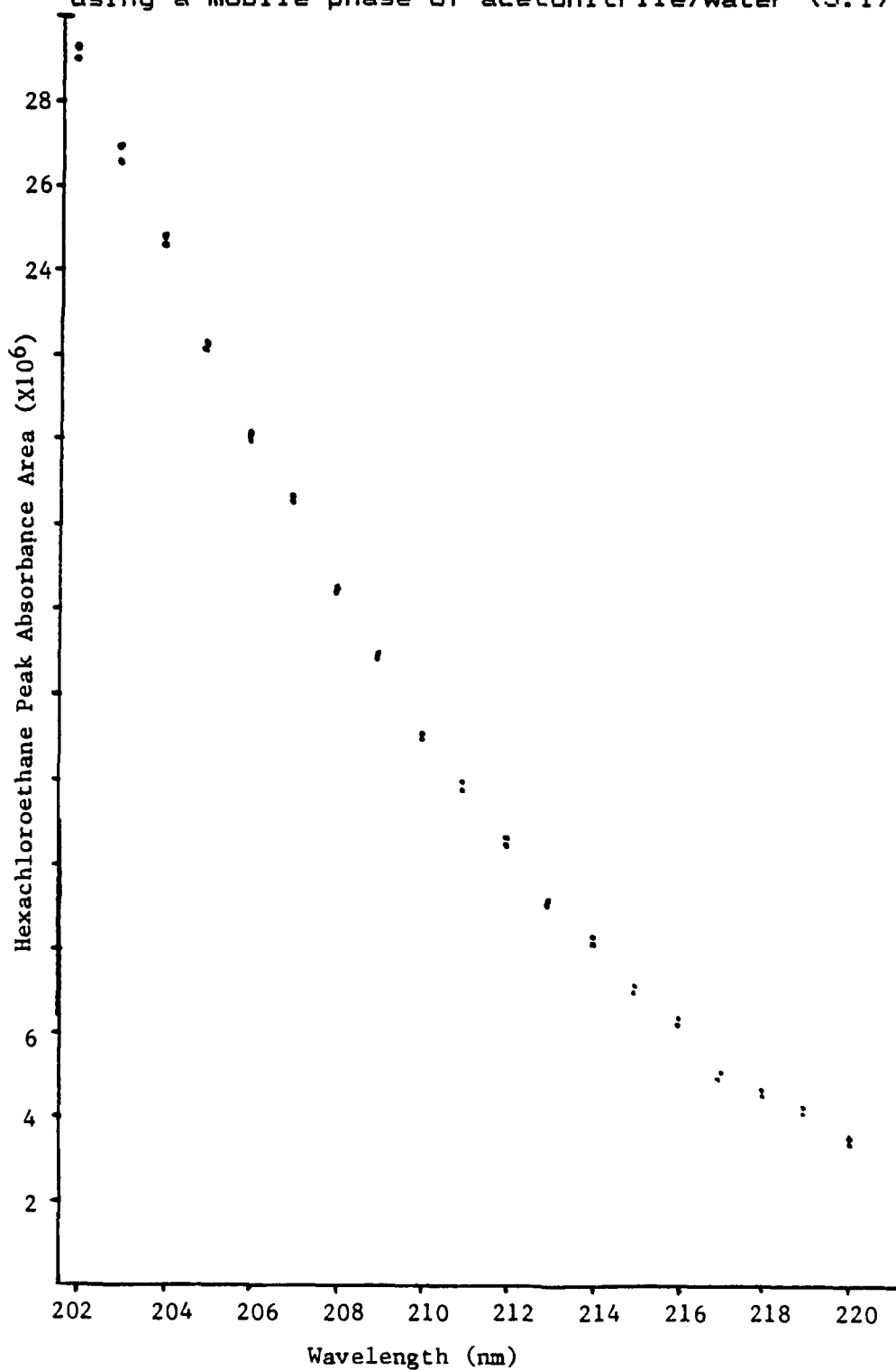


FIGURE 3 Mass Spectrum of Hexachloroethane

MID LIBRARY SEARCH
05/05/89 13:12:00 + 11:33
SAMPLE: SUBLIMED HEX
CONDOS.: 1/20L
ENHANCED (S 158 2N 0T)

DATA: 00K743 #12/3
CALI: 00K743 # 2

BASE N 2: 117
R10: 452047.

