US EPA ARCHIVE DOCUMENT

Sponsor

BASF Corporation
Consumer Products and life Sciences Division
Agricultural Products Group
P.O. Box 13528
Research Triangle Park, NC 27709-3528

FINAL REPORT

STUDY TITLE

METHOD VALIDATION REPORT:

BASF Analytical Method A9207: Determination of Vinclozolin Metabolite BF 352-?5 in Animal Matrices

Data Requirement

Pesticide Assessment Guidelines, Subdivision O
Residue Chemistry
Guideline 171-4(d): Analytical Method

Authors

Laura Sears, BASF Corporation
Paul E. Vorndam, Ph.D.,
Colorado Analytical Research & Development Corporation

Final Report Date

December 18, 1992

Performing Laboratory

Colorado Analytical Research & Development Corporation
4720 Forge Road, Suite 108
Colorado Springs, CO 80907
(719) 593-1165

Laboratory Project ID

BASF-1170

This report contains a total of 77 pages.

PR 86-5 DATA CONFIDENTIALITY CLAIM

No claim of confidentiality is made for any information contained in this study on the basis of its falling within the scope of FIFRA 10 (d) (1) (A), (B) or (C).

Company: BASF Corporation, Agricultural Chemicals

Company Agent: Rodney C. Akers Date: Dec. 21, 1992

Title: Senior Registration Specialist Signature: Rodney C. Akers

GOOD LABORATORY PRACTICES STATEMENT

This study was conducted according to 40 CFR, Part 160 FIFRA Good Laboratory Practices.

Submitter: Rodney C. Akers Dec. 21, 1992

Sponsor: Rodney C. Akers Dec. 21, 1992

Study Director: Laura Sears 12/18/92

CERTIFICATION OF GOOD LABORATORY PRACTICE

Project number BASF-1170, (BASF Study No. 92159) was performed in accordance with the U.S. Environmental Protection Agency Good Laboratory Practice (GLP) standards set forth in 40 CFR Part 160, and printed in the Federal Register Vol. 54 No. 158, August 17, 1989, pp. 34052-34074 and the Standard Operating Procedures of Colorado Analytical Research & Development Corporation.

Upon completion of the study, laboratory notebooks, chain of custody forms, certified copies of raw data and reports generated during the conduct of this study will be retained in the Archives of Colorado Analytical Research & Development Corporation, 4720 Forge Road, Suite 108, Colorado Springs, CO 80907 for a period of ten years. Copies of laboratory notebooks, copies of chain of custody forms and original raw data will be archived by BASF Corporation, P.O. Box 13528, 26 Davis Drive, Research Triangle Park, NC 27709-3528.

Paul E. Vorndam Date 12/14/92

Paul E. Vorndam, Ph.D.
Colorado Analytical Research & Development Corporation
Study Project Director

William D. Rhoads Date 12/14/92

William D. Rhoads, Ph.D.
President

Colorado Analytical Research & Development Corporation
Laboratory Conducting Study

QUALITY ASSURANCE STATEMENT OF INSPECTIONS
(Page 1 of 2)

Method Title: BASF Analytical Method A9207: Determination of Vinclozolin Metabolite BF 352-25 in Animal Matrices

Test/Reference Substance:

BF 352-25, (2,3,4-trihydoxy-2-methyl-butanoic acid (3,5-dichloroanilide)

This final report has been reviewed by the Colorado Analytical Research & Development Corporation Quality Assurance Department in accordance with the United States Environmental Protection Agency FIFRA Good Laboratory Practice Standards as outlined in 40 *CFR* part 160, Federal Register Notice, August 17, 1989, Vol. 54, No. 158, effective October 16, 1989.

Review or signature by QAU and the Study Director took place as follows:

Protocol	Date of QAU Review/Signature	Date Signed by Study Director
Protocol	9/14/92	9/11/92
Report		
Draft Report	11/17,18,20/92	

Audits on the study were conducted and reported as follows:

Type[a]	Date/s of Inspection	Date Reported to Management/Study Director		QAU Signature
Sample Tracking	9/17/92	9/25/92	10/ 2/92	9/25/92
Personnel	9/17/92	↓	↓	↓
Lab. Functions - General	9/22/92	↓	↓	↓
Lab. Functions -Experimental	9/17,22/92	↓	↓	↓
Record Keeping	9/17,22/92	↓	↓	↓
Critical Phases	9/17,22,23/92	↓	↓	↓
Raw Data	10/20-21/92	10/22/92	10/30/92	10/22/92

[a] Specific phase of audit referenced on original audit.

QUALITY ASSURANCE STATEMENT OF INSPECTIONS
(Page 2 of 2)

This final report was reviewed on December 11, 1992. The final report accurately reports the data generated during the conduct of the study and accurately reflects the conduct of the study.

Approved and submitted by: Kenneth R. Weissenfluh 12/14/92

Kenneth R. Weissenfluh, B.S.
Director of Quality Assurance

Date

STATEMENT OF THE QUALITY ASSURANCE UNIT

Method Number: A9207

Protocol Number: 92159

Test Substance and Reference Substance:

BASF Code: BF 352 25
Chemical Name: 2,3,4-trihydroxy-2-methylbutanoic acid (3,5-dichloroanilide)
Lot Number: L31/141-6
Purity: 99.4%

The quality assurance unit of the testing facility at the ARC audited the protocol and reported its findings to the study director and to management.

Date of Inspection	Report to Study Director and to Management
(Protocol)	
September 10, 1992	September 10, 1992
(Report Review)	
December 18, 1992	December 18, 1992

David W Haughey
Signature of QAU

CERTIFICATION OF AUTHENTICITY

The subsequent pages of this document are presented as they were received from the performing laboratory.

Sponsor: BASF Corporation

Study Director: Laura Sears
Analytical Contracts Group

Signature: Laura Sears

Date: 12/18/92

Inquiries

Inquiries should be addressed to:

Laura Sears
Analytical Contracts Group
BASF Corporation
Agricultural Research Center
P.O. Box 13528
26 Davis Drive
Research Triangle Park, NC 27709-3528
(919) 248-6500

TABLE OF CONTENTS

Title Page..... 1
Confidentiality Statement..... 2
BASF Compliance Statement..... 3
Certification of Good Laboratory Practice 4
Quality Assurance Statement of Inspections..... 5
BASF Quality Assurance Protocol Audit Statement..... 7
Certification of Authenticity..... 8
Table of Contents..... 9
Study Personnel.....10
Study Certification.....11
Introduction and Summary.....12
Principle.....14
Apparatus.....15
Reagents.....15
General Procedure for the Determination
of BF 352-25 in Animal Matrices.....16
Preparation of Quality Assurance Procedural Recovery
Fortification Samples.....19
High Performance Liquid Chromatographic Analysis.....20
Miscellaneous Items Related to the Method.....24
Results and Discussion.....25
Conclusions.....27
References.....28

Table I, Typical High Performance Liquid Chromatographic Conditions for Determination of Vinclozolin Metabolite BF 352-25 in Animal Matrices ANALYTICAL METHOD: BASF A9207.....29
Table II, Linear Response Data: BF 352-25 10 to 150 nanogram Standard Curve.....30
Table III, Method Validation: Recoveries from Cow Milk.....31
Table IV, Method Validation: Recoveries from Cow Muscle....33
Table V, Method Validation: Recoveries from Cow Liver.....35
Table VI, Method Validation: Recoveries from Cow Fat.....37
Table VII, Method Validation: Recoveries from Cow Kidney....39
Table VIII, Method Validation: Recoveries from Chicken Eggs..41

Figure 1, Summary Flow Diagram of the Method.....43
Figure 2, Typical High Performance Liquid Chromatograms of Animal Matrices Analyzed using ANALYTICAL METHOD BASF A9207.....45
Figure 3, Linear Response Data, BF 352-25.....62

Attachments.....63
1 Matrices and Standards Receipt and Storage.....64
2 Liquid Chromatographic Bench Sheets.....66

STUDY PERSONNEL

The following Colorado Analytical Research & Development Corporation personnel were associated with this study:

	Signature	Initials
Roberta Montgomery Laboratory Technician I	Roberta Montgomery	RMM
Ingram L. Nichols Laboratory Aide	Ingram Nichols	ILN
Eadie L. Darrow Laboratory Technician II	Eadie L. Darrow	ELD
Micheal G. Classen Laboratory Technician II	Micheal Classen	MGC
Leonard L. Liker Laboratory Technician II	ON FILE	LL
Deidre Uhden Senior Laboratory Technician	Deidre Uhden	DU
Susan E. Kane, B.S. Senior Laboratory Technician	Susan E Kane	SEK
Paul E. Vorndam, Ph.D. Group Leader	Paul E Vorndam	PEV
Kendy L. Keatley, B.S. Quality Assurance Officer Director of Quality Assurance October 22, 1991 to November 6, 1991	Kendy L. Keatley	KLK
Kenneth R. Weissenfluh, B.S. Director of Quality Asssurance Prior to July 5, 1991 and After November 6, 1991	Kenneth R. Weissenfluh	KRW
William D. Rhoads, Ph.D. President	William D. Rhoads	WDR

STUDY CERTIFICATION

To the best of my knowledge, there were no significant deviations from the Good Laboratory Practice Regulations which affected the quality or the integrity of this method validation study.

This final report accurately reflects the raw data obtained for the study.

Approved and Submitted by: Paul E. Vorndam Date 12/14/92

Paul E. Vorndam, Ph.D.
Laboratory Study Project Director
Colorado Analytical Research & Development Corporation

COLORADO ANALYTICAL RESEARCH & DEVELOPMENT CORPORATION
4720 Forge Road, Suite 108
Colorado Springs, Colorado 80907
(719) 593-1165

ANALYTICAL METHOD:

BASF A9207

Title: Determination of Vinclozolin Metabolite BF 352-25 in Animal Matrices.

Developed by: Deidre Uhden and Paul E. Vorndam, Ph.D.

Approved by: William D. Rhoads, Ph.D.
President

Date: September 11, 1992

1.0 Introduction and Summary

1.1 Introduction

This method was designed to confirm the presence of vinclozolin and vinclozolin metabolite residues in animal matrices. The current methodology for the determination of vinclozolin residues in animal tissues (Reference 1) incorporates an alkaline hydrolysis step to release the 3,5-dichloroaniline moiety common to parent and its metabolites. While this method gives an excellent accountability of vinclozolin residues, it is not specific in the presence of other pesticides containing the 3,5-dichloroaniline moiety.

Method A9207 has been designed to quantitate the major vinclozolin residue in animal matrices, BF 352-25, in order to retain selectivity in the presence of other dichloroaniline containing pesticides. BF 352-25 is a significant component of the total vinclozolin derived residue in animal matrices. The percentage of BF 352-25 of the total radioactive residue (TRR) found in goat and hen metabolism studies is indicated below (References 2 and 3).

	%BF 352-25 of TRR					
	Muscle	Fat	Milk	Eggs	Liver	Kidney
Goat	77	41	53	N/A	39	28
Hen	59	35	N/A	57	40	N/A

In addition, 23% and 14% of the TRR in fat (goat and hen respectively) can be determined as vinclozolin using existing methodologies (References 4 and 5).

1.2 Scope

This method is used for the determination of Vinclozolin Metabolite BF 352-25 in cow milk, muscle, liver, fat, kidney and in chicken eggs. The limit of quantitation for all matrices is 0.05 ppm BF 352-25. The method was developed by Colorado Analytical Research & Development Corporation for BASF Corporation.

Substance:

BASF Code: BF 352-25

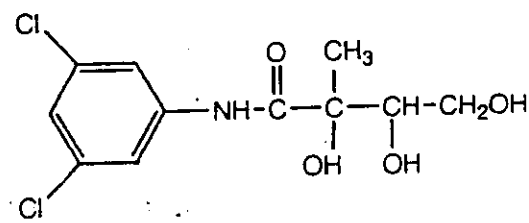
Chemical Name: 2,3,4-trihydroxy-2-methylbutanoic acid-(3,5-dichloroanilide)

Molecular Formula: $C_{11}H_{13}Cl_2NO_4$

Molecular Weight: 294.14

Melting Point: 125.5-127° C

Structural Formula:



2.0 Principle

all matrices!

A 20 g aliquot of the homogenized sample (50 g for milk) is weighed and refluxed for 30 minutes with 100 mL of methanol (chicken eggs) or 50/50 v/v deionized water/methanol (cow kidney) or blended with 100 mL of methanol (cow milk, muscle, fat) or blended with 100 mL of 50/50 methanol/water (liver). Methanol was shown to be an effective extractant of incorporated residue in the goat and hen metabolism studies (References 1 and 2). The liquid portion of the blend/reflux is separated from solid matrix residue by a series of centrifuge or centrifuge/filtration steps. The liquid is concentrated to aqueous and the aqueous layer is then partitioned 3 times with 150 mL portions of dichloromethane. The dichloromethane is dried by rinsing through a bed of anhydrous sodium sulfate and then evaporated to dryness at 40° C. The residue is then brought up in 5 mL of ethyl acetate. The ethyl acetate is applied to a 10 mm silica gel (5 g) column previously wetted with 10 mL hexane. The column is eluted, in sequence, with 150 mL hexane, 50 mL ethyl acetate and 100 mL of 15/85 methanol/ethyl acetate. The first two eluates are discarded and the third is evaporated to dryness at 40° C. The resultant residue is then brought up in 60/40 (v/v) water/methanol for HPLC analysis using UV detection at 254 nm against a minimum 5 point BF 352-25 standard curve. The flow diagram for this method is presented in Figure 1.

3.0 Apparatus

3.1 Analytical Balance

3.2 High performance liquid chromatographic column, Whatman Partisphere C18, 4.6 mm x 25 cm, or equivalent

3.3 High Performance Liquid Chromatograph, UV detector, Hewlett Packard 1090, or equivalent

3.4 Waring Blender, or equivalent

3.5 Sample vials, HPLC autosampler

3.6 Centrifuge, Beckman Model TJ-6, or equivalent

3.7 Rotary Evaporator, Büchi Rotovapor R, or equivalent

3.8 General Laboratory Glassware (including reflux condensor)

3.9 Heating mantles

4.0 Reagents

4.1 Acetonitrile UV, distilled in glass, Baxter B&J ChromPure (Cat. No. 015-4), or equivalent

4.2 Deionized water (filtered for HPLC)

4.3 Dichloromethane, distilled in glass, Baxter B&J ChromPure (Cat. No. CP80175-4), or equivalent

4.4 Ethyl acetate, distilled in glass, Baxter B&J ChromPure (Cat. No. CP80132-4), or equivalent

4.5 Hexane, distilled in glass, Baxter B&J ChromPure (Cat. No. CP80145-4), or equivalent

4.6 Methanol, distilled in glass, Baxter B&J ChromPure HPLC Solvent (Cat. No. CP18050-4), or equivalent

4.7 Silica Gel, Mallinckrodt, 100-200 mesh, Type 60 A Special (Cat. No. 6447), or equivalent

4.8 Sodium Sulfate, anhydrous, Mallinckrodt analytical reagent (Cat. No. 8024), or equivalent

5.0 General Procedure for the Determination of BF 352-25 in Animal Matrices

5.1 Homogenization

Place the sample into a blender and homogenize for approximately 5 minutes at room temperature. Store the liquified sample in the freezer until just prior to use.

5.2 Extraction

5.2.1 Cow kidney or chicken eggs

Weigh a 20g sample of homogenized matrix into a 250 mL round bottom flask on a top loading analytical balance. Add 100 mL of methanol (MeOH) for eggs. For kidney use 100 mL 50/50 (v/v) deionized water/methanol. Gently reflux the mixture with a heating mantle for 30 minutes (stirring is unnecessary) and then cool to room temperature. Scrape and pour the contents of the reflux flask into 250 mL centrifuge bottle. Save flask for further rinsing. Centrifuge at 3000 rpm for approximately 2 minutes. Decant the liquid into a 1000 mL round bottom flask. Rinse the reflux flask with 100 mL of 50/50 (v/v) methanol/deionized water and add to the residue in the centrifuge bottle and shake (by hand or mechanically) vigorously for approximately 2 minutes. Recentrifuge and decant as before and combine all of the liquid extractions into the 1000 mL round bottom flask. Add an additional 100 mL of 50/50 (v/v) methanol/deionized water and repeat the centrifuge step. Discard the sediment. Proceed to paragraph 5.2.4.

5.2.2 Cow liver, muscle or fat

Weigh a 20g sample of homogenized matrix into a 100 mL beaker. Rinse/scrape the contents of the beaker into a blender with 100 mL of methanol (muscle or fat). For liver use 100 mL of 50/50 (v/v) methanol/water. Blend for approximately 2 minutes and then transfer into a 250 mL centrifuge bottle. Centrifuge for approximately 2 minutes at 3000 rpm. For liver and muscle, decant liquid into 1000 mL round bottom flask. For fat, vacuum filter through Büchner funnel with #4 Whatman filter paper and transfer to the 1000 mL round bottom flask. Repeat 2 times with 100 mL of 50/50 (v/v) methanol/deionized water each time (liver, muscle and fat) adding the solvent to either the centrifuge bottle (liver, muscle), or returning the marc to the blender (fat) and adding solvent to the blender. Proceed to paragraph 5.2.4.

