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MOBAY CHEMICAL CORPORATION
AGRICULTURAL CHEMICALS DIVISION

Research and Development Department

TITLE: A Gas Chromatographic Method for BAYTHROID™ Residue in Crops

AUTHORS: A. M. Harbin, R. G. Minor, P. L. Freeseman, and L. K. Pfankuche

ABSTRACT: In this gas chromatographic method for determining the residue of BAYTHROID in crops, samples are blended with methanol/water (4:1). The filtered extract is reduced to the water and partitioned with acetone/chloroform (1:2), concentrated, and passed through a Florisil column prior to gas chromatography utilizing an electron capture detector. Recovery of BAYTHROID from cotton fortified at 0.05 to 1.00 ppm ranged from 70 to 92%. Recoveries from soybeans ranged from 82 to 98% when fortified at 0.05 ppm and 80 to 89% when fortified at 0.10 ppm. Recoveries from peanuts fortified at 0.05 ppm BAYTHROID ranged from 74 to 84%. Cotton, soybean, and peanut controls were all <0.01 ppm.

DATE: June 14, 1983

APPROVED BY: D. R. Flint
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NOTEBOOK REFERENCE: 80-R-293, 83-R-04, 83-R-07, 83-R-83 and 83-R-85

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A Gas Chromatographic Method for BAYTHROID Residue in Crops

INTRODUCTION

The method for determining BAYTHROID [BAY FCR 1272, cyfluthrin, cyano(4-fluoro-3-phenoxyphenyl)methyl-3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropanecarboxylate] residues in crops is presented and includes recoveries from cotton, soybeans, and peanuts. Due to the persistence of BAYTHROID in crops as shown in the metabolism work with cotton[1] and soybeans[2], the method has been devised to determine residues of parent compound only.

ANALYTICAL METHOD

Equipment

Gas chromatograph equipped with an electron capture detector. (Coulson or Hall detectors may also be used.)
Rotary vacuum evaporator with 32°C water bath.

Glassware

Standard laboratory glassware.

Reagents

Acetone, pesticide grade.
Acetone/chloroform solution, pesticide grade, 1:2 mixture.
Acetonitrile, pesticide grade.
Florisil (2.5% water).
Formic Acid (88%).
Glass wool, Pyrex.
Hexane, pesticide grade.
Hyflo Super-Cel.
Methanol/water solution, pesticide grade, 4:1 mixture.
Methylene chloride, pesticide grade.
Sodium sulfate, powdered, anhydrous, analytical reagent grade.
Standard solution: BAYTHROID, 0.25 µg/ml in hexane.

Detailed Procedure

A. Preparation of Sample

1. Grind the sample with an equal weight of dry ice in a Waring Blendor jar.

2. Place the sample in a freezer to allow the dry ice to sublime.

B. Extraction

1. Weigh a 25 g aliquot of the sample into a Waring Blendor jar.

2. Add 200 ml methanol/water mixture (4:1), and blend the sample at moderate speed for 2 min.

3. Filter the sample under vacuum through a 1 cm layer of Hyflo Super-Cel, on a 9-cm Whatman No. 1 filter paper in a Büchner funnel, into a 1000-ml flask.

4. Reblend the filter cake and filter paper with another 200 ml methanol/water (4:1).

5. Filter the sample under vacuum through a 9-cm Whatman No. 1 filter paper into the 1000-ml flask in step II-3.

6. Add 100 ml acetonitrile to form an azeotrope, and evaporate the solution in the flask until water condensation is visible in the condenser.

7. Using 300 ml acetone/chloroform mixture (1:2) and 1 ml formic acid, transfer the contents of the flask to a 500-ml separatory funnel. Shake the separatory funnel for 60 sec (shake carefully to avoid emulsions).

8. Allow the phases to separate, and drain the lower layer through sodium sulfate into a 1000-ml flask.

9. Add 300 ml acetone/chloroform (1:2) to the aqueous phase in the separatory funnel, shake the funnel for 60 sec, and repeat step 8.

10. Evaporate the solution in the flask to dryness.

C. Hexane/Acetonitrile Partitioning

1. Add 200 ml hexane to the flask from step II-10. Swirl flask to mix hexane and residue, and transfer solution to a 500-ml separatory funnel.

2. Rinse the flask with 200 ml acetonitrile. Swirl acetonitrile in the flask, and add this to the separatory funnel.

3. Shake the separatory funnel for 30 sec, allow the layers to separate, and drain the lower layer back into the 1000-ml flask. Discard the hexane layer

4. Return the acetonitrile fraction to the separatory funnel, and add 100 ml hexane.

5. Shake the separatory funnel for 30 sec, allow the phases to separate, and drain the lower layer into the flask.

6. Evaporate the solution in the flask to dryness.

D. Column Cleanup

1. With a stream of air, force the cotton plug from the top of a 10 ml disposal serological pipet to the bottom of the pipet.

2. Slowly pour 1 g Florisil (2.5% water) into the column. Add 10 ml methylene chloride to the column, and drain the solvent through the column, discarding the 10 ml of methylene chloride.

The formation of emulsions during extraction of oily crops was eliminated by the addition of formic acid during partitioning. Step C, the hexane/acetonitrile partitioning, was included to reduce the large amount of oil present in peanuts and cotton. This cleanup procedure was omitted from the method in the case of soybeans, and satisfactory recoveries were still obtained. In general, it may be concluded that this partitioning need only be performed for exceptionally oily crops.

Recoveries from cotton fortified at various concentrations of BAYTHROID ranged from 70 to 92% (Table I). For soybeans, recoveries in meat, forage and dry vines ranged from 82 to 98% when fortified at 0.05 ppm, and 80 to 89% when fortified at 0.10 ppm (Table II). Recoveries from peanut shells, meat and dry vines ranged from 74 to 84% when fortified at the 0.05 ppm level (Table III). Control values in all three crops were <0.01 ppm. A representative scan of soybean meat control fortified with 0.05 ppm BAYTHROID is shown in Figure 1.

