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RH-9090 Residue Analytical Method and Validation Data for Meat, Milk and Eggs

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SUBJECT: RH-9090 RESIDUE ANALYTICAL METHOD AND VALIDATION DATA FOR MEAT, MILK AND EGGS

The attached report describes the residue analytical method for RH-9090 in meat, milk and eggs. Validation data at 3 fortification levels are also reported.

RH-9090 is a metabolite of RH-3866 (SYSTHANE™), a fungicide being developed for use on grapes, apples and other crops. Since residues of RH-9090 may occur in processed commodities used in animal feed, a validated residue analytical method is required for this compound in meat, milk and eggs.

The method involves extraction with methanol, partition with hexane to remove interferences and then with methylene chloride. The methylene chloride is evaporated and the samples are purified on silica gel. Liver samples require a further cleanup on reversed phase LC-18 disposable cartridges. Quantitation is performed by gas chromatography on a 0.53 mm ID SPB-608 capillary column with a thermionic specific detector optimized for nitrogen.

Average recoveries for all substrates at all fortification levels range from 79% at 0.01 ppm in the liver to 118% at 0.01 ppm in the chicken. The sensitivity of the method is 0.01 ppm for all substrates.

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JJM/mad
attachment

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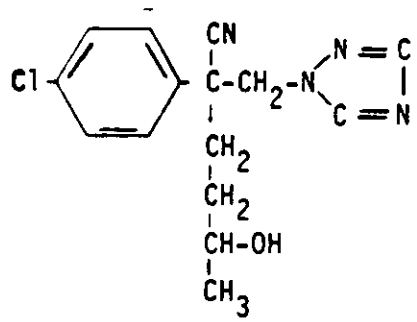
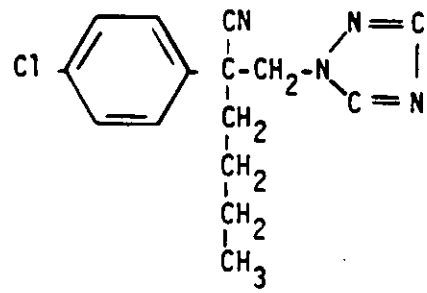
I. INTRODUCTION

RH-9090 is a metabolite of RH-3866 (SYSTHANE™), a fungicide being developed for use on grapes, apples and other crops. Since residues of RH-9090 may occur in processed commodities used in animal feed, a validated residue analytical method is required for this compound in meat, milk and eggs. The structure of these compounds are shown below:



RH-9090



RH-3866

α-(3-hydroxybutyl)-α-(4-chlorophenyl) -1H-1,2,4-triazole propanenitrile

α-Butyl-α-(4-chlorophenyl)-1H-1,2,4-triazole propanenitrile

II. SUMMARY

RH-9090 is extracted from meat, milk and eggs with methanol. The extract is first partitioned with hexane to remove interferences and then with methylene chloride. The methylene chloride is evaporated and the samples are cleaned up on silica gel. Liver samples require a further cleanup on reversed phase LC-18 disposable cartridges. RH-9090 is determined by gas chromatography using a 0.53 mm ID capillary column (SPB-608) and a capillary thermionic specific detector optimized for nitrogen selectivity.

The sensitivity of the method is 0.01 ppm for all substrates. Recoveries were obtained at 3 fortification levels for each substrate. Average recoveries for each substrate are shown in Table I.

TABLE I

SUMMARY OF RECOVERY DATA
% RECOVERY ± STANDARD DEVIATION

Added ppm	Eggs	Liver	Beef	Chicken	Kidney	Milk
0.00	*0.00	0.00	0.00	0.00	0.00	0.00
0.01	87 ± 7	79 ± 8	89 ± 12	118 ± 33	111 ± 18	85 ± 2
0.05	91 ± 4	86 ± 19	93 ± 8	102 ± 13	92 ± 2	95 ± 5
0.20	97 ± 3	93 ± 15	93 ± 15	97 ± 3	92 ± 7	95 ± 3

*Values are ppm found.

III. METHOD

A. Chemicals

ITEM	GRADE	SOURCE
Acetone	Pesticide	Fisher
Bio-Sil A, 100-200 Mesh	Activate at 200°C for 48 hours	Bio-Rad
	Cap, cool and store in a desicator	
Celite-545	-	Johns Manville
Cotton	Sterile, absorbent	Johnson & Johnson
Hexanes	Pesticide	Baker
Isooctane	Pesticide	Baker
Isopropyl alcohol	Pesticide	Baker
LC-18 Supelclean tubes, 6 ml, 1 g		Supelco
Methanol	Pesticide	Baker
Methylene chloride	Pesticide	Baker
Sodium Chloride	Certified A.C.S.	Fisher
Sodium sulfate, anhydrous	granular AR	Mallinckrodt
Toluene	Pesticide	Baker
RH-9090	Analytical Standard	Rohm and Haas Co.
Water	Milli-Q	Millipore

B. Preparation of Solutions

1. Prepare a 10% sodium chloride solution by dissolving 400 g of sodium chloride in 4 liters of Milli-Q water.

2. Prepare a 2% sodium chloride solution by diluting 100 ml of the 10% solution above to 500 ml with Milli-Q water.

3. Prepare 100/20, 100/35 and 100/100 toluene/acetone solutions by adding 200, 350 and 1000 ml of acetone to 1000 ml of toluene.

4. Prepare 100/30 isooctane/isopropyl alcohol solution by addition 300 ml of isopropyl alcohol to 1000 ml of isooctane.

5. Prepare the 100/20, 100/60 and 100/100 water/methanol solutions by adding 20, 60 and 100 ml of methanol to 100 ml of Milli-Q water.

6. Prepare standard and fortification solutions by carefully weighing on an analytical balance 0.01g of RH-9090 analytical standard into a 100 ml volumetric flask. Add approximately 80-90 ml of isooctane/isopropyl alcohol (100/30) and sonicate until dissolution occurs. Bring to volume with isooctane/isopropyl alcohol. This is the primary standard of 100 g/ml. Make serial dilutions to 4, 1, 0.5, 0.2, 0.1, 0.05 and 0.02 µg/ml for working standards.

C. Equipment

Blenders - Explosion-resistant, Waring EPI - Thomas
Buchner funnels - Porcelain, 83 mm ID - Thomas
Beakers - 150 ml - Fisher
Chromatography columns - 17mm OD, 14.5 mm ID with 250 ml reservoir - Kontes
Erlenmeyer flasks - 250 ml - Fisher
Filter flasks - 500 ml - Fisher
Filter paper - 7.5 cm, reeve angel - Thomas
Graduated cylinders - 500, 250, 100, 50, 10 ml - Fisher
Glass stirring rods - Fisher
Glass vial - Wheaton, 1 oz. with caps - Thomas
N-Evap concentrator - Organomation Associates, Inc.
Pasteur pipets - Fisher
Pipets - 1 -10 ml - Fisher
Rotary evaporator - Buchi Rotovapor R, Brinkman
Round bottom flasks - 500, 250, 100 ml - Fisher
Separatory funnels - 500 ml - Fisher
Solid Phase Extraction Vacuum Manifold with 20 ml reservoirs and adaptors - Supelco
Test Tubes - Culture, 15 x 125 mm - Kimble
Volumetric flasks - 100 ml - Fisher

D. Instrumentation

1. Varian 3500 Capillary Gas Chromatrograph equipped with a Varian Model 8035 Autosampler and a Capillary Thermionic Specific Detector. Data is obtained with a HP 300 Data Acquisition and Processing Station with Hewlett-Packard Extrachrom Software. Data is processed by Nelson Analytical software.

Column: Fused silica capillary, SPB-608, 0.53 mm ID, 15 meters, 0.50 µm df - Supelco

Temperatures: Column - 220°C
Injector - 250°C
Detector - 250°C

Flows: Air (Zero Grade) - 175 ml/min.
Hydrogen (UPC) - 4.5 ml/min.
Helium (UPC) - 20 ml/min., 10 ml/min. purge

Bead current: 3.5 amps

Under these conditions the retention time of RH-9090 is 4.7 minutes.

2. Confirmatory Analysis is performed on a Hewlett-Packard Model 5890 gas chromatograph equipped with a capillary injector, Model 7673A Automatic sampler, Model 3393A Integrator and a ^{63}Ni ECD. Data is collected and processed by a Nelson Analytical System.

Column: Fused silica capillary, DB-17, 0.53 mm ID, 30 meters, 1.0 µm df - J & W Scientific.

Temperatures: Column - Initial 220°C - 5 minutes
Program rate 10°/minute
Final 260°C - 21 minutes
Injector - 270°C
Detector - 325°C

Flows: Set at oven temperature of 50°C
Split - 45 ml/min.
Column - 15 ml/min.
Septum - 1 ml/min.
Purge - 60 ml/min.
Total flow through the detector = 75 ml/min.
At an oven temperature of 170°C the flow through the column is 8.7 ml/min.

Under these conditions the retention time for RH-9090 is 16.1 minutes.

E. Analytical Procedure

1. Sample Processing

All samples are purchased fresh at local markets. Each day whole fresh eggs are cracked and the contents poured into a Waring blender jar. The eggs are gently blended into a

homogeneous sample. The liver, kidney and chicken samples are cut into small pieces with a sharp knife, then blended with dry ice into a powder and thoroughly mixed. The dry ice is allowed to sublime in the freezer and the samples kept frozen until analysis. The beef samples are ground chuck. Fresh, pasteurized, homogenized milk is used for the milk samples.

2. Extraction

a. Meat, Eggs and Poultry

Weigh 20 g of the representative sample into a 1 qt. blender jar. Add the fortifications when appropriate. For all substrates but milk add 15 g of Celite-545, 100 ml of methanol and blend for 5 minutes. Vacuum suction filter the blended mixture through 7.5 cm filter paper using a porcelain filter funnel and a 500 ml filter flask. Wash the blender jar with 25 ml of methanol and pour over the filter cake into the 500 ml flask. Add the filtrate to the 500 ml separatory funnel. Wash the filter flask with 10 ml methanol and add it to the separatory funnel.

b. Milk

Weigh 20 g of fresh milk into a 150 ml beaker. Add the fortifications when appropriate. Add 100 ml methanol and stir with a glass stirring rod. Let stand for 30 minutes. Add 10 g of Celite-545 to the beaker and stir with the glass stirring rod. Vacuum suction filter the mixture through 7.5 cm filter paper using a porcelain filter funnel and a 500 ml filter flask. Wash the beaker with 25 ml of methanol and pour over the filter cake into the 500 ml filter flask. Transfer the filtrate to a 500 ml separatory funnel. Wash the filter flask with 10 ml of methanol and add it to the separatory funnel.

3. Partition

To the separatory funnel containing the extract add 30 ml of 2% sodium chloride solution, 100 ml of hexanes and shake gently 10-15 times (be careful not to shake too vigorously or an emulsion forms). When the phases separate, draw down the bottom aqueous-methanol solution into a second 500 ml separatory funnel. Discard the hexane phase. Add 150 ml of methylene chloride, 130 ml of 10% sodium chloride solution and shake vigorously for 1 minute. When the phases separate, draw down the lower methylene chloride phase into a 500 ml round bottom flask. Add 75 ml of methylene chloride to the separatory funnel and shake 30 seconds. When the phases separate, draw down the methylene chloride phase into the 500 ml round bottom flask containing the first extract. Evaporate the combined methylene chloride phases on the rotovap at 45-50°C at atmospheric pressure. Remove the final traces of methylene chloride under reduced pressure. Add 25 ml of toluene/acetone: 100/20 and swirl to dissolve the residue.

4. Cleanup

a. Bio-Sil A Column Chromatography

Insert a small cotton plug into a 14.5 mm ID chromatographic column and dry pack the column with 8cc of the activated Bio-Sil A (measure in a 10 cc graduated cylinder). Top the column with 1 inch of anhydrous sodium sulfate. Add the 25 ml of toluene/acetone: 100/20 solution in the 500 ml round bottom flask from the partition step to the column and collect the eulate in a 250 ml Erlenmeyer flask. Rinse the 500 ml round bottom flask with 10 ml of toluene/acetone: 100/20 and add to the column when the previous addition just enters the top of the column. Add 60 ml of toluene/acetone: 100/35 to the column when the previous addition just enters the top of the column and collect the eluate in the 250 ml Erlenmeyer flask. Discard the combined eluate. Collect 100 ml of toluene/acetone: 100/100 in a 250 ml round bottom flask and concentrate the eluate to dryness on the rotary evaporator at 70°C under reduced pressure. For all samples, except liver, add the appropriate volume of isooctane/isopropyl alcohol: 100/30 for gas chromatography. Four ml for controls, 0.01 ppm and 0.05 ppm samples. Ten ml for 0.2 ppm samples. Liver samples must be further cleaned up on the LC-18 solid phase extraction tube.

b. LC-18 Solid Phase Extraction

Transfer the dry liver residue in the 250 ml round bottom flask to a 1 oz. glass vial with three 5 ml washes using a Pasteur pipet to repeatedly wash down the sides of the flask and make the transfer. Evaporate the methanol to dryness on the N-Evap at 45°C under a gently stream of nitrogen. Add 2 ml of methanol to dissolve the residue in the 1 oz. vial.

Following the manufacturer's instructions for use of the Solid Phase Extraction Vacuum Manifold, condition an LC-18 SPE 1 g extraction tube with 4 ml of methanol followed by 6 ml of Milli-Q water. Allow 1-2 mm of water to remain above the top of the tube frit. Connect the 20 ml reservoir to the tube with an adapter.

Add 10 ml of Milli-Q water to the 1 oz. vial containing the 2 ml methanol sample, cap and shake. Pour the contents of the vial into the 20 ml reservoir. Wash the 1 oz. vial with 2 ml of Milli-Q water/methanol: 100/20 and add it to the 20 ml reservoir. Aspirate the sample through the tube. In a like manner, aspirate 8 ml of Milli-Q water/methanol: 100/60 through the tube. Discard the eulate. Position the collection tube (15 x 125 mm culture

tube) in the vacuum manifold and aspirate 10 ml of Milli-Q water/methanol: 100/100 through the tube. Turn off the vacuum and pour the contents of the collection tube into a 100 ml round bottom flask. In a like manner, collect an additional 5 ml of the Milli-Q water/methanol: 100/100 in the collection tube. Add this eluate to the 100 ml round bottom flask and concentrate to dryness on the rotary evaporator at 70°C under reduced pressure. Add the appropriate volume of isooctane/isopropyl alcohol: 100/30 for gas chromatography. Four ml for controls, 0.01 ppm and 0.05 ppm samples. Ten ml for 0.2 ppm samples.

