BASF CORPORATION CHEMICALS DIVISION
Agricultural Chemicals
100 Cherry Hill Road
Parsippany, New Jersey 07054

410635-36

Study Title

GLC Method for residue determinations of Quinclorac in cow and chicken matrices, Method NO. 268 and method amendment no. 268/1

Data Requirements

EPA Guideline Number: 171-4

Author

Dr. Frank Mayer

Study Completed On

April 8, 1988

Performing Laboratory

BASF Aktiengesellschaft
Agricultural Research and Development
Environmental Research, LI 445

Registration Document No. BASF:

88/0542

This report consists of 80 pages.

Method 268 comprises pages 88/0542 0001 to 0056
Method amendment 268/1 comprises page 88/0542 0056 to 0080

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 Group

Company Agent: Jack R. Graham 2/28/89
Jack R. Graham Date
Manager, Registration

GOOD LABORATORY PRACTICES STATEMENT

This study meets the requirements for 40 CFR 160, Good Laboratory Practices.

Company: BASF CORPORATION
Agricultural Chemicals Group

Submitter and Sponsor: Jack R. Graham 2/28/88
Jack R. Graham Date
Manager, Registration

Study Director: See page 88/0542 0005 and 88/0542 0057 of amendment

Study Title

GLC Method for Residue Determinations of Quinclorac in Cow and Chicken Matrices

Author

Dr. Frank Mayer

Study Completed On

April 8, 1988

Performing Laboratory

BASF Aktiengesellschaft
Agricultural Research and Development
Environmental Research Li 445
Residue Analysis

Laboratory Study Code

Methode 268

BASF Report No:

Method No. 268

GOOD LABORATOPY PRACTICE

S T A T E M E N T O F C O M P L I A N C E

Study number: Methode 268

Test substance: Quinclorac

Study director: Dr. Frank Mayer

Title: GLC Method for Residue Determinations of Quinclorac in Cow and Chicken Matrices

To the best of my knowledge and belief, this study was conducted in compliance with Good Laboratory Practice Regulations.

Study Director ..... Mayer

Date: 25 May 1988

..... Sponsor

..... 21.02.89 Date

..... Submitter

..... Date

Method No. 268

Quality Assurance Statements

We hereby attest to the authenticity of the study and guarantee that the data are correct and accurate to the best of our knowledge and that the study was performed by the procedures described.

Study Director:	Dr. Frank Mayer APE/CU BASF Landwirtschaftliche Versuchsstation Postfach 2 20, D-6703 Limburgerhof (FRG) Tel.: 0 62 36 / 68 - 24 32
Laboratory Analysts:	Karla Schadt (method development, adaption cow matrices) APE/CU Address see above Tel.: 0 62 36 / 68 - 24 41 Wolfgang Laik (adaption chicken tissues) Silvia Holz (adaption eggs) APE/CU Address see above Tel.: 0 62 36 / 68 - 22 85
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BASF Aktiengesellschaft

BASF Landw. Versuchsstation Postfach 220 D-6703 Limburgerhof

STATEMENT

of the quality assurance unit

Study Code : METHODE 268

Test Article : QUINCLORAC

Titel : GLC Method for Residue Determinations of Quinclorac in Cow and Chicken Matrices

The quality assurance unit of the testing facility audited the final report and reported findings to the study director and to management.

Date of inspection	Report to study director and to management
06-Aug-87	-
14-Jun-88	14-Jun-88
21-Jun-88	-

D- 6703 Limburgerhof

21-Jun-88

Signature QAU

Table of Contents

1 INTRODUCTION AND SUMMARY

1.1 Scope and Source of the Method

1.1.1 Scope

Metabolism investigations (see ref. 1.2) so far have shown that quinclorac residues in animal matrices consist almost exclusively of the parent compound. This method was therefore based on the determination of the active ingredient.

1.1.2 Source

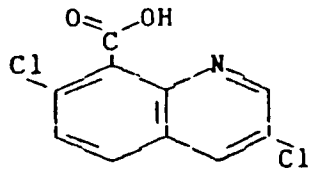
The method was developed by the author.

1.2 Substance

Common name: Quinclorac
Laboratory number: 150 732
BAS-number: BAS 514 ..H (.. = 00-99: number of formulation)

Chemical name:
3,7-Dichloro-8-quinolinecarboxylic acid

Structural formula:



Empirical formula: $C_{10}H_5Cl_2NO_2$
Molecular weight: 242.1
Melting point: Above 237° C decomposition
Appearance: Crystalline, colourless
Odour: Weak

Solubility: (g substance in 100 g solvent at 20° C)

Water	$6.2 \cdot 10^{-3}$
Ethanol	0.2
Acetonitrile	< 0.1
Acetone	0.2
Ethylacetate	0.1
Dichloromethane	< 0.1
Diethylether	0.1
Toluene	< 0.1
n-Hexane	< 0.1
Olive oil	< 0.1

1.3 Principle of Method

The sample is extracted with acetone/sodium hydroxide solution first, then with acetone/sulfuric acid. The extract is cleaned up by an Extrelut column. Interferences are removed by $NaHCO_3$/ethyl acetate partition at pH = 8. The sample is acidified and the active ingredient is partitioned into ethyl acetate. After C_{18} modified silica column clean-up, Quinclorac is methylated with diazomethane and determined by capillary gas liquid chromatography with electron capture detection (ECD).

Limits of determination and sensitivity: 0.05 mg/kg.

2 MATERIALS AND METHODS

Equipment and reagents in the following lists are examples and may be replaced by equivalent ones.

2.1 Equipment

Wide neck bottles	500 ml
Volumetric cylinders	250 ml, 100 ml, 50 ml, 25 ml
Funnels	10 cm, 7 cm i. d.
Round bottom flasks, standard ground joint 29	250 ml, 100 ml
Volumetric flasks	500 ml
Separatory funnels	100 ml
Centrifuge	Varifuge st., Heraeus - Christ, Osterode, FRG
Centrifugation tubes	250 ml
Volumetric pipettes	50 ml, 25 ml, 10 ml, 3 ml, 2 ml
Ultra Turrax T 25	Janke + Kunkel GmbH u. Co. KG, 7813 Staufen im Breisgau, FRG
Rotary evaporator	"
Ultrasonic bath	Elma Transsonic T 460 Hans Schmidbauer KG, Singen /HTW, FRG
N-EVAP (nitrogen stream evaporator)	Labotec, Wiesbaden, FRG, or Organomation Associates Inc., P.O. Box 159, South Berlin MA 01549
Solid phase extraction system (SPE)	Visiprep Vacuum Manifold No. 5-7030, Supelco Inc., Bellefonte, PA 16823-0048
Gas liquid chromatograph with ^{63}Ni-ECD	Varian 3700