Chromatograms of all recovery samples are included with Mobay Ag Chem Reports No. 8431 84351 and 84360.

SUMMARY

A gas chromatographic method for the determination of BAYTHROID in crops has been devised. Recoveries after fortification at 0.05 to 1.00 ppm averaged 82% for cotton. BAYTHROID recovery from soybeans, forage, and dry vines ranged from 82 to 98% when fortified at 0.05 ppm, and 80 to 89% when fortified at 0.10 ppm. Recoveries from peanut shells, meat, and dry vines fortified at 0.05 ppm ranged from 74 to 84%. Control values on all three crops were <0.01 ppm.

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TABLE I

Recovery of BAYTHROID from Cotton

	Ppm		
	Fortified	Recovered	% Recovery[1]
Cottonseed	0.00	0.013	-
	1.00	0.937	92
	0.00	<0.010	-
	0.10	0.082	82
	0.00	<0.010	-
	0.05	0.042	80
	0.10	0.087	85
	0.20	0.170	84
	1.00	0.761	76
	1.00	0.829	83
	1.00	0.895	89
Hulls	0.00	0.010	-
	0.05	0.045	70
	0.50	0.421	82

[1]Percent recovery = $\frac{\text{Ppm Gross Residue - Ppm Control Blank}}{\text{Ppm Fortified}} \times 100$

TABLE II

Recovery of BAYTHROID from Soybeans[1]

	Ppm		
	Fortified	Recovered	% Recovery[2]
Soybeans	0.00	<0.010	-
	0.05	0.043	80
	0.10	0.085	82
Forage	0.00	<0.010	-
	0.05	0.049	98
	0.10	0.089	89
Dry Vines	0.00	<0.010	-
	0.05	0.045	84
	0.10	0.083	80

[1]See Appendix I.

[2]Percent recovery = $\frac{\text{Ppm Gross Residue - Ppm Control Blank}}{\text{Ppm Fortified}} \times 100$

TABLE III

Recovery of BAYTHROID from Peanuts[1]

	Ppm		
	Fortified	Recovered	% Recovery[2]
Shells	0.00	<0.010	-
	0.05	0.039	78
Meat	0.00	<0.010	-
	0.05	0.037	74
Dry Vines	0.00	<0.010	-
	0.05	0.042	84

[1]See Appendix II.

[2]Percent recovery = $\frac{\text{Ppm Gross Residue - Ppm Control Blank}}{\text{Ppm Fortified}} \times 100$

