BIOCHEMISTRY DEPARTMENT

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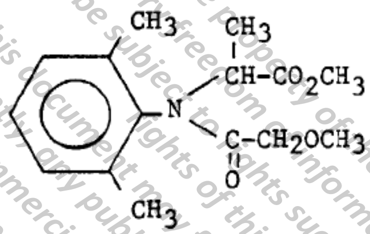
PAGE	METHOD No.	SUBJECT
1 of 14	AG-325	Gas Chromatographic Residue Determination of CGA-48988 in Crop Samples
EDITION 4/17/78		
SUBMITTED BY: 5.1.2.e Woo		APPROVED BY: 5.1.2.e Woo

1.0 SCOPE

This method is used for the extraction, cleanup and final determination of CGA-48988 residues in crop samples. The limit of detection is 1.0 ppm for tobacco and 0.05 ppm for potato samples.

CGA-48988:

N-(2,6-dimethylphenyl)-N-(2'-methoxyacetyl)alanine methyl ester.



2.0 PRINCIPLE

Residues of parent CGA-48988 are extracted from crop samples by blending with 20% water in methanol for 10 minutes. An aliquot of the extract is evaporated, acidified and partitioned with dichloromethane. The organic phase is evaporated to dryness and cleaned up by chromatography on a Grade V Alumina column. Final determination is made using a gas chromatograph equipped with an alkali flame ionization detector operating in the nitrogen-specific mode.

3.0 APPARATUS

- Bottle, Boston round narrow mouth, 16 oz.
- Flask, round bottom, 250-ml, 500-ml, 1000-ml.
- Flask, erlenmeyer, 250-ml.
- Waring Blender

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- Funnel, 12.5-cm size.
- Filter paper, Whatman 2V, 32-cm.
- Separatory funnel, 250-ml with Teflon stopcock.
- Column, chromatographic, 19 mm i.d., with Teflon stopcock.
- Air manifold, N-Evap. by Organomation or equivalent.
- Rotary evaporator, Büchi or equivalent.
- Vortex mixer.
- Hobart Food Cutter, Hobart Manufacturing Co., Troy, Ohio.
- Sample Vial, 20-ml.

4.0 REAGENTS

- Alumina, basic, (Woelm) W200; Activity Grade V (prepared by addition of 76 ml water to 324 g activity grade Super I alumina).
- Ethyl ether, anhydrous, reagent grade.
- Hexane, distilled in glass, Burdick and Jackson
- Acetone, distilled in glass, Burdick and Jackson
- Methanol, reagent grade.
- 2N Hydrochloric acid
- CGA-48988, analytical standard

5.0 PROCEDURE

5.1 Preparation of Sample

A representative sample of 300-400g of the crop is chopped in a Hobart Food Cutter.

5.2 Extraction

5.2.1 Tobacco

5.2.1.1 Weigh 15 g of the finely chopped sample into a Waring blender jar. Add 300 ml of 20% water in methanol. Blend the sample for 10 minutes using slow speed (use a Variac to regulate the speed).

5.2.1.2 Filter the extract through a Whatman 2V filter paper into a 16-oz. bottle (Boston round, narrow mouth).

5.2.1.3 Transfer a 5-g aliquot (100 ml) of the extract to a 1000-ml round bottom flask. Evaporate to about 5 ml using the rotary evaporator. Add 150 ml of distilled water and 3 ml of 2N hydrochloric acid.

5.2.2 Potatoes

5.2.2.1 Weigh 50 g of the finely chopped sample in a 100-ml beaker. Transfer the sample to a Waring blender using 500 ml of 20% water in methanol. Blend the sample for 10 minutes using slow speed.

5.2.2.2 Filter the extract through a Whatman 2V filter paper into a 16-oz. bottle (Boston round, narrow mouth).

5.2.2.3 Transfer a 20-g aliquot (200 ml) to a 1000-ml round bottom flask. Evaporate to a small volume using the rotary evaporator. Add 100 ml of distilled water and 3 ml of 2N hydrochloric acid.

5.3 Partition

Transfer the solution in Step 5.2.1.3 or 5.2.2.3 to a 250-ml separatory funnel. Rinse the flask with 50 ml of dichloromethane and transfer to the separatory funnel.