5. Gas Chromatography

a. Preparation of Standard Curves

A 2 µl aliquot of each standard in the range of 0.5 to 0.02 µg/ml is injected into the gas chromatograph. The resulting peak heights are measured and plotted vs. concentration (µg/ml) of RH-9090 to obtain a standard curve. Standard curves are prepared for each analysis day.

b. Sample Analysis

A 2 µl aliquot of the final GLC sample is injected into the gas chromatograph. If necessary, the sample is diluted with isooctane/isopropyl alcohol: 100/30 to give a response with the standard curve range. The peak height is measured and the concentration determined from the standard curve. The limit of detection is set by the data system at one-half the value of the peak height of the lowest standard injected (0.5 x lowest peak height of 0.02 µg/ml standard).

6. Method of Calculation

The RH-9090 residue concentration is determined as follows:

a. Fortification Recovery

For samples fortified with known amounts of RH-9090 prior to extraction measure the peak height, determine the concentration (µg/ml) from the standard curve and calculate the % recovery from equation 1.

Eq. 1 $$\frac{(\text{µg/ml Found}) \times \text{Final Sample Volume (ml)}}{\text{Fortification (µg)}} \times 100 = \%\ \text{Recovery}$$

b. RH-9090 Residue Concentration

The RH-9090 residue concentration is determined as follows:

Eq. 2 $$\frac{\text{Final Sample Volume(ml)} \times \text{Concentration(µg/ml)}}{\text{Average Recovery (\%)} \times \text{Sample Weight (g)}} \times 100 = \text{ppm}$$

IV. RESULTS

Recoveries of fortifications of RH-9090 added to meat, milk and eggs are given below in Table II. Detailed analytical results are appended in Tables III through VIII. Sample chromatograms and calibration curves are shown in Figures 1 through 24. Sample confirmatory analysis chromatograms are shown in Figures 25 through 28. The residue method described above can detect RH-9090 at the 0.01 ppm level for each substrate analyzed and should be suitable for enforcement purposes.

TABLE II

Recovery Data (%)

Added ppm	Eggs	Liver	Beef	Chicken	Kidney	Milk
0.00	*0.00	0.00	0.00	0.00	0.00	0.00
0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.00	0.00	0.00	0.003	0.00		0.00
0.00	0.00	0.00		0.00		
0.01	81	79	87	161	88	86
0.01	81	88	104	87	119	84
0.01	90	80	89	100	105	87
0.01	94	69	75	125	130	83
Ave. ± σ	87 ± 7	79 ± 8	89 ± 12	118 ± 33	111 ± 18	85 ± 2
0.05	88	78	96	114	94	91
0.05	91	77	100	88	92	91
0.05	88	76	96	104	90	96
0.05	97	114	81	—	91	102
Ave. ± σ	91 ± 4	86 ± 19	93 ± 8	102 ± 13	92 ± 2	95 ± 5
0.20	94	90	86	99	101	92
0.20	94	80	96	99	86	95
0.20	100	88	99	93	87	98
0.20	98	115	91	—	92	—
Ave. ± σ	97 ± 3	93 ± 15	93 ± 6	97 ± 3	92 ± 7	95 ± 3

* Values are ppm found.

TABLE III

Detailed Recovery Data for Eggs

ppm added	Peak Height	g/ml RH-9090 Found	Final Volume	Sample Wt. (g)	ppm Found	% Recovery
0.00	0.0	0.00	2.0	20	0.00	-
0.00	0.0	0.00	2.0	20	0.00	-
0.00	0.0	0.00	4.0	20	0.00	-
0.00	0.0	0.00	4.0	20	0.00	-
0.01	51.4	0.0811	2.0	20	0.0081	81
0.01	51.1	0.0807	2.0	20	0.0081	81
0.01	30.7	0.0448	4.0	20	0.00896	90
0.01	28.3	0.0472	4.0	20	0.00944	94
0.05	279.0	0.441	2.0	20	0.0441	88
0.05	287.0	0.454	2.0	20	0.0454	91
0.05	151.0	0.220	4.0	20	0.044	88
0.05	145.0	0.241	4.0	20	0.0483	97
0.20	257.0	0.374	10.0	20	0.187	94
0.20	257.0	0.375	10.0	20	0.187	94
0.20	276.0	0.401	10.0	20	0.201	100
0.20	234.0	0.391	10.0	20	0.195	98

TABLE IV

Detailed Recovery Data for Liver

ppm added	Peak Height	g/ml RH-9090 Found	Final Volume	Sample Wt. (g)	ppm Found	% Recovery
0.00	0.0	0.00	4.0	20	0.00	-
0.00	0.0	0.00	4.0	20	0.00	-
0.00	0.0	0.00	4.0	20	0.00	-
0.00	0.0	0.00	4.0	20	0.00	-
0.01	30.2	0.0396	4.0	20	0.00792	79
0.01	33.5	0.044	4.0	20	0.0879	88
0.01	29.3	0.0399	4.0	20	0.00797	80
0.01	24.2	0.0344	4.0	20	0.00689	69
0.05	148.0	0.194	4.0	20	0.0389	78
0.05	147.0	0.193	4.0	20	0.0386	77
0.05	140.0	0.191	4.0	20	0.0382	76
0.05	201.0	0.286	4.0	20	0.0572	114
0.20	263.0	0.358	10.0	20	0.179	90
0.20	234.0	0.318	10.0	20	0.159	80
0.20	260.0	0.354	10.0	20	0.177	88
0.20	325.0	0.462	10.0	20	0.231	115

TABLE V

Detailed Recovery Data for Beef

ppm added	Peak Height	g/ml RH-9090 Found	Final Volume	Sample Wt. (g)	ppm Found	% Recovery
0.00	0.0	0.00	4.0	20	0.00	-
0.00	0.0	0.00	4.0	20	0.00	-
0.00	10.8	0.0152	4.0	20	0.003	-
0.01	35.5	0.0437	4.0	20	0.00873	87
0.01	42.1	0.0519	4.0	20	0.0104	104
0.01	37.6	0.053	4.0	20	0.00755	75
0.01						
0.05	195.0	0.240	4.0	20	0.048	96
0.05	203.0	0.250	4.0	20	0.050	100
0.05	154.0	0.218	4.0	20	0.0405	81
0.05	751.0	0.371	4.0	20	0.0742	148
0.20	264.0	0.372	10.0	20	0.183	91
0.20	759.0	0.375	10.0	20	0.188	94

TABLE VI

Detailed Recovery Data for Chicken

ppm added	Peak Height	g/ml RH-9090 Found	Final Volume	Sample Wt. (g)	ppm Found	% Recovery
0.00	0.0	0.00	4.0	20	0.00	-
0.00	0.0	0.00	4.0	20	0.00	-
0.00	0.0	0.00	4.0	20	0.00	-
0.00	0.0	0.00	4.0	20	0.00	-
0.01	64.3	0.0804	4.0	20	0.0161	161
0.01	34.6	0.0433	4.0	20	0.00867	87
0.01	40.5	0.050	4.0	20	0.010	100
0.01	50.6	0.0625	4.0	20	0.0125	125
0.05	228.0	0.285	4.0	20	0.0571	114
0.05	117.0	0.221	4.0	20	0.0442	88
0.05	210.0	0.259	4.0	20	0.0519	104
0.20	317.0	0.396	10.0	20	0.198	99
0.20	236.0	0.394	10.0	20	0.197	99
0.20	300.0	0.370	10.0	20	0.185	93

TABLE VII

Detailed Analytical Data for Kidney

ppm added	Peak Height	g/ml RH-9090 Found	Final Volume	Sample Wt. (g)	ppm Found	% Recovery
0.00	0.0	0.00	4.0	20	0.00	-
0.00	0.0	0.00	4.0	20	0.00	-
0.01	107.0	0.0526	4.0	20	0.0105	105
0.01	131.0	0.0649	4.0	20	0.013	130
0.01	88.8	0.0439	4.0	20	0.00878	88
0.01	120.0	0.0595	4.0	20	0.0119	119
0.05	457.0	0.226	4.0	20	0.0452	90
0.05	460.0	0.227	4.0	20	0.0454	91
0.05	476.0	0.235	4.0	20	0.047	94
0.05	466.0	0.230	4.0	20	0.046	92
0.20	286.7	0.404	10.0	20	0.202	101
0.20	702.0	0.347	10.0	20	0.173	87
0.20	741.0	0.366	10.0	20	0.183	92
0.20	699.0	0.345	10.0	20	0.173	86

TABLE VIII

Detailed Analytical Data for Milk

ppm added	Peak Height	g/ml RH-9090 Found	Final Volume	Sample Wt. (g)	ppm Found	% Recovery
0.00	13.7	0.00	4.0	20	0.00	-
0.00	9.2	0.00	4.0	20	0.00	-
0.00	0.0	0.00	4.0	20	0.00	-
0.01	78.8	0.0487	4.0	20	0.0086	86
0.01	76.8	0.0474	4.0	20	0.00835	84
0.01	53.4	0.0434	4.0	20	0.00868	87
0.01	51.3	0.0416	4.0	20	0.00833	83
0.05	377.0	0.233	4.0	20	0.0455	91
0.05	376.0	0.232	4.0	20	0.0453	91
0.05	297.0	0.241	4.0	20	0.0482	96
0.05	313.0	0.254	4.0	20	0.0508	102
0.20	604.0	0.373	10.0	20	0.185	92
0.20	618.0	0.382	10.0	20	0.190	95
0.20	482.0	0.392	10.0	20	0.196	98

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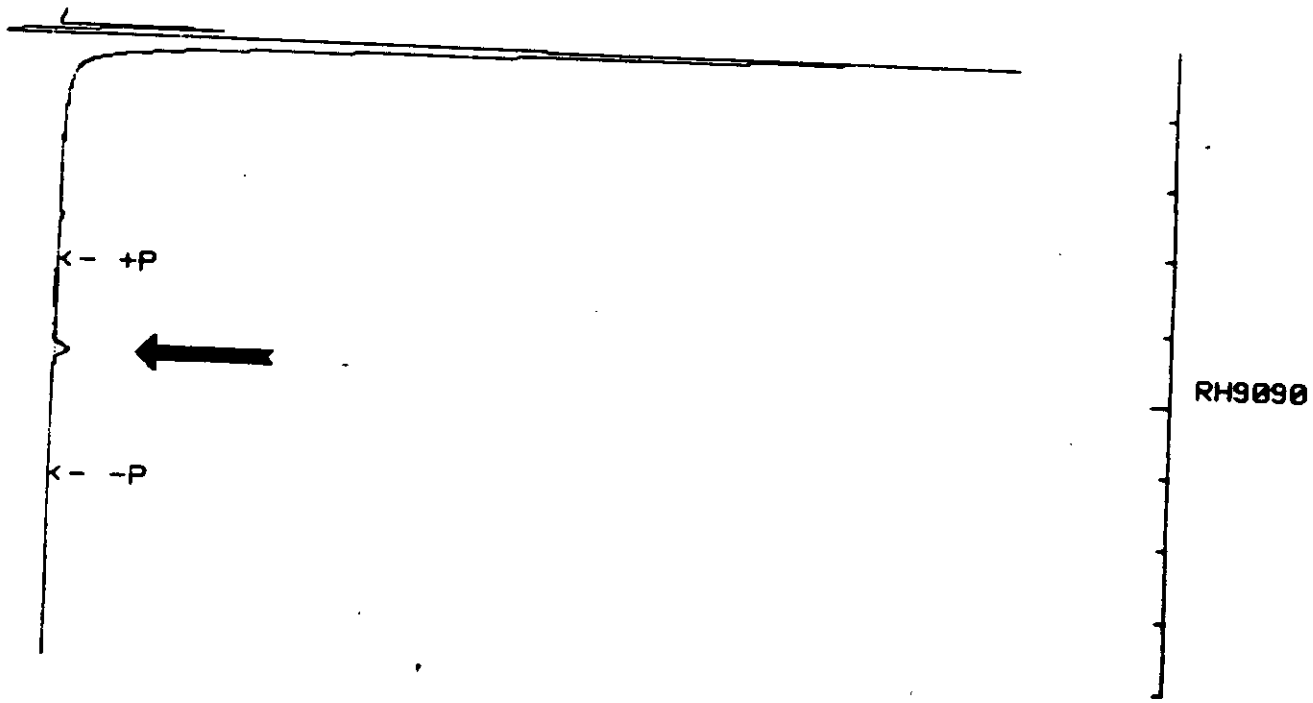
FIGURE 1

0.05 ug/ml RH-9090 Standard

Data file: M902875
Method file: JM9090m
Type: STANDARD

Plot times: 0 to 9 minutes
Plot range: 30 millivolts (-2 mv offset)



Retention Time	Compound Name	PPM Injected	Area	Height
4.75	RH9090	0.050	.25	29.71

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FIGURE 2

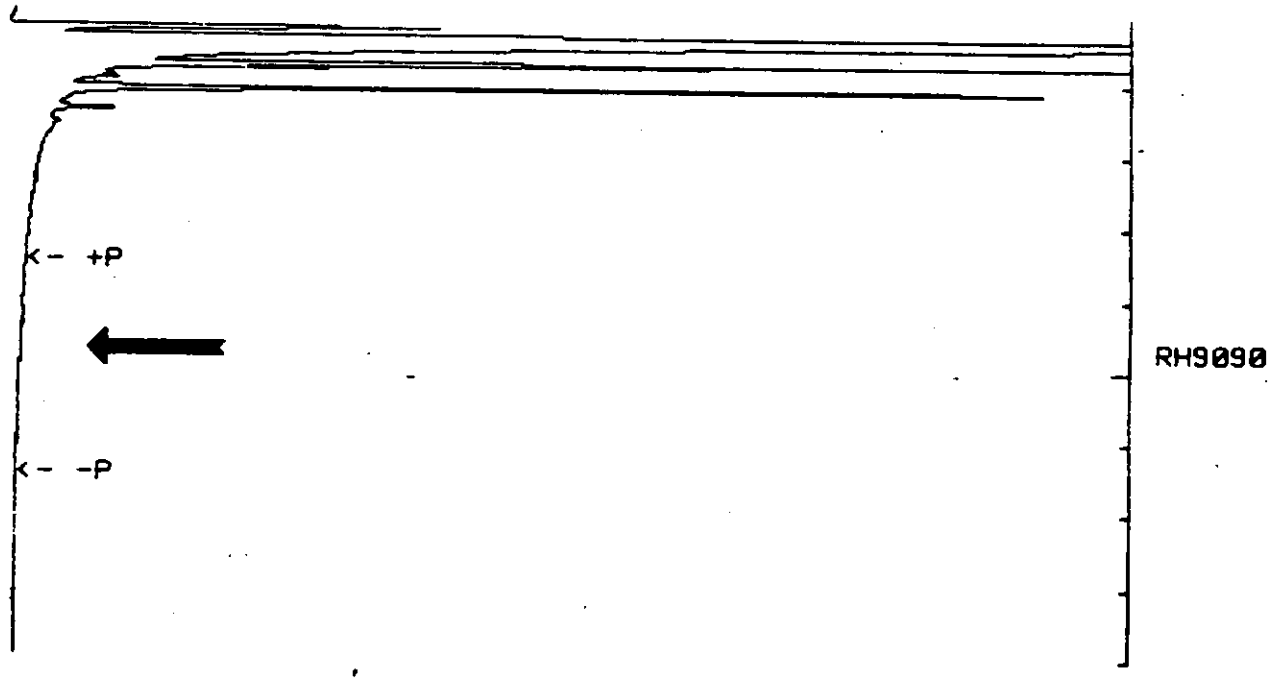
Control Egg

0f 0C21

Data file: M902877
Method file: JM9090m
Type: SAMPLE

RAR number: JM3652
Sample No: 1
Component: EGG

Plot times: 0 to 9 minutes
Plot range: 30 millivolts (-2 mv offset)



