MOBAY CHEMICAL CORPORATION
AGRICULTURAL CHEMICALS DIVISION

Research and Development Department

TITLE: Gas Chromatographic Method for Determination of ®GUTHION Residues in Plant Material

AUTHORS: G. L. Westberg[1] and B. D. Becker

ABSTRACT: An analysis procedure is described for GUTHION and GUTHION oxygen analog residues in various crops. A silica gel column is used to separate GUTHION and its oxygen analog. Residues are determined by gas liquid chromatography with flame photometric detection.

DATE: February 13, 1981

APPROVED BY: D. R. Flint
D. R. Flint

REASON FOR REVISION: To add Addendum I

DATE REVISED: March 21, 1984

GUTHION is a Reg. TM of the Parent Company of Farbenfabriken Bayer GmbH, Leverkusen
[1]Assistant Director of Morse Laboratories, Inc., Sacramento, California

Gas Chromatographic Method for Determination of GUTHION Residues in Plant Material

INTRODUCTION

The analysis procedure for GUTHION and its oxygen analog describes extraction procedures for moist crops containing chlorophyll and oilseed crops. A chromatographic column clean-up is required for both crop types, utilizing alumina, Super-Cel and Sea Sorb. Separation of GUTHION and its oxygen analog is achieved by use of a silica gel column.

REAGENTS REQUIRED

1. Acetone - pesticide grade.
2. Acetonitrile - pesticide grade.
3. Alumina - chromatographic grade, acid washed (Merck).
4. Benzene - pesticide grade.
5. Chloroform - pesticide grade.
6. Ethyl acetate - pesticide grade.
7. Glass wool - Pyrex.
8. Hyflo Super-Cel - Filter aid (Johns - Manville).
9. Isopropyl alcohol - pesticide grade.
10. Petroleum ether - pesticide grade.
11. Sea Sorb 43 - magnesium oxide (Westvaco).
12. Silica gel - (10% water deactivated) (Fisher).
13. Sodium sulfate - anhydrous.
14. Standard solutions - GUTHION (0.10 ppm in ethyl acetate) and GUTHION PO (0.20 ppm in ethyl acetate) (Mobay Chemical Corporation, Kansas City, Missouri).

EQUIPMENT REQUIRED

1. MT-220 gas chromatograph (or equivalent) equipped with a flame photometeric detector in the phosphorus mode.
2. Rotary vacuum evaporator with water bath set at 40°C.
3. Waring Blendor (2 speed) equipped with a 1 qt. jar.
4. Hobart Food Chopper (for moist crops).

5. Wiley Mill (for oilseed crops).

6. Chromatographic columns, 2 cm i.d. x 45 cm, modified with 300 ml bell top reservoir.

7. Standard laboratory glassware.

PROCEDURE

I. Extraction

A. Moist Crops

1. Weigh 50 grams of chopped frozen sample into a 1 qt. Waring Blendor jar.

2. Add 200 ml of acetone and blend at high speed for 10 minutes.

3. Make to the 300 ml mark with distilled water.

4. Blend for an additional 2 minutes.

5. Filter through a 32 cm Whatman No. 12 fluted filter paper into a 1000 ml separatory funnel.

6. Rinse the funnel and filter paper with 20 ml of acetone.

7. Extract filtrate vigorously for one minute with 400 ml chloroform.

8. Drain the chloroform through a 32 cm Whatman No. 12 fluted filter paper containing 2 tablespoonfuls of Super-Cel in the bottom of the cone. Collect in a 1000 ml round bottom flask.

9. Extract a second time with 100 ml of chloroform.

10. Use this chloroform to wash the filter paper and Super-Cel.

11. Evaporate the combined chloroform extracts on a rotary evaporator to an approximate volume of 2 ml.

12. Evaporate just to dryness with an air jet. Proceed to step II.B.

B. Oilseed Crops

1. Weigh 50 grams of finely ground sample into a 500 ml boiling flask.

2. Add 300 ml of petroleum ether and attach to a reflux condenser. Reflux for 1 hour (boiling range 30° to 60°C).

3. Filter the extract through a pledget of glass wool in a funnel. Rinse with two 50 ml portions of petroleum ether.

4. Collect the filtrate in a 500 ml separatory funnel. Extract the petroleum ether with 50 ml of acetonitrile.

5. Extract the petroleum ether again with three successive 50 ml portions of acetonitrile. Collect acetonitrile extracts in a 500 ml separatory funnel.

6. Rinse the acetonitrile with 200 ml petroleum ether. Collect acetonitrile in a 500 ml round-bottom flask.

7. Extract the petroleum ether with four successive 10 ml washes of acetonitrile.

8. Combine acetonitrile portions and evaporate to an approximate volume of 2 ml.

9. Evaporate just to dryness with a stream of air. Proceed to step II.B.

II. Chromatographic Column Clean-Up

A. Preparation of the Chromatographic Column

1. The column is maintained under suction by means of an aspirator and suction flask at all times during preparation and operation, with the exception that as each layer of adsorbent is introduced, the column is removed from suction and it is tamped until visual observation indicates that all of the trapped air has been liberated. Tamping is accomplished by tapping the lower end of the column on a wooden block. Satisfactory tamping is indicated when no air is observed to escape from the powder layer and no channels are visible along the sides of the column.