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5.3.1 Shake the separatory funnel for 30 seconds and allow the layers to separate. Drain the dichloromethane phase through a funnel containing absorbant cotton into a 250-ml round bottom flask.

5.3.2 Repeat step 5.3.1 with two additional 50-ml portions of dichloromethane. Rinse the cotton with 15 ml of dichloromethane and combine the organic extracts.

5.3.3 Evaporate the dichloromethane solution to dryness using a rotary evaporator (bath temperature 40°C).

5.4 Column Cleanup

5.4.1 Fill a chromatographic column (19 mm i.d. containing glass wool at the bottom) with hexane. Measure 30 ml of Grade V Alumina using a graduated cylinder and add to the column. Gently tap to remove any trapped air bubbles. (The column is approximately 11 cm in height.)

5.4.2 Drain the hexane until the liquid layer reaches the top of the alumina.

5.4.3 Load the sample (step 5.3.3) onto the column using three 5-ml portions of hexane. (Use an ultrasonic bath to aid dissolving the sample in the first portion of hexane.) Discard the eluate.

5.4.4 Rinse the sample flask with 100 ml of 1:9 ethyl ether:hexane . Load onto the column and discard the eluate.

5.4.5 Add 100 ml of 1:2 ethyl ether:hexane to the column. Collect the eluate in a 250-ml round bottom flask.

5.4.6 Evaporate the sample eluate to dryness using a rotary evaporator (bath temperature 40°C).

5.4.7 Quantitatively transfer the residue to a 20-ml sample vial using two 5-ml portions of acetone.

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5.4.8 Evaporate the sample to dryness using a gentle stream of air in an N-Evap.

6.0 <u>GAS CHROMATOGRAPHIC ANALYSIS</u>

Sample residues (Section 5.4.8) are dissolved in acetone for analysis. CGA-48988 residues are detected by gas chromatography, using an alkali flame ionization detector in the nitrogen-specific mode. Gas chromatographic conditions are given in Table 1.

6.1 Standardization

6.1.1 Prepare a stock solution containing 100 mg of CGA-48988 in 100 ml of acetone. Serial dilutions should be made with acetone until a working solution containing 1 ng/µl is achieved.

6.1.2 Standardize the gas chromatograph, operating under the conditions specified in Table 1, by injecting 2- to 8-µl aliquots of the 1 ng/µl solution. This represents a 2 to 8 nanogram working range.

6.1.3 Determine the peak height or area for injected standards. Typical chromatograms of standards are shown in Figure 3.

6.1.4 Construct a standard curve, plotting detector response (peak height or area) versus nanograms injected. A typical standard curve is presented in Figure 2.

6.2 Detection of Sample Residues

6.2.1 Dissolve the residue from Section 5.4.8 in an appropriate volume of acetone.

6.2.2 Inject an aliquot into the gas chromatograph. Compare peak heights or areas of unknown samples with the standard curve to determine

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the CGA-48988 amounts in the aliquot injected. Typical chromatograms of treated tobacco are shown in Figures 4 and 5. Typical chromatograms of treated potato are shown in Figure 6.

6.2.3 Calculate residue results in ppm by the following equation:

$$\text{ppm} = \frac{\text{CGA-48988 found (ng)}}{\text{mg crop injected}} \div R$$

The recovery factor (R) is determined using a fortified control sample carried through the procedure and is expressed as a decimal (100% = 1.00, etc.).

7.0 DISCUSSION

The percent recovery of CGA-48988 from tobacco samples (green and cured) spiked at the 1.0 to 60 ppm level ranged from 71 to 95 percent, with an average of 86 ± 7% (n = 13).

The percent recovery of CGA-48988 from potato samples spiked at the 0.05 to 5 ppm level ranged from 74 to 97 percent, with an average of 86 ± 8% (n = 10).