5.2.3 Cow milk

Weigh a 50g sample into a 100 mL beaker and transfer to a blender with 100 mL of methanol. Blend for approximately 2 minutes. Transfer contents of blender to a 250 mL centrifuge bottle. Rinse the blender contents into the centrifuge bottle with an additional 10-15 mL of methanol. Centrifuge at 3000 rpm for approximately 2 minutes. Decant liquid into Büchner funnel equipped with #54 Whatman filter paper and, approximately, a 10 g bed of Celite and vacuum filter. Add an additional 100 mL of methanol to the centrifuge bottle and shake (by hand or mechanically) vigorously for approximately 2 minutes. Centrifuge at 3000 rpm for approximately 2 minutes and vacuum filter the contents through the same Büchner funnel as before. Rinse on the filter with 2 additional 50 mL portions of methanol. Transfer the liquid to a 1000 mL round bottom flask. Rinse the filter flask with an additional 10 mL of methanol and transfer to the 1000 mL round bottom flask. Proceed to paragragh 5.2.4.

5.2.4 Extract Concentration

Concentrate the contents of the 1000 mL round bottom flask (this size of flask is necessary to prevent loss of material due to foaming) to aqueous on a rotary evaporator with a water bath maintained at 40°±5° C. The rotary evaporator rpm or vacuum may be adjusted necessary to prevent undue foaming. An anti-bump trap may also be used, if necessary.

5.3 Partition

Transfer the aqueous phase (approximate volume of 100 mL) to a 500 mL separatory funnel. Partition 3 times with 150 mL portions of methylene chloride (DCM). Rinse the 1000 mL round bottom flask contents into the separatory funnel with the first 150 mL DCM portion. Centrifuge separatory funnel contents to break emulsions if necessary prior to separation of layers (typically necessary for liver). Place a loose plug of glass wool in a funnel followed by approximately 25 g of anhydrous sodium sulfate. Combine the DCM partitions and slowly pour through the bed of sodium sulfate into a 500 mL round bottom flask. Rinse the sodium sulfate bed into the round bottom flask with an additional 25 mL of DCM. Concentrate on a rotary evaporator until just dry at 40°±5° C.

5.4 Silica Gel Chromatography

Prepare a chromatography column by placing a small plug of glass wool followed by 5g of silica gel (non-activated) into a 10 mm diameter column. Prewet the column with 10 mL of hexane. Dilute the concentrated residue to a volume of approximately 5 mL with ethyl acetate (EtOAc). Transfer the ethyl acetate with a disposable pipette to the column and rinse the round bottom flask into the column with two 1 mL portions of EtOAc. Drain the solvent just to the top of the column after each transfer. Elute the column with:

A 150 mL hexane
B 50 mL EtOAc
C 100 mL 15/85 (v/v) MeOH/EtOAc

Discard elutes A & B. Collect eluate C in a 250 mL round bottom flask and concentrate on a rotary evaporator just to dryness at 40°±5° C.

Note: Each new batch of silica gel should be tested with standards to be sure the elution profile is correct. Eluate C may be modified in strength or volume if required to elute BF 352-25.

5.5 Dilution for Analysis

Dilute the residue with 5.0 mL of 60/40 (v/v) water/methanol for HPLC analysis (gently swirl the flask to insure that the residue dissolves). This volume is generally adequate for detection levels of 0.05 to 0.25 ppm (for milk at 0.05 ppm only). Further dilutions can be made if required.

6.0 Preparation of Quality Assurance Procedural Recovery Fortification Samples

Procedural recovery samples ~~are~~ to be analyzed concurrently by the analytical method to provide quality assurance for the analytical data set. An untreated control matrix is fortified with a known quantity of BF 352-25 standard solution.

A stock 100 µg/mL BF 352-25 fortification sample is typically prepared from the BF 352-25 standard by weighing out a 10.0 mg equivalent amount of BF 352-25 and diluting to 100 mL with ethyl acetate. Serial dilutions of this stock solution with ethyl acetate are prepared to achieve specific working standard solutions. The solutions are stored under refrigeration with an expiration date of six months from the date of preparation.

Fortification levels for the method validation were from 0.05 ppm to 1.0 ppm. An unfortified control matrix is also analyzed to allow any background analyte level found in the control to be accounted for in fortified control matrices.

7.0 High Performance Liquid Chromatographic Analysis

The samples are analyzed by high performance liquid chromatography employing UV detection at 254 nanometers with a C18 25 cm x 4.6 mm i.d. liquid chromatographic column against a minimum five point standard curve. Typical liquid chromatographic conditions are presented in Table I.

7.1 Preparation of Standard BF 352-25

Prepare a stock standard solution of 100 µg/mL BF 352-25 in ethyl acetate by weighing 10.0 mg BF 352-25, corrected for purity, into a 100 mL volumetric flask and bringing to volume with ethyl acetate.

For a 10 ng to 150 ng standard curve, serially dilute this stock solution with 60/40 (v/v) deionized water/MeOH to achieve working standard solutions of 100, 250, 500, 1000 and 1500 ng/mL, or similar concentrations, to cover a minimum five point standard curve range (from, typically, 100 µL injected).

These solutions are stored under refrigeration with an expiration date of six months from the date of preparation.

7.2 Standardization of the High Performance Liquid Chromatograph

7.2.1 Standardize the liquid chromatograph for the 10 ng to 150 ng standard curve by injecting (typically) 100 µL aliquots of the BF 352-25 standard dilutions equivalent to 10, 25, 50, 100 and 150 ng from the 100 µL liquid chromatograph injections. Typical liquid chromatograms of standards are shown in Figure 2.

7.2.2 Generate a least squares best-fit linear regression standard curve line using peak heights or areas observed for the injection standards. Using a calculator or computer with statistical linear regression capability, enter the peak values versus the standard nanograms injected to determine slope and y-intercept for the best-fit line. Typical linear regression best-fit data obtained from these standards are provided in Table II, and schematically in Figure 3.

7.3 Determination of BF 352-25 in Animal Matrix Samples

7.3.1 Alternating standard and sample injections, inject 100 µL aliquots of the samples for the chosen matrix and standards prepared as in step 7.1 into the liquid chromatograph. The sequence can be ended with a blank (mobile phase), low, middle and high standards. If the BF 352-25 chromatogram peak height (area) exceeds the peak height (area) of the 150 ng standard of the standard curve, the sample must be diluted using an appropriate amount of 60/40 (v/v) water/methanol and reanalyzed. Typical chromatograms of Vinclozolin Metabolite BF 352-25 samples for each matrix are provided in Figure 2.

7.3.2 Using the integrator peak height or area observed for the test substance (see above), calculate the nanograms of BF 352-25 found, using the standard curve, with the slope and y-intercept calculated as in 7.2.2. The nanograms of BF 352-25 observed for the sample are calculated from the signal value using the equation,

ng BF 352-25
= [Pk signal value - (y-Intercept)] ÷ Slope

Example Calculation:

For a BF 352-25 fortified sample, an integrator peak height value of 67.86 was obtained from liquid chromatographic analysis. The y-Intercept (constant) = -0.4474693 and the Slope (X-coefficient) = 1.6420546.

7.3.3 Calculate the Gross residue, BF 352-25, in ppm, for a given matrix as follows:

Gross Residue ppm,

=(Sample Peak Height (Area) - Constant)
÷X Coefficient
=nanograms found, BF 352-25
÷ milligrams injected

where Constant = Y-intercept from Least Squares Linear Regression Line for Standard

X Coefficient= Slope from Least Squares Linear Regression line for Standard

and

milligrams injected

$$= \frac{\text{Sample Wt in g}}{\text{Final Volume, mL}} \times \frac{1000\text{mg/g}}{1000\mu\text{L/mL}}$$

X 100 µL injected

For treated samples the ppm BF 352-25 is converted to ppm of vinclozolin equivalents by multiplying with the molecular weight conversion factor of 0.97.

$$\frac{\text{molecular wt., vinclozolin}}{\text{molecular wt., BF 352-25}} \quad \frac{286.11}{294.14} = 0.97$$

ppm BF 352-25 X 0.97 = ppm vinclozolin equivalents

7.3.4 Calculate the percent recovered BF 352-25 in fortified sample as follows:

$$\%\text{Recovery} = \frac{\text{Gross Residue ppm} - \text{Control Residue ppm,}}{\text{fortification level, ppm}} \times 100$$

9.0 Results and Discussion

9.1 Matrix Fortification

The validity of Analytical Method: BASF A9207 was demonstrated by recoveries of the test subtance from fortified control matrices. Fortifications of matrix samples were done at the screening level of the method, 0.05 µg/g and at 5 and 20 times the screening level, or at 0.25 µg/g and 1.0 µg/g, respectively for the test substance. After fortification, analysis was conducted as outlined under paragraph 5.0, General Procedure for the Determination of BF 352-25 in Animal Matrices. BF 352-25 results are presented in Tables III-VIII. Representative chromatograms of control and fortified matrix samples are presented in Figure 2. Chromatographic analytical bench sheets are included as an Attachment.

9.2 Linearity

Table II presents representative linear response data for the BF 352-25 standard. From the data outlined in the table, a correlation coefficient (r) of 0.9995 for a 10 nanogram to 150 nanogram BF 352-25 standard curve was obtained. Also presented in the table are nanogram values obtained by back calculation using least squares linear regression. These values demonstrate reasonable linearity for the analytical method.

Standard linearity back calculations were done using a least squares linear regression as follows:

ng found = (STD Response, Peak Height - Constant)
X Coefficient

An example of a standard linearity back calculation is as follows for 150 nanogram standard 6816, BF 352-25:

nanograms found

= STD Response, Peak Height, 240.04
- Constant, -0.4474693
÷ X Coefficient, 1.6420546

= 146.5

Figure 3 presents the response of the standards in graphic form, including the least squares best fit line. Representative chromatograms resulting from injection of standards are presented in Fig. 2.

The chromatographic analytical data set is included in this report as an Attachment.

9.3 Recoveries through the Method

The method was validated at the laboratories of Colorado Analytical Research & Development Corporation. BF 352-25 recoveries for 0.05, 0.25 and 1.0 ppm fortifications ranged from 78.6 to 88.4% for cow milk, 64.4 to 94.5% for cow muscle, 60.1 to 101.2% for cow liver, 54.9 to 86.2% for cow fat, 46.6 to 107.8% for cow kidney and 51.0 to 72.5 for chicken eggs. The quantitation limit for all matrices was 0.05 ppm. The limit of detection for this method, using the conditions described herein, is 0.025 ppm. Example chromatograms of unfortified and fortified samples for each matrix are provided in Figure 2. Detailed tables of results are shown in Tables III-VIII. A summary of results, by matrix, is provided below:

Matrix	Average % Recovery	Standard Deviation	N
Bovine			
Liver	69.2	10.9	12
Muscle	75.5	8.0	12
Fat	75.9	7.8	12
Kidney	68.9	16.0	12
Milk	81.7	2.8	12
Chicken			
Eggs	64.6	6.2	12

10.0 Conclusions

This method has been shown to give good recoveries of BF 352-25 in bovine and hen matrices. The limit of quantitation at 0.05 ppm demonstrates no interferences due to matrix, and has been validated through recovery studies. The limit of detection, using the chromatographic conditions described herein, is 0.025 ppm.

The use of this method for enforcement purposes will allow the selective detection of vinclozolin related residues without interference from other 3,5-dichloroaniline containing pesticides. BF 352-25 represents a significant, though not comprehensive portion of the total vinclozolin-derived residues. Analysis of BF 352-25 is sufficient to confirm the presence of over-tolerance residues of vinclozolin and its metabolites.

Comparing the currently proposed tolerances for animal commodities with the %TRR represented by BF 352-25, we find that all levels are above the 0.025 ppm limit of detection (LOD), with the exception of chicken liver and chicken fat, which fall just below the LOD.

Commodity		Proposed Tolerance	X	%TRR for BF 352-25	=	ppm BF 352-25 at tolerance
GOAT	Milk	0.2		0.53		0.11
	Fat	0.6		0.41		0.25
	Liver	1.2		0.39		0.47
	Kidney	1.2		0.28		0.34
	Muscle	0.3		0.77		0.23
HEN	Eggs	0.05		0.57		0.03
	Fat	0.05		0.35		<0.025 (0.018)
	Liver	0.05		0.40		<0.025 (0.02)
	Muscle	0.05		0.59		0.03

This method should adequately serve as a confirmatory method for vinclozolin-derived residues in animal commodities, at the proposed (12/88) tolerance levels.

11.0 References

1) Single, Yvonne H., "GC Method for Residue Determination of Vinclozolin (BAS 352 F) and its Metabolites in Bovine and Chicken Tissues, Milk and Eggs", BASF Method No. A9004, December, 1990. MRID No. 749601.

2) Kohl, W., "The Metabolism of ^{14}C-Vinclozolin in the Goat: Part III Further Isolation and Identification of Metabolites", BASF Report No. 2570, September, 1988. MRID No. 40854402

3) Kohl, Dr. Werner, "1st Addendum to HRC Report BSF 468/88223 Biokinetics and Metabolism of ^{14}C-Vinclozolin (^{14}C-BAS 352 F) in the Laying Hen, Part II", BASF Report No. 2589, April, 1989. MRID No. 4140005

4) Pesticide Analytical Manual, Volume I, Methods Which Detect Multiple Residues, U.S. Department of Health and Human Services, Food and Drug Administration, PB88-911899

5) Mayer, Dr. Frank, "GLC Method for Residue Determinations of Vinclozolin (Parent) and its Metabolites BF 352-23 (E) and BF 352-41 (S) in Animal Matrices", BASF Method No. 272, April, 1988.

TABLE I

Typical High Performance Liquid Chromatographic Conditions for Determination of Vinclozolin Metabolite BF 352-25 in Animal Matrices
ANALYTICAL METHOD: BASF A9207

Instrument:	Hewlett Packard Model 1090 HPLC with programmable variable volume auto-injector, programmable variable filter-photometric detector or equivalent.
Precolumn:	Zorbax Reversed Phase (RP) C18.
Column:	Whatman Partisphere ODS, 25 cm, 4.6 mm i.d.
Temperature:	Ambient
Mobile Phase:	Acetonitrile/Water 70/30 (isocratic)
Flow Rate:	1.45 mL/minute
Injection Vol:	100 µL
Run Time:	13.5 minutes
Post Time:	10 minutes
Detection:	UV @ 254 nanometers

The post time may be adjusted to avoid interference carryover, if necessary, depending on the matrix.

A typical retention time for BF 352-25 was 7.35±0.1 minutes, depending on column chromatographic conditions.