2.2 Reagents and Standards

Acetone dist.	
Water deionised	
Dichloromethane dist.	
Ethyl acetate dist.	
n-Hexane dist.	
Sodium hydroxide	No. 0288
analytical grade	Baker, Groß-Gerau, FRG
Sodium hydroxide solution	
0.1 N (0.1 mol/l) in water	
Extrelut 20 columns	No. 11737
Extrelut, refill package	No. 11738
	Merck, Darmstadt, FRG
Empty SPE columns, 3 ml	No. 7121-3
	Baker, Groß-Gerau, FRG
C_{18}-modified silica (40 µm)	No. 7025-0
gel column 3 ml	Baker, Groß-Gerau, FRG
Filter aid e.g.	Serva Feinbiochemica
Celite Type 545	Heidelberg / New York
Universal indicator	No. 9535
sticks pH 0 - 14	Merck, Darmstadt, FRG
Sodium hydrogen carbonate	No. 6323
powdered, analytical grade	Merck, Darmstadt, FRG
Sodium hydrogen carbonate	
solution, saturated	
Sodium sulphate anhydrous	No. 6645
fine powder extra pure	Merck, Darmstadt, FRG
Methanol dist.	
Sulfuric acid conc.	No. 731
analytical grade	Merck, Darmstadt, FRG
Sulfuric acid, 0.1 ml/l	
in water	
Formic acid conc., anal.	No. 264
grade	Merck, Darmstadt, FRD

N-Methyl-N-nitroso-p-toluenesulfonamide (Diactin)	No. 803406 Merck-Schuchardt 8011 Hohenbrunn. FRG
Diethylether. anal. grade	No. 32 203. Riedel-de-Haen 3016 Seelze 1. FRG
Potassium hydroxide pellets. anal. grade	No. 30 603. Riedel-de-Haen 3016 Seelze 1. FRG
Filter 0.45 µm 1.5 cm diam. Braunrand/Ha Spartan 13/20	No. 463 030 Schleicher + Schull. D-3354 Dassel. FRG

Preparation of diazomethane solution:

6 g N-methyl-N-nitroso-p-toluenesulfonamide (Diactin) are dissolved in 100 ml diethylether. A flask containing 3 g KOH dissolved in 5 ml water. diluted with 45 ml methanol is placed in a 60° C water bath. The solution in diethyl ether is added dropwise to the KOH solution within 30 minutes. Diazomethane is distilled along with ether by the means of a descendant Liebig condenser and collected in a flask containing 10 ml ether chilled in an ice bath. Addition and distillation of ether solutions should be adjusted in such a way that the volume of the reaction mixture stays constant in order to avoid concentration of diazomethane.
At the end 10 ml ether are added through the dropping funnel and distilled with the rest of diazomethane.
The solution is stable for 2 - 3 days if stored in the refrigerator in a bottle loosely closed by a plastic stopper.
When handling Diactin or diazomethane solution. latex or rubber gloves should be worn. All work should be carried out under a well operating hood.

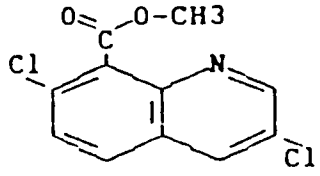
Purification of Extrelute:

Transfer the contents of 40 Extrelut refill packages into a 5 l beaker. Add 2.4 l methanol and 20 ml conc. formic acid. Allow to stand for 1 h. Stir occasionally.

Filter with suction. Wash with 200 ml dichloromethane and twice with 200 ml methanol. Continue with suction until the material is dry. Keep on drying in a drying cabinet at 80 °C until the weight remains constant.

Standard substances:

Quinclorac (str. formula see 1.2)	> 99.5 %
3.7-Dichloro-8-quinolinecarboxylic acid methylester	> 99.5 %



(both standards supplied by: Dr. Pawliczek, BASF Aktiengesellschaft, APE/CU Agricultural Research Center D-6703 Limburgerhof Tel. 06236/68-2422)

Standard solutions for fortifications:
Quinclorac 100: 1 µg/ml in acetone

Standard solutions for GLC analysis:
3.7-Dichloro-8-quinolinecarboxylic acid methylester
12.5: 25: 50: 100 ng/ml in acetone

Stability of standard solutions:

Storage Days	Room temperature Daylight	4° C Refrigerator
	Quinclorac 200 µg/ml in acetone	1)
71	100 %	100 %
	3,7-Dichloro-8-quinolinecarboxylic acid methylester 2) 0.2 µg/ml in acetone	
7	99.6 %	103.8 %
35	100.1 %	100.8 %
106	-	101.4 %
200	-	99.6 %

1) Raw data see logbook no. 00089, page 160, 161 (development method no. 222).
2) Raw data see logbook no. 00180, page 18 and corresponding chromatogram file.

2.3 Analytical Procedure

Note: The symbols in brackets are identical with the symbols used in the formula for the calculation of the analytical results (see 2.9.2).
The analytical procedure is described for one sample.

2.3.1 Preparation of Samples

Tissues are cut into pieces and minced. Milk and eggs are homogenized using an Ultra Turrax. The samples are stored at -20° C until analysis.

2.3.2 Extraction

Weigh 20 g (= G) sample material in a 500 ml wide neck bottle. Add 150 ml acetone and 100 ml 0.1 M sodium hydroxide solution. Macerate for 5 minutes using an Ultra Turrax. Add approximately 20 g of filter aid (e. g. celite) and macerate again for 5 seconds.
Add 3 ml conc. sulfuric acid (for better centrifugation). Transfer the content of the bottle into two centrifuge tubes using acetone. Centrifuge for 10 minutes with appr. 3000 rpm. Plug the outlet of a 10 cm funnel with some cotton wool and filter the supernatant into a 500 ml volumetric flask.
Add 50 ml acetone and 50 ml 0.1 M sulfuric acid to the sediment in the centrifugation tube. Macerate for 3 minutes. Centrifuge for 10 minutes with appr. 3000 rpm. Filter as above into the same flask and fill up to 500 ml with acetone.

2.3.3 Extrelut column

Transfer an aliquot of 50 ml (A = 10 %) from the extract in 2.3.2. into a 250 ml round bottom flask. Evaporate the acetone using a rotary evaporator with appr. 30° C water bath temperature until appr. 15 ml aqueous solution remain in the flask. Transfer this solution into a 25 ml volumetric cylinder. Rinse the flask with appr. 3 ml water.
Take a new Extrelut column or ~~fill an empty Extrelut~~ column with Extrelut material for the absorption of 20 ml water (equivalent to the content of one refill package. purified according to 2.2). Transfer the aqueous extract into the column. Rinse the volumetric cylinder with appr. 2 ml of water. Do not give more then 20 ml water in total onto the column.