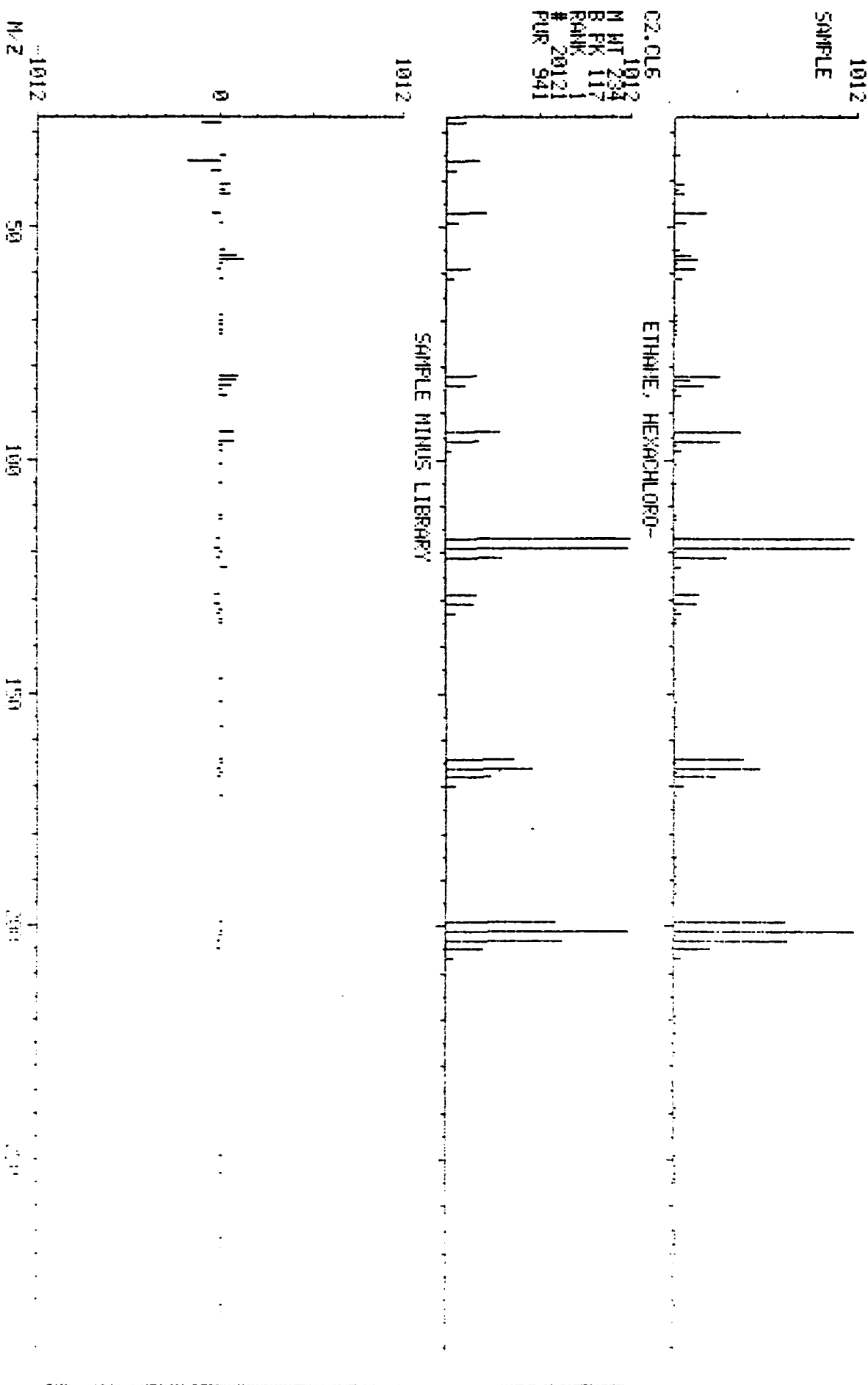
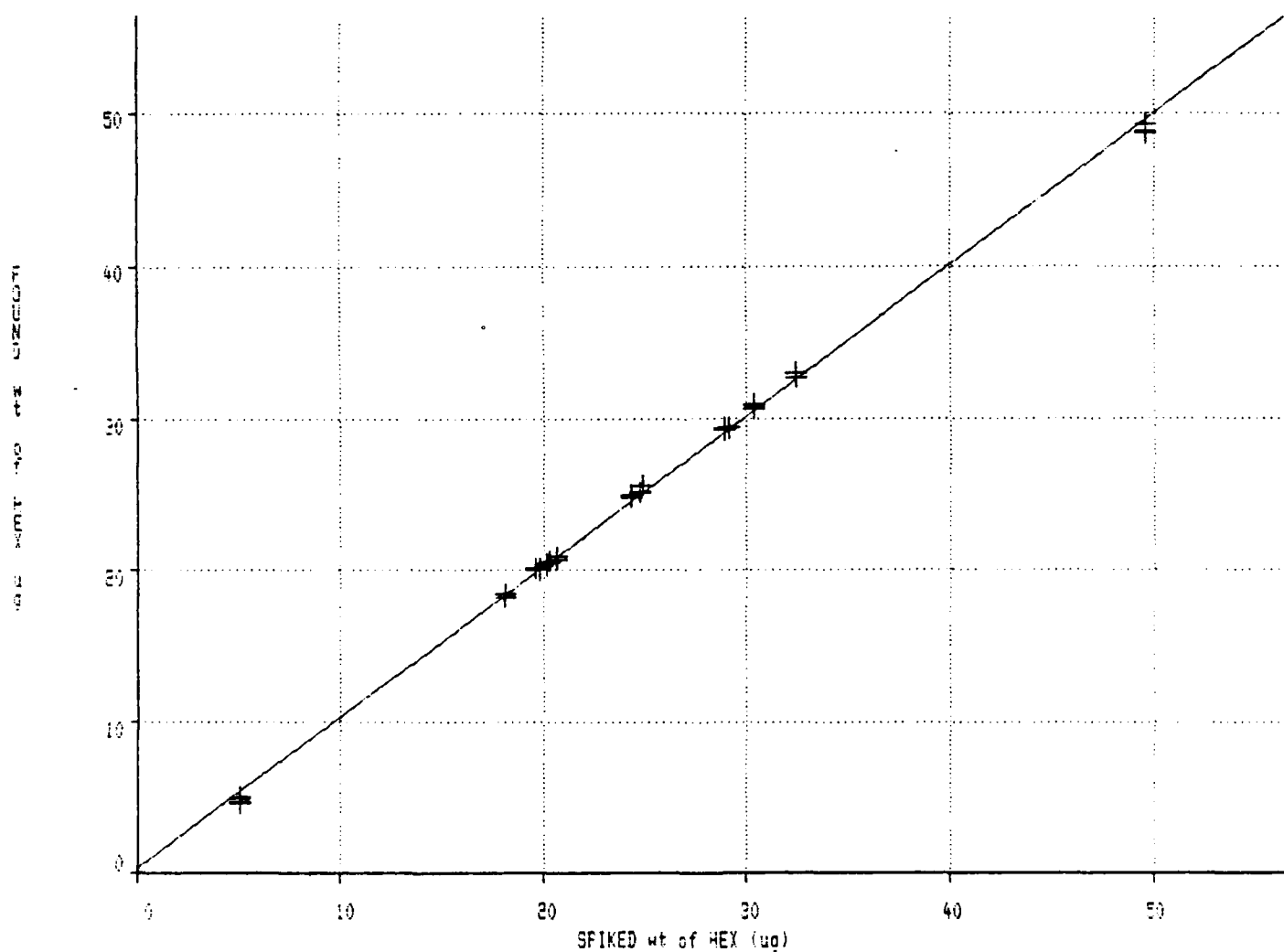


