

Cover Sheet for

ENVIRONMENTAL CHEMISTRY METHOD

Pesticide Name: Lambda-Cyhalothrin

MRID #: 405159-01

Matrix: Water

Analysis: GC/ECD

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ICI AGROCHEMICALS RESIDUE ANALYTICAL METHOD NUMBER 125b

THE DETERMINATION OF RESIDUES OF PP321 IN WATER

FOLLOWING SAMPLING BY A SOLID-PHASE EXTRACTION TECHNIQUE

- A gas-liquid chromatographic method using an internal standard

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Date of Issue : September '89

The contents of this method are confidential and should not be disclosed to third parties without the prior agreement of the study director concerned.

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CHEMICAL PROPERTIES

CYHALOTHRIN (PP563)

Chemical Name : (RS)- α -cyano-3-phenoxybenzyl-(Z)-(1RS, 3RS)(2-chloro-3,3,3-trifluoroprop-1-enyl)-2,2-dimethylcyclopropanecarboxylate
 PP563 consists of two enantiomer pairs designated A and B.

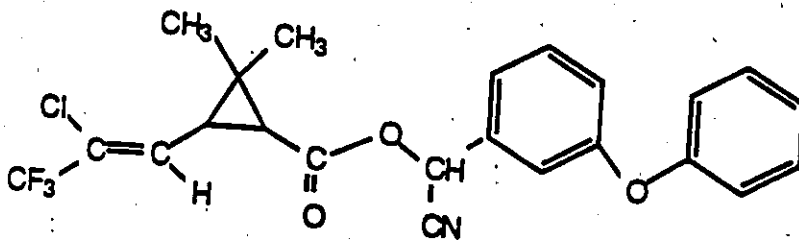
LAMBDA-CYHALOTHRIN (PP321)

Chemical Name : α -cyano-3-phenoxybenzyl-3-(2-chloro-3,3,3-trifluoroprop-1-enyl)-2,2-dimethylcyclopropanecarboxylate
 a 1:1 mixture of the (Z)-(1R,3R), S-ester and (Z)-(1S,3S), R-ester
 PP321 consists of one enantiomer pair designated B.

Empirical Formula : $C_{23}H_{19}ClF_3NO_3$

Molecular Weight : 449.9

Structural Formula :



1. SCOPE

The analytical procedures described are suitable for the determination of residues of the synthetic pyrethroid insecticides cyhalothrin - PP563, and lambda-cyhalothrin - PP321 in natural waters including pond water.

In water at pH values greater than 7, PP321 (enantiomer pair B) rapidly epimerises to enantiomer pair A. Therefore the determination of both enantiomer pairs is necessary.

The internal standard used, R171554 has also been found to epimerise during the analytical procedure. This method is suitable for the determination of both epimers found.

Since pyrethroids are of low water solubility and will readily adsorb onto glassware, sampling and subsequent storage in water bottles is not recommended.

The recommended sampling procedure is to sample at source as shown in Fig 2.

The limit of determination for the method has been set at 1 ng/litre water (for each of the enantiomer pairs A and B).

2. SUMMARY

The water sample is loaded onto a pre-conditioned solid phase extraction cartridge, (cartridge as shown in Figure 1). The cartridges are frozen immediately after sampling and should be maintained frozen during transportation and storage, prior to analysis.

After thawing, the cartridges are accurately fortified with a known amount of internal standard. Cartridges are then eluted with organic solvent, and co-extractives removed and the analyte concentrated by further solid-phase sorbent extraction.

Final quantitative determination is by on-column capillary gas-liquid chromatography using internal standardisation.

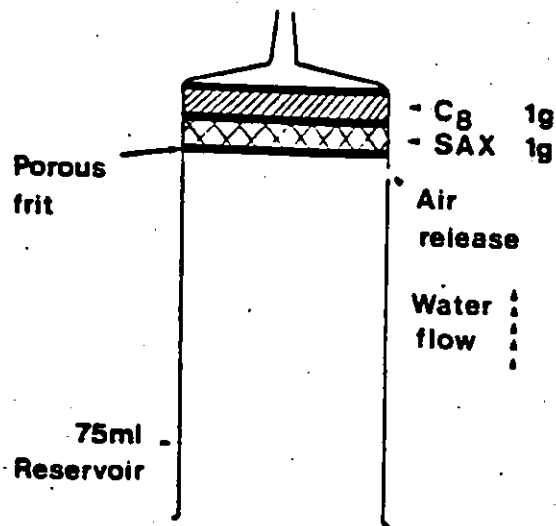
A flow chart summary of the method is shown in Appendix 5.