2. Successive layers of two grams Super-Cel, five grams of a mixture of Sea Sorb 43:Super-Cel 1:2, ten grams alumina (acid-washed), and twenty grams Na_2SO_4 (anhydrous) are introduced into the column. Each layer of adsorbent is tamped in turn.

3. Fifty milliliters of isopropyl alcohol are introduced to equilibrate the column.

B. Clean-Up Procedure

1. Dissolve the remaining residue (from step I A or B) in 25 ml of isopropyl alcohol. Boil gently if necessary to effect solution. Cool.

2. Pour the sample into the chromatographic column, just as the last of the equilibrating isopropyl alcohol passes into the sodium sulfate layer.

3. Rinse the beaker with an additional 25 ml of isopropyl alcohol and pour this into the column.

4. Wash with isopropyl alcohol until 160 ml of effluent have been collected.

5. Transfer the effluent into a 250 ml round-bottom flask and evaporate just to dryness on a rotary evaporator.

III. Silica Gel Column for Separation of GUTHION (PS) and GUTHION Oxygen Analog (PO)

1. Tamp a plug of glass wool into a glass chromatographic tube (20 x 400 mm with a 300-ml integral reservoir).

2. Fill the glass column with benzene. While stirring, add 25 g of silica gel (10% water deactivated). Tap column gently to dislodge air bubbles.

3. Cover the silica gel layer with 1/2 inch Superbrite beads.

4. Transfer the sample to the column using three 10-ml portions of benzene, allowing each portion to pass into the top layer before adding the next portion.

5. Add 250-ml benzene to column. Discard the benzene eluant.

6. Add 250-ml of 2% acetonitrile in benzene. Collect total eluant in a 500-ml flask (PS fraction). Change receivers.

7. Add 250-ml of 20% acetonitrile in benzene. Collect total eluant in a 500-ml flask (PO fraction).

8. Evaporate the solvent from each fraction on a rotary vacuum evaporator using a water bath at 40°C.

9. Remove the last traces of solvent from each residue with a stream of dry air.

10. Dissolve each residue in 5 ml of ethyl acetate.

IV. Gas Chromatographic Measurement of GUTHION and its Oxygen Analog

1. Use an MT-220 gas chromatograph (or equivalent) equipped with a flame-photometeric detector in the phosphorus mode.

2. Column description: 20-cm x 2 mm I.D. glass column packed with 15% QF-1 on 100/120 mesh Gas Chrom Q.

3. Carrier gas: Nitrogen, 100 ml/min for PS, 120 ml/min for PO.

4. Operating temperatures:

   Column oven : for PS - 235°C; for PO - 240°C
   Detector : 200°C
   Injection port: 230°C

5. Inject a 5 µl aliquot of a 0.10 ppm GUTHION standard solution (or a 0.2 ppm GUTHION oxygen analog solution). Adjust the instrument attenuation to achieve a standard response >50% of scale. Inject a standard aliquot for each sample aliquot injected.

6. Compare the response of the sample injection to it's corresponding standard. Identify the compounds by their retention times on the strip chart.

7. Measure the peak response in cm. The general equation for calculation of ppm is:

$$ppm = \frac{\text{Response (sample)}}{\text{Response (standard)}} \times \frac{\text{ng of Standard injection}}{\text{Sample Weight (gm)}} \times \frac{\text{Final Vol. (ml)}}{\text{Spl Inj Vol. } (\mu l)}$$

8. To convert GUTHION oxygen analog residue to GUTHION-equivalent residue:

   ppm (from above) x 1.053 = ppm GUTHION equivalents.

LITERATURE CITED

1. Adams, J. M. 1964. "Colorometric Determination of GUTHION Residues in Plant Material," Mobay Ag Chem Report No. 13534.

2. Meagher, W. R., Adams, J. M., Anderson, C. A., MacDougall, D. 1960. Agricultural and Food Chemistry, Vol. 8, No. 4, Page 282, July/Aug. 1960.

ADDENDUM No. 1

The purpose of this Addendum is to give modifications to Method No. 69523 which simplify the procedure for analysis of residues of GUTHION and its oxygen analog in various crops. The modifications change the oil seed partition (step IB); eliminate the chromatographic column clean-up (step II); and change the collection of the GUTHION PS fraction (step III, 6).

I. Extraction

A. Moist Crops - No changes.

B. Oilseed Crops

1. Weigh 50 grams of finely ground sample into a 500 ml boiling flask.

2. Add 300 ml of petroleum ether and attach to a reflux condenser. Reflux for 1 hour (boiling range 30° to 60°C).

3. Filter the extract through a pledget of glass wool in a funnel. Rinse with two 50 ml portions of petroleum ether.

4. Collect the filtrate from step 3, in a 1 liter separatory funnel.

5. Put 100 ml of petroleum ether into a 500 ml separatory funnel.

6. Add 100 ml of acetonitrile to the 1-liter separatory funnel (step 4); shake the funnel for 30 seconds. Allow the layers to separate. Drain the lower layer into the 500 ml separatory funnel (step 5). Shake this funnel for 30 seconds. After the layers have separated, drain the bottom layer into a 1-liter round bottom flask.

