



UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

October 18, 1995

MEMORANDUM

SUBJECT: **Rimsulfuron and Nicosulfuron**
Validation of Environmental Chemistry Methods
Water Matrix- ECM 0019W; Rimsulfuron and Nicosulfuron by HPLC (MRID #43023103)
Soil Matrix- ECM 001851; Rimsulfuron by Liquid Chromatography (MRID #43023101)
Registrant- E.I. du Pont de Nemours

FROM: ✓ Silvia C. Termes, Ph.D., Chemist
Environmental Fate and Ground Water Branch
Environmental Fate and Effects Division (7507C)

TO: Robert J. Taylor
Product Manager #25
Registration Division (7507C)

THRU: Henry M. Jacoby, Chief
Environmental Fate and Ground Water Branch
Environmental Fate and Effects Division (7507C)

General Comment

Both of the submitted studies cited above carried Confidential Business Information classification. However, there is a clear declaration of non-confidentiality by the registrant on the second page of the methods' packages. The EFGWB had requested earlier from the Information Service Branch of PMSD that the CBI classification be lifted in order to publish the reformatted methods in the ECM manual. The Product Manager should follow up on this issue and notify the Analytical Chemistry Branch (BEAD).

A. Water Matrices (Rimsulfuron; Nicosulfuron)

Fortification levels for both rimsulfuron and nicosulfuron were 0 (blank), 0.1, 1.0 and 10.0 ppb. According to the tests performed by the EPA laboratory, the analytical procedure seems acceptable and no major problems were encountered during the laboratory work.

The validation report pointed out a step which may cause a potential major deficiency in the results. The registrant's method specifies that the water samples be filtered prior to extraction. It was the EPA's analyst concern that rimsulfuron and/or nicosulfuron could adsorb onto suspended particulates found in natural waters and lower the recoveries (accuracy) of the method.



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However, it is known from studies of the adsorptivity of sulfonylurea herbicides to particulates (soils) that this family of chemicals adsorb weakly to soils/sediments and, therefore, the pesticide will be partitioned primarily into the aqueous phase.

The Minimum Detection Limit (MDL, 3x noise) and the Limit of Quantitation (LOQ, 10x noise) were,

	Nicosulfuron	Rimsulfuron
MDL (ppb)	0.02	0.05
LOQ (ppb)	0.07	0.17

The Limit of Detection (lowest level of fortification), LOD, is 0.1 ppb for each of the analytes in water.

Summary of Method: The method involves filtration, concentration of analytes by Solid Phase Extraction (SPE) and elution with acetone. The acetone eluate is evaporated to dryness and reconstituted with the HPLC mobile phase (water/acetonitrile, 15/85 v/v and tetrabutylammonium hydrogen sulfate. Determination of analytes is made by HPLC, with UV detection at 240 nm.

B. Soil Matrices (Rimsulfuron)

Fortification levels of 0 (blank), 0.2, 0.5 and 1.0 ppb in samples of silt loam soil gave recovery value ranges from 74 to 93%, with an average value of 83.2%, standard deviation of 6.3% and relative standard deviation of 7.6%. At the lowest level of fortification (0.2 ppb), the average recovery value was 79.8% (SD 4.3%, RSD 5.4%).

The LOD (as lower level of fortification) was 0.2 ppb, the Minimum Detection Limit, MDL, is 0.15 ppb (3x noise) and the Level of Quantitation, LOQ, is 0.26 ppb (5x noise).

Summary of Method: Soil is extracted with a 1:1 acetonitrile:methylene chloride, centrifuged and shaken. Extracts and decant (done twice) are combined and evaporated to near dryness and reconstituted with pH 7 phosphate buffer solution. Clean-up is performed by HPLC (phenyl column) and quantitation is done using column switching and solvent switching technique. Detection is by UV (254 nm).



UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

ENVIRONMENTAL CHEMISTRY SECTION
BUILDING 1105—JOHN C. STENNIS SPACE CENTER
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SEP 29 1994

MEMORANDUM

SUBJECT: Environmental Chemistry Method Evaluation Report - Rimsulfuron (DPX-E9636) in Soil (MRID 430231-01) Report No. ECM 0018S1

FROM: Aubry E. Dupuy, Chief *Aubry E. Dupuy*
BEAD/ACB/Environmental Chemistry Section

TO: Sylvia C. Termes, Chemist
EFED/Environmental Fate and Ground Water Branch (7507C)

THRU: Donald A. Marlow, Chief *DM*
BEAD/Analytical Chemistry Branch (7503W)

THRU: Henry M. Jacoby, Chief
EFED/Environmental Fate and Ground Water Branch (7507C)

The Environmental Fate and Ground Water Branch (EFGWB) has requested an Environmental Chemistry Method Validation on Rimsulfuron (DPX-E9636) in soil. The analytical method, "Analytical Method for the Quantitation of DPX-E9636 in Soil by Liquid Chromatography," was submitted by DuPont Company, DuPont Report No. AMR 2231-91, Revision No. 1 (MRID 430231-01). This report has been classified as FIFRA Confidential Business Information.

We feel that this method should not have been declared FIFRA CBI, since there is a clear declaration of non confidentiality by the registrant on the second page of the method package. Please ask the technical reviewer to contact the Information Service Branch in PMSD and/or the registrant to remove the CBI Classification on this method in order for us to publish the reformatted method in the new ECM manual.

As requested, the fortification levels were the same as in the submitted report at 0.2, 0.5 and 1.0 part per billion (ppb) in soil. Four replicate analyses were performed at each level. A matrix blank (four replicates) was also run. The attached Lab Evaluation Analysis Report contains a Summary, Analysis Results and Experimental Section, including copies of representative chromatograms, calibration curves and examples of calculations.

If you have any questions concerning this report, please contact Han Tai (601-688-3252) or Aubry E. Dupuy, Jr. (601-688-3212).

cc: Danny McDaniel, QA Coordinator, ECS
Han Tai, Chemist, ECS

Environmental Chemistry Method Evaluation Report
Rimsulfuron (DPX-E9636) in Soil
ECM 0018S1

Environmental Chemistry Section
Analytical Chemistry Branch
Biological and Economic Analysis Division

Prepared by: Han Tai

Date: 9/30/94

Reviewed by:

Dan McDaniel

Approved by:

Aubrey E. Dwyer, Jr.

Part I

Summary and Conclusion

The Environmental Chemistry Laboratory validated the environmental chemistry method for Rimsulfuron (DPX-E9636) in soil from Dupont and this report contains the results of our analysis. The Rimsulfuron method is described in DuPont Report No. AMR 2231-91, Revision No. 1 (MRID 430231-01), "Analytical Method for the Quantitation of DPX-E9636 in Soil by Liquid Chromatography." The Report is classified as FIFRA Confidential Business Information.

The soil sample is extracted with a 1:1 mixture of acetonitrile: methylene chloride. The extract is evaporated to near dryness and reconstituted in a pH 7 phosphate buffer solution. High pressure liquid chromatography (HPLC) with a phenyl column is then employed for column clean-up. Quantitation is done on a C-18 column, using the column switching and the solvent switching technique. The HPLC analytical peak is detected by a multi-wavelength UV detector at 254 nm.

Fortification levels are 0 (blank), 0.2, 0.5 and 1.0 part per billion (ppb) in soil. Four replicate samples were analysed for each fortification level. Precision and accuracy data at each spiking level is presented in the report. The percent recovery values range from 74 to 93%, with an average value of 83.2%, standard deviation of 6.5% and relative standard deviation of 7.6% (12 determinations over-all).

With the present instrument set-up, the analytical peak of DPX-E9636 elutes at about 43 minutes. Each HPLC run requires about 65 minutes. A set of four standards and six samples can be completed in about 10 hours.

Part II
Analysis Results

Method: "Analytical method for the Quantitation of DPX-E9636 in Soil by Liquid Chromatography," by Jennifer S. Amoo, DuPont Report No. AMR 2231-91, Revision 1 (MRID 430231-01), October 18, 1993

Results:	ppb added	ppb Found(1)	% Recovery	\bar{x} (2)	SD	RSD	
	1.0	0.741	74.1				
	1.0	0.897	89.7				
	1.0	0.914	91.4				
	1.0	0.793	79.3	83.6	8.3	9.9	
	0.5	0.466	93.1				
	0.5	0.448	89.6				
	0.5	0.397	79.3				
	0.5	0.414	82.8	86.2	6.3	7.3	
	0.2	0.155	77.6				
	0.2	0.155	77.6				
	0.2	0.172	86.2				
	0.2	0.155	77.6	79.8	4.3	5.4	
	0 (blank)	ND(3)	_____				
		ND	_____				
		ND	_____				
		ND	_____				
				Overall	83.2	6.5	7.8

- Notes: (1) Values are results of four replicate analyses, including extraction and HPLC
 (2) \bar{X} = mean; SD = Standard Deviation; RSD = Relative Standard Deviation ($100SD/\bar{X}$)
 (3) ND = Not Detected for the present procedure. Minimum Detection Limit (MDL) is 0.15 ppb (3 x noise). Limit of Quantitation (LOQ) is 0.26 ppb (5 x noise).

Part III Experimental

General Description of Method

A. Extraction:

An aliquot of 50 gm. of soil sample is extracted with 50 ml 1:1 Acetonitrile:methylene chloride in a centrifuge bottle and on a wrist-action shaker for 10 minutes. Centrifuge and decant extract to a round bottom flask. Repeat extraction once again and combine extracts. Four aliquots are combined for a sample size of 200 gm, and 2 aliquots for 100 gm.

Evaporate combined extracts to about 2 ml. Transfer to a 15 ml centrifuge tube. Rinse bottle with a 10 mM phosphate solution and combine in the same tube (5 ml rinse for 200 gm. sample, 2 ml rinse for 100 gm. sample). Dilute with phosphate solution to 10.0 ml. (200 gm. sample) or 5.0 ml (100 gm. sample). Extracts are kept in an ice bath and acidified with 85% phosphoric acid (10 ul or 5 ul) immediately before HPLC.

B. High Pressure Liquid Chromatography (HPLC):

- a. Waters Automated Valve Station (WAVS) for valve connections.
- b. Waters Model 590 Programable Solvent Delivery System (Pump A) for the control of solvent switching and column switching. Six Event-Out terminals are connected to WAVS. The Event-In #1 terminal is connected to Waters model 712 Automated Injector.

Figure 1 shows the WAVS connections and the Model 590 Event-Out settings.

