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

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

JUN 9 1987

MEMORANDUM

SUBJECT: PP #6F3431 "Harmony" (DPX-M6316) on Wheat and Barley.
Evaluation of Method Trial Report dated 5/4/87

FROM: Cynthia Deyrup, Ph.D., Chemist *Cynthia Deyrup*
Tolerance Petition Section 2
Residue Chemistry Branch
Hazard Evaluation Division (TS-769)

THRU: Charles L. Trichilo, Ph.D., Chief
Residue Chemistry Branch
Hazard Evaluation Division (TS-769)

TO: Robert Taylor, Product Manager No. 15
Registration Division (TS-767)

and

Toxicology Branch
Hazard Evaluation Division (TS-769)

EPA's Analytical Chemistry Laboratory (ACS, COB, BUD) has completed a method trial on "Harmony" (DPX-M6316) on wheat grain and straw. The methods tested were Du Pont's AMR-646-86, used on wheat grain, and AMR-761-87, which was used on wheat straw.

Wheat grain and straw were fortified at levels of 0.05 and 0.10 ppm with DPX-M6316. The recoveries are tabulated below.

Recovery of DPX-M6313 (Harmony) from Wheat Grain and Straw

Commodity	Chemical Added	Method	PPM Added	% Recovered
Wheat grain	DPX-M6316	AMR-646-86	0.05	92.9, 92.9
			0.10	86.5, 90.3
Wheat straw	DPX-M6316	AMR-761-87	0.05	76.6, 86.6
			0.10	50.0, 83.3

All controls were N.D. (none detected) at a limit of detection of <0.05 ppm.

The photoconductivity detector in Beltsville was not sensitive enough to carry out the analyses. Beltsville has been having problems with this detector for some time and has sent the detector back to Tracor for repair. The analyses were carried out at the Du Pont Agricultural Experiment Station. Du Pont has indicated that the sensitivity of the column increases with aging. According to Ron Thomas (ACS) the column response increases upon seasoning with both DPX-M6316 and crop extract.

Mr. Thomas has pointed out that the photoconductivity detector is not a common piece of laboratory equipment.

RCB Conclusions

Methods AMR-646-86 and AMR-761-87 for the analysis of parent DPX-M6316 are adequate for the collection of residue data.

Additional methodology for metabolites and meat and milk may be needed prior to the establishment of permanent tolerances. Also, a confirmatory method may be needed prior to the establishment of a permanent tolerance.

Recommendations

Methods AMR-646-86 and AMR-761-87 should be made available to interested parties. RCB is therefore sending copies of these methods to FDA so that these methods may be used for monitoring, and so that the enforcement agencies have the opportunity to comment on the suitability of these methods for enforcement purposes prior to the establishment of a permanent tolerance. These methods should not be published in the Pesticide Analytical Manual, Volume II (PAM II) until the permanent tolerances have been established. A copy of these methods is also being sent to PMSD so that they are more readily available under FOI.

RCB continues to recommend against the establishment of tolerances for DPX-M6316 on wheat and barley grain and straw; the data gaps

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cited in the Harmony® Registration Standard need to be resolved.

Attachments: 1-PAM II Cover Sheet
2-Methods (AMR-646-86 and AMR-761-87)
3-Method Trial Report from ACS/COB/BUD
4-Method Trial Request

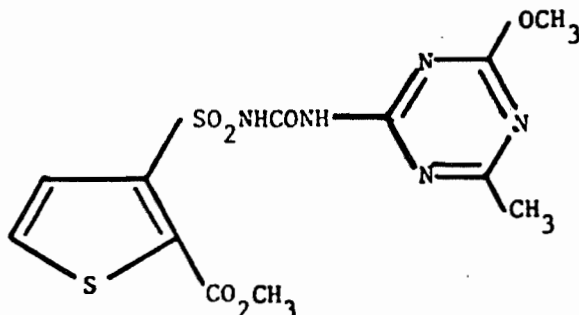
cc (with Attachment 1 only): PM #15, PP #6F3431, D.Marlow,
RF, Circu, Reviewer-C.Deyrup, TOX, Robert E. Thompson (RTP)
cc (with Attachments 1-4): PMSD/ISB-Eldredge, M.Bradley, FDA

RDI:J.H.Onley:6/8/87:R.D.Schmitt:6/8/87
TS-769:RCB:CM#2:RM810:X7484:C.Deyrup:cd:6/8/87

Acceptable Name: Methyl 3-[[[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)amino]carbonyl]amino]-sulfonyl]-2-thiophene-carboxylate

Pesticide Reg. Sec. 180

Structure:



Other names: Harmony
DPX-M6316
INM-6316

Petitioner: Du Pont de Nemours & Co. (Inc.)
Barley Mill Plaza
Walkers Mill Building
Wilmington, DE, 19899

Method No.: AMR-646-86, Analysis of the herbicide DPX-M6316
in wheat or barley grain by liquid chromatography;
AMR-761-87, Analytical Method for Residues of
DPX-M6316 in Wheat and Barley Straw

Pesticide Petition: PP #6F3431

Product Application: Wheat and Barley

Detection Limit: 0.05 ppm

Method Trial Report: PP #6F3431. Harmony on Wheat and Barley.
Method Trial Report 3/20/87

Commodity	Chemical Added	PPM Added	PPM Recovered	% Recovered
Wheat grain	DPX-M6316	0.05	0.046, 0.046	92.9, 92.9
		0.10	0.087, 0.090	86.5, 90.3
Wheat straw	DPX-M6316	0.05	0.038, 0.043	76.6, 86.6
		0.10	0.050, 0.083	50.0, 83.3

Notes:

1. Seasoning the column with crop extract and DPX-M6316 increases the column response.
2. One chemist can run a set of 4-6 samples in 1.5-2 days.

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Attachment 2

Study Title

ANALYSIS OF THE HERBICIDE, DPX-M6316,
IN WHEAT AND BARLEY GRAIN BY LIQUID CHROMATOGRAPHY

Data Requirement

U.S. EPA Pesticide Assessment Guidelines
Subdivision O, 171-4

Authors

E. W. Zahnow
S. L. Kintner

Study Completed On

December 18, 1986

Performing Laboratory

E. I. du Pont de Nemours & Company, Inc.
Agricultural Products Department
Research and Development Division
Experimental Station
Wilmington, Delaware 19898

Laboratory Project ID

AMR-646-86

STATEMENT OF NO DATA CONFIDENTIALITY CLAIMS

No claim of confidentiality is made for any information contained in this study on the basis of its falling within the scope of FIFRA Section 10(d) (1)(A), (B), or (C).

Company E. I. du Pont de Nemours and Company, Inc.

Company Agent Robert W. Freerksen Date January 8, 1987
(Typed Name)

Registration Specialist Robert W. Freerksen
(Title) (Signature)

GOOD LABORATORY PRACTICE STATEMENT

The GLP requirements specified in 40 CFR Part 160 are not applicable to residue data chemistry requirements at the time of submission.

Study Director E. W. Zahnow Date January 8, 1987

Submitter E. I. du Pont de Nemours and Company, Inc.

Sponsor E. I. du Pont de Nemours and Company, Inc.

E. W. Zahnow, Research Associate E. W. Zahnow

S. L. Kintner, Contract Chemist S. L. Kintner per E. W. Zahnow

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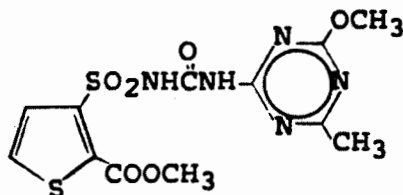
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Introduction

An analytical method based on the use of liquid chromatography and a photoconductivity detector is described for methyl 3-[[[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)aminocarbonyl]amino sulfonyl]-2-thiophenecarboxylate, otherwise known as DPX-M6316. As little as 500 pg can be detected and measured after passage through the HPLC column. Coupled with extraction, cleanup and isolation procedures, the method provides a means of determining DPX-M6316 in wheat or barley grain with a detection limit of 20 ng/g (20 ppb), based on a 25-g sample. Using this method, it is possible to process four samples in an eight-hour day.



DPX-M6316

Equipment and Reagents

The liquid chromatograph used was a Du Pont Model 8800 liquid chromatograph equipped with a column compartment. The column was a Zorbax® SIL, 4.6 mm i.d. x 25 cm. Both the column and liquid chromatograph were purchased from Analytical Instruments Division, E. I. du Pont de Nemours and Co., Inc., Wilmington, Delaware.

The Tracor® Model 965 photoconductivity detector used was purchased from Tracor® Instruments, Austin, Texas.

For homogenization and extraction of samples, a Tekmar Tissumizer®, Model SDT-1810, with a Model SDT-182 EN shaft and generator was employed. The Tissumizer® was purchased from Tekmar Company, Cincinnati, Ohio.

A Vortex-Genie® mixer, purchased from Fisher Scientific, Pittsburgh, Pennsylvania, was used for mixing of samples in centrifuge tubes.

A Millipore® all-glass filter apparatus, No. XX15 04700, with a 0.5- μ m Teflon® filter, No. FHUP 04700, was used for filtering of the mobile phase, Solution A1 and Solution D. The Millipore equipment was obtained from Millipore Corporation, Bedford, Massachusetts.

For concentration of samples, a vacuum rotary evaporator with a water bath (Fisher Scientific, Pittsburgh, Pennsylvania) and pear-shaped flasks, No. K608700, purchased from Kontes, Vineland, New Jersey, were used.

An N-EVAP®, Organomation Associates, Worcester, Massachusetts, (water bath set at room temperature) concentrated the samples to dryness under nitrogen.

The HPLC grade solvents were obtained from Fisher Scientific Company, Pittsburgh, Pennsylvania. All other chemicals were ACS reagent grade and also obtained from Fisher Scientific.

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Analytical Grade DPX-M6316 was obtained from the Agricultural Products Department, E. I. Du Pont, Wilmington, DE.

The centrifuge used was an International Centrifuge (Model EXD) which was obtained from International Equipment Company, Boston, Massachusetts.

The Corning pH Meter (Model 7) used in determining pH was obtained from Fisher Scientific Co., Pittsburgh, Pennsylvania.

Experimental

Preliminary Treatment

The kernels of grain were stored in a freezer until needed.

Isolation and Cleanup Procedure

Section I - Extraction and Isolation

Part A - Wheat

A representative 25-g sample was weighed into a 250-mL centrifuge bottle, 50 mL of ethyl acetate was added, and the mixture was homogenized for 2 minutes with the Tekmar Tissumizer®..

The Tissumizer® probe was rinsed with about 10 mL of ethyl acetate from a Pasteur capillary pipet, and the liquid was collected in the centrifuge bottle containing the homogenized sample. The sample was then centrifuged at 2500 rpm for 10 minutes. The liquid layer was decanted into a 500-mL separatory funnel, and the extraction and centrifugation steps were repeated again with another 50-mL portion of ethyl acetate. The liquid layer was then combined in the separatory funnel with the liquid from the first extraction.

Several samples were run simultaneously. However, between samples the Tissumizer® probe was cleaned successively with hot water, distilled water, and ethyl acetate, and it was then run for one minute in a centrifuge bottle containing 80 mL of ethyl acetate to prevent cross-contamination.

Part B - Barley

A representative 25-g sample was weighed into a 250-mL centrifuge bottle, 100 mL of aqueous 0.1M NaHCO₃ solution was added, and the mixture was homogenized for 2 minutes with the Tekmar Tissumizer®.

The Tissumizer® probe was rinsed with about 10 mL of aqueous 0.1M NaHCO₃ solution from a Pasteur capillary pipet, and the liquid was collected in the centrifuge bottle containing the homogenized sample. The sample was then centrifuged at 2500 rpm for

10 minutes. The liquid layer was decanted into a 600-mL beaker, and the extraction and centrifugation steps were repeated twice more with 100-mL portions of aqueous 0.1M NaHCO solution. These liquid layers were all combined in the 600-mL beaker.

Several samples were run simultaneously. However, between samples the Tissumizer® probe was rinsed first with hot water and then with distilled water. Then it was run for 1 minute in a centrifuge bottle containing 80 mL of aqueous 0.1 M NaHCO solution to prevent cross-contamination.

Section II - Cleanup

Part A - Wheat

The ethyl acetate extract was extracted 3 times with 100-mL portions of aqueous 0.1 M NaHCO₃ solution, shaking vigorously (1 cycle per second) for 1 minute each time. The aqueous extracts (bottom layers) were combined in a 600-mL beaker. After the third extraction the ethyl acetate layer was discarded, and the aqueous solution was then adjusted to pH 3.5 ±0.2 (pH meter) with 1.0 N HCL which was added dropwise, with continuous stirring, to prevent localized excesses of acid which could cause decomposition of the DPX-M6316. Once this pH change has been made, it is important to continue on to the next step within an hour.

The acidified aqueous sample was transferred to a 500-mL separatory funnel, and the beaker was rinsed with 75 mL of methylene chloride. The methylene chloride was added to the separatory funnel, and the sample was gently (1 cycle per two seconds) shaken for one minute. If the shaking is too vigorous, severe emulsions may form. The methylene chloride (lower layer) was drained into a 125-mL separatory funnel and then drained into a 500-mL pear-shaped flask. This intermediate step was necessary to obtain a better separation from the aqueous phase. The aqueous solution was extracted twice more with additional 75-mL portions of methylene chloride which were also added to the 500-mL pear-shaped flask, again using the 125-mL separatory funnel as an intermediate container. The aqueous solution was discarded at this point. The methylene chloride solution may be kept overnight in a refrigerator, if necessary.

Part B - Barley

The aqueous solution was then adjusted to pH 3.5 ± 0.2 (pH meter) with 1.0 N HCl which was added dropwise, with continuous stirring, to prevent localized excesses of acid which could cause decomposition of the DPX-M6316. Once this pH change has been made, it is important to continue on to the next step within an hour.