3. SAMPLING PROCEDURE

3.1 Extraction Cartridge Preparation

Prepare extraction cartridges as shown in Figure 1 overleaf:-

Figure 1



- a) Place one 20 μm porous polyethylene frit in the base of a 75 cm^3 polypropylene reservoir.
- b) Add C₈ Sepralyte (reverse-phase) sorbent (1 g).
- c) Place another frit on top of the C₈ sorbent and add SAX (strong anion exchange) sorbent (1 g).
- d) Add a further frit to the top of the sorbent bed.

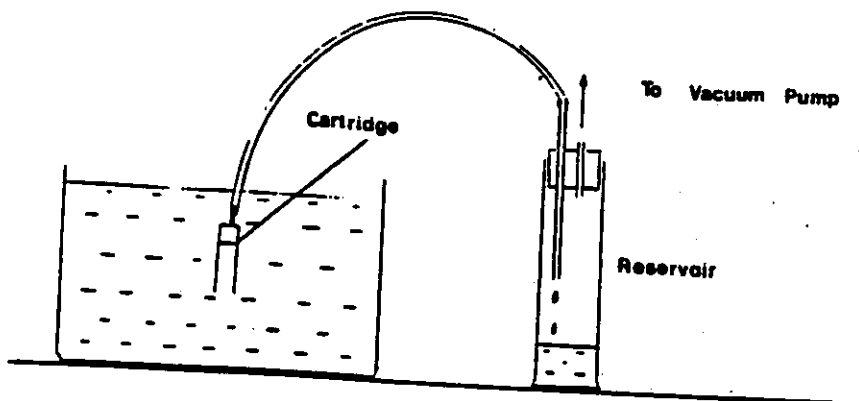
3.2 Extraction Cartridge Conditioning

- a) Before use all cartridges must be conditioned with methanol followed by deionised water.
- b) All cartridges must be used as soon as possible after conditioning (within one hour).
- c) Cartridges can be conditioned by placing in a rack supported over a collection vessel and allowing the solvent to pass through under gravity.
- d) Apply methanol (25 cm^3) to each cartridge and allow to drain under gravity.
- e) When the methanol has drained from each cartridge (i.e. has stopped dripping) apply deionised water (5 cm^3) and allow to drain under gravity.
- f) A hole must be drilled in the side of each cartridge (1 cm from the sorbent bed) in order to release air when the cartridge is lowered into the water. This prevents desolvation of the cartridge prior to the water reaching the sorbent bed, when pumping commences.
- g) The cartridges should then be transferred into individual plastic bags until required for sampling.

3.3 Water Sampling

Water can be sampled through the cartridge using the apparatus shown below:

Figure 2



- b) Water can be pumped through the cartridge using a hand held vacuum pump (approximately $25 \text{ cm}^3/\text{stroke}$). A flow of $50 \text{ cm}^3/\text{min}$ should be achieved.
- c) The total volume of water sampled through the cartridge can then be measured on the cylinder and recorded.

Desolvation of the sorbent bed reduces the retention efficiency of the analyte on the cartridge and therefore there is a maximum volume of water that can be sampled. This maximum is 350 cm^3 water.

Since for a limit of 1 ppt (1 ng/litre), one litre of water is required, then three cartridges are taken per sample. For higher limits of determination fewer cartridges can be used.

- d) Immediately following sampling the cartridge should be removed carefully from the tubing and placed in its original plastic bag. The bags should be sealed, labelled and placed in a freezer as soon as possible after sampling.

3.4 Prevention of Contamination

In order to avoid contamination of samples during the course of sampling the following procedures are necessary.

- a) Cartridges should be conditioned and drilled in a contamination free zone (i.e. an area well away from the sampling area).
- b) All equipment used for drilling or conditioning should also be stored away from the sampling area.
- c) Immediately after sampling, the cartridges should be replaced in their individual plastic bags, labelled appropriately, then sealed and stored in a temperature-monitored freezer at $<-18^\circ\text{C}$ until required for analysis.

- d) Handling of the cartridges should be minimised and contact with the insides of the cartridges avoided at all times.