FIGURE 4

Analysis of Precision and Accuracy of the Method

$$y = A*x + B$$

A = 0.994485712, B = 0.356352829
Correlation=0.9994348



HEXACHLOROETHANE, SPIKED vs FOUND

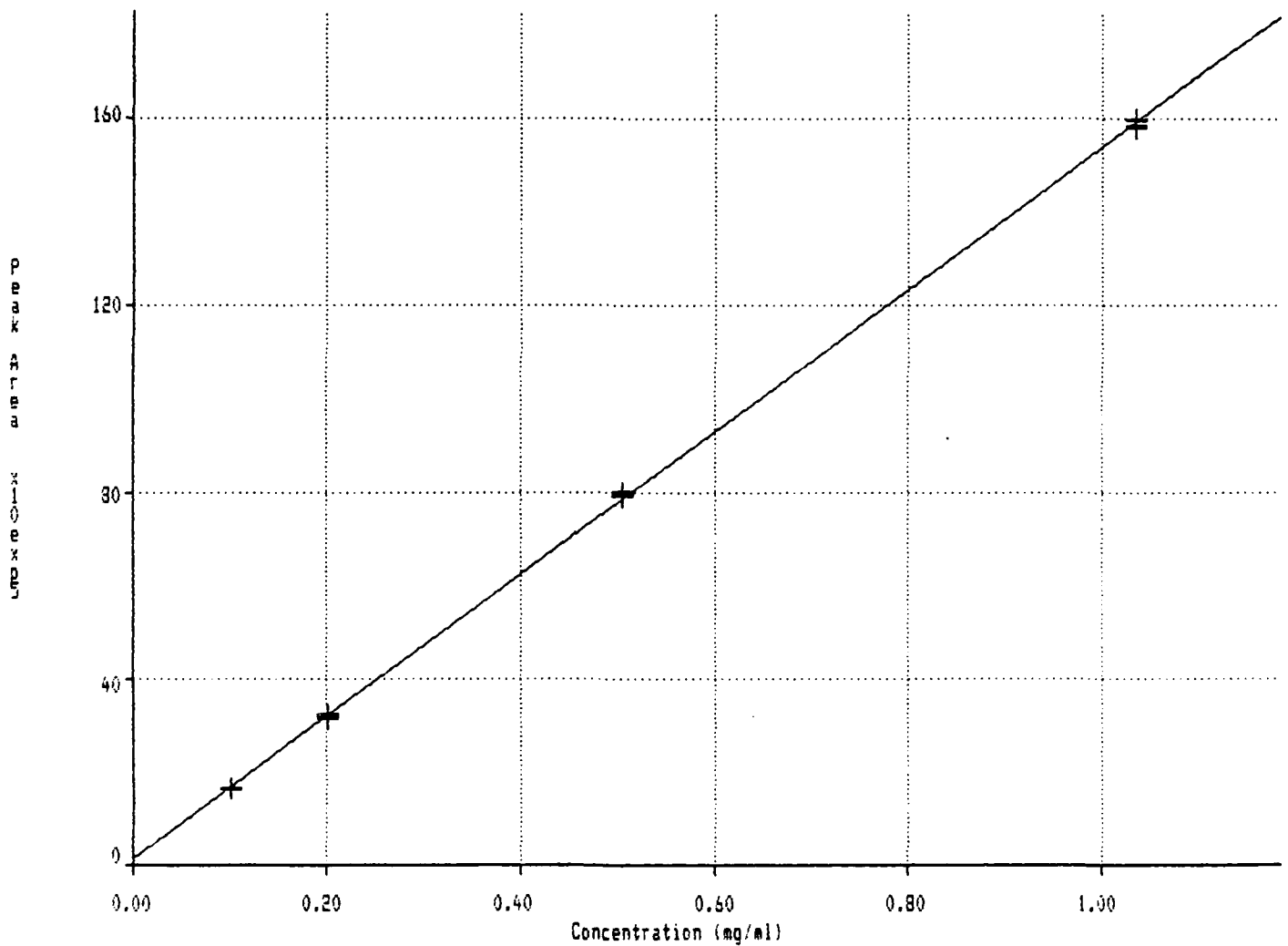
FIGURE 5

Results of Injecting 4 Concentrations of Hexachloroethane in Quadruplicate

X	Y	X	Y	X	Y	X	Y
0.10020	16.46800	0.20130	32.38100	0.50550	80.18700	1.03550	158.11000