The acidified aqueous sample was transferred to a 500-mL separatory funnel, and the beaker was rinsed with 75 mL of methylene chloride. The methylene chloride was added to the separatory

funnel, and the sample was gently (1 cycle per two seconds) shaken for 1 minute. The methylene chloride (lower layer) was drained into a 600-mL beaker. The aqueous solution was extracted twice more with additional 75-mL portions of methylene chloride which were combined in the 600-mL beaker. The aqueous solution was discarded at this point. The methylene chloride solution may be kept overnight in a refrigerator, if necessary.

From the 600-mL beaker the methylene chloride was transferred into a 500-mL separatory funnel, and then from this it was drained into a 250-mL centrifuge bottle, taking care to remove any water phase which formed. The methylene chloride was then centrifuged at 2500 rpm for 10 minutes, and if another water layer formed, the methylene chloride was drawn off with a 50-mL glass syringe and put into a 500-mL pear-shaped flask.

Part C - Wheat and Barley

The methylene chloride was evaporated to 4-5 mL on a rotary evaporator at 20-25°C, and this was then transferred to a 15-mL centrifuge tube with a Pasteur capillary pipet. The evaporation should be performed carefully to avoid bumping of the solution, and frequent observation of this operation is highly recommended. The flask was washed twice with 2-mL portions of methylene chloride, and these washings were combined in the 15-mL centrifuge tube with the sample. The sample was evaporated to dryness under a gentle stream of nitrogen on a N-EVAP® at room temperature.

If the samples will not be analyzed on the same day as they are processed, the dry extracts should be stored in a freezer.

The rotary evaporator should be cleaned daily by evaporating 150 mL of methylene chloride to dryness. This aids in preventing cross-contamination.

Section III - Analysis

For analysis, the sample was dissolved in Solution A1 (see Appendix A), with dilution to a final volume of 5 mL. The sample was mixed for one minute using a Vortex® mixer. The entire sample was then filtered into a small bottle using a Millipore® Millex®-SR 0.5- μ m filter unit mounted on a 5-mL hypodermic syringe. After fitting the filter unit to the syringe (plunger removed), the sample was put into the syringe, the plunger was inserted, and the liquid was squeezed through the filter.

The filter units were discarded after each use. The syringe was cleaned with a solution of 50% methanol-50% isopropanol and then dried between samples.

Liquid Chromatography

The liquid chromatograph used in this method was a Du Pont Model 8800 with a Tracor Model 965 photoconductivity detector.

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The photoconductivity detector must be used for this analysis to obtain adequate sensitivity and selectivity. The mercury lamp was used in the detector since it provided much greater sensitivity than the zinc lamp. The flow of the mobile phase through the reference and analytical loops was balanced to within $\pm 2\%$. This was accomplished by means of the metering valve (Nupro Model SS-2SA-TFE) in the solvent line which exits from the reference compartment of the conductivity cell. Also, the ion-exchange resin tube was not needed to purify the mobile phase and might actually have introduced unwanted materials into the system if it had not been removed.

The mobile phase composition is given in Appendix A. This mobile phase was sparged with helium for 15-30 minutes before starting the pump as well as during operation.

The column was a Du Pont Zorbax SIL (25 cm x 4.6 mm) controlled at 35°C by an oven or column heater. A new column had to be conditioned by pumping Solution D (see Appendix A) through it for several hours at 1 mL/min. The conditioning solution should also be sparged with helium as noted above for the mobile phase. After a number of samples have been injected, the column may begin to lose its efficiency, and the conditioning solvent can be used in such a situation to restore efficiency. Loss of efficiency can be recognized by broad peaks which tail, loss of peak height, and by shifting retention times. This conditioning solvent must be

thoroughly flushed from the column with the mobile phase. An hour of flushing at 0.5 mL/min was usually sufficient.

The sample valve was a Rheodyne Model 7125 for manual injection of standards and samples. The loop volume used was 10 μ L to minimize contamination of the HPLC column and broadening of the chromatographic peaks.

During normal operation, mobile phase was pumped through the column at 0.5 mL/min which was judged to be the minimum practical rate. At this flow rate, DPX-M6316 eluted from the column in 16-18 minutes, depending on the extent of column deactivation. This rate was selected because the detector response increases with decreasing flow rate due to the longer residence time of the sample in the quartz reactor coil.

Standardization

A standard stock solution of DPX-M6316 was prepared by weighing out 10.0 mg into a 100-mL volumetric flask and dissolving the solid in methylene chloride. This solution was quite stable and could be stored for many months in a refrigerator. It should be noted that long-term storage and repeated use of this solution may result in the evaporation of some methylene chloride, thereby increasing the concentration of DPX-M6316.

A 1.0 μ g/mL standard was prepared by pipetting 1.0 mL of the stock solution into a clean, dry, 100-mL volumetric flask,

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evaporating the methylene chloride with a gentle nitrogen stream, dissolving the residue in Solution A1, (see Appendix A) and diluting to volume with Solution A1. Standards with concentrations of 0.50, 0.20, 0.10 and 0.05 µg/mL were prepared by appropriate dilution of the 1.0 µg/mL standard with Solution A1. The set of standards prepared in Solution A1 was replaced with a fresh set every week. Over this time period no change in detector response was observed. All standards should be stored in a refrigerator when not in use.

The detector output was linear over this particular weight range of DPX-M6316. Response factors for a series of standards are shown in Table 1. The average is 60.7 mm/ng, and the standard deviation is 2.6 mm/ng. A detector response curve is shown in Figure 1. A chromatogram of the 0.05 µg/mL standard is shown in Figure 2. Normally, the short term noise is <1mm (peak to peak).

Calculations

The amount of DPX-M6316 in a given sample can be calculated from the following equation:

$$\text{ng/g (ppb)} = H \times \frac{1}{\text{RF}} \times E \times \frac{1}{S} \times \frac{1}{W}$$

- (a) H is the peak height in millimeters.
- (b) RF is the response factor in millimeters per nanogram.
- (c) E is the total volume of the sample extract in milliliters.
- (d) S is the volume of sample extract injected in milliliters.
- (e) W is the sample weight in grams.

The HPLC standards, prepared as described earlier, cover a range of 10-200 ppb for the method as written. In the event that the sample contains more than the amount of DPX-M6316 equivalent to the standard of highest concentration, dilute the final extract to a volume greater than 5 mL or make a serial dilution of an aliquot. This reduces the amount of extraneous material put onto the HPLC column and will result in a longer column life.

Results and Discussion

Recoveries for wheat and barley grain, fortified at 20 ppb, are summarized in Table 2.

Figure 3 is a chromatogram of an extract of a wheat grain control, and Figure 4 is the corresponding chromatogram of the extract of the fortified control. The measured recovery was 82%.

Similarly, Figure 5 is a chromatogram of an extract of a barley grain control, and Figure 6 is a chromatogram of the extract of the fortified control. In this case the recovery was 100%.

With the exception of the photoconductivity detector, it is possible to make reasonable substitutions for the equipment and apparatus described in this report. For example, a μ Porasil column™ (Waters Part No. 27477) has been found to be a suitable replacement for the Zorbax® SIL. It is important to keep in mind that trade-offs may result from any such substitutions. Again, in the

case of the μ Porasil™ column, longer column life is accompanied by lower sensitivity which, in turn, can be compensated by injection of a larger sample.

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TABLE 1Response Factors

<u>DPX-M6316 (ng)</u>	<u>Response Factor (mm/ng)</u>
0.5	60.0
1.0	64.0
2.0	63.0
5.0	57.0
10.0	62.5
50.0	58.8

Average - 60.7 mm/ng

Standard Deviation - 2.6 mm/ng

TABLE 2Recovery of DPX-M6316 from Grain

<u>Type</u>	<u>Fortification</u>	<u>Number of Recoveries</u>	<u>Recovery (%)</u>		
			<u>Mean</u>	<u>SD</u>	<u>Range</u>
Wheat	20	16	85	8.4	71-98
Barley	20	6	93	7.8	87-105

APPENDIX AMobile Phase

Mix 750 mL of cyclohexane, 125 mL of methanol and 125 mL of isopropanol.

Filter through a 0.5- μ m Millipore® filter (FHUP 04700) held in a Millipore® filter apparatus (XX15 04700).

Add 1 mL of a solution of 9 parts glacial acetic acid and 1 part water.

Keep this solution in a sealed container.

Solution A1

Mix 750 mL of cyclohexane and 250 mL of isopropanol.

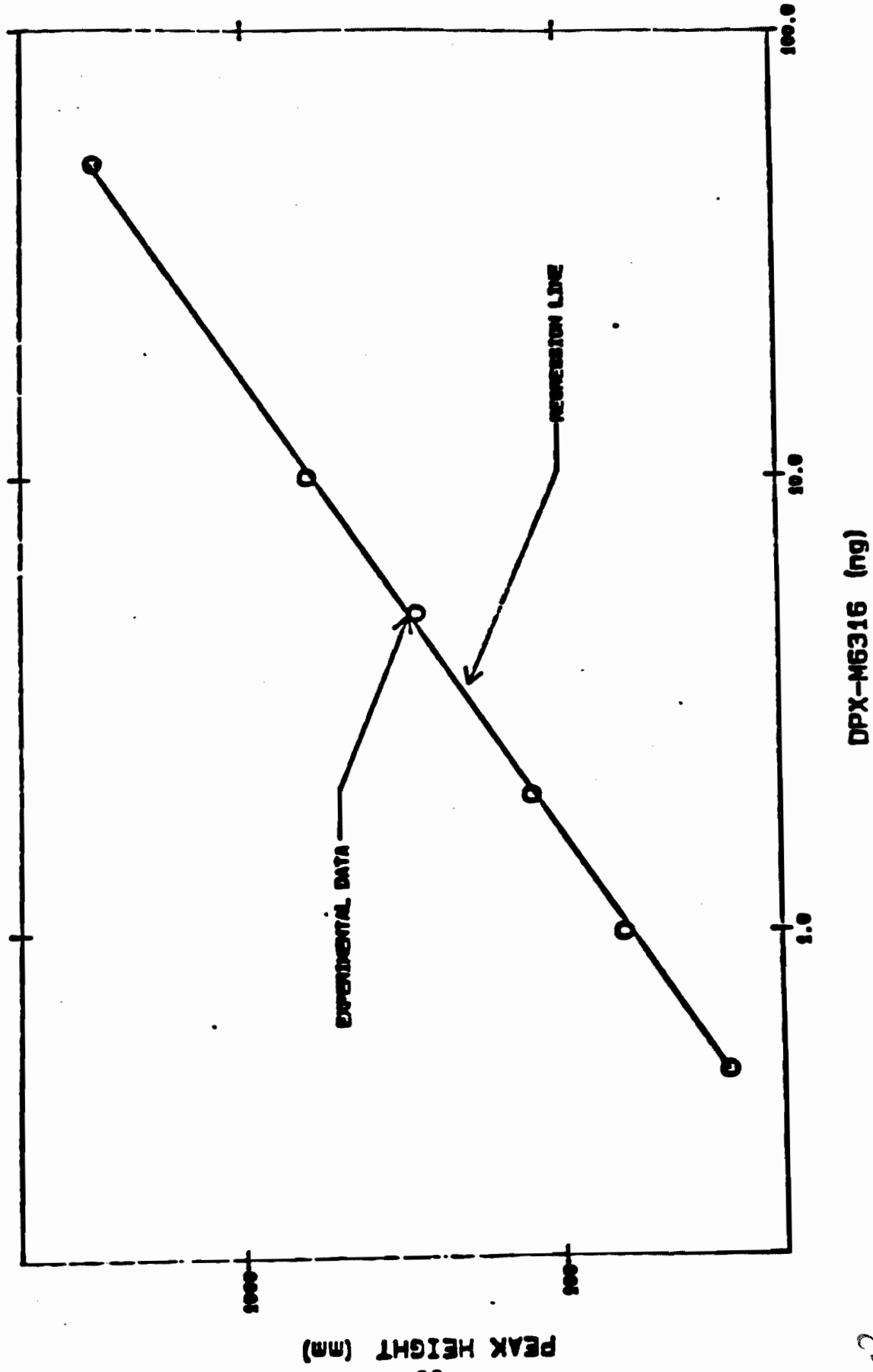
Filter through a 0.5- μ m Millipore® filter (FHUP 04700) held in a Millipore® filter apparatus (XX15 4700).

Solution D

Mix 400 mL isopropanol, 400 mL methanol, 200 mL glacial acetic acid and 40 mL H₂O.

Filter through a 0.5- μ m Millipore® filter (FHUP 04700) held in a Millipore® filter apparatus (XX15 04700).