Allow the water to penetrate the column for 30 minutes. Then elute with 55 ml ethyl acetate. Collect the eluate in a 100 ml round bottom flask.

2.3.4 Ethyl acetate / $NaHCO_3$ partition

Transfer the eluate from the extrelut column (see 2.3.3) into a 100 ml separatory funnel using a 7 cm i.d. funnel. Shake for appr. 1 minute first with 30 ml, then with 20 ml saturated sodium hydrogen carbonate solution. Collect both aqueous solutions in a 100 ml round bottom flask.

2.3.5 H_2SO_4 / Ethyl acetate partition

Acidify the alkaline aqueous phase of 2.3.4. carefully with concentrated sulfuric acid (CO_2-evolution!) to pH = 2 (check pH!). Approximately 1-2 ml sulfuric acid are needed. Transfer the acid solution into a 100 ml separatory funnel and shake with 20 ml ethyl acetate for appr. 1 minute. Release the aqueous phase into the same 100 ml round bottom flask mentioned in 2.3.4.. Filter the organic phase through a 7 cm i.d. funnel plugged with some cotton wool and filled with 3 - 5 g anhydrous sodium sulphate into a 100 ml round bottom flask. Repeat the shaking procedure with further 20 ml of ethyl acetate and filter as above.
Evaporate the combined organic phases to dryness using a rotary evaporator at 30° C water bath temperature.

2.3.6 C_{18} column clean-up

Note:
The properties of the C_{18} material for solid phase extraction may vary between manufactures as well as between different lots from the same manufacturer. Quantity and

composition of solvent mixtures for washing and eluting must therefore be checked and, if necessary, adjusted. If more than 25 % of added Quinclorac are irreversibly bound to the C_{18} material, this lot is not suitable for this method.

Fill 1 g C_{18} material into a 3 ml empty column. Insert the column into the solid phase extraction system. Wash the column with 10 ml methanol and 10 ml water by means of 10 kPa vacuum (difference to atmospheric pressure). Do not run the column dry between and after the individual washings.

Dissolve the residue in the flask from 2.3.5 in 0.3 ml methanol and 25 ml water of pH = 2 (H_2SO_4). Allow the solution to percolate slowly through the column (within 20 minutes, using slight (5 - 10 kPa) or no vacuum). Suck air through the column with full vacuum (appr. 40 kPa) for 2 minutes.

Wash the column with 3 ml acetone + n-hexane = 3 + 97. Insert a 3 ml column with 0.5 g anhydrous Na_2SO_4 between the C_{18} column and the extraction system.

Elute Quinclorac with 12 ml acetone + n-hexan = 25 + 75. Collect the eluate in a 20 ml flask (sketch see att. 13). Evaporate to dryness in a stream of nitrogen using an N-EVAP with a water bath temperature of 30°C.

2.3.7 Methylation

(All operations with diazomethane under the hood!)
Dissolve the residue in the 20 ml flask from 2.3.6 in 0.5 ml acetone. Dip the flask into an ultrasonic bath for

about 5 seconds. Add 2 ml of diazomethane solution (preparation see 2.2). Allow to stand for 10 minutes. If the solution becomes colourless during this period, add another 2 ml of diazomethane solution and allow to stand for another 10 minutes.

Evaporate to dryness using the N-EVAP with a water bath temperature of 20° C. Dissolve the residue in 2 ml (= V_E for the determination limit) acetone. Inject 1 µl (= V_I) of this solution into the gas liquid chromatograph. Dilute the final volume of the determination limit if necessary.

2.4 Instrumentation

Equipment and operating conditions in the following lists are examples and may be changed if necessary.

2.4.1 Description

Gas liquid chromatograph with ^{63}Ni-ECD:	e. g. 1)	Perkin Elmer F 22
	2)	Varian 3 700
	3)	Perkin Elmer 3 920

Capillary column:	WCOT glass
Length:	25 m
Internal diameter:	0.28 mm
Stationary phase:	SE 54

2.4.2 Operating conditions

Injection volume :	1 µl
Recorder chart speed:	0.5 cm/min
Carrier gas:	He: 1000 mbar

	1)	2)	3)
Injection port temp.:	270° C	280° C	270° C
Detector temp.:	350° C	300° C	270° C
Septum purge:	———	5 ml/min	———
Split:	15 ml/min	20 ml/min	10 ml/min
Make-up gas:	Ar/CH_4: 30 ml/min	N_2: 30 ml/min	Ar/CH_4: 30 ml/min
Retention time (appr.):	23 min	13 min	33 min

1), 2) and 3) refer to the instrument (see 2.4.1).

2.4.3 Calibration procedures

Calculation of results is based on peak height measurements using a calibration curve. To obtain this standard curve inject e. g. 12.5, 25, 50, 100 pg quinclorac methyl ester into the gas chromatograph. For routine analysis at least every third injection should be a standard. Plot peak height (mm) versus amount (ng) of injected standard.

2.5 Interferences

2.5.1 Sample matrices

If interfering peaks occur in the chromatogram, change oven temperature or determine the residue by means of HPLC or GC-MS (see 2.6).

2.5.2 Other pesticides

Not known to date.

2.5.4 Labware

Not known to date.

B90-9

Study Title

GLC Method for Residue Determinations of Quinclorac in Animal Matrices

Data Requirement

EPA Guideline 171 - 4

Author

Dr. Frank Mayer

Study Completed On

February 16th, 1989

Performing Laboratory

BASF AG
Agricultural Research and Development
Environmental Research
Residue Analysis

Laboratory Study Code

Methode 268

BASF Report No.

Method Amendment No. 268/1

GOOD LABORATORY PRACTICE

S T A T E M E N T O F C O M P L I A N C E

Study number: Methode 268

Test substance: Quinclorac

Study Director: Dr. Ulrich Schepers

Title: GLC Method for Residue Determinations of Quinclorac in Animal Matrices

To the best of my knowledge and belief, this study was conducted in compliance with Good Laboratory Practice Regulations.

Study Director: U. Schepers

Date: 20. 2. 89

QUALITY ASSURANCE STATEMENTS

We hereby attest to the authenticity of the study and guarantee that the data are correct and accurate to the best of our knowledge and that the study was performed by the procedures described.

