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FMC Agricultural Chemical Group
Richmond

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Title: Analytical Method for the Analysis
of FMC 54800 Residues in/on Cottonseed

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ABSTRACT:

A routine residue method for the analysis of FMC 54800 residues in/on ginned cottonseed has been developed. The method involved an initial acetone extraction, a hexane/water partition and cleanup by gel permeation and Florisil® column chromatography prior to analysis by capillary gas chromatography using a ⁶³Ni electron capture detector.

Method sensitivity was determined to be 0.05 ppm and method detectability was estimated to be 0.01 ppm. Average method recovery was 74±8%.

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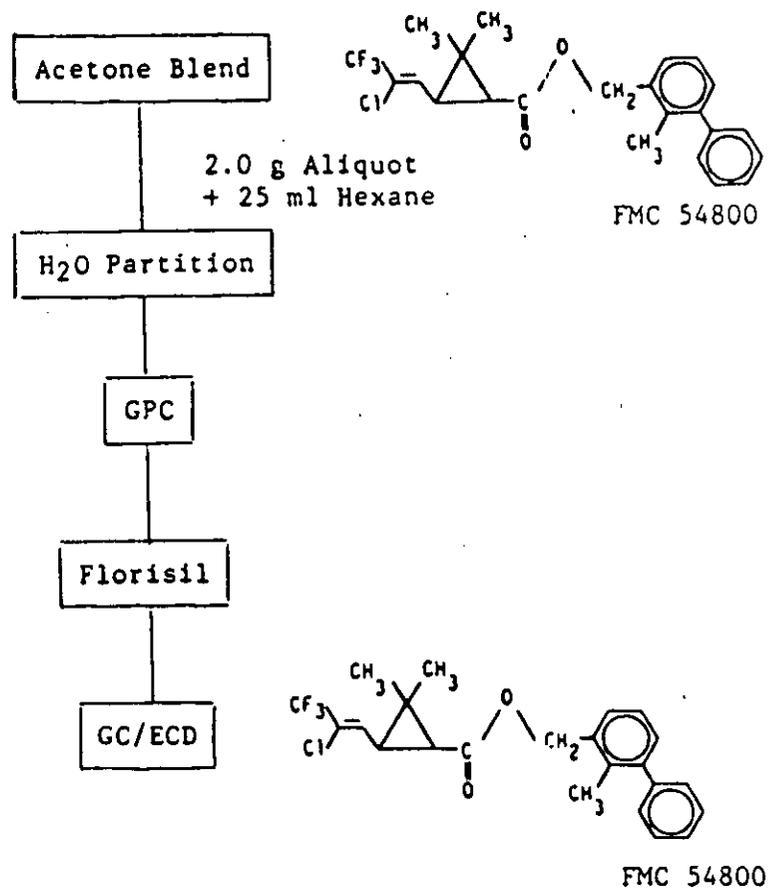
I. INTRODUCTION

An analytical method for the routine determination of FMC 54800 residues in/on ginned cottonseed has been developed. This method has been validated utilizing fortifications of untreated ginned cottonseed samples.

II. ANALYTICAL METHOD

A schematic diagram of the analytical procedure is shown in Figure 1.

FIGURE 1
ANALYTICAL PROCEDURE



A. Apparatus

Automatic Liquid Sampler, Hewlett-Packard 7672A
Autoprep 1001® GPC, ABC Laboratories
Bio Beads® S-X3, Bio Rad Laboratories
Chromatographic column, 15 mm o.d. x 175 mm, 50 ml reservoir
Gas Chromatograph, Hewlett-Packard 5840
Fused Silica Capillary Column, 530 μ coated with methyl silicone,
Hewlett-Packard
Hobart Food Processor
 ^{63}Ni Electron Capture Detector, Hewlett-Packard 18803B
Standard laboratory glassware and equipment
Waring Blender

B. Reagents

Acetone, Distilled in Glass®, Burdick and Jackson
Analytical Standards, FMC Corporation, Agricultural Chemical Group,
Princeton, NJ
Cyclohexane, Distilled in Glass, Burdick and Jackson
Florisil®, PR Grade 60/100 mesh, Floridin Co.
Hexane, Nanograde®, Mallinckrodt
Methylene Chloride, Distilled in Glass, Burdick and Jackson
Methyl t-Butyl Ether, Distilled in Glass, Burdick and Jackson
Sodium Chloride, Reagent Grade
Sodium Sulfate, anhydrous, Reagent Grade
Water, Deionized

C. Procedure

Sample Preparation

The cottonseed samples were macerated by using a Hobart Meat Grinder. Dry ice was added as needed to keep the sample cold. Macerated samples were stored at -18°C until analysis.