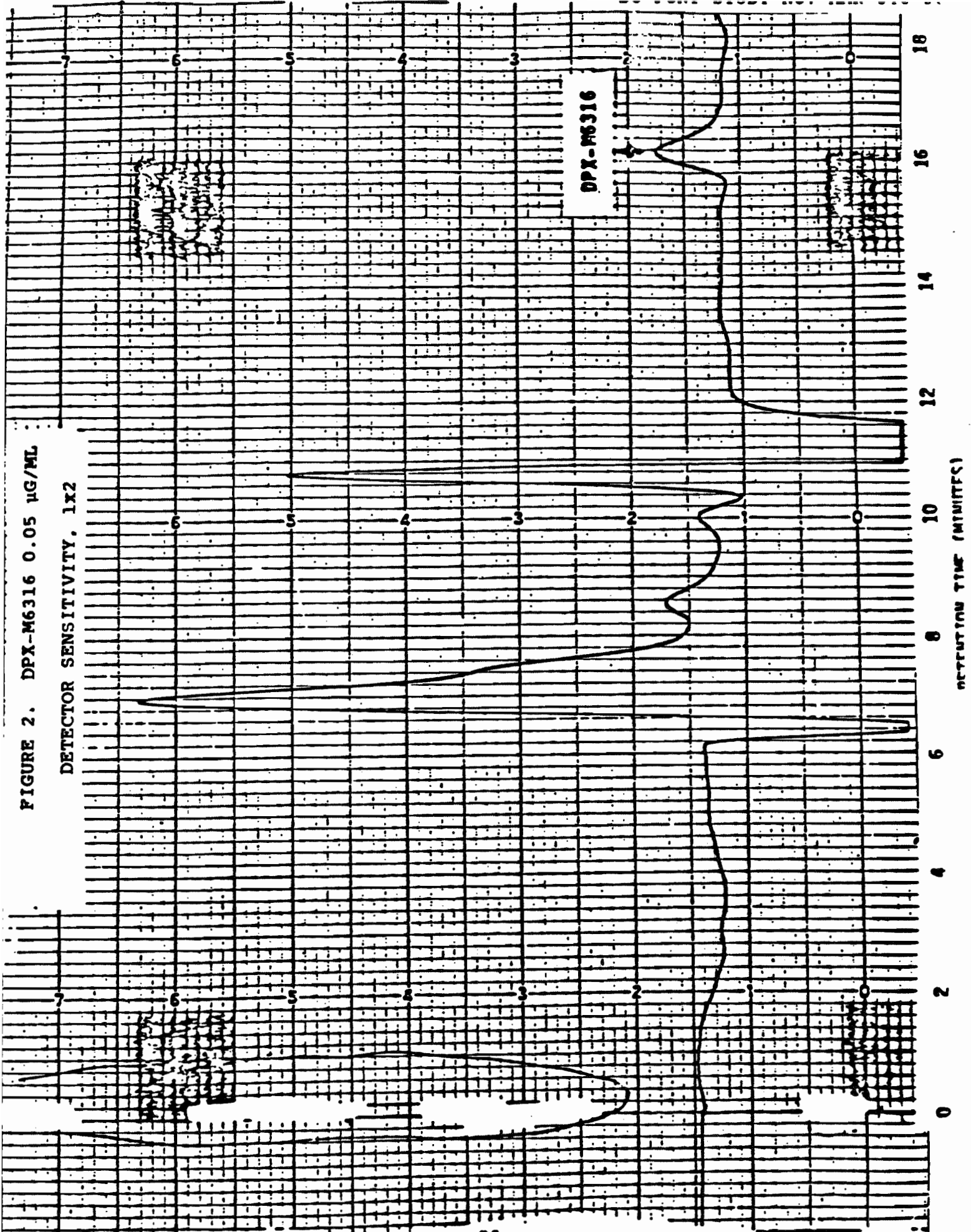
FIGURE 1
DETECTOR RESPONSE



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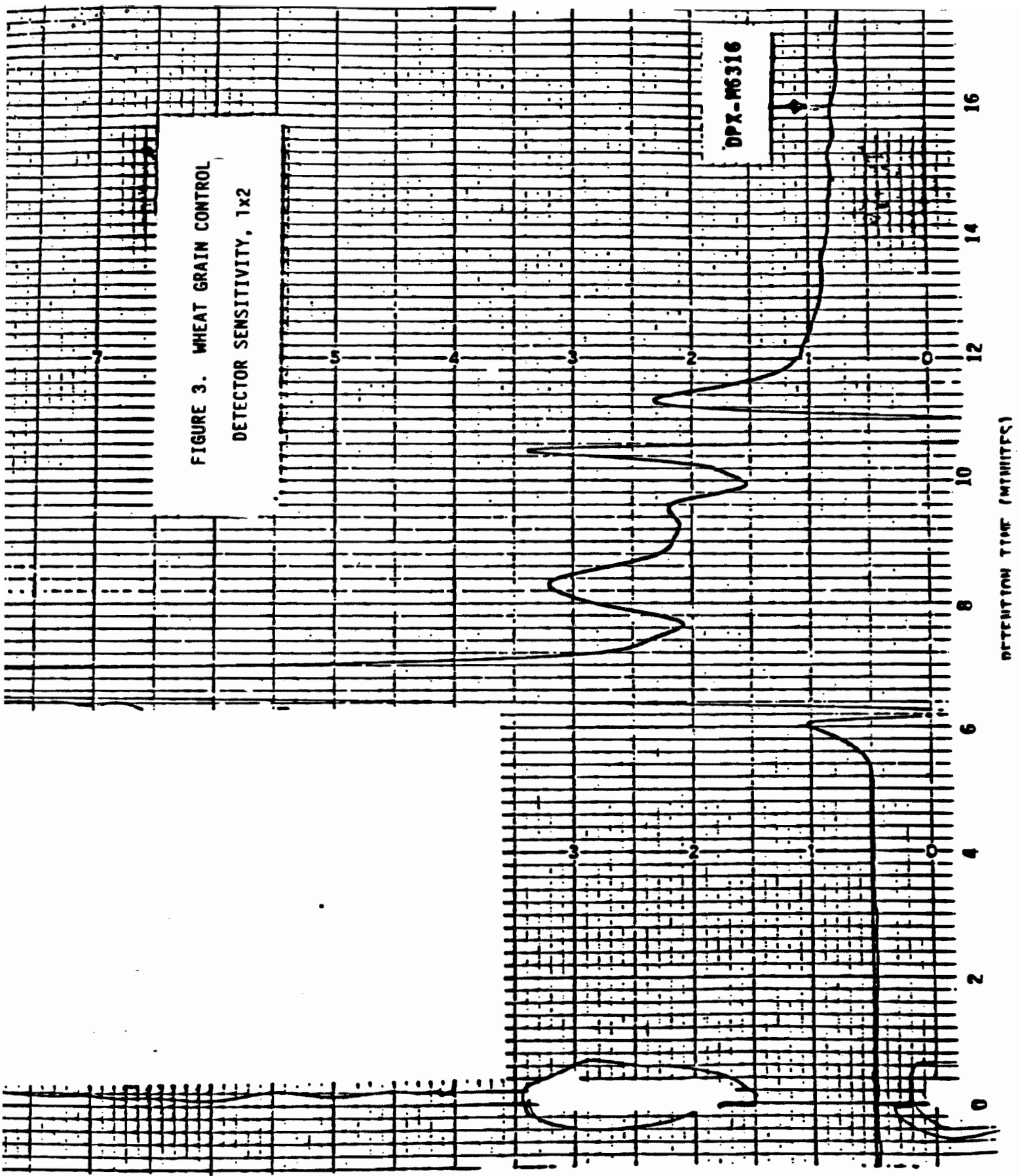
FIGURE 2. DPX-M6316 0.05 µG/ML

DETECTOR SENSITIVITY, 1x2



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FIGURE 3. WHEAT GRAIN CONTROL
DETECTOR SENSITIVITY, 1x2



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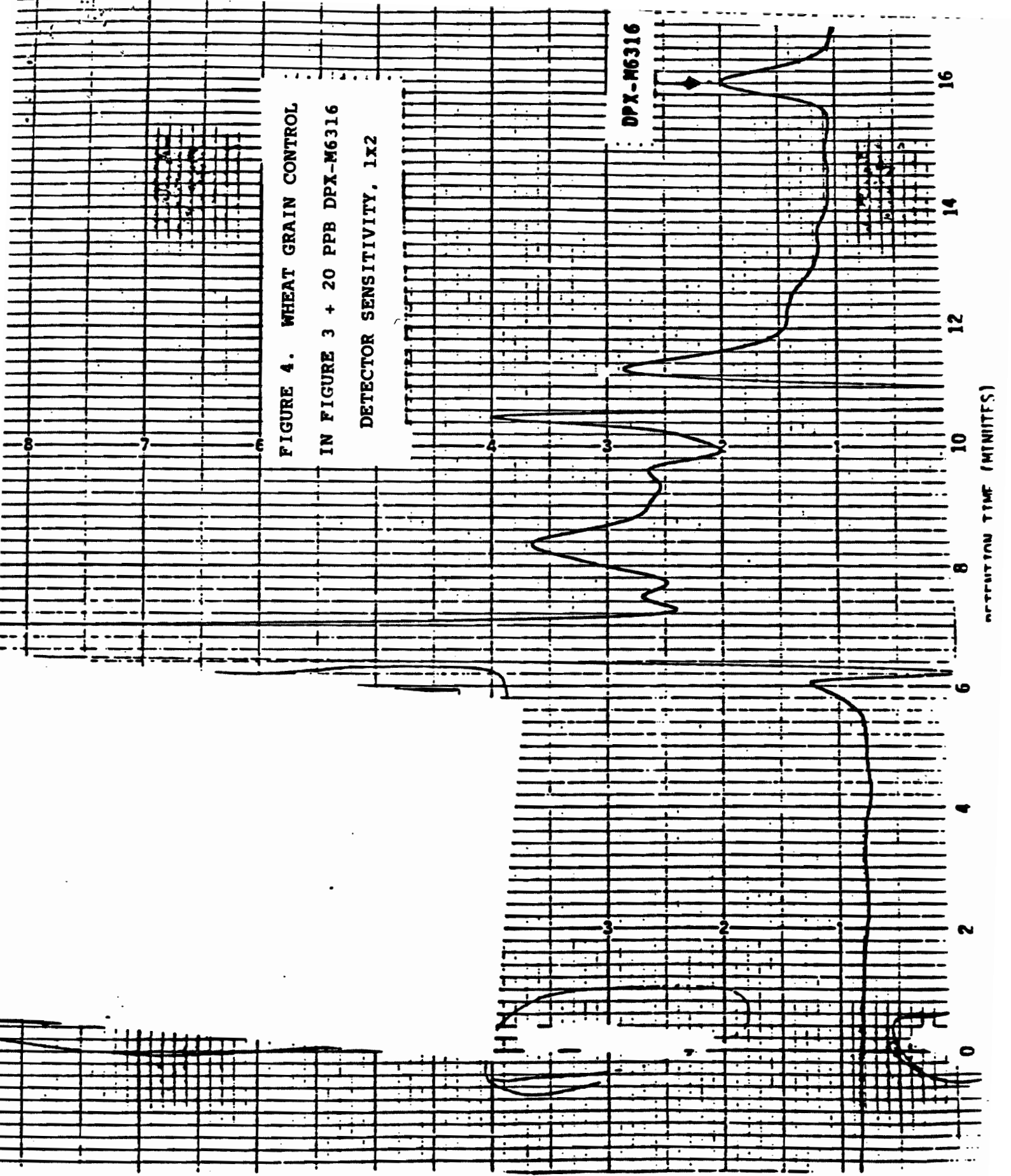
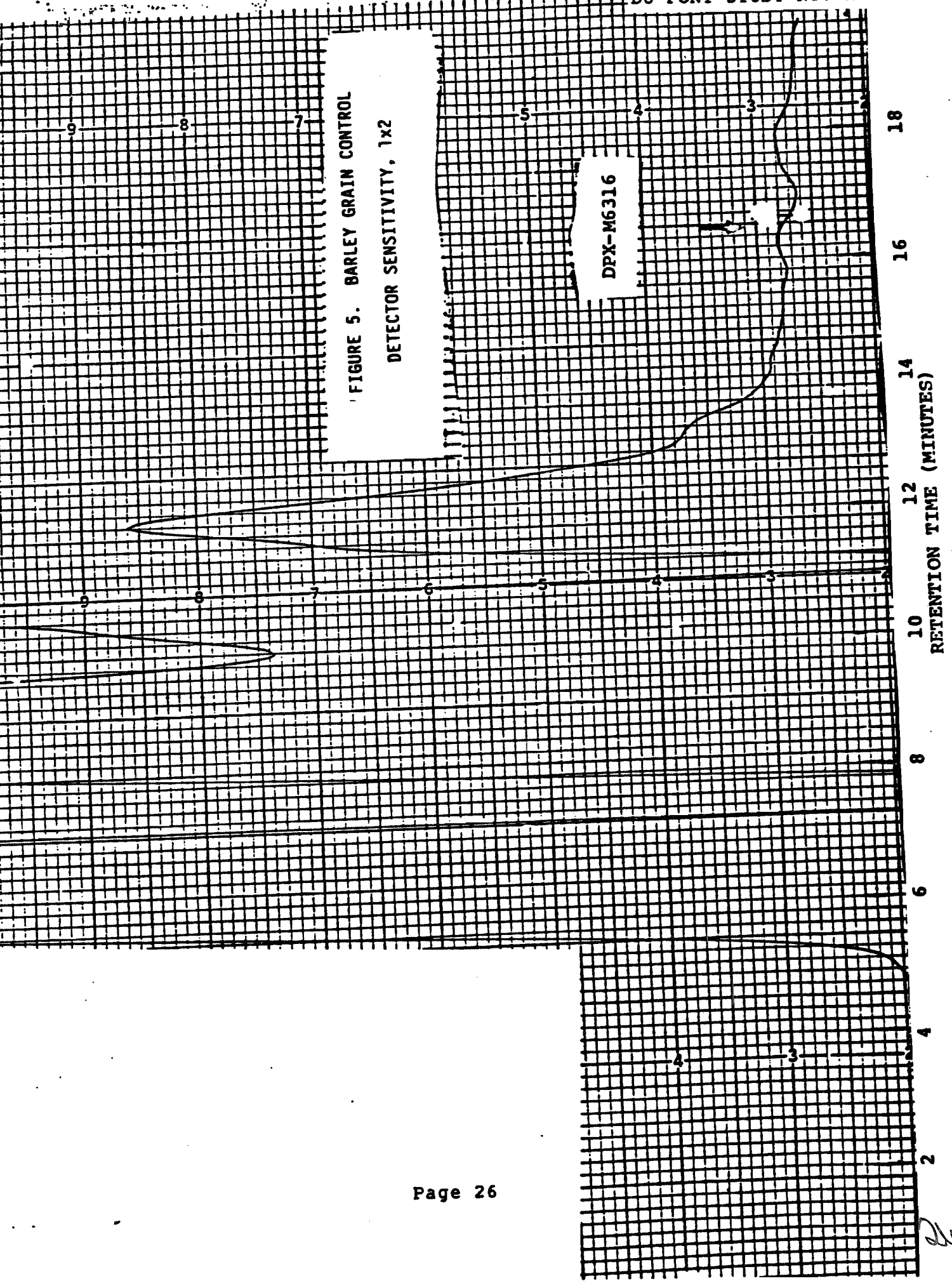