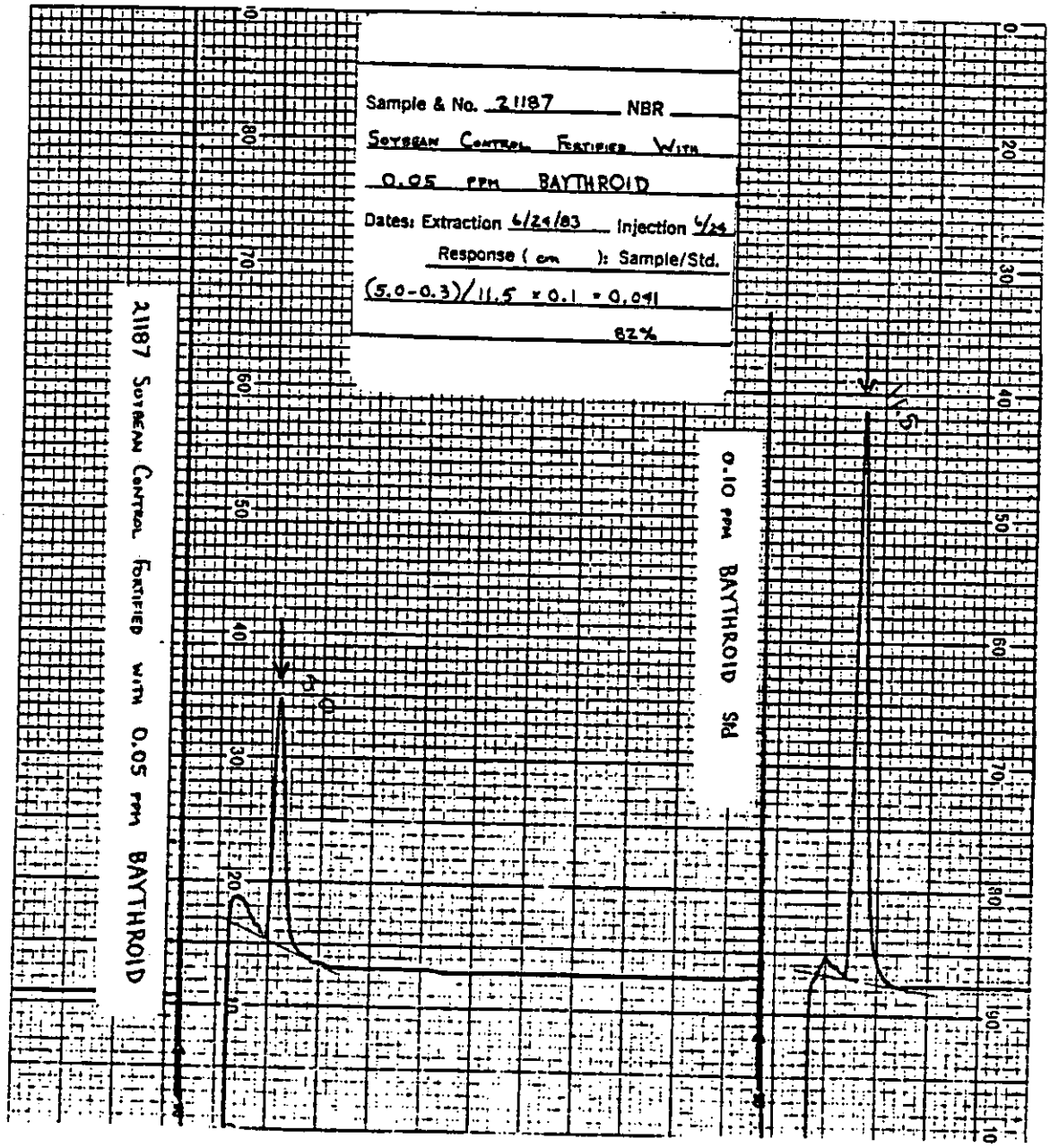

Figure 1. Representative scan of soybean meat control fortified with 0.05 ppm BAYTHROID.

LITERATURE CITED

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APPENDIX I

Method Modifications for the Analysis of Soybeans

The method when used to determine recoveries on soybeans was modified so that 2.0 ml of extract was applied to the Florisil column in step D-5. This was done to increase sensitivity for gas chromatographic analysis, which was done with a Hall detector in the halogen mode.

Step C, the hexane/acetonitrile partitioning, was omitted from the method when used for soybeans. This was because the amount of oil extracted did not present as great a problem as was observed with cotton and peanuts.

A more polar column, 1 m x 2 mm i.d. glass 3% OV-3 on 80/100 mesh Gas Chrom Q was use in the analysis of soybeans and showed no interferences. However, an even more polar column, 7% OV-17 on Chromosorb GHP, was also tried and failed to resolve a small interference.

APPENDIX II

Method Modifications for the Analysis of Peanuts

The method when employed to determine recoveries on peanuts was modified so that the entire sample (10 ml) was transferred onto the Florisil column in step D-5, increasing the column weight and eluting volumes by a corresponding factor of 10. This increased the sensitivity of the gas chromatographic analysis, which utilized a Coulson chloride cell detector. A 4' x 2 mm i.d. 3% OV-101 on 80/100 mesh GCQ glass column was used in the analysis.

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MOBAY CHEMICAL CORPORATION
AGRICULTURAL CHEMICALS DIVISION

Research and Development Department

TITLE: An Analytical Method for BAYTHROID™ in Bovine and Poultry Tissues, Milk and Eggs

AUTHORS: H. R. Shaw II, H. M. Chopade, J. E. Ayers and C. C. Gentile

ABSTRACT: A gas chromatographic method for measuring the intact residues of BAYTHROID in animal products has been developed. BAYTHROID is removed from the sample matrix by organic solvent extraction. The organosoluble extract is partitioned with various solvents to remove lipids and polar and non-polar interferences. The final purification step is chromatography of the sample on either a silica gel column or a Florisil Sep-Pak. The purified sample is subjected to gas chromatography utilizing an electron capture detector. The range of recovery of BAYTHROID from milk fortified at 0.02 ppm was 90 to 125%. Recovery of BAYTHROID from bovine tissues, poultry tissues, and eggs fortified at 0.05 ppm ranged from 76 to 85%, 74 to 94% and 70 to 78%, respectively. Control values were <0.01 ppm.

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APPROVED BY: D. R. Flint
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NOTEBOOK REFERENCE: 82-R-99, 82-R-103, 82-R-224, 83-R-03 and 83-R-101

BAYTHROID is a TM of the Parent Company of Farbenfabriken Bayer GmbH, Leverkusen

An Analytical Method for BAYTHROID in Bovine and Poultry Tissues, Milk and Eggs

INTRODUCTION

A method for the quantitation of BAYTHROID [BAY FCR 1272, cyfluthrin, cyano(4-fluoro-3-phenoxyphenyl)-methyl-3-(2,2-dichloroethenyl)2,2-dimethylcylcopropane-carboxylate], in bovine and poultry tissues, milk, and eggs is described in this report.

ANALYTICAL METHOD

Apparatus Required

Assorted laboratory glassware.
Tekmar Tissuemizer or Waring Blendor, explosion proof or equivalent.
Chromatography columns, 1.9 cm i.d. x 45 cm, bell-type.
Gas chromatograph, Hewlett Packard Model 5750B or equivalent equipped with a electron capture detector.
Rotary vacuum evaporator with water bath maintained at 34°C ± 1°C.

Materials Required

Acetone, pesticide grade.
Acetonitrile, pesticide grade.
Chloroform, pesticide grade.
Ethyl acetate, pesticide grade.
Florisil, Fisher Scientific, 60-100 mesh, partially deactivated. Prepare by heating Florisil overnight at 120 to 130°C. Add 2.5% (w/w) water to the Florisil and m well before using.
Florisil Sep-Pak cartridge, Waters Associates.
Formic acid, analytical reagent grade.
Glass fiber filter (GF/A), Whatman.
Glass wool, Pyrex.
Hexane, pesticide grade.
Hydrochloric acid, concentrated, analytical reagent grade.
Hyflo Super-Cel, Johns-Manville.
Sea sand, washed and ignited, Fisher Scientific Company.
Sodium chloride solution, 5% (w/v) in deionized water.
Sodium sulfate, granular, anhydrous, analytical reagent grade.
Silica gel, Fisher Scientific, 100-200 mesh, reagent grade, partially deactivated. Prepare by heating silica gel overnight at 120 to 130°C. Add 7% (w/w) water to the silica gel and mix well before using.

Standard Solution

BAYTHROID, 1.25 µg/ml omi ethyl acetate. For standard injection solution, dilute 2 m to 5 ml with ethyl acetate (0.5 µg/ml).