HPLC Instrumentation

- Columns: (1) Zorbax Phenyl, Reliance Cartridge, 4.0 x 80 mm, 820662-942
(Mac Mod Analytical, Chadds Ford, PA)
- (2) Zorbax Rx-C8, 4.6 x 250 mm. Serial No. 13488, 880967-901
(Mac Mod Analytical, Chadds Ford, PA)

Mobile Phases: Flow rate 1.5 ml/min; Isocratic; 4 Eluents, as listed below:

<u>Eluent</u>	<u>Methanol (%)</u>	<u>Phosphate Buffer Molar</u>	<u>pH</u>	<u>Purpose</u>
A	43	0.03	3.5	Retain DPX on Phenyl
B	10	0.01	7.5	Transfer DPX from Phenyl to C8
C	20	0.01	7.0	Chromatography of DPX on C8
D	90	—	—	Column cleaning

Detector: Waters Model 490 Multi-Wavelength Detector
Absorbance, UV 254 nm; 0.005 AUFS (Absorbance Units Full Scale)
time constant, 10.0

Temperature: Waters Temperature Control System
2 Columns in the same oven; Temp Control Module (TCM) set at 35.0°C

Pump: Pump A - Waters Model 590 Programmable Pump
Pump B - Waters Model 510 pump

Injector: Waters Model 712 Intelligent Sample Processor (WISP)
Injection volume - 2.0 ml

Recorder: Kipp-Zonan Model BD-41
Full scale 10 mv; chart speed 5 mm/min.

Retention Time for DPX - E9636:

Phenyl column, RT1 - about 20 min.
Phenyl column, RT2 - about 22 min.
C8 Column - about 43 min. (analytical peak)

Brief Description of HPLC Operation

1. All samples and standards to be run are placed in an ice bath.
2. Acidify, centrifuge and syringe filter 3-3.5 ml of the solution into a 4-ml sample vial (Steps 13-17, Method p.16). The sample vial is loosely capped (screw cap with Teflon septum). Tight capping may cause a partial vacuum in the vial during syringe plunger travel and prevent withdrawal of a full 2.0 ml.

3. The present 712 system is not equipped with a cooling accessory. One vial is processed at a time for each HPLC run. Injection volume 2000 μ l (2.0 ml).
4. Determine Eluent Switch Time RT1 and TB (Method p.18)
 - a. Set 590 Segment (Seg) 01 - Event in #1 and time 0.00 minute.
 - b. Set Seg 02 at 47.00 minutes; Seg 03 at 48.00 minutes; Seg 04 at 49.00 minutes; Seg 05 thru 07 as in Figure 1.
 - c. Run one vial of standard 0.02 μ g/ml.
 - d. Record Retention time RT1 of DPX-E9636. $TB = 1/2 (RT1)$
 - e. Change Seg 02 time from 47.00 to TB.
 - f. Reset 590 to Seg 01.
5. Determine Column Switch Time RT2, TS, TE (Method p.18)
 - a. Repeat steps 3a, 3b, 3c
 - b. Record retention time RT2 of DPX-E9636. $TS = RT2 - 2.00$;
 $TE = RT2 + 3.00$
 - c. Change Seg 03 time from 48.00 to TS. Change Seg 04 Time from 49.00 to TE.
 - d. Reset 590 to Seg 01
6. Run standards and samples

The retention times RT1 and RT2 are determined daily for each set of HPLC runs. (Method p.17. p.18). A standard is run between every 2 samples (Method p.22). For the present instrument set-up, each HPLC run takes about 65 minutes.

Modification of Methods:

The supply of file soil sample was about 2 Kg. In order to carry out four replicate analyses for each set of four samples, set one used a sample size of 200 gm. with final extract volume of 10.0 ml. and sets two, three and four used a sample size of 100 gm, with final extract volume of 5.0 ml. The analysis results were found to be comparable between the sets.

Source of Analytical Reference Standard:

DPX-E9636, F-1008, Lot AHM-1, Purity 99.68%, 29 July 1993.

Received from: Pesticides Repository. U.S. EPA (MD-8)

c/o Man-Tech Environmental Technology, Inc.
P.O. Box 123, 2 Triangle Drive
Research Triangle Park, NC 27709

Source of Sample Matrix:

ECS File Soil, from Ms. Flynt's home, Pearl River County, MS. Sieved through 5.6 mm sieve, stored in freezer. Characterization of this soil is listed in Appendix 1.

Comments:

1. For the present HPLC instrument set-up the run time for each standard or sample was about 65 minutes. A set of 4 standards and 6-8 samples would require about 10-12 hours for the HPLC run.
2. In order to bring intensity of the analytical peak to an adequate scale deflection of the recorder response, the detector was set at a sensitivity range of 0.005 Absorption Unit Full Scale (AUFS). Under this range the peak height of the standard of the highest concentration, 0.02 ug/ml, was about 30% on the recorder chart. A Time Constant of 10.0 for the detector was necessary to reduce extensive noise.

Chromatograms and calibration curve:

A. Standards, DPX-E9636

- 1a 0.02 ug/ml, start to 30 minutes
- 1b 0.02 ug/ml, 30 to 65 minutes
- 2 0.01 ug/ml
- 3 0.004 ug/ml
- 4 0.002 ug/ml
- 5 Calibration curve

B. Sample, soil, 30 to 60 minutes

- 1. Fortification 1.0 ppb
- 2. Fortification 0.5 ppb
- 3. Fortification 0.2 ppb
- 4. Blank (no fortification)

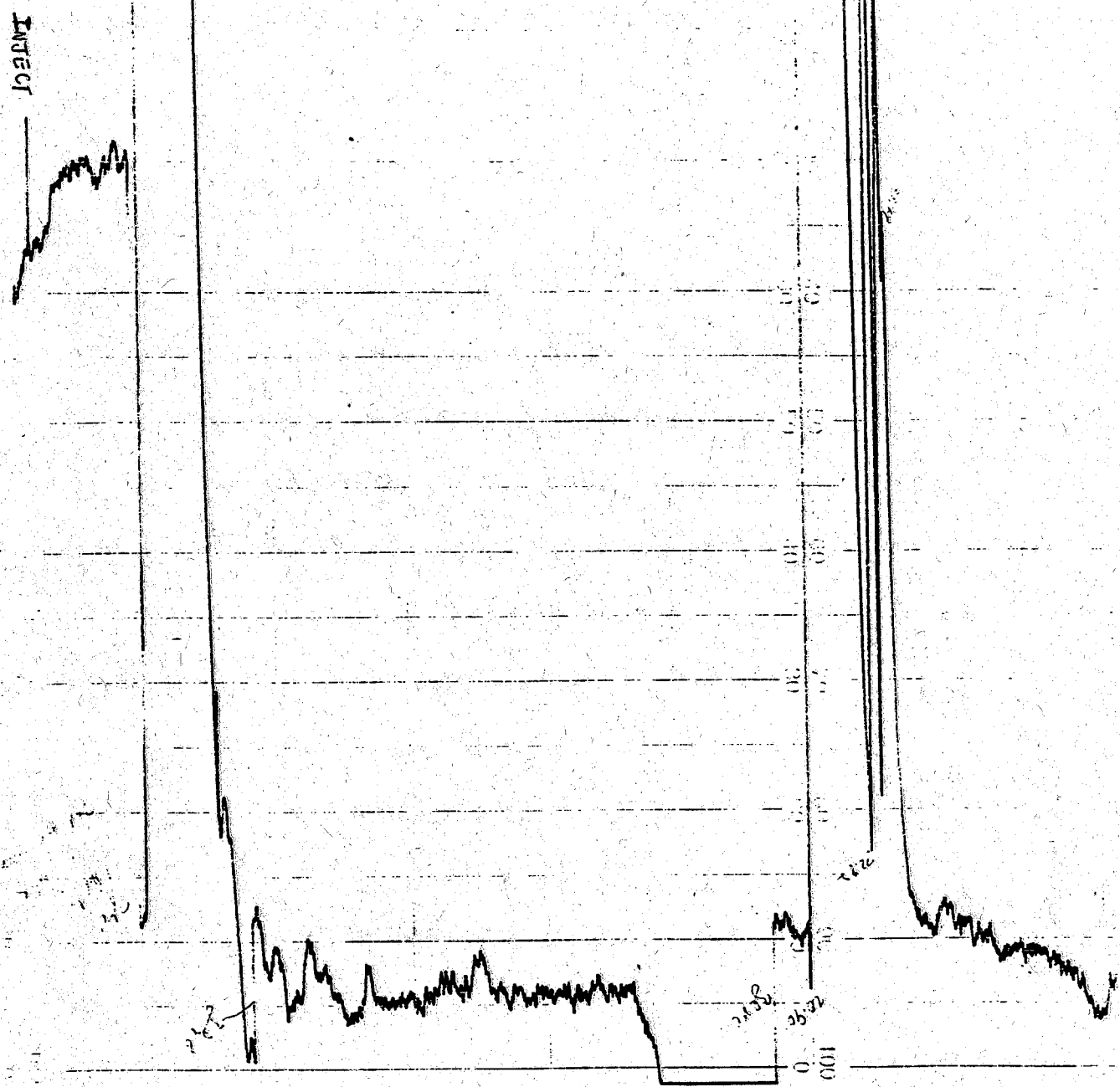
Notes:

- 1. An arrow indicates the position of the DPX-E9636 peak on the chromatogram.
- 2. On the chromatogram, the detector response to the eluent/column switching is recorded. (start to 30 minutes, and 50 to 65 minutes). The patterns are essentially the same for all runs, regardless of a standard or a sample. The full run (start to 65 minutes) is shown in Figures A1a and A1b as an illustration. Other representative chromatograms show the segment between 30 and 60 minutes where the peak of DPX-E9636 is located.