Extraction

A 20 g subsample of macerated crop was weighed into a 1 qt blending jar and blended for 5 minutes with 200 ml of acetone. The sample was filtered through a Buchner suction funnel lined with Whatman GF/A glass fiber filter paper. The blending jar and filter cake were rinsed with 50 ml of acetone and the rinse added to the filtrate. The filtrate was transferred to a graduated cylinder and the volume was adjusted to 250 ml.

Partition

A 2 g sample aliquot (25 ml) was transferred to a 250 ml separatory funnel and 25 ml of hexane, ca 25 g salt and 50 ml deionized water were added to the sample. The sample was shaken for one minute and the phases allowed to separate. The lower, aqueous phase was transferred to a second separatory funnel and extracted

with 50 ml of hexane. The hexane fractions were dried by passing through a bed of anhydrous sodium sulfate and combined in a 250 ml Phillips beaker. The sample was concentrated on a warm steam bath under a gentle stream of nitrogen to ca 1 ml, transferred to a 13 ml graduated centrifuge tube using cyclohexane/methylene chloride (85/15, v/v) as the transfer solvent and the volume adjusted to 10.0 ml.

Gel Permeation Column Cleanup - Autoprep 1001 GPC

The Autoprep 1001 GPC is an automated gel permeation column cleanup system. Twenty-three samples can be loaded into sample loops (each with a 5.0 ml capacity) at one time and the system programmed to sequentially elute each sample through the column, discarding and collecting the appropriate fractions from the column eluate. Operating conditions for the Autoprep 1001 GPC are described below:

Column:	25 mm i.d. x 300 mm glass packed with ca 50 g Bio-Beads S-X3 (200/400 mesh) compressed to a bed length of 240 mm with a Kontes organic solvent plunger assembly
Solvent System:	Cyclohexane/Methylene Chloride (85/15, v/v)
Flow Rate:	3.6 ml/min
Dump Time:	20 min
Collect Time:	8 min
Wash Time:	5 min

Approximately 8 ml of the sample from the partition step was injected into the sample introduction valve of the Autoprep 1001. Excess solvent (over 5.0 ml) was automatically discarded by the sample loop system. Each of the individual samples was loaded into the system and the last loop loaded with 5.0 ml of the cyclohexane/methylene chloride (85/15) solvent mixture. The appropriate parameters were set on the control panel of the instrument and the Autostart button engaged. The fractions of the column eluate containing FMC 54800 residues were collected in 125 ml Phillips beakers. The "dump" and "wash" fractions were collected in a waste reservoir attached to the instrument.

The column eluates containing FMC 54800 residues were concentrated on a steam bath under a gentle stream of nitrogen to a volume of 2-3 ml.

Florisil Column Cleanup

A 15 mm o.d. x 175 mm chromatographic column was packed with 4.0 g of activated Florisil (activated by drying in a 105°C oven for 16 hrs). The Florisil was capped with a 0.5 inch layer of anhydrous sodium sulfate. In preparing this column, the narrow stem of the chromatographic column was packed with glass wool to reduce the flow rate of the column eluate. Also the column had hexane in it when adding the Florisil to insure an even, air-free bed of packing.

The sample was transferred to the column using two 5 ml hexane rinses. The column was allowed to elute to the top of the packing between each addition of the solvent. The column was then eluted with 35 ml hexane/methyl t-butyl ether (9/1, v/v) which was collected in a 125 ml Phillips beaker. The sample was concentrated on a steam bath under a gentle stream of nitrogen to 2-3 ml. The sample was transferred to a centrifuge tube with hexane and concentrated in a warm water bath to 2.0 ml for glc analysis.