0.10020	16.51200	0.20130	32.01500	0.50550	79.46400	1.03550	159.53000
0.10020	16.31600	0.20130	31.34600	0.50550	79.55800	1.03550	159.87000
0.10020	16.33700	0.20130	31.91600	0.50550	79.10600	1.03550	158.46000

Hexachloroethane Mix

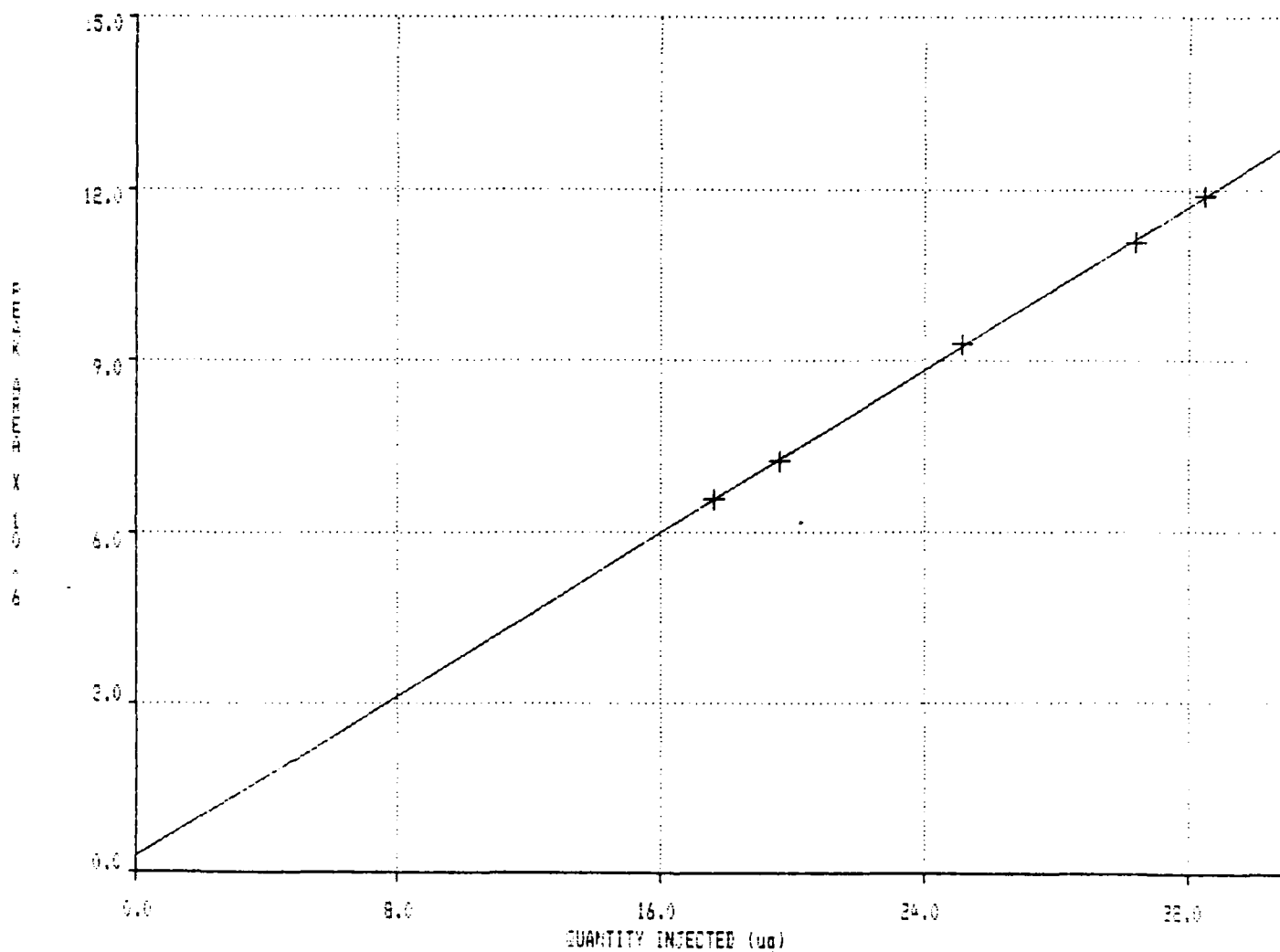
$y = A*x + B$
 $A = 152.5189027, B = 1.469480441$
 Correlation=0.9999107