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Figure 1: Flow Diagram of the Analytical Procedure for the Determination of CGA-48988 in Crop

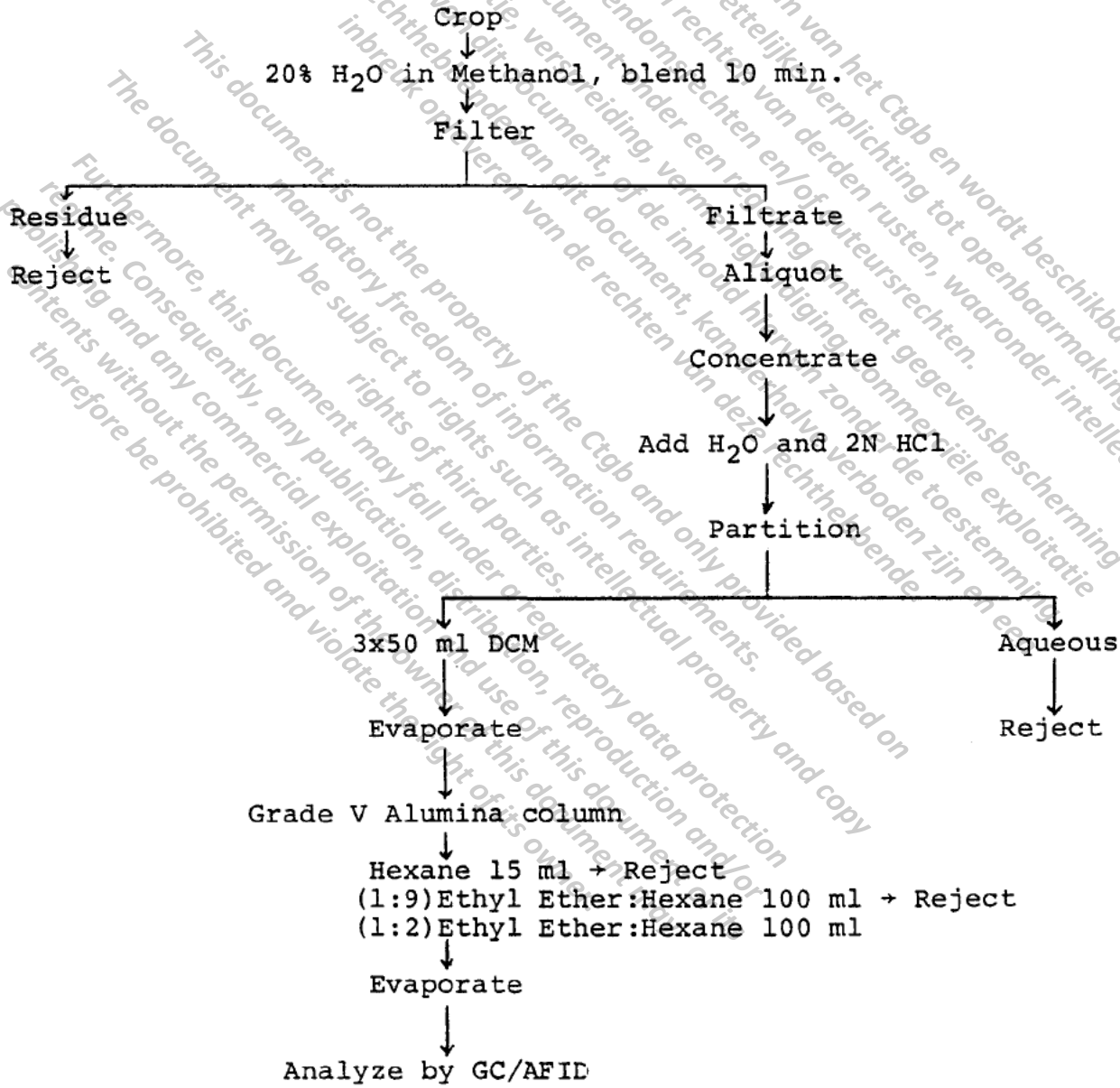

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Figure 2: Typical Standard Curve of CGA-48988 by Alkali Flame Ionization Detector (See Table 2 for Data)

