ATTACHMENT-6

FMC Agricultural Chemical Group
Princeton

REISSUED: 11/17/83
Pages 4, 7, 8

P-0757
11/14/83

TITLE: ANALYTICAL METHOD FOR THE RESIDUE ANALYSIS OF FMC 54800 IN APPLES

Project No. & Title:	G182 - FMC 54800
Author:	S. Witkonton
Supervisory Personnel:	R.F. Cook
Work Done By:	S. Witkonton and C.A. Lang
Notebook Reference:	E2698:117-120
Study/Raw Data File No.:	S 182 83 14
Period Covered:	October 26-November 14, 1983

ABSTRACT

A routine residue method for the analysis of FMC 54800 in apples was developed which involved an acetone extraction, evaporation, hexane/aqueous partition and Florisil® column clean-up of hexane extract. Quantification was accomplished by gas chromatography with electron capture detection.

Method sensitivity was determined as 0.05 ppm and method detectability was estimated as 0.01 ppm. Average method recovery was 93 ±9%.

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I. INTRODUCTION

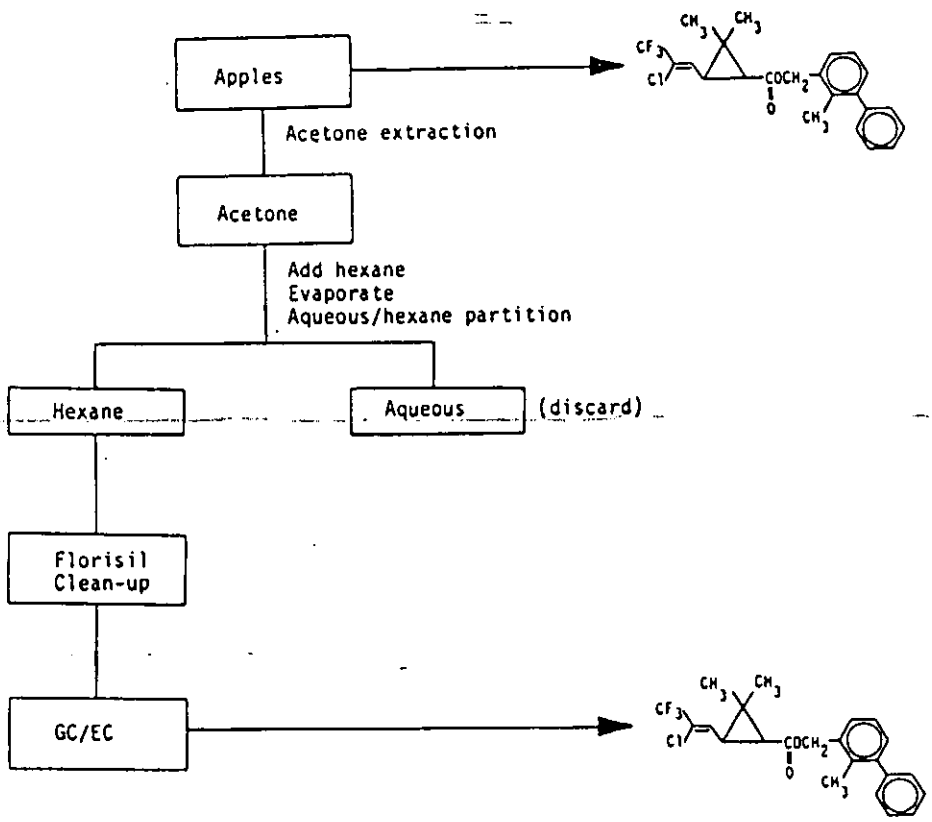
A FMC 54800 analytical method for apples was developed for routine residue analysis. This method has been validated utilizing fortifications of untreated apples.

II. ANALYTICAL METHOD

Figure 1 diagrams the analytical method employed in the analysis of FMC 54800 in apples.