Study Director: Dr. Ulrich Schepers U. Schepers 20.2.89
APE/CU
BASF Landwirtschaftliche Versuchsstation
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Author of Report: Dr. Frank Mayer Mayer 16.2.89
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Quality Assurance Officer: Dr. H. Morgenthaler Morgenthaler 20.2.89
APE/CQ
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Phone: 0 62 36 / 68 - 2 522

Statistician: Not involved

Report approved by: Dr. S. Otto SOtto 20.2.89
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BASF Aktiengesellschaft

BASF Landw. Versuchsstation Postfach 220 D-6703 Limburgerhof

STATEMENT

of the quality assurance unit

Study Code : METHODE 268

Test Article : QUINCLORAC

Title : GLC Method for Residue Determinations of Quinclorac in Animal Matrices

The quality assurance unit of the testing facility audited the final report and reported findings to the study director and to management.

Date of inspection	Report to study director and to management
20-Feb-89	20-Feb-89

D- 6703 Limburgerhof

20-Feb-89

Signature QAU

Table of Contents

1. INTRODUCTION AND SUMMARY

1.1 Scope and Source of the Method Amendment

1.1.1 Scope

In Method No. 268, a C_{18} solid phase extraction column is used as a clean-up step. However, method accountability studies (ref. 1,2) revealed a loss of approximately 20 % recovery on that column, probably due to irreversible binding of the active ingredient to unalkylated residual silanol groups of the column material. At this time, a better quality of C_{18} material either from a different lot or from a different manufacturer has not been found on the market.

Therefore, an alternative clean-up using an amino solid phase extraction column was developed. Because of the ion exchange separation mechanism on that column, a buffer solution is required for the elution of Quinclorac, and this is followed by an additional buffer/dichloromethane partition.

A validation of the new procedure in goat liver, goat muscle and cow milk is reported in this study. Also, some experience regarding chemicals used in the method is mentioned. Further fortification trials were carried out along with cold animal feeding studies (ref. 3, 4).

An accountability study using the new procedure showed that the average recovery loss on the amino column is only 5 - 10 %, including the subsequent partition (ref. 5).

1.1.2 Source

The method modification was developed by the study director.

1.2 Substance

See Method No. 268

1.3 Principle of the Method

The sample is initially extracted with acetone/sodium hydroxide solution, then with acetone/sulfuric acid. The extract is cleaned up by an Extrelut column. Interferences are removed by $NaHCO_3$/ethyl acetate partition at pH = 8. The sample is acidified and the active ingredient is partitioned into dichloromethane. After an amino solid phase extraction column clean-up and subsequent extraction into dichloromethane, Quinclorac is methylated with diazomethane and determined by capillary gas liquid chromatography with electron capture detection (ECD).

Limit of quantitation: 0.05 mg/kg

2. MATERIALS AND METHODS

Equipment and reagents in the following lists are examples and may be replaced by equivalent ones.

2.1 Equipment

See Method No. 268.

Additional equipment:

Round bottom flasks	50 mL
Separatory funnels	50 mL

2.2 Reagents and Standards

See Method No. 268.

Additional reagents:

Amino SPE columns, 3 mL	No. 7088-3 Baker, D-6080 Groß-Gerau, FRG
Citric acid monohydrate	No. 244 Merck, D-6100 Darmstadt, FRG
Hydrochloric acid 12 N	No. 30721 Riedel-de Haen, D-3016 Seelze 1, FRG

Solvent mixtures:

0.025 % (w/v) Hydrochloric acid in methanol:
0.68 g of concentrated hydrochloric acid in 1 L of methanol

Citrate buffer pH = 1.5:

Dissolve 21 g of citric acid monohydrate in 500 mL of water. Add 200 mL of 1 M NaOH solution. Add 1 M hydrochloric acid so that the pH is adjusted to pH = 1.5 (approximately 295 mL). Add water to 1 L and check pH once again.

Notes:

1. Quinclorac is an acid and is therefore adsorbed by alkaline reagents. Thus, the use of glass wool for filtration is not recommended, since it may contain alkaline sites which would adsorb the Quinclorac.

2. Check the pH-value of a 5 % solution of the sodium sulfate. If it is higher than the pH-value of the water, this lot of sodium sulfate must not be used without the following acidification procedure:

3. Add 0.1 mL of concentrated sulfuric acid to 100 g of sodium sulfate, slurried with enough ether to cover the solid. Remove the ether on a steam bath. Air dry. This ensures that the sodium sulfate is acidic.

2.3 Analytical Procedure

See Method No. 268

2.3.1 Preparation of Samples

See Method No. 268

2.3.2 Extraction

See Method No. 268

2.3.3 Extrelut Column

See Method No. 268

Notes: Since the clean-up capability of the amino column is better compared to the C_{18} column, the following partition steps 2.3.4 and 2.3.5 may be omitted in most cases, depending on the actual matrix. However, if the chromatogram indicates interferences, GLC column overload or low recovery due to amino column overload, analyze another extract aliquot of this sample and include the partition steps.

If the partition steps 2.3.4 and 2.3.5 are omitted, filter the ethyl-acetate eluate from the Extrelut column through a 7 cm i. d. funnel plugged with some cotton wool and filled with 3 - 5 g of anhydrous sodium sulfate. Rinse the sodium sulfate with approximately 5 mL of the organic solvent. This drying step removes from the organic extract residual sulfuric acid that might neutralize the amino groups of the amino column and prevent them from adsorbing Quinclorac.

2.3.4 Ethyl Acetate / $NaHCO_3$ Partition

See Method No. 268

2.3.5 H_2SO_4 / Dichloromethane Partition

Carefully acidify the alkaline aqueous phase from 2.3.4 with concentrated sulfuric acid (CO_2-evolution!) to pH = 2. Add the acid dropwise while gently swirling the flask. Approximately 2.6 mL of sulfuric acid are needed. Transfer the contents of the flask into a 100 mL separatory funnel. Rinse the flask with 2 mL of water.

Before adding any dichloromethane, shake the funnel carefully several times. Consider the pressure in the funnel caused by CO_2 evolution. After neutralisation (check pH), shake twice with 20 mL of dichloromethane for approximately one minute.

Seal the outlet of a 5.5 cm glass funnel with a plug of cotton wool. Add approximately 1 g of anhydrous sodium sulphate into the funnel. Filter the organic phases through the funnel and combine them in a 100 mL round bottom flask. Wash the sodium sulphate with approximately 2 mL of dichloromethane. Do not evaporate the dichloromethane.

2.3.6 Amino Column Clean-up (replaces C_{18} column clean-up)

Note: The properties of the amino material for solid phase extraction may vary between different manufacturers as well as between different lots from the same manufacturer. Quantity and composition of solvent mixtures for washing and eluting must be checked and if necessary adjusted, for each lot used.

Insert the 3 mL amino column (0.5 g adsorbent) into a Luer fitting on the cover of the extraction system. Apply a moderate vacuum of approximately 5 - 10 kPa (= 1 in Hg difference to atmospheric pressure) for all washings and the elution procedure. Always suck the solution into the column until the level of the liquid has reached the surface of the adsorbent. Do not let the column run dry.