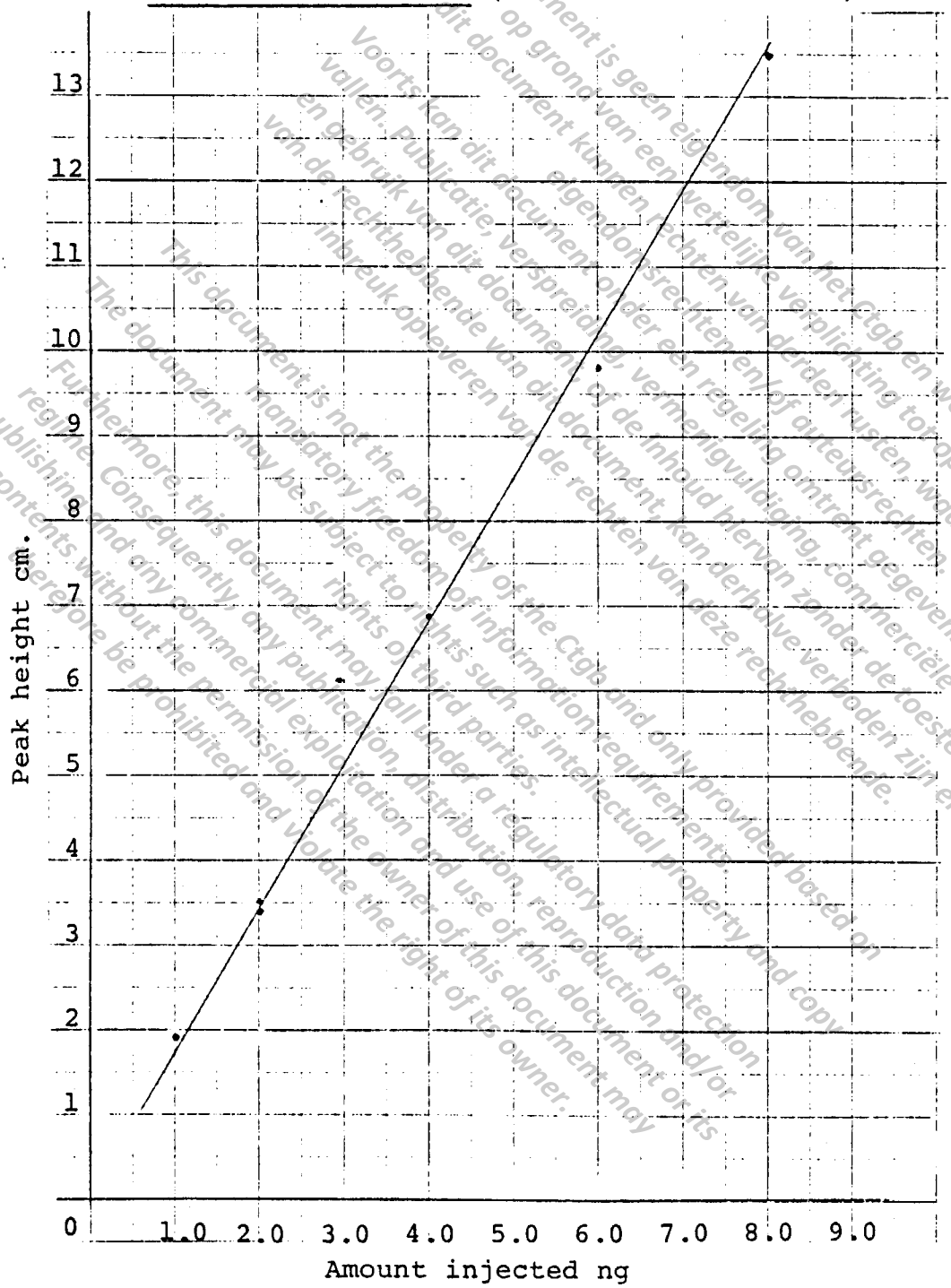