7. Repeat step 6 two more times with fresh 100 ml portions of acetonitrile.

8. The acetonitrile is evaporated just to dryness on a rotary vacuum evaporator. The last traces of solvent are removed with a gentle stream of nitrogen.

9. Add 10 ml of ~~benzene~~ toluene to the round bottom flask.

10. Proceed to step III.

II. Chromatographic Column Clean-up - Eliminate

III. Silica Gel Column for Separation of GUTHION (PS) and GUTHION Oxygen Analog (PO)

1. Tamp a plug of glass wool into a glass chromatographic tube (20 x 400 mm with a 300-ml integral reservoir).

2. Fill the glass column with ~~benzene~~ toluene. While stirring, add 25 g of silica gel (10% water deactivated). Tap column gently to dislodge air bubbles.

3. Cover the silica gel layer with 1/2 inch ~~Superbrite beads~~ anhydrous Na_2SO_4.

4. Transfer the sample to the column using three 10-ml portions of ~~benzene~~ toluene, allowing each portion to pass into the top layer before adding the next portion.

5. Add 150 ml ~~benzene~~ toluene to the column. Discard the ~~benzene~~ toluene eluant.

6. Add 100 ml of ~~benzene~~ toluene. Collect the eluant in a 500 ml flask (PS fraction). Add ~~250~~ 300 ml of 2% acetonitrile in ~~benzene~~ toluene. Collect the total eluant in the 500 ml flask (PS fraction). Change receivers.

7. Add ~~250~~ 300-ml of 20% acetonitrile in ~~benzene~~ toluene. Collect total eluant in a 500-ml flask (PO fraction).

8. Evaporate the solvent from each fraction on a rotary vacuum evaporator using a water bath at ~~40°C~~ 58°C.

9. Remove the last traces of solvent from each residue with a stream of dry air.

10. Dissolve each residue in 5 ml of ethyl acetate. Proceed to step IV.

IV. Gas Chromatographic Measurement of GUTHION and its Oxygen Analog

1. Use an MT-220 gas chromatograph (or equivalent) equipped with a flame photometric detector in the phosphorus mode.

2. Column description: 20-cm x 2 mm I.D. glass column packed with 15% QF-1 on 100/120 mesh Gas Chrom Q.

3. Carrier gas: Nitrogen, 100 ml/min for PS, 150 ml/min for PO.

4. Operating temperatures:

Column oven	:	for PS - 235°C; for PO - 240°C
Detector	:	200°C
Injection Port	:	240°C

5. Inject a 5 µl aliquot of a 0.10 ppm GUTHION standard solution (or a 0.2 ppm GUTHION oxygen analog solution). Adjust the instrument attenuation to achieve a standard response >50% of scale. Inject a standard aliquot for each sample aliquot injected.

6. Compare the response of the sample injection to it's corresponding standard. Identify the compounds by their retention times on the strip chart.

7. Measure the peak response in cm. The general equation for calculation of ppm is:

$$ppm = \frac{\text{Response (sample)}}{\text{Response (standard)}} \times \frac{\text{ng of Standard injection}}{\text{Sample Weight (gm)}} \times \frac{\text{Final Vol. (ml)}}{\text{Spl Inj Vol. (µl)}}$$

8. To convert GUTHION oxygen analog residue to GUTHION-equivalent residue:

   ppm (from above) x 1.053 = ppm GUTHION equivalents.

DISCUSSION

Recovery experiments were run with GUTHION (PS) and GUTHION oxygen analog (PO) added to strawberries and peanut meats by fortifying the samples with 1.0 ppm GUTHION PS and GUTHION PO before extraction. Recoveries ranged from 82 to 102% for GUTHION PS. The GUTHION PO recoveries were 108 to 111%. Chromatograms for the controls and recoveries on strawberries and peanut meats are shown in Figures 1-8.