TABLE II

LINEAR RESPONSE DATA: BF 352-25
10 to 150 nanogram Standard Curve

Analysis Location: Colorado Analytical Research & Development Corporation
Analysis Method: BASF A9207
Analysis Type: High Performance Liquid Chromatography
Matrix: Standards

(Lab) Sample No.	Date ANALYZED	STD Conc (ng)	STD Response Pk Height Integrator	ng Found*	Matrix Present (Y/N)**
6819 STD	9/14/92	10	14.54	9.1	N
6816 STD	9/14/92	150	240.04	146.5	N
6822 STD	9/14/92	25	39.76	24.5	N
6814 STD	9/14/92	100	163.93	100.1	N
6815 STD	9/14/92	50	82.40	50.5	N
6819 STD	9/14/92	10	15.42	9.7	N
6815 STD	9/14/92	50	84.66	51.8	N
6816 STD	9/14/92	150	250.59	152.9	N

*Back calculated by Least Squares Linear Regression
** No sample matrices present; standards only

Regression Output:

Constant, Slope	1.6420546
Std Err of Y Est	1.9199202
R	0.9995
No. of Observations	8
Degrees of Freedom, Total	7
X Coefficient, Y-intercept	-0.4474693
Std Err of Coef.	0.0220185

Table III, page 1 of 2

Method Validation:
Recoveries from Cow Milk

Colorado Analytical Research & Development Corporation
4720 Forge Road, Suite 108, Colorado Springs, CO 80907

LIQUID CHROMATOGRAPHIC ANALYSIS

Compound: BF 352-25
Recoveries, Cow Milk

Least Sqrs Linear Regresssion
from Standard Curve:

Date Received: 5/13/92
Date Processed: 9/14/92
Date Analyzed: 9/1·/92

Correlation: 0.9995
Y-intercept: -0.4474693
Slope: 1.6420546

CARDC ID CODE	SAMPLE Wt. g	INJECTED mg	Corr ppm	PK HGHT INTG	% Recovery
1170-181	50	1000	0.00000	<0	
1170-182+.05ppm	50	1000	0.04160	67.86	83.2
1170-183+.05ppm	50	1000	0.04419	72.12	88.4
1170-184+.25ppm	50	250	0.19892	81.21	79.6
1170-185+.25ppm	50	250	0.20108	82.10	80.4
1170-186+1.0ppm	50	66.66667	0.80184	87.33	80.2
1170-187+1.0ppm	50	66.66667	0.80499	88.77	81.5
				Average	82.2%
				± Standard Deviation	3.3%
				N	6

Table III, page 2 of 2

Method Validation:
Recoveries from Cow Milk

Colorado Analytical Research & Development Corporation
4720 Forge Road, Suite 108, Colorado Springs, CO 80907

LIQUID CHROMATOGRAPHIC ANALYSIS

Compound: BF 352-25
Recoveries, Cow Milk

Date Received: 5/13, 9/14/92
Date Processed: 9/15/92
Date Analyzed: 9/15/92

Least Sqrs Linear Regresssion from Standard Curve:

Correlation: 0.9998
Y-intercept: -1.1927997
Slope: 1.8807567

CARDC ID CODE	SAMPLE Wt. g	INJECTED mg	Corr ppm	PK HGHT INTG	% Recovery
1170-188	50	1000	0.00000	<0	
1170-189+.05ppm	50	1000	0.04194	77.68	83.9
1170-190+.05ppm	50	1000	0.04198	77.76	84.0
1170-191+.25ppm	50	250	0.20458	95.00	81.8
1170-192+.25ppm	50	250	0.19761	91.72	79.0
1170-193+1.0ppm	50	66.66667	0.78633	97.40	78.6
1170-194+1.0ppm	50	66.66667	0.80340	99.54	80.3
				Average	81.3%
				± Standard Deviation	2.4%
				N	6

Table IV, page 1 of 2

Method Validation:
Recoveries from Cow Muscle

Colorado Analytical Research & Development Corporation
4720 Forge Road, Suite 108, Colorado Springs, CO 80907

LIQUID CHROMATOGRAPHIC ANALYSIS

Compound: BF 352-25
Recoveries, Cow Muscle

Least Sqrs Linear Regresssion from Standard Curve:

Date Received: 6/18/92
Date Processed: 9/16/92
Date Analyzed: 9/16,17/92

Correlation: 0.9991
Y-intercept: 0.4762640
Slope: 1.7555411

CARDC ID CODE	SAMPLE Wt. g	INJECTED mg	Corr ppm	PK HGHT INTG	% Recovery
1170-195	20	400	0.00000	<0	
1170-196+.05ppm	20	400	0.04723	33.64	94.5
1170-197+.05ppm	20	400	0.04162	29.70	83.2
1170-198+.25ppm	20	400	0.18889	133.12	75.6
1170-199+.25ppm	20	400	0.19255	135.69	77.0
1170-200+1.0ppm	20	80	0.71384	100.73	71.4
1170-201+1.0ppm	20	80	0.69490	98.07	69.5
				Average	78.5%
				± Standard Deviation	9.2%
				N	6

Table IV, page 2 of 2

Method Validation:
Recoveries from Cow Muscle

Colorado Analytical Research & Development Corporation
4720 Forge Road, Suite 108, Colorado Springs, CO 80907

LIQUID CHROMATOGRAPHIC ANALYSIS

Compound: BF 352-25
Recoveries, Cow Muscle

Date Received: 6/18/92
Date Processed: 9/17/92
Date Analyzed: 9/17,18/92

Least Sqrs Linear Regresssion from Standard Curve:

Correlation: 0.9998
Y-intercept: -0.7013939
Slope: 1.7110663

CARDC ID CODE	SAMPLE Wt. g	INJECTED mg	Corr ppm	PK HGHT INTG	% Recovery
1170-202	20	400	0.00000	<0	
1170-203+.05ppm	20	400	0.03872	25.80	77.4
1170-204+.05ppm	20	400	0.03687	24.53	73.7
1170-205+.25ppm	20	400	0.18930	128.86	75.7
1170-206+.25ppm	20	400	0.19450	132.42	77.8
1170-207+1.0ppm	20	80	0.64420	87.48	64.4
1170-208+1.0ppm	20	80	0.65457	88.90	65.5
				Average	72.4%
				± Standard Deviation	6.0%
				N	6

Table V, Page 1 of 2

Method Validation:
Recoveries from Cow Liver

Colorado Analytical Research & Development Corporation
4720 Forge Road, Suite 108, Colorado Springs, CO 80907

LIQUID CHROMATOGRAPHIC ANALYSIS

Compound: BF 352-25
Recoveries, Cow Liver

Least Sqrs Linear Regresssion
from Standard Curve:

Date Received: 3/5/92
Date Processed: 9/23/92
Date Analyzed: 9/24/92

Correlation: 0.9998
Y-intercept: 176.8751154
Slope: 186.6550442

CARDC ID CODE	SAMPLE Wt. g	INJECTED mg	Corr ppm	PK HGHT INTG	% Recovery
1170-230	20	400	0.01495	1293	
1170-231+.05ppm	20	400	0.05060	5071	101.2
1170-232+.05ppm	20	400	0.03300	3757	66.0
1170-233+.25ppm	20	400	0.17447	14319	69.8
1170-234+.25ppm	20	400	0.18727	15275	74.9
1170-235+1.0ppm	20	80	0.69446	10770	69.4
1170-236+1.0ppm	20	80	0.68488	10627	68.5
				Average	75.0%
				± Standard Deviation	13.2%
				N	6

Table V, Page 2 of 2

Method Validation:
Recoveries from Cow Liver

Colorado Analytical Research & Development Corporation
4720 Forge Road, Suite 108, Colorado Springs, CO 80907

LIQUID CHROMATOGRAPHIC ANALYSIS

Compound: BF 352-25
Recoveries, Cow Liver

Date Received: 9/24/92
Date Processed: 9/25/92
Date Analyzed: 9/25,26/92

Least Sqrs Linear Regresssion from Standard Curve:

Correlation: 0.9992
Y-intercept: 190.7388896
Slope: 179.3286035

CARDC ID CODE	SAMPLE Wt. g	INJECTED mg	Corr ppm	PK HGHT INTG	% Recovery
1170-244	20	400	0.00000	<0	
1170-245+.05ppm	20	400	0.03038	2370	60.8
1170-246+.05ppm	20	400	0.03332	2581	66.6
1170-247+.25ppm	20	400	0.16610	12105	66.4
1170-248+.25ppm	20	400	0.16250	11847	65.0
1170-249+1.0ppm	20	80	0.60087	8811	60.1
1170-250+1.0ppm	20	80	0.62046	9092	62.0
				Average	63.5%
				± Standard Deviation	2.9%
				N	6

Table VI, Page 1 of 2

Method Validation:
Recoveries from Cow Fat

Colorado Analytical Research & Development Corporation
4720 Forge Road, Suite 108, Colorado Springs, CO 80907

LIQUID CHROMATOGRAPHIC ANALYSIS

Compound: BF 352-25
Recoveries, Cow Fat

Least Sqrs Linear Regresssion from Standard Curve:

Date Received: 9/15/92
Date Processed: 9/22/92
Date Analyzed: 9/22,23/92

Correlation: 0.9996
Y-intercept: 0.5511790
Slope: 1.5726799

CARDC ID CODE	SAMPLE Wt. g	INJECTED mg	Corr ppm	PK HGHT INTG	% Recovery
1170-223	20	400	0.00000	<0	
1170-224+.05ppm	20	400	0.04311	27.67	86.2
1170-225+.05ppm	20	400	0.03427	22.11	68.5
1170-226+.25ppm	20	400	0.19945	126.02	79.8
1170-227+.25ppm	20	400	0.13714	86.82	54.9
1170-228+1.0ppm	20	80	0.78655	99.51	78.7
1170-229+1.0ppm	20	80	0.78337	99.11	78.3
				Average	74.4%
				± Standard Deviation	11.1%
				N	6

Table VI, Page 2 of 2

Method Validation:
Recoveries from Cow Fat

Colorado Analytical Research & Development Corporation
4720 Forge Road, Suite 108, Colorado Springs, CO 80907

LIQUID CHROMATOGRAPHIC ANALYSIS

Compound: BF 352-25
Recoveries, Cow Fat

Date Received: 9/15/92
Date Processed: 9/24/92
Date Analyzed: 9/24,25/92

Least Sqrs Linear Regresssion from Standard Curve:

Correlation: 0.9999
Y-intercept: 207.0360016
Slope: 190.5737835

CARDC ID CODE	SAMPLE Wt. g	INJECTED mg	Corr ppm	PK HGHT INTG	% Recovery
1170-237	20	400	0.00000	<0	
1170-238+.05ppm	20	400	0.03787	3094	75.7
1170-239+.05ppm	20	400	0.03911	3188	78.2
1170-240+.25ppm	20	400	0.18831	14562	75.3
1170-241+.25ppm	20	400	0.20358	15726	81.4
1170-242+1.0ppm	20	80	0.76893	11930	76.9
1170-243+1.0ppm	20	80	0.77384	12005	74.4
				Average	77.0%
				± Standard Deviation	2.5%
				N	6

Table VII, Page 1 of 2

Method Validation:
Recoveries from Cow Kidney

Colorado Analytical Research & Development Corporation
4720 Forge Road, Suite 108, Colorado Springs, CO 80907

LIQUID CHROMATOGRAPHIC ANALYSIS

Compound: BF 352-25
Recoveries, Cow Kidney

Least Sqrs Linear Regresssion
from Standard Curve:

Date Received: 10/26/92
Date Processed: 11/9/92
Date Analyzed: 11/9,10/92

Correlation: 0.9996
Y-intercept: -213.0830806
Slope: 189.7681920

CARDC ID CODE	SAMPLE Wt. g	INJECTED mg	Corr ppm	PK HGHT INTG	% Recovery
1170-300	20	400	0.00000	<0	
1170-301+.05ppm	20	400	0.03685	2584	73.7
1170-302+.05ppm	20	400	0.03882	2734	77.6
1170-303+.25ppm	20	400	0.15646	11663	62.6
1170-304+.25ppm	20	400	0.18955	14175	75.8
1170-305+1.0ppm	20	80	0.46617	6864	46.6
1170-306+1.0ppm	20	80	0.53434	7899	53.4
				Average	65.0%
				± Standard Deviation	12.9%
				N	6

Table VII, Page 2 of 2

Method Validation:
Recoveries from Cow Kidney

Colorado Analytical Research & Development Corporation
4720 Forge Road, Suite 108, Colorado Springs, CO 80907

LIQUID CHROMATOGRAPHIC ANALYSIS

Compound: BF 352-25
Recoveries, Cow Kidney

Date Received: 10/26/92
Date Processed: 11/17/92
Dat: Analyzed: 11/18,19/92

Least Sqrs Linear Regresssion from Standard Curve:

Correlation: 0.9981
Y-intercept: 87.1077410
Slope: 131.2241066

CARDC ID CODE	SAMPLE Wt. g	INJECTED mg	Corr ppm	PK HGHT INTG	% Recovery
1170-307	20	400	0.00000	<0	
1170-308+.05ppm	20	400	0.03092	1710	61.8
1170-309+.05ppm	20	400	0.05391	2917	107.8
1170-310+.25ppm	20	400	0.18583	9841	74.3
1170-311+.25ppm	20	400	0.18870	9992	75.5
1170-312+1.0ppm	20	80	0.62602	6659	62.6
1170-313+1.0ppm	20	80	0.54734	5833	54.7
				Average	72.8%
				± Standard Deviation	18.9%
				N	6

Table VIII, Page 1 of 2

Method Validation:
Recoveries from Chicken Eggs

Colorado Analytical Research & Development Corporation
4720 Forge Road, Suite 108, Colorado Springs, CO 80907

LIQUID CHROMATOGRAPHIC ANALYSIS

Compound: BF 352-25
Recoveries, Chicken Eggs

Least Sqrs Linear Regresssion
from Standard Curve:

Date Received: 9/15/92
Date Processed: 9/29/92
Date Analyzed: 9/30/92

Correlation: 0.9995
Y-intercept: 79.3958855
Slope: 206.9629962

CARDC ID CODE	SAMPLE Wt. g	INJECTED mg	Corr ppm	PK HGHT INTG	% Recovery
1170-251	20	400	0.00000	<0	
1170-252+.05ppm	20	400	0.03324	2831	66.5
1170-253+.05ppm	20	400	0.03471	2953	69.4
1170-254+.25ppm	20	400	0.15476	12891	61.9
1170-255+.25ppm	20	400	0.14921	12432	59.7
1170-256+1.0ppm	20	80	0.68029	11343	68.0
1170-257+1.0ppm	20	80	0.66368	11068	66.4
				Average	65.3%
				± Standard Deviation	3.7%
				N	6

Table VIII, Page 2 of 2

Method Validation:
Recoveries from Chicken Eggs

Colorado Analytical Research & Development Corporation
4720 Forge Road, Suite 108, Colorado Springs, CO 80907

LIQUID CHROMATOGRAPHIC ANALYSIS

Compound: BF 352-25
Recoveries, Chicken Eggs

Least Sqrs Linear Regresssion from Standard Curve:

Date Received: 9/15/92
Date Processed: 9/30/92
Date Analyzed: 9/30,10/1/92

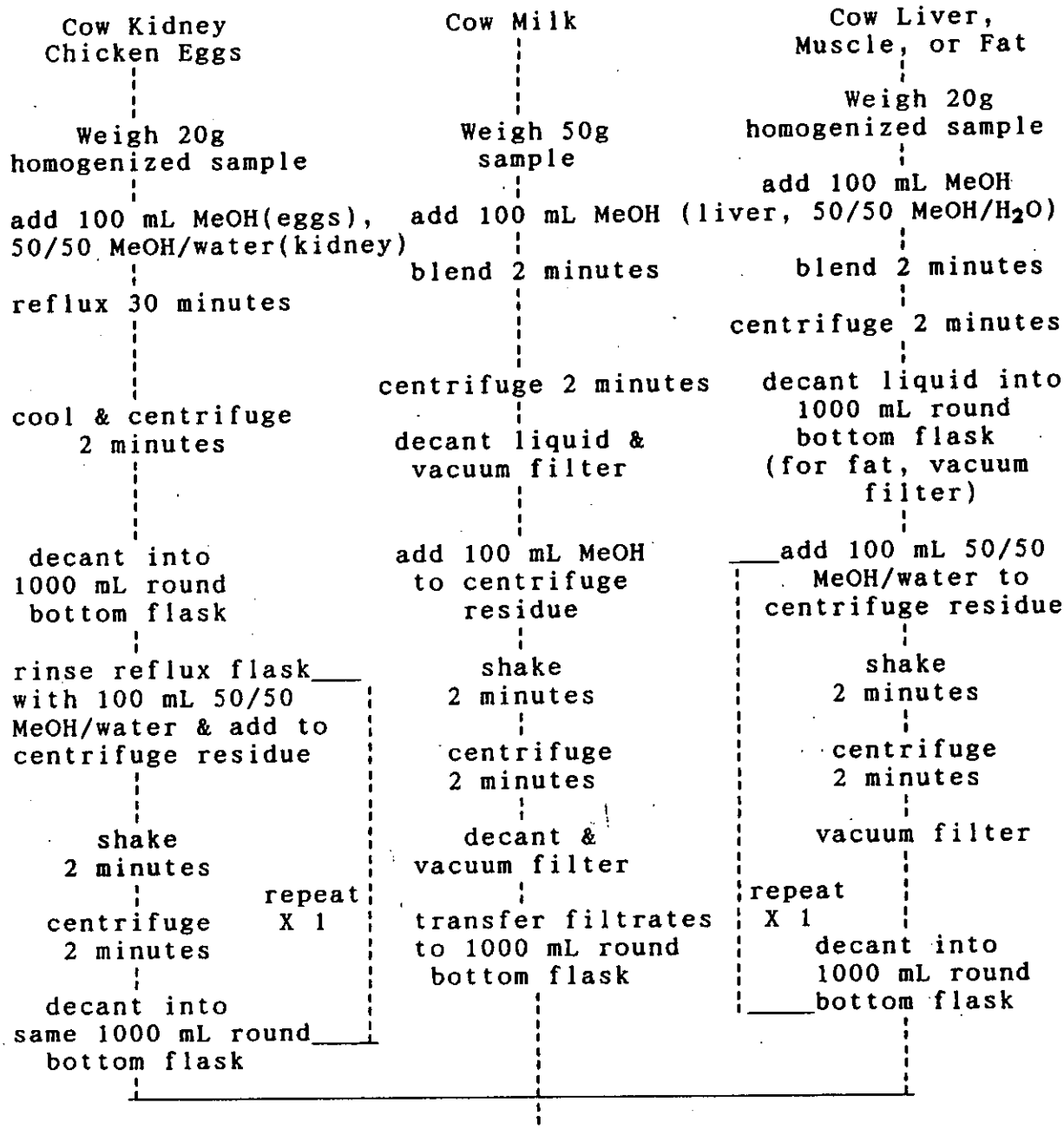
Correlation: 0.9998
Y-intercept: 346.2434393
Slope: 194.7560596

CARDC ID CODE	SAMPLE Wt. g	INJECTED mg	Corr ppm	PK HGHT INTG	% Recovery
1170-258	20	400	0.00000	<0	
1170-259+.05ppm	20	400	0.02548	2331	51.0
1170-260+.05ppm	20	400	0.02849	2566	57.0
1170-261+.25ppm	20	400	0.16021	12827	64.1
1170-262+.25ppm	20	400	0.18135	14474	72.5
1170-263+1.0ppm	20	80	0.70022	11256	70.0
1170-264+1.0ppm	20	80	0.69136	11118	69.1
				Average	64.0%
				± Standard Deviation	8.4%
				N	6

Figure 1
(Page 1 of 2)

Summary Flow Diagram of the Method

ANALYTICAL METHOD: BASF A9207



continued on following page

Figure 1
(Page 2 of 2)

Summary Flow Diagram of the Method
(for cow milk, muscle, liver, fat, kidney and chicken eggs)

ANALYTICAL METHOD: BASF A9207

Continued from preceding page.