Ret. Time	Compound Name	Peak Area	Peak Height	ug/ml Found	Volume (ml)	Samp Wt.	Recov Fact.	PPM
4.72	RH9090	0.0	0.0	0.00	4.00	20.0	1.00	0.00

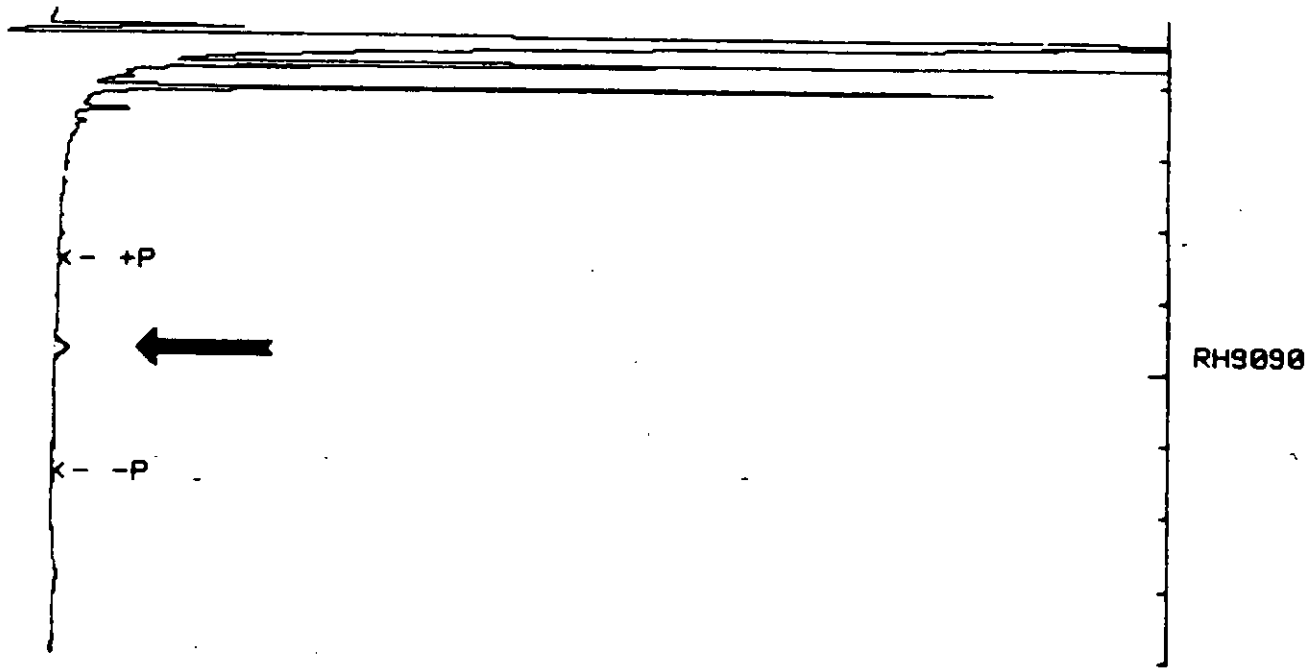
FIGURE 3

Control Egg Fortified with 0.01 ppm RH-9090

Data file: M902878
Method file: JM9090m
Type: FORTIFICATION

RAR number: JM3652
Sample No: 1
Component: EGG

Plot times: 0 to 9 minutes
Plot range: 30 millivolts (-2 mv offset)



Ret. Time	Compound Name	Peak Area	Peak Height	ug/ml Found	Volume (ml)	Ctl Corr.	ug Added	Pct Recov
4.74	RH9090	.3	28.3	0.0472	4.00	0.000	0.200	94

FIGURE 4

Calibration Curve
090287

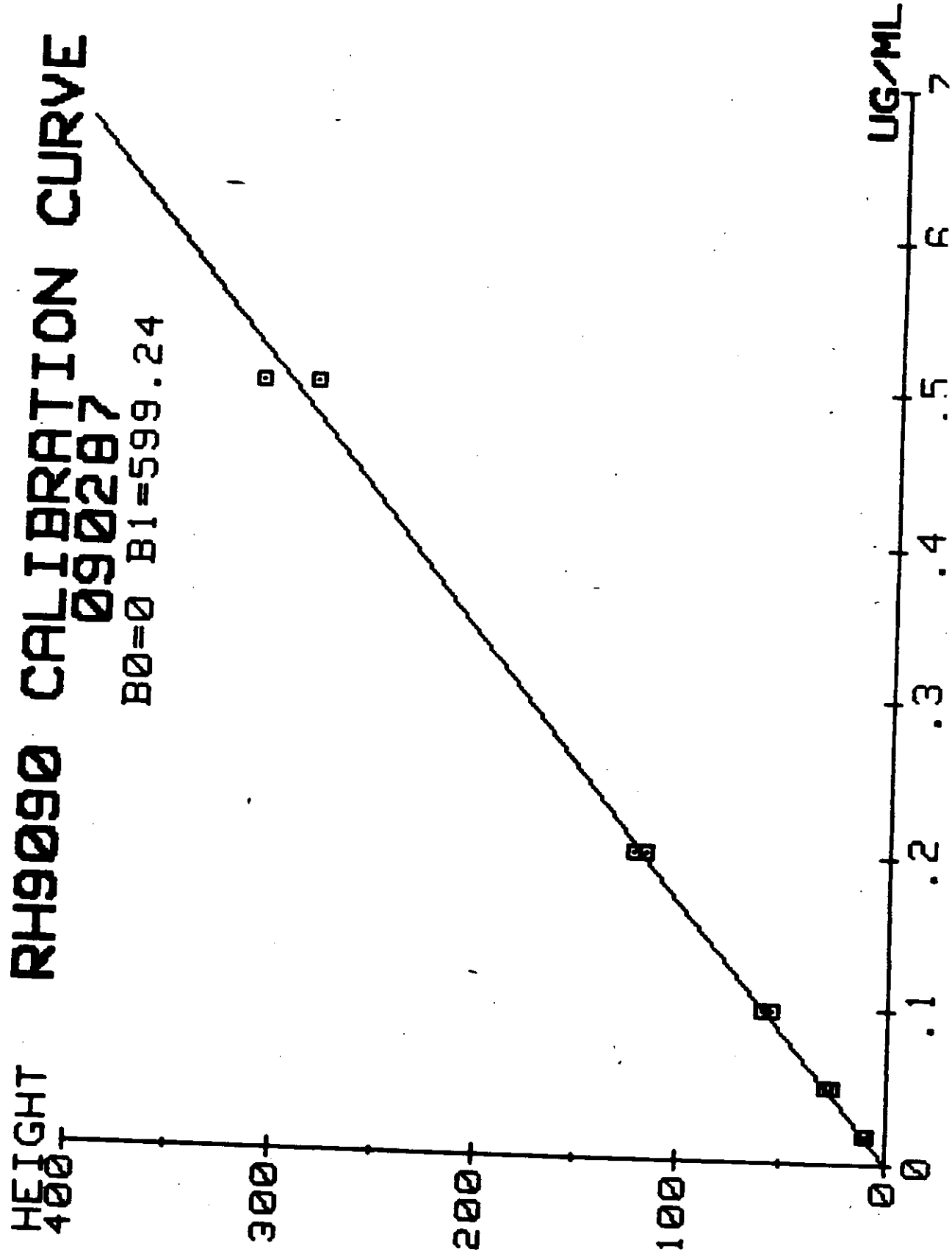


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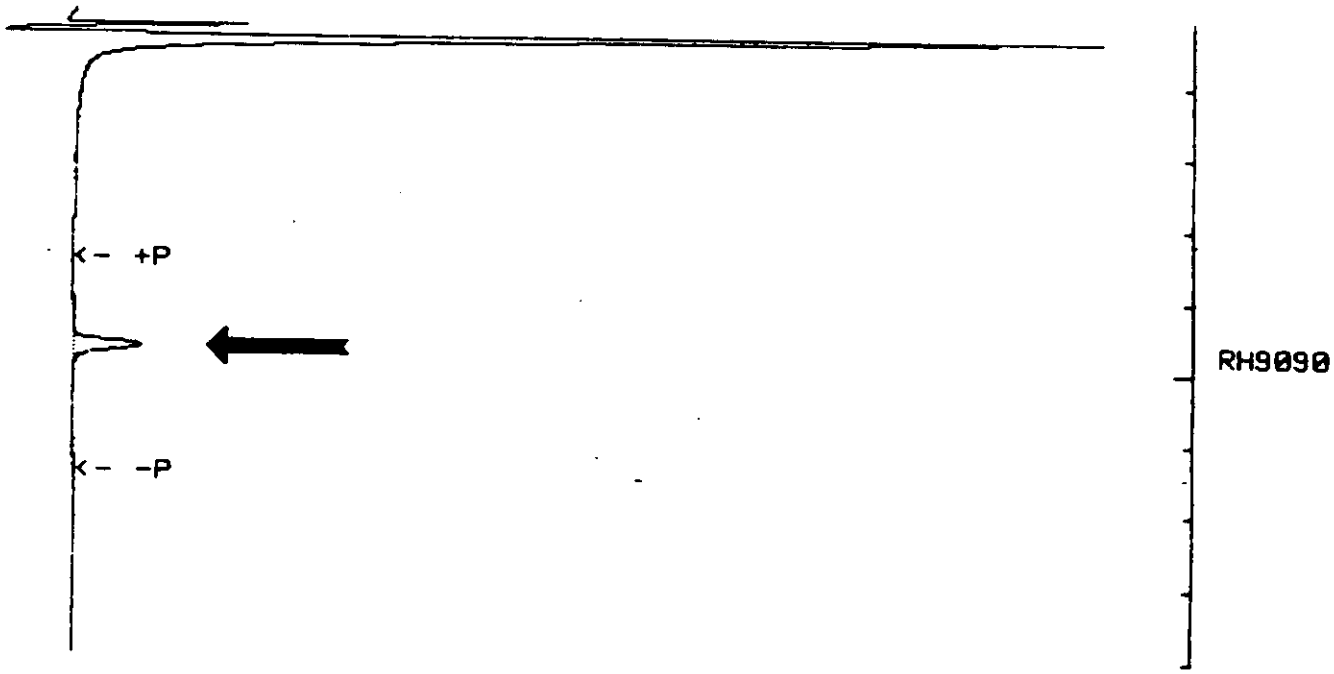
FIGURE 5

0.2 µg/ml RH-9090 Standard

0024

Data file: M825872
Method file: JM9090m
Type: STANDARD

Plot times: 0 to 9 minutes
Plot range: 30 millivolts (-2 mv offset)



Retention Time	Compound Name	PPM Injected	Area	Height
4.73	RH9090	0.200	1.34	148.71

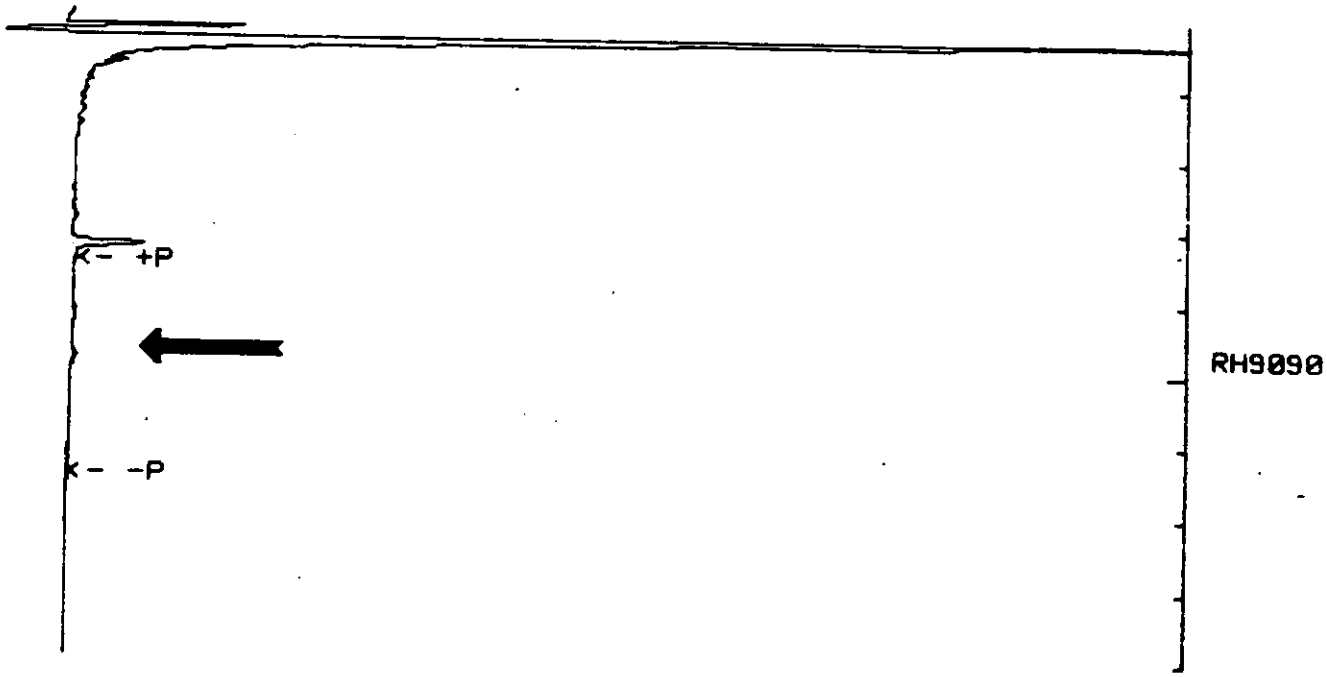
FIGURE 6

Control Liver

Data file: M825879
Method file: JM9090m
Type: SAMPLE

RAR number: 3645
Sample No: 2
Component: LIVER

Plot times: 0 to 9 minutes
Plot range: 30 millivolts (-2 mv offset)