Pre-wash the column with dichloromethane, if the partition steps 2.3.4 and 2.3.5 were done, or with ethyl-acetate, if the Extrelut eluate from 2.3.3 is used directly. Wash the column with 3 mL of the appropriate solvent. Add another 2 mL of the solvent into the column.

Connect a 75 mL reservoir to the column by means of an adapter. Pour the dichloromethane solution from 2.3.5 or the ethylacetate solution from 2.3.3 into the reservoir. Allow the solution to percolate through the column. Apply a moderate vacuum (5 kPa) if necessary. Percolation should take at least 20 minutes. Rinse the flask and the reservoir with 2 mL of the appropriate solvent and suck it through the column.

Wash the column with 5 mL (2 mL directly onto the column, 3 mL through the reservoir) of 0.025 % (w/v) hydrochloric acid in methanol (see 2.2).

Elute the Quinclorac with 15 ml (2 mL directly onto the column, 13 mL through the reservoir) of citrate buffer solution of pH = 1.5 (see 2.2). Collect the eluate using the stainless steel rack in an appropriate container (e.g. 15 mL elongated flask, 15 mL centrifuge tube, 25 mL volumetric flask, scintillation vial).

2.3.7 Citrate buffer / Dichloromethane Partition

Transfer the eluate from 2.3.6 into a 50 mL separatory funnel. Rinse twice with approximately 1 mL of water. Extract the compound from the buffer solution by partitioning with 20 mL of dichloromethane. Repeat the extraction. Filter the organic phases through a 5.5 cm funnel sealed with a plug of cotton wool and containing approximately 1 g of anhydrous sodium sulfate into a 50 mL round bottom flask. Rinse the sodium sulfate with approximately 2 mL of dichloromethane.

Concentrate the dichlormethane extract to approximately 2 - 3 mL using a rotary evaporator with a 20°C water bath temperature. Do not evaporate to dryness. Dichloromethane may contain impurities that attack the Quinclorac molecule if they are concentrated down along with it. Use this dichloromethane extract for the methylation in step 2.3.8.

2.3.8 Methylation

See under 2.3.7 in Method No. 268.

2.4 Instrumentation

See Method No. 268.

Alternative conditions:

2.4.1 Description

Gas-liquid chromatograph with ^{63}Ni-ECD	Varian 6000
Column:	Fused silica megabore
Length:	30 m
Internal diameter:	0.53 mm
Stationary phase:	DB 5
Film thickness:	1.5 µm

2.4.2 Operating Conditions

Injection volume:	1 µL
Carrier gas:	N_2; 9 mL/min
Make-up gas:	N_2; 20 mL/min
Injection port temperature:	230°C
Oven temperature:	205°C
Detector temperature:	300°C
Retention time (appr.):	7.8 min

2.4.3 Calibration Procedures

See Method No. 268.

2.5 Interferences

See Method No. 268.

2.6 Confirmatory Techniques

See Method No. 268.

2.7 Time Required for Analyses

See Method No. 268.

Omitting the partition steps 2.3.4 and 2.3.5 saves approximately 2 hours.

2.8 Potential Problems

1. Funnel becomes clogged during extract filtration (2.3.2):
   Use the fastest possible centrifuge speed. Filter 95 % of the supernatant before decanting the remaining milliliters.
   Internal diameter of funnel stem should be 1 cm. Do not use too much cotton wool. Do not pack the cotton wool too tightly.

2. Alkaline sodium sulfate adsorbs Quinclorac:
   Do not use this lot or acidify it (see 2.2).

3. Alkaline glass wool also adsorbs Quinclorac:
   Always use cotton wool.

4. Extrelut column becomes clogged. Extract aliquot does not completely enter the column material:
   Force with moderate vacuum (5 kPa) using a SPE vacuum system.

5. Water breaks through Extrelut column along with ethyl acetate eluate:
   Shake for 30 seconds and discard water phase before adding sodium hydrogen carbonate solution. To avoid break through, apply aqueous extract in a smaller volume or use more Extrelut.

6. Elution time from Extrelut too long:
   Extrelut contains too many fines. Remove by decantation during purification or use a different lot.

7. Methylation not complete:
   Concentration of diazomethane solution too low. Use more solution or prepare a more concentrated solution. Diaktin (= "Diazald") impure or too old. Use a different lot for diazomethane preparation.

8. Late eluting peaks from GC column have an impact on the following injection:
   Inject pure acetone after each concentrated (i. e. V_e = 2 mL) sample.

9. Detector response not linear:
   Use second order calibration.

10. Detector sensitivity increases during run:
    Inject concentrated sample (i. e. Ve = 2 mL) two or three times before starting the set. Use several calibration curves.

2.9 Methods of Calculation

See Method No. 268.

3. RESULTS AND DISCUSSION

3.1 Accuracy and Precision

The following recovery experiments were carried out using the method as described in this amendment including the partition steps 2.3.4 and 2.3.5.

Matrix	Fortification mg/kg	Recovery %	Mean value %	Standard deviation ±	Variation coefficient ±%	Date of analytical report
Cow milk	0.05*	91.2; 89.2 91.3; 92.1 87.4;	90.2	1.9	2.1	13Feb89
	5	81.8; 82.1 81.7; 85.0 82.6	82.6	1.4	1.7	13Feb89
Goat muscle	0.05*	64.8; 71.7; 76.0; 77.4; 86.3	75.2	7.9	10.5	13Sep88
	5	84.0; 84.3; 88.8; 84.3; 83.8	85.0	2.1	2.5	13Sep88
Goat liver	0.05*	74.1; 91.5; 77.3; 76.8; 87.2	81.4	7.5	9.3	02Sep88
	5	66.3; 66.9; 57.3; 57.8; 69.5	63.6	5.6	8.8	02Sep88

* Quantitation limit

Fortification trials at each level were run in parallel.

3.2 Quantitation limit

Quantitation limit for Quinclorac residues in animal matrices is 0.05 mg/kg. This is the lowest amount which is supported by recovery data.

3.3 Ruggedness Testing

Not performed.

3.4 Limitations

Not known to date.

4. CONCLUSIONS

To overcome recovery losses due to inconsistent quality of C_{18} solid phase extraction material, an alternative amino column clean-up was described. It was shown in the typical matrices cow milk, goat muscle and goat liver, that the new analytical procedure is applicable for measuring residues of Quinlcorac in animal matrices.