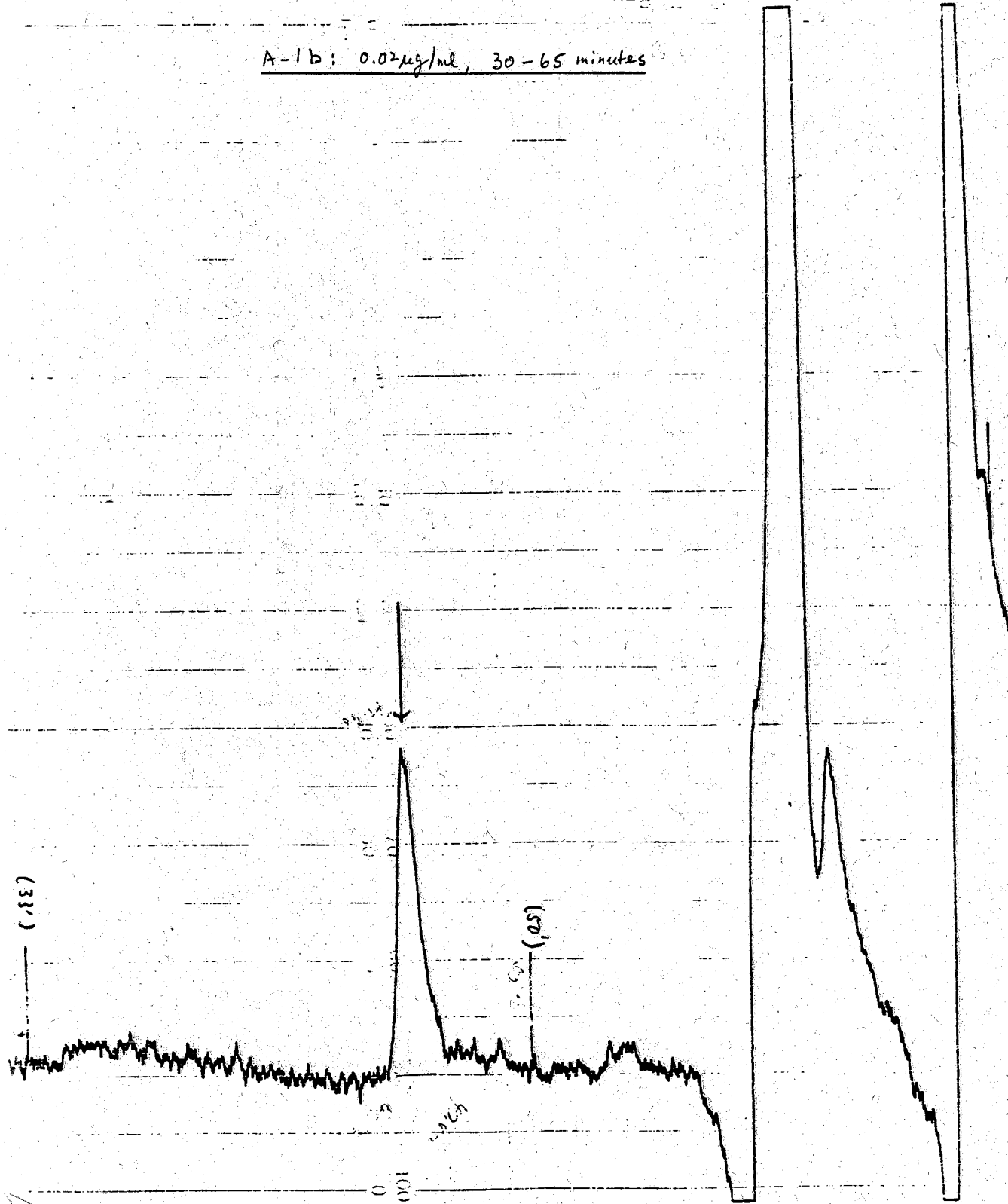
A. DPX - E9636 STANDARDS

INJECTION VOLUME: 2.0 μ l

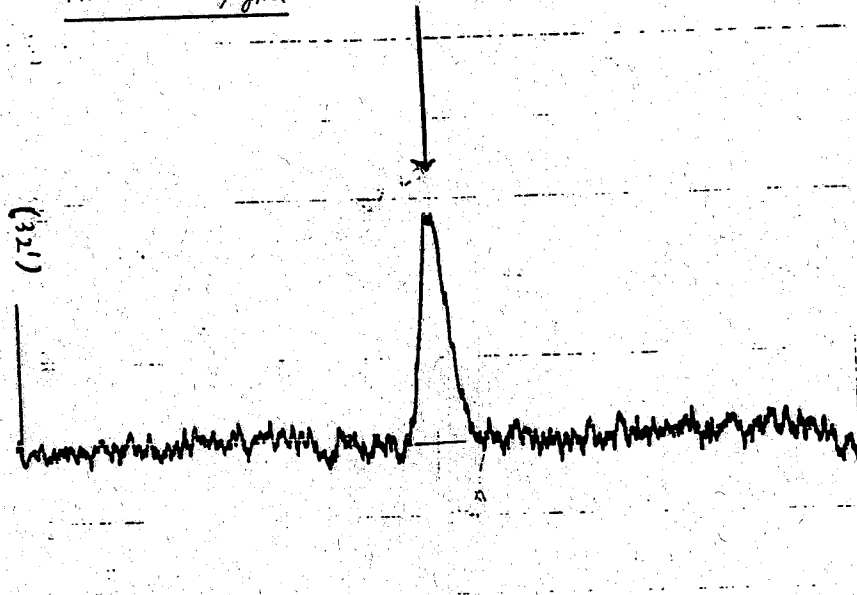
A-1a : 0.02 μ g/ μ l, start - 30 minutes