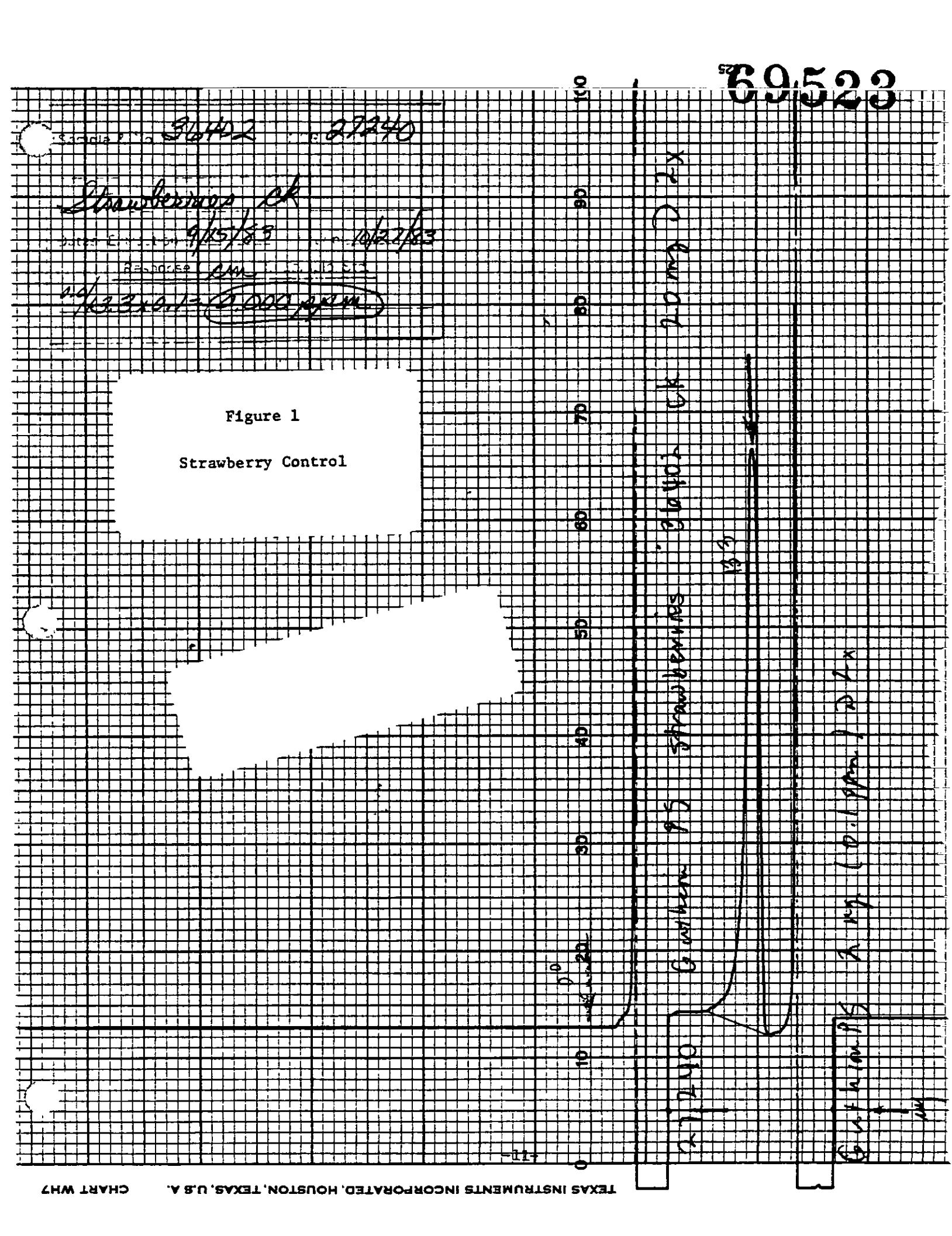
69523

Strawberries ck

9/15/83 — 10/22/83

Figure 1

Strawberry Control