Gas-Liquid Chromatography (GLC)

Samples were analyzed on a Hewlett-Packard 5840A Gas Chromatograph equipped with a ⁶³Ni electron capture detector and a Hewlett-Packard 7672A Automatic Liquid Sampler. Operating conditions are described below:

Column:	10 meter x 530 μ fused silica capillary column coated with methyl silicone (Hewlett-Packard)
Injector Temp.:	250°C
Column Oven Temp.:	245°C
Detector Temp.:	350°C
Carrier Gas Flow (He):	3.0 ml/min
Detector Make Up Flow (N ₂):	29 ml/min

Using the above parameters, FMC 54800 elutes as a single peak with a retention time of ca 15.0 minutes.

III. QUANTITATION

Sample analysis was automated by using a Hewlett-Packard 7672A Automatic Liquid Sampler. Quantitation of residues was automatically performed by peak area integration and comparison to external standard calibrations. By using the appropriate

dilution factor for the sample size injected, results of each analysis were reported on a ppm ($\mu\text{g/g}$) basis. By using a run programming mode, external standard calibrations were constantly updated during the analysis of each series of samples. In practice, standards were analyzed following every two or three samples and the results of each standard analysis were averaged with the results of previous standard analyses to obtain updated calibration information for subsequent sample analyses. The following standards were used for quantitation:

Solution No.	Concentration	Solvent
48-4 or 48-6	50 $\text{pg}/\mu\text{l}$	Toluene
48-5	20 $\text{pg}/\mu\text{l}$	Toluene
48-3	100 $\text{pg}/\mu\text{l}$	Toluene

IV. ANALYTICAL LIMITS

Method sensitivity was determined by fortifying and recovering FMC 54800 from untreated crop samples. Method sensitivity was 0.05 ppm. Method detectability was 0.01 ppm for cottonseed analyses.

V. FORTIFICATION RECOVERIES

Untreated cottonseed was spiked with FMC 54800 prior to the addition of solvent in the initial blend extraction. A fortification solution of FMC 54800 in ethanol (10 $\text{ng}/\mu\text{l}$, Sol. No. 48-1) was used for this purpose. Individual recovery data is shown in Table 1, page 7.

VI. SIGNATURES

We, the undersigned, hereby declare that this study was performed under our supervision according to the procedures herein described, and that this report provides a true and accurate record of the results obtained.

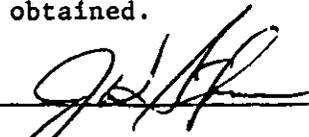
AUTHOR: DATE: 11/14/84TITLE: Senior ChemistSUPERVISOR: DATE: 11/14/84TITLE: Manager, Western Development

TABLE 1
RECOVERY DATA

Sample ID	Fortification (ppm)	Recovery	
		(ppm)	%
RRZ 214 83-JJK-Check	0.05	0.036	72
RRZ 218 EVG-83-100A	0.05	0.039	78
RRZ 219 RIS-83-102A	0.05	0.035	70
RRZ 282 AEP-83-95A	0.05	0.037	74
RRZ 323 CDA-83-12A	0.05	0.033	66
RRA 011 83-KMR-29A	0.05	0.032	64
RRA 012 83-KMR-30A	0.05	0.034	68
RRA 019 Control	0.05	0.046	92
RRA 021 85-83-2	0.1	0.078	78
Average ± Standard Deviation			74±8

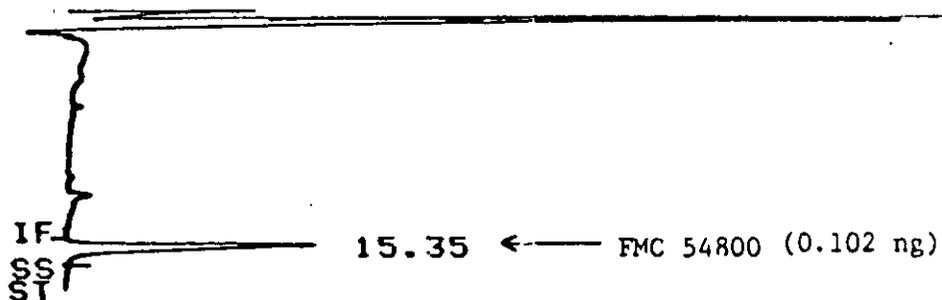
VII. CHROMATOGRAMS

INDEX TO CHROMATOGRAMS

Figure	Description	Injection
2	Standard	2 μ l x 50 pg/ μ l
3	Cottonseed, Untreated RRZ 214 84-JJK-Check	1 mg
4	Cottonseed, Untreated + 0.05 ppm RRZ 214 83-JJK-Check + 0.05 ppm	1 mg
5	Cottonseed, Treated RRZ 214 83-JJK-13-1	1 mg

