PAGE 1 of 10	METHOD NO. AG-63	SUBJECT DETERMINATION OF AMETRYNE RESIDUES IN BANANAS, PINEAPPLE, POTATO TUBERS AND SUGAR CANE
EDITION 6/7/68	SUPERSEDES	
Submitted by: A.M. Mattson, R. Kahrs		APPROVED BY: R.T. Murphy

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1.0 SCOPE

This method is for use in determining residues of Ametryne in bananas, pineapple, potato tubers and sugar cane at a level of 0.25 ppm. Ametryne is determined without interference from other s-triazine herbicides (Atrazine and Simazine) which may also be used for weed control in pineapple and sugar cane.

2.0 PRINCIPLE

The crops are extracted by blending with acetonitrile. The acetonitrile is diluted with water and the Ametryne is extracted into dichloromethane. Column clean-up is done with Woelm Alumina of Activity Grade V with two eluants, hexane and benzene : hexane. Final determination is done using gas chromatography employing a Microcoulometric Detector.

3.0 REAGENTS

3.1 Acetonitrile : Reagent grade
3.2 Benzene : Nanograde
3.3 Hexane : Nanograde
3.4 Distilled Water :
3.5 Dichloromethane : Spectroquality
3.6 Sodium Sulfate : Anhydrous granular reagent
3.7 Aluminum Oxide : Woelm Basic Grade I
3.8 Ametryne : Standard
3.9 Aluminum Oxide : Woelm Basic Grade V. Prepared by mixing 85 grams Grade I with 15 grams water and allowed to stand at least 2 hours.

3.10 Filter paper Whatman 2V, 32 cm.
3.11 Filter paper Reeves Angel 802, 32 cm.

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4.0 EQUIPMENT

4.1 Chromatographic Column: These columns have an inner diameter of 18 mm and are 200 mm long. A 100 ml reservoir is attached by joining a 100 ml round bottom flask to the top of the column. The bottom is equipped with a perforated plate.

4.2 Flash Evaporator : Buchi or equivalent

4.3 Air Manifold : N-EVAP by Organomation or equivalent

4.4 Blendor : Osterize or equivalent

5.0 PROCEDURE

5.1 Preparation of the samples

5.1.1 Sugar Cane

Samples of 300-500 grams of sugar cane are chopped using a Hobart Food Cutter.

5.1.2 Bananas

Samples of 300-500 grams of bananas are sectioned with a knife and thoroughly mixed.

5.1.3 Pineapple

After the crown portion is removed, the remainder of the pineapple is sectioned with a knife and thoroughly mixed.

5.1.4 Potatoes

300-500 grams of potatoes are cubed with a knife and thoroughly mixed.

5.2 Extraction of the Sample

A 200 gram subsample of crop is placed in a blendor with 600 mls of acetonitrile. The mixture is thoroughly blended for 5 minutes, let stand for 1 minute, and reblended for 2 minutes.

The liquid extract is then filtered through a double layer of filter paper into a 32 oz bottle. The inner paper is a coarse filter (Reeves Angel 802). The outer paper is a finer filter (Whatman 2V). Both papers are fluted. Assuming 80% water in the commodities, the final solution contains 2 grams of crop sample per 7.6 ml of solvent.

5.3 Partition

A 20 gram aliquot (76 ml) is placed in a 500 ml separatory funnel and 350 ml of distilled water are added. A small amount of sodium sulfate (either granules or solution) is added to prevent emulsion formation. The solution is then extracted with 50 ml of dichloromethane by shaking vigorously for 1 minute. After the layers have separated the dichloromethane is drawn off through a small pad of anhydrous sodium sulfate into a 250 ml Erlenmeyer flask equipped with a 24/40 neck. The extraction is repeated with a 25 ml portion of dichloromethane. The filter pad is rinsed with a small amount of dichloromethane. The extract is then taken just to dryness using a flash evaporator.

5.4 Column Clean-up

A dry packed column is prepared by adding 12.5 grams of Grade V basic alumina to a column which has a small glass wool plug at the bottom. The

column is tapped gently to eliminate channeling. Another glass wool plug is placed at the top of the alumina.

The sample residue is dissolved in 2 ml of benzene. This solution is transferred to the column and allowed to penetrate into the alumina. The flask is rinsed with small portions of hexane which are also allowed to penetrate into the column. The remainder of the hexane is added so that a total of 75 ml of first eluant is used.

When the last of the hexane has gone into the column a clean receiver flask is placed under the column. One hundred and fifty (150) ml of 1:1 benzene : hexane are added to the column to quantitatively elute the Ametryne. This second eluant is evaporated just to dryness on a flash evaporator. The residue is then transferred quantitatively to a small glass vial (2 dram) with dichloromethane. This solution is taken to dryness under a small stream of nitrogen or air.

6.0 DETERMINATION OF AMETRYNE

The residue in the vial is dissolved in an appropriate amount of Nanograde benzene for injection into a gas chromatograph equipped with a Dohrmann Microcoulometric Detector sensitive to sulfur. The conditions used for gas chromatography are given in Table I.

The gas chromatography method is standardized by injecting known amounts of Ametryne. A stock solution of 100 mg of Ametryne standard dissolved in 100 ml of chloroform is prepared. Dilutions are made from this using benzene.

to obtain solutions containing 10 ng/µl.

Peak areas are calculated by triangulation and plotted against the amount of Ametryne injected. Peak areas of unkown samples are compared directly with this graph to obtain the amount of Ametryne present. Results are calculated in terms of ppm by dividing the nanograms of Ametryne found by the milligrams of crop equivalent injected.

7.0 RECOVERY STUDIES

The percent recovery of Ametryne added at 0.25 and 0.50 ppm to pineapple, sugar cane, bananas, and potatoes are given in Table II. Recoveries are given for Ametryne added to the raw agricultural commodity prior to extraction. Recoveries of Ametryne in the presence of 1 ppm of Atrazine and Simazine in pineapples and sugar cane show no decrease. Typical chromatograms obtained for recovery studies are shown in Figures 1 and 2.

8.0 NOTES

8.1 Ametryne is a volatile substance which can be lost if residues are heated or left under air or nitrogen flow too long.

8.2 This procedure can be used if mixed residues of Atrazine or Simazine and Ametryne are expected. Chlorotriazines do not appear to have any effect on the Ametryne recoveries. Atrazine and Simazine can be used in weed control in pineapple and sugar cane. There is some evidence that Prometryne may suppress the recovery of Ametryne.

8.3 If sugar cane samples are dry the extracting solvent should contain a percentage of water to ensure deactivation of the crop material.

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Table I

GAS CHROMATOGRAPHIC CONDITIONS

Instrument:	Microtek MT220 equipped with Dohrmann Microcoulometric Detector.
Column:	3 % Reoplex 400 on Gas Chrom "Q" (60/80 mesh) packed in Pyrex tubing (4 ft x 1/4 in)
Injector Temperature:	225° C
Column Temperature:	210° C
Transfer Temperature:	250° C
Furnace Temperature:	875° C
N_2 carrier:	100 ml/min
O_2 flow:	50ml/min
N_2 purge:	20 ml/min
Attenuation:	100 ohms
Cell:	T-300 sulfur specific
Minimum detection limit:	10 nanograms
Volumes injected:	2 to 8 microliters
Retention time:	3.8 minutes

Table II

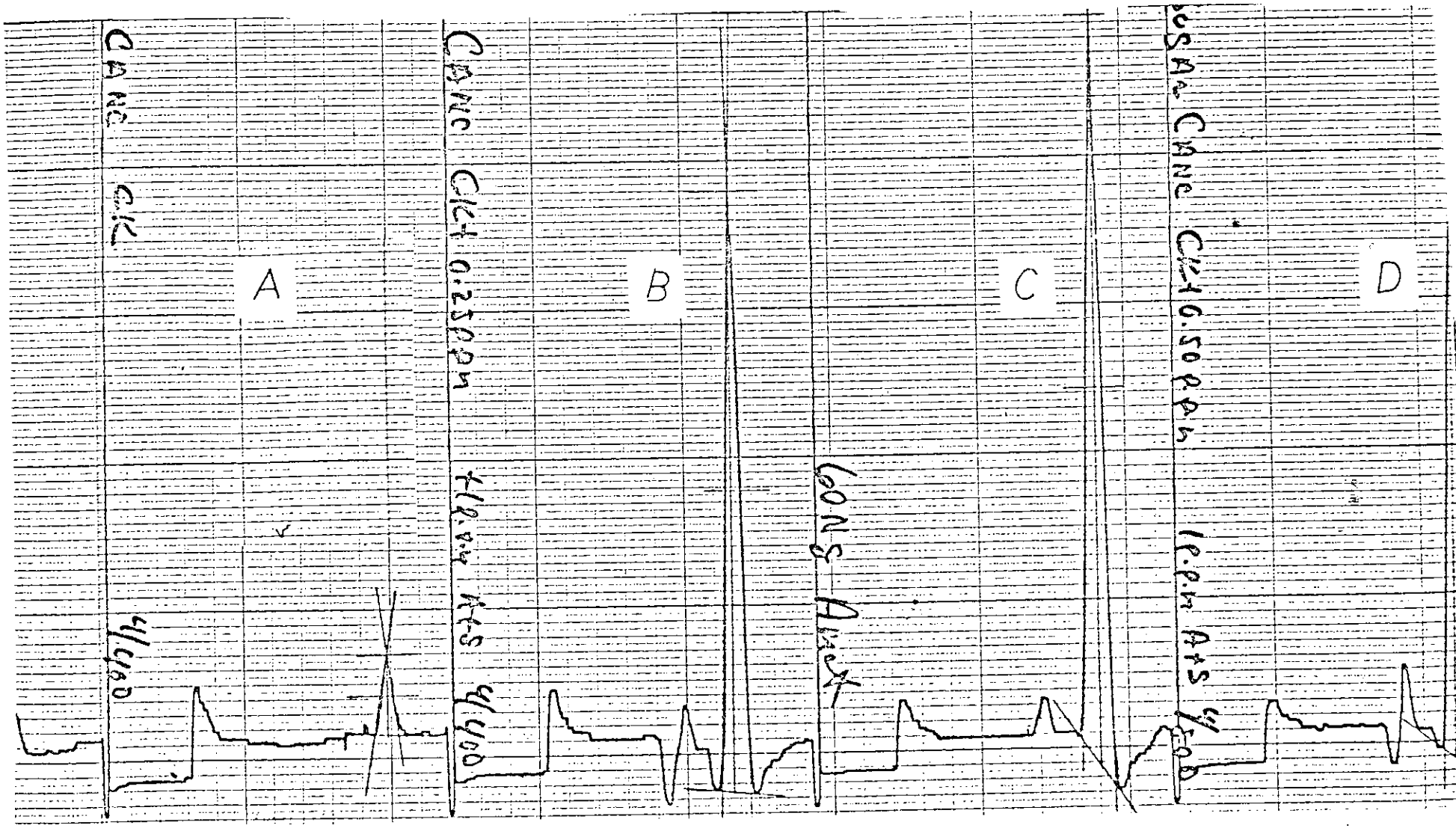
RECOVERIES OF AMETRYNE ADDED TO CROPS BEFORE EXTRACTION

Crop	Ametryne Added (ppm)	Crop Equivalent Injected (mg)	Area of Peak(in^2) Gross	Area of Peak(in^2) Corr.	Ametryne Found (ng)	Ametryne Found (ppm)	Recovery (%)
Banana	0.00	200	0.060	-	-	<0.10	-
	0.25	200	0.318	0.258	51	0.26	104
	0.50	100	0.340	0.310	48	0.48	96
Potatoes	0.00	200	0.075	-	-	<0.10	-
	0.25	200	0.545	0.470	46	0.23	92
	0.50	100	0.572	0.534	52	0.52	104
Pineapple	0.00	200	0.065	-	-	<0.10	-
	0.25	200	0.335	0.270	53	0.27	108
	0.50	100	0.280	0.247	50	0.50	100
Pineapple	0.00*	200	0.000	-	-	<0.10	-
	0.25*	200	0.265	0.265	55	0.28	112
	0.50*	100	0.275	0.275	57	0.57	114
Sugar Cane	0.00	200	0.050	-	-	<0.10	-
	0.25	200	0.290	0.240	30	0.15	60
	0.50	100	0.440	0.415	38	0.38	76
Sugar Cane	0.00*	200	0.055	-	-	<0.10	-
	0.25*	200	0.405	0.345	31	0.16	64
	0.50*	100	0.420	0.392	36	0.36	72

* 1 ppm each of Atrazine and Simazine were also added.

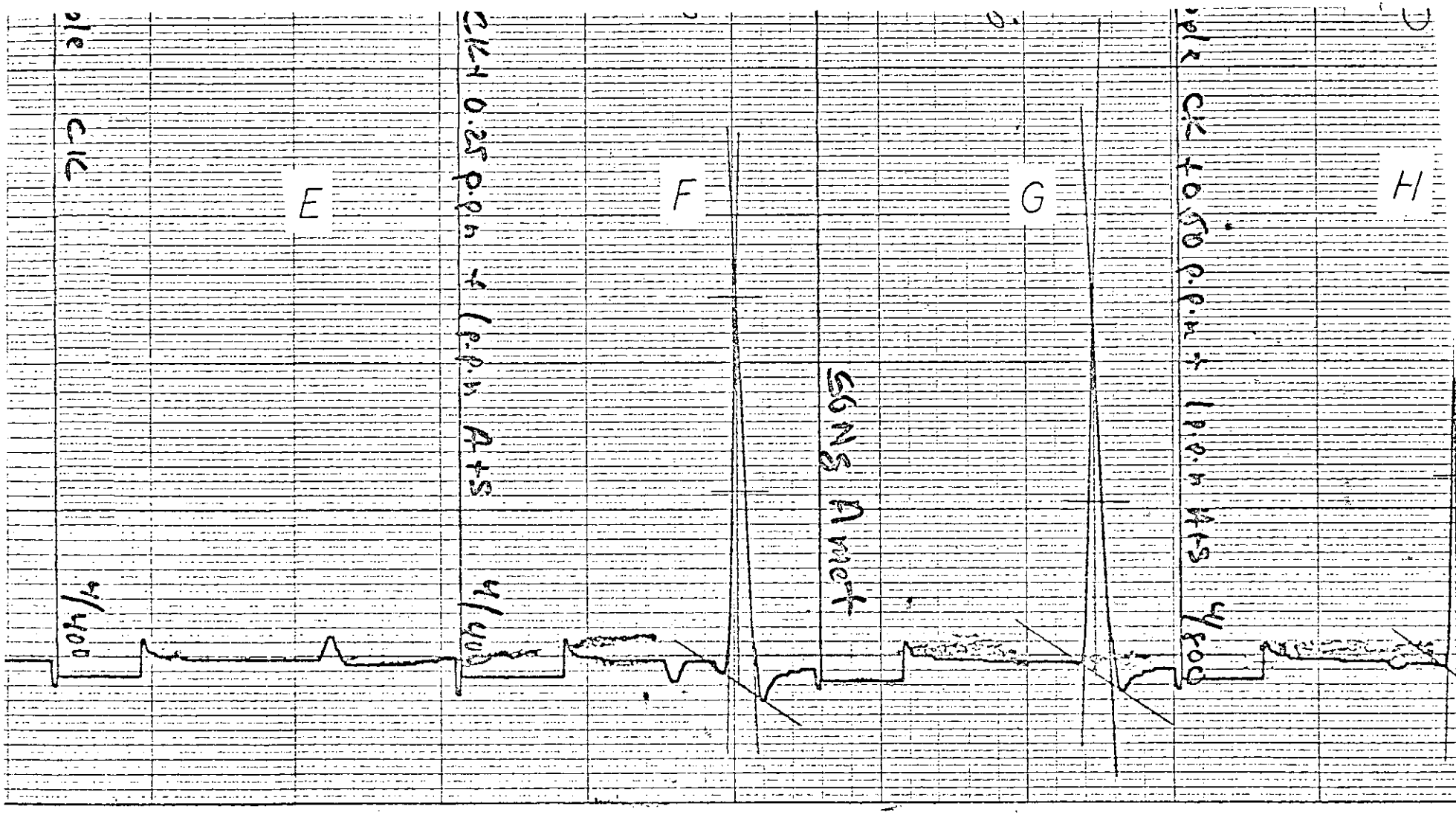
Figure 1

GAS CHROMATOGRAMS OF RECOVERY OF AMETRYNE ADDED TO SUGAR CANE



A. Sugar cane check (200 mg crop injected).
B. Check plus 0.25 ppm of Ametryne and 1 ppm each of Atrazine and Simazine (200 mg crop injected).
C. Ametryne standard, 60 ng.
D. Check plus 0.50 ppm of Ametryne and 1 ppm each of Atrazine and Simazine (100 mg crop injected).

GAS CHROMATOGRAMS OF RECOVERY OF AMETRYNE ADDED TO PINEAPPLE



E. Pineapple check (200 mg crop injected).
F. Check plus 0.25 ppm Ametryne and 1ppm each of Atrazine and Simazine (200 mg crop injected).
G. Ametryne standard, 50 ng.
H. Check plus 0.50 ppm Ametryne and 1 ppm each of Atrazine and Simazine (100 mg crop injected).