FIGURE 4. WHEAT GRAIN CONTROL
 IN FIGURE 3 + 20 PPB DPX-M6316
 DETECTOR SENSITIVITY, 1x2

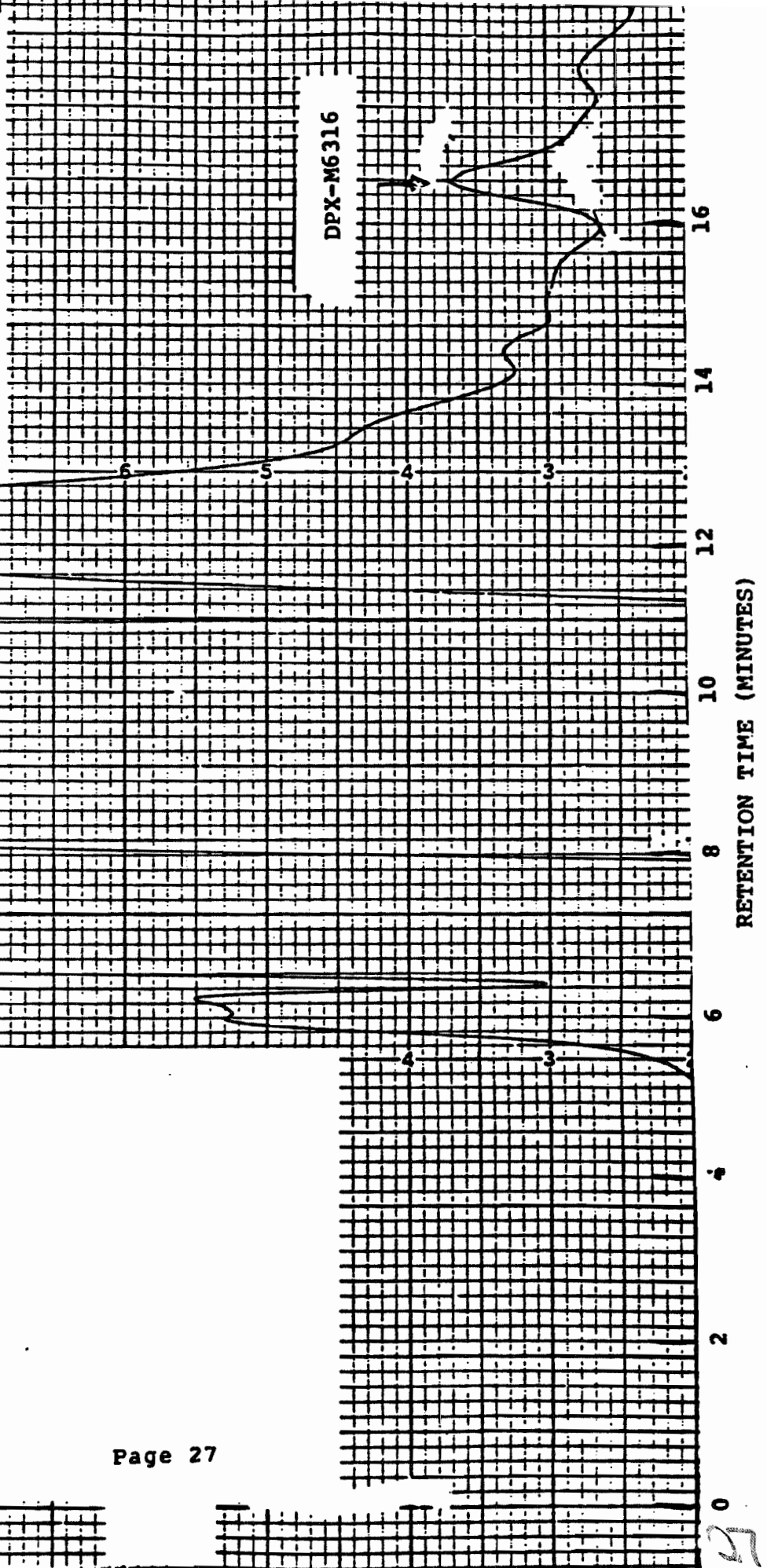
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FIGURE 5. BARLEY GRAIN CONTROL
DETECTOR SENSITIVITY, 1x2



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FIGURE 6. BARLEY GRAIN CONTROL
IN FIGURE 5 + 20 PPB DPX-M6316
DETECTOR SENSITIVITY 1x2



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STORAGE LOCATION OF RAW DATA, REPORTS

E. I. du Pont de Nemours and Company, Inc.
Agricultural Products Department
Experimental Station
Residue Studies Groups' Archives
Wilmington, Delaware 19898

Study Title

ANALYSIS OF THE HERBICIDE, DPX-M6316 IN
WHEAT AND BARLEY STRAW BY LIQUID CHROMATOGRAPHY

Data Requirement

U.S. EPA Pesticide Assessment Guidelines
Subdivision O, 171-4

Authors

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F. M. Rimkus, B.S.

Study Completed On

January 23, 1986

Performing Laboratory

E. I. du Pont de Nemours & Company, Inc.
Agricultural Products Department
Research and Development Division
Experimental Station
Wilmington, Delaware 19898

Laboratory Project ID

AMR-761-87

STATEMENT OF NO DATA CONFIDENTIALITY CLAIMS

No claim of confidentiality is made for any information contained in this study on the basis of its falling within the scope of FIFRA Section 10(d) (1)(A), (B), or (C).

Company E. I. du Pont de Nemours and Company, Inc.

Company Agent Robert W. Freerksen Date 2/3/87
(Typed Name)

Registration Specialist *Robert W. Freerksen*
(Title) (Signature)

GOOD LABORATORY PRACTICE STATEMENT

The GLP requirements specified in 40 CFR Part 160 are not applicable to residue data chemistry requirements.

This study was conducted in the spirit of good laboratory practices and meets all requirements to the best of our knowledge.

Study Director E. W. Zahnow Date February 3, 1987
E. W. Zahnow

Submitter E. I. du Pont de Nemours and Company, Inc.

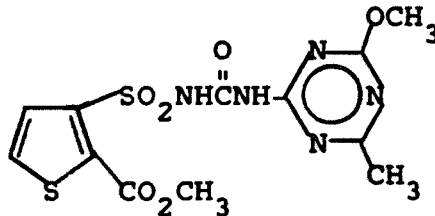
Sponsor E. I. du Pont de Nemours and Company, Inc.

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INTRODUCTION

An analytical method based on the use of liquid chromatography and a photoconductivity detector is described for methyl 3-[[[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)aminocarbonyl] aminosulfonyl]-2-thiophenecarboxylate, otherwise known as DPX-M6316. As little as 1 ng can be detected and measured after passage through the HPLC column. Coupled with extraction, cleanup and isolation procedures, the method provides a means of determining DPX-M6316 in wheat and barley straw with a detection limit of 50 ng/g (50 ppb), based on a 10-g sample. Using this method, it is possible to process four samples in an eight-hour day.

DPX-M6316EQUIPMENT AND REAGENTS

The liquid chromatograph used was a Du Pont Model 8800 or a Du Pont Model 850 equipped with a column compartment, purchased from E. I. du Pont de Nemours and Co., Inc., Wilmington, Delaware.



The Tracor[®] Model 965 photoconductivity detector used was purchased from Tracor[®] Instruments, Austin, Texas.

The column was a Waters[™] μ Porasil[™] (3.9 mm x 30 cm), purchased from Waters Chromatography Division of Millipore Corp., Milford, Massachusetts.

For homogenization and extraction of samples, a Tekmar[®] Tissumizer[®], Model SDT-1810, with a Model SDT-182 EN shaft and generator was employed. The Tissumizer[®] was purchased from Tekmar Company, Cincinnati, Ohio.

A Vortex-Genie[®] mixer, purchased from Fisher Scientific, Pittsburgh, Pennsylvania, was used for mixing of samples in centrifuge tubes.

A Millipore[®] all glass filter apparatus, No. XX15 04700, with a 0.5- μ m Teflon[®] filter, No. FHUP 047 00, was used for filtering of the mobile phase. The Millipore equipment was obtained from Millipore Corporation, Bedford, Massachusetts.

For concentration of samples, a vacuum rotary evaporator with a water bath and pear-shaped flasks, No. K-608700, purchased from Kontes, Vineland, New Jersey were used. An N-EVAP[®], Organomation Assoc., Worcester, Massachusetts (water bath set at room temperature) concentrated the samples to dryness under

nitrogen. The vacuum rotary evaporator was purchased from Fisher Scientific, Pittsburgh, Pennsylvania.

The HPLC grade solvents were obtained from Fisher Scientific Company, Pittsburgh, Pennsylvania. All other chemicals were ACS reagent grade, also obtained from Fisher Scientific.

Analytical Grade DPX-M6316 was obtained from the Agricultural Products Department, E. I. Du Pont de Nemours and Co., Inc., Wilmington, Delaware.

The centrifuge used was an International Centrifuge (Model EXD, serial no. 48091H) which was obtained from International Equipment Company, Boston, Massachusetts, or a Sorvall RC-3B refrigerated centrifuge, SN:8601372 which was obtained from E. I. du Pont de Nemours and Co., Inc., Wilmington, Delaware.

The Hobart cutter (Model 84142) used for cutting samples was obtained from the Hobart Manufacturing Company, Troy, Ohio.

The Corning pH Meter (Model 7) used in determining pH was obtained from Fisher Scientific Co., Pittsburgh, Pennsylvania.

EXPERIMENTALPreliminary Treatment

The samples were cut up to a powder using a Hobart cutter and dry ice. These were then stored in a freezer until needed. The dry ice was evaporated in the freezer. Between samples, the cutter was cleaned with hot water and distilled water and the parts were rinsed with a mixture of 50% methanol and 50% isopropanol to prevent contamination.

Isolation and Cleanup ProcedureSection I - Extraction and Isolation

A representative 10-g sample of wheat or barley straw was weighed into a 250-mL centrifuge bottle, and 25 mL of pH 3.0 water was added to soak the sample for one hour. The water was adjusted to pH 3.0 with 1.0 N HCl using a calibrated pH meter.

After soaking for one hour, the sample (solids and water) was extracted for two minutes with 75 mL of ethyl acetate using the Tekmar Tissumizer®.

The Tissumizer[®] probe was rinsed with about 10 mL of ethyl acetate from a Pasteur capillary pipette, and the liquid was collected in the centrifuge bottle containing the homogenized sample. The sample was then centrifuged at 2700 rpm for 10 minutes. The liquid layer was decanted through a glass wool plug held in a glass funnel and collected in a 500-mL separatory funnel, and the extraction and centrifugation steps were repeated three more times with 75-mL portions of ethyl acetate. The liquid layers were then combined in the separatory funnel with the liquid from the first extraction.

Several samples were run simultaneously. However, between samples the Tissumizer[®] probe was cleaned successively with hot water, distilled water, and ethyl acetate, and it was then run for one minute in a centrifuge bottle containing 80 mL of ethyl acetate to prevent cross-contamination.

Section II - Cleanup

The liquid contained in the 500-mL separatory funnel was extracted three times with 100-mL portions of aqueous 0.1 M NaHCO₃ solution, shaking vigorously (1 cycle per second) for one minute each time. The aqueous extracts (bottom layers) were combined in a 600-mL beaker. After the third extraction, the ethyl acetate layer was discarded, and the aqueous solution was then transferred to a 500-mL separatory funnel.

9

The aqueous solution was extracted three times with 100-mL portions of hexane, shaking vigorously (1 cycle per second) for one minute. The hexane layers (upper) were separated and discarded.

The aqueous solution was then adjusted to pH 3.5 ± 0.2 (pH meter) with 1.0 N HCl which was added dropwise, with continuous stirring, to prevent localized excesses of acid which could cause decomposition of the DPX-M6316. Once this pH change has been made, it is important to continue on to the next step within an hour.

The acidified aqueous sample was transferred to a 500-mL separatory funnel, and the beaker was rinsed with 75 mL of methylene chloride. The methylene chloride was added to the separatory funnel, and the sample gently (1 cycle per two seconds) shaken for one minute. If the shaking is too vigorous, severe emulsions may form. The methylene chloride (lower layer) was drained into a 125-mL separatory funnel and then drained into a 500-mL pear-shaped flask. This intermediate step was necessary to obtain a better separation from the aqueous phase. The aqueous solution was extracted twice more with additional 75-mL portions of methylene chloride which were also added to the 500-mL pear-shaped flask, again using the 125-mL separatory funnel as an intermediate container. The methylene chloride solution may be held overnight in a refrigerator, if necessary.

The methylene chloride was evaporated to 4-5 mL on a rotary evaporator at 20-25°C, and this was then transferred to a 15-mL centrifuge tube with a Pasteur capillary pipette. The evaporation should be performed carefully to avoid bumping of the solution and frequent observation of this operation is highly recommended. The flask was washed twice with 2-mL portions of methylene chloride, and these washings were combined in the 15-mL centrifuge tube with the sample. The sample was evaporated to dryness under a gentle stream of nitrogen on a N-EVAP[®] at room temperature.

If the samples will not be analyzed on the same day as they are processed, the dry extracts should be stored in a freezer.

The rotary evaporator should be cleaned daily by evaporating 150 mL of methylene chloride to dryness. This aids in preventing cross-contamination.

Section III - Analysis

For analysis, the sample was dissolved in Solution A1 (see Appendix A), with dilution to a final volume of 5 mL. The sample was mixed for one minute using a Vortex[®] mixer. The entire sample was then filtered into a small bottle using a Millipore[®] Millex[®]-SR 0.5- μ m filter unit mounted on a 5-mL hypodermic syringe. After fitting the filter unit to the syringe

(barrel removed), the sample was put into the syringe, the barrel was inserted, and the liquid was squeezed through the filter.

