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
Postfach 2 20, D-6703 Limburgerhof (FRG)
Phone: 0 62 36 / 68 - 2 614

Author of Report: Dr. Frank Mayer Mayer 16.2.89
APE/CU
Adress see above
Phone: 0 62 36 / 68 - 2 432

Laboratory Analyst: Heinz Sträßner H. Sträßner 20.2.89
APE/CU
Phone: 0 62 36 / 68 - 2 441

Klaus Adler
APE/CU
Phone: 0 62 36 / 68 - 2 285 20.2.89
Adresses see above

Quality Assurance Officer: Dr. H. Morgenthaler Morgenthaler 20.2.89
APE/CQ
Adress see above
Phone: 0 62 36 / 68 - 2 522

Statistician: Not involved

Report approved by: Dr. S. Otto SOtto 20.2.89
APE/CU
Adress see above
Phone: 0 62 36 / 68 - 2 201

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

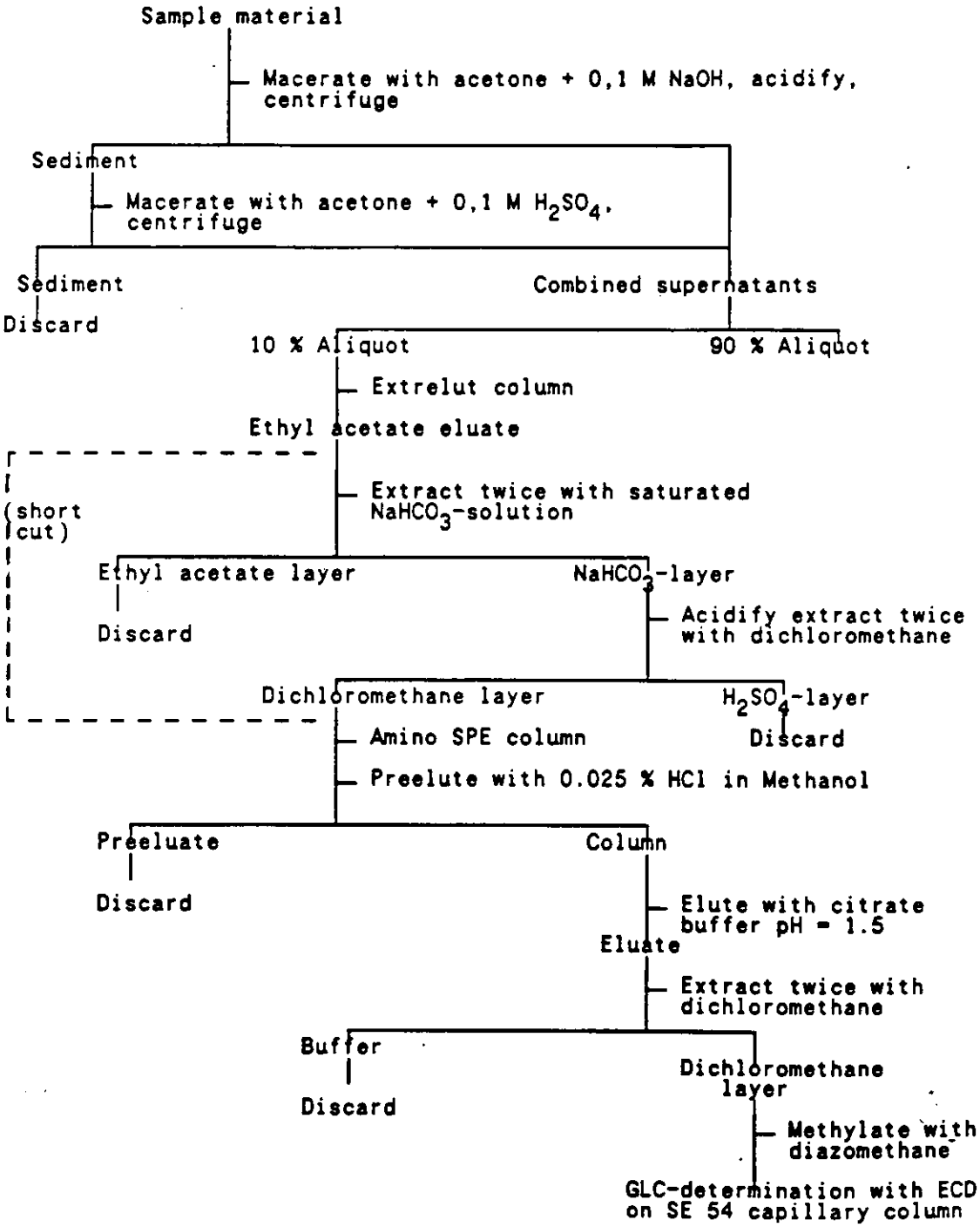
Reporting: February 1989/ss

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

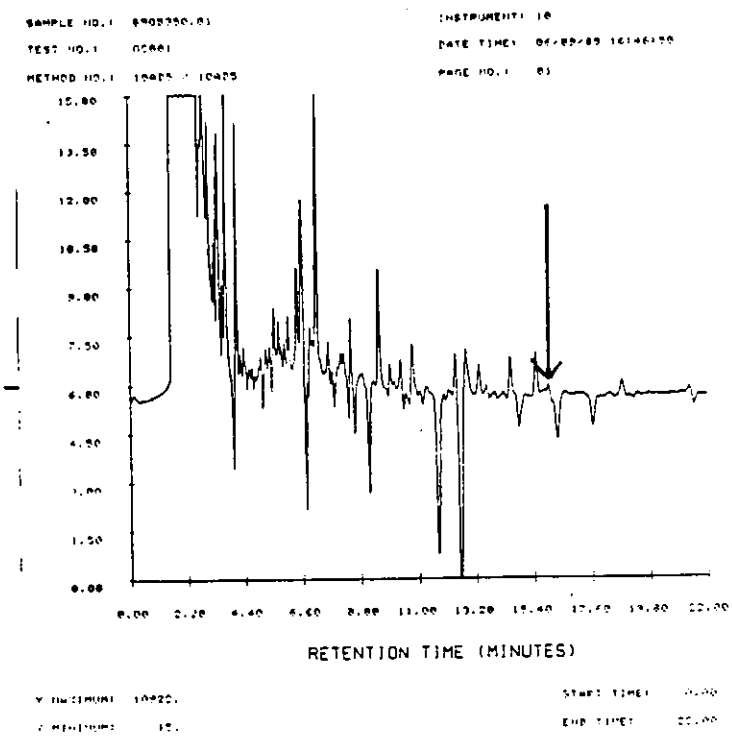
3. Typical chromatograms from recovery trials with goat liver

Attachment 1:

Typical chromatograms from recovery trials with cow milk

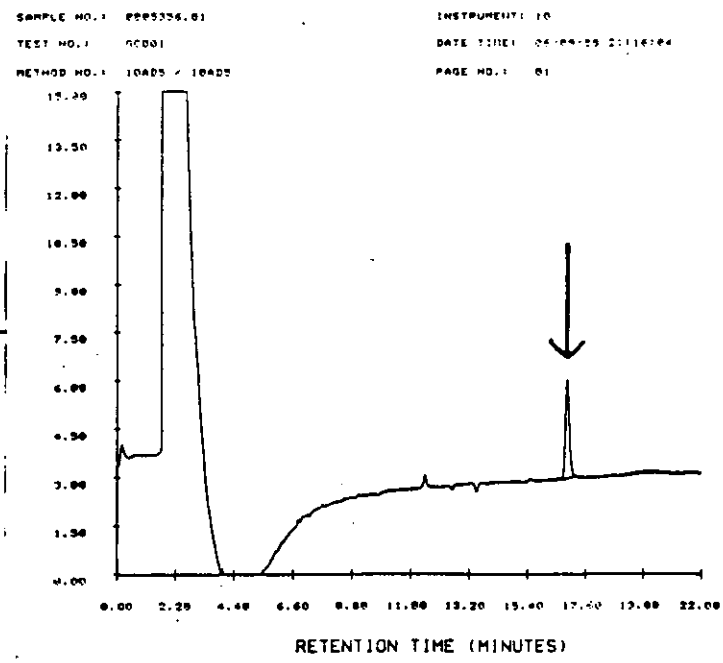
Control

UNBEH.



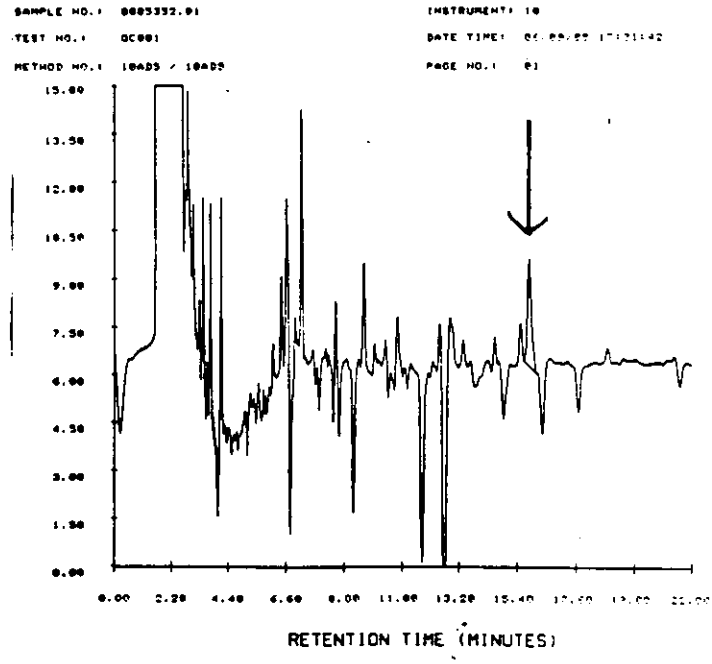
Fortification 5 mg/kg

ZUS. 1



Fortification 0,05 mg/kg

ZUS. 2



Attachment 2:

Typical chromatograms from recovery trials with goat muscle

Control

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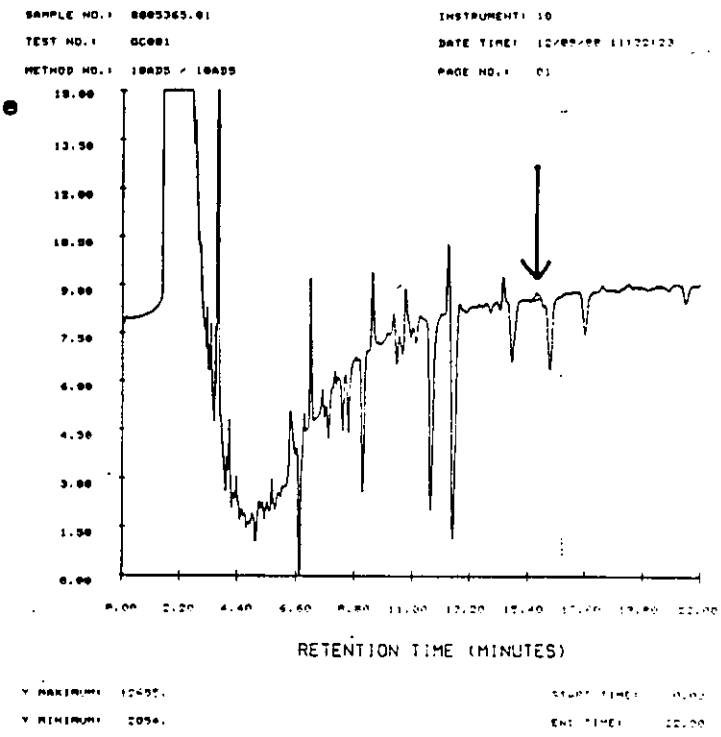
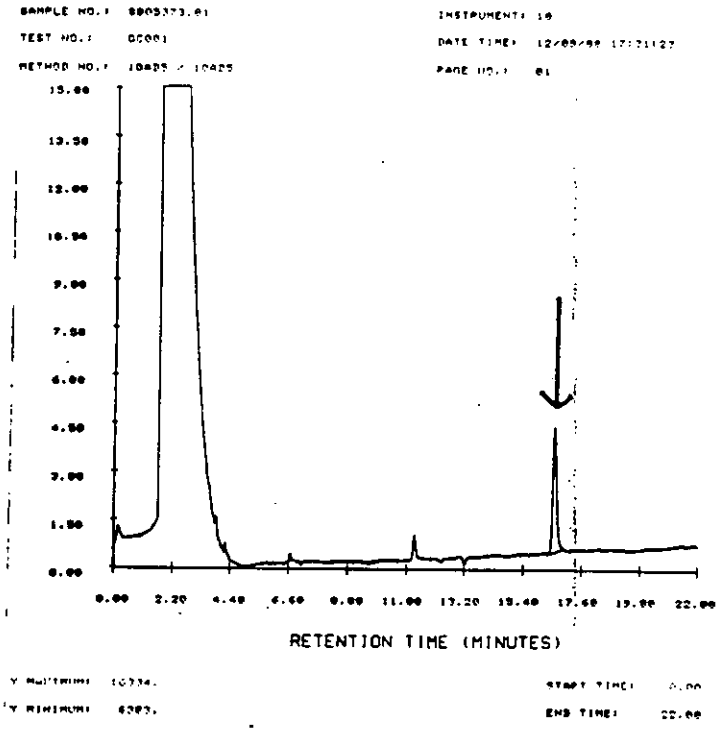
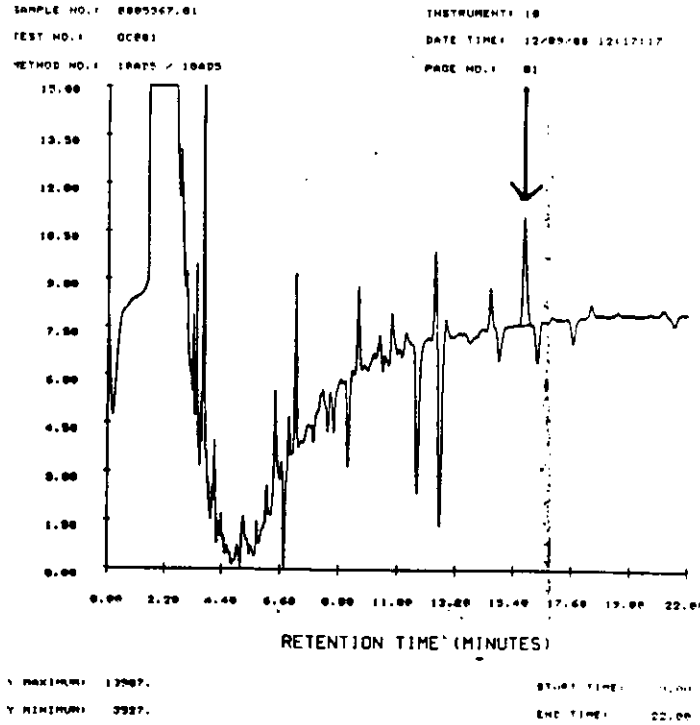