17.65000	6.58980
19.80000	7.24200
25.15000	9.30160

30.40000	11.07800
32.50000	11.89700

$y = A * x + B$
 $A = 0.356682539, B = 0.283215577$
 Correlation=0.9998543



HEXACHLOROETHANE MTT, DAY #1, BLANK EXCL

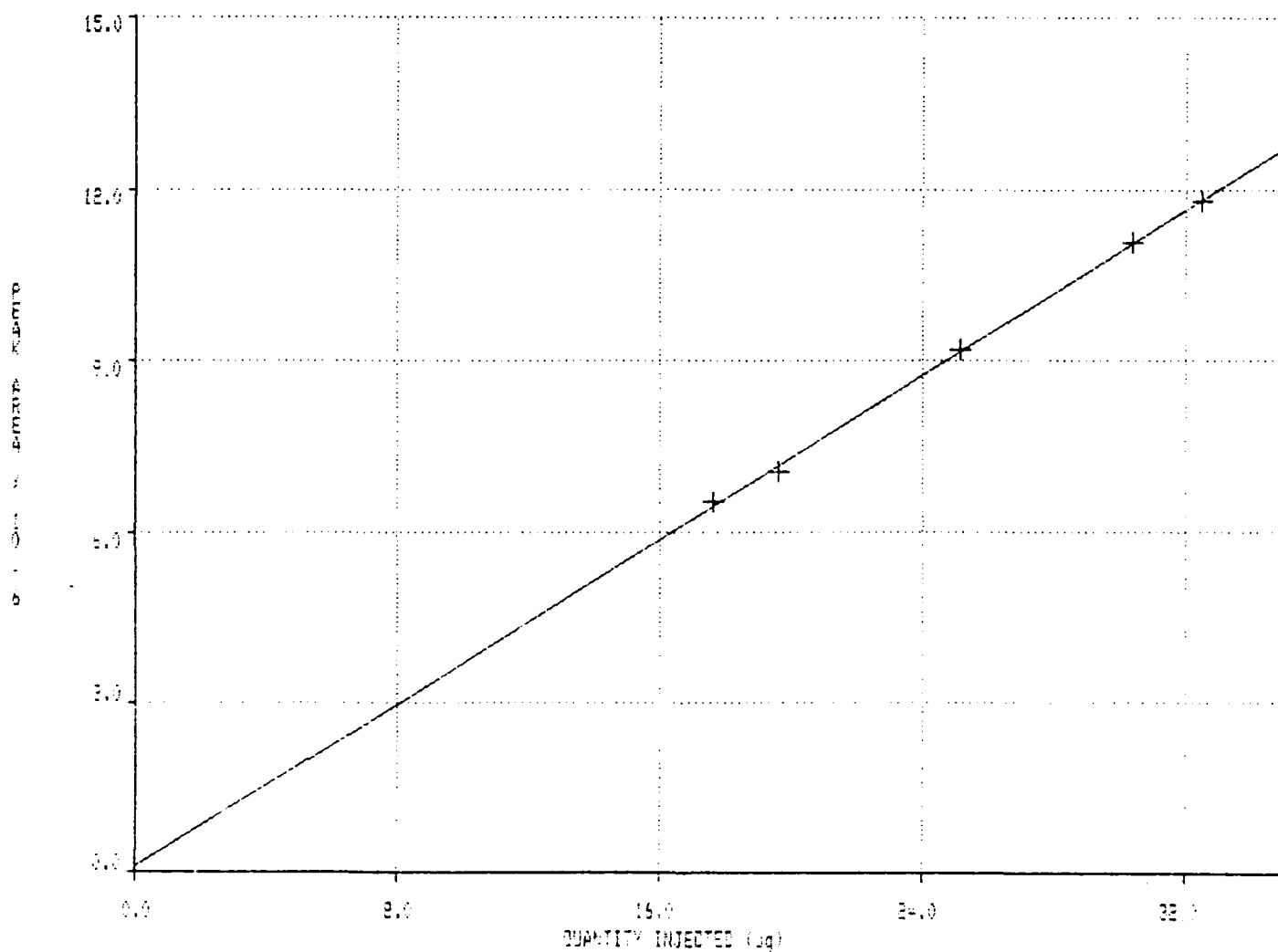
Calibration Curve for Day 1, Smoke Mix P & A Study