The filter units were discarded after each use. The syringe was cleaned with a solution of 50% methanol-50% propanol and then dried between samples.

Liquid Chromatography

The liquid chromatograph used in this method was a Du Pont Model 8800 or Du Pont Model 850 with a Tracor Model 965 photoconductivity detector.

The photoconductivity detector (Tracor Model 965) must be used for this analysis to obtain adequate sensitivity and selectivity. The mercury lamp was used in the detector since it provided much greater sensitivity than the zinc lamp. The flow of the mobile phase through the reference and analytical loops was balanced to within $\pm 2\%$. This was accomplished by means of the metering valve (Nupro Model SS-2SA-TFE) in the solvent line which exits from the reference compartment of the conductivity cell. Also, the ion exchange resin tube was not needed to purify the mobile phase and might actually have introduced unwanted materials into the system if it had not been removed.

The mobile phase composition is given in Appendix A. This mobile phase was sparged with helium for 15-30 minutes before starting the pump as well as during operation.

The column was a Waters™ μ Porasil® (3.9 mm x 30 cm) controlled at 35°C by an oven or column heater. A new column had to be conditioned by pumping Solution D (see Appendix A) through it for several hours at 1 mL/min. The conditioning solution should also be sparged with helium as noted above for the mobile phase. After a number of samples have been injected, the column may begin to lose its efficiency, and the conditioning solvent can be used in such a situation to restore efficiency. Loss of efficiency can be recognized by broad peaks which tail, loss of peak height, and by shifting retention times. This conditioning solvent must be thoroughly flushed from the column with the mobile phase. An hour of flushing at 0.5 mL/min was usually sufficient.

The sample valve was a Rheodyne Model 7125 or Valco CV-6-UHPa-N60 for manual injection of standards and samples. The loop volume used was either 20 μ L or 25 μ L.

During normal operation, mobile phase was pumped through the column at 0.5 mL/min which was judged to be the minimum practical rate. At this flow rate, DPX-M6316 eluted from the column in 12-14 minutes, depending on the extent of column deactivation. This rate was selected because the detector response increases with decreasing flow rate due to the longer residence time of the sample in the quartz reactor coil.

Standardization

A standard stock solution of DPX-M6316 was prepared by weighing out 10.0 mg into a 100-mL volumetric flask and dissolving it in methylene chloride. This solution was quite stable and could be stored for many months in a refrigerator. It should be noted that long-term storage and repeated use of this solution may result in the evaporation of some methylene chloride, thereby increasing the concentration of DPX-M6316.

A 1.0 $\mu\text{g/mL}$ standard was prepared by pipetting 1.0 mL of the stock solution into a clean, dry 100-mL volumetric flask, evaporating the methylene chloride with a gentle nitrogen stream, dissolving the residue in Solution A1, (see Appendix A) and diluting to volume with Solution A1. Standards with concentrations of 0.50, 0.20, 0.10 and 0.05 $\mu\text{g/mL}$ were prepared by appropriate dilution of the 1.0 $\mu\text{g/mL}$ standard with Solution A1. The set of standards prepared in Solution A1 was replaced with a fresh set every week. Over this time period no change in detector response was observed. All standards should be stored in a refrigerator when not in use.

The detector output was linear over this particular weight range of DPX-M6316. Response factors for a series of standards are shown in Table 1. The average is 27.4 mm/ng, and the standard deviation is 0.65 mm/ng. These results were obtained with a new column. However, with increasing use the response factor will increase, and after several months it will be about

40 mm/ng. A chromatogram of the 0.05 µg/mL standard is shown in Figure 1. Normally, the short term noise is <1mm (peak to peak).

CALCULATIONS

The amount of DPX-M6316 in a given sample can be calculated from the following equation:

$$\text{ng/g (ppb)} = H \times \frac{1}{\text{RF}} \times E \times \frac{1}{S} \times \frac{1}{W}$$

- (a) H is the peak height in millimeters
- (b) RF is the response factor in millimeters per nanogram
- (c) E is the total volume of the sample extract in milliliters
- (d) S is the volume of sample extract injected in milliliters
- (e) W is the sample weight in grams

In the event that the sample contains more than the amount of DPX-M6316 equivalent to the standard of highest concentration, dilute the final extract to a volume greater than 5 mL or make a serial dilution of an aliquot. This reduces the amount of extraneous material put onto the HPLC column and will result in a longer column life.

RESULTS AND DISCUSSION

Recoveries for wheat and barley straw, fortified at 50 ppb, are summarized in Table 2.

Figure 2 is a chromatogram of an extract of wheat straw control, and Figure 3 is the corresponding chromatogram of the extract of the fortified control. The measured recovery was 91%.

Similarly, Figure 4 is a chromatogram of an extract of a barley straw control, and Figure 5 is a chromatogram of the extract of the fortified control. In this case, the measured recovery was 79%.

APPENDIX A

Mobile Phase

Mix 750 mL of cyclohexane, 125 mL of methanol and 125 mL of isopropanol.

Filter through a 0.5- μ m Millipore[®] filter (FHUP 04700) held in a Millipore[®] filter apparatus (XX15 04700).

Add 1 mL of a solution of 9 parts glacial acetic acid and 1 part water.

Keep this solution in a sealed container.

Solution A1

Mix 750 mL of cyclohexane and 250 mL of isopropanol.

Filter through a 0.5- μ m Millipore[®] filter (FHUP 04700) held in a Millipore[®] filter apparatus (XX15 4700).

Solution D

Mix 400 mL isopropanol, 400 mL methanol, 200 mL glacial acetic acid, and 40 mL H₂O.

Filter through a 0.5- μ m Millipore[®] filter (FHUP 04700) held in a Millipore[®] filter apparatus (XX15 4700).

TABLE 1

RESPONSE FACTORS

<u>DPX-M6316 (ng)</u>	<u>Response Factor (mm/ng)</u>
1.0	28.0
2.0	28.0
4.0	27.0
10	27.5
20	26.5

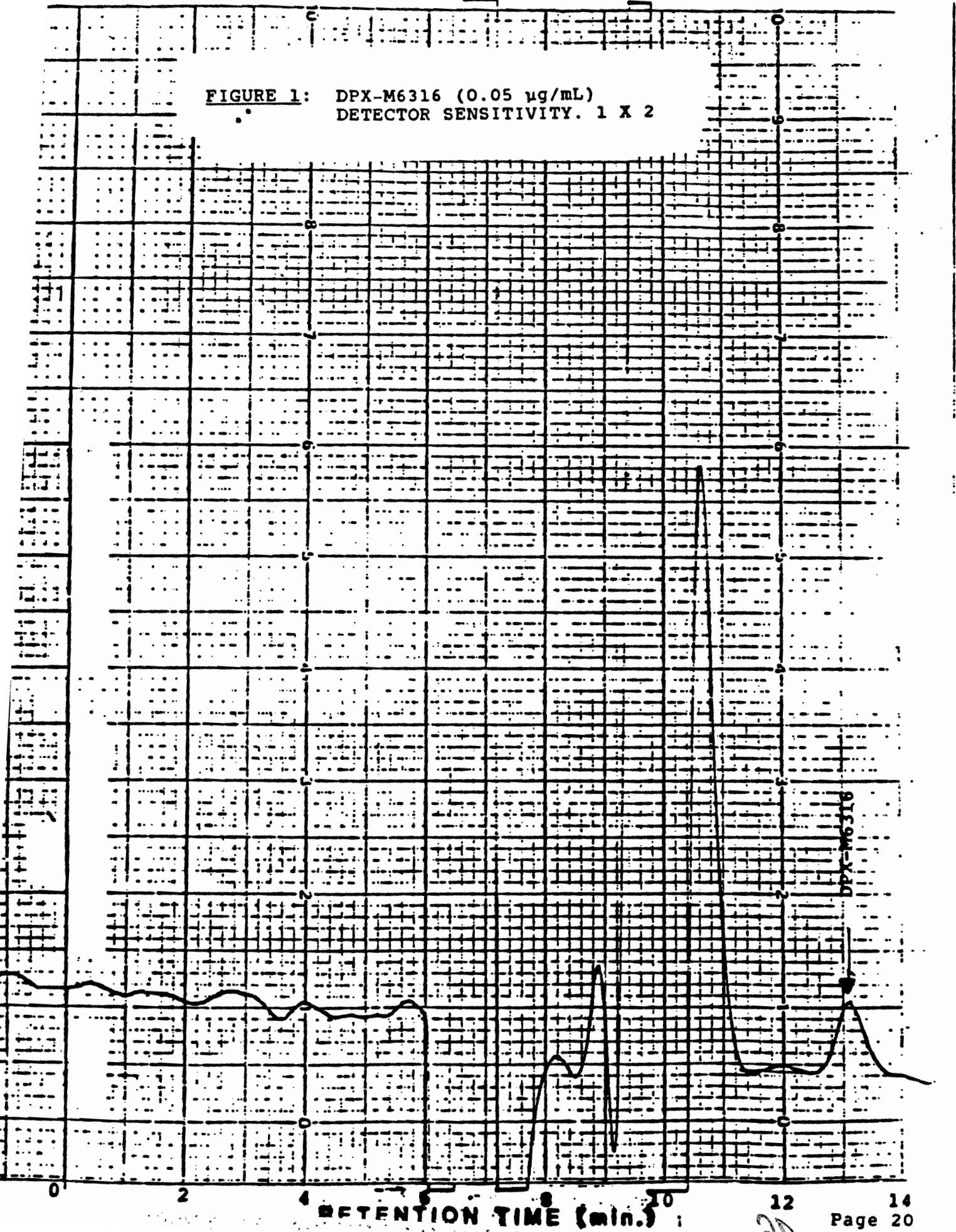
Average - 27.4 mm/ng
Standard Deviation - 0.65 mm/ng

18

TABLE 2RECOVERY OF DPX-M6316 FROM STRAW

<u>Type</u>	<u>Fortification (ppb)</u>	<u>Number of Recoveries</u>	<u>Recovery (%)</u>		
			<u>Mean</u>	<u>SD</u>	<u>Range</u>
Wheat	50	4	77	19	55-94
Barley	50	3	79	1.5	78-81

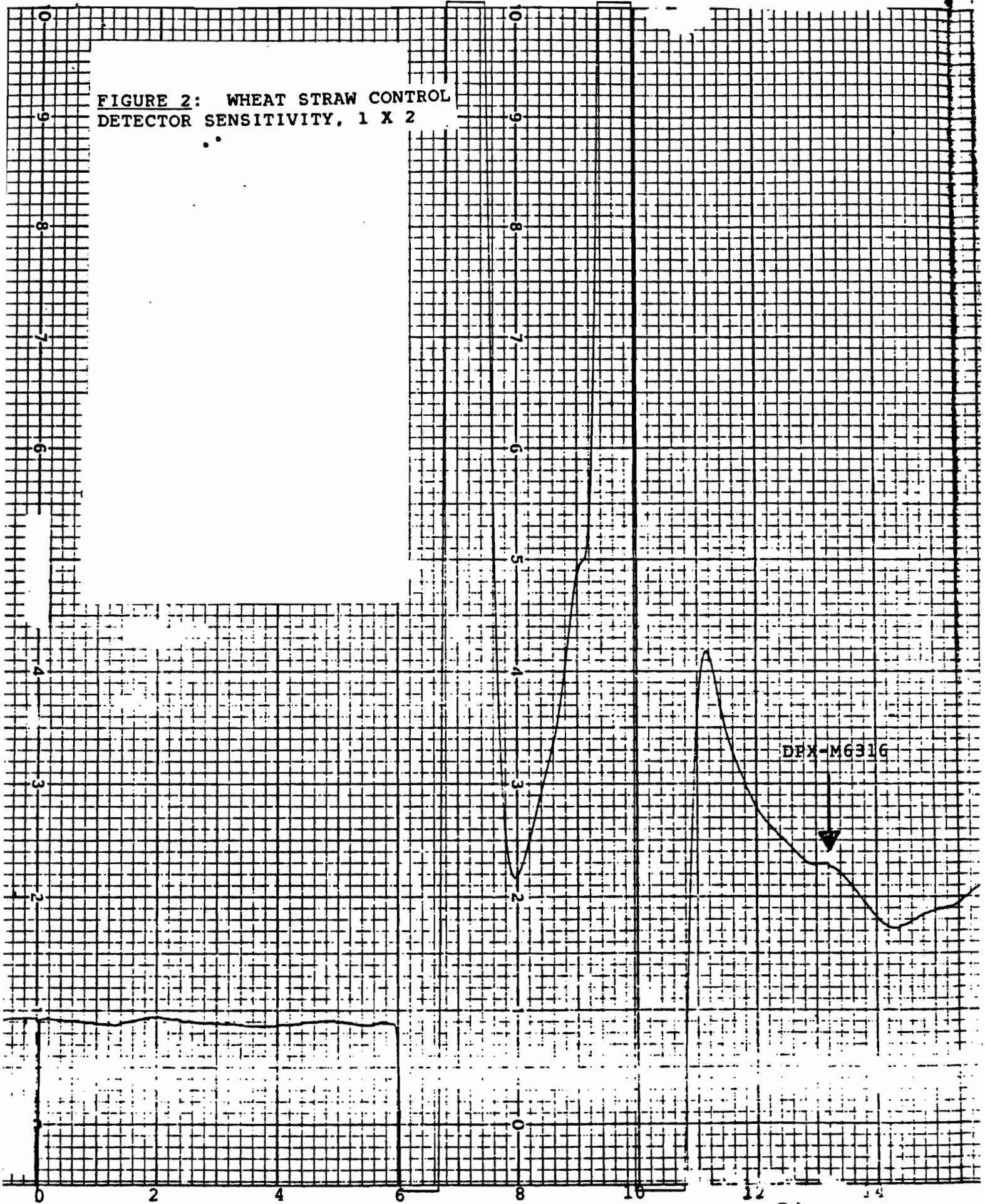
FIGURE 1: DPX-M6316 (0.05 µg/mL)
DETECTOR SENSITIVITY. 1 X 2



RETENTION TIME (min.)