4. ANALYTICAL PROCEDURE

4.1 Sample Preparation

- a) After removal from the freezer and thawing, sample matrix cartridges should be taken to dryness under vacuum on a vacuum manifold system (eg. Supelco) for 30 minutes.
- b) Sample matrix cartridges should be fortified with the internal standard (R171554 see Appendix 3.2) at an appropriate level.

4.2 Extraction

- a) Extract matrix cartridges with acetonitrile ($3 \times 5 \text{ cm}^3$) on a manifold system, aiding percolation of solvent through matrix with a vacuum if necessary. Collect all the eluate.
- b) Apply a vacuum to cartridges (approximately 10 minutes) to remove any remaining eluate.
- c) Add buffer (Clark and Lubs pH 1, see Appendix 3.2) (2 cm^3) to cartridge, allow to percolate into cartridge then take to dryness under vacuum. Discard all eluate from this step.
- d) Continue extraction with diethyl ether: hexane 70:30 (5 cm^3) followed by acetonitrile (5 cm^3). Remove any excess eluate by applying a vacuum and then combine extracts from steps a, b, and d.
- e) Repeat steps a-d for any further cartridges remaining for each sample. (see 3.3c)
- f) Combine all organic eluates in a round bottom flask and concentrate by rotary evaporation until between $5\text{-}10 \text{ cm}^3$ remain.
- g) Ultrasonicate round bottom flask, measure volume remaining and make to 15 cm^3 with acetonitrile.
- h) Add ultra-pure water (50 cm^3) to each flask and shake thoroughly (this should only be done immediately before application to the C_8 sorbent cartridge in 4.3).

4.3 Sorbent Clean-Up

- a) Prepare matrix cartridges (1g of C_8 Sepralyte sorbent) in a reservoir (75 cm^3). Place on a vacuum manifold system with individual valve control eg. Supelco.
- b) Condition cartridges with methanol (25 cm^3) followed by water (5 cm^3) allowing each to percolate under gravity.

(N.B. Cartridges must be conditioned within half an hour of use and must not be allowed to dry out before use).

- c) Apply analyte solution 4.2 (h) to C₈ cartridge and allow to percolate under gravity, controlling the rate, (no greater than 1 cm³/min). Discard the eluate.
- d) Wash cartridge with water (5 cm³) followed by acetonitrile:water 50:50 (2 x 2.5 cm³). Discard the eluate.
- e) Take cartridges to dryness under vacuum (approximately 1 hour).
- f) Elute cartridges with hexane (4 x 2.5 cm³) and collect the eluate.
- g) Make the hexane eluate to an appropriate volume for analysis by GLC.

5. GAS-LIQUID CHROMATOGRAPHY

The conditions for the analysis by GLC will depend upon the equipment available. A highly sensitive detector is required for analysis at low levels, and the following conditions have been found to be satisfactory.

5.1 Instrumentation

Varian 3500 capillary gas chromatograph with automated on-column injector and fitted with a Ni⁶³ (8 mCi) electron capture detector.

5.2 GLC Conditions

- a) SE54 bonded phase fused silica open tubular capillary column 25m x 0.32mm ID.
- b) Temperature programs:-
 Column temperature:- 89°C (hold 1 minute) program at 10°C/min to 240°C (hold 21 minutes) program at 10°C/min to 250°C (hold 10 minutes).

 Injector temperature:- 40°C program at 150°C/min to 250° (hold 35 minutes).
- c) Detector temperature 300°C.
- d) Carrier gas, helium at 2 cm³/min. Make up gas nitrogen at 28 cm³/min.

Under these conditions PP563 is resolved into its two enantiomer pairs, A with retention time 24.1 mins and B (PP321) with retention time 24.85. The internal standard used -R171554 (see Appendix 3.2) has a retention time of 27.1 minutes. Sensitivity is such that 2.4 pg enantiomer pair B (PP321) injected on column with electrometer attenuation at 10 x 8 and recorder on 1mV gives approximately 40% full scale deflection.

6. CALCULATION OF RESIDUES

Residues of enantiomer pairs A and B in the final extract are calculated using the internal standardisation procedure.

Prior to extraction each sample is accurately fortified with a known concentration of internal standard. Each sample residue can thus be individually corrected by measuring the percentage recovery of internal standard through the analytical method.