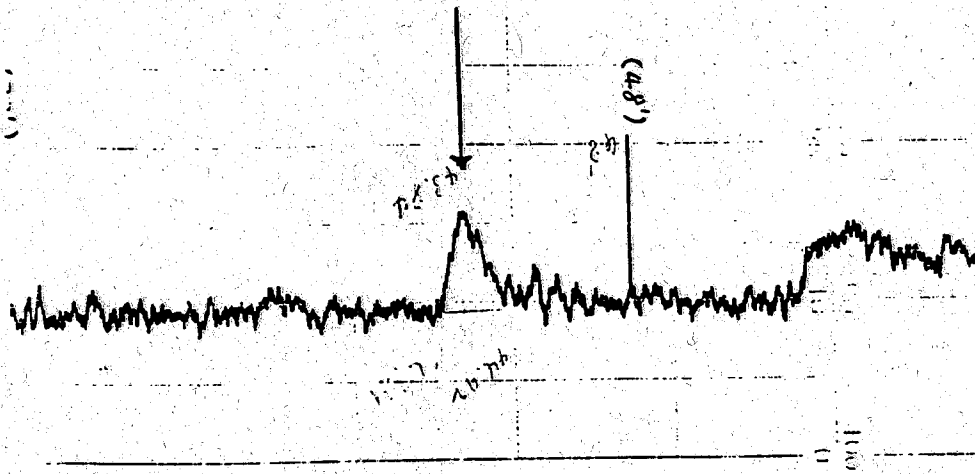
A-1b: 0.02 μg/ml, 30-65 minutes



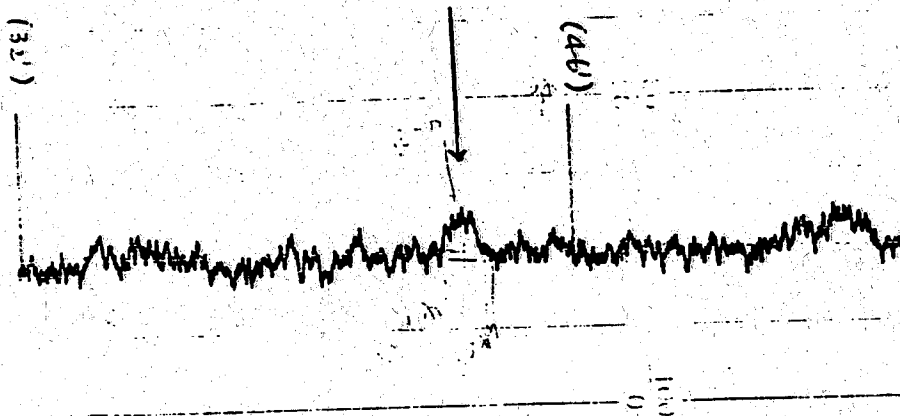
A-2: 0.01 µg/ml



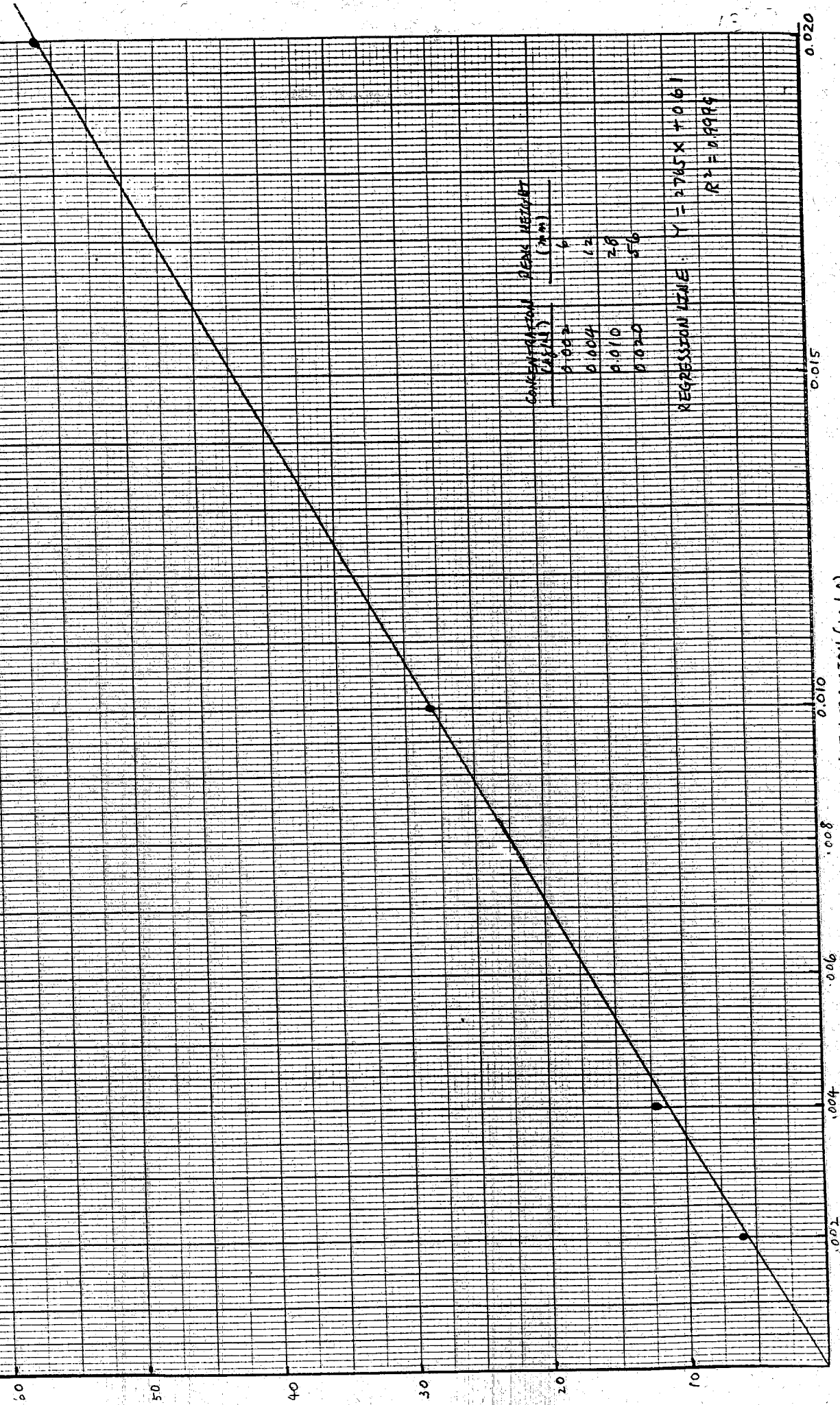
A-3: 0.004 µg/ml



A-4: 0.002 µg/ml



A-5 DPX-E936 CALIBRATION CURVE



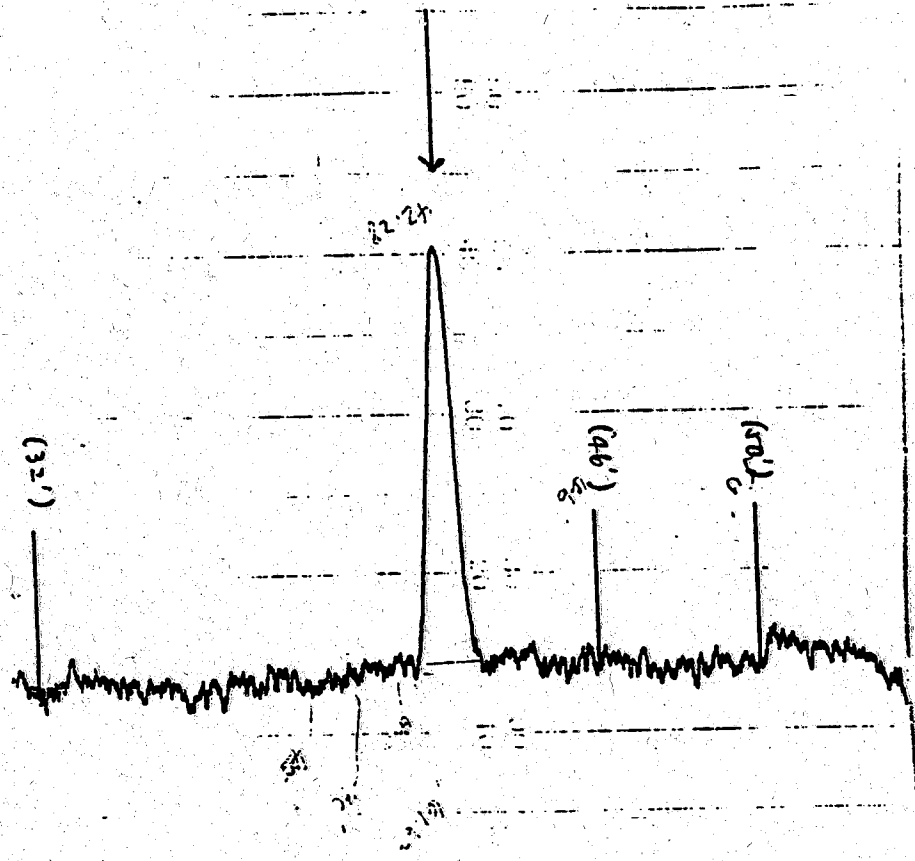
CONCENTRATION (ug/ml)

0.020
0.015
0.010
0.008
0.006
0.004
0.002

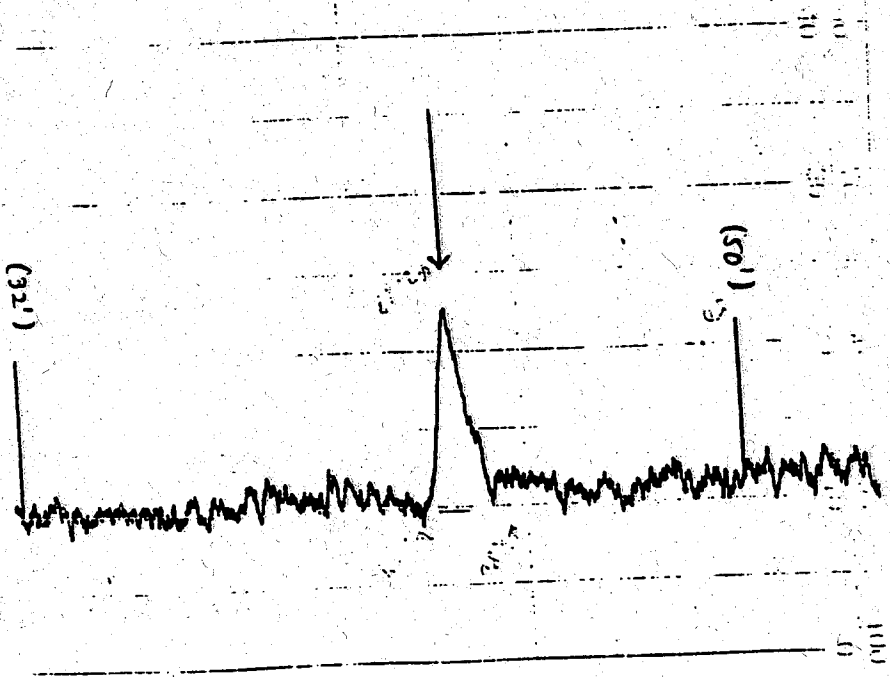
60
50
40
30
20
10

B SOIL SAMPLES - DPX-E9636 FORTIFICATION (35

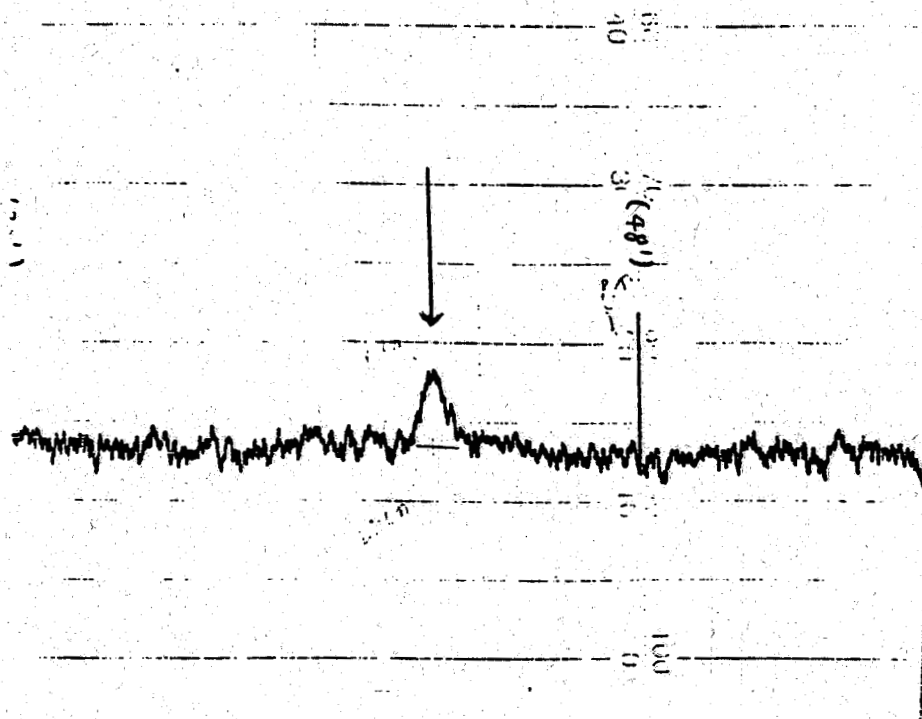
B-1: FORTIFIED 1.0 ppb



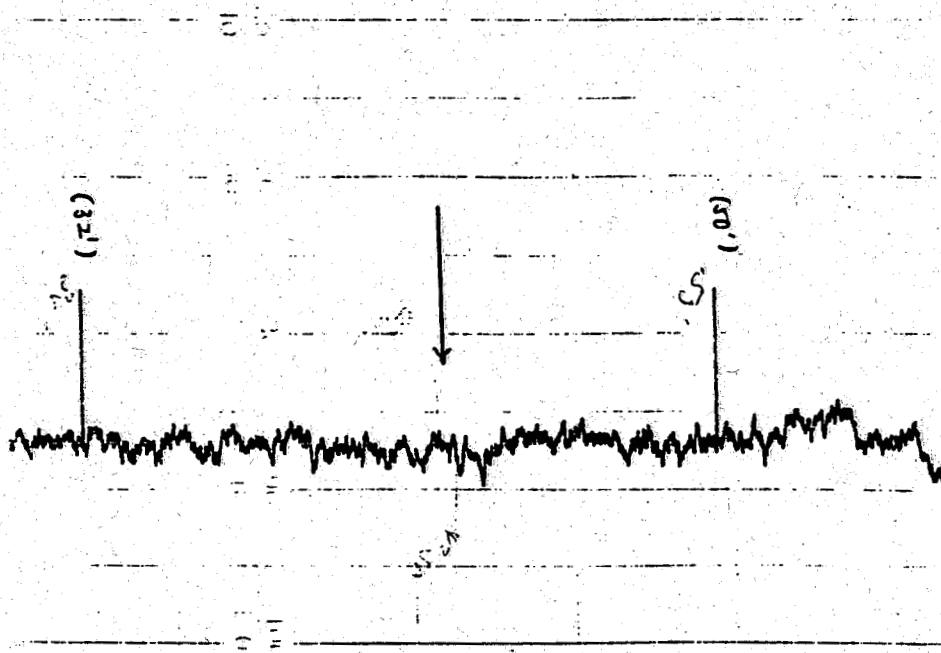
B-2 FORTIFIED 0.5 ppb



B-3: FORTIFIED 0.2 ppb



B-4: BLANK - NO FORTIFICATION



Example of Calculation (Method. p.22, p.23)

1. Peak Intensity:

The peak intensity is expressed in terms of peak height. The peak height in millimeters (mm) is measured manually from the apex of the analytical peak to the baseline drawn across the base of peak (mid-point between top and valley of noise tracing). Injection volume for all standards and samples are 2.0 ml each, therefore, injection volume adjustment is not needed in calculation.