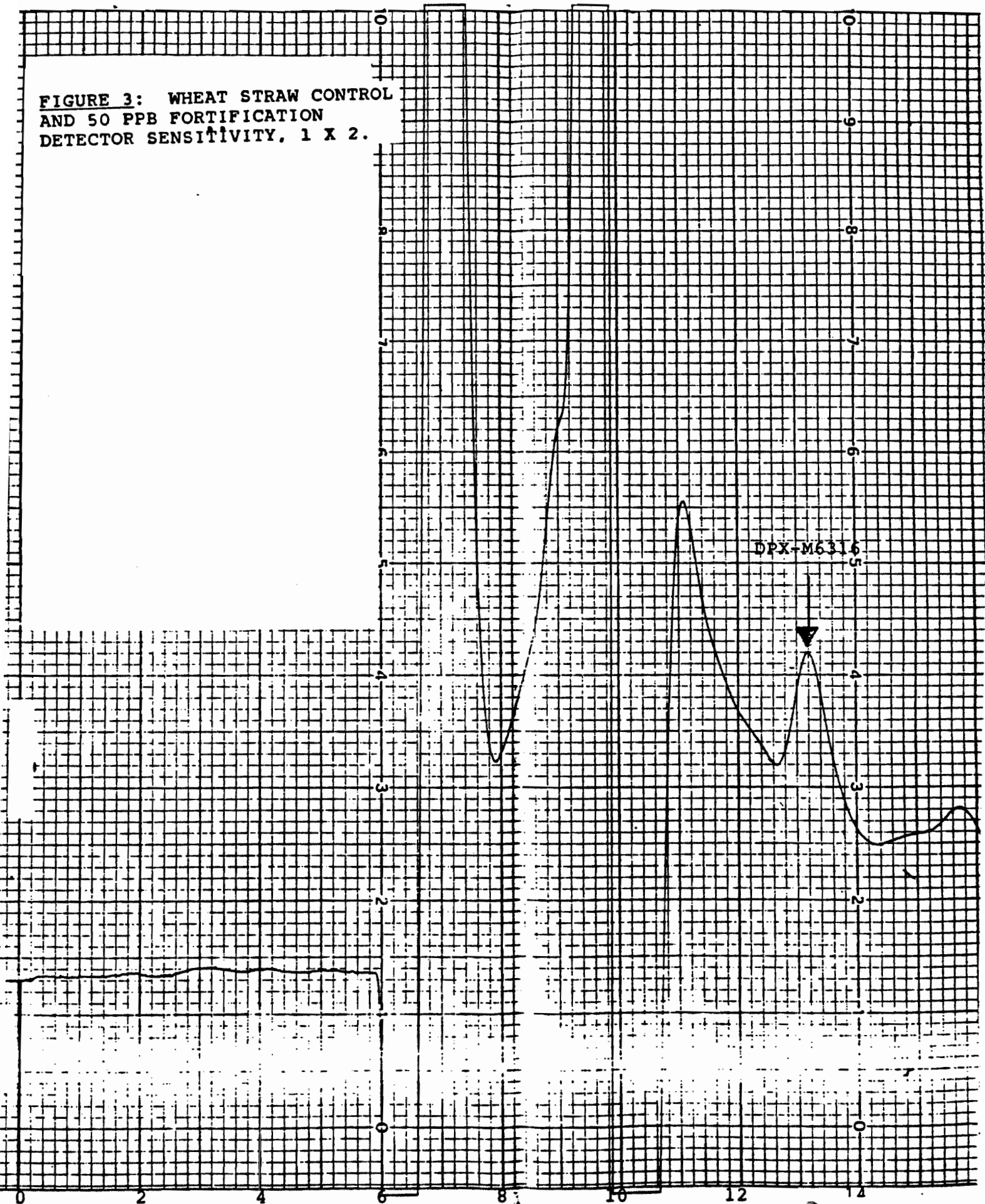
20

FIGURE 2: WHEAT STRAW CONTROL
DETECTOR SENSITIVITY, 1 X 2



RETENTION TIME (min)

FIGURE 3: WHEAT STRAW CONTROL
AND 50 PPB FORTIFICATION
DETECTOR SENSITIVITY, 1 X 2.



RETENTION TIME (min)

FIGURE 4: BARLEY STRAW CONTROL
DETECTOR SENSITIVITY, 1 X 2

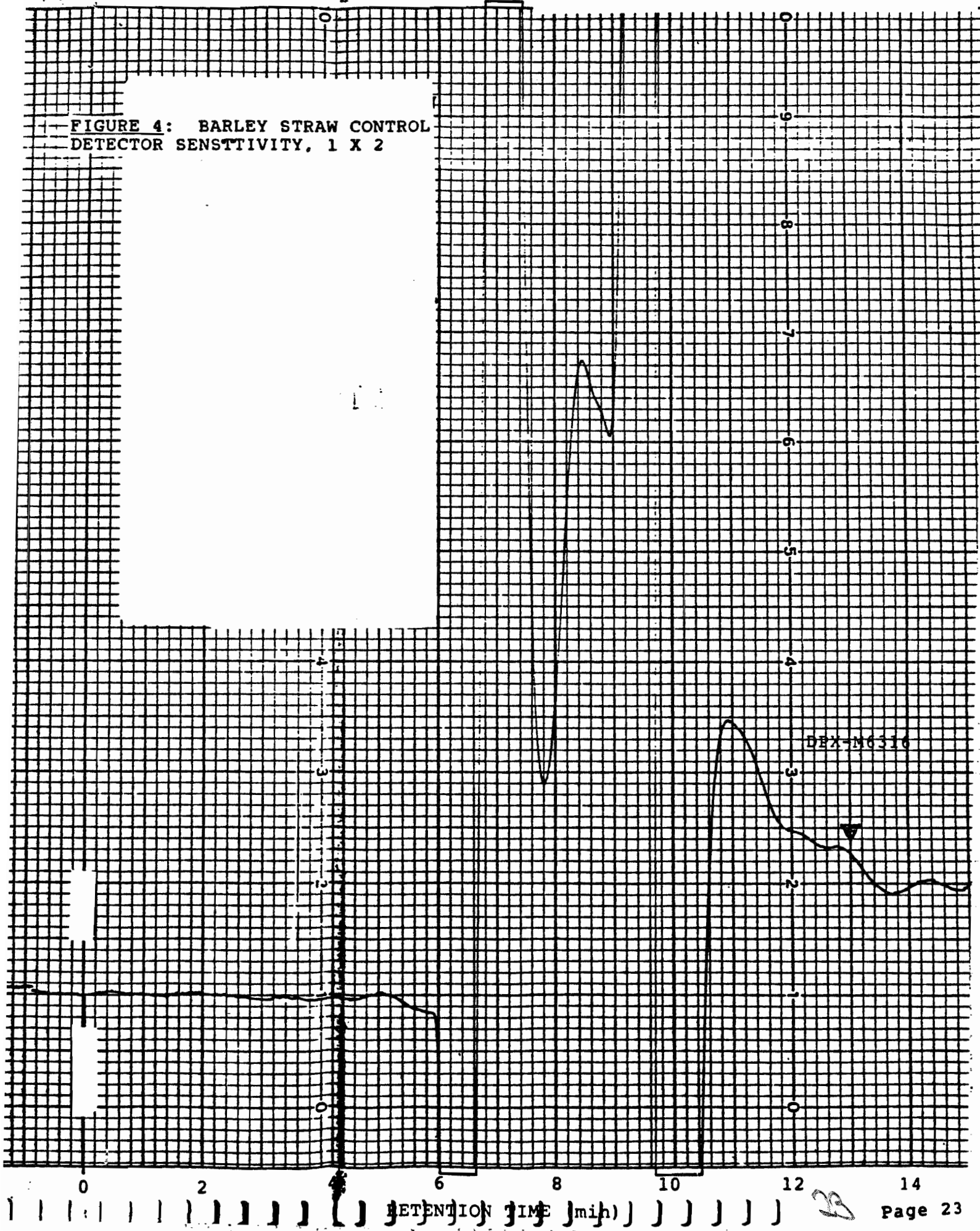
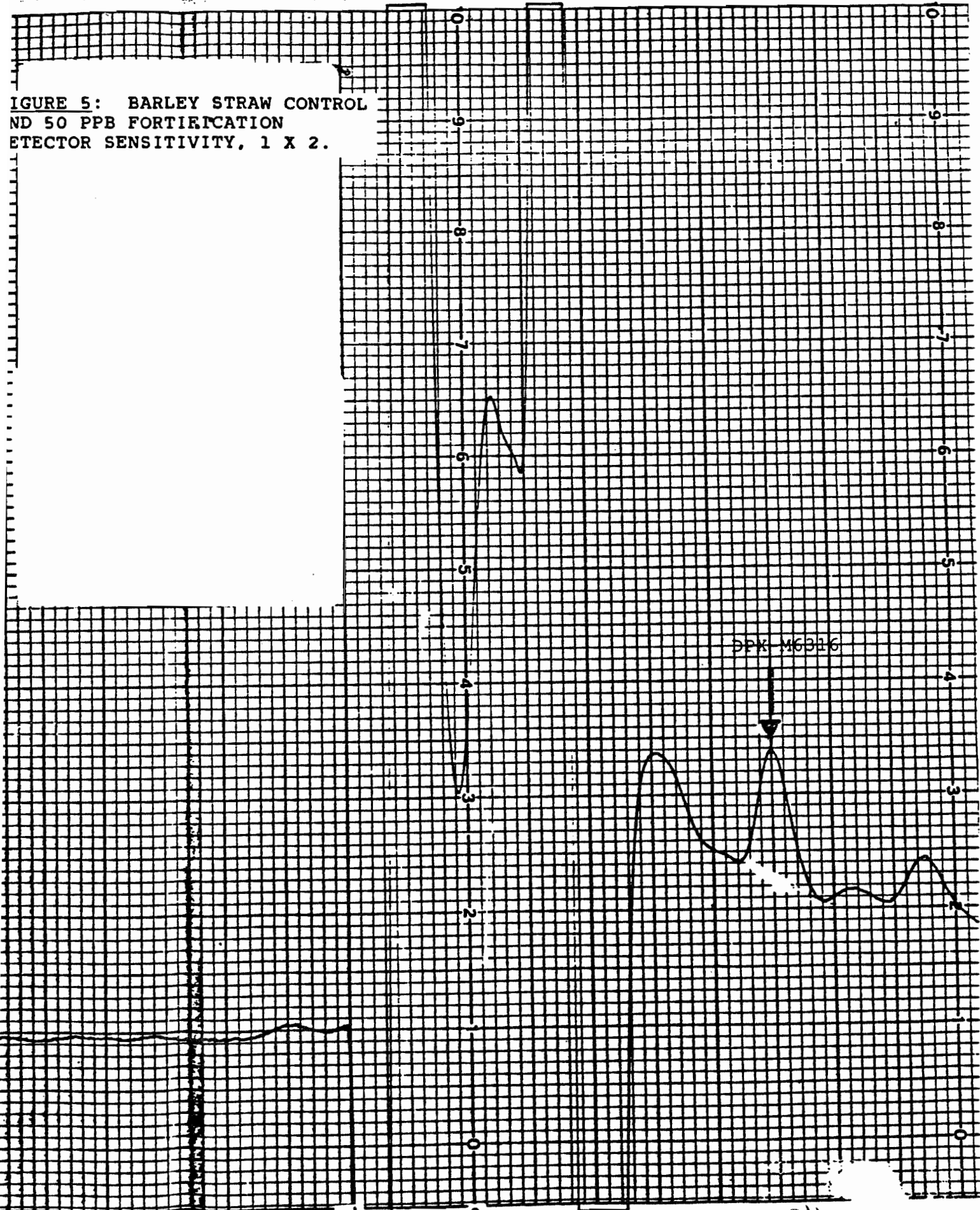


FIGURE 5: BARLEY STRAW CONTROL
ND 50 PPB FORTIFICATION
DETECTOR SENSITIVITY, 1 X 2.



STORAGE LOCATION OF RAW DATA, REPORTS

E. I. du Pont de Nemours and Company, Inc.
Agricultural Products Department
Experimental Station
Residue Studies Groups' Archives
Wilmington, Delaware 19898



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

Analytical Chemistry Section
Building 306, ARC-East
Beltsville, Maryland 20705

MAY 4 1987

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

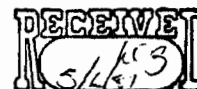
SUBJECT: PP#6F3431 (Harmony) on Wheat and Barley
FROM: Ronald F. Thomas, Chemist ^{DPW for} RFT 4-29-87
Analytical Chemistry Laboratory
THRU: Warren R. Bontoyan, Head *WRB*
Analytical Chemistry Section
THRU: Donald A. Marlow, Chief *DAM*
Chemical Operations Branch
TO: Charles Trichilo, Chief
Residue Chemistry Branch
Hazard Evaluation Division

Introduction

The Analytical Chemistry Section was requested by the Residue Chemistry Branch, Hazard Evaluation Division, to conduct a method trial on the herbicide "Harmony" (DPX-M6316). Analytical methods AMR-646-86 and AMR-761-87 (DuPont Company) were used for wheat grain and straw.

Method Summary

The analytical methods used are HPLC methods using the Tracor photoconductivity detector. Finely ground wheat grain and straw are extracted with ethyl acetate. The ethyl acetate extracts are partitioned with sodium bicarbonate solution and the ethyl acetate discarded. The bicarbonate solution is adjusted to pH 3.5 and extracted with methylene chloride. The methylene chloride is evaporated to dryness using a roto-vap and nitrogen. The residue is dissolved in the HPLC mobile phase and injected into the HPLC which is connected to the PCD.