5. QUALITY ASSURANCE PROCEDURES

Testing Facility:

BASF Aktiengesellschaft
Agricultural Research and Development
Environmental Research-Li 445
Residue Analysis
D-6703 Limburgerhof
Federal Republic of Germany

Guidelines covered: Residue Chemistry
Guideline 171-4
For Good Laboratory Practice:
40CFR Part 160

Location of data:

Logbook No. 00209 Page 240 - 243
(Development)
Logbook No. 00246 Page 149 - 152
(Validation)

Schedule:

Start of experiments: July 28th, 1988

Date of completion: February 16th, 1989

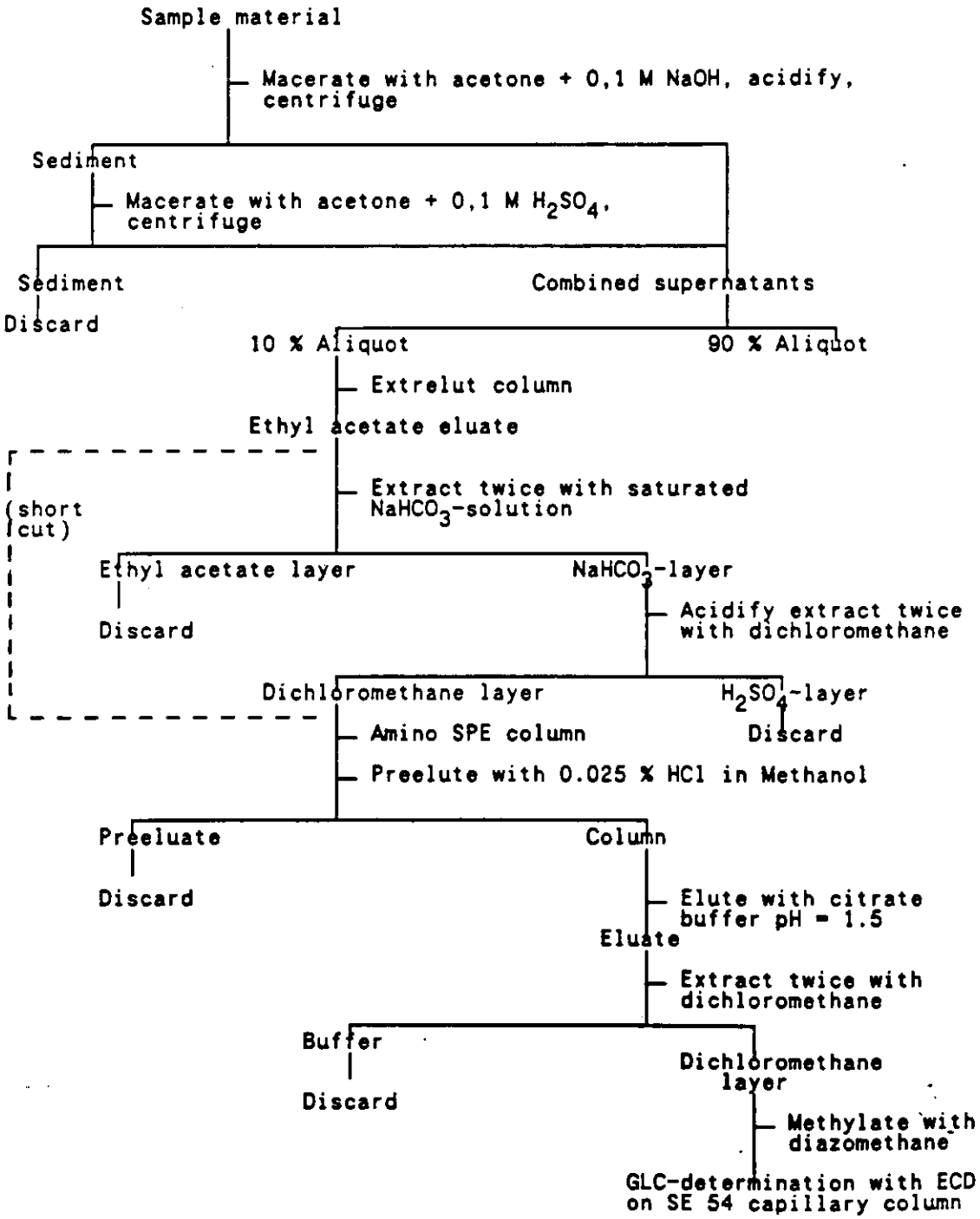
Reporting: February 1989/ss

Archiving

Protocol, raw data, original report and duplicate of report will be retained at BASF AG, Landwirtschaftliche Versuchsstation D-6703 Limburgerhof, at least for the period of time specified in the GLP regulations.

6 TABLES AND FIGURES

6.1 Flow Chart of Analytical Procedure



7. REFERENCES

1. BASF Report No. 2523 (Laboratory Study Code VAL-02-88): Quinclorac - Accountability of Method No. 268 in Chicken Tissues and Eggs

2. BASF Report No. 2539 (Laboratory Study Code VAL-01-88): Quinclorac - Accountability of Method No. 268 in Goat Tissues and Milk

3. BASF Corporation Agricultural Chemicals Group Protocol No. A8830: Residue Analysis of Tissues and Eggs from Chicken fed with Quinclorac

4. BASF Corporation Agricultural Chemicals Group Protocol No. A8831: Residue Analysis of Tissues and Milk from Cows fed with Quinclorac

5. BASF Report No. 2539 (Laboratory Study Code VAL-06-88): Quinclorac - Accountability of Method No. 268 in Goat Tissues and Milk

8. APPENDICES

1. Typical chromatograms from recovery trials with cow milk

2. Typical chromatograms from recovery trials with goat muscle

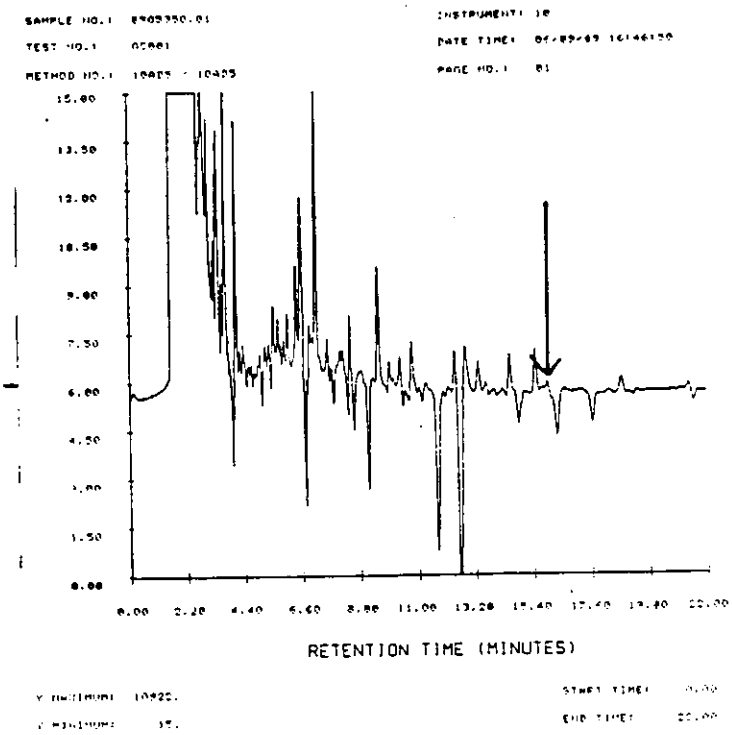
3. Typical chromatograms from recovery trials with goat liver