2. Calculation Formula:

$$\text{ng/g (ppb) DPX-E9636 in sample} = \frac{H \times FV \times 1000}{\text{Avg CF} \times SW}$$

where:

H = Peak height of DPX-E9636

FV = Final volume of extract

SW = Sample weight in grams

Avg CF = Average Calibration Factor for standards

3. % Recovery:

$$\% \text{ Recovery} = \frac{\text{ppb Found}}{\text{ppb Added}} \times 100$$

3. Example:

a. standard:

<u>concentration(ug/ml)</u>	<u>peak height (mm)</u>	<u>CF</u>	<u>chromatograms</u>
0.02	58	2900	A-1b
0.01	28	2800	A-2
0.004	12	3000	A-3
0.002	6	*	A-4
	Average	<u>2900</u>	

Regression Line $Y(\text{mm}) = 2765 X (\text{ug/ml}) + 0.61$; $R = 0.9999$

* peak intensity near MDL, not included for averaging F

b. Sample:

Fortification level = 1.0 ppb (Chromatogram B-1)

Peak height (H) = 52 mm

Final volume (FV) = 10 ml

Sample weight (SW) = 200 gm

Avg. Calibration
Factor (CF) = 2900

ppb DPX-E9636 Found = $\frac{52 \times 10 \times 1000}{2900 \times 200} = 0.897$ ppb

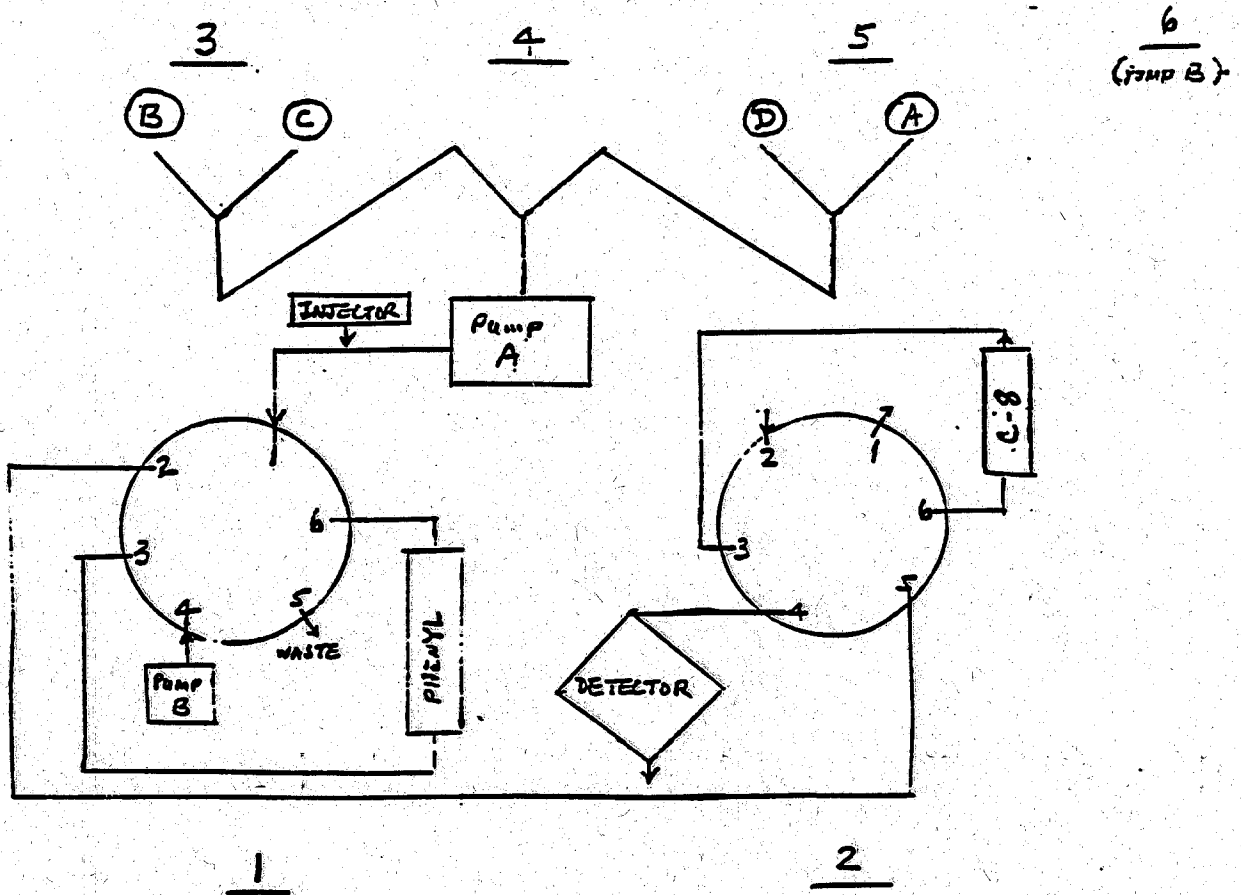
% Recovery = $\frac{0.897}{1.0} \times 100 = 89.7\%$

Figure 1

Waters Automated Valve System (WAVS) Connections

Waters Model 590 Event-Out Switch Settings

Figure 1. WAYS CONNECTION



Model 590 Events Out Switch Settings

N = ON
 F = OFF

<u>SEGMENT</u>	<u>TIME (min.)</u>	<u>1</u>	<u>2</u>	<u>3</u>	<u>4</u>	<u>5</u>	<u>6</u>
01	0.00	F	F	F	F	F	F
02	TB	F	F	N	N	F	F
03	TS	F	N	F	N	F	F
04	TE	N	N	F	N	F	N
05	50.00	N	N	F	F	N	N
06	55.00	N	N	F	N	F	F
07	62.00	F	F	F	F	F	F

MECHANICAL (TEXTURE) ANALYSIS
 Mississippi Cooperative Extension Service, Soil Testing Lab
 P.O. Box 9610
 Mississippi State, MS 39762

U.S. EPA / E. Flynt

NAME:
Building 1105, Stennis Space, MS 39529

ADDRESS:
November 15, 1993

DATE:
UNCL

COUNTY:

<u>LAB#</u>	<u>FIELD#</u>	<u>%CLAY</u>	<u>%SILT</u>	<u>%SAND</u>	<u>TEXTURE</u>
<u>EPA1</u>	<u>1</u>	<u>15.5</u>	<u>20.3</u>	<u>64.2</u>	<u>SILT LOAM</u>
<u>EPA2</u>	<u>1</u>	<u>14.8</u>	<u>22.0</u>	<u>63.2</u>	<u>SILT LOAM</u>
<u>(Average)</u>	<u>1</u>	<u>15.2</u>	<u>21.2</u>	<u>63.7</u>	<u>SILT LOAM</u>

pH 4.8
 % Organic Matter 2.79%



UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

ENVIRONMENTAL CHEMISTRY SECTION
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FEB 10 1995

MEMORANDUM

SUBJECT: Environmental Chemistry Method Validation - Report - ECM 0019W Rimsulfuron (DPX-E9636) and Nicosulfuron (DPX-V9360) in Water by HPLC (MRID 430231-03)

FROM: Aubry E. Dupuy, Chief *Aubry E. Dupuy, Jr.*
BEAD/ACB/Environmental Chemistry Section

TO: Sylvia C. Termes, Chemist
EFED/Environmental Fate and Ground Water Branch (7507C)

THRU: Donald A. Marlow, Chief *DM*
BEAD/Analytical Chemistry Branch (7503W)

THRU: Henry M. Jacoby, Chief
EFED/Environmental Fate and Ground Water Branch (7507C)

The Environmental Fate and Ground Water Branch has requested an Environmental Chemistry Method Lab evaluation on Rimsulfuron (DPX-E9636) and Nicosulfuron (DPX-V9360) in water. The DuPont Company has submitted an analytical method, "Analytical Method for the Determination of E9636 and V9360 in water by HPLC," DuPont Report No. AMR 2186-91 (MRID 430231-03). This report is classified as FIFRA Confidential Business Information.

We feel that this method should not have been declared FIFRA CBI, since there is a clear declaration of non confidentiality by the registrant on the second page of the method package. Please ask the technical reviewer to contact the Information Service Branch in PMSD and/or the registrant to remove the CBI Classification on this method in order for us to publish the reformatted method in the new ECM manual.

As requested, the fortification levels were the same as in the submitted report at 0.1, 1.0 and 10.0 part per billion (ppb). Four replicate analyses were performed at each level and sample blank. The attached Analysis Report contains a Summary, Analysis Results and Experimental Sections which including copies of representative chromatograms, calibration curves, and an examples of calculations.

If you have any questions concerning this report, please contact Han Tai (601 688-3252) or Aubry E. Dupuy, Jr. (601-688-3212).

cc: Dany McDaniel, QA Coordinator, ECS
Han Tai, Chemist, ECS

Environmental Chemistry Method Validation Report

ECM 0019W

Rimsulfuron (DPX-E9636) and Nicosulfuron
(DPX-V9360) in Water

Environmental Chemistry Section

Analytical Chemistry Branch

Biological and Economic Analysis Division

Prepared by:

Han Tai
Han Tai, Chemist/ECS

Date: 01/20/95

Reviewed by:

Danny McDaniel
Danny McDaniel, QA Coordinator, ECS

Part I
 Summary and Conclusion

An Environmental Chemistry Method Validation has been performed on the analysis of Rimsulfuron (DPX-E9636) and Nicosulfuron (DPX-V9360) in water. The analytical method is described in DuPont Report No. AMR 2186-91 (MRID-430231-03), "Analytical Method for the Determination of E9636 and V9360 in water by HPLC." The report is classified as FIFRA Confidential Business Information.

The analytes in water are concentrated by Solid Phase Extraction (SPE) on a Bond Elute C-18 column. The SPE column is eluted with acetone. The acetone eluant is evaporated to dryness and reconstituted with the HPLC mobile phase. High Pressure Liquid Chromatography (HPLC) is employed for the determination of the analytes on a Nova Pak C-18 column with detection by UV absorption at 240 nm.

Fortification levels are 0 (blank), 0.1, 1.0 and 10.0 part per billion (ppb) in water. Four replicate analyses have been made for each fortification level. The analysis results, in terms of percent recovery are summarized as follows (a total of 12 determinations on each analyte):

ppb Added	V9360			E9636		
	Range	Mean	Std. Dev.	Range	Mean	Std. Dev.
0.1	73.9 - 77.3	75.6	2.0	83.7 - 100.5	89.8	7.4
1.0	77.3 - 83.7	81.7	2.7	71.1 - 76.6	73.2	2.4
10.0	79.7 - 85.7	82.9	2.5	78.8 - 84.9	83.0	2.9

The analysis procedure seems to be acceptable. No major problems were encountered during the laboratory work.

We have also observed one step in the procedure which may cause major deficiency in the analysis result. The DuPont method specifies that the water samples be filtered before extraction. However, suspended particulate matter is normally considered part of a water sample and is usually extracted along with the water. We have no data regarding the adsorptivity of sulfonyl ureas to particulates, and would like to point out that this method might be biased toward lower recoveries (accuracy) should these analytes adsorb on particulates. Since we received limited information, did the registrant address this issue? If so, we would like to receive a copy for reference with future work with sulfonyl urea methods.

Part II
 Analysis Results

Method: "Analytical Method for the Determination of E9636 and V9360 in Water by HPLC," by Edward W. Zahnow and David B. Orescan, DuPont Report No. 2186-91 (MRID 430231-03). September 11, 1991

Results: ppb added	V9360					E9636				
	ppb Found(1)	% Recovery	$\chi(2)$	SD	RSD	ppb Found(1)	% Recovery	$\chi(2)$	SD	RSD
0.1	0.0739	73.9				0.0837	83.7			
0.1	0.0739	73.9				0.1005	100.5			
0.1	0.0773	77.3				0.0875	87.5			
0.1	0.0773	77.3	75.6	2.0	2.6	0.0875	87.5	89.8	7.4	8.2
1.0	0.8128	81.3				0.7262	72.6			
1.0	0.8374	83.7				0.7262	72.6			
1.0	0.7732	77.3				0.7658	76.6			
1.0	0.8200	82.0	81.1	2.7	3.3	0.7112	71.1	73.2	2.4	3.3
10.0	8.374	83.7				8.492	84.9			
10.0	8.571	85.7				8.492	84.9			
10.0	7.966	79.7				7.877	78.8			
10.0	8.247	82.5	82.9	2.5	3.0	8.315	83.2	83.0	2.9	3.5

Part II
 Analysis Results Con't.