Concentrate (rotary evaporator) to aqueous @ 40±5°C

Transfer aqueous phase to 500 mL separatory funnel. Partition 3 times with 150 mL portions of DCM. Rinse 1000 mL flask into the separatory funnel with the first 150 mL portion of DCM.

combine DCM portions & dry by passing through 25g bed of anhydrous Na_2SO_4

rinse Na_2SO_4 with additional 25mL DCM

combine DCM portions & concentrate (rotary evaporator) until just dry @ 40±5° C

bring up residue in 5mL EtOAc

transfer EtOAc to 10 mm i.d. column containing 5g of silica gel prewet with 10mL hexane

elute column with:
A 150 mL hexane
B 50 mL EtOAc
C 100 mL 15/85 MeOH/EtOAc

discard eluates A & B. Concentrate eluate C (rotary evaporator) until just dry @ 40±5° C

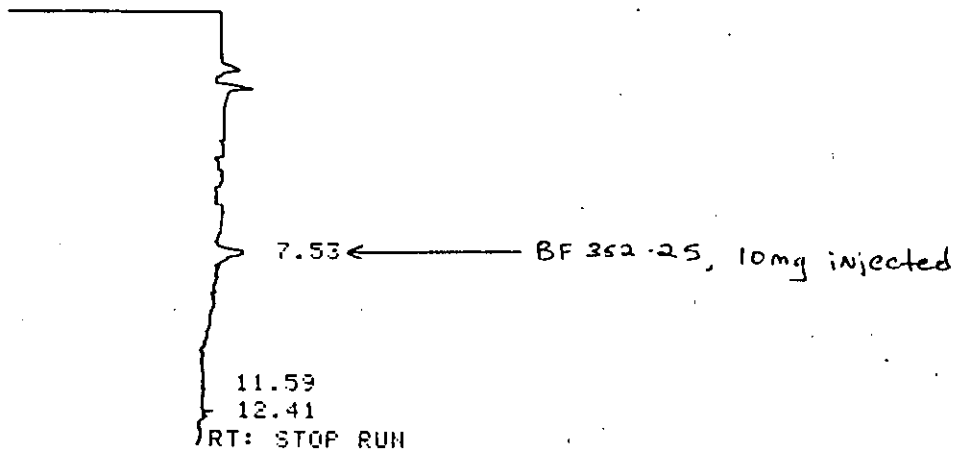
bring residue up in 60/40 H_2O/MeOH for HPLC analysis

FIGURE 2, Page 1 of 17

Typical High Performance Liquid Chromatograms of
Animal Matrices Analyzed using
ANALYTICAL METHOD: BASF A9207

Standard Curve

10 nanogram Standard 6819:



[hp] 5880A MANUAL INJECTION @ 17:37 SEP 22, 1992
AREA %

RT	AREA	TYPE	WIDTH	HEIGHT	BASELINE	AREA %
0.00			BASELINE @ START RUN = 5086.35			
0.00			THRESHOLD @ START RUN = 2			
0.00			PEAK WIDTH @ START RUN = 0.26			
0.00			RP: REJECT → 0			
0.10			RT: INTG → OFF			
5.00			RT: INTG → ON			
7.53	316.62	BV	0.344	14.43	5077.52	88.374
11.59	8.10	BB	-----	1.15	5071.35	2.262
12.41	33.55	BP	-----	2.17	5070.82	9.364
13.00			RT: INTG → OFF			

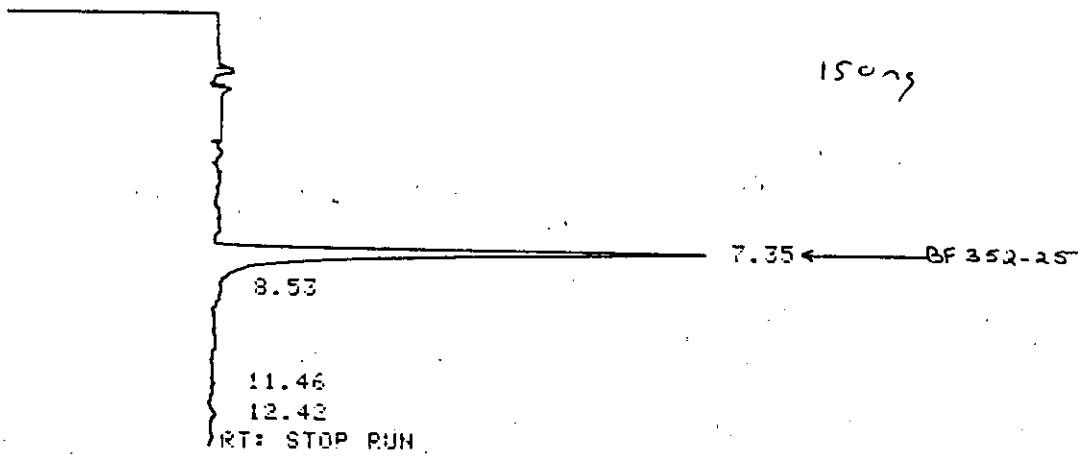
TOTAL AREA = 358.27
MULTIPLIER = 1

FIGURE 2, Page 2 of 17

Typical High Performance Liquid Chromatograms of
Animal Matrices Analyzed using
ANALYTICAL METHOD: BASF A9207

Standard Curve

150 nanogram Standard 6816:



[hp] 5880A MANUAL INJECTION @ 18:39 SEP 14, 1992
AREA %

RT	AREA	TYPE	WIDTH	HEIGHT	BASELINE	AREA %
0.00			BASELINE @ START RUN = 5092.72			
0.00			THRESHOLD @ START RUN = 2			
0.00			PEAK WIDTH @ START RUN = 0.30			
0.00			RP: REJECT → 0			
0.10			RT: INTG → OFF			
5.00			RT: INTG → ON			
7.35	4091.04	BV	0.27 *	240.04	5083.62	96.774
8.53	65.34	VV	-----	3.68	5083.05	1.546
11.46	16.96	BP	-----	1.95	5081.32	0.401
12.42	54.07	PB	-----	3.26	5080.59	1.279
13.00			RT: INTG → OFF			

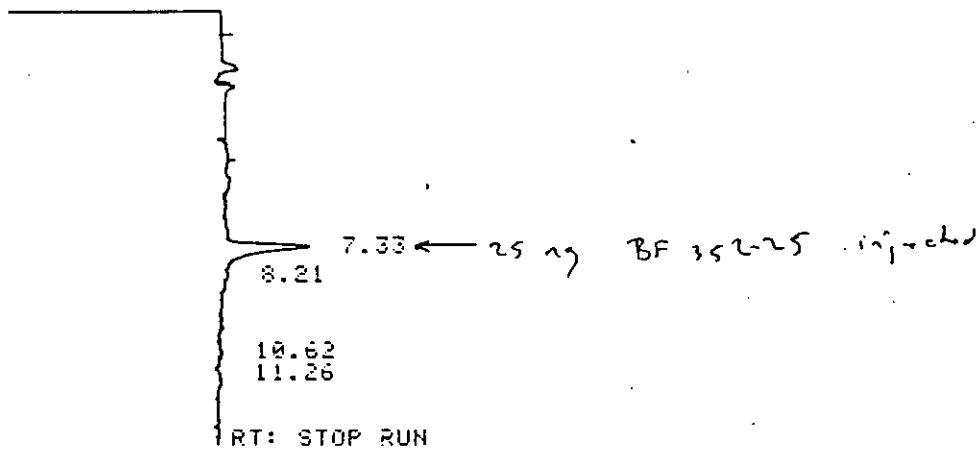
TOTAL AREA = 4227.41
MULTIPLIER = 1

FIGURE 2, Page 3 of 17

Typical High Performance Liquid Chromatograms of
Animal Matrices Analyzed using
ANALYTICAL METHOD: BASF A9207

Standard Curve

25 nanogram Standard 6822:



[hp] 5880A MANUAL INJECTION @ 19:51 SEP 14, 1992
AREA %

RT	AREA	TYPE	WIDTH	HEIGHT	BASELINE	AREA %
0.00			BASELINE @ START RUN = 5092.72			
0.00			THRESHOLD @ START RUN = 2			
0.00			PEAK WIDTH @ START RUN = 0.30			
0.00			RP: REJECT → 0			
0.10			RT: INTG → OFF			
5.00			RT: INTG → ON			
7.33	665.44	BV	0.262	39.76	5089.73	91.989
8.21	30.22	VP	-----	2.20	5088.06	4.178
10.62	7.43	BB	-----	1.48	5085.55	1.027
11.26	20.30	BB	-----	2.50	5084.14	2.806
13.00			RT: INTG → OFF			

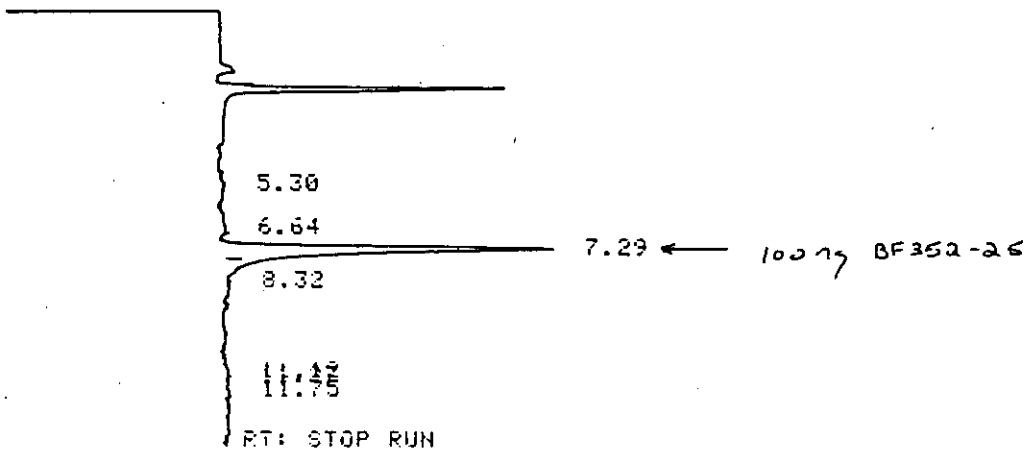
TOTAL AREA = 723.39
MULTIPLIER = 1

FIGURE 2, Page 4 of 17

Typical High Performance Liquid Chromatograms of
Animal Matrices Analyzed using
ANALYTICAL METHOD: BASF A9207

Standard Curve

100 nanogram Standard 6814:



[hp] 5880A MANUAL INJECTION @ 21:03 SEP 14, 1992
AREA %

RT	AREA	TYPE	WIDTH	HEIGHT	BASELINE	AREA %
0.00			BASELINE @ START RUN = 5086.35			
0.00			THRESHOLD @ START RUN = 2			
0.00			PEAK WIDTH @ START RUN = 0.30			
0.00			RP: REJECT → 0			
0.10			RT: INTG → OFF			
5.00			RT: INTG → ON			
5.30	9.34	BB	-----	1.43	5089.23	0.323
6.64	35.17	BP	-----	2.18	5088.17	1.216
7.29	2783.40	PP	0.266*	163.93	5087.62	96.273
8.32	1.00	PB	-----	1.18	5090.91	0.035
11.12	6.65	BV	-----	1.42	5089.95	0.230
11.27	11.79	VP	-----	1.29	5089.59	0.408
11.75	43.81	PH	-----	2.41	5089.19	1.515
13.00			RT: INTG → OFF			

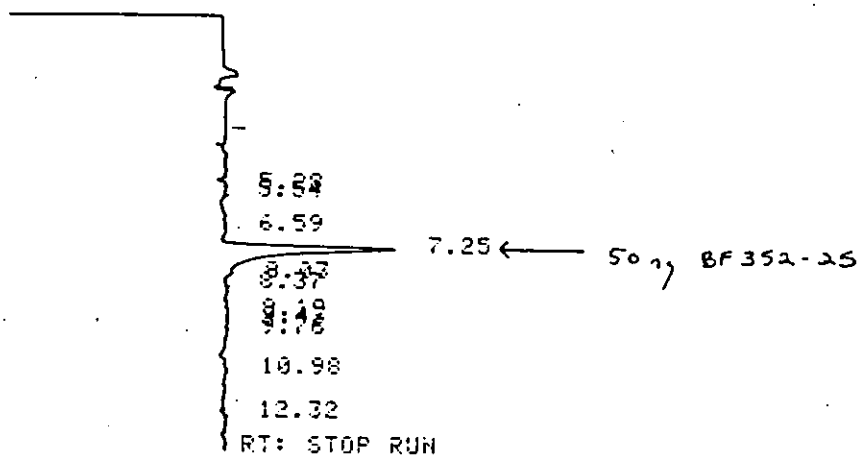
TOTAL AREA = 2891.16
MULTIPLIER = 1

FIGURE 2, Page 5 of 17

Typical High Performance Liquid Chromatograms of
Animal Matrices Analyzed using
ANALYTICAL METHOD: BASF A9207

Standard Curve

50 nanogram Standard 6815:



[hp] 5880A MANUAL INJECTION @ 21:51 SEP 14, 1992
AREA %

RT	AREA	TYPE	WIDTH	HEIGHT	BASELINE	AREA %
0.00			BASELINE @ START RUN = 5086.35			
0.00			THRESHOLD @ START RUN = 2			
0.00			PEAK WIDTH @ START RUN = 0.30			
0.00			RP: REJECT → 0			
0.10			RT: INTG → OFF			
5.00			RT: INTG → ON			
5.28	17.54	BB	-----	1.50	5086.75	1.241
5.54	6.89	BB	-----	1.30	5086.58	0.487
6.59	7.64	BB	-----	1.96	5085.52	0.540
7.25	1311.48	BB	0.249*	82.40	5086.79	92.811
8.03	6.58	BB	-----	2.06	5088.01	0.465
8.37	7.33	BB	-----	1.69	5085.78	0.519
9.19	15.71	BV	-----	2.07	5086.24	1.112
9.43	11.89	VP	-----	2.52	5085.89	0.841
9.76	12.02	BV	-----	2.52	5085.56	0.850
10.98	3.42	BB	-----	1.24	5086.35	0.242
12.32	12.58	BH	-----	2.60	5084.17	0.890
13.00			RT: INTG → OFF			

TOTAL AREA = 1413.07
MULTIPLIER = 1

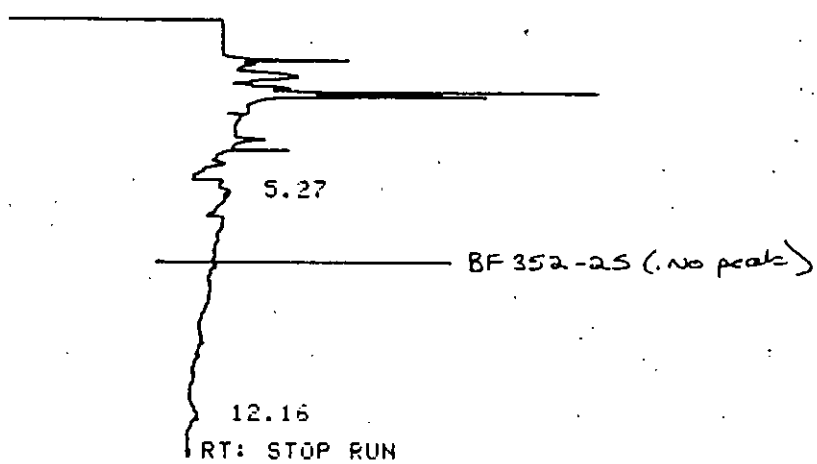
FIGURE 2, Page 6 of 17

Typical High Performance Liquid Chromatograms of
Animal Matrices Analyzed using
ANALYTICAL METHOD: BASF A9207