69523

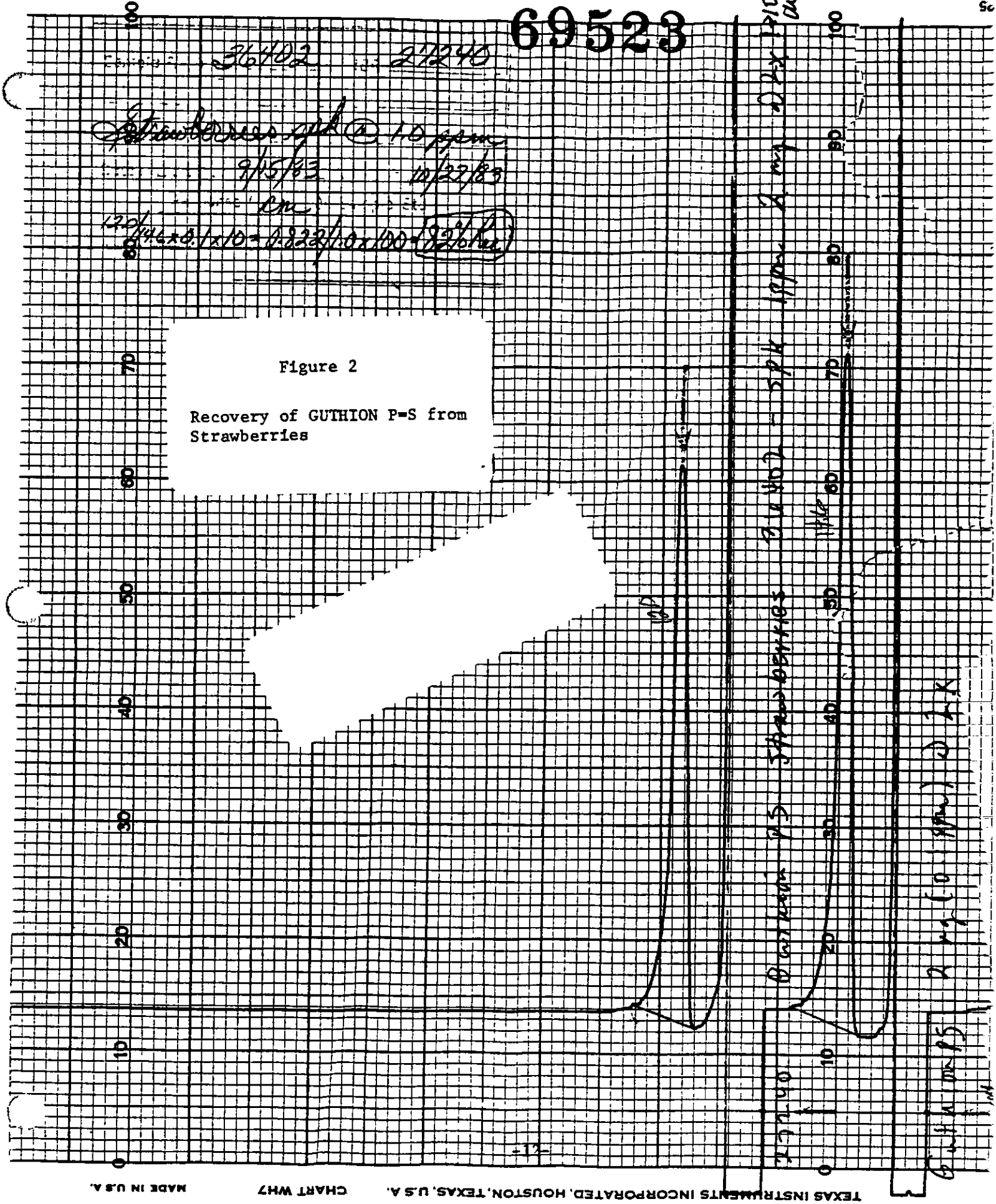


Figure 2

Recovery of GUTHION P=S from Strawberries