The calculation for the determination of cyhalothrin (PP563) residues may be performed using a 'single point ratio calibration' (Cardone and Palermo, 1980). It should be noted that such calibrations are feasible only when the chosen internal standard meets certain criteria (see Ref 1). The calculations below are shown for PP321 (enantiomer pair B) but are equally valid for enantiomer pair A.

- a) Using the GLC operated under conditions described in 5.2 above, make repeated injections of 2 μ l of a standard solution containing a mixture of R171554 and PP563 each at 2.5 ng/cm³ until a consistent response is obtained.

- b) Measure the peak heights or area obtained and calculate the detector 'Response factor ratio'.

$$\text{i.e. Rf ratio} = \frac{\text{Peak ht PP321 (mm)}}{\text{(Std) Conc'n PP321 (ng/cm}^3\text{)}} \div \frac{\text{Peak ht internal standard (mm)}}{\text{Conc'n internal standard (ng/cm}^3\text{)}}$$

- c) Make an injection of the sample solution (SA) and measure the peak heights or area obtained for PP321 and internal standard (R171554).

- d) Since the response factor ratio will be constant for a detector with a linear response, the PP321 residue in the sample can thus be calculated:-

$$\text{Rf ratio} = \frac{\text{Peak ht PP321 SA}}{\text{(Std) Conc'n PP321 SA}} \div \frac{\text{Peak ht R171554 SA}}{\text{Conc' R171554 SA}}$$

$$\text{Therefore PP321 conc'n} = \frac{\text{Peak ht PP321 SA} \times \text{Conc'n R171554 SA}}{\text{Rf} \times \text{Peak ht R171554}}$$

$$\text{Units (ng/cm}^3\text{)} = \frac{\text{mm} \times \text{ng/cm}^3}{\text{mm}}$$

- e) This concentration must then be corrected in order to express the residue in terms of the concentration of analyte in a known amount of matrix.

$$\text{Residue in ng/l} = \frac{\text{PP321 conc'n}}{\text{(Sample/matrix) conc'n in final solution}}$$

$$\text{Units} = \frac{\text{ng/cm}^3}{\text{(ng/l) 1/cm}^3}$$

Note:- Injections of sample solutions (3 maximum) should be bracketed between standards.

7.

LIMIT OF DETERMINATION

A true assessment of the limit of determination of the method may be determined by fortification of untreated samples at low levels and subjecting them to the complete analytical procedure. The chromatographic response obtained for these recoveries at the retention time of PP321 (enantiomer pair B) or enantiomer pair A should exceed the background signal noise by a factor of at least four to be considered an acceptable quantitative limit of determination. In addition the precision of measurement at this level should not exceed a coefficient of variation of $\pm 15\%$.

In these laboratories the LOD for each of enantiomer pairs A and B has been set at 1 ng/l.

8.

CONTROL EXPERIMENTS

- a) To ensure that no observed contamination of the samples occurs during analysis at least one untreated sample should be analysed alongside a set of samples using exactly the same procedure.
- b) In these laboratories in order to ensure that any interference peaks observed at the retention time of PP321 or PP563 are not due to solvent/reagents a reagent blank sample is analysed alongside any set of samples ie. complete procedure carried out in absence of sample matrix.

9.

RECOVERY EXPERIMENTS

- a) Before extraction each sample must be accurately fortified with a concentration of internal standard within the range of the residue levels expected.
- b) At least one untreated sample should be accurately fortified with suitable concentrations of PP321 and R171554 within the range of the expected residue level in order to check the correlation between the analyte and internal standard recoveries during analysis.

10.