10. Shake the second separatory funnel for 1 min. Allow the organic phases to separate.

11. Draw off the lower phase (acetonitrile) into a 1-l boiling flask.

12. Repeat steps 6, 7 and 8.

13. Transfer the lower phase (acetonitrile) into the second separatory funnel and shake the funnel for 1 min. Allow the layers to separate.

14. Draw off the lower phase (acetonitrile) into the same 1-l flask from step 11.

15. Repeat steps 12, 13 and 14.

16. Concentrate the combined organic extract to dryness on the rotary evaporator.

17. Proceed to step II.

C. Milk

1. Add 25 ml milk to a 500 ml separatory funnel.

2. Add 1 ml concentrated HCl and shake funnel to mix contents.

3. Add 150 ml acetone/chloroform (2:1), and shake mixture for 1 min.

4. Allow phases to separate, and draw off lower layer into a 1-l boiling flask.

5. Add 10 ml water to the separatory funnel.

6. Repeat steps 3 and 4 twice.

7. Discard aqueous layer.

8. Concentrate the organic fraction to dryness on the rotary evaporator.

9. Proceed to step II.

D. Eggs

1. Weigh 5 g homogenized egg into a 250 ml beaker.

2. Add 12 g Florisil (2.5% H_2O) to the beaker, and stir the sample with a glass rod until a homogenous, free flowing powder is formed.

3. Add a glass wool plug to the bottom of a 1.9 cm i.d. x 45 cm chromatographic column.

4. Add a 1 cm layer of sea sand to the column.

5. Add 100 ml hexane (saturated with acetonitrile)/chloroform (90:10) to the column.

6. Add 16 g Florisil to the column, and allow the Florisil to settle.

7. Drain the solvent from column until a 5 cm layer of solvent remains on the top of the column.

8. Add the sample from step 2 to the column.

9. Add 1 cm layer of sea sand to the top of the column.

10. Rinse the beaker and glass rod with 15 ml of the same solvent mixture (step 5), and transfer the solvent to the column.

11. Elute the column with 250 ml hexane (saturated with acetonitrile)/ chloroform (90:10) into a 250 ml graduated cylinder.

12. Transfer the eluant to a 500 ml round bottom flask, and concentrate the organic extract to dryness on the rotary evaporator.

13. Proceed to step III.

II. Clean-Up for All Tissues and Milk

A. Methanol/water ethyl acetate partition.

1. Add 25 ml water, 5 ml methanol and 90 ml ethyl acetate (water saturated) to the flask (IA - 8 or IB - 16 or IC - 8).

2. Stopper the flask, and shake it for 1 min.

3. Transfer contents of the flask to a 250 ml separatory funnel.

4. Allow the phases to separate, and then draw off the lower phase into the same flask.

5. Drain the ethyl acetate (phase still in the separatory funnel) into a 500 ml boiling flask through a funnel containing anhydrous granular sodium sulfate (50 g).

6. To the flask from step 4, add 90 ml fresh ethyl acetate (water saturated), and repeat steps 2, 3, 4 and 5.

7. Repeat step 6.

8. Discard the aqueous solution.

9. Concentrate the combined ethyl acetate fractions to dryness on the rotary evaporator.

B. Hexane/acetonitrile partition.

For the following steps use hexane saturated with acetonitrile and acetonitrile saturated with hexane.

1. To the residue in the flask (step IIA-9), add 150 ml hexane and 150 ml acetonitrile. Stopper the flask, and shake it for 1 min.

2. Transfer contents of the flask into a 500 ml separatory funnel.

3. Add 150 ml hexane into a second separatory funnel.

4. Draw off the lower layer (step 2) into the second separatory funnel.

5. Stopper and shake the second separatory funnel for 1 min.

6. Draw off the lower layer into a ~~500 ml~~ 1 liter boiling flask.

7. Add 150 ml acetonitrile into the first separatory funnel from step 2, and shake the funnel for 1 min.

8. Repeat steps 4, 5 and 6.

9. Repeat steps 7 and 8.

10. Discard hexane in both separatory funnels.

11. Concentrate acetonitrile to dryness on the rotary evaporator.

C. Hexane/acetone/water partition.

1. Add 100 ml acetone and 100 ml hexane to the flask from step IIB-11, and shake the flask to mix the contents.

2. Transfer contents of flask to 500 ml separatory funnel.

3. Add 200 ml deionized or distilled water to the flask. Swirl flask, and then transfer contents to the separatory funnel.

4. Shake the separatory funnel for 1 min.

5. Allow the phases to separate.

6. Drain the lower layer (acetone/water) and discard.

7. Drain the layer (acetone/hexane) into a 500 ml boiling flask through a funnel containing anhydrous granular sodium sulfate (50 g).

8. Evaporate the layer (acetone/hexane) to dryness on the rotary evaporator.

NOTE: Do not attempt to use this acetone/hexane in the next step for it contains enough acetone to disrupt the column conditions.

D. Column

1. Add glass wool to the bottom of a 1.9 cm diameter x 45 cm column, and pack the glass wool flat with a rod.

2. Add a 0.25 cm layer of granular anhydrous sodium sulfate.

3. Add 100 ml hexane to the column.

4. Add 30 g silica gel (7% H_2O) to the column, and allow the silica gel to settle.

5. Add a 0.25 cm layer of granular anhydrous sodium sulfate to top of the column.

6. Drain hexane down to top of column packing.

7. Use 20 ml hexane to dissolve the dry residue from step IIC-8, and add the sample to the column.

8. Let the column drain at a flow rate of 3 to 5 ml per min. Use 80 ml more of hexane to rinse the flask, and then add this hexane to the column after the initial 20 ml hexane has reached the top of the column.