FIGURE 2 - Standard

WAIT
START



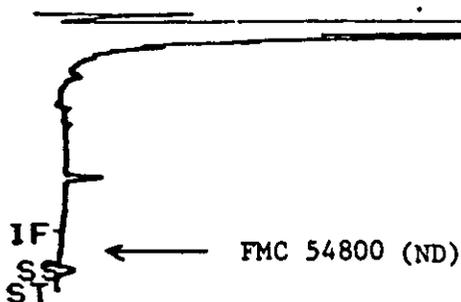
HP RUN # 4 JUL/24/84 TIME 16:49:09
 BOTTLE 4
 ESTD

RT	EXP RT	AREA	CAL #	AMT
15.35	15.20	82800	(R) 1	0.102

DIL FACTOR: 1.0000 E+ 0

FIGURE 3 - Cottonseed, Untreated

START



HP RUN # 2 JUL/24/84 TIME 16:02:09
 BOTTLE 2
 NO PEAKS IN WDOS

RT	AREA	AREA %
0.66	90220	100.000

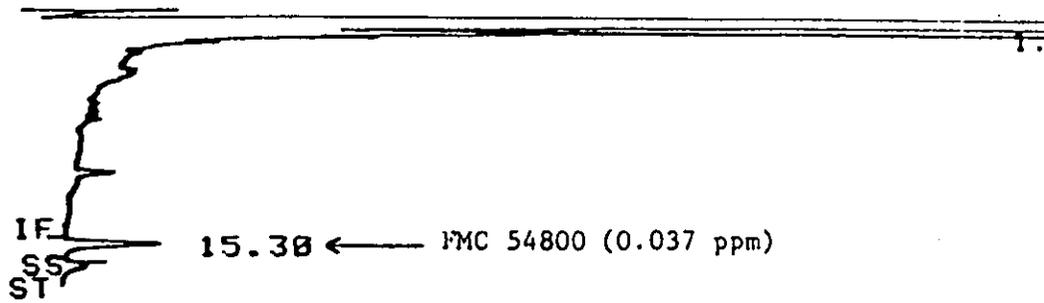
DIL FACTOR: 1.0000 E+ 0

HP 8430A 7480B/100 07700A/09

FIGURE 4 - Cottonseed, Untreated + 0.05 ppm

10
RAN-0140

WAIT
START



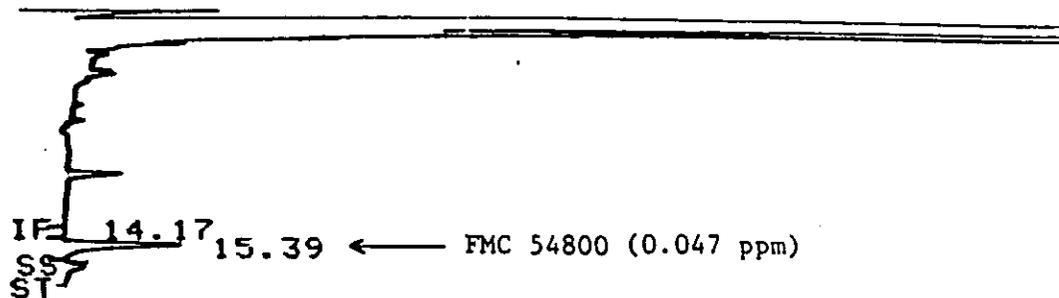
HP RUN # 3 JUL/24/84 TIME 16:25:33
BOTTLE 3
ESTD