Fortification 5 mg/kg
ZUS. 3



Fortification 0,05 mg/kg
ZUS. 2



Attachment 3:

Typical chromatograms from recovery trials with goat liver

Control

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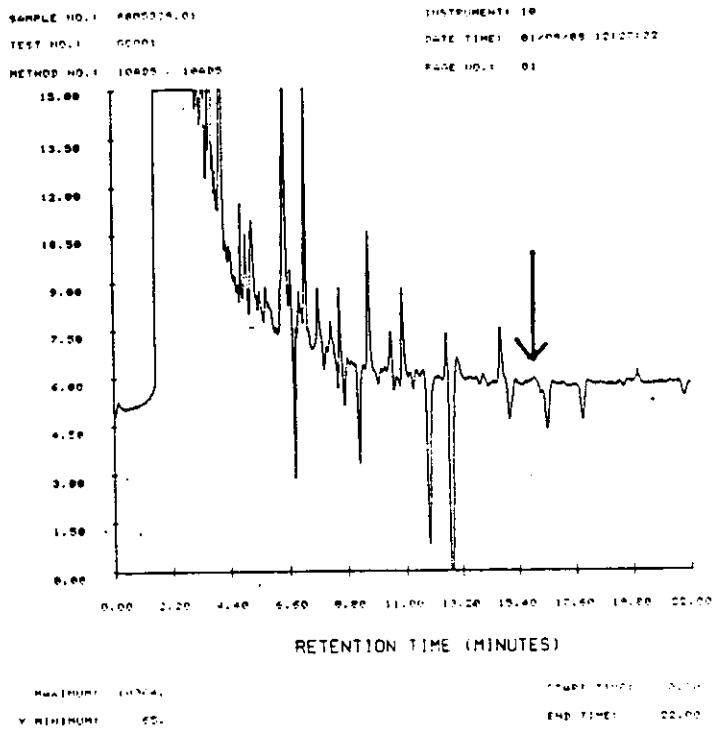
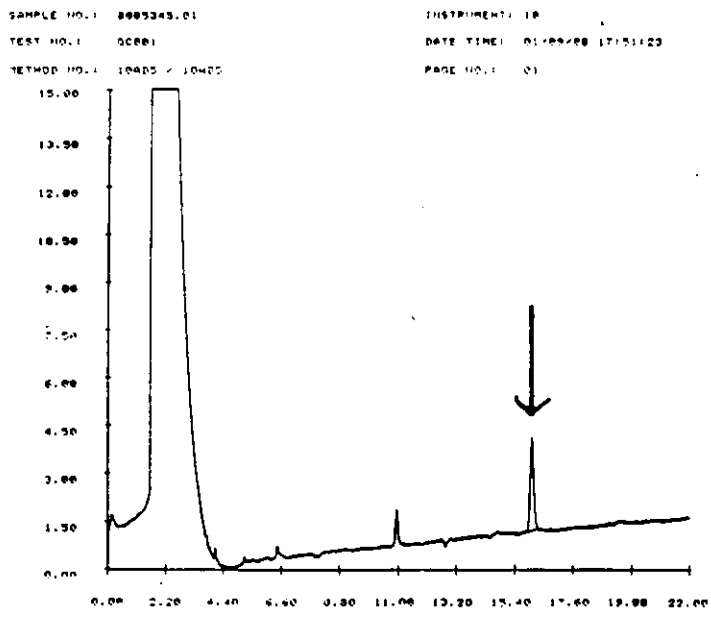


Fortification 5 mg/kg

ZUS. 1



Fortification 0,05 mg/kg

ZUS. 3