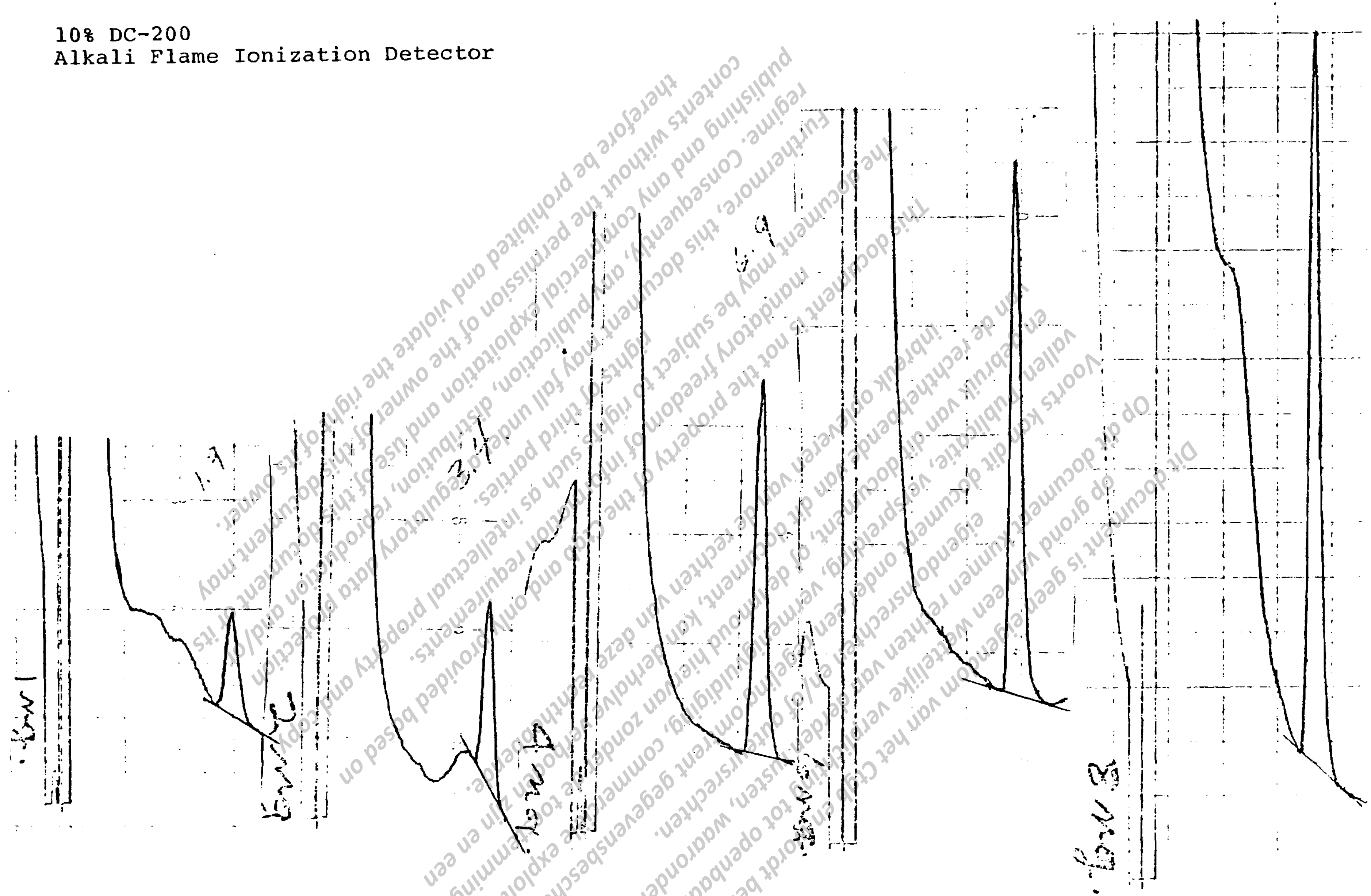