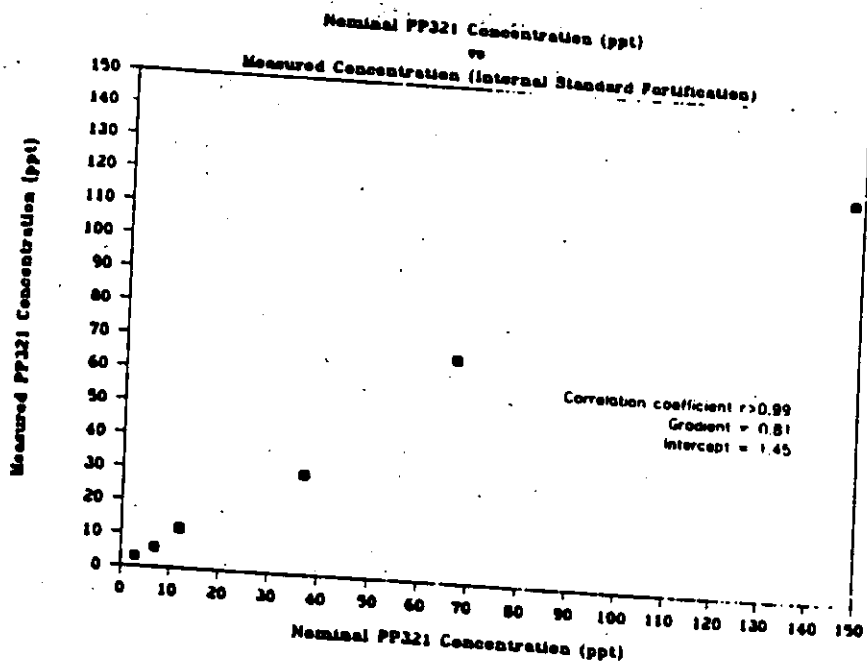
METHOD VALIDATION

10.1

Internal Standardisation Procedure

The validity of the internal standardisation procedure has been demonstrated by plotting a calibration graph for the residues calculated by the internal standardisation procedure, against concentration of PP321 in accurately fortified control samples with constant internal standard concentration.

The resultant plot is shown overleaf.



10.2 Recovery Experiments

For authentic water samples (115) fortified with internal standard R171554 at 10 ng/l (10 ppt) and analysed using this analytical procedure, the mean internal standard recovery was found to be 70% with a standard deviation of ± 15 .

10.3 Estimation of the Precision of the Method

For twenty six untreated samples accurately fortified with PP321 and internal standard R171554 at 10 ng/l (10 ppt) and then taken through the analytical method, the mean recovery by internal standardisation was calculated to be 10 ng/l with a standard deviation of <2 .

10.4 Extractability

Extractability of ^{14}C -PP321 from authentic sample extraction cartridges sampled at source was found to be greater than 88% in all cases.

10.5 Linearity

For accurate quantitation of residue concentrations, all analyses should be carried out within the linear range of the detector response.

In these laboratories the detector responses for PP321 and R171554 were shown to be linear over the concentration range 1.25 ng/ml - 100 ng/ml (Appendix 4).

11. CONFIRMATION OF RESIDUES

Capillary gas chromatography-mass spectrometry (GCMS) operated in the selected ion monitoring mode (SIM) may be used for the qualitative and quantitative confirmation of residues. Samples obtained from the residue analytical method are examined by SIM; ie. 2 or more of the most abundant m/z values present in the mass spectrum are continuously monitored throughout the gas chromatographic run and collected on a data capture system.

Qualitative confirmation of residues is given by the appearance of a peak at the correct gas chromatographic retention time for all the specific m/z values monitored.

In these laboratories GCMS was used to qualitatively and semi-quantitatively confirm the presence of residues of PP563 and also the epimer of the internal standard R171554, formed by inversion of the optically active α -CN position.

11.1 GC-MS Operating Conditions

a) Chromatography

Instrument : Finnigan 9610 Gas Chromatograph

Column : RSL 150, 25 m column, ID 0.25 mm

Conditions : 45°C (hold 2 minutes); program at 15°C minute⁻¹ to 220°C (hold 17.5 minutes); program at 5°C minute⁻¹ to 260°C (hold 8 minutes)

Carrier Gas : Helium, at 10 psi back pressure

The mass chromatogram of PP563 is shown in Appendix 2.

b) Mass Spectrometer

Instrument : Finnigan 4600 quadrapole mass spectrometer

Mode : Negative ion methane electron capture

Conditions :

Source Pressure : 2.1×10^{-5} TORR

Electron energy : 100 eV

Filament current : 0.3 AMPS

EM voltage : 1300 VOLTS

Ioniser pressure : 0.22 TORR

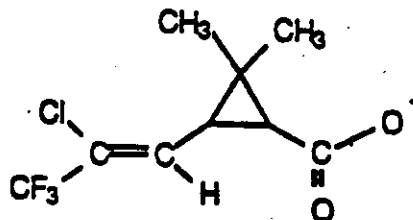
Ioniser temp : 120°C

Sensitivity : 10^{-7} AMPS/VOLT

C) Selected Ion Monitoring

Compound PP563

m/z 241 structure of ion:

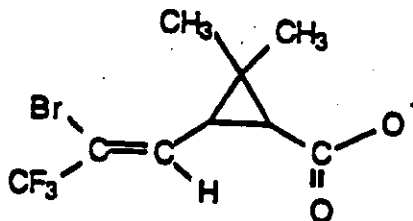


m/z 205 structure of ion:

241-HCl

Compound R171554

m/z 285 structure of ion



m/z 205 structure of ion

285 - HBr

m/z 79/81 structure of ion

Br-

The mass spectrum of PP563 is shown in Appendix 2.

12.

EXAMPLES OF CHROMATOGRAPHIC TRACES

These are shown in Appendix 1.

13.

REFERENCES

1. Cardone M J, Palermo P J and Sybrandt L B : Potential error in single point ratio calculations based on linear calibration curves with a significant intercept, Anal. Chem., 52, pp 1187-1191, 1980.

APPENDIX 1

Typical Gas Chromatograms for PP563 Residue Determination in Pond Water

Figure 1 : 2.5 ng/cm³ cyhalothrin and internal standard.

Figure 2 : Untreated sample at 0.25 l/cm³

Figure 3 : Water residue sample at 0.25 l/cm³

FIGURE 1 : 2.5 ng/cm³ PP563 and Internal Standard

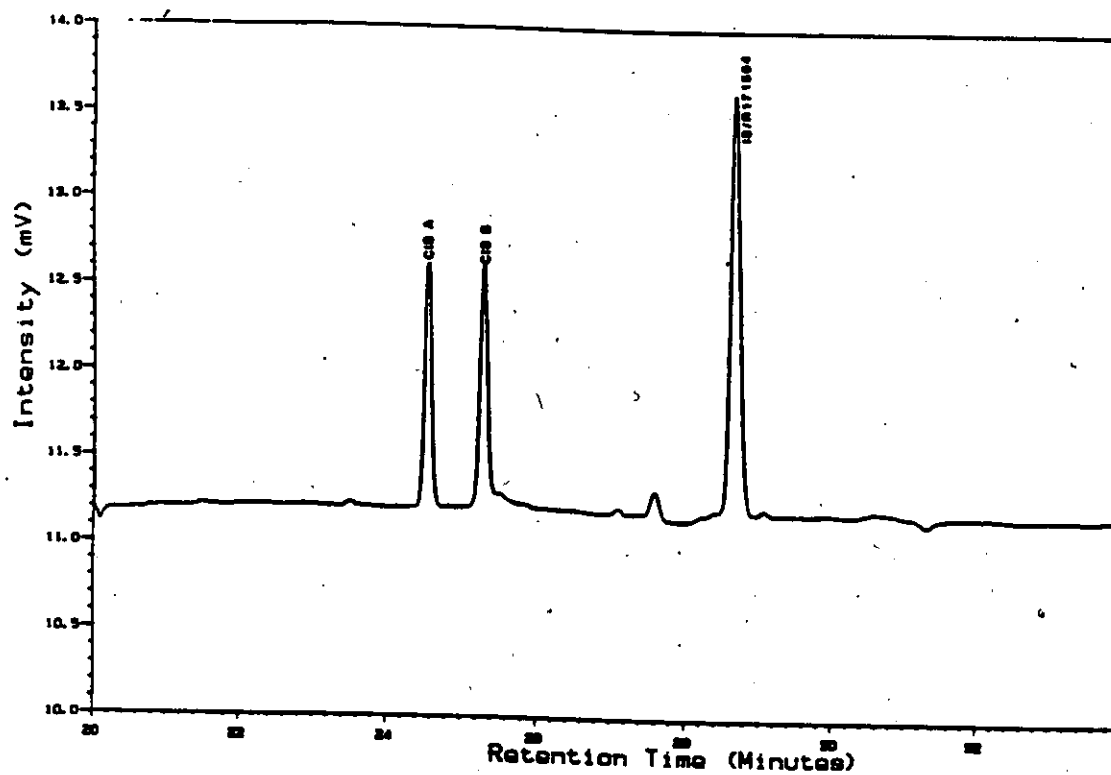


FIGURE 2 : Untreated Sample at 0.25 1/cm³