Ret. Time	Compound Name	Peak Area	Peak Height	ug/ml Found	Volume (ml)	Samp Wt.	Recov Fact.	PPM
4.72	RH9090	0.0	0.0	0.00	4.00	20.0	1.00	0.00

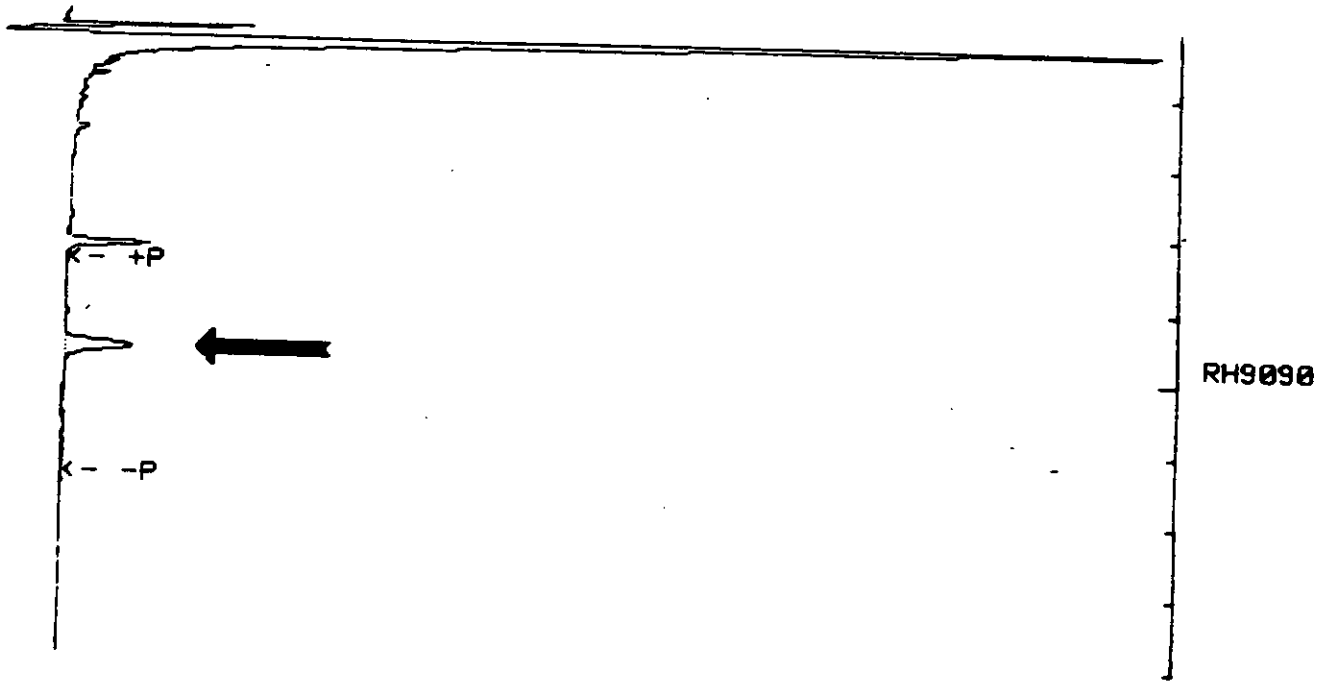
FIGURE 7

Control Liver Fortified with 0.05 ppm RH-9090

Data file: M8258711
Method file: JM9090m
Type: FORTIFICATION

RAR number: 3645
Sample No: 2
Component: LIVER

Plot times: 0 to 9 minutes
Plot range: 30 millivolts (-2 mv offset)



Ret. Time	Compound Name	Peak Area	Peak Height	ug/ml Found	Volume (ml)	Ctl Corr.	ug Added	Pct Recov
4.74	RH9090	1.3	147.2	0.193	4.00	0.000	1.00	77

FIGURE 8

Calibration Curve
08/25/87
Liver

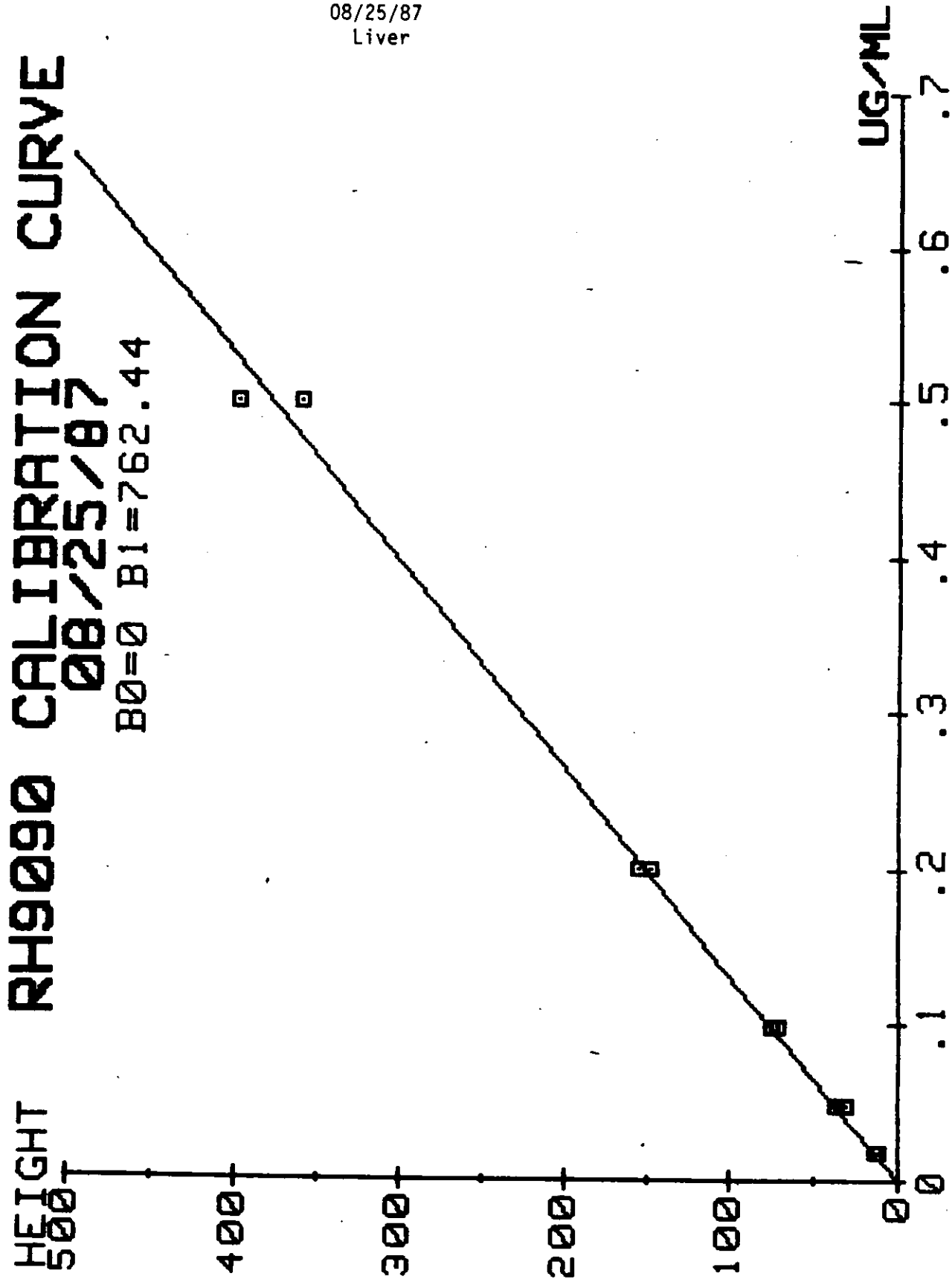
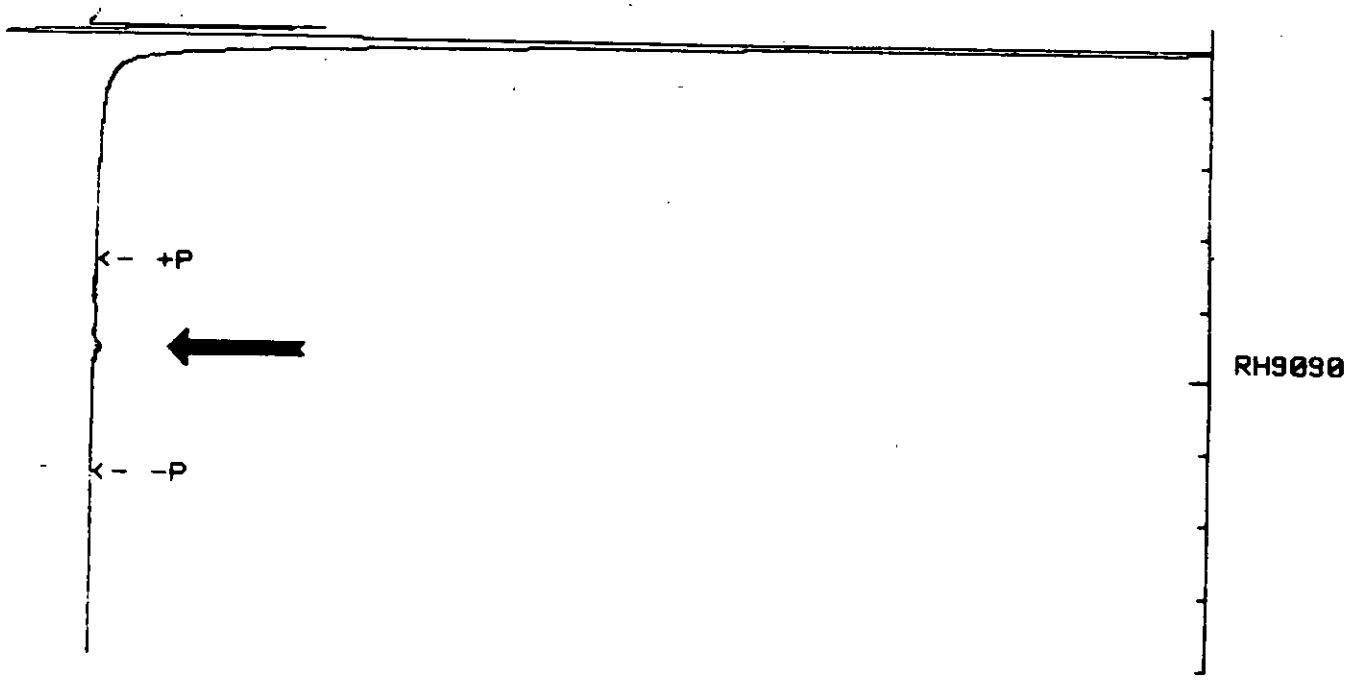


FIGURE 9

0.02 µg/ml RH-9090 Standard

Data file: N915875
Method file: JM9090m
Type: STANDARD

Plot times: 0 to 9 minutes
Plot range: 30 millivolts (-2.1 mv offset)



Retention Time	Compound Name	PPM Injected	Area	Height
4.73	RH9090	0.020	.11	12.99

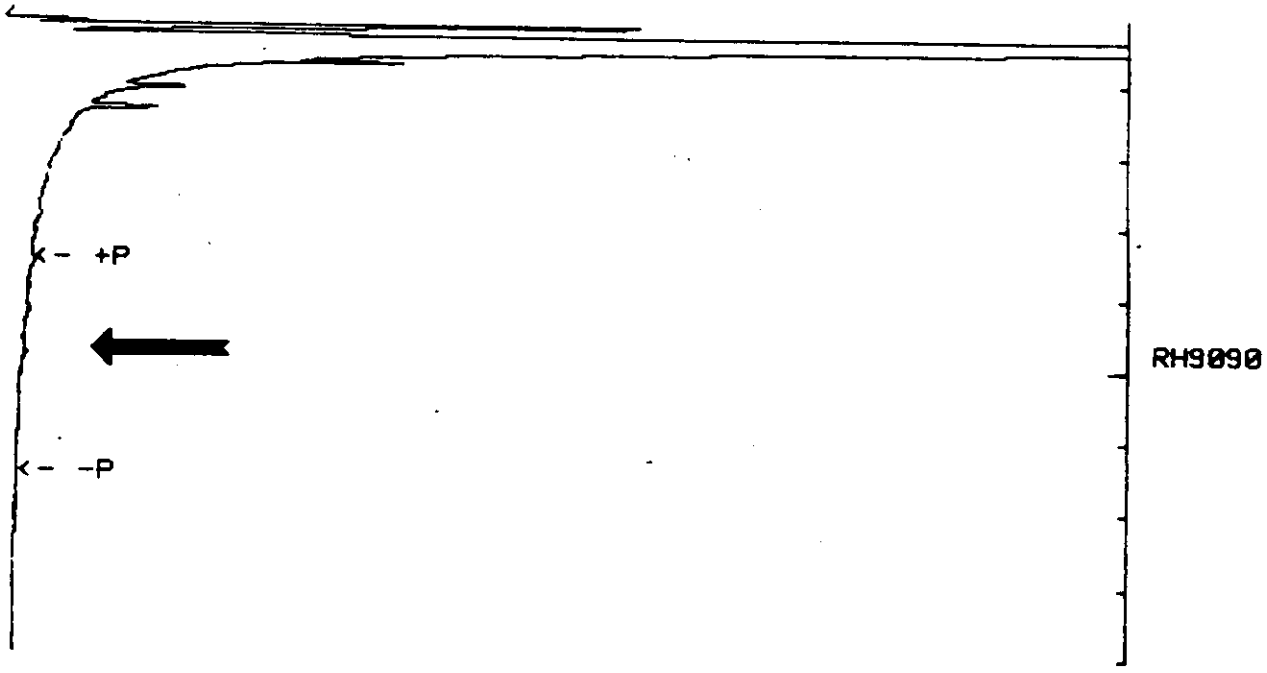
FIGURE 10

Control Chicken

Data file: N915876
Method file: JM9090m
Type: SAMPLE

RAR number: JM3654-
Sample No: 1
Component: CHICK

Plot times: 0 to 9 minutes
Plot range: 30 millivolts (-2.1 mv offset)



Ret. Time	Compound Name	Peak Area	Peak Height	ug/ml Found	Volume (ml)	Samp Wt.	Recov Fact.	PPM
4.75	RH9090	0.0	0.0	0.00	4.00	20.0	1.00	0.00