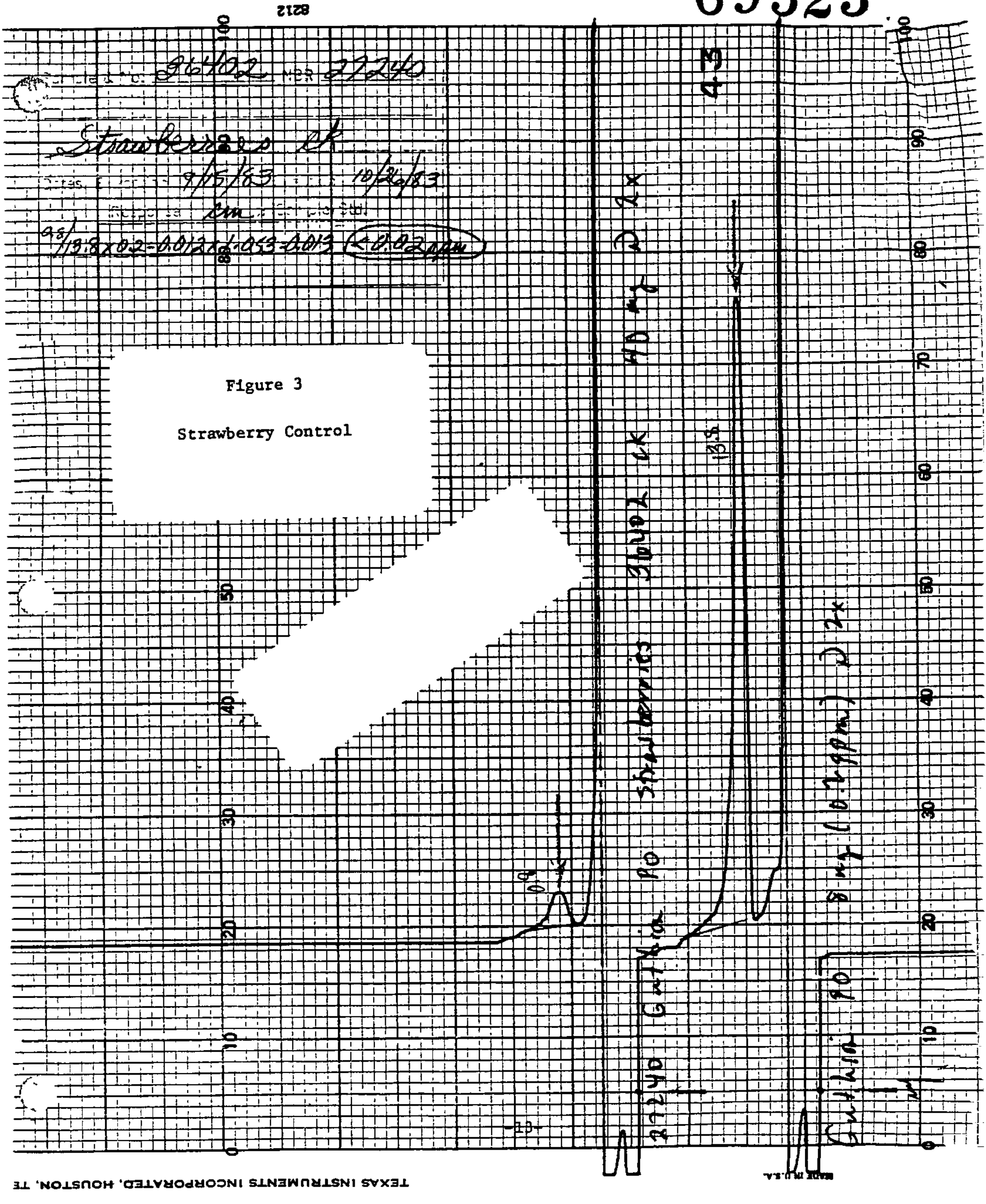


Figure 3

Strawberry Control

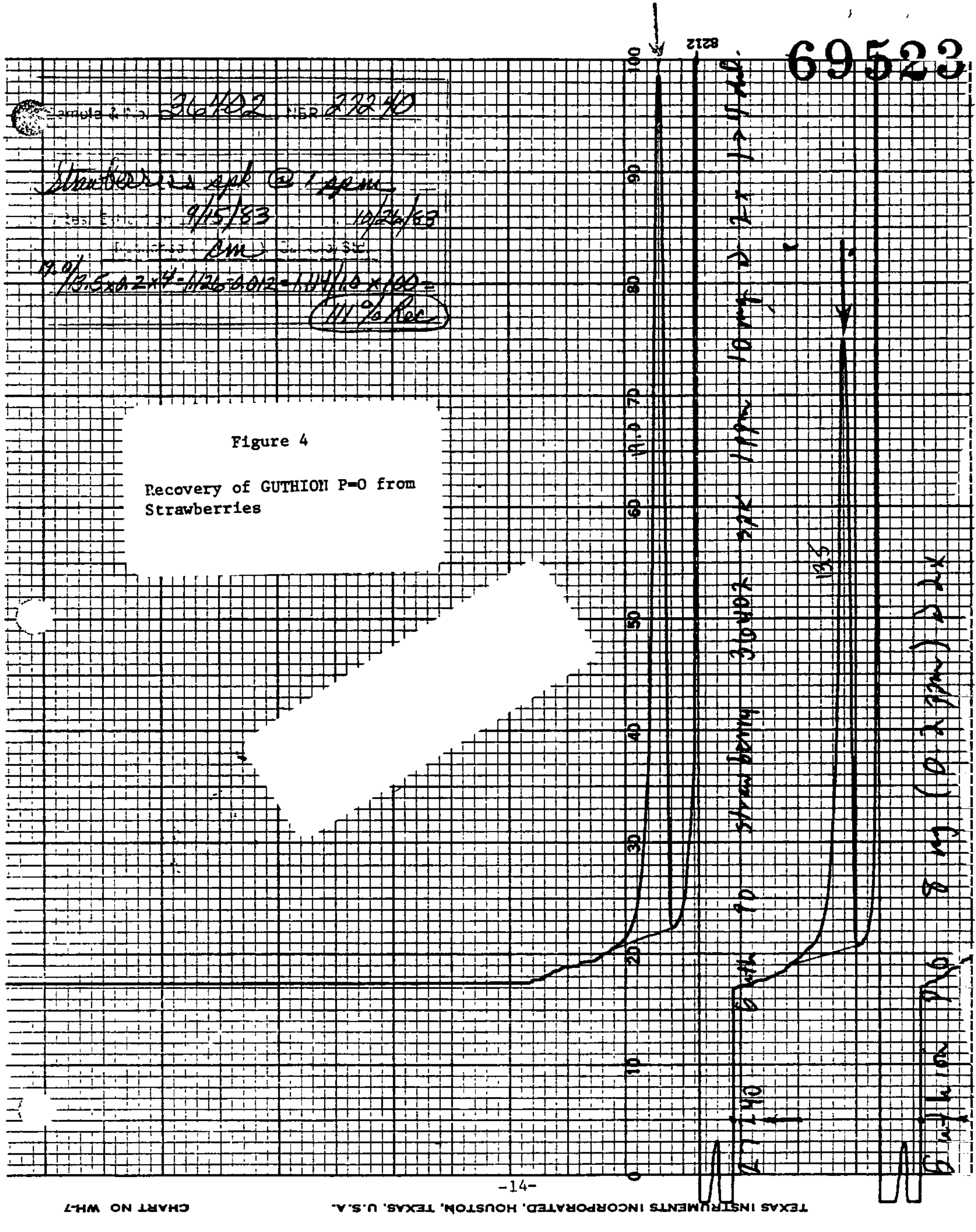


Figure 4