Figure 3: Typical Gas Chromatography of CGA-4 ,88 Standards

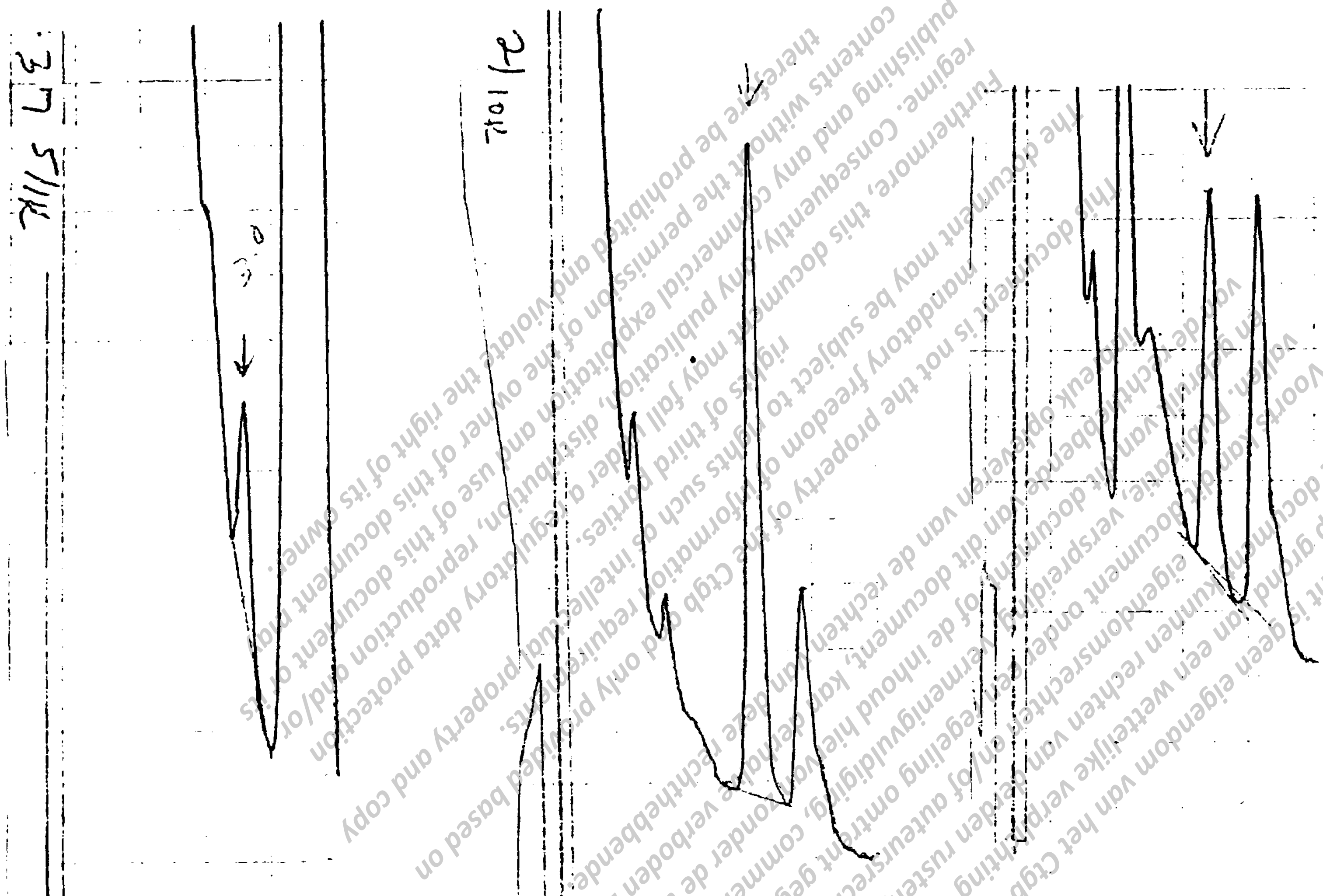
10% DC-200
Alkali Flame Ionization Detector