Cow Milk

Untreated Control (UTC), <0.05 ppm:

#1170-181



Control Milk
5ml Final Volume
100ung injected

[hp] 5880A MANUAL INJECTION @ 17:51 SEP 14, 1992
AREA %

RT	AREA	TYPE	WIDTH	HEIGHT	BASELINE	AREA %
0.00			BASELINE @ START RUN = 5086.35			
0.00			THRESHOLD @ START RUN = 2			
0.00			PEAK WIDTH @ START RUN = 0.30			
0.00			RP: REJECT → 0			
0.10			RT: INTG → OFF			
5.00			RT: INTG → ON			
5.27	213.88	BB	-----*	7.41	5101.13	74.661
12.16	72.59	BB	0.335	3.40	5079.42	25.339
13.00			RT: INTG → OFF			

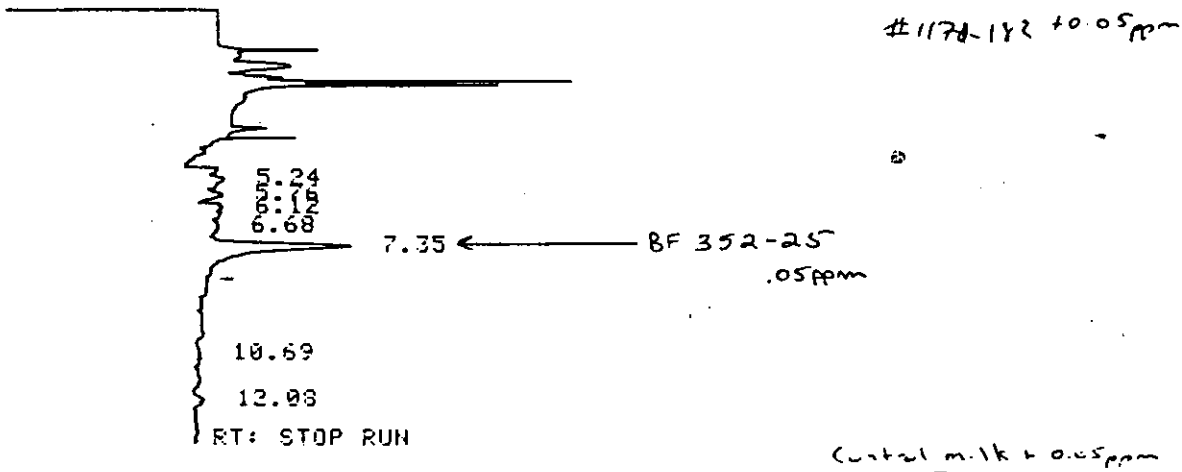
TOTAL AREA = 286.46
MULTIPLIER = 1

FIGURE 2, Page 7 of 17

Typical High Performance Liquid Chromatograms of
Animal Matrices Analyzed using
ANALYTICAL METHOD: BASF A9207

Cow Milk

UTC + 0.05 ppm, 83.2% Recovery:



Control milk + 0.05ppm
5ml Final Volume
100ung injected

[hp] 5880A MANUAL INJECTION @ 18:15 SEP 14, 1992
AREA %

RT	AREA	TYPE	WIDTH	HEIGHT	BASELINE	AREA %
0.00			BASELINE @ START RUN = 5084.22			
0.00			THRESHOLD @ START RUN = 2			
0.00			PEAK WIDTH @ START RUN = 0.30			
0.00			RP: REJECT → 0			
0.10			RT: INTG → OFF			
5.00			RT: INTG → ON			
5.24	151.54	BV	------*	7.67	5098.20	9.595
5.76	165.48	VP	-----	11.72	5093.63	10.478
6.12	11.03	PB	0.076	2.28	5091.07	0.698
6.68	38.45	BP	-----	2.08	5089.20	2.434
7.35	1150.49	PB	-----	67.86	5086.98	72.845
10.69	21.46	PB	-----	1.41	5080.83	1.359
12.08	40.92	BB	-----	4.21	5079.66	2.591
13.00			RT: INTG → OFF			

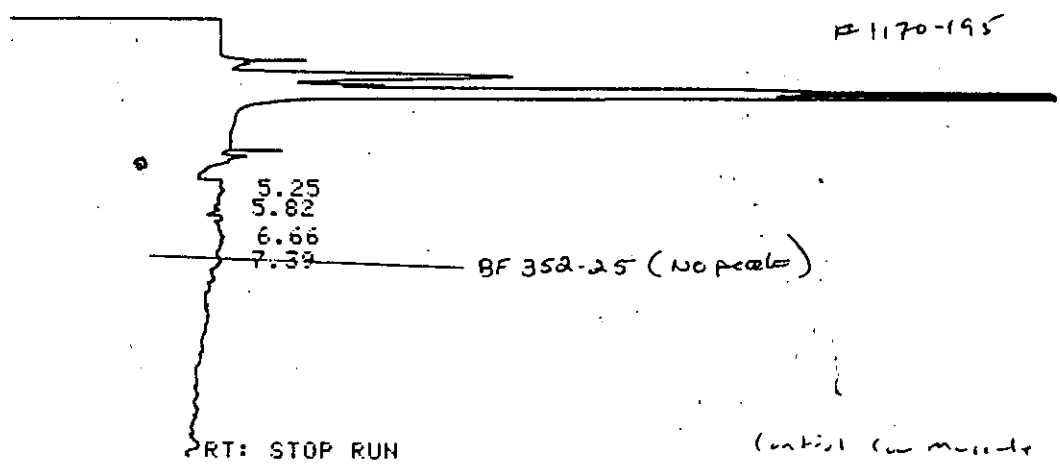
TOTAL AREA = 1579.36
MULTIPLIER = 1

FIGURE 2, Page 8 of 17

Typical High Performance Liquid Chromatograms of
Animal Matrices Analyzed using
ANALYTICAL METHOD: BASF A9207

Cow Muscle

Untreated Control (UTC), <0.05 ppm:



(initial cow muscle
5 ml final volume
400 mg injected)

[hp] 5880A MANUAL INJECTION @ 18:13 SEP 16, 1992
AREA %

RT	AREA	TYPE	WIDTH	HEIGHT	BASELINE	AREA %
0.00			BASELINE @ START RUN = 5086.35			
0.00			THRESHOLD @ START RUN = 2			
0.00			PEAK WIDTH @ START RUN = 0.30			
0.00			RP: REJECT → 0			
0.10			RT: INTG → OFF			
5.00			RT: INTG → ON			
5.25	37.01	BV	-----	4.26	5100.62	18.546
5.82	37.98	BP	-----	3.54	5098.46	19.034
6.66	78.66	PB	-----	4.12	5093.09	39.421
7.39	45.89	SB	-----	3.05	5091.88	22.999
13.00			RT: INTG → OFF			

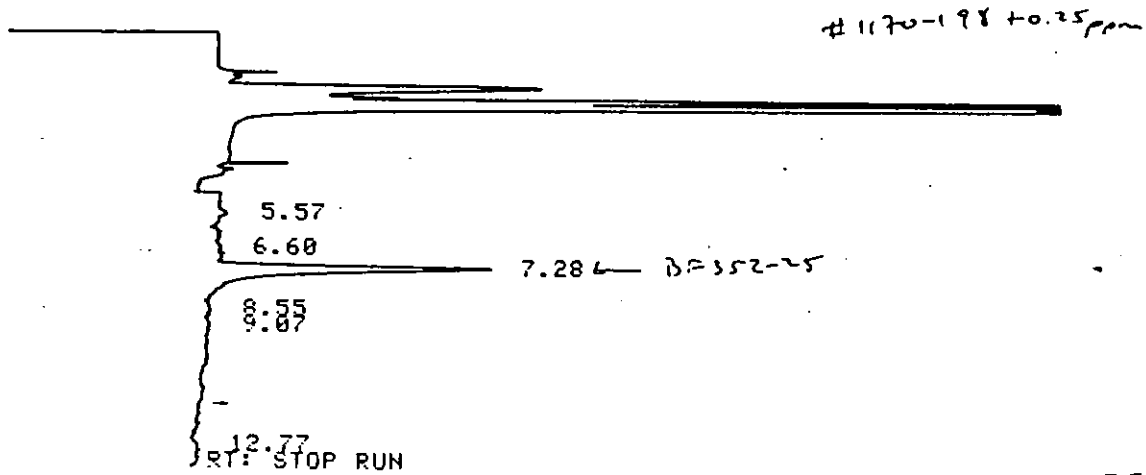
TOTAL AREA = 199.53
MULTIPLIER = 1

FIGURE 2, Page 9 of 17

Typical High Performance Liquid Chromatograms of
Animal Matrices Analyzed using
ANALYTICAL METHOD: BASF A9207

Cow Muscle

UTC + 0.25 ppm, 75.6% Recovery:



[hp] 5880A MANUAL INJECTION @ 19:49 SEP 16, 1992
AREA %

Control + 0.25ppm BF352-25
5 ml Final Volume
400 mg injected

RT	AREA	TYPE	WIDTH	HEIGHT	BASELINE	AREA %
0.00			BASELINE @ START RUN = 5090.58			
0.00			THRESHOLD @ START RUN = 2			
0.00			PEAK WIDTH @ START RUN = 0.30			
0.00			RP: REJECT → 0			
0.10			RT: INTG → OFF			
5.00			RT: INTG → ON			
5.57	140.66	BP	-----*	6.68	5107.44	5.804
6.60	19.58	BB	-----	1.56	5102.69	0.808
7.28	2124.41	BP	0.25	133.12	5101.73	87.650
8.55	63.94	PP	/SYM	3.33	5096.24	2.638
9.07	40.80	PB	-----	2.63	5096.48	1.683
12.77	34.35	A BH	-----	3.67	5089.46	1.417
13.00			RT: INTG → OFF			

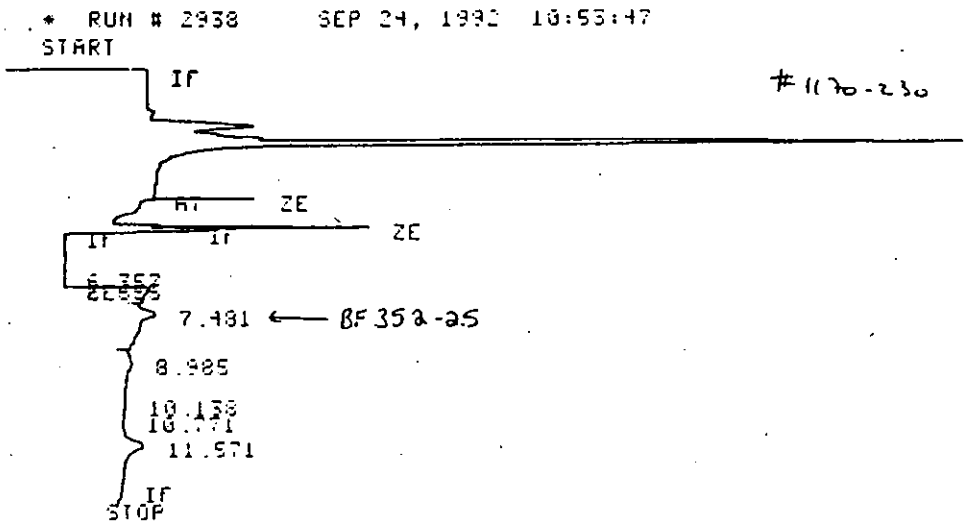
TOTAL AREA = 2423.75
MULTIPLIER = 1

FIGURE 2, Page 10 of 17

Typical High Performance Liquid Chromatograms of
Animal Matrices Analyzed using
ANALYTICAL METHOD: BASF A9207

Cow Liver

Untreated Control (UTC), <0.05 ppm:



Control liver
Spiked Final Volume
Young injected

RUN# 2938 SEP 24, 1992 10:53:47

METHOD NAME: M+TRI.MET

HEIGHT%

RT	HEIGHT	TYPE	WIDTH	HEIGHT%
6.357	419	BP	.158	9.45610
6.555	276	PP	.233	6.22884
7.481	1293	PP	.425	29.18077
8.985	432	PP	.377	9.74949
10.138	237	PP	.402	5.34863
10.771	191	PU	.255	4.31054
11.571	1583	UU	.488	35.71557

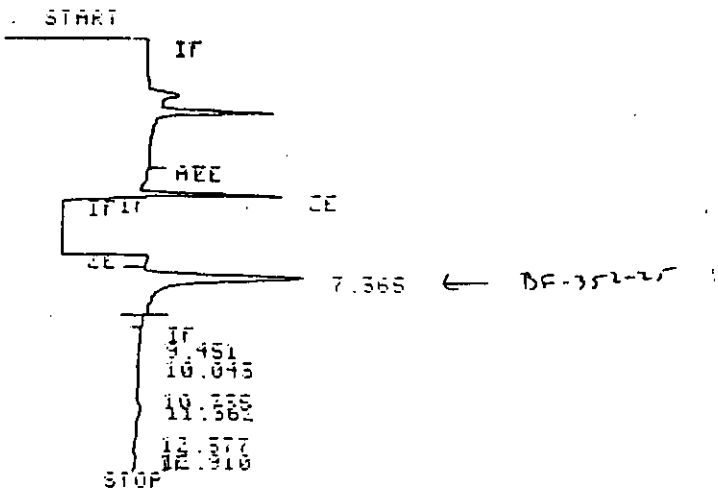
TOTAL HEIGHT= 4431
MUL FACTOR=1.0000E+00

FIGURE 2, Page 11 of 17

Typical High Performance Liquid Chromatograms of
Animal Matrices Analyzed using
ANALYTICAL METHOD: BASF A9207

Cow Liver

UTC + 1.0 ppm, 69.4% Recovery:



#1170-235+1.0ppm

BF-352-25

RUN# 2945 SEP 24, 1992 13:42:15

METHOD NAME: M*TRI.MET

Control liver + 1.0ppm BF352-25
80 mg injected
25ml Final Volume

HEIGHT%

RT	HEIGHT	TYPE	WIDTH	HEIGHT%
7.365	10770	BB	[illegible]	[illegible]
9.451	182	PV	.149	1.48208
10.045	148	PV	.156	1.20621
10.735	278	VV	.337	2.26334
11.362	466	VV	.426	3.79479
12.377	220	VP	.324	1.79183
12.910	216	I PP	.103	1.75896

TOTAL HEIGHT= 12280
MUL FACTOR=1.0000E+00

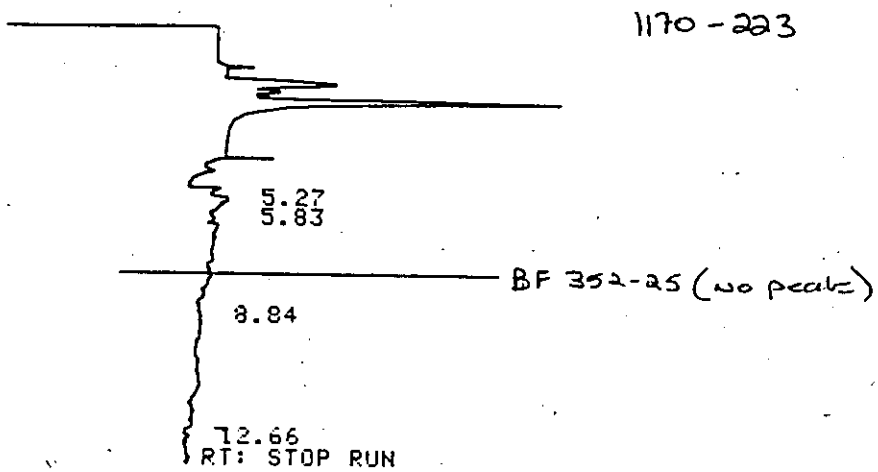
FIGURE 2, Page 12 of 17

Typical High Performance Liquid Chromatograms of
Animal Matrices Analyzed using
ANALYTICAL METHOD: BASF A9207

Cow Fat

Untreated Control (UTC), <0.05 ppm:

1170-223



[hp] 5880A MANUAL INJECTION @ 18:01 SEP 22, 1992 Control Fat
AREA %
5mL Final volume
400 mg injected

RT	AREA	TYPE	WIDTH	HEIGHT	BASELINE	AREA %
0.00			BASELINE @ START RUN = 5092.72			
0.00			THRESHOLD @ START RUN = 2			
0.00			PEAK WIDTH @ START RUN = 0.26			
0.00			RP: REJECT → 0			
0.10			RT: INTG → OFF			
5.00			RT: INTG → ON			
5.27	191.56	BV	-----*	9.50	5101.19	63.011
5.83	86.31	VP	-----	5.13	5098.65	28.392
8.84	5.52	BB	-----	1.05	5090.22	1.814
12.66	20.62	PH	0.142	2.28	5082.31	6.782
13.00			RT: INTG → OFF			