Recovery of GUTHION P=O from Strawberries

69523

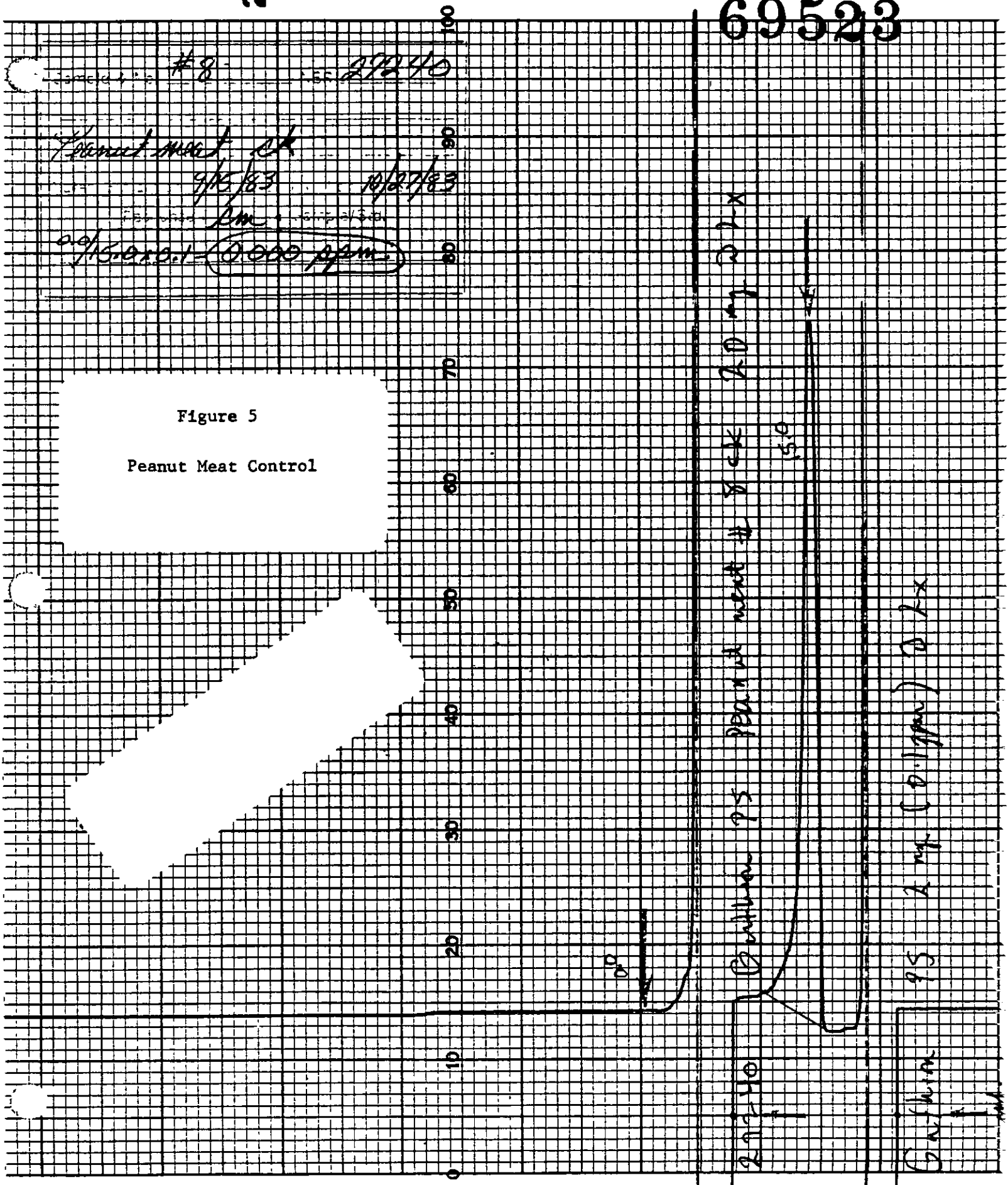
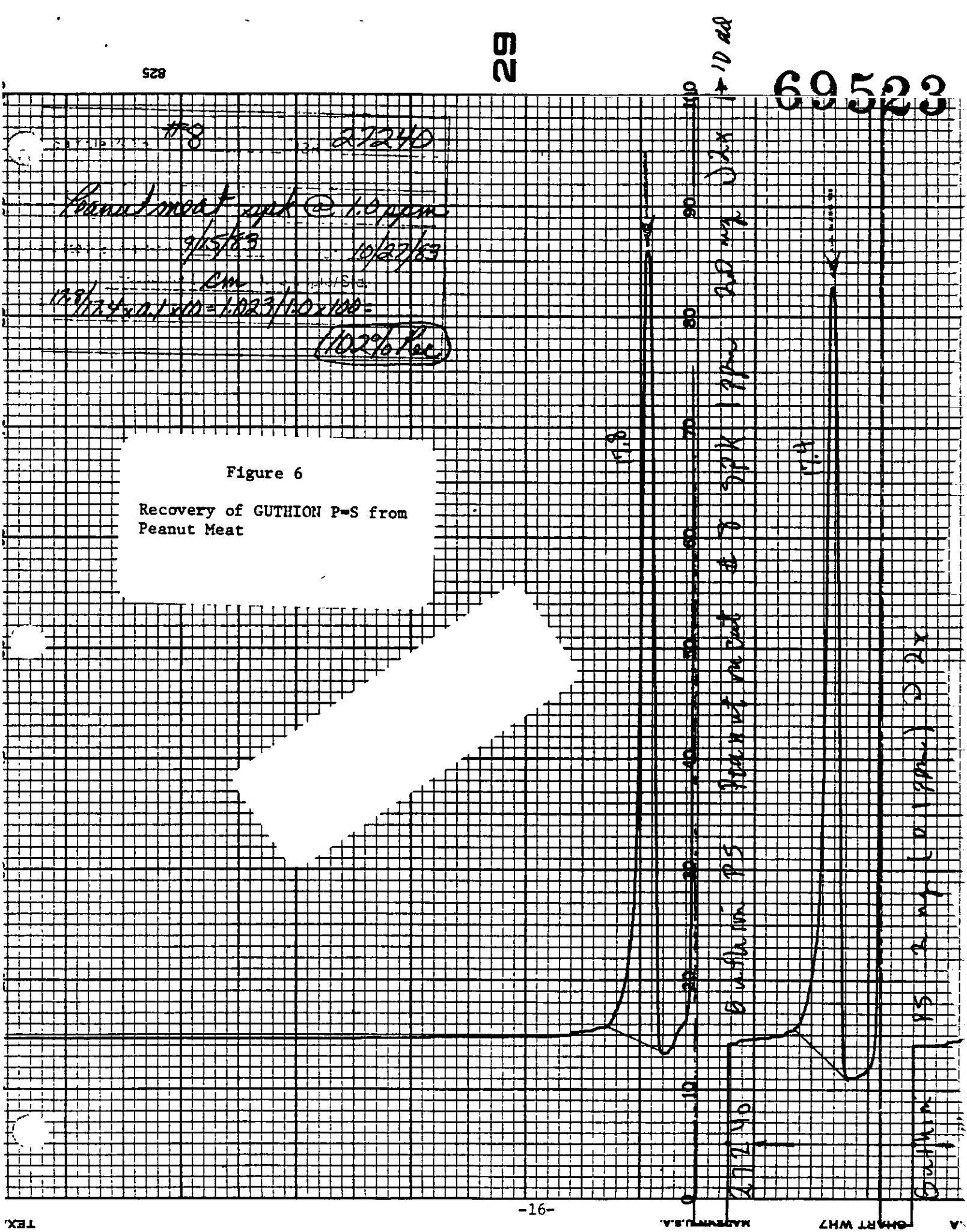