Results

Recovery of DPX-M6316 (Harmony) from Wheat Grain and Straw

<u>Commodity</u>	<u>Chemical Added</u>	<u>PPM Added</u>	<u>PPM Found</u>	<u>% Recovery</u>
Wheat Grain	DPX M6316	0	ND*	-
		0	ND	-
		0.05	.046	92.9
		0.05	.046	92.9
		0.10	.090	90.3
Wheat Straw	DPX M6316	0	ND	-
		0	ND	-
		0.05	.043	86.6
		0.05	.038	76.6
		0.10	.083	83.3
		0.10	.050	50.0

* ND = <0.05 ppm

Comments

1. The photoconductivity detector at Beltsville ACL was not sensitive enough to do the analysis. The extracts were taken to the Agriculture Experiment Station, DuPont, and were analyzed using their instrument.

2. One chemist can run a set of 4 - 6 samples in 1.5-2 days

3. The method appears to be satisfactory for enforcement purposes, however, the photoconductivity detector is not in common use in many laboratories.

4. Note the quote from the method concerning the HPLC column.

" The detector output was linear over this particular weight range of DPX-M6316. Response factors for a series of standards are shown in Table 1. The average is 27.4 mm/ng, and the standard deviation is 0.65 mm/ng. These results were obtained with a new column. However, with increasing use the response factor will increase, and after several months it will be about 40 mm/ng. A chromatogram of the 0.05 ug/ml standard is shown in Figure 1. Normally, the short term noise is <1mm (peak to peak)."

5. The report and project file were audited by the ACS OA Committee on April 28, 1987.

USE SEPARATE FORM FOR EACH METHOD

Modifications to method (major or minor):

None

Special Precautions to be taken:

None

Source of analytical reference standards:

DuPont Company
Research Triangle Park, N.C.

If derivatized standard used, give source:

N/A

Instrumentation for quantitation:

Tracor Photo Conductivity Detector

Instrumentation for confirmation:

N/A

If instrument parameters differ from method given, list parameters used.

N/A

Commercial source for any special chemicals or apparatus:

N/A

Comments:

See attachments

Chromatograms:

Submitted

3

Wheat Germ

09/15/87

Samples A, B, C, D, E, F

Sample wts - 25 gm

Final Volume 5.0 ml

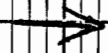
Amount Infected 20k

STANDARD Amount 409

Operating conditions as per method

Control

B



125

100

100



100

INSECT

10 20 30 40 50 60 70 80 90

20

30

40

50

60

70

80

90

100

Wheat Grain

09/15/87

Samples A, B, C, D, E, F

Sample wts - 25 gm

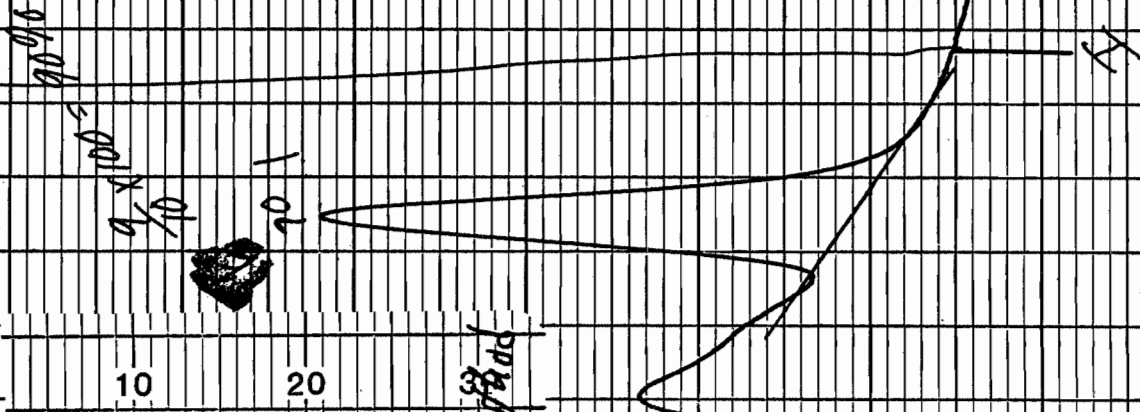
Funnel Volume 5.0 ml

Amount Infected 20k

STANDARD AMOUNT 409

Operating conditions as per method

0.1 PPM Fortification



Infected

Samples A, B, C, D, E, F
 Sample wts - 2.5 gm
 Final Volume 5.0 ml
 Amount Infected 20h
 STANDARD Amount 409
 operating conditions as per method

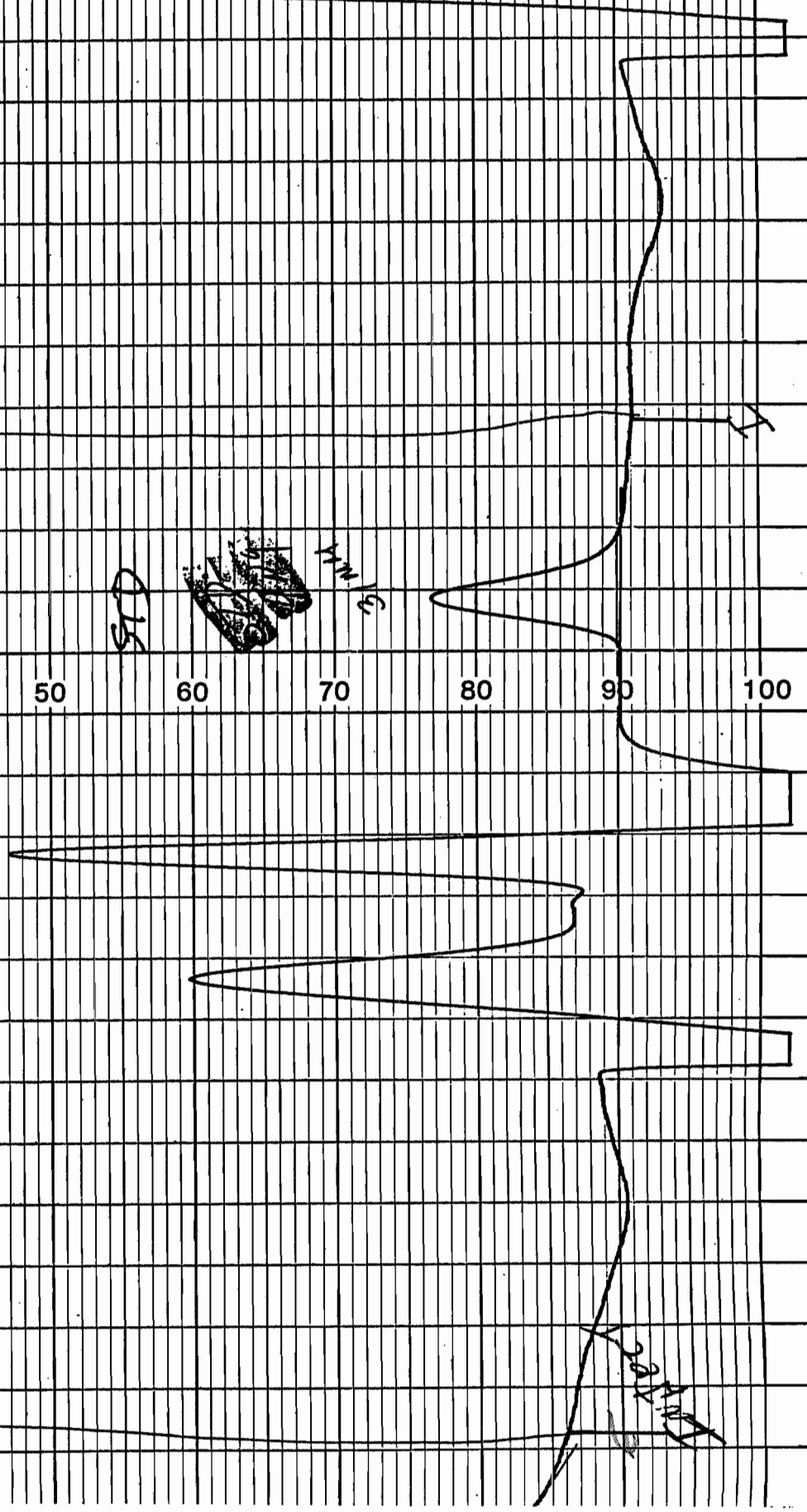
0.2 ng / μ l

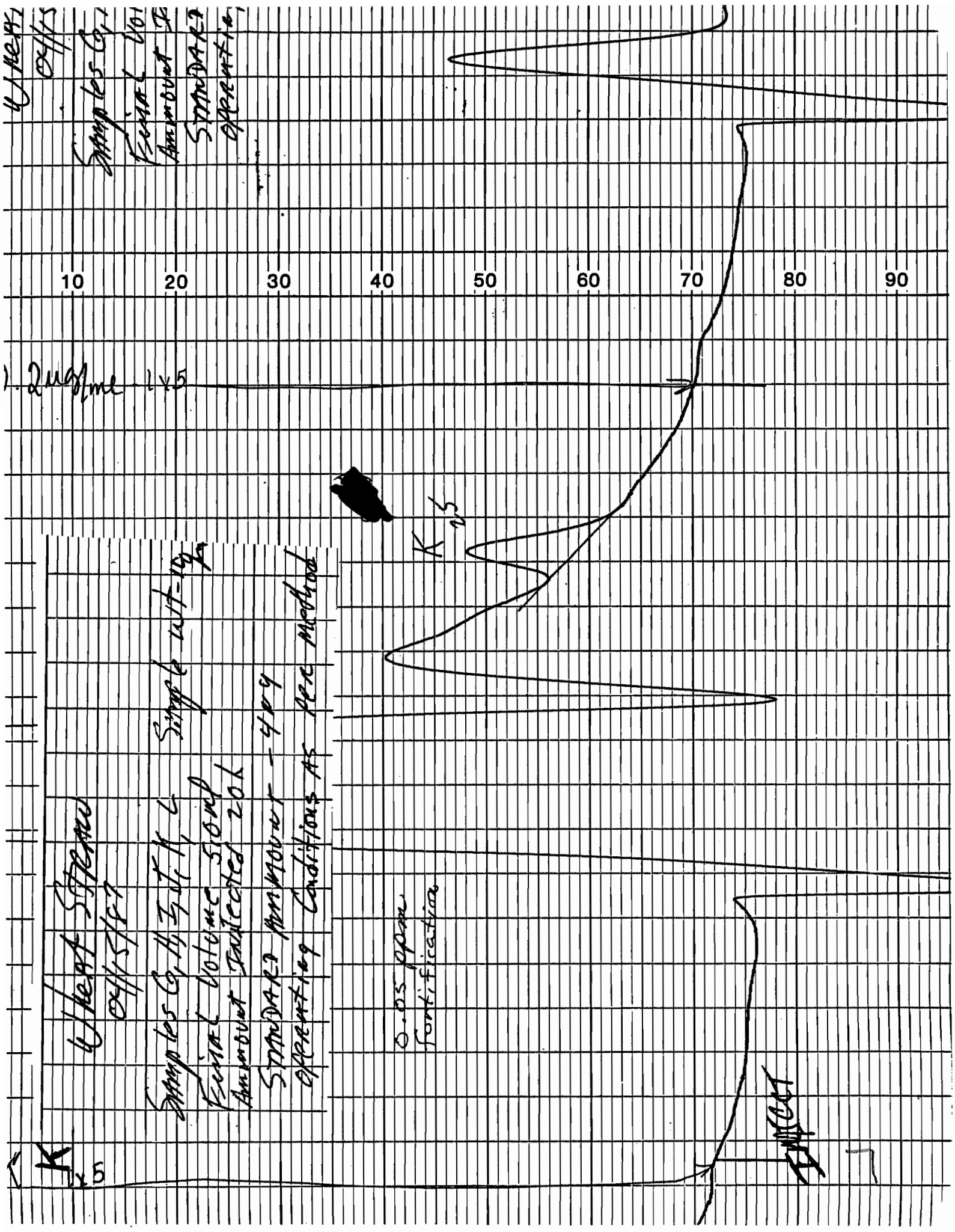
20 40 50 60 70 80 90 100

STD

~~STD~~

AMOUNT





Wheat
0.05

Samples Co. 1
Final Vol
Amount 20g
STANDARD
Operating

10 20 30 40 50 60 70 80 90

2.2 mg/ml 1x5

K 25

Wheat Standard
0.05

Samples Co. 1, 2, 3, 4, 6
Final Volume 50ml
Amount Indicated 20g
STANDARD amount - 40g
Operating Conditions AS PER Method