FIGURE 1

METHOD OF ANALYSIS OF FMC 54800 IN APPLES



A. Apparatus

Flask, Erlenmeyer, 250 and 500 ml
Flask, round-bottom, 50 ml with 24/40 ȝ neck opening
Funnel, glass 4" diameter
Funnel, separatory, 500 ml
Graduated cylinder, 10, 50, 100, 500 ml
Hobart® food chopper
Kuderna-Danish evaporative concentrator, 500 ml with a 24/40 ȝ top joint and a 24/25 ȝ bottom joint and retaining hooks
N-EVAP® evaporator
Snyder column, 3 ball, 250 mm
Steam bath
Syringe, Hamilton, 10, 25, 50, 100 µl
Turbomatic Jr. glassware washer, series 7000, Better-Built Machinery Corp.
Ultrasonic Extractor-Tissumizer® with SDT-182 EN Generator, Tekmar Co.

B. Instrumentation

Gas chromatograph Hewlett-Packard 5713 equipped with H-P-5880A Series GC Terminal and an electron capture detector
GC column, 122 cm x 2 mm (I.D.) glass silanized with Sylon-CT reagent. Packed with 4% SE-30/6% OV-210 on Chromosorb W HP 100/120 mesh.

C. Reagents

Acetone, Resi-Analyzed, Baker
Distilled water (house still)
Ethyl acetate, distilled in glass, Burdick and Jackson
Fluted filter paper, grade 515, size 18.5 cm, Eaton-Dikeman
Florisil, 100/200 mesh, Floridin, deactivated with 3% water
Hexane, Resi-Analyzed, Baker
Sodium chloride, Fisher
Sodium sulfate, anhydrous, Fisher. Prewashed with acetone and hexane.
Versatone®, glassware cleaning agent, VWR

D. Procedures

All glassware was thoroughly washed with a water rinse, non-phosphorous detergent wash, water rinse, and final distilled water rinse in a Better-Built Model 7000 laboratory glassware washer and rinse with acetone before used.

1. Extraction

Twenty grams of apples were extracted with 200 ml of acetone for two minutes with an ultrasonic extractor (Tissumizer). The mixture was filtered through fluted filter paper which had been washed with 100 ml of acetone. The blending jar and filter cake were washed with 50 ml of acetone. The combined acetone (~250 ml) was adjusted to 280 ml with acetone. Seventy ml (5 g aliquot of apples equivalent) was transferred into a 500 ml Kuderna-Danish concentrator equipped with 50 ml round-bottom flask. One hundred ml of hexane was added. The acetone/hexane mixture was evaporated in a steam bath to ~20 ml. The concen-

trated solution was transferred into a 500 ml separatory funnel which contains 50 ml of sodium chloride solution (∿5 g NaCl + 50 ml distilled H_2O). The Kuderna-Danish flask was washed with 100 ml of hexane followed by 50 ml of distilled H_2O and transferred successively to the 500 ml separatory funnel. The mixture was shaken vigorously and left to stand for phase separation. The aqueous (lower phase) was drained into a 250 ml Erlenmeyer flask. The hexane extract (upper phase) was transferred into another 500 ml Kuderna-Danish concentrator. The remaining aqueous phase was re-extracted with 2 x 100 ml of hexane. The combined hexane (300 ml) was evaporated to ∿10 ml using steam bath and further concentrated using N-EVAP under N_2 to ∿2 ml. This concentrated solution was then cleaned up by Florisil column.

2. Florisil Column Clean-up

The glass chromatographic column (14 mn I.D.) was filled with about 100 ml hexane. Ten grams of 3% H_2O deactivated Florisil was slowly added. The Florisil was allowed to settle and a few grams of anhydrous sodium sulfate was added on top of the packing.

The solvent was drained to about 1/16 inch above sodium sulfate. The hexane extract (∿2 ml) in a concentration tube was transferred quantitatively to the Florisil column. The concentration tube was washed with about 2 x 2 ml of hexane and loaded in the column. The column was eluted with 100 ml of hexane. The hexane eluate was discarded. The column was then eluted for FMC 54800 with 100 ml of 5% ethyl acetate in hexane (v/v). The solution was evaporated using a Kuderna-Danish to about 5 ml. This solution was transferred quantitatively to a 13 ml centrifuge tube and evaporated using N_2 to the appropriate volume for GC analysis.