9. Discard the hexane.

10. Elute BAYTHROID from the column with 200 ml hexane/ethyl acetate (90:10).

11. Concentrate the hexane/ethyl acetate to dryness on the rotary evaporator.

12. Transfer the residue to a centrifuge tube with four or five rinses of acetone (5 ml each).

13. Concentrate the sample on the rotary evaporator as follows according to the initial weight of sample. For 25 g sample the final volume is 5 ml, and for 10 g sample, the final volume is 2 m

14. Proceed to step [redacted] C

III. Clean-Up for Eggs

A. Hexane/5% NaCl partition.

1. Dissolve the dry residue from step ID-11 in 100 ml hexane.

2. Transfer the hexane to a 500 ml separatory funnel.

3. Add 100 ml 5% NaCl to the separatory funnel, shake the contents for 1 min and allow the layers to separate.

4. Drain and discard the lower aqueous layer.

B. Hexane/acetonitrile partition.

Follow the procedure in steps IIB-1 to 11 using solvent volumes of 100 ml instead of 150 ml.

C. Florisil Sep-Pak.

1. Pass 5 ml hexane through a Sep-Pak cartridge.

2. Dissolve the residue from step IIIB-11 in 5 ml hexane.

3. Load the sample onto the cartridge using a 10 ml syringe with a Luer end fitting.

4. Rinse the flask with 5 ml hexane, and pass the hexane through the Sep-Pak cartridge.

5. Collect and discard about 10 ml hexane eluate.

6. Elute the Sep-Pak cartridge with 8 ml hexane (saturated with acetonitrile)/chloroform (90:10). Collect eluate in a 13 ml graduated centrifuge tube. This fraction contains BAYTHROID.

7. Evaporate the solvent mixture under a slow stream of nitrogen making sure that all traces of chloroform are removed.

8. Take up the dry residue in 1 ml acetone.

9. Proceed to step IV.

IV. Gas Chromatography

1. Use the following conditions:

Standard Column : 55 cm x 2 mm i.d. glass column packed with 15% UCW 98 on 80/100 mesh Chromosorb W, acid washed, silanized.?

Carrier Gas : Argon/methane (95:5), 30 ml/min.

Temperature : Column Oven : 240°C
Detector : 300°C
Injection Port: 250°C

Retention Time: 4 min for BAYTHROID

Confirmatory Column: 55 cm x mm i.d. glass column packed with 5% SE 30 on 80-100 mesh Chromosorb W(HP).

Carrier Gas : Argon/methane (95:5), 32 ml/min.

Temperature : Column Oven : 238°C
Detector : 300°C
Injection Port: 300°C

Retention Time: 6 min for BAYTHROID

2. Inject 4 µl of a 0.5 µg/ml standard of BAYTHROID (equivalent to 0.1 ppm) or inject 4 µl of a sample.

3. Identify the compound by its retention time, and measure the peak height on the recorder chart.

4. Calculate the ppm of BAYTHROID by comparing the response obtained for a sample to the response for a corresponding standard as follows:

$$\text{Ppm} = \frac{\text{Peak height (sample)}}{\text{Peak height (standard)}} \times \frac{\text{Std. inj. (µg)}}{\text{Spl. wt. (g)}} \times \frac{\text{Final vol. (µl)}}{\text{Spl. inj. (µl)}}$$

RESULTS AND DISCUSSION

The range of recovery values from milk fortified at 0.02 ppm with BAYTHROID was 90 to 125% when analyzed by the standard or confirmatory procedure (Table I). Representative graphs for control and BAYTHROID fortified milk samples are shown in Figures 1 and 2.

The range of recovery values from bovine tissues fortified at 0.05 ppm with BAYTHROID was 76 to 85% (Table II).

The range of recovery values from eggs fortified at 0.05 ppm with BAYTHROID was 70 to 78% (Table III). Representative graphs for control and BAYTHROID fortified egg samples are shown in Figures 3 and 4.

Recovery of BAYTHROID from poultry tissues fortified at 0.05 ppm ranged from 74 to 94 (Table IV).

Control values were <0.01 ppm for all tissues, milk and eggs.

CONCLUSION

A gas chromatographic method has been devised which will quantitate residues of BAYTHROID in bovine tissues, poultry tissues, milk and eggs. Recovery of BAYTHROID from bovine milk ranged from 90 to 125%. Recovery of BAYTHROID from bovine tissues ranged from 76% to 85% and recovery from poultry tissues ranged from 74 to 94%. Recovery of BAYTHROID from eggs ranged from 70 to 78%. Control values were all <0.01 ppm.

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TABLE I

Recovery of BAYTHROID from Milk[1]

Sample	BAYTHROID Fortification Level (Ppm)	Recovery (%)
	Standard Column	
218C	0	0
219C	0	0
223C	0	0
218R	0.02	100
219R	0.02	125
223R	0.02	110
	Confirmatory Column	
218C	0	0
219C	0	0
223C	0	0
218R	0.02	95
219R	0.02	110
223R	0.02	90

[1]For individual data see Mobay Ag Chem Report No. 86038.

TABLE II

Recovery of BAYTHROID from Bovine Tissues[1,2]

Sample	BAYTHROID Fortification Level (Ppm)	Recovery (%)
Muscle		
100C	0	0
100R	0.05	84
Fat		
101C	0	0
101R	0.05	66
Kidney		
102C	0	0
102R	0.05	78
Liver		
103C	0	0
103R	0.05	76

[1]For individual data see Mobay Ag Chem Report No. 85981.

[2]Analyses described in Appendix I.