0.05 ppm
Fortification

STANDARD

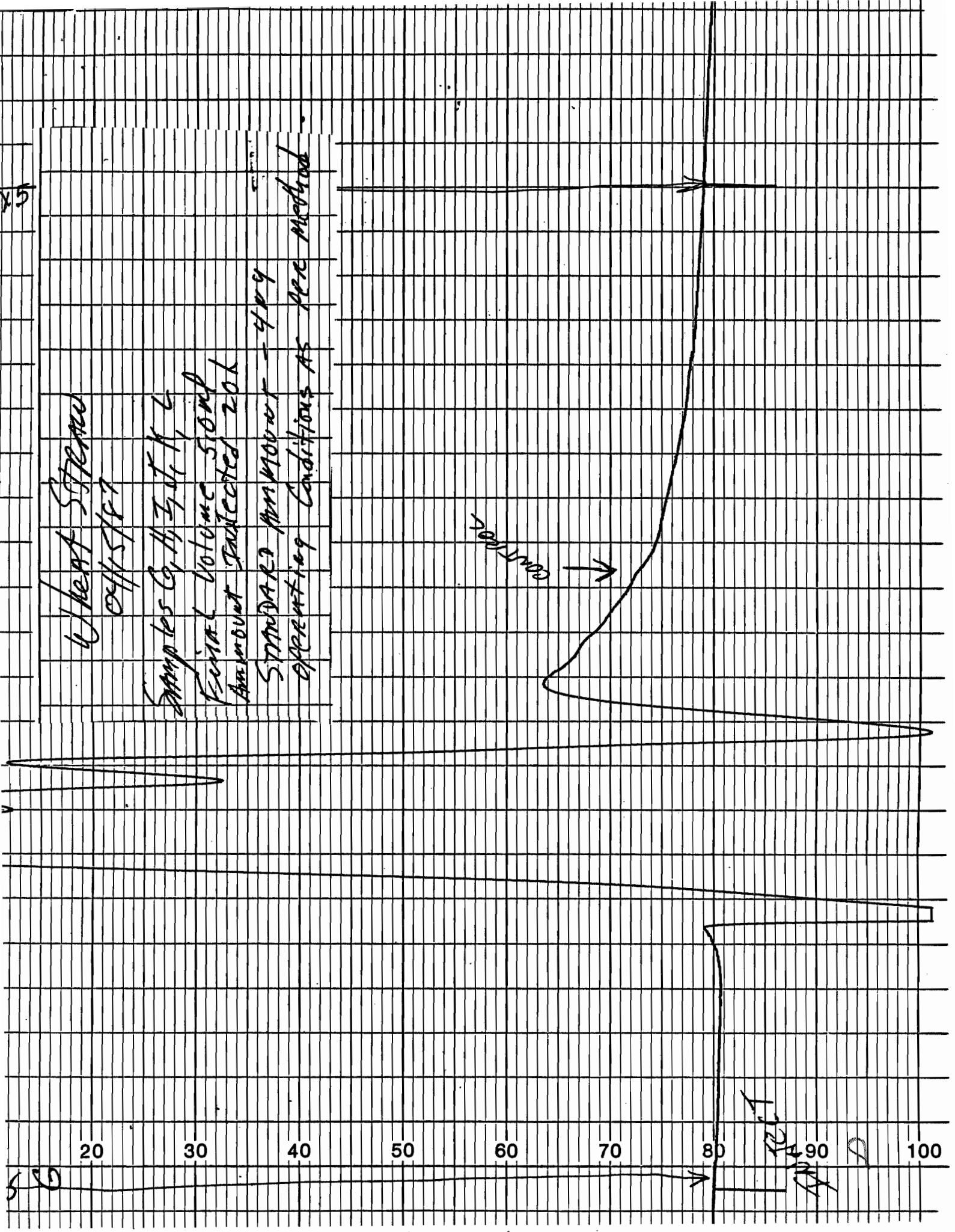
K 25

15

Wheat Stream
04/15/87

Samples C, H, I, J, K, L
Final Volume 50ml
Amount Injected 20ul

STANDARD AMOUNT - 4ug
Operating Conditions As per Method



20

30

40

50

60

70

80

90

100

AU

PEAK

CONTAMINANT

~~Final Volume 50 ml~~
~~Amount Diluted 20 l~~
Standard Preparation = 4 ug
~~Operating Conditions see method~~

0.2 ng/ml
std

4.18

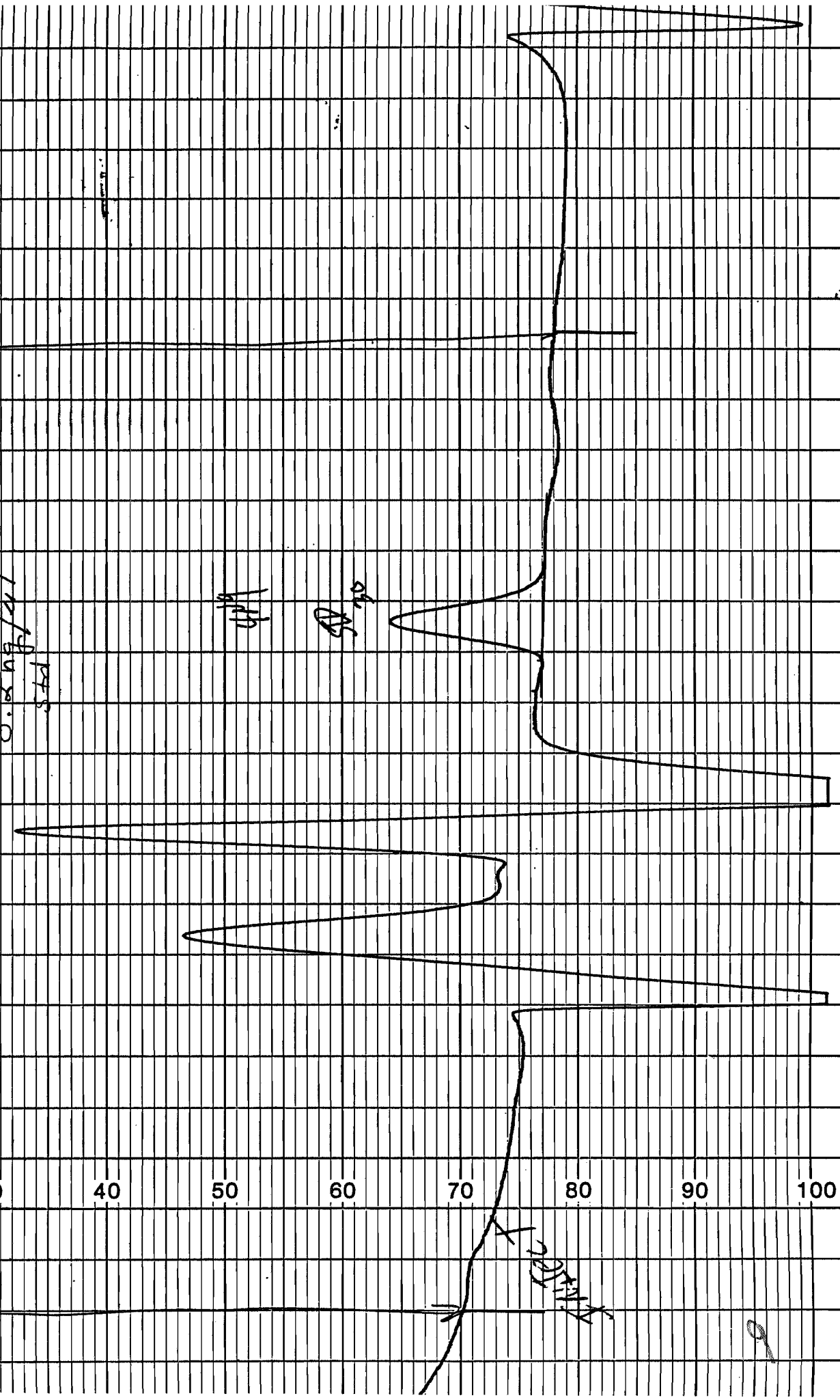
5.75

20 30 40 50 60 70 80 90 100

2 x 5
std

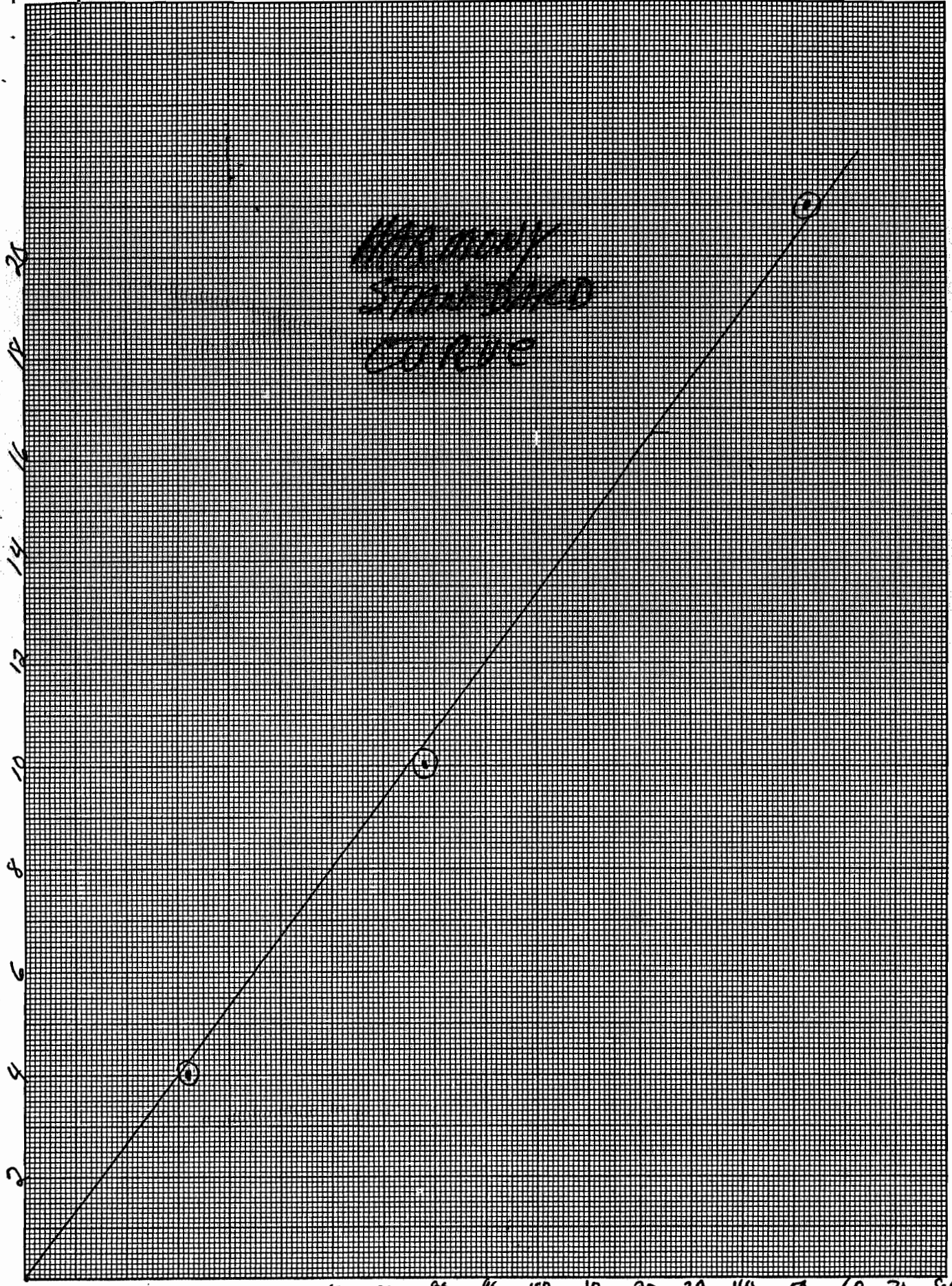
2.21
2.21

9



NG HARMONY

HARMONY
STANDARD
CURVE



10 X 10 TO THE CENTIMETER 18 X 25 CM.
UNITED CONVERTERS & PRINTERS

PK HT - MM

10

Attachment 4



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

MAR 17 1987

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: PP #6F3431. DPX-M6316 (Harmony) on wheat and barley.
Method Trial Request

FROM: Cynthia Deyrup, Ph.D., Chemist
Tolerance Petition Section 2
Residue Chemistry Branch
Hazard Evaluation Division (TS-769) *John Only for*

THRU: Charles L. Trichilo, Ph.D., Chief
Residue Chemistry Branch
Hazard Evaluation Division (TS-769) *MB*

TO: Donald A Marlow, Chief
Chemical Operations Branch, BUD (TS-768)

E.I. du Pont de Nemours & Company (Inc.) is proposing tolerances for DPX-M6316 on wheat and barley.

In your 10/31/86 memo, you pointed out the difficulties encountered in attempting to carry out a method trial of E.I. du Pont de Nemours & Co.'s Method No. AMR-235-84 for Harmony on wheat and barley. DuPont has now submitted revised copies of its methodology. Therefore, a method trial on the revised methodology is requested for one chemical on two commodities. Samples should be run in duplicate at the requested fortification levels (see attached table). Two copies of the appropriate method along with recoveries and sample chromatograms are attached.

Please return the requested information on the attached forms and any other information concerning the method trial that we should be aware of including copies of chromatograms for representative controls and fortified samples, standard curves, and examples of sample calculations.

The standard for DPX-M6316 is available at the EPA Repository (FTS 629-3951). The Repository code is F-855.

The Registration Division "projected return date" for this action is unknown at this time. You may want to contact the Registration Division on the priority of this action.

Please forward results of this method trial directly to C. Deyrup.

Attachment: Two copies of methods
TS769:RCB:C.Deyrup:cd:CM#2:Rm810:x77484
cc: (with Attachment): Don Marlow only
cc: (without Attachment): RF, Circ., M. Bradley,
PP#6F3431, PM#25, W. Bontoyan, MTO F., A. Barton/HED
RDI: JH Onley:3/16/87:RDSchmitt:3/16/87

Method: Document No. AMR-646-86, Analysis of the herbicide DPX-M6316 in wheat or barley grain by liquid chromatography. and Document No. AMR-761-87, Analytical Method for residues of DPX-M6316 in Wheat and Barley Straw.

Do not use control values for recovery corrections.

Do not report control values as 0; if less than limit of detection, report as such.

<u>Commodity</u>	<u>Chemical Added</u>	<u>PPM added</u>	<u>PPM found</u>	<u>% Recovery</u>
Wheat grain	DPX-M6316	0.05 0.10		
Wheat straw	DPX-M6316	0.05 0.10		

3

USE SEPARATE FORM FOR EACH METHOD

Modifications to method (major or minor):

Special precautions to be taken:

Source of analytical reference standards:

If derivatized standard used, give source:

Instrumentation for quantitation:

Instrumentation for confirmation:

If instrument parameters differ from method given, list parameters used.

Commercial source for any special chemicals or apparatus:

Comments:

Chromatograms:

4