0 (Blank)	ND (3)				ND
0 (Blank)	ND				ND
0 (Blank)	ND				ND
0 (Blank)	ND				ND

Overall (12 values)		79.9	3.9	4.9					
						82.0	8.3	10.1	

Notes:

(1) Four replicate analyses, which include extraction and HPLC

(2) x - mean

SD - Standard Deviation

RSD - % Relative Standard Deviation (100 x SD/X)

(3) ND - Not Detected

Minimum Detection Limit (MDL, 3 x noise) and Limit of Quantitation (LOQ, 10 x noise), for 200 gm sample, 1.0 ml final extract, are calculated as follows:

	<u>V9360</u>	<u>E9636</u>
MDL (ppb)	0.02	0.05
LOQ (ppb)	0.07	0.17

Part III
Experimental

General Description of Method

A. Extraction:

Solid Phase Extraction (SPE) cartridge Bond Elut (500 mg) is prewashed with 5 ml each of acetonitrile and water. Water sample, 200 gm, is passed through the SPE cartridge, which is then dried by drawing air through for 60 minutes. The analytes, V9360 and E9636, are eluted with 5 ml acetone into a graduated 15 ml centrifuge tube. The acetone solution is evaporated to dryness under a gentle stream of nitrogen. The residue is dissolved in HPLC mobile phase by vortex and ultrasonic mixing and filtered (ACRO-13 0.2 um syringe filter) for HPLC analysis. The final extract volume is 1.0 ml for blank, 0.1 ppb and 1.0 ppb fortified samples; 4.0 ml for 10.0 ppb fortified samples. Samples can be stored in refrigerator as dry residues if not for immediate HPLC analysis.

B. High Pressure Liquid Chromatography (HPLC):

The parameters are the same as the DuPont method, and summarized as follows:

Column: Waters NOVA Pak C-18, 4.6 x 150 mm, T-40563, at 35°C

Mobile Phase: 15/85 (v/v) acetonitrile/water, containing an ion-pairing reagent Waters Pic A, low UV. 0.005M (Waters 84189, Tetra-butyl ammonium hydrogen sulfate); Isocratic; Flow Rate 1.5 ml/min.

Pump: Waters Model 590 HPLC Pump

Detector: Waters Model 490 Multi-Wavelength Detector, Absorbance at UV 240 nm; 0.01 AUFS (Absorbance Unit Full Scale); Time Constant 2.0

Injector: Waters Model U6k Manual Injector

Inj. Volume: 200 ul - standard 0.02 ug/ml; sample blank and 0.1 ppb

70 ul - standard 1.00 ug/ml

100 ul - for all other standards and samples

The injector volume was chosen so that the detector response would give readable records per deflection on the chart paper (2-80%) while covering the concentration range as listed in the submitted method p.9). Hamilton syringe, 250 ul, was used for injection. The DuPont method did specify injection volume of 100 ul.

Recorder: Kipp-Zonan Model BD-41

Full scale 10.0 mv; chart speed 0.5 cm/min.

Retention Time: V9360 - about 6 minutes (5.9-6.1 minutes)

E9636 - about 17 minutes (16.9-17.1 minutes)

(A stopwatch was used for timing)

Source of Analytical Reference Standards

Standards were obtained from EPA Pesticides Repository (MD-8), Man-Tech Environmental Technology, Inc., P.O. Box 123, Research Triangle Park, N.C. 27709.

V9360 - 03946/F946, Lot AEG-1, Purity 99.15%, 5 Aug. 1993.

E9636 - 31008/F-1008, Lot AHM-1 Purity 99.68%. 29 July 1993.

About 50 mg each in separate vials.

Source of Sample Matrix

Water, grab sample, 2 gallons in clean glass bottle, at the bank of Pearl River, near Bldg. S-2423, southwestern corner of Stennis Space Center, MS. The sample was filtered through Water type HA 0.45 um filter before fortification and analysis (method, p. 8)

Comment

The standards were prepared as per method which resulted in the lowest fortification (0.1 ppb) with less than 100% recovery being below the lowest prepared calibration standard (0.02 ug/ml).

Chromatograms:

A. Standards:

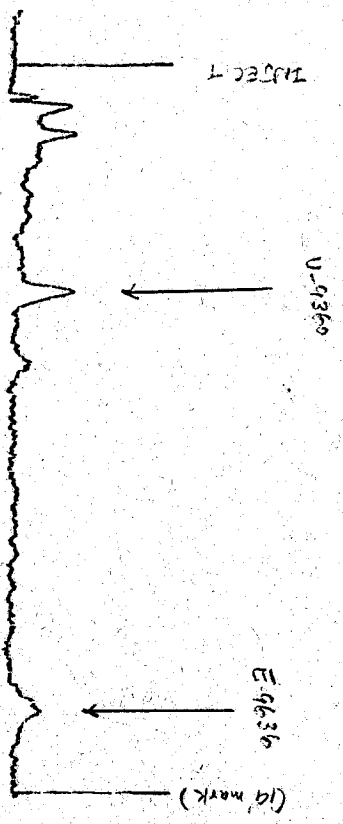
1. 0.02 ug/ml, 200 ul
2. 0.05 ug/ml, 100 ul
3. 0.10 ug/ml, 100 ul
4. 0.20 ug/ml, 100 ul
5. 0.50 ug/ml, 100 ul
6. 1.00 ug/ml, 70 ul
7. Calibration Curve

B. Samples:

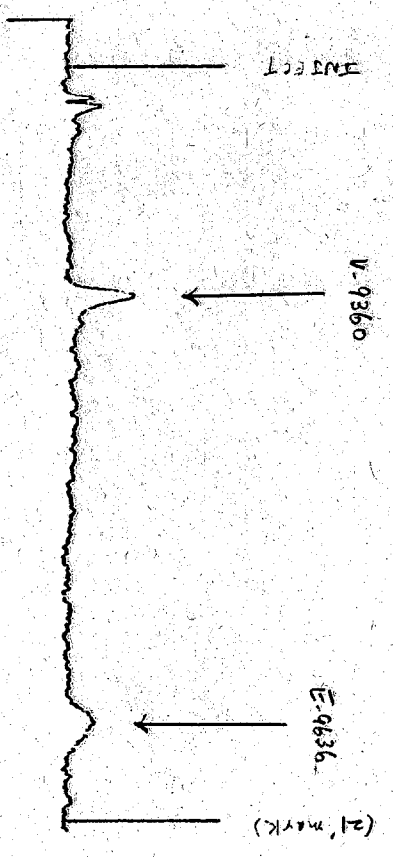
1. Fortification Level 0.1 ppb
2. Fortification Level 1.0 ppb
3. Fortification Level 10.0 ppb
4. Blank (no fortification)

Notes: Arrows indicate the locations of the analytical peaks.