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TABLE III

Recovery of BAYTHROID from Eggs[1]

Sample	BAYTHROID Fortification Level (Ppm)	BAYTHROID Recovery (%)
	Standard Column	
11C	0	0
12R	0.05	70
13R	0.05	71
14R	0.05	78
	Confirmatory Column	
11C	0	0
12R	0.05	64
13R	0.05	62
14R	0.05	80

[1]For individual data see Mobay Ag Chem Report No. 85983.

TABLE IV

Recovery of BAYTHROID from Poultry Tissues[1,2]

Sample	BAYTHROID Fortification Level (Ppm)	Recovery (%)
Muscle		
1C	0	0
1R	0.05	94
Fat		
2C	0	0
2R	0.05	84
Skin		
3C	0	0
3R	0.05	88
Liver		
4C	0	0
4R	0.05	76
Kidney		
5C	0	0
5R	0.05	74

[1]For individual data see Mobay Ag Chem Report No. 85982.

[2]Analyses described in Appendix I.

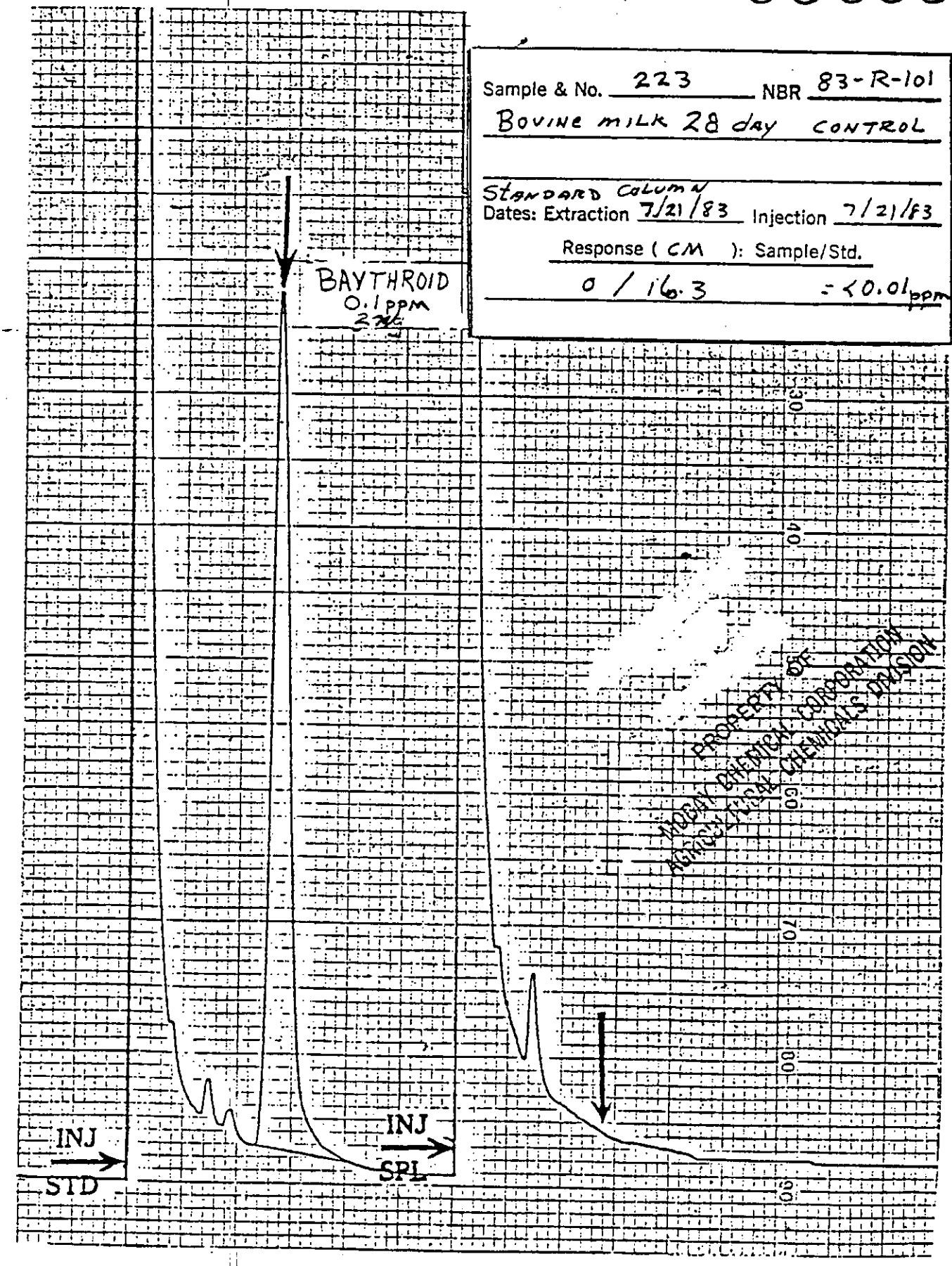

Figure 1. Representative chromatogram for control milk.

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Sample & No. 223 R NBR 83-R-1

Bovine milk fortified wit

0.02 ppm BAYTHROID

STANDARD COLUMN

Dates: Extraction 2/27/83 Injection 7/25/

Response (CM): Sample/Std.