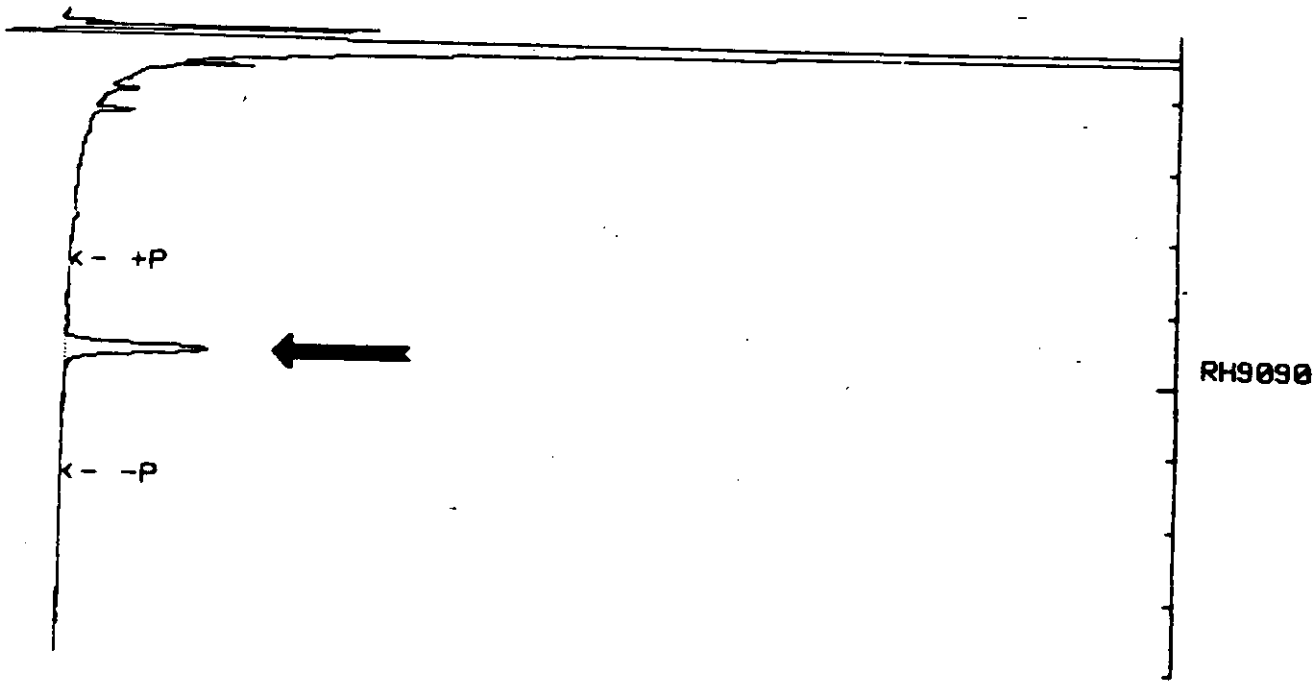
FIGURE 11

Control Chicken Fortified with 0.2 ppm RH-9090

Data file: N9158711
Method file: JM9090m
Type: FORTIFICATION

RAR number: JM3654
Sample No: 1
Component: CHICK

Plot times: 0 to 9 minutes
Plot range: 30 millivolts (-2.1 mv offset)



Ret. Time	Compound Name	Peak Area	Peak Height	ug/ml Found	Volume (ml)	Ctl Corr.	ug Added	Pct Recov
4.73	RH9090	3.0	316.6	0.396	10.0	0.000	4.00	99

FIGURE 12

Calibration Curve
091587
Chicken

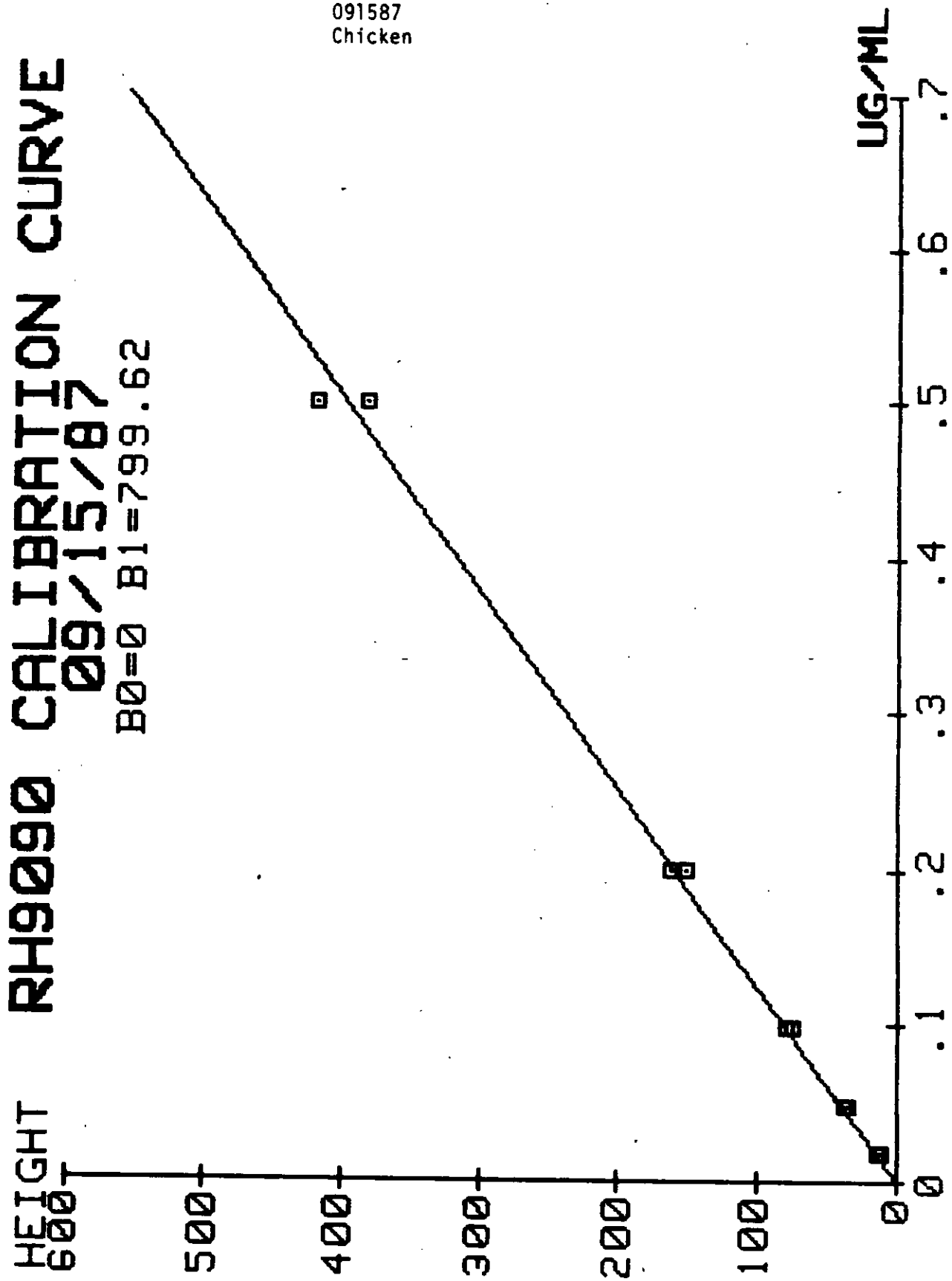


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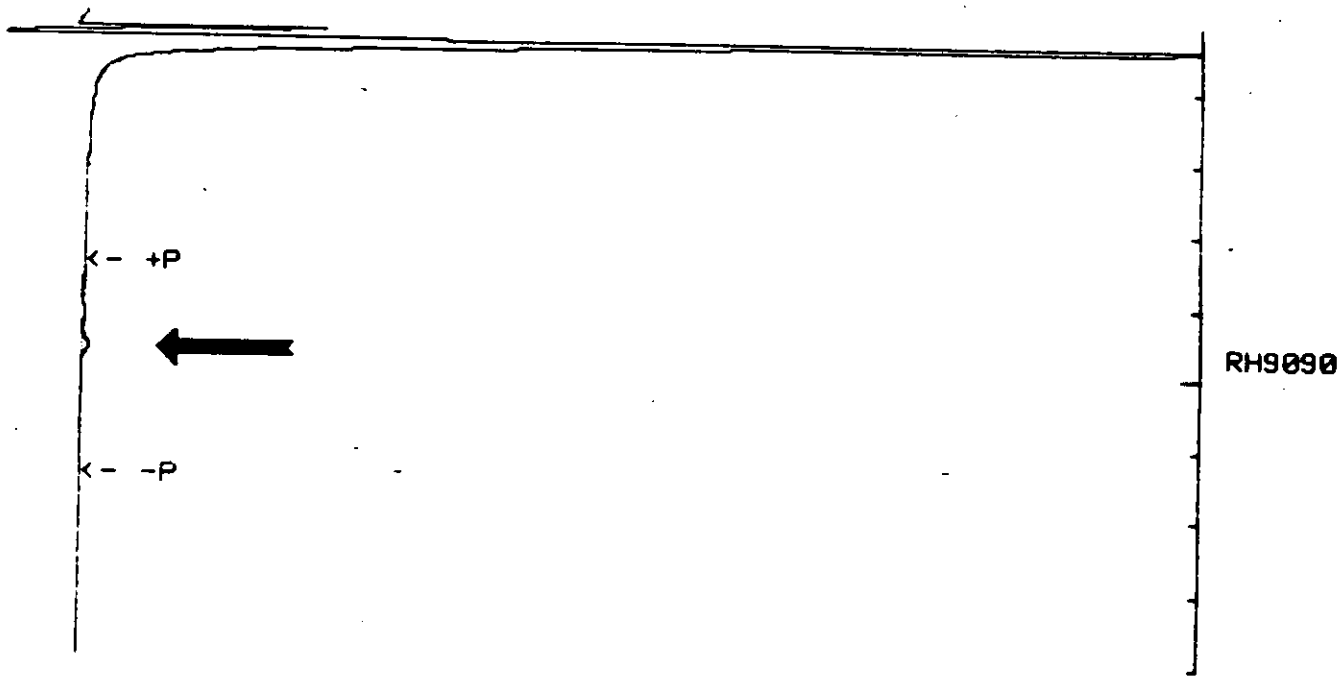
FIGURE 13

0.02 µg/ml RH-9090 Standard

C 0032

Data file: M918875
Method file: JM9090m
Type: STANDARD

Plot times: 0 to 9 minutes
Plot range: 30 millivolts (-2.1 mv offset)



Retention Time	Compound Name	PPM Injected	Area	Height
4.72	RH9090	0.020	.13	14.24

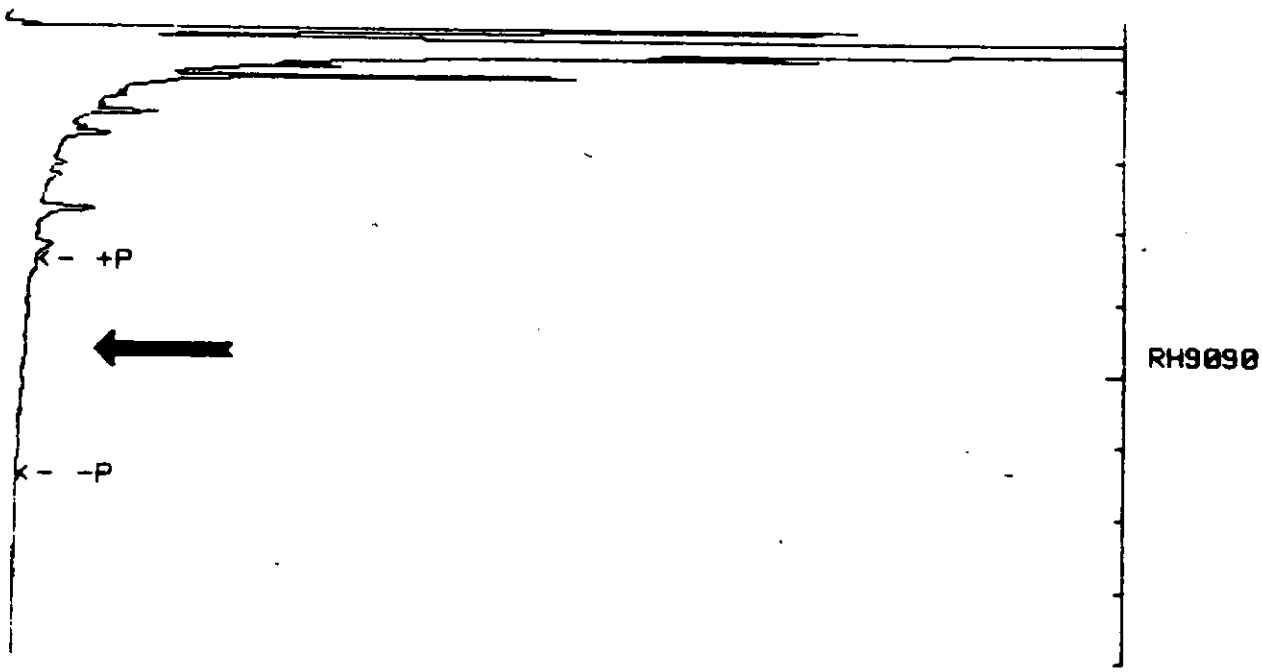
FIGURE 14

Control Beef

Data file: M918876
Method file: JM9090m
Type: SAMPLE

RAR number: JM3656
Sample No: 1
Component: BEEF

Plot times: 0 to 9 minutes
Plot range: 30 millivolts (-2.1 mv offset)



Ret. Time	Compound Name	Peak Area	Peak Height	ug/ml Found	Volume (ml)	Samp Wt.	Recov Fact.	PPM
4.72	RH9090	0.0	0.0	0.00	4.00	20.0	1.00	0.00