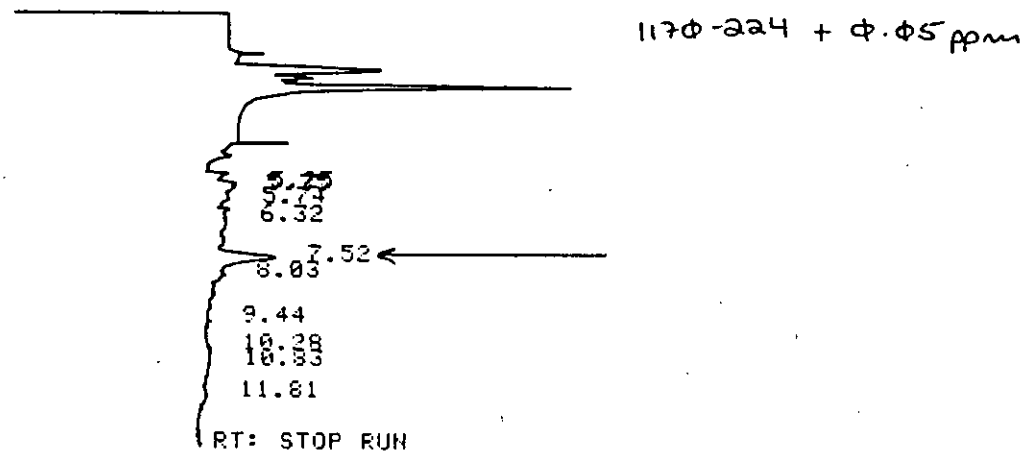
TOTAL AREA = 304.01
MULTIPLIER = 1

FIGURE 2, Page 13 of 17

Typical High Performance Liquid Chromatograms of
Animal Matrices Analyzed using
ANALYTICAL METHOD: BASF A9207

Cow Fat

UTC + 0.05 ppm, 86.2% Recovery:



[hp] 5880A MANUAL INJECTION @ 18:25 SEP 22, 1992
AREA %

Control + 0.05 ppm BF352-25
5mL Final Volume
400 mg injected

RT	AREA	TYPE	WIDTH	HEIGHT	BASELINE	AREA %
0.00			BASELINE @ START RUN = 5090.58			
0.00			THRESHOLD @ START RUN = 2			
0.00			PEAK WIDTH @ START RUN = 0.26			
0.00			RP: REJECT → 0			
0.10			RT: INTG → OFF			
5.00			RT: INTG → ON			
5.25	53.65	BV	-----	8.33	5099.97	8.978
5.35	47.83	VB	-----*	5.79	5100.85	8.005
5.74	22.60	BB	*-----	3.41	5101.67	3.783
6.32	23.89	BV	-----	3.17	5093.98	3.999
7.52	409.12	BB	0.232	27.67	5092.48	68.468
8.03	5.99	BB	-----	2.15	5093.10	1.003
9.44	4.20	BB	-----	1.00	5086.93	0.703
10.28	7.65	BP	-----	1.42	5086.80	1.280
10.83	2.76	BP	-----	1.42	5087.48	0.462
11.81	19.83	BB	-----	1.98	5084.67	3.319
13.00			RT: INTG → OFF			

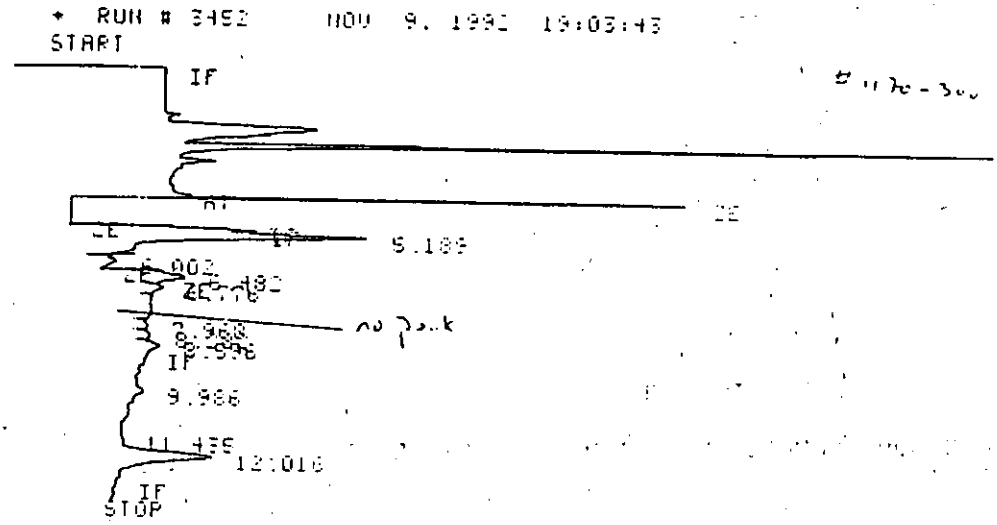
TOTAL AREA = 597.53
MULTIPLIER = 1

FIGURE 2, Page 14 of 17

Typical High Performance Liquid Chromatograms of
Animal Matrices Analyzed using
ANALYTICAL METHOD: BASF A9207

Cow Kidney

Untreated Control (UTC), <0.05 ppm:



RUN# 3452 NOV 9, 1992 19:03:43

METHOD NAME: N:TRI.MET

HEIGHT%

RT	HEIGHT	TYPE	WIDTH	HEIGHT%
5.189	4722	BB	.012	40.28667
6.002	413	BP	.188	3.52359
6.482	901	PU	.172	7.68706
6.776	270	UP	.160	2.30356
7.968	204	UU	.162	1.74047
8.282	357	UU	.211	3.04582
8.596	761	UU	.461	6.49262
9.986	600	UU	.478	5.11902
11.435	245	PU	.353	2.09027
12.016	3248	UP	.372	27.71095

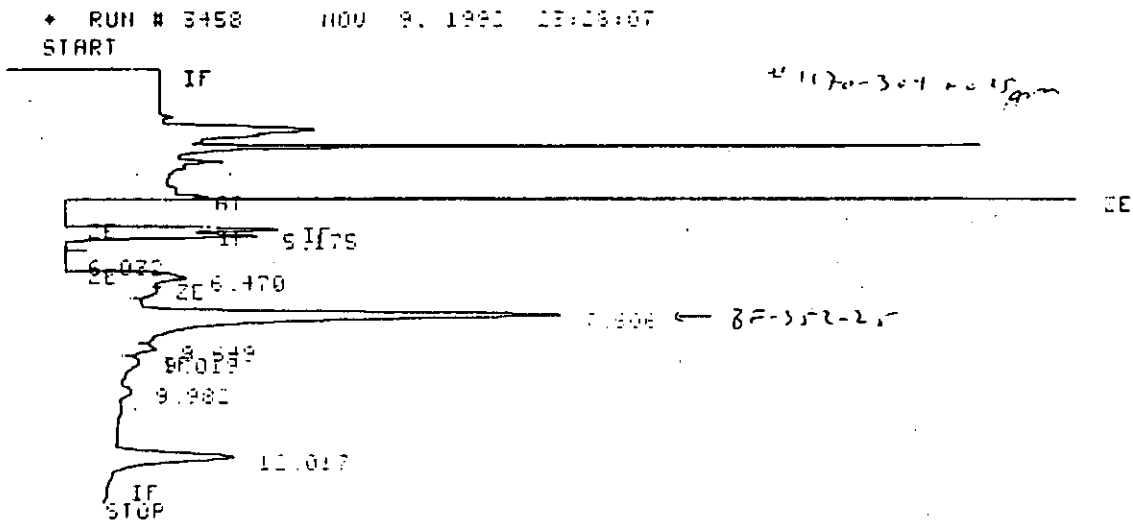
TOTAL HEIGHT= 11721
NOL FACTOR=1.0000E+00

FIGURE 2, Page 15 of 17

Typical High Performance Liquid Chromatograms of
Animal Matrices Analyzed using
ANALYTICAL METHOD: BASF A9207

Cow Kidney

UTC + 0.25 ppm, 75.8% Recovery:



RUN# 3458 NOV 9, 1991 13:18:10

METHOD NAME: H:TFI.MET

HEIGHT%

RT	HEIGHT	TYPE	HIGHT	HEIGHT.
6.032	703	BV	.133	3.17167
6.470	1191	VP	.188	5.37334
7.606	14175	PV	.313	63.99118
8.649	987	VV	.274	4.45157
9.019	515	VP	.413	2.32348
9.982	476	PV	.553	2.14752
12.017	4118	VV	.391	18.57334

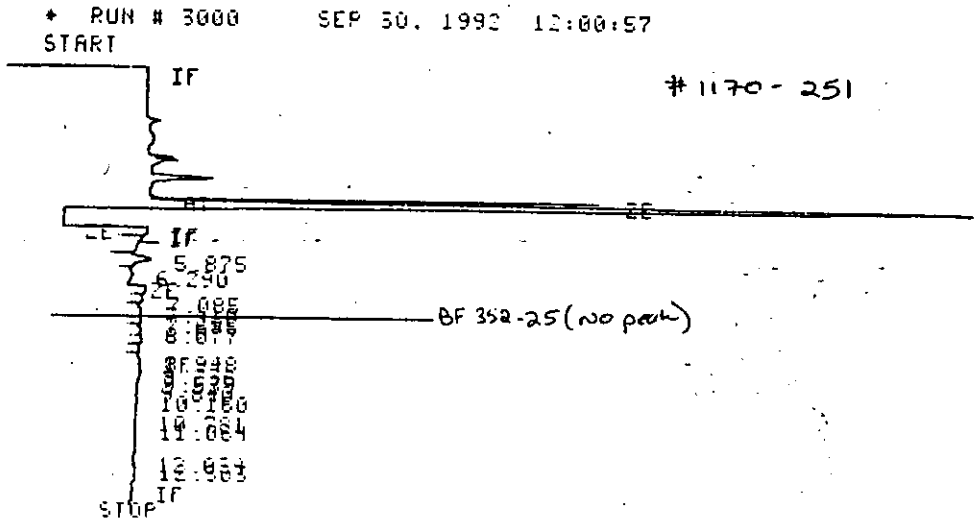
TOTAL HEIGHT= 22165
MUL FACTOR=1.0000E+00

FIGURE 2, Page 16 of 17

Typical High Performance Liquid Chromatograms of Animal Matrices Analyzed using ANALYTICAL METHOD: BASF A9207

Chicken Eggs

Untreated Control (UTC), <0.05 ppm:



RUN# 3000 SEP 30, 1992 12:00:57

METHOD NAME: M+TPI.MET

HEIGHT%

PT	HEIGHT	TYPE	WIDTH	HEIGHT%
5.875	1456	BV	.186	25.05047
6.290	399	VV	.360	6.87576
7.085	230	VP	.176	3.99741
7.410	300	PV	.158	5.16974
7.695	319	VV	.171	5.49716
8.077	353	VV	.211	6.06583
8.948	382	VV	.209	6.58280
9.236	387	VV	.209	6.66896
9.539	284	VV	.179	4.89402
9.840	249	VV	.176	4.29088
10.160	262	VV	.160	4.51491
10.781	226	VV	.403	3.89454
11.064	290	VV	.358	4.99741
12.034	246	VV	.157	4.23919
12.303	361	VP	.313	6.22092

Control eggs
5mL Final volume
400mg injected

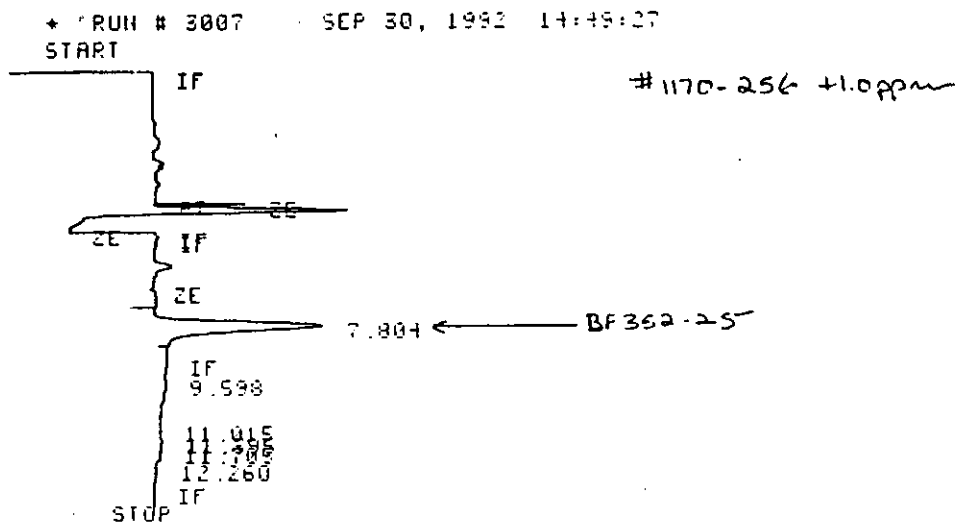
TOTAL HEIGHT= 5803
MUL FACTOR=1.0000E+00

FIGURE 2, Page 17 of 17

Typical High Performance Liquid Chromatograms of
Animal Matrices Analyzed using
ANALYTICAL METHOD: BASF A9207

Chicken Eggs

UTC + 1.0 ppm, 68.0% Recovery:



RUN# 3007 SEP 30, 1992 14:49:27

METHOD NAME: M+TPI.MET

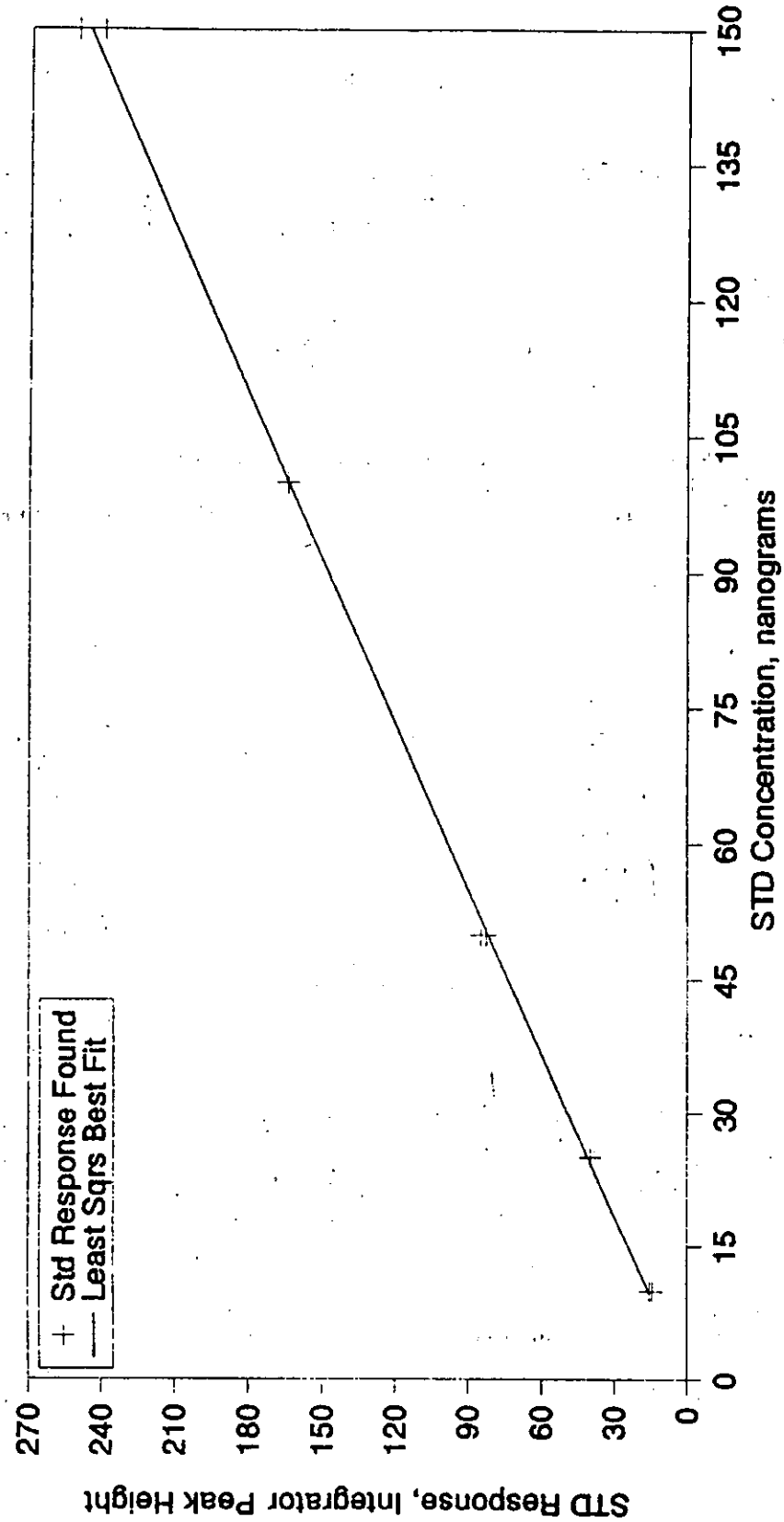
HEIGHT%

RT	HEIGHT	TYPE	WIDTH	HEIGHT%
7.804	11343	BV	.363	81.62135
9.598	842	VV	.523	6.05886
11.015	481	VV	.517	3.46113
11.395	584	VV	.262	4.20235
11.705	500	VP	.343	3.59790
12.260	147	PB	.102	1.05778

control eggs + 1.0ppm BF352-25
25 ml final volume
80 uug injected

TOTAL HEIGHT= 13897
NUL FACTOR=1.0000E+00

FIGURE 3
LINEAR RESPONSE DATA
BF 352-25



10 to 150 ng Standard Curve
Y-intercept (constant) = -0.4474693
Slope (X-coefficient) = 1.6420546

ATTACHMENTS

Matrices and Standards Receipt and Storage p 64-65

Liquid Chromatographic Bench Sheets p 66-77

MATRICES AND STANDARDS RECEIPT AND STORAGE

The analytical method was validated using animal matrices purchased at a local grocery store or butcher on the following dates:

Matrix	Master Log Book Number	Date Received
Bovine		
Liver	11701157	9/15/92
	11771080	3/ 5/92
	11701158	9/24/92
Muscle	11701134	6/18/92
Fat	11701157	9/15/92
Kidney	11701175	10/26/92
Milk	11701120	5/13/92
	11701157	9/15/92
Chicken		
Eggs	11701157	9/15/92

All matrices were received at refrigerator temperatures, blended and stored at an average of -23°C until analysis. Samples were thawed immediately prior to analysis.