3.7/16.5 X 0.1 = 0.02 PPM

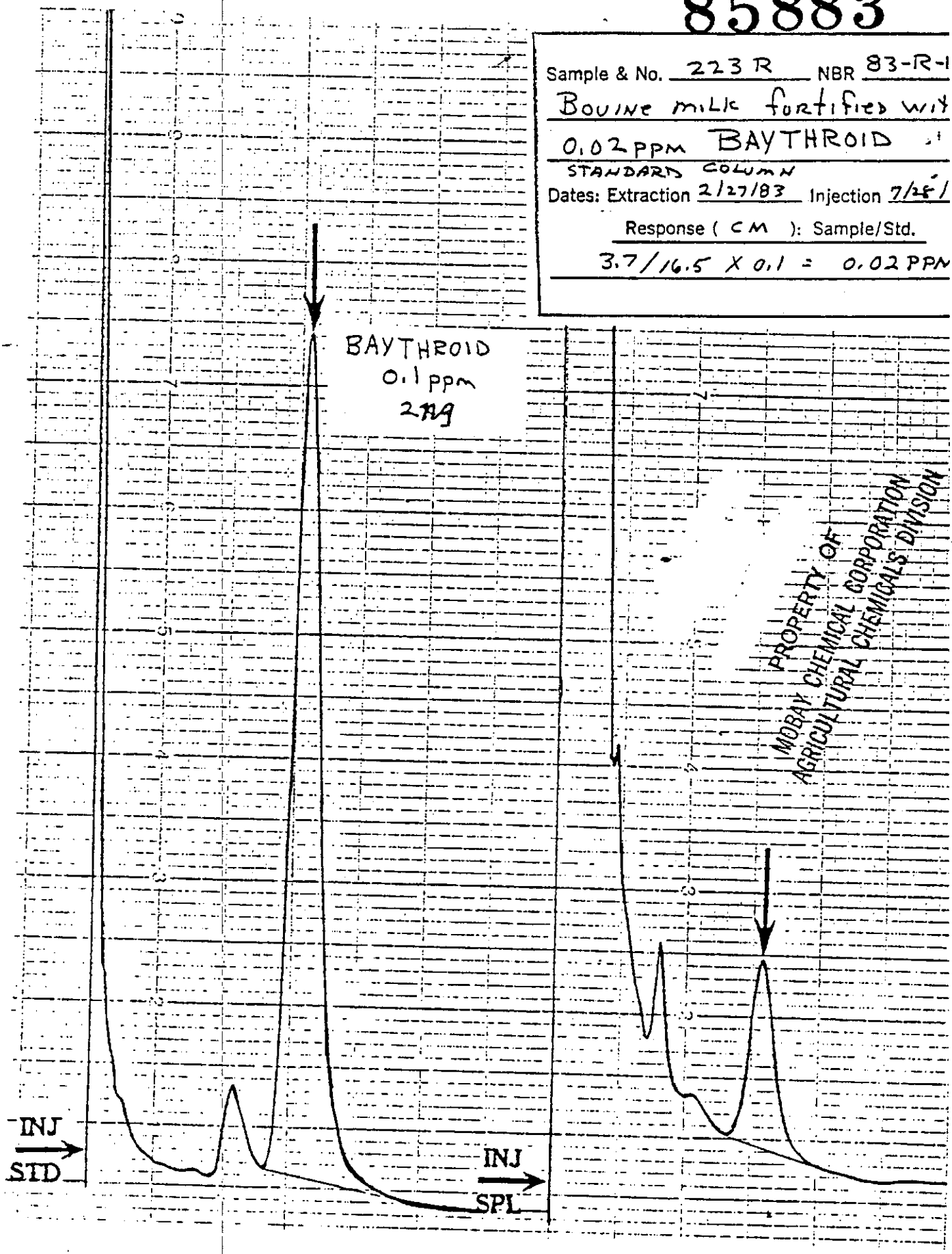

Figure 2. Representative chromatogram for recovery of BAYTHROID from milk fortified with 0.02 ppm BAYTHROID.

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Sample & No. 11C NBR 82-R-99

Chicken eggs Control

STANDARD COLUMN

Dates: Extraction 8/16/83 Injection 8/18/83

Response (cm): Sample/Std.

0/15.6 = <0.01 ppm

BAYTHROID
0.1 ppm
2 Ng

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INJ
STD

INJ
SPL

Figure 3. Representative chromatogram for control egg.

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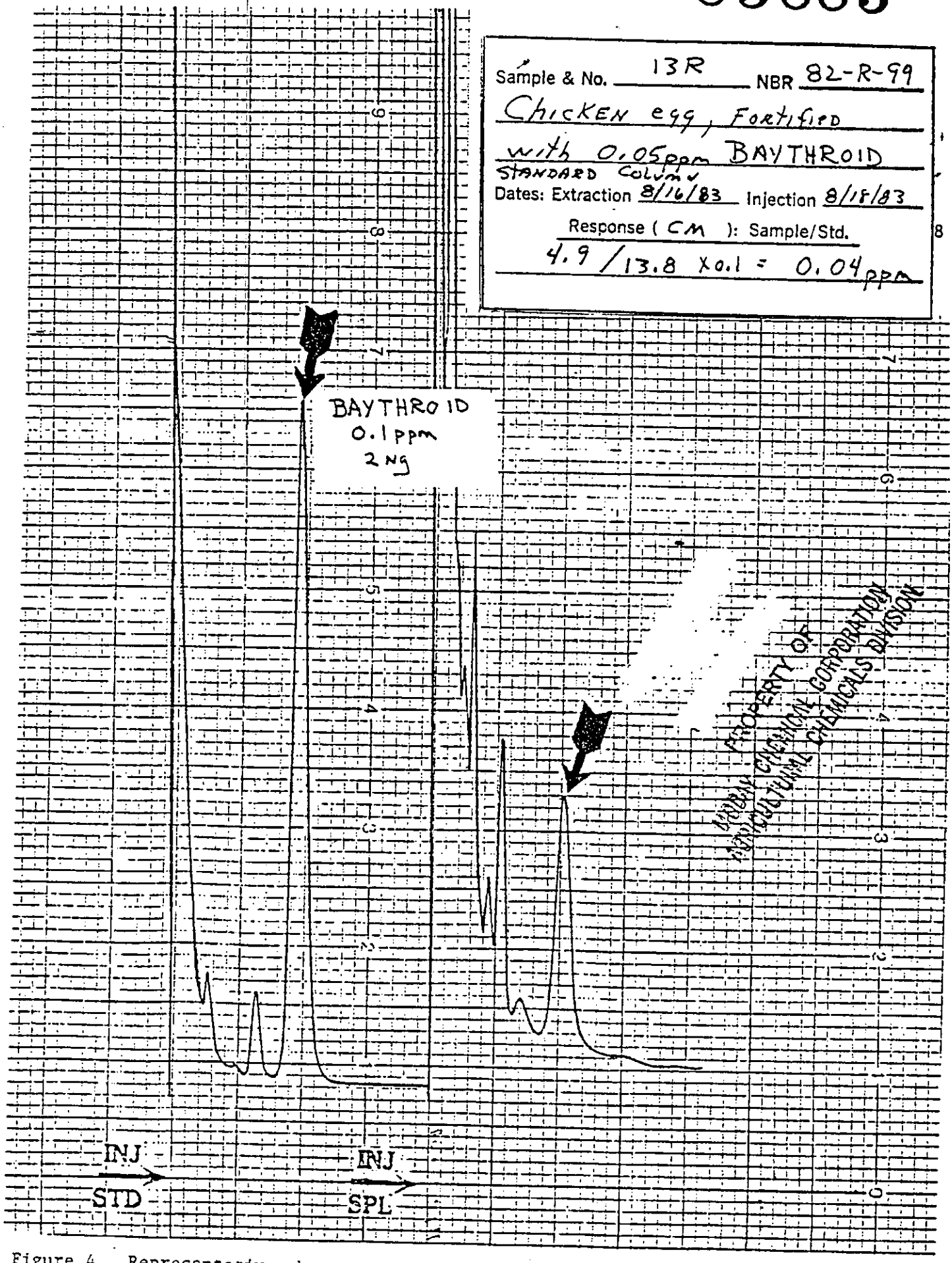