FIGURE 6

X	Y
17.85000	6.53750
19.80000	7.06410
25.15000	9.18980

X	Y
30.40000	11.06900
32.50000	11.81300

$y = A*x + B$
 $A = 0.360690884, B = 0.095766448$
 Correlation=0.9996228



HEXACHLOROETHANE MTT, DAY #2, BLANK EXCL

Calibration Curve For Day 2, Smoke Mix P & A Study

FIGURE 7

PRECISION AND ACCURACY FOR HEXACHLOROETHANE

TARGET VALUE ug	INPUT VALUES	AVERAGE	STD DEV	PERCENT COEFF VAR	PERCENT INACCURACY
-----------------------	-----------------	---------	------------	-------------------------	-----------------------

20	19.732	19.843	.102	.5	-.8
	19.737				
	19.795				
	19.861				
	19.95				
	19.827				
	20.0287				
	19.8204				

25.05	25.2426	24.964	.204	.8	-.3
	24.9877				
	25.15				
	24.917				
	25.138				
	24.818				
	24.813				
	24.646				

29.7	29.865	29.629	.216	.7	-.2
	29.691				
	29.773				
	29.435				
	29.572				
	29.226				
	29.8355				
	29.641				

LEAST SQUARES FIT *** Y INTERCEPT = -.327425
 VARIANCE = 4.653931E-04
 SLOPE = 1.00894

FOR 1 REPLICATIONS

LOWER DETECT LIMIT X(D)= 1.028966

WITH Y INTERCEPT Y(D)= .1916576

FIGURE 8

FIGURE 9
One-Way Analysis of Variance

Data: known

Level codes: spike

Labels:

Range test: Conf. Int. Confidence level: 95

Analysis of variance

Source of variation	Sum of Squares	d.f.	Mean square	F-ratio	Sig. level
Between groups	383.33259	2	191.66629	999.999	.0000
Within groups	.68973	21	.03284		
Total (corrected)	384.02232	23			

0 missing value(s) have been excluded.

Table of means for known by spike

Level	Count	Average	Std. Error (internal)	Std. Error (pooled s)	95 Percent Confidence intervals for mean	
20	8	19.843888	.0360751	.0640745	19.710605	19.977170
25.05	8	24.964038	.0720245	.0640745	24.830755	25.097320
29.7	8	29.629812	.0763393	.0640745	29.496530	29.763095
Total	24	24.812579	.0369934	.0369934	24.735629	24.889530