A-1 STANDARD, 0.02 µg/µl
INJECTION VOLUME: 200 µl

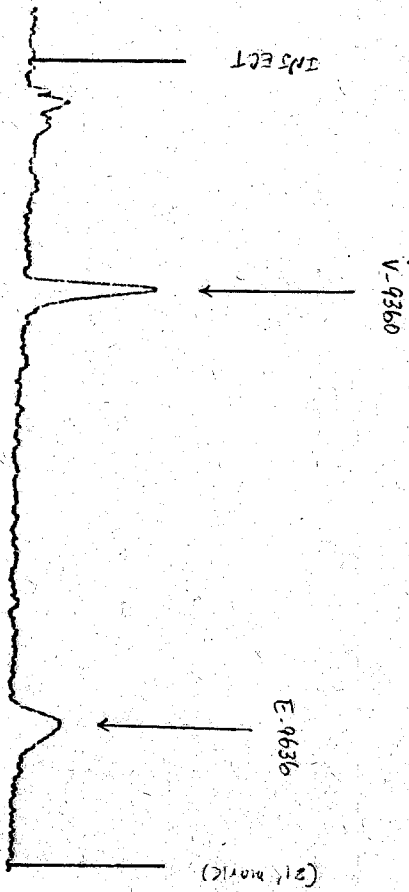


A-2 STANDARD, 0.05 µg/µl
INJECTION VOLUME: 100 µl



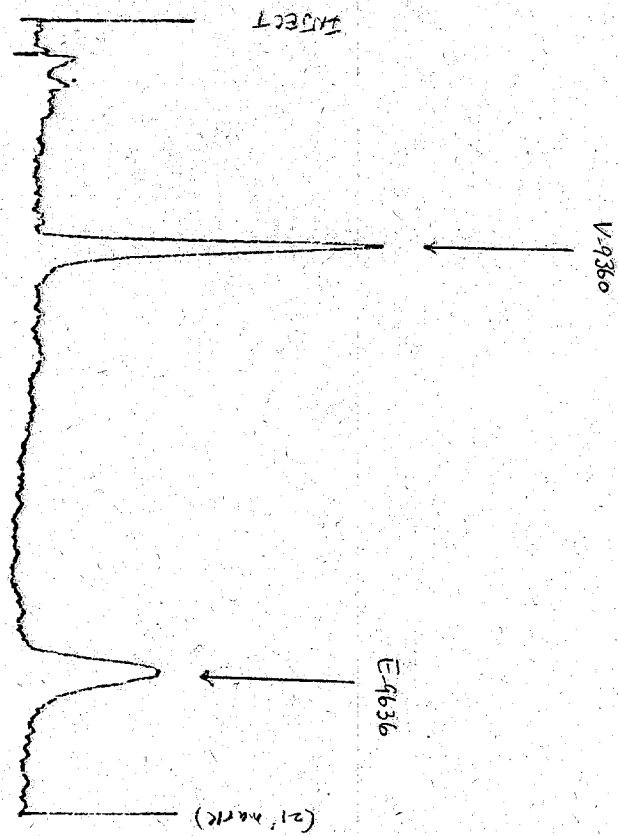
A.3 STANDARD, 0.10 µg/ml

INJECTION VOLUME: 100 µl

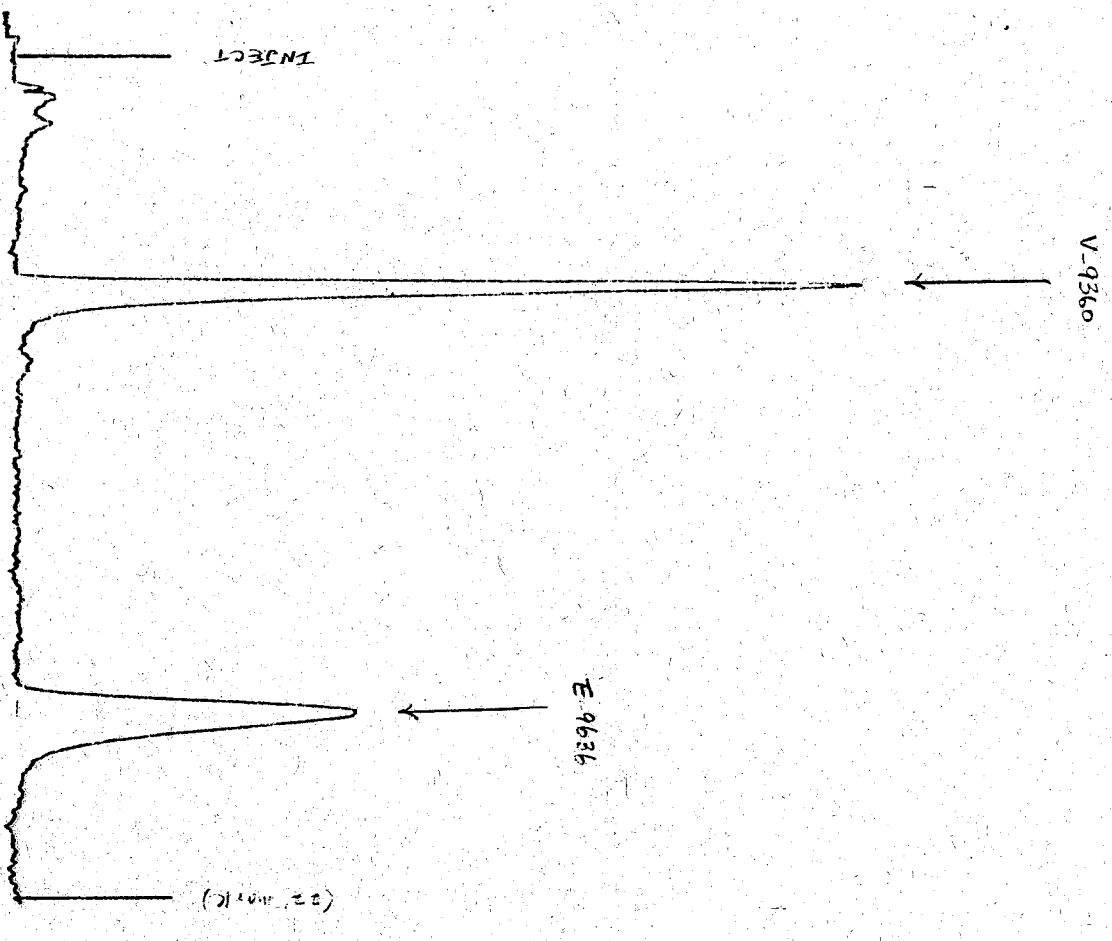


A.4 STANDARD, 0.20 µg/ml

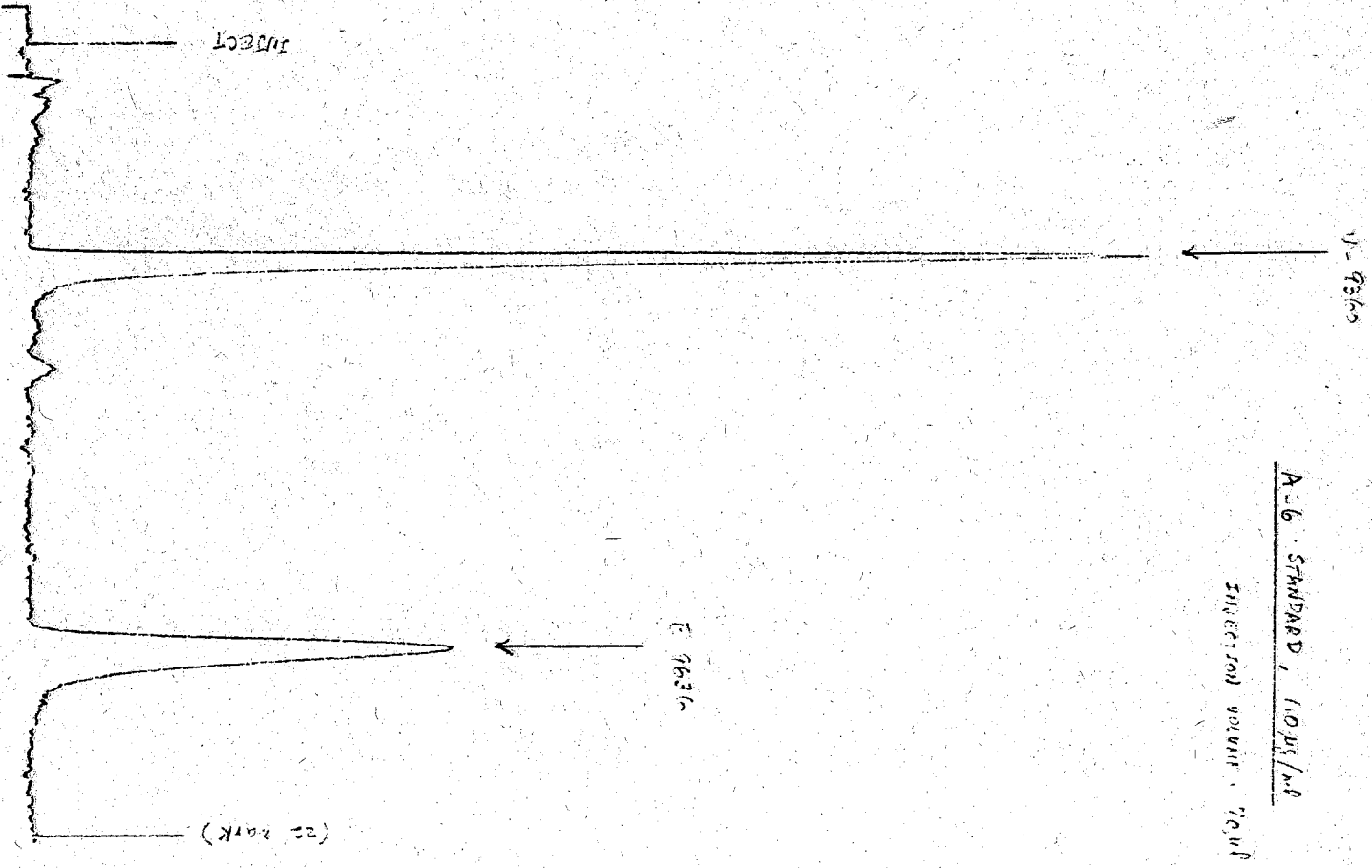
INJECTION VOLUME: 100 µl



A-5 STANDARD, 0.50 µg/ml
INJECTION VOLUME: 100 µl

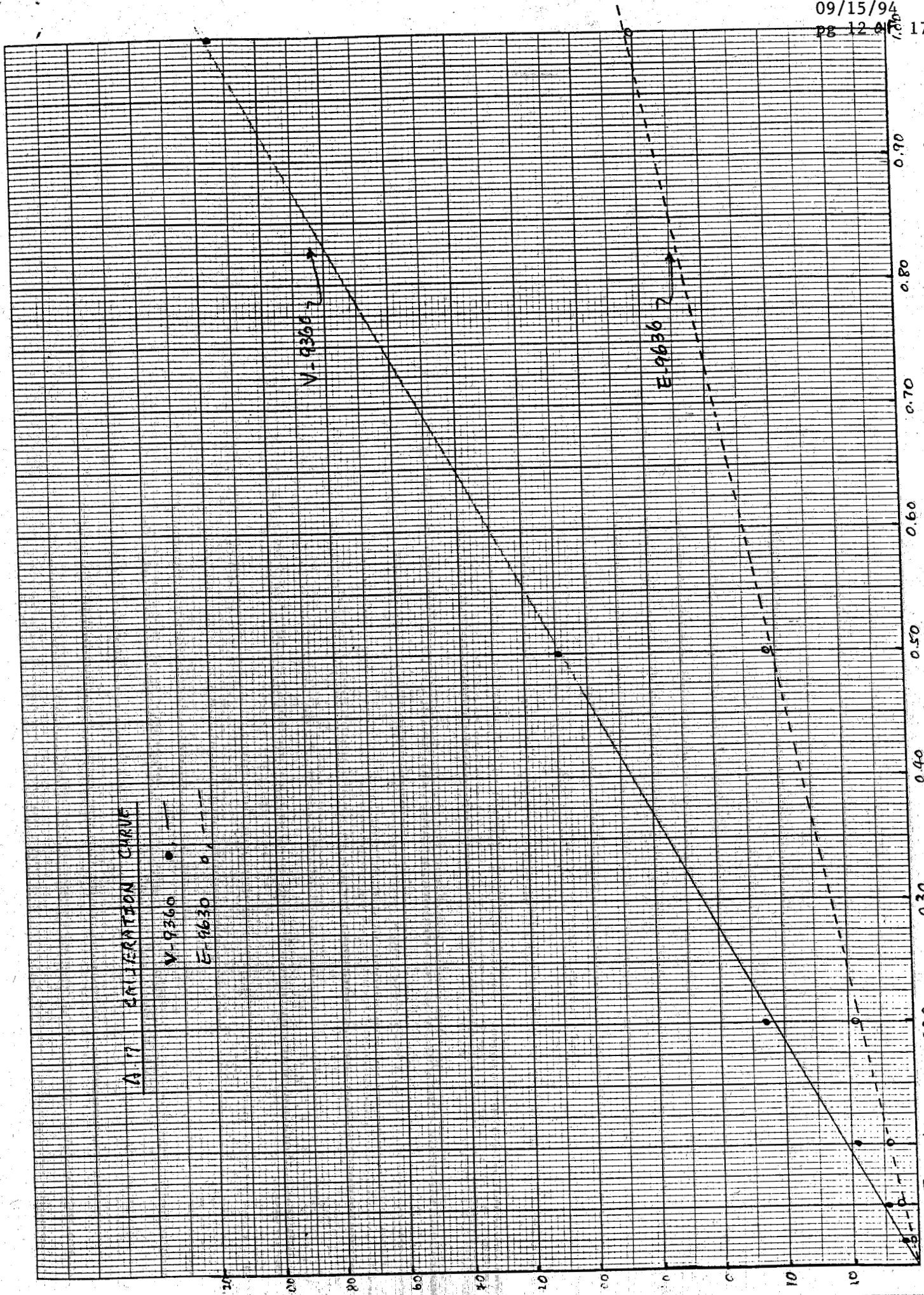


A-6 STANDARD, 1.0 µg/ml
INJECTION VOLUME: 100 µl



A.P. CALIBRATION CURVE

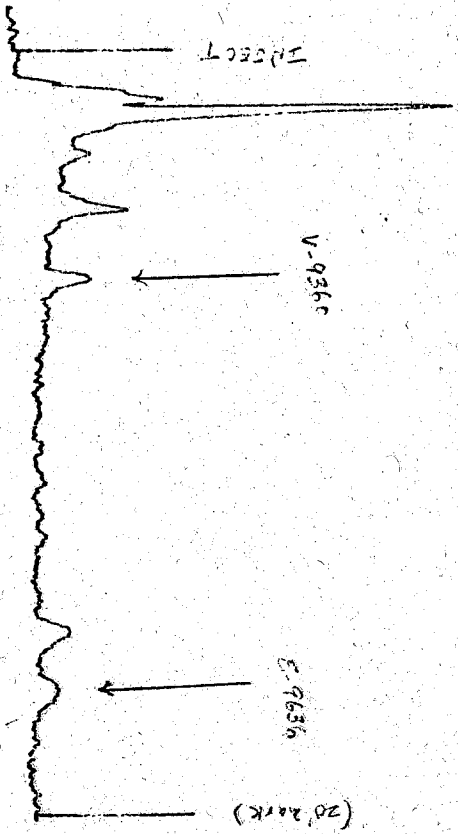
V-9360 ● —
E-9636 ○ - - -



CONCENTRATION (µg/l)