RT	EXP RT	AREA	CAL #	AMT
15.30	15.20	30090	(R) 1	0.037

DIL FACTOR: 1.0000 E+ 0

FIGURE 5 - Cottonseed, Treated

WAIT
START



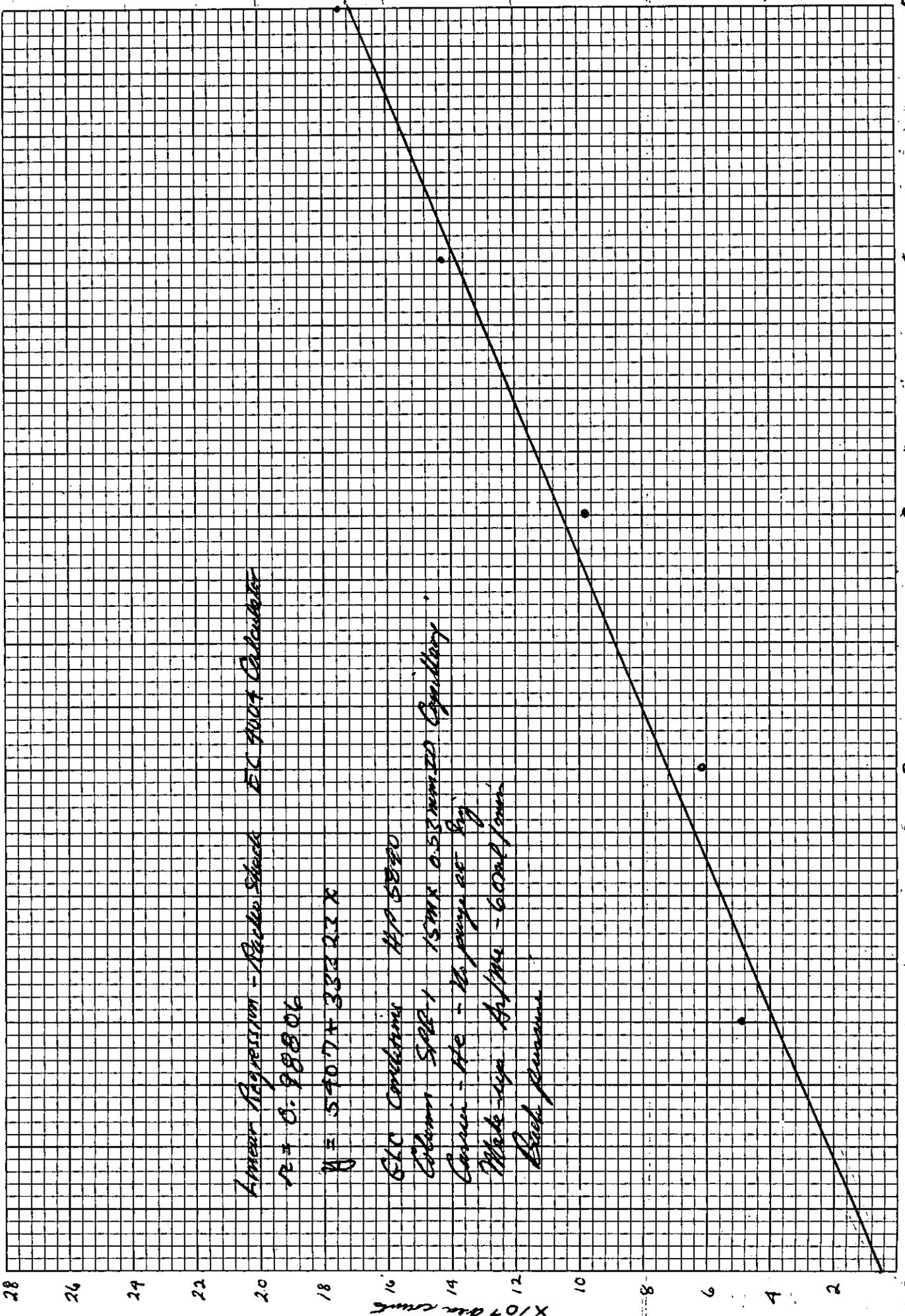
HP RUN # 5 JUL/24/84 TIME 18:13:16
BOTTLE 5
ESTD

RT	EXP RT	AREA	CAL #	AMT
15.39	15.27	38350	(R) 1	0.047

DIL FACTOR: 1.0000 E+ 0

QUALITY CONTROL CORPORATION
 1000 WEST 10TH AVENUE
 DENVER, COLORADO 80202

Experiments PMW B 87-93



Linear Regression - Parker School EC 7004 Calculator

$R = 0.98806$

$Y = 5.407 + 332.22 X$

GLO conditions 4795000
 Column SPB-1 15Mx 0.53mm ID Capillary
 Carrier - He - 10.00 ml/min
 Make-up gas Ar/He - 60ml/min
 Peak Parameters

ul injected (Conc 0.252 mg ul)