Figure 5

Peanut Meat Control


29
69523
#8
Peanut meat spk @ 1.0 ppm
9/5/83
10/27/83
Figure 6
Recovery of GUTHION P=S from
Peanut Meat

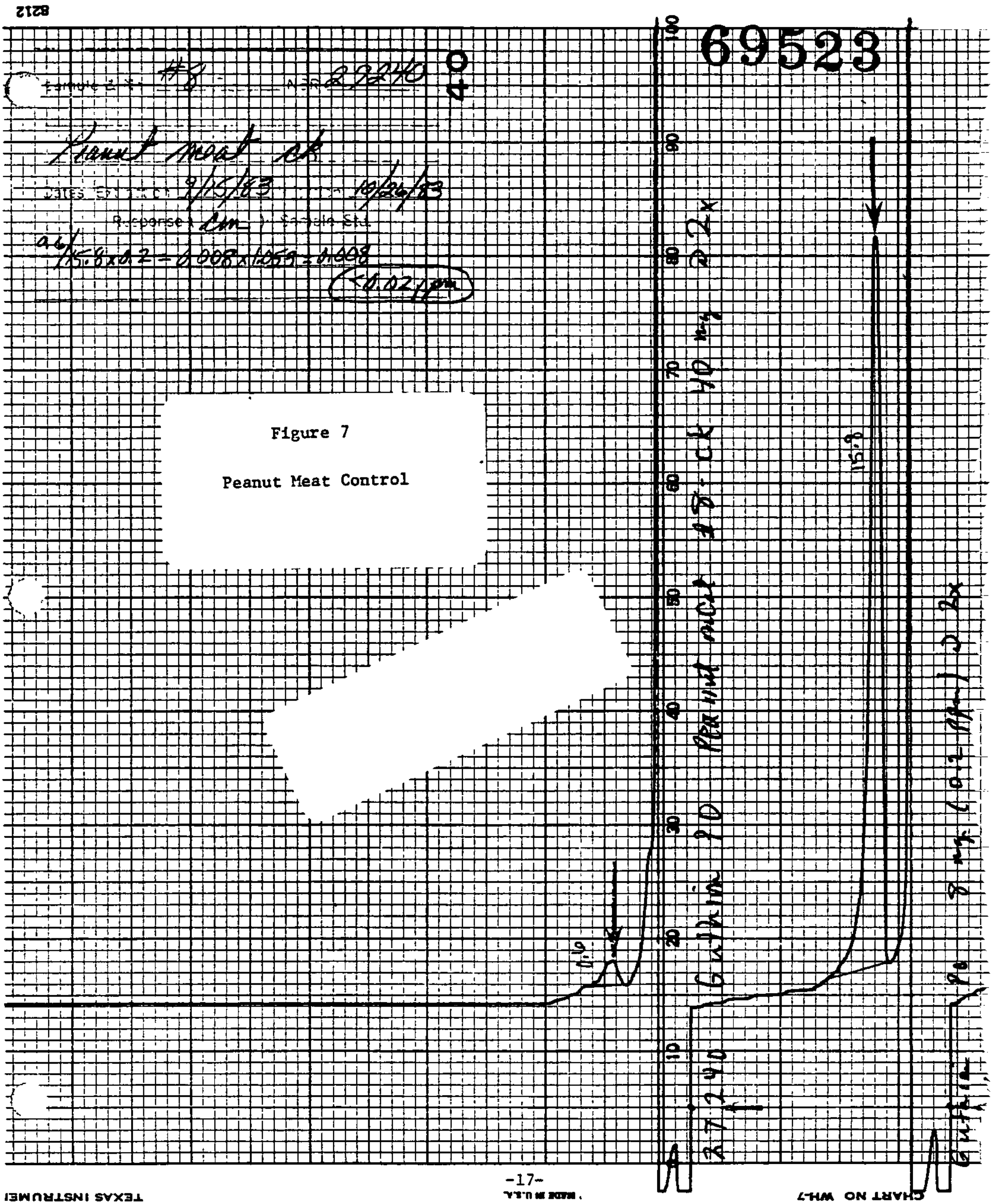


Figure 7

Peanut Meat Control