The reference standard used during the course of this method validation was shipped frozen from BASF Corporation Chemicals Division, Agricultural Research Center, 26 Davis Drive, Research Triangle Park, NC 27709 on October 30, 1991 by Federal Express overnight shipment. After receipt by Colorado Analytical Research & Development Corporation on October 31, 1991 the standard vials were stored at an average of -26°C until used in analysis.

The analytical standard was received and logged into Colorado Analytical Research & Development Corporation's Master Log Book as follows:

CARDC Master Log Book Number and Date Received	Standard Label Description
11701000 10/31/91 Bag Containing Vials:	BF 352-25 lot # L31/141-6 PCP0061
Vials:	230865 PCP00067 ca. 50mg 19.77.90

Protocol No.: 92159

BENCH SHEET

PROJECT BASF-1170
METHOD NO BASF A9207
SAMPLE MATRIX COW MILK
COMPOUND(S) BF352-25

SET # MILK - 1
METHOD VALIDATION

INITIATED BY PEV DATE 9/15/92
REVIEWED BY SEK 9/16/92

NOTEBOOK 94 PAGE 65 MLB# 11701120

STORAGE	SAMPLE PREP	WEIGHING	FORTIFY	EXTRACT	CLEANUP	PARTITION	LC		
PEV 5/13/92	NA	DU 9/14/92	DU 9/14/92	DU 9/14/92	DU 9/14/92	DU 9/14/92	SEK 9/14/92		
Freezer #6		BALANCE # 20303446					SERIAL # 2422G00611		

COMMENTS

SAMPLE DATA CARDC	BASF	STD #	SAMP WT. (g)	PPM	mg inj	ng FND	FORTIFICATION STD - DATA STD.VOL.	STD.CONC.	FINAL VOL (mL)	FND ppm	CORR ppm	PK HT PK AREA MAN(mm)	% REC
	BLANK											< 0	
	STD	6819						10 ng				14.54	
1170-181	UTC		50		1000				5	0	0	< 0	
1170-182	UTC	7207	50	0.05	1000	41.59878	2.5mL of	1 µg/mL	5	0.04160	0.04160	67.86	83.2
	STD	6816						150 ng				240.04	
1170-183	UTC	7207	50	0.05	1000	44.19309	2.5mL of	1 µg/mL	5	0.04419	0.04419	72.12	88.4
1170-184	UTC	6810	50	0.25	250	49.7288	1 mL of	12.5 µg/mL	20	0.19892	0.19892	81.21	79.6
	STD	6822						25 ng				39.76	
1170-185	UTC	6810	50	0.25	250	50.27084	1 mL of	12.5 µg/mL	20	0.20108	0.20108	82.10	80.4
1170-186	UTC	6808	50	1.0	66.66667	53.45588	0.5mL of	100 µg/mL	75	0.80184	0.80184	87.33	80.2
	STD	6814						100 ng				163.93	
1170-187	UTC	6808	50	1.0	66.66667	54.33283	0.5mL of	100 µg/mL	75	0.80499	0.80499	88.77	81.5
	STD	6815						50 ng				82.40	
	BLANK											< 0	
	STD	6819						10 ng				15.42	
	STD	6815						50 ng				84.66	
	STD	6816						150 ng				250.59	
												Average	82.2
												Std Dev	3.3

CORR: 0.9995 INTCPT: -0.4474693 SLOPE: 1.6420546

Protocol No.: 92159

BENCH SHEET

PROJECT BASF-1170
METHOD NO BASF A9207
SAMPLE MATRIX COW MILK
COMPOUND(S) BF352-25

SET # MILK - 2
METHOD VALIDATION

INITIATED BY SEK DATE 9/16/92
REVIEWED BY PEV 9/16/92

NOTEBOOK 94 PAGE 66 MLB# 11701120, 11701157

STORAGE	SAMPLE PREP	WEIGHING	FORTIFY	EXTRACT	CLEANUP	PARTITION	LC		
PEV 5/13/92 9/15/92 Freezer #6	NA	DU 9/14,15/92 BALANCE # 20303446	DU 9/15/92	DU 9/15/92	DU 9/15/92	DU 9/15/92	SEK 9/15/92 SERIAL # 2422G00611		

COMMENTS

SAMPLE DATA CARDC	BASF	STD #	SAMP WT. (g)	PPM	mg inj	ng FND	FORTIFICATION STD - DATA STD.VOL.	STD.CONC.	FINAL VOL (mL)	FND ppm	CORR ppm	PK HT PK AREA MAN(mm)	% REC
	BLANK											< 0	
	STD	6819						10 ng				18.34	
1170-188	UTC		50		1000				5	0	0	< 0	
1170-189	UTC	7207	50	0.05	1000	41.93674	2.5mL of	1 µg/mL	5	0.04194	0.04194	77.68	83.9
	STD	6816						150 ng				284.82	
1170-190	UTC	7207	50	0.05	1000	41.97927	2.5mL of	1 µg/mL	5	0.04198	0.04198	77.76	84.0
1170-191	UTC	6810	50	0.25	250	51.14580	1 mL of	12.5 µg/mL	20	0.20458	0.20458	95.00	81.8
	STD	6822						25 ng				46.27	
1170-192	UTC	6810	50	0.25	250	49.40182	1 mL of	12.5 µg/mL	20	0.19761	0.19761	91.72	79.0
1170-193	UTC	6808	50	1.0	66.66667	52.42188	0.5mL of	100 µg/mL	75	0.78633	0.78633	97.40	78.6
	STD	6814						100 ng				184.68	
1170-194	UTC	6808	50	1.0	66.66667	53.55972	0.5mL of	100 µg/mL	75	0.80340	0.80340	99.54	80.3
	STD	6815						50 ng				93.31	
	BLANK											< 0	
	STD	6819						10 ng				17.53	
	STD	6815						50 ng				92.03	
	STD	6816						150 ng				278.49	
												Average	81.3
												Std Dev	2.4

CORR: 0.9998 INTCPT: -1.1927997 SLOPE: 1.8807567

Protocol No.: 92159

BENCH SHEET

PROJECT BASF-1170
METHOD NO BASF A9207
SAMPLE MATRIX COW MUSCLE
COMPOUND(S) BF352-25
NOTEBOOK 94 PAGE 67

SET # COW MUSCLE - 1
METHOD VALIDATION

INITIATED BY SEK DATE 9/17/92
REVIEWED BY PEV 9/17/92

MLB# 11701134

STORAGE	SAMPLE PREP	WEIGHING	FORTIFY	EXTRACT	CLEANUP	PARTITION	LC		
PEV 6/18/92	DU 6/18/92	DU 9/16/92	DU 9/16/92	DU 9/16/92	DU 9/16/92	DU 9/16/92	SEK 9/16,17/92		
Freezer #6		BALANCE # 20303446					SERIAL # 2422G00611		

COMMENTS

SAMPLE DATA CARDC	BASF	STD #	SAMP WT. (g)	PPM	mg inj	ng FND	FORTIFICATION STD - DATA STD.VOL.	STD.CONC.	FINAL VOL (mL)	FND ppm	CORR ppm	PK HT PK AREA MAN(mm)	% REC
	BLANK											< 0	
	STD	6819						10 ng				18.05	
1170-195	UTC		20		400				5	0	0	< 0	
1170-196	UTC	7207	20	0.05	400	18.89089	1 mL of	1 µg/mL	5	0.04723	0.04723	33.64	94.5
	STD	6816						150 ng				271.59	
1170-197	UTC	7207	20	0.05	400	16.64657	1 mL of	1 µg/mL	5	0.04162	0.04162	29.70	83.2
1170-198	UTC	6811	20	0.25	400	75.55718	0.5mL of	10 µg/mL	5	0.18889	0.18889	133.12	75.6
	STD	6822						25 ng				43.77	
1170-199	UTC	6811	20	0.25	400	77.02112	0.5mL of	10 µg/mL	5	0.19255	0.19255	135.69	77.0
1170-200	UTC	6811	20	1.0	80	57.10703	2 mL of	10 µg/mL	25	0.71384	0.71384	100.73	71.4
	STD	6814						100 ng				175.68	
1170-201	UTC	6811	20	1.0	80	55.59183	2 mL of	10 µg/mL	25	0.69490	0.69490	98.07	69.5
	STD	6815						50 ng				90.23	
	BLANK											< 0	
	STD	6819						10 ng				20.51	
	STD	6815						50 ng				83.70	
	STD	6816						150 ng				257.05	
												Average	78.5
												Std Dev	9.2

CORR: 0.9991 INTCPT: 0.4762640 SLOPE: 1.7555411

Protocol No.: 92159

BENCH SHEET

PROJECT BASF-1170
METHOD NO BASF A9207
SAMPLE MATRIX COW MUSCLE
COMPOUND(S) BF352-25

SET # COW MUSCLE - 2
METHOD VALIDATION

INITIATED BY PEV DATE 9/18/92
REVIEWED BY SEK 9/18/92

NOTEBOOK 94 PAGE 68 MLB# 11701134

STORAGE	SAMPLE PREP	WEIGHING	FORTIFY	EXTRACT	CLEANUP	PARTITION	LC		
PEV 6/18/92	DU 6/18/92	DU 9/17/92	DU 9/17/92	DU 9/17/92	DU 9/17/92	DU 9/17/92	SEK 9/17,18/92		
Freezer #6		BALANCE # 20303446					SERIAL # 2422G00611		

COMMENTS

SAMPLE DATA CARDC	BASF	STD #	SAMP WT. (g)	PPM	mg inj	ng FND	FORTIFICATION STD - DATA STD.VOL.	STD.CONC.	FINAL VOL (mL)	FND ppm	CORR ppm	PK HT PK AREA MAN(mm)	% REC
	BLANK											< 0	
	STD	6819						10 ng				17.00	
1170-202	UTC		20		400				5	0	0	< 0	
1170-203	UTC	7207	20	0.05	400	15.48823	1 mL of	1 µg/mL	5	0.03872	0.03872	25.80	77.4
	STD	6816						150 ng				257.80	
1170-204	UTC	7207	20	0.05	400	14.74601	1 mL of	1 µg/mL	5	0.03687	0.03687	24.53	73.7
1170-205	UTC	6811	20	0.25	400	75.71968	0.5mL of	10 µg/mL	5	0.18930	0.18930	128.86	75.7
	STD	6822						25 ng				40.13	
1170-206	UTC	6811	20	0.25	400	77.80025	0.5mL of	10 µg/mL	5	0.19450	0.19450	132.42	77.8
1170-207	UTC	6811	20	1.0	80	51.53593	2 mL of	10 µg/mL	25	0.64420	0.64420	87.48	64.4
	STD	6814						100 ng				169.32	
1170-208	UTC	6811	20	1.0	80	52.36582	2 mL of	10 µg/mL	25	0.65457	0.65457	88.90	65.5
	STD	6815						50 ng				86.61	
	BLANK											< 0	-
	STD	6819						10 ng				15.26	
	STD	6815						50 ng				86.85	
	STD	6816						150 ng				253.95	
												Average	72.4
												Std Dev	6.0

CORR: 0.9998 INTCPT: -0.7013939 SLOPE: 1.7110663

Protocol No.: 92159

BENCH SHEET

PROJECT BASF-1170 | SET # LIVER - 3 | INITIATED BY SEK | DATE 9/25/92
METHOD NO BASF A9207 | METHOD VALIDATION | REVIEWED BY PEV | 9/28/92
SAMPLE MATRIX BEEF LIVER
COMPOUND(S) BF352-25
NOTEBOOK 94 PAGE 72 MLB# 11771180, 11701157

STORAGE	SAMPLE PREP	WEIGHING	FORTIFY	EXTRACT	CLEANUP	PARTITION	LC		
PEV	MGC	MGC,3/5/92		DU					
3/5/92	3/5/92	BALANCE #	DU	9/23/92	DU	DU	SEK		
DU	DU	8148	9/23/92	MGC	9/23/92	9/23/92	9/24/92		
9/15/92	9/15/92	DU,9/23/92		3/5/92					
Freezer		BALANCE #					SERIAL #		
#6		20303446					2650A01211		

COMMENTS

SAMPLE DATA CARDC	BASF	STD #	SAMP WT. (g)	PPM	mg inj	ng FND	FORTIFICATION STD - DATA STD.VOL.	STD.CONC.	FINAL VOL (mL)	FND ppm	CORR ppm	PK HT PK AREA MAN(mm)	% REC
	BLANK											< 0	
	STD	6819						10 ng				1861	
1170-230	UTC		20		400				5	0.01495	0.01495	1293	
1170-231	UTC	7207	20	0.05	400	26.22016	1 mL of	1 µg/mL	5	0.06555	0.05060	5071	101.2
	STD	6816						150 ng				27932	
1170-232	UTC	7207	20	0.05	400	19.18043	1 mL of	1 µg/mL	5	0.04795	0.03300	3757	66.0
1170-233	UTC	6811	20	0.25	400	75.76610	0.5mL of	10 µg/mL	5	0.18942	0.17447	14319	69.8
	STD	6822						25 ng				4580	
1170-234	UTC	6811	20	0.25	400	80.88758	0.5mL of	10 µg/mL	5	0.20222	0.18727	15275	74.9
1170-235	UTC	6811	20	1.0	80	56.75242	2 mL of	10 µg/mL	25	0.70941	0.69446	10770	69.4
	STD	6814						100 ng				18967	
1170-236	UTC	6811	20	1.0	80	55.98630	2 mL of	10 µg/mL	25	0.69983	0.68488	10627	68.5
	STD	6815						50 ng				9681	
	BLANK											< 0	
	STD	6819						10 ng				2068	
	STD	6815						50 ng				9826	
	STD	6816						150 ng				28227	
												Average	75.0
												Std Dev	13.2

CORR: 0.9998 INTCPT: 176.8751154 SLOPE: 186.6550442

Protocol No.: 92159

BENCH SHEET

PROJECT BASF-1170 | SET # LIVER - 4 | INITIATED BY PEV | DATE 9/28/92
METHOD NO BASF A9207 | METHOD VALIDATION | REVIEWED BY SEK | 9/28/92
SAMPLE MATRIX BEEF LIVER
COMPOUND(S) BF352-25
NOTEBOOK 94 PAGE 74 MLB# 11701158

STORAGE	SAMPLE PREP	WEIGHING	FORTIFY	EXTRACT	CLEANUP	PARTITION	LC		
DU 9/24/92	DU 9/24/92	DU 9/25/92	DU 9/25/92	DU 9/25/92	DU 9/25/92	DU 9/25/92	SEK 9/25,26/92		
Freezer #6		BALANCE # 20303446					SERIAL # 2650A01211		