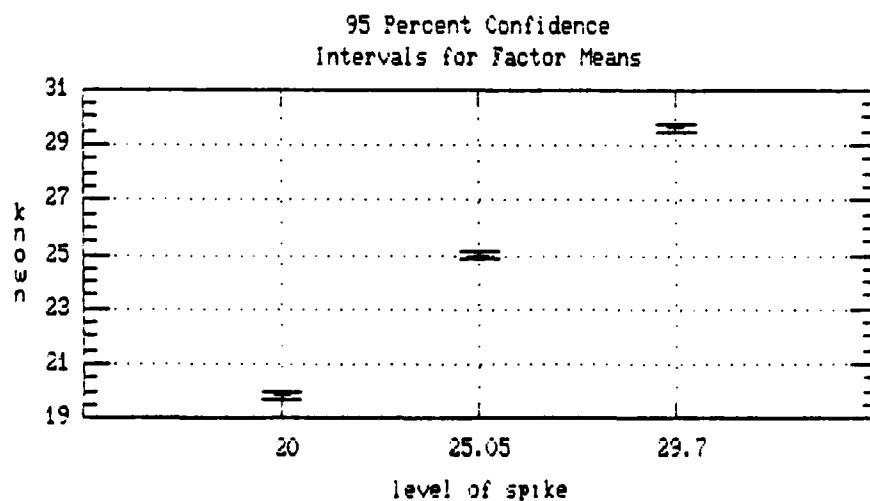


FIGURE 9 Continued

Regression Analysis - Linear model: $Y = a + bX$

Dependent variable: known

Independent variable: spike

Parameter	Estimate	Standard Error	T Value	Prob. Level
Intercept	-0.326599	0.230778	-1.41521	0.171008
Slope	1.00893	9.14714E-3	110.3	0

Analysis of Variance

Source	Sum of Squares	Df	Mean Square	F-Ratio	Prob. Level
Model	383.329	1	383.329	12166.111	.00000
Error	.693175	22	.031508		
Total (Corr.)	384.02232	23			

Correlation Coefficient = 0.999097
 Std. Error of Est. = 0.177505

R-squared = 99.82 percent

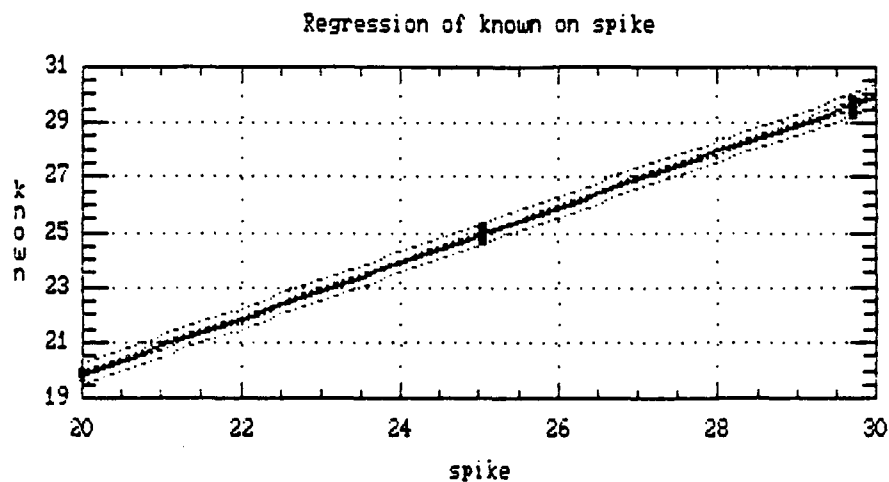


TABLE 1

Comparison of peak areas obtained for hexachloroethane using 2 different mobile phases and 3 different wavelengths.

<u>WAVELENGTH</u>	<u>RETENTION TIMES</u>			
	<u>8.4 minutes</u>		<u>13.6 minutes</u>	
	<u>Area x 10⁷</u>	<u>S.D.</u>	<u>Area x 10⁷</u>	<u>S.D.</u>
210	1.719	0.013	1.759	0.003
215	0.921	0.005	0.954	0.010
220	0.465	0.006	0.480	0.001
<u>RATIOS</u>				
210/215	1.866	0.025	1.843a	0.023
220/215	0.504	0.009	0.503	0.006

a. Not significantly different from the ratio at 8.4 minutes at the 95% confidence level.

TABLE 2

P&A STUDY FOR HEXACHLOROETHANE ASSAY IN SMOKE MIXES

Order of Injection	Quantity Injected (ug)	PEAK RESPONSE			
		Day	Residence	Day	Residence
		1	Time (min)	2	Time (min)
		(X10 ⁶)		(X10 ⁶)	
Std A	17.65	6.5898	9.32	6.5375	8.38
" B	19.60	7.242	9.34	7.0641	8.41
" C	25.15	9.3016	9.34	9.1898	8.40
" D	30.40	11.078	9.34	11.069	8.40
" E	32.50	11.897	9.30	11.813	8.42
" F	0.0	0	0	0	0
QP G	19.95	7.3038	9.34	7.1969	8.43
" H	25.05	9.2868	9.32	9.1086	8.42
" I	29.45	10.846	9.33	10.715	8.42
QL C	25.15	9.2849	9.32	9.1239	8.43
QP J	19.85	7.2908	9.32	7.7059	8.43
" K	25.10	9.2717	9.31	9.10120	8.39
" L	29.55	10.849	9.31	1.0659	8.37
QL C	25.15	9.3095	9.31	9.0806	8.39
QP M	20.20	7.4701	9.31	7.3186	8.38
" N	25.15	9.2854	9.32	9.083	8.38
" O	30.15	10.991	9.32	10.797	8.39
QL C	25.15	9.2738	9.31	9.0582	8.38
QP P	20.00	7.4271	9.28	7.2448	8.37
" Q	24.85	9.063	9.30	8.9144	8.38
" R	29.70	10.925	9.31	10.787	8.38
QL C	25.15	9.2979	9.32	9.1278	8.39