Amount Injected: 1 ng 2 ng 4 ng 6 ng 8 ng

Figure 4: Typical GC Scans for CGA-48988 Analyses of Green Tobacco Samples

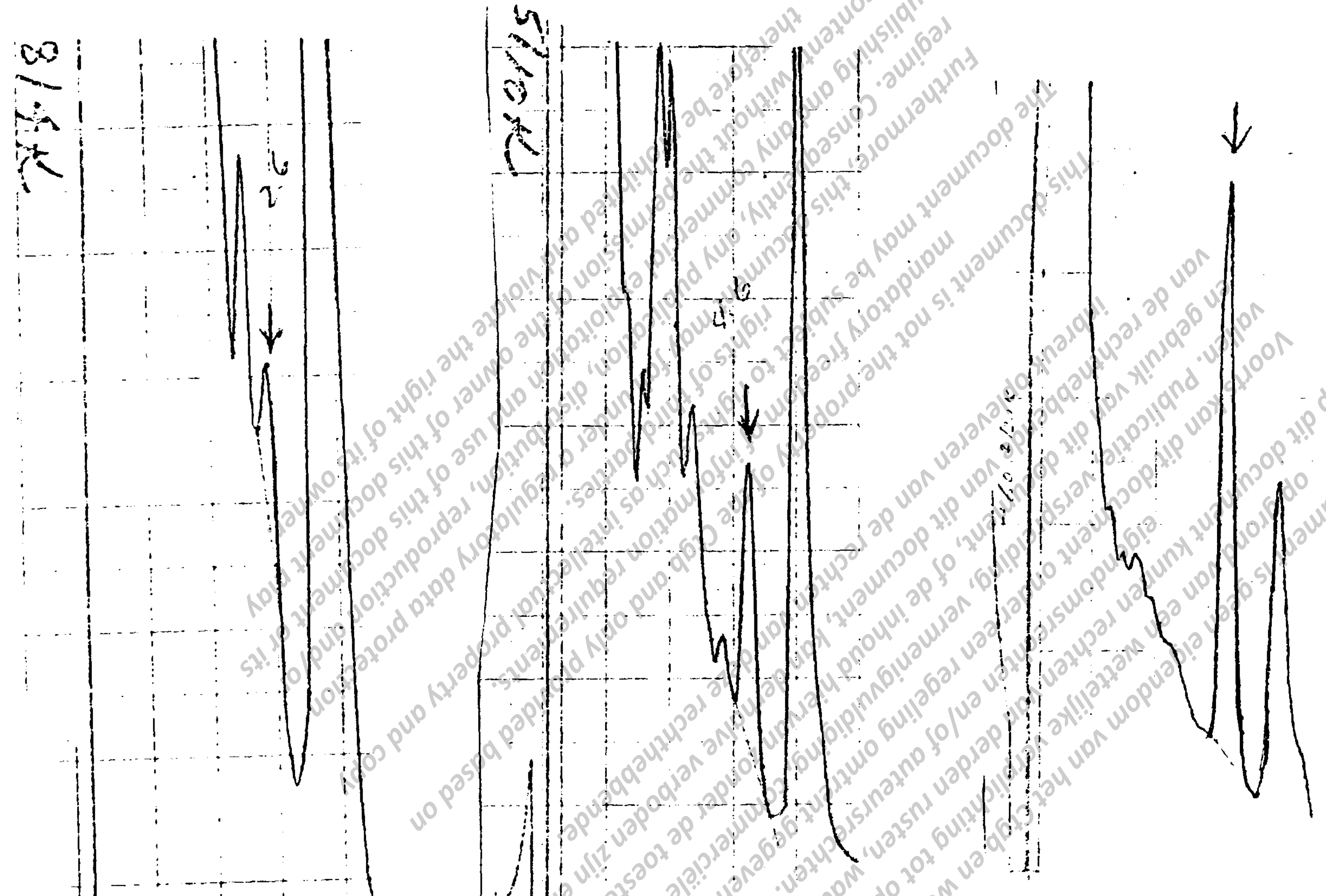
AG-A 4732 IV



A	B	C
Check	Check + 5.0 ppm	3 lb. a.i./A treated
25 mg injected	1.0 mg injected	1.0 mg injected
Found 1.3 ng	Found 4.4 ng	Found 3.1 ng
<1.0 ppm	4.4 ppm	3.1 ppm
	88% Rec.	