Figure 4. Representative chromatogram for recovery of BAYTHROID from eggs fort[illegible]

APPENDIX I

Method Modifications in the Analysis of Bovine and Poultry Tissues

(Made by Morse Laboratories, Sacrament, California in order to use the Coulson Detector)

The following modifications were made to the method in analyzing bovine and poultry tissues.

1. The amount of fat analyzed was increased from 10 g to 20 g in step IB-1.

2. ~~The silica gel column~~ in step IID-1 to 14 was ~~changed to~~ a Florisil (2.5% water) column.

   a. Place plug of glass wool in bottom of a 20 x 400 mm chromatographic column. Small amount of sea sand
   b. Slowly pour 10 g Florisil (2.5% water) into the column. ~~Add 100 ml methylene chloride~~ to the column, ~~and drain the solution through~~ the column, ~~discarding the 100 ml methylene chloride.~~
   c. Add 100 ml hexane to the column, and drain the solution through the column, discarding the 100 ml hexane.
   d. Pipet ~~100~~ ml (all) of the sample onto the column. Small hexane
   e. Drain the hexane from the column, and discard the hexane.
   f. Add ~~80~~ ml hexane to the column, and drain the solution through the column, discarding the 80 ml hexane. Small - Same sample flask
   g. Add 20 ml hexane/acetone (90:10) to the column, and drain the solution through the column, discarding the 20 ml hexane/acetone.
   h. Place a 125 or 250 ml round bottom flask under the column, add 40 ml hexane/acetone (90:10) to the column, and collect all 40 ml hexane/acetone.
   i. Evaporate the 40 ml hexane/acetone to dryness; pipet ~~1.25~~ ml ~~hexane~~ acetone, and retain sample for gas chromatography. 2nd - 10 g sample; Sml - 25 g sample

   NOTE: Liver and kidney were subjected to this procedure twice.

3. The gas-liquid chromatography column in step IV was changed to a 120 cm x 0.6 cm O.D., and the column packing was changed to 3% OV-101 on Gas Chrom Q (100/120 mesh). → go to GC section

4. A Coulson (Cl) detector was used.

Detailed Procedure

I. Extraction

A. All tissues except fat and skin.

1. Weigh 10 g Hyflo Super-Cel and 25 g tissue into a Griffin tall form beaker (500 ml). In the case of bovine liver or kidney, use 10 g tissue plus 2 ml concentrated HCl.

2. Add 150 ml acetone/chloroform (2:1).

3. Homogenize the mixture for 2 to 3 min at a medium speed with a Tekmar Tissuemizer.

4. Filter the homogenate under vacuum through a 9 cm glass fiber filter in a Büchner funnel into a 1-l boiling flask.

5. Homogenize the filter cake in the same beaker with another 150 ml acetone/chloroform (2:1).

6. Repeat step 4 using the same flask.

7. Repeat steps 5 and 6.

8. Concentrate the combined organic extracts to dryness under vacuum on the rotary evaporator.

9. Proceed to step II.

B. Fat and skin.

1. Weigh 10 g Hyflo Super-Cel, 10 g tissue, and 50 g anhydrous sodium sulfate (granular) into a Griffin tall form beaker (500 ml).

2. Add 150 ml hexane to the beaker.

3. Homogenize the mixture for 2 to 3 min at a medium speed with a Tekmar Tissuemizer.

4. Filter the sample under vacuum through a 9-cm glass fiber filter in a Büchner funnel into a 1-l boiling flask.

5. Transfer the filtrate into a 500 ml separatory funnel.

6. Homogenize the filter cake with 150 ml acetonitrile.

7. Repeat steps 4 and 5.

8. Shake the separatory funnel for 1 min. Allow the two phases to separate.

9. Transfer the lower phase (acetonitrile) into a second 500 ml separatory funnel, and add 150 ml fresh hexane.