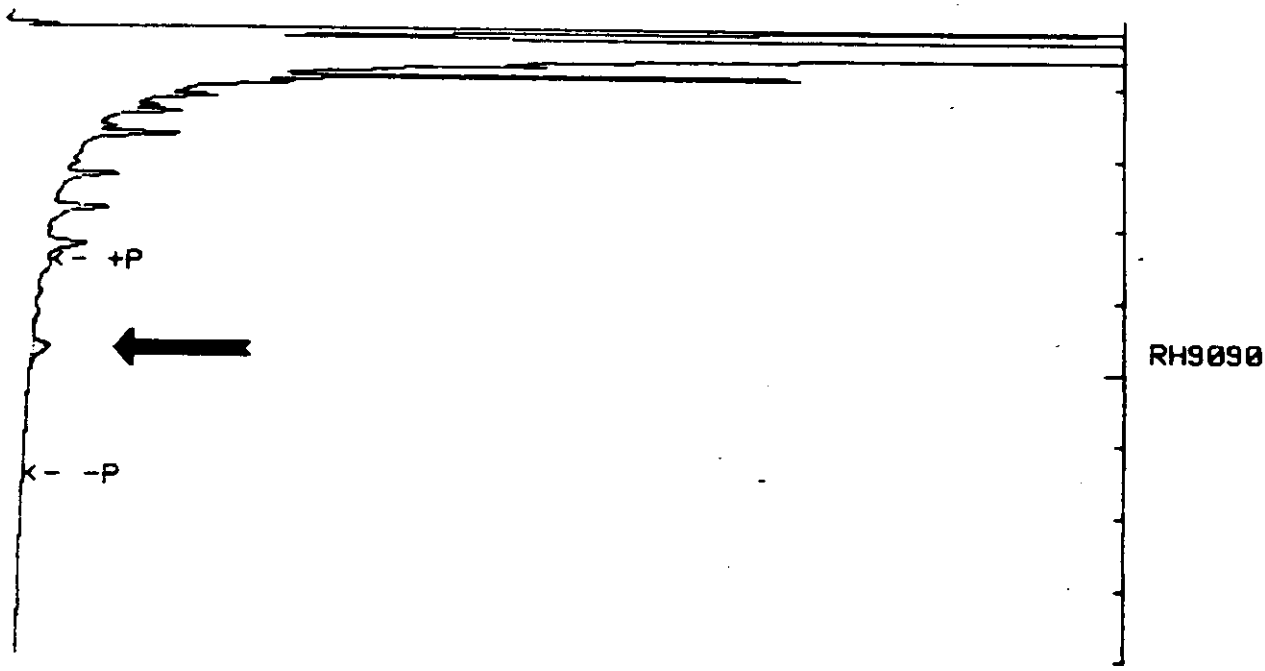
FIGURE 15

Control Beef Fortified with 0.01 ppm RH-9090

Data file: M918877
Method file: JM9090m
Type: FORTIFICATION

RAR number: JM3656
Sample No: 1
Component: BEEF

Plot times: 0 to 9 minutes
Plot range: 30 millivolts (-2.1 mv offset)



Ret. Time	Compound Name	Peak Area	Peak Height	ug/ml Found	Volume (ml)	Ctl Corr.	ug Added	Pct Recov
4.70	RH9090	.4	35.5	0.0437	4.00	0.000	0.200	87

FIGURE 16

Calibration Curve
091887
Beef

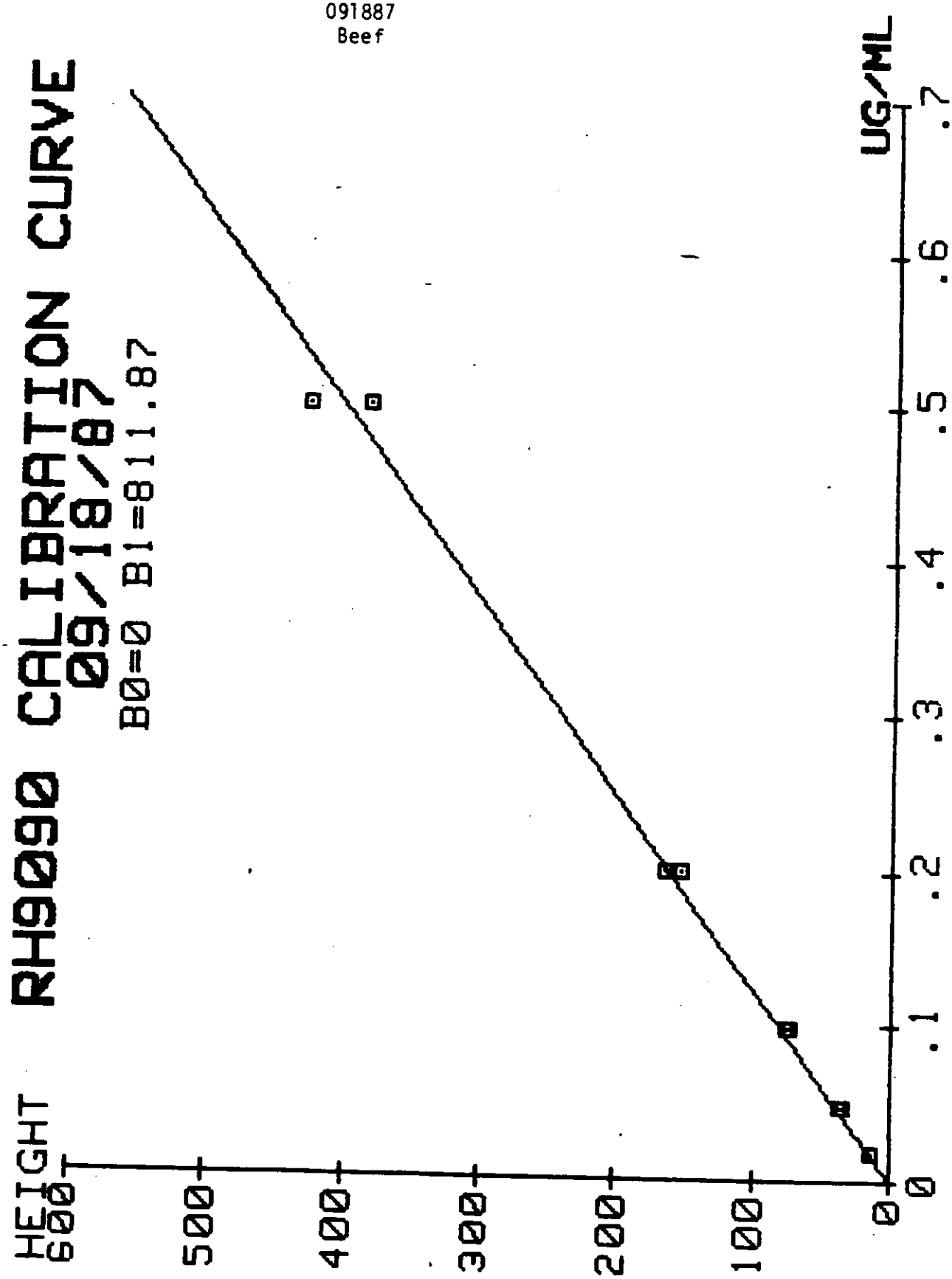
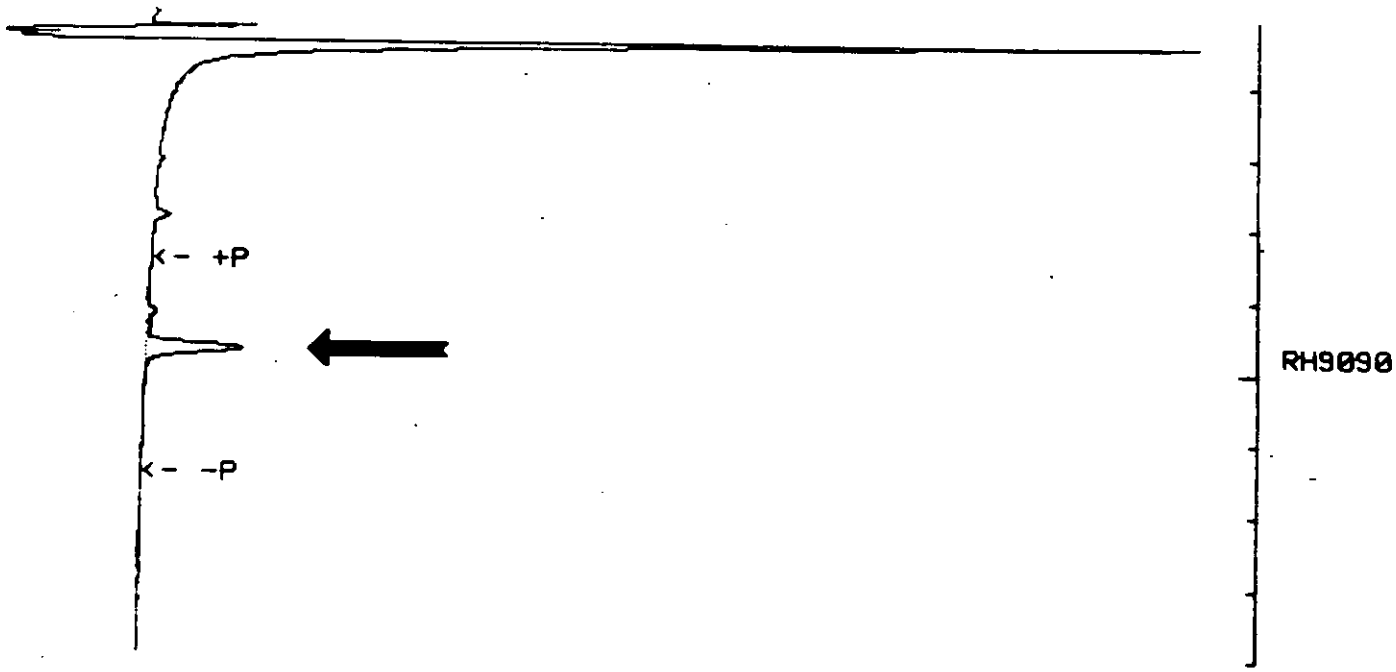


FIGURE 17

0.10 µg/ml RH-9090 Standard

Data file: M0028721
Method file: JM9090m
Type: STANDARD

Plot times: 0 to 9 minutes
Plot range: 30 millivolts (-1.8 mv offset)



Retention Time	Compound Name	PPM Injected	Area	Height
4.73	RH9090	0.100	2.04	217.01

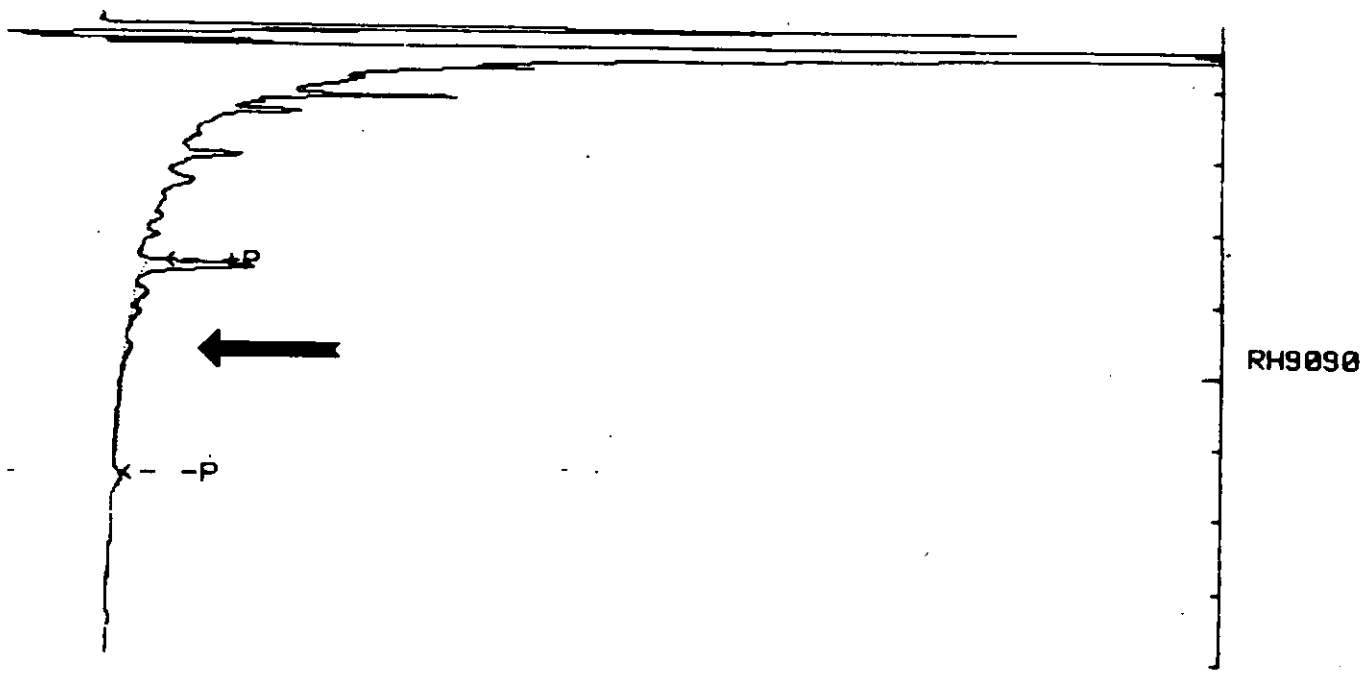
FIGURE 18

Control Kidney

Data file: M002878
Method file: JM9090m
Type: SAMPLE

RAR number: 3659
Sample No: 1
Component: KIDNEY

Plot times: 0 to 9 minutes
Plot range: 30 millivolts (-2.1 mv offset)



Ret. Time	Compound Name	Peak Area	Peak Height	ug/ml Found	Volume (ml)	Samp Wt.	Recov Fact.	PPM
4.72	RH9090	0.0	0.0	0.00	4.00	20.0	1.00	0.00

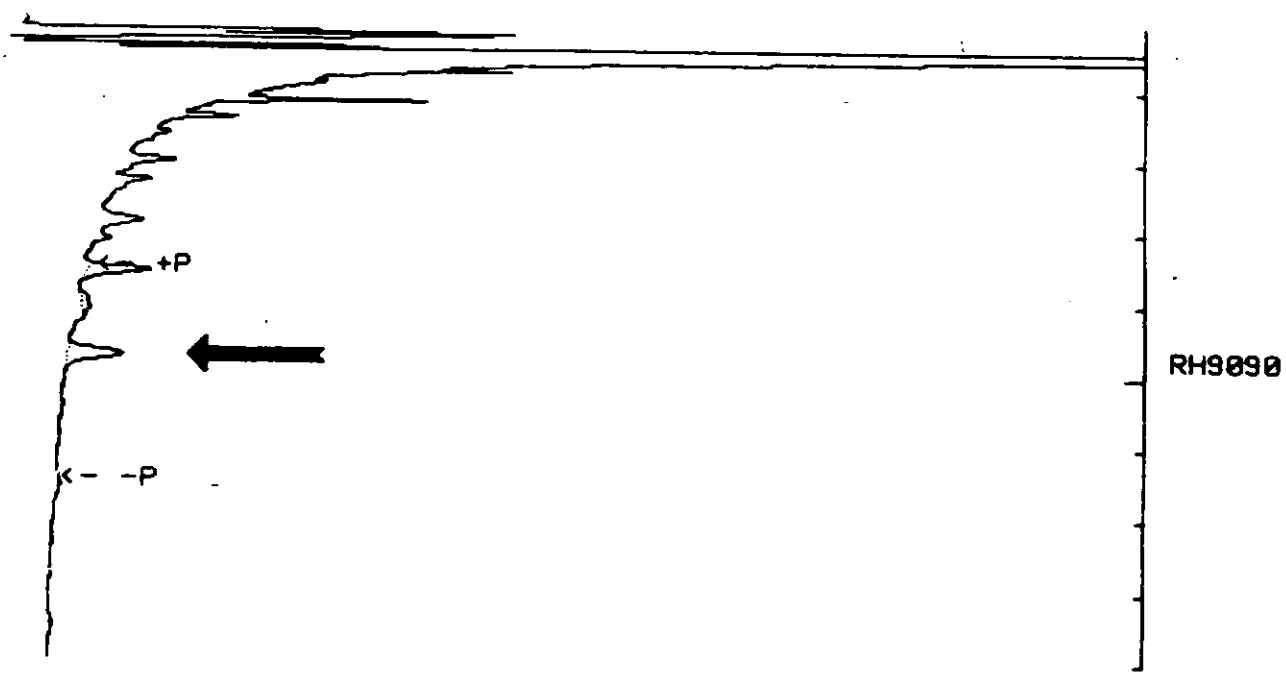
FIGURE 19

Control Kidney Fortified with 0.05 ppm RH-9090

Data file: M0028711
Method file: JM9090m
Type: FORTIFICATION

RAR number: 3659
Sample No: 1
Component: KIDNEY

Plot times: 0 to 9 minutes
Plot range: 30 millivolts (-2.1 mv offset)