E. Analysis

The FMC 54800 was quantitated by gas chromatography employing an electron capture detector. A Hewlett-packard 5880A Level 4 terminal and HP 5713 gas chromatograph was used.

Column Parameters-

Length:	122 cm
Diameter:	2 mm I.D.
Packing:	4% SE-30/6% OV-210 on 100/200 Chromosorb W HP (Supelco)

Instrument Parameters

Injection Port Temperature:	250°C
Column Temperature:	235°C
Detector Temperature:	350°C
Carrier Gas:	5% methane 95% argon at 30 ml/min
Attenuation:	2^{10}

III. QUANTITATION

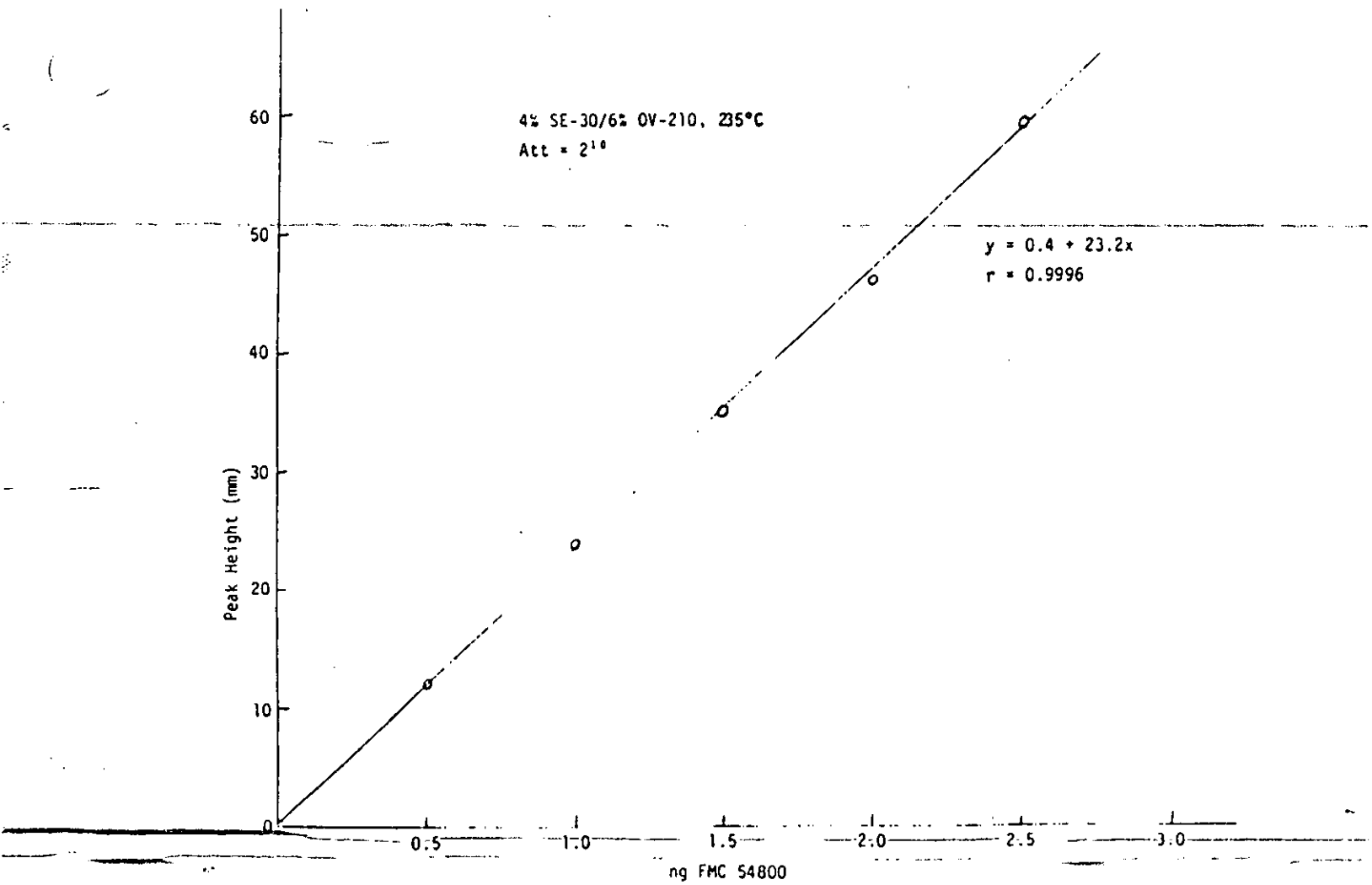
Quantitation of FMC 54800 was either by hand calculation or by the Hewlett-Packard 5880A terminal. Peak height measurement and comparison with a FMC 54800 external standard (1 ng, injected after every two samples) was used to determine ng in the sample injected. By using the appropriate dilution factor to the sample size injected, ppm values were obtained. In essense, the Hewlett-Packard 5880A calculated ppm by using the formula:

$$\text{ppm FMC 54800} = \frac{\text{Peak height unknown x ng standard}}{\text{Peak height standard x mg crop injected}}$$

A standard curve of FMC 54800 was prepared to determine detector linearity over a 0.5 to 2.5 ng injected range (Figure 2). The standard curve was drawn by linear regression analysis (r = 0.9996 y = 0.4 + 23.2x).

FIGURE 2

STANDARD CURVE OF FMC 54800



IV. ANALYTICAL LIMITS

Quantitatively reliable measure of response (method sensitivity) was determined by satisfactory recovery of FMC 54800 from fortified check samples (0.05 ppm). Visual recognition of detector response (method detectability) was possible when the response began to exceed 2 mm in height for FMC 54800 (0.01 ppm). Any apparent response below 2 mm was considered non-detectable (ND). Residue values between method detectability and method sensitivity would be reported as estimated values, as their quantitative reliability was untested.

V. FORTIFICATION RECOVERIES

A. Standard Preparation

The following standards were used for fortification and quantitation.

Ref. No.	Compound	Concentration	Solvent
E2823:2	FMC 54800	(A) 1.0 µg/µl	~~Hexane~~
		10 ng/µl	Hexane
		0.5 ng/µl	Hexane

B. Fortification

Untreated apple samples were fortified before any analytical manipulation. The fortification standard (1.0 and 0.01 µg/µl) was added with an appropriately sized µl syringe directly onto the sample. Table 1 lists recovery data for apples. Representative chromatograms are give in Figure 3.

TABLE 1

RECOVERY OF FMC 54800 FROM FORTIFIED APPLE CHECK SAMPLES

Check Sample I.D.	Fortification Level (ppm)	Recovery Level (ppm)	Percent Recovery
Reagent Blank	0.00	ND[1/]	-
Apple, Check PRZ-222-CJW-83-22A	0.00	ND	-
Apple, Check PRZ-222-CJW-83-22A	0.05	0.057	114
Apple, Check PRZ-222-CJW-83-22A	0.10	0.090	90
Apple, Check PRZ-222-CJW-83-22A	0.20	0.190	95
Apple, Check PRZ-236-8352-3	0.00	ND	-
Apple, Check PRZ-236-8352-3	0.05	0.048	96
Apple, Check PRZ-236-8352-3	0.10	0.088	88
Apple, Check PRZ-236-8352-3	0.20	0.180	90
Apple, Check PRZ-236-8352-3	1.00	0.840	84
Apple, Check PRZ-236-8352-3	2.00	1.720	86
Average ± Standard Deviation			93 ± 9

[1/] ND = <0.01 ppm

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: Sigrid Wilkerson Date: 11/14/83

Title: Research Chemist

Supervisor: R E Cook Date: 14 NOV 83

Title: Manager, Residue Chemistry

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attachments

VII. CHROMATOGRAMS

FIGURE 3

GAS CHROMATOGRAMS FOR FMC 54800 STANDARD, APPLE CHECK AND FORTIFIED APPLE SAMPLES