COMMENTS

SAMPLE DATA CARDC	BASF	STD #	SAMP WT. (g)	PPM	mg inj	ng FND	FORTIFICATION STD - DATA STD.VOL.	STD.CONC.	FINAL VOL (mL)	FND ppm	CORR ppm	PK HT PK AREA MAN(mm)	% REC
	BLANK											< 0	
	STD	6819						10 ng				2001	
1170-244	UTC		20		400				5	0	0	< 0	
1170-245	UTC	7207	20	0.05	400	12.15233	1 mL of	1 µg/mL	5	0.03038	0.03038	2370	60.8
	STD	6816						150 ng				27519	
1170-246	UTC	7207	20	0.05	400	13.32895	1 mL of	1 µg/mL	5	0.03332	0.03332	2581	66.6
1170-247	UTC	6811	20	0.25	400	66.43815	0.5mL of	10 µg/mL	5	0.16610	0.16610	12105	66.4
	STD	6822						25 ng				4277	
1170-248	UTC	6811	20	0.25	400	64.99945	0.5mL of	10 µg/mL	5	0.16250	0.16250	11847	65.0
1170-249	UTC	6811	20	1.0	80	48.06964	2 mL of	10 µg/mL	25	0.60087	0.60087	8811	60.1
	STD	6814						100 ng				18504	
1170-250	UTC	6811	20	1.0	80	49.63659	2 mL of	10 µg/mL	25	0.62046	0.62046	9092	62.0
	STD	6815						50 ng				9268	
	BLANK											< 0	
	STD	6819						10 ng				1893	
	STD	6815						50 ng				9456	
	STD	6816						150 ng				26342	
												Average	63.5
												Std Dev	2.9

CORR: 0.9992 INTCPT: 190.7388896 SLOPE: 179.3286035

Protocol No.: 92159

BENCH SHEET

PROJECT BASF-1170
METHOD NO BASF A9207
SAMPLE MATRIX BEEF FAT
COMPOUND(S) BF352-25
NOTEBOOK 94 PAGE 71

SET # FAT - 1
METHOD VALIDATION

INITIATED BY PEV DATE 9/23/92
REVIEWED BY SEK 9/23/92

MLB# 11701157

STORAGE	SAMPLE PREP	WEIGHING	FORTIFY	EXTRACT	CLEANUP	PARTITION	LC		
DU 9/15/92	DU 9/15/92	DU 9/22/92	DU 9/22/92	DU 9/22/92	DU 9/22/92	DU 9/22/92	SEK 9/22,23/92		
Freezer #6		BALANCE # 20303446					SERIAL # 2422G00611		

COMMENTS

SAMPLE DATA CARDC	BASF	STD #	SAMP WT. (g)	PPM	mg inj	ng FND	FORTIFICATION STD - DATA STD.VOL.	STD.CONC.	FINAL VOL (mL)	FND ppm	CORR ppm	PK HT PK AREA MAN(mm)	% REC
	BLANK											< 0	
	STD	6819						10 ng				14.43	
1170-223	UTC		20		400				5	0	0	< 0	
1170-224	UTC	7207	20	0.05	400	17.24370	1 mL of	1 µg/mL	5	0.04311	0.04311	27.67	86.2
	STD	6816						150 ng				240.54	
1170-225	UTC	7207	20	0.05	400	13.70833	1 mL of	1 µg/mL	5	0.03427	0.03427	22.11	68.5
1170-226	UTC	6811	20	0.25	400	79.78027	0.5mL of	10 µg/mL	5	0.19945	0.19945	126.02	79.8
	STD	6822						25 ng				39.15	
1170-227	UTC	6811	20	0.25	400	54.85466	0.5mL of	10 µg/mL	5	0.13714	0.13714	86.82	54.9
1170-228	UTC	6811	20	1.0	80	62.92369	2 mL of	10 µg/mL	25	0.78655	0.78655	99.51	78.7
	STD	6814						100 ng				158.58	
1170-229	UTC	6811	20	1.0	80	62.66935	2 mL of	10 µg/mL	25	0.78337	0.78337	99.11	78.3
	STD	6815						50 ng				79.87	
	BLANK											< 0	
	STD	6819						10 ng				17.51	
	STD	6815						50 ng				79.88	
	STD	6816						150 ng				231.56	
												Average	74.4
												Std Dev	11.1

CORR: 0.9996 INTCPT: 0.5511790 SLOPE: 1.5726799

Protocol No.: 92159

BENCH SHEET

PROJECT BASF-1170
METHOD NO BASF A9207
SAMPLE MATRIX BEEF FAT
COMPOUND(S) BF352-25

SET # FAT - 2
METHOD VALIDATION

INITIATED BY SEK DATE 9/25/92
REVIEWED BY PEV 9/28/92

NOTEBOOK 94 PAGE 73 MLB# 11701157

STORAGE	SAMPLE PREP	WEIGHING	FORTIFY	EXTRACT	CLEANUP	PARTITION	LC		
DU 9/15/92	DU 9/15/92	DU 9/24/92	DU 9/24/92	DU 9/24/92	DU 9/24/92	DU 9/24/92	SEK 9/24,25/92		
Freezer #6		BALANCE # 20303446					SERIAL # 2650A01211		

COMMENTS

SAMPLE DATA CARDC	BASF	STD #	SAMP WT. (g)	PPM	mg inj	ng FND	FORTIFICATION STD - DATA STD.VOL.	STD.CONC.	FINAL VOL (mL)	FND ppm	CORR ppm	PK HT / PK AREA MAN(mm)	% REC
	BLANK											< 0	
	STD	6819						10 ng				2133	
1170-237	UTC		20		400				5	0	0	< 0	
1170-238	UTC	7207	20	0.05	400	15.14880	1 mL of	1 µg/mL	5	0.03787	0.03787	3094	75.7
	STD	6816						150 ng				29044	
1170-239	UTC	7207	20	0.05	400	15.64205	1 mL of	1 µg/mL	5	0.03911	0.03911	3188	78.2
1170-240	UTC	6811	20	0.25	400	75.32497	0.5mL of	10 µg/mL	5	0.18831	0.18831	14562	75.3
	STD	6822						25 ng				4899	
1170-241	UTC	6811	20	0.25	400	81.43284	0.5mL of	10 µg/mL	5	0.20358	0.20358	15726	81.4
1170-242	UTC	6811	20	1.0	80	61.51404	2 mL of	10 µg/mL	25	0.76893	0.76893	11930	76.9
	STD	6814						100 ng				19263	
1170-243	UTC	6811	20	1.0	80	61.90759	2 mL of	10 µg/mL	25	0.77384	0.77384	12005	77.4
	STD	6815						50 ng				9851	
	BLANK											< 0	
	STD	6819						10 ng				2068	
	STD	6815						50 ng				9746	
	STD	6816						150 ng				28515	
												Average	77.5
												Std Dev	2.2

CORR: 0.9999 INTCPT: 207.0360016 SLOPE: 190.5737835

Protocol No.: A9207

BENCH SHEET

PROJECT BASF-1170
METHOD NO BASF A9207
SAMPLE MATRIX BEEF KIDNEY
COMPOUND(S) BF352-25

SET # MV-6 KIDNEY
METHOD VALIDATION

INITIATED BY SEK DATE 11/10/92
REVIEWED BY PEV 11/10/92

NOTEBOOK 94 PAGE 82

MLB# 11701175

STORAGE	SAMPLE PREP	WEIGHING	FORTIFY	EXTRACT	CLEANUP	PARTITION	LC		
DU 10/26/92	DU 10/27/92	DU 11/9/92	DU 11/9/92	DU 11/9/92	DU 11/9/92	DU 11/9/92	SEK 11/9,10/92		
Freezer #6		BALANCE # 20303446					SERIAL # 2650A01211		

COMMENTS

SAMPLE DATA CARDC	BASF	STD #	SAMP WT. (g)	PPM	mg inj	ng FND	FORTIFICATION STD - DATA STD.VOL.	STD.CONC.	FINAL VOL (mL)	FND ppm	CORR ppm	PK HT PK AREA MAN(mm)	% REC
	BLANK											< 0	
	STD	7421						10 ng				1636	
1170-300	UTC		20		400				5	0	0	< 0	
1170-301	UTC	7207	20	0.05	400	14.73947	1 mL of	1 µg/mL	5	0.03685	0.03685	2584	73.7
	STD	7360						150 ng				27898	
1170-302	UTC	7207	20	0.05	400	15.52991	1 mL of	1 µg/mL	5	0.03882	0.03882	2734	77.6
1170-303	UTC	6811	20	0.25	400	62.58205	0.5mL of	10 µg/mL	5	0.15646	0.15646	11663	62.6
	STD	6822						25 ng				4138	
1170-304	UTC	6811	20	0.25	400	75.81926	0.5mL of	10 µg/mL	5	0.18955	0.18955	14175	75.8
1170-305	UTC	7445	20	1.0	80	37.29331	1 mL of	20 µg/mL	25	0.46617	0.46617	6864	46.6
	STD	6814						100 ng				18796	
1170-306	UTC	7445	20	1.0	80	42.74733	1 mL of	20 µg/mL	25	0.53434	0.53434	7899	53.4
	STD	7446						50 ng				9109	
	BLANK											< 0	
	STD	7421						10 ng				1871	
	STD	7446						50 ng				9724	
	STD	7360						150 ng				28547	
												Average	65.0
												Std Dev	12.9

CORR: 0.9996 INTCPT: -213.0830806 SLOPE: 189.7681920

92/5181 074

Protocol No.: 92159

BENCH SHEET

PROJECT BASF-1170 SET # MV-7 BEEF KIDNEY INITIATED BY SEK DATE 11/19/92

METHOD NO BASF A9207 METHOD VALIDATION REVIEWED BY PEV 11/18/92

SAMPLE MATRIX BEEF KIDNEY

COMPOUND(S) BF352-25

NOTEBOOK 94 PAGE 84 MLB# 11701175

STORAGE	SAMPLE PREP	WEIGHING	FORTIFY	EXTRACT	CLEANUP	PARTITION	LC		
DU 10/26/92	DU 10/27/92	DU 11/17/92	DU 11/17/92	DU 11/17/92	DU 11/17/92	DU 11/17/92	SEK 11/18,19/92		
Freezer #6		BALANCE # 20303446					SERIAL # 2650A01211		

COMMENTS

SAMPLE DATA CARDC	BASF	STD #	SAMP WT. (g)	PPM	mg inj	ng FND	FORTIFICATION STD - DATA STD.VOL.	STD.CONC.	FINAL VOL (mL)	FND ppm	CORR ppm	PK HT PK AREA MAN(mm)	% REC
	BLANK											< 0	
	STD	7478						10 ng				1422	
1170-307	UTC		20		400				5	0	0	< 0	
1170-308	UTC	7473	20	0.05	400	12.36733	1 mL of	1 µg/mL	5	0.03092	0.03092	1710	61.8
	STD	7476						150 ng				19102	
1170-309	UTC	7473	20	0.05	400	21.56534	1 mL of	1 µg/mL	5	0.05391	0.05391	2917	107.8
1170-310	UTC	7474	20	0.25	400	24.33003	0.5mL of	10 µg/mL	5	0.18583	0.18583	9841	74.3
	STD	7479						25 ng				3208	
1170-311	UTC	7474	20	0.25	400	75.48074	0.5mL of	10 µg/mL	5	0.18870	0.18870	9992	75.5
1170-312	UTC	7474	20	1.0	80	50.08144	2 mL of	10 µg/mL	25	0.62602	0.62602	6659	62.6
	STD	7475						100 ng				12742	
1170-313	UTC	7474	20	1.0	80	43.78687	2 mL of	10 µg/mL	25	0.54734	0.54734	5833	54.7
	STD	7477						50 ng				6710	
	BLANK											< 0	
	STD	7478						10 ng				1421	
	STD	7477						50 ng				6958	
	STD	7476						150 ng				20651	
												Average	72.8
												Std Dev	18.9

CORR: 0.9981 INTCPT: 87.1077410 SLOPE: 131.2241066

Protocol No.: 92159

BENCH SHEET

PROJECT BASF-1170
METHOD NO BASF A9207
SAMPLE MATRIX CHICKEN EGGS
COMPOUND(S) BF352-25
NOTEBOOK 94 PAGE 75

SET # EGGS - 1
METHOD VALIDATION

INITIATED BY PEV DATE 10/1/92
REVIEWED BY SEK 10/8/92

MLB# 11701157

STORAGE	SAMPLE PREP	WEIGHING	FORTIFY	EXTRACT	CLEANUP	PARTITION	LC		
DU 9/15/92	DU 9/15/92	DU 9/29/92	DU 9/29/92	DU 9/29/92	DU 9/29/92	DU 9/29/92	SEK 9/30/92		
Freezer #6		BALANCE # 20303446					SERIAL # 2650A01211		

COMMENTS

SAMPLE DATA CARDC	BASF	STD #	SAMP WT. (g)	PPM	mg inj	ng FND	FORTIFICATION STD - DATA STD.VOL.	STD.CONC.	FINAL VOL (mL)	FND ppm	CORR ppm	PK HT PK AREA MAN(mm)	% REC
	BLANK											< 0	
	STD	6819						10 ng				2700	
1170-251	UTC		20		400				5	0	0	< 0	
1170-252	UTC	7207	20	0.05	400	13.29515	1 mL of	1 µg/mL	5	0.03324	0.03324	2831	66.5
	STD	6816						150 ng				31527	
1170-253	UTC	7207	20	0.05	400	13.88463	1 mL of	1 µg/mL	5	0.03471	0.03471	2953	69.4
1170-254	UTC	6811	20	0.25	400	61.90287	0.5mL of	10 µg/mL	5	0.15476	0.15476	12891	61.9
	STD	6822						25 ng				5135	
1170-255	UTC	6811	20	0.25	400	59.68509	0.5mL of	10 µg/mL	5	0.14921	0.14921	12432	59.7
1170-256	UTC	6811	20	1.0	80	54.42328	2 mL of	10 µg/mL	25	0.68029	0.68029	11343	68.0
	STD	6814						100 ng				20421	
1170-257	UTC	6811	20	1.0	80	53.09454	2 mL of	10 µg/mL	25	0.66368	0.66368	11068	66.4
	STD	6815						50 ng				9754	
	BLANK	--	--									< 0	
	STD	6819						10 ng				2247	
	STD	6815						50 ng				10518	
	STD	6816						150 ng				31128	
												Average	65.3
												Std Dev	3.7

CORR: 0.9995 INTCPT: 79.3958855 SLOPE: 206.9629962

Protocol No.: 92159

BENCH SHEET

PROJECT BASF-1170
METHOD NO BASF A9207
SAMPLE MATRIX CHICKEN EGGS
COMPOUND(S) BF352-25

SET # EGGS - 2
METHOD VALIDATION

INITIATED BY PEV DATE 10/1/92
REVIEWED BY SEK 10/8/92

NOTEBOOK 94 PAGE 76 MLB# 11701157

STORAGE	SAMPLE PREP	WEIGHING	FORTIFY	EXTRACT	CLEANUP	PARTITION	LC		
DU 9/15/92	DU 9/15/92	DU 9/30/92	DU 9/30/92	DU 9/30/92	DU 9/30/92	DU 9/30/92	SEK 9/30/92 10/1/92		
Freezer #6		BALANCE # 20303446					SERIAL # 2650A01211		

COMMENTS

SAMPLE DATA CARDC	BASF	STD #	SAMP WT. (g)	PPM	mg inj	ng FND	FORTIFICATION STD - DATA STD.VOL.	STD.CONC.	FINAL VOL (mL)	FND ppm	CORR ppm	PK HT PK AREA MAN(mm)	% REC
	BLANK											< 0	
	STD	6819						10 ng				1999	
1170-258	UTC		20		400				5	0	0	< 0	
1170-259	UTC	7207	20	0.05	400	10.19099	1 mL of	1 µg/mL	5	0.02548	0.02548	2331	51.0
	STD	6816						150 ng				29333	
1170-260	UTC	7207	20	0.05	400	11.39763	1 mL of	1 µg/mL	5	0.02849	0.02849	2566	57.0
1170-261	UTC	6811	20	0.25	400	64.08405	0.5mL of	10 µg/mL	5	0.16021	0.16021	12827	64.1
	STD	6822						25 ng				5038	
1170-262	UTC	6811	20	0.25	400	72.54078	0.5mL of	10 µg/mL	5	0.18135	0.18135	14474	72.5
1170-263	UTC	6811	20	1.0	80	56.01755	2 mL of	10 µg/mL	25	0.70022	0.70022	11256	70.0
	STD	6814						100 ng				20089	
1170-264	UTC	6811	20	1.0	80	55.30897	2 mL of	10 µg/mL	25	0.69136	0.69136	11118	69.1
	STD	6815						50 ng				10313	
	BLANK											< 0	
	STD	6819						10 ng				2350	
	STD	6815						50 ng				10277	
	STD	6816						150 ng				29513	
												Average	64.0
												Std Dev	8.4

CORR: 0.9998 INTCPT: 346.2434393 SLOPE: 194.7560596

92/5181 077