Ret. Time	Compound Name	Peak Area	Peak Height	ug/ml Found	Volume (ml)	Ctl Corr.	ug Added	Pct Recov
4.73	RH9090	1.1	120.3	0.0595	4.00	0.000	0.200	119

FIGURE 20

Calibration Curve
10/02/87

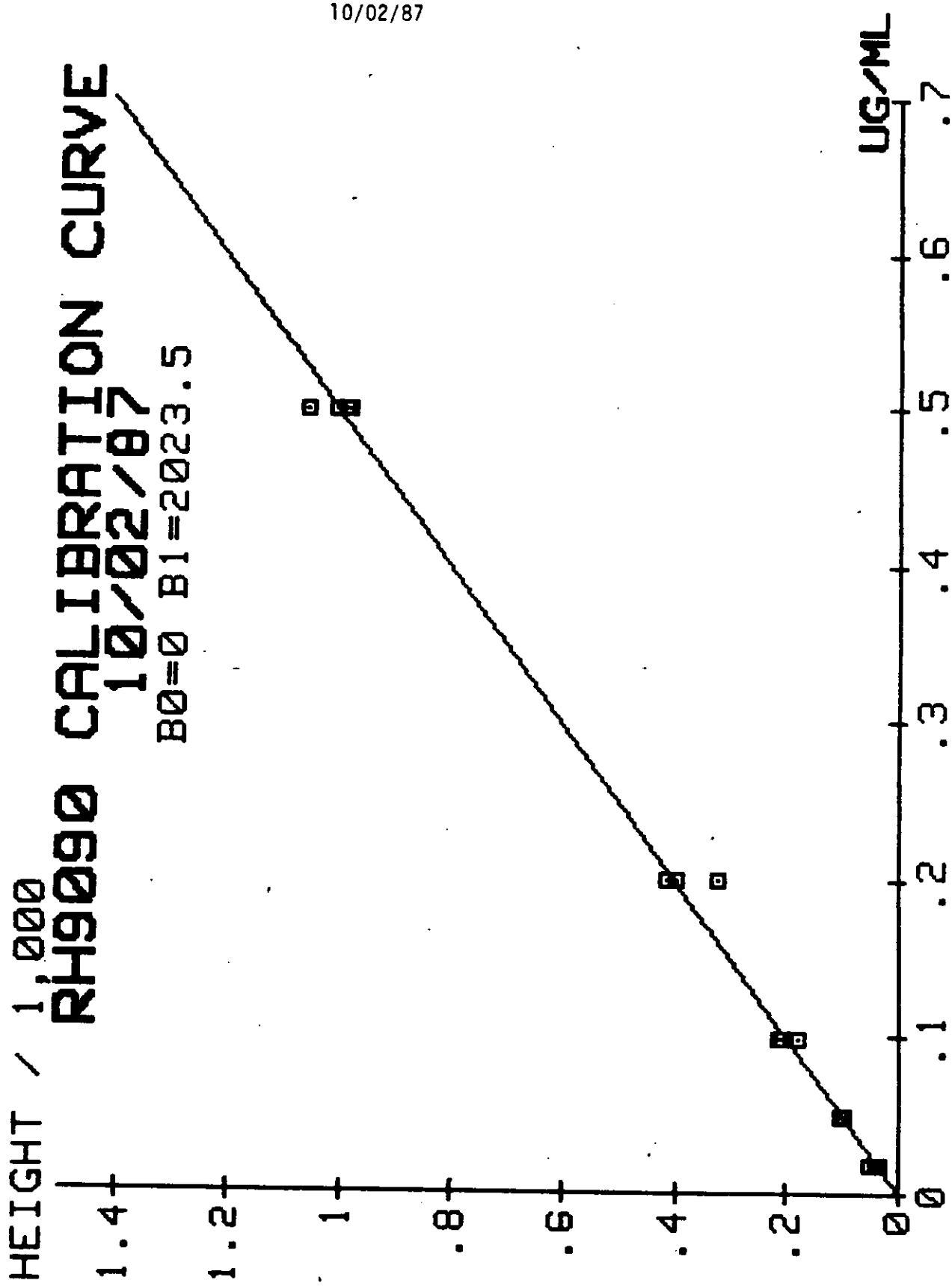


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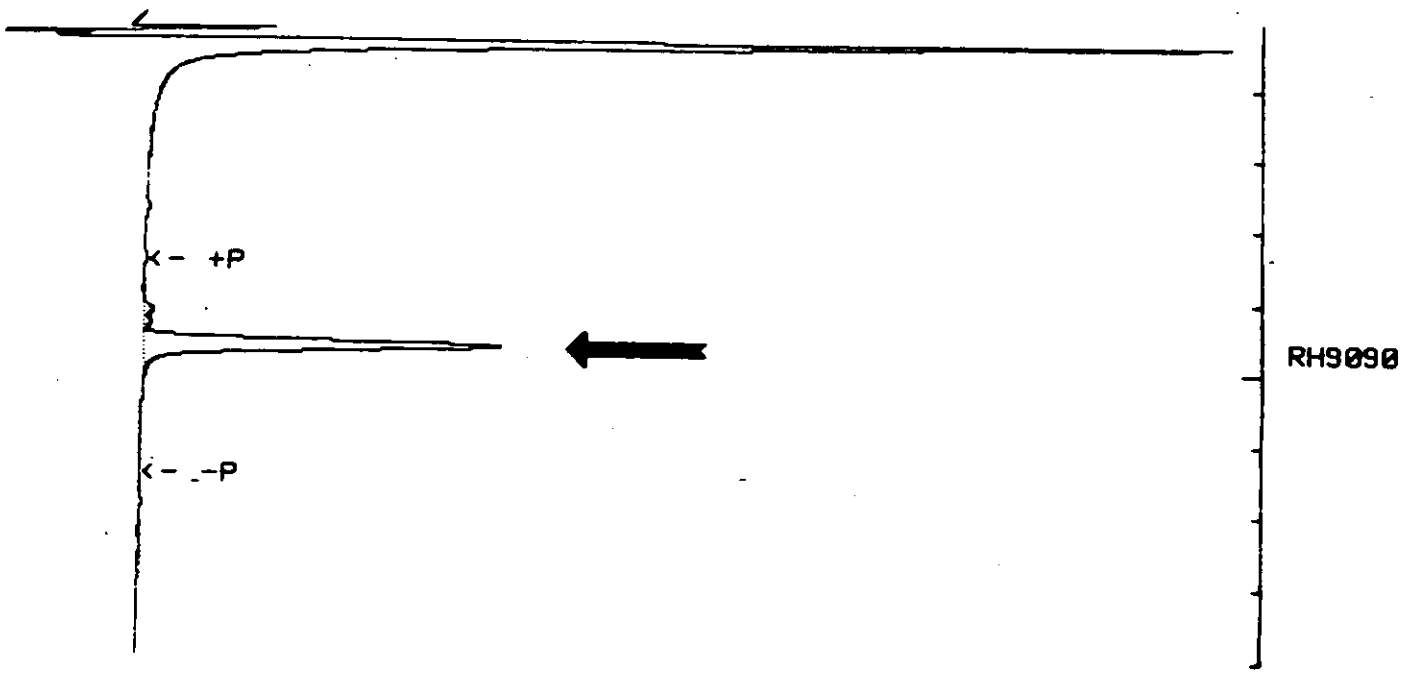
FIGURE 21

0.50 µg/ml RH-9090 Standard

0040

Data file: M0158714
Method file: JM9090m
Type: STANDARD

Plot times: 0 to 9 minutes
Plot range: 30 millivolts (-2 mv offset)



Retention Time	Compound Name	PPM Injected	Area	Height
4.69	RH9090	0.500	7.72	811.96

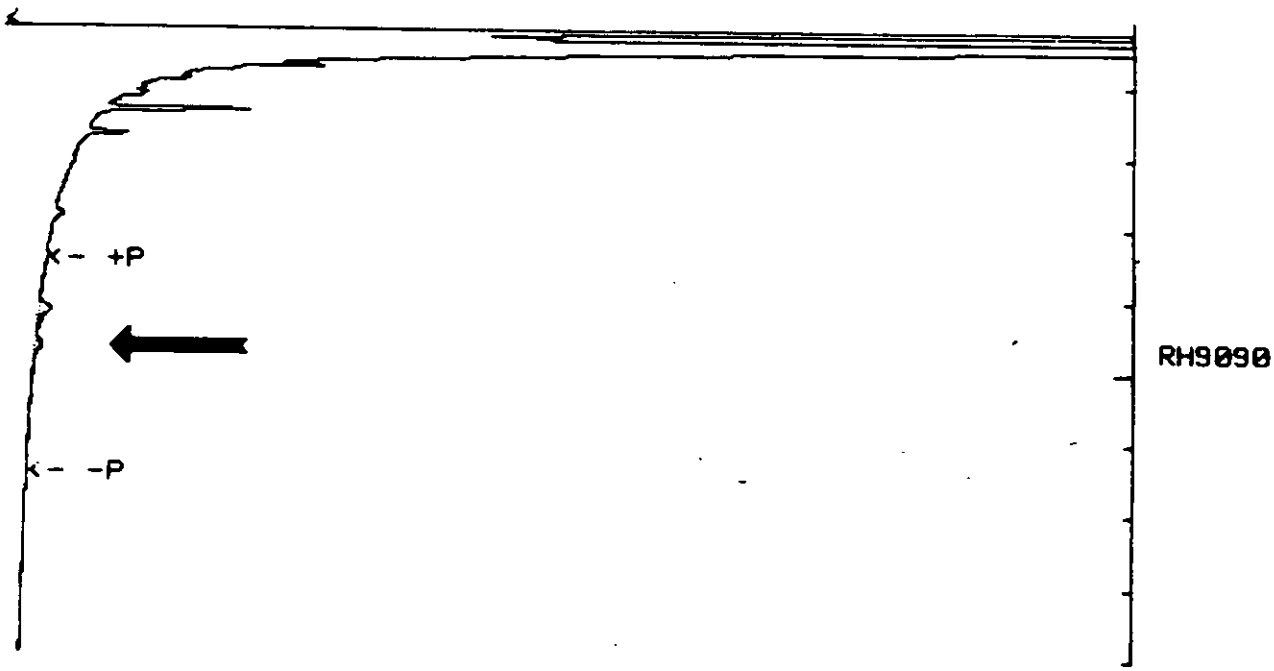
FIGURE 22

Control Milk

Data file: M0158717
Method file: JM9090m
Type: SAMPLE

RAR number: 3662
Sample No: 1
Component: MILK

Plot times: 0 to 9 minutes
Plot range: 30 millivolts (-2 mv offset)



Ret. Time	Compound Name	Peak Area	Peak Height	ug/ml Found	Volume (ml)	Samp Wt.	Recov Fact.	PPM
4.69	RH9090	.1	13.7	0.00	4.00	20.0	1.00	0.00

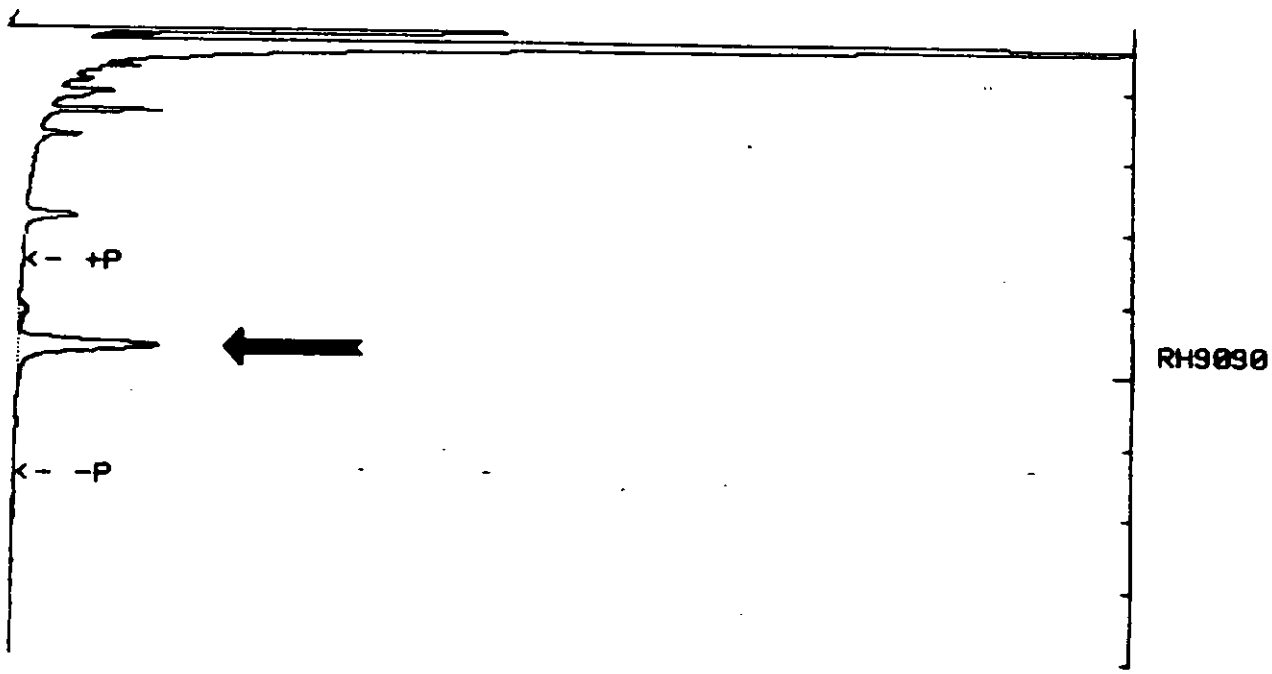
FIGURE 23

Control Milk Fortified with 0.05 ppm RH-9090

Data file: M019871'1
Method file: JM9090m
Type: FORTIFICATION

RAR number: 3664
Sample No: 1
Component: MILK

Plot times: 0 to 9 minutes
Plot range: 30 millivolts (-2 mv offset)