TABLE 3

CALCULATED QUANTITIES OF HEXACHLOROETHANE IN SMOKE MIXES

SAMPLE ID	QTY INJECTED (ug)	DAY 1 CALCULATED (ug)	DAY 2 CALCULATED (ug)
QP G	19.95	19.68	19.69
" A	25.05	25.24	24.99
" I	29.45	29.61	29.44
QL C	25.15	25.24	25.03
QP J	19.85	19.65	19.71
QP K	25.10	25.20	24.97
" L	29.55	29.62	29.29
QL C	25.15	25.31	24.91
QP M	20.20	20.15	20.03
" N	25.15	25.24	24.92
" O	30.15	30.02	29.67
QL C	25.15	25.21	24.85
QP P	20.00	20.03	19.82
QP Q	24.85	24.62	24.45
QP R	29.70	29.84	29.64
QL C	25.15	25.27	25.04

APPENDIX A

Statistical Analysis of Calibration Data

1. Assume the calibration curve is linear, and can be described by the equation:

$$Y = Y_0 + bX$$

$$b = \frac{N(\sum X_i Y_i) - (\sum X_i)(\sum Y_i)}{N(\sum X_i^2) - (\sum X_i)^2}$$

$$Y_0 = \frac{(\sum Y_i) - b(\sum X_i)}{N}$$

Source of Variation	Sum of Squares (SS)	Degrees of Freedom (df)	Mean Square (MS)
Residual	0	N-2	(ResidualSS) / (N-2)
Total Error	(sum d ²)/2	N-2	(Total ErrorSS)/df Total Error
Lack of Fit (LOF)	Residual SS -Total SS	dfResidual -dfTotal	LOFSS/df LOF

$$F\text{-Ratio} = (\text{MS LOF}) / \text{MS Total Error}$$

$$Q = ((\sum Y_i^2) - (\sum Y_i)^2 / N) - b^2 ((\sum X_i^2) - (\sum X_i)^2 / N)$$

N = number of data points
 X_i = i-th target concentration
 Y_i = i-th value of dependent variable
 d = difference between duplicates

APPENDIX B

STATISTICAL TREATMENT OF DATA

The detection limit and confidence limits will be determined in either of two acceptable approaches. The first approach is to use a computer program that the Product Assurance Laboratory possesses that utilizes the mathematical treatment of Hubaux and Vos (Ref. 5). The second approach is described as follows.

For $Y = Y_0 + bX$

$$b = \frac{N(\sum X_i Y_i) - (\sum X_i)(\sum Y_i)}{N(\sum X_i^2) - (\sum X_i)^2} \quad Y_0 = \frac{(\sum Y_i) - b(\sum X_i)}{N}$$

Upper confidence limit = Y_u

$$Y_u = Y_0 + bX + S_{x.y} T \left(\frac{1 + \frac{(X_i - \bar{X})^2}{N \sum (X_i - \bar{X})^2}}{N} \right)^{(1/2)}$$

Lower confidence limit = Y_l

$$Y_l = Y_0 + bX - S_{x.y} T \left(\frac{1 + \frac{(X_i - \bar{X})^2}{N \sum (X_i - \bar{X})^2}}{N} \right)^{(1/2)}$$

Where:

$$S_{x.y} = \left(\frac{\sum (Y_i - (Y' + b(X_i - \bar{X})))^2}{N-2} \right)^{(1/2)}$$

T = student's T for 2-tailed $P=0.10$ and $N-2$ degrees of freedom

N = number of data points

X_i = i -th target concentration

Y_i = i -th value of dependent variable

$\bar{X}$ = mean value of X_i 's

$\bar{Y}$ = mean value of Y_i 's

The calculated reporting limit is determined by drawing a horizontal line from the Y -intercept of the upper confidence curve to its corresponding value on the lower confidence curve and reading the X value for this point on the lower confidence curve. This value is the certified reporting limit as long as one of the tested concentrations was at or below this value, otherwise, the lowest tested value is the certified reporting limit.

APPENDIX B Continued

The slope of the least squares linear regression line of a plot of found versus target concentrations is a measure of the accuracy of the method.

Standard deviation = S

$$S = \left(\frac{\sum(Y_i^2) - ((\sum Y_i)^2/N)}{N-1} \right)^{1/2}$$

$$\text{Percent inaccuracy} = \frac{Y_t' - X}{X} (100)$$

X = target concentration

Y_t' = average found concentration at the target concentration

$$\text{Percent imprecision} = \frac{S}{Y_t'} (100)$$