Attachment 1:

Typical chromatograms from recovery trials with cow milk

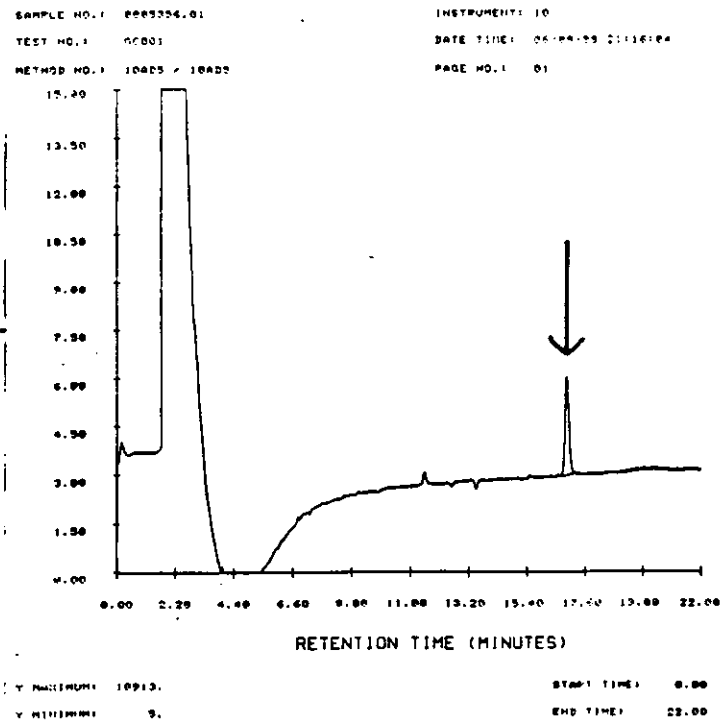
Control

UNBEH.



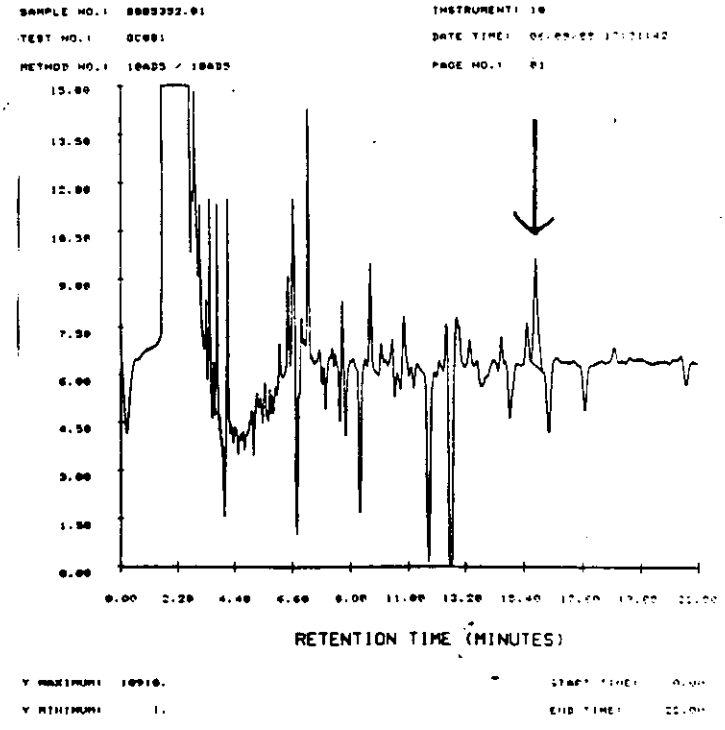
Fortification 5 mg/kg

ZUS. 1



Fortification 0,05 mg/kg

ZUS. 2

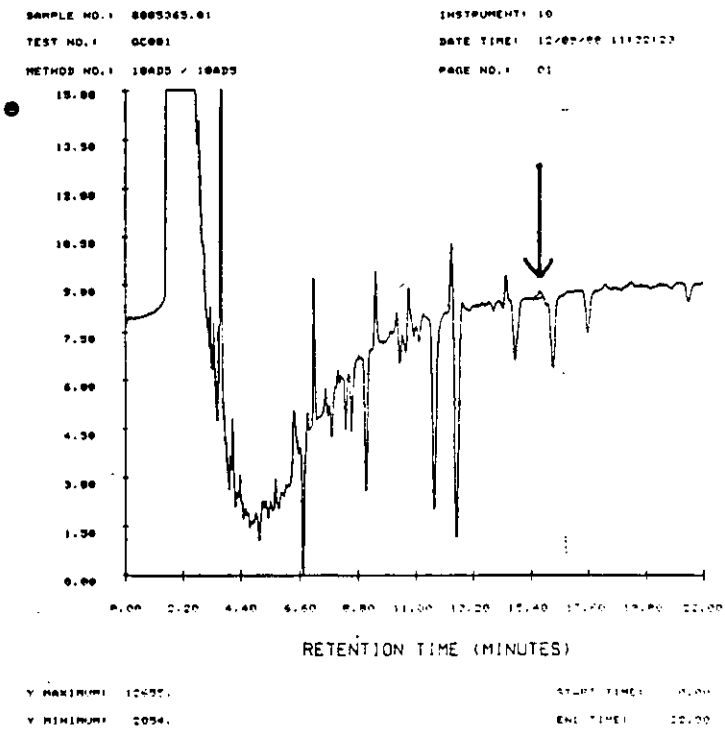


Attachment 2:

Typical chromatograms from recovery trials with goat muscle

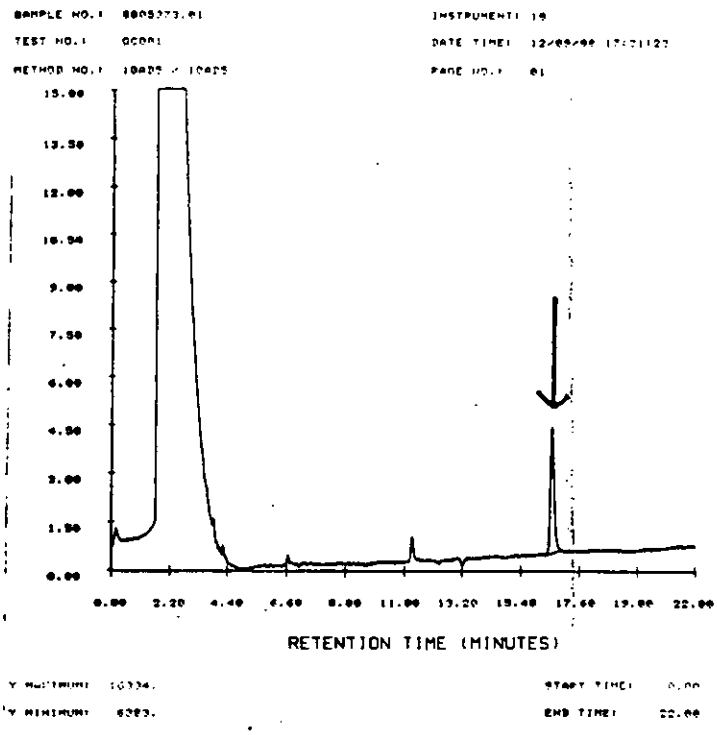
Control

UNBEH.



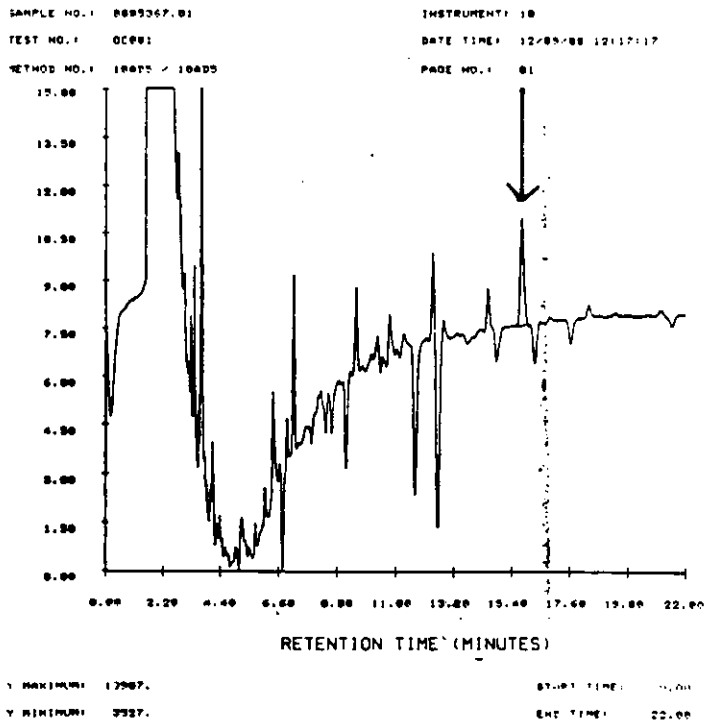
Fortification 5 mg/kg

ZUS. 3



Fortification 0,05 mg/kg

ZUS. 2

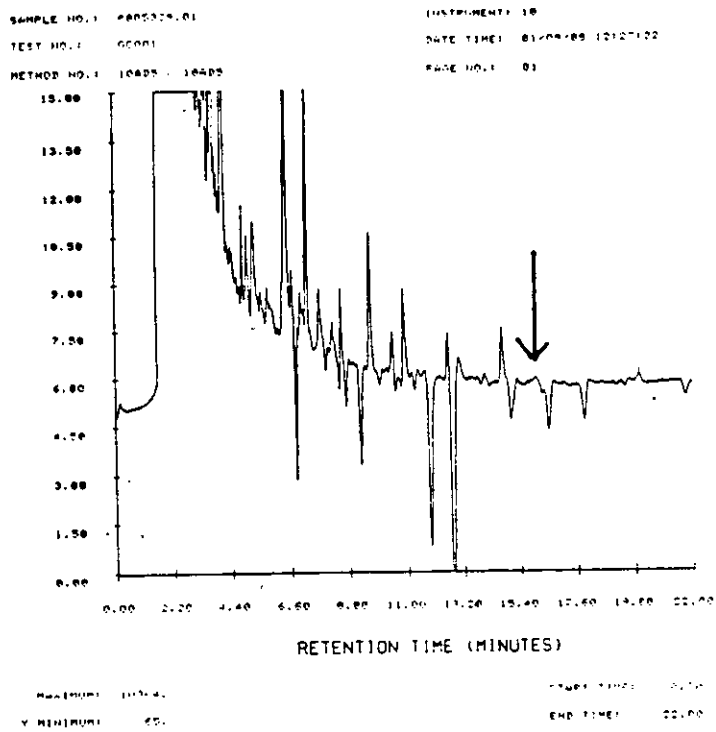


Attachment 3:

Typical chromatograms from recovery trials with goat liver

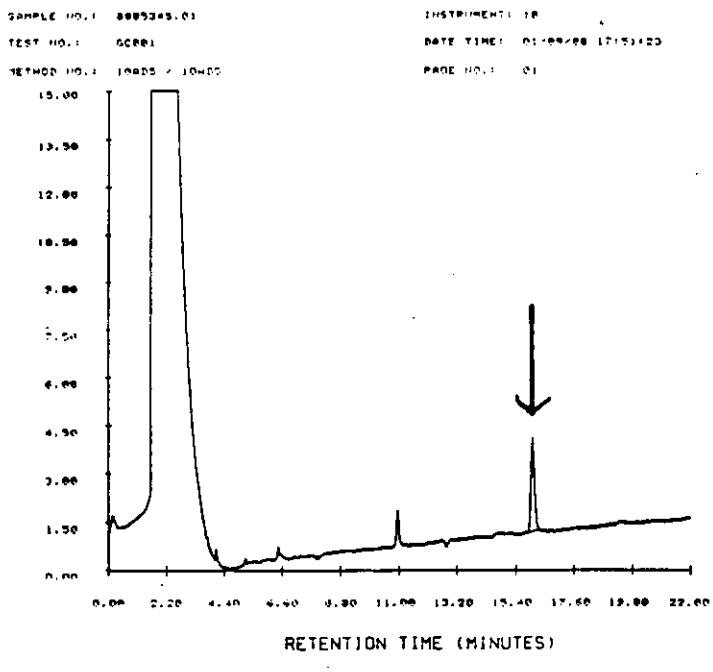
Control

UNBEH.



Fortification 5 mg/kg

ZUS. 1



Fortification 0,05 mg/kg

ZUS. 3