Ret. Time	Compound Name	Peak Area	Peak Height	ug/ml Found	Volume (ml)	Ctl Corr.	ug Added	Pct Recov
4.70	RH9090	3.2	312.7	0.254	4.00	0.000	1.00	102

FIGURE 24

Calibration Curve
10/15/87
Milk

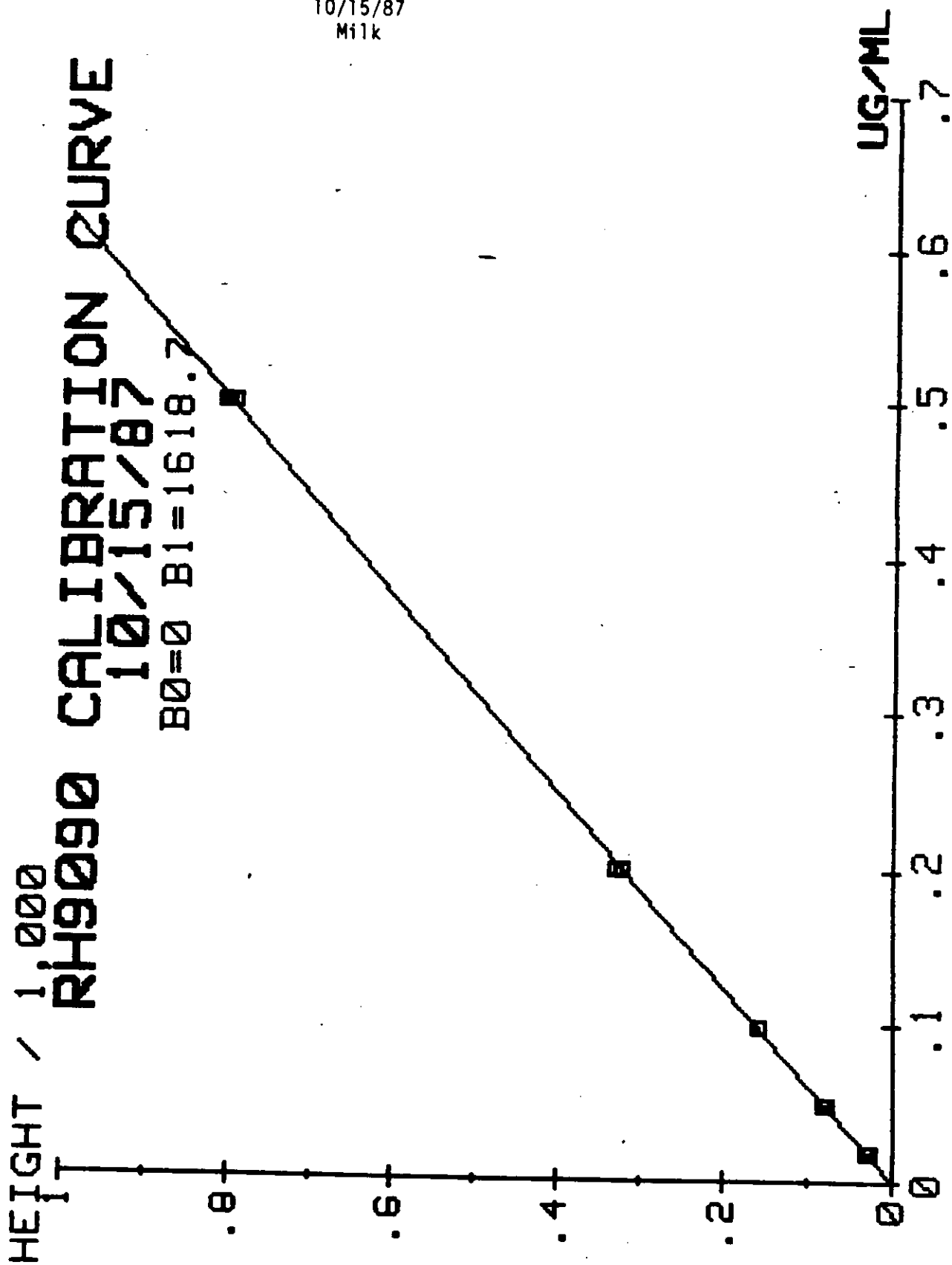
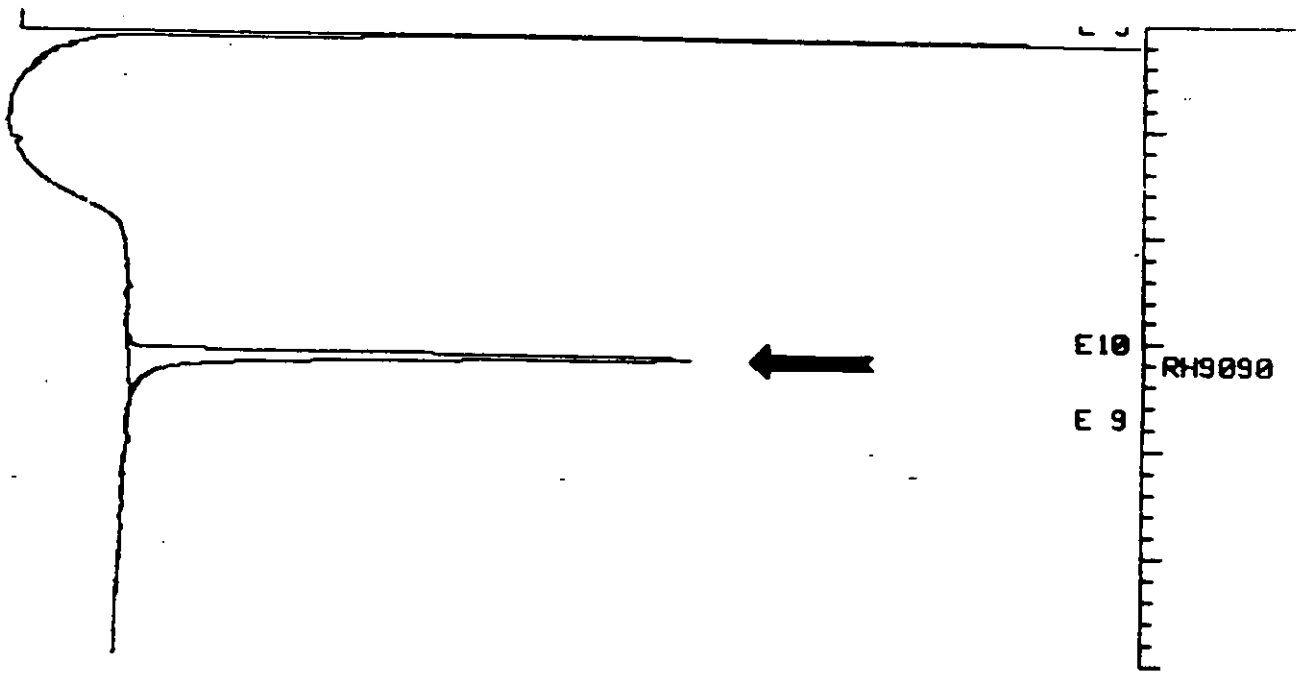


FIGURE 25

0.5 µg/ml RH-9090 Standard
Confirmatory Column

DATA FILE: R10298728
METHOD FILE: TISHA_1
TYPE: STANDARD

Start time= 0.00 Stop time= 30.00 minutes Offset= -50 mv
Full Range = 300 millivolts



RETENTION TIME	COMPOUND NAME	PPM INJECTED	AREA	HEIGHT
16.01	RH9090	.5000	33258.95	12693.05

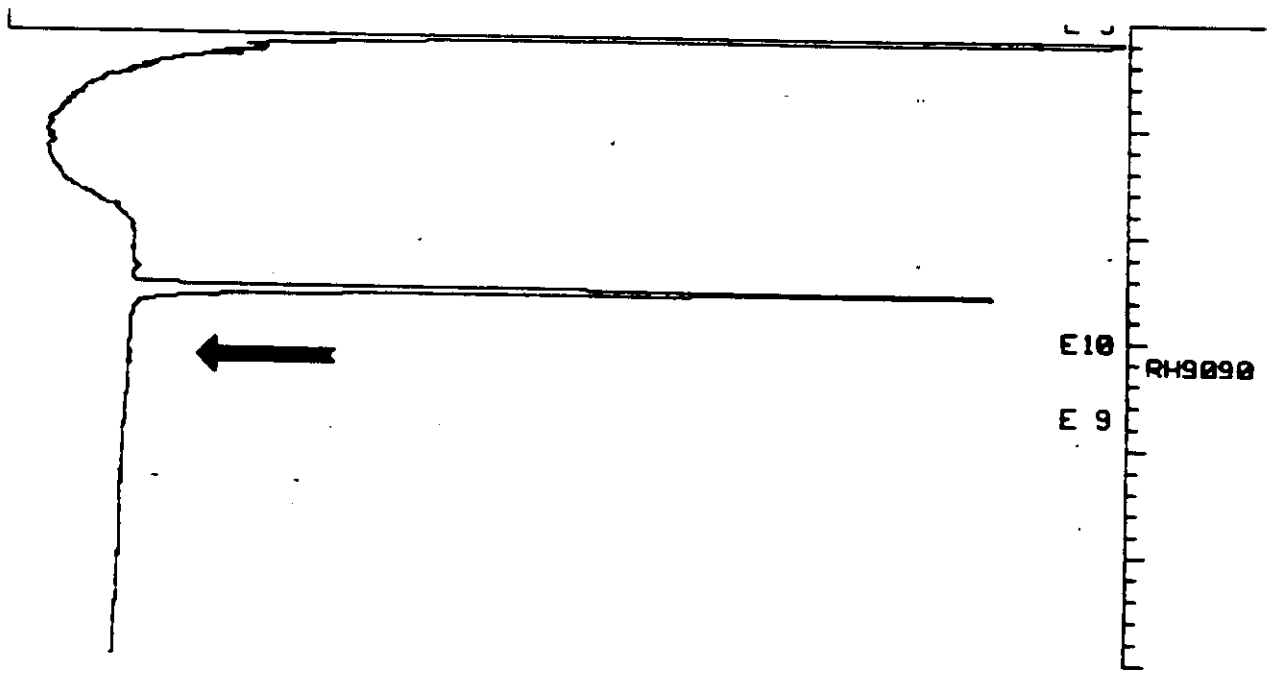
FIGURE 26

Control Milk
Confirmatory Column

DATA FILE: R10298719
METHOD FILE: TISHA_1
TYPE: SAMPLE

RAR NUMBER: 3662
SAMPLE NO: 1

Start time= 0.00 Stop time= 30.00 minutes Offset= -50 mv
Full Range = 300 millivolts



RET. TIME	COMPOUND NAME	PEAK AREA	PEAK HEIGHT	UG/ML	VOLUME (ML)	SAMP WT.	RECOV FACT.	PPM
16.06	RH9090	114.0	40.0	0.0000	4.00	20.0	1.000	0.0000

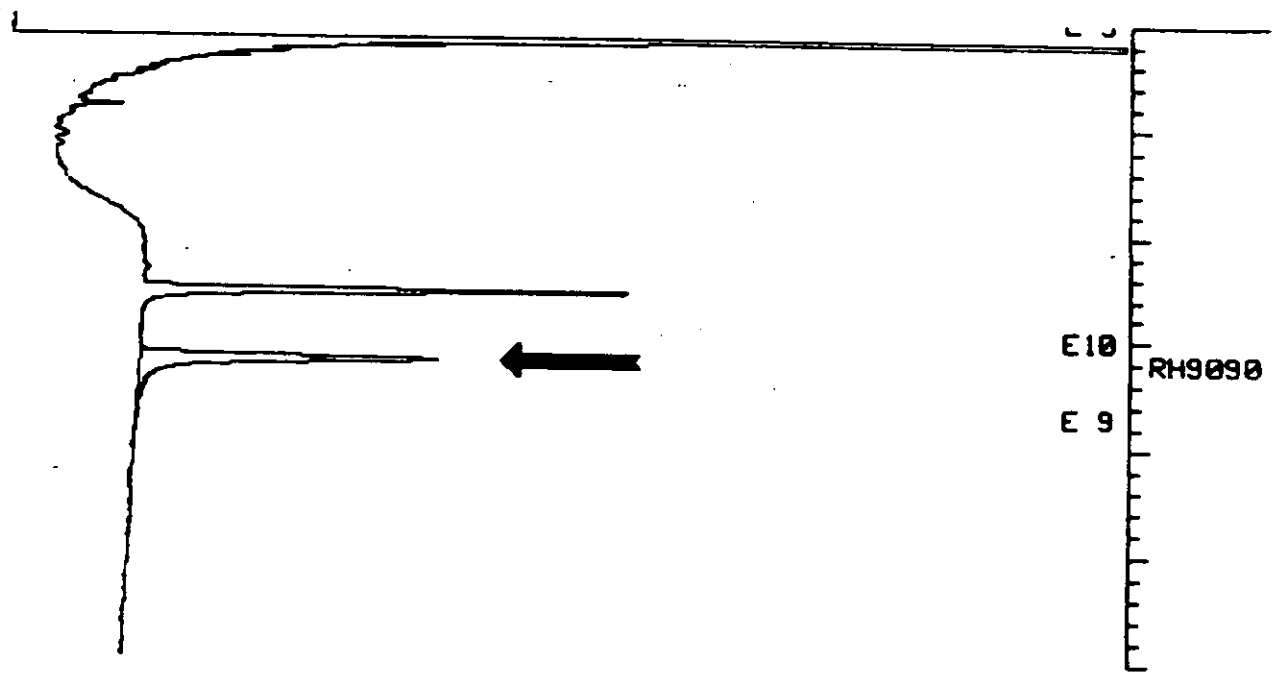
FIGURE 27

Control Milk Fortified with 0.05 ppm RH-9090
Confirmatory Column

DATA FILE: R10298725
METHOD FILE: TISHA_1
TYPE: FORTIFICATION

RAR NUMBER: 3664
SAMPLE NO: 1

Start time= 0.00 Stop time= 30.00 minutes Offset= -51 mv
Full Range = 300 millivolts



RET. TIME	COMPOUND NAME	PEAK AREA	PEAK HEIGHT	UG/ML	VOLUME (ML)	CTL CORR.	UG ADDED	PCT RECOV
16.01	RH9090	16894.3	6533.5	.2578	4.00	0.000	1.000	103.1