B-1 WATER SAMPLE, TORTIFIED 0.1 PPB

FINAL EXTRACT VOLUME: 1.0 ml
INJECTION VOLUME: 200 µl



B-2 WATER SAMPLE, TORTIFIED 1.0 PPB

FINAL EXTRACT VOLUME: 1.0 ml
INJECTION VOLUME: 100 µl

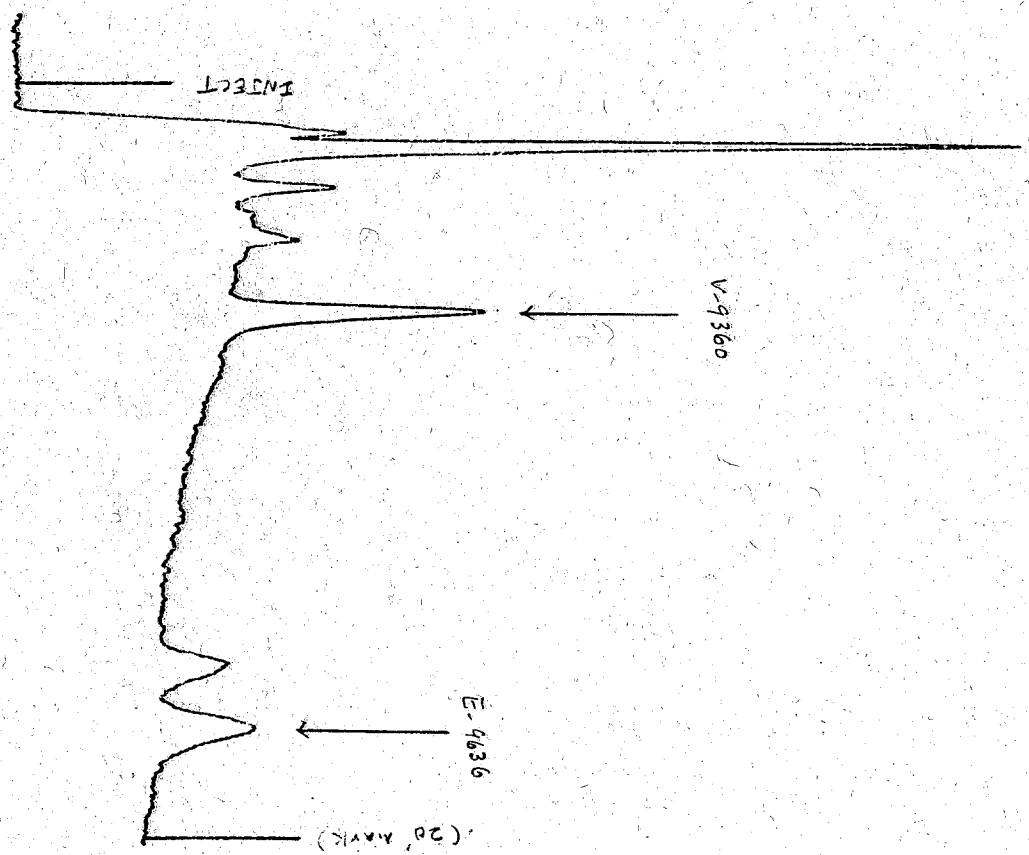
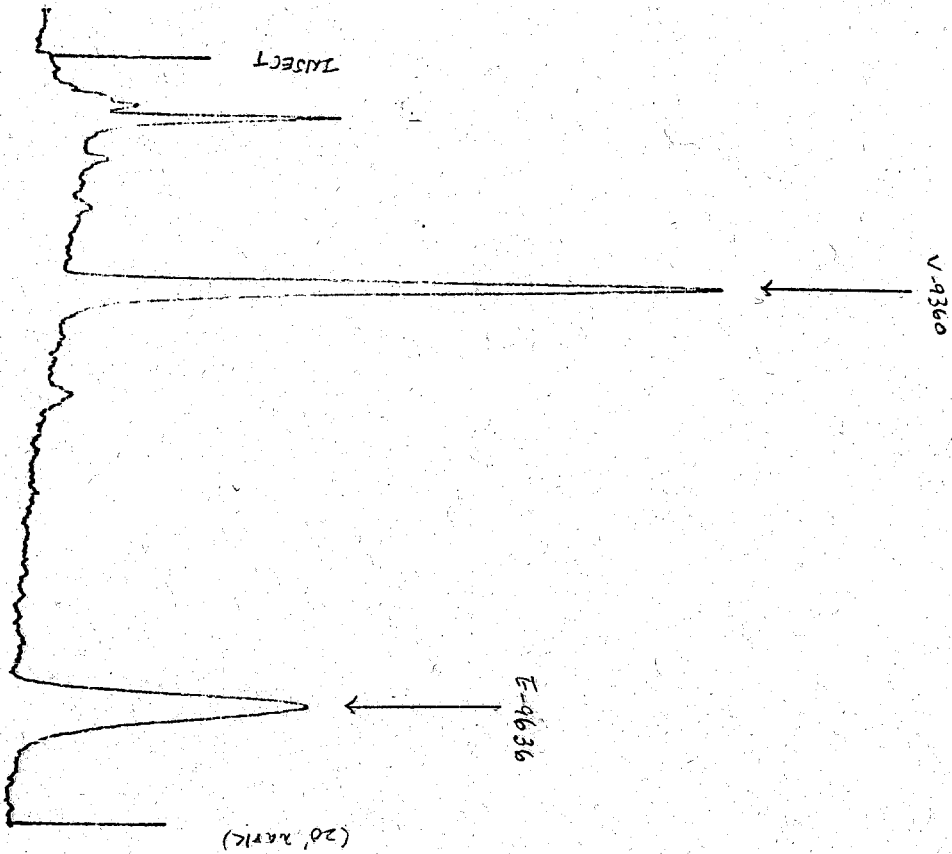


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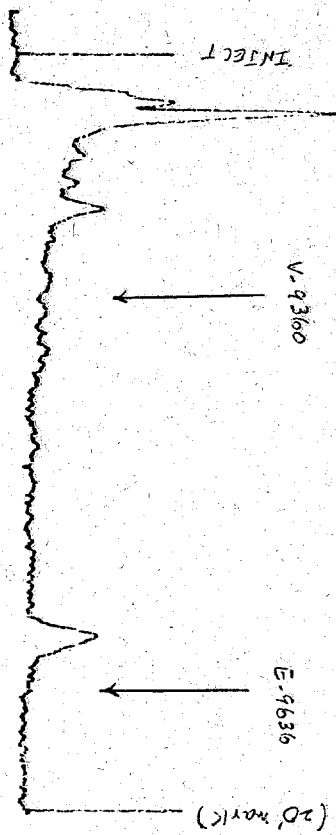
B-3 WATER SAMPLE, FORTIFIED 10.0 PPB

FINAL EXTRACT VOLUME: 4.0 ml
INJECTION VOLUME: 100 µl



B-4 WATER SAMPLE, BLANK

FINAL EXTRACT VOLUME: 1.0 ml
INJECTION VOLUME: 200 µl



Examples of Calculation:

A. Peak Intensity:

The peak intensity is expressed in terms of peak height. The peak height in millimeters (mm) is the distance measured manually from apex of the HPLC analytical peak to the baseline drawn across the base of the peak.

The calculations use peak height based on an injection volume of 100 μ l. For injection volume other than 100 μ l, the peak height is adjusted by direct proportion, as in the following equation:

$$\text{peak height, mm} = \frac{(\text{measured peak height, mm}) (100 \mu\text{l})}{(\text{injection volume, } \mu\text{l})}$$

B. Calculation Formulas:

1. Response Factor (RF) of standards:

$$\text{RF} = (\text{peak height, mm}) / (\text{concentration, } \mu\text{g/ml})$$

2. ppb Found = (H)(V)(1000)/(RF)(W) (method, p.18)

where H = Peak height, mm, adjusted to inj. vol. 100 μ l

V = Volume, final extract, ml

RF = Response Factor

W = Weight of water sample, gm

3. % Recovery = (ppb Found)(100)/(ppb added)

C. Example

1. Standards

Concentration C = ug/ml	Injection Volume ul	Peak Height Measured mm	Peak Height Adj. to 100 ul mm	RF (mm)/(c)	Chromatogram No.
(a) <u>V9360</u>					
0.02	200	8	4	200	A-1
0.05	100	9	9	180	A-2
0.10	100	18	18	180	A-3
0.20	100	45	25	225	A-4
0.50	100	109	109	218	A-5
1.00	70	151	215	215	A-6
				Mean	<u>203.0</u>
(b) <u>E9636</u>					
0.02	200	4	2	100	A-1
0.05	100	5	5	100	A-2
0.10	100	8	8	80	A-3
0.20	100	18	18	90	A-4
0.50	100	43	43	86	A-5
1.00	70	57	81	81	A-6
				Mean	<u>89.5</u>

The mean values of RF are used for calculation.
 Regression Lines: (calculated by HP 41CX STAT-PAK)
 V9360: $Y \text{ (mm)} = 216.9 X \text{ (ug/ml)} - 0.9$ $R = 0.9995$
 E9636: $Y \text{ (mm)} = 80.9 X \text{ (ug/ml)} + 1.0$ $R = 0.9988$

2. Water Samples: (Set #1) Weight of Sample, W = 200 gm (200 ml)
 H (mm)

ppb added	Inj.vol. ul	V ml	H (mm)		RF	ppb Found	% Recovery	Chromatogram No.
			measured	100ul				
(a)								
<u>V9360</u>								
0.1	200	1.0	6	3	203	0.0739	73.9	B-1
1.0	100	1.0	33	33	203	0.8128	81.3	B-2
10.0	100	4.0	85	85	203	8.374	83.7	B-3
Blank	200	1.0	ND	-	-	ND	-	B-4
(b)								
<u>E9636</u>								
0.1	200	1.0	3	15	89.5	0.0837	83.7	B-1
1.0	100	1.0	13	13	89.5	0.7262	72.6	B-2
10.0	100	4.0	38	38	89.5	8.492	84.9	B-3
Blank	200	1.0	ND	-	-	ND	-	B-4