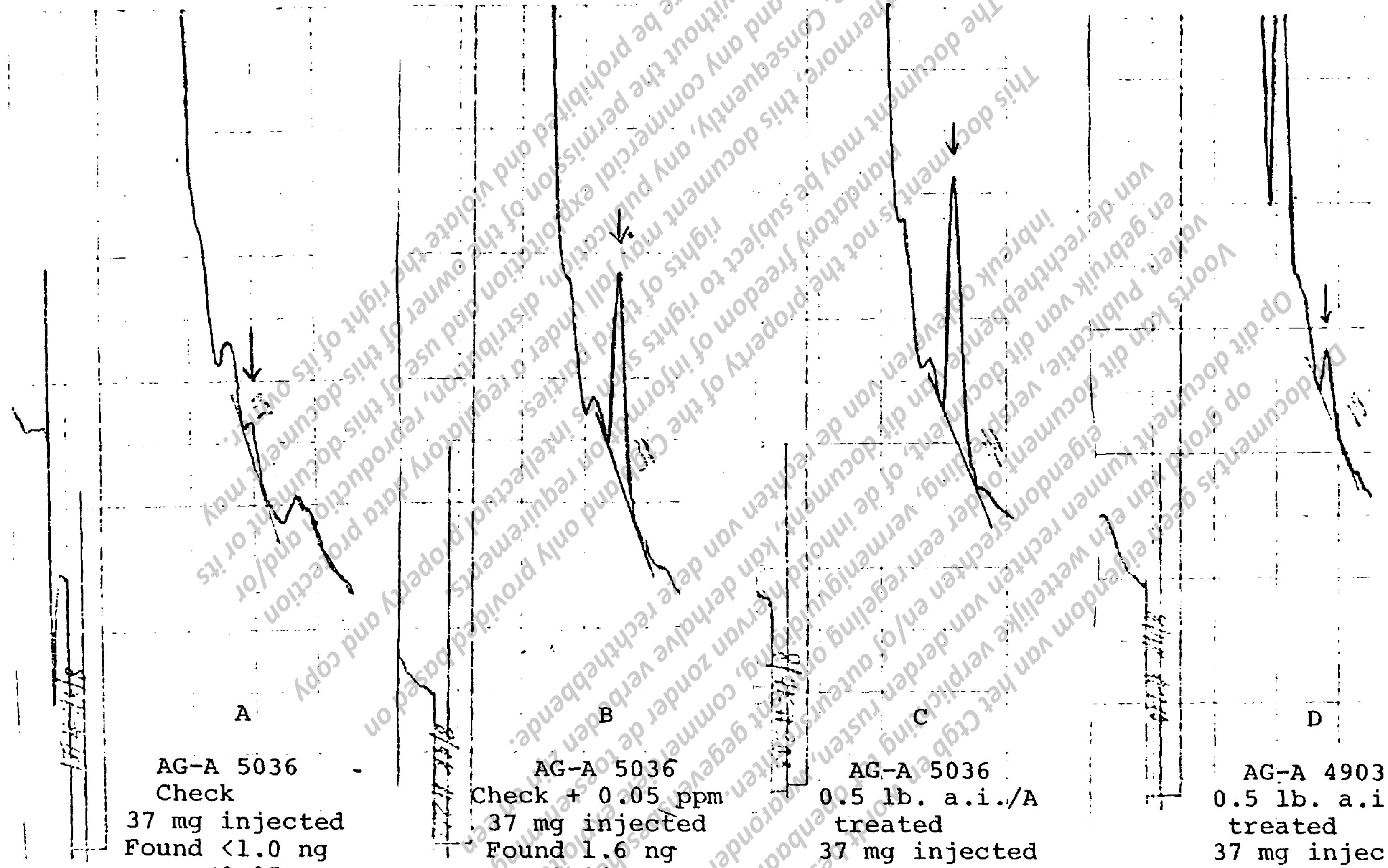
Figure 5: Typical GC Scans for CGA-48988 Analysis of Cured Tobacco Samples

AG-A-4732 IV



A	B	C
Check 10 mg injected Found 1.5 ng <1.0 ppm	Check + 1.0 ppm 2.5 mg injected Found 2.7 ng 0.93 ppm 92.5% Rec.	3 lb. a.i./A treated 2.5 mg injected Found 5.2 ng 2.07 ppm

Figure 6: Typical GC Scans for CGA-48988 Analyses of Potato Samples



A	B	C	D
AG-A 5036	AG-A 5036	AG-A 5036	AG-A 4903
Check	Check + 0.05 ppm	0.5 lb. a.i./A treated	0.5 lb. a.i./A treated
37 mg injected	37 mg injected	37 mg injected	37 mg injected
Found <1.0 ng	Found 1.6 ng	Found 2.2 ng	Found <1.0 ng
<0.05 ppm	0.04 ppm	0.06 ppm	<0.05 ppm
	86.1% Rec.		

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TABLE I: GAS CHROMATOGRAPHIC CONDITIONS

Instrument	Tracor 220 equipped with an Alkali flame ionization detector (Perkin Elmer)
Column Packing	10% DC 200 on Gras Chrom Q (80/100 mesh)
Column	Pyrex 6' x 4 mm i.d.
Temperatures	
Column	220°C
Injector	265°C
Detector	255°C
Gas Flows	
He carrier	60 ml/min.
H_2 reaction gas	3.0 ml/min (regulated)
Compressed air	100 ml/min.
Attenuation	1 x 8
Bead Current Setting	745
Minimum Detection Limit	1 nanogram
Volumes Injected	2-8 µl
Chart Speed	1 cm/minute
Retention Time	3.5 minutes

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TABLE II: TYPICAL STANDARD CURVE

Amount CGA-48988 injected (ng)	Peak height (cm)
1.0	1.9
2.0	3.4
4.0	6.9
8.0	13.5
2.0	3.5
6.0	9.8

See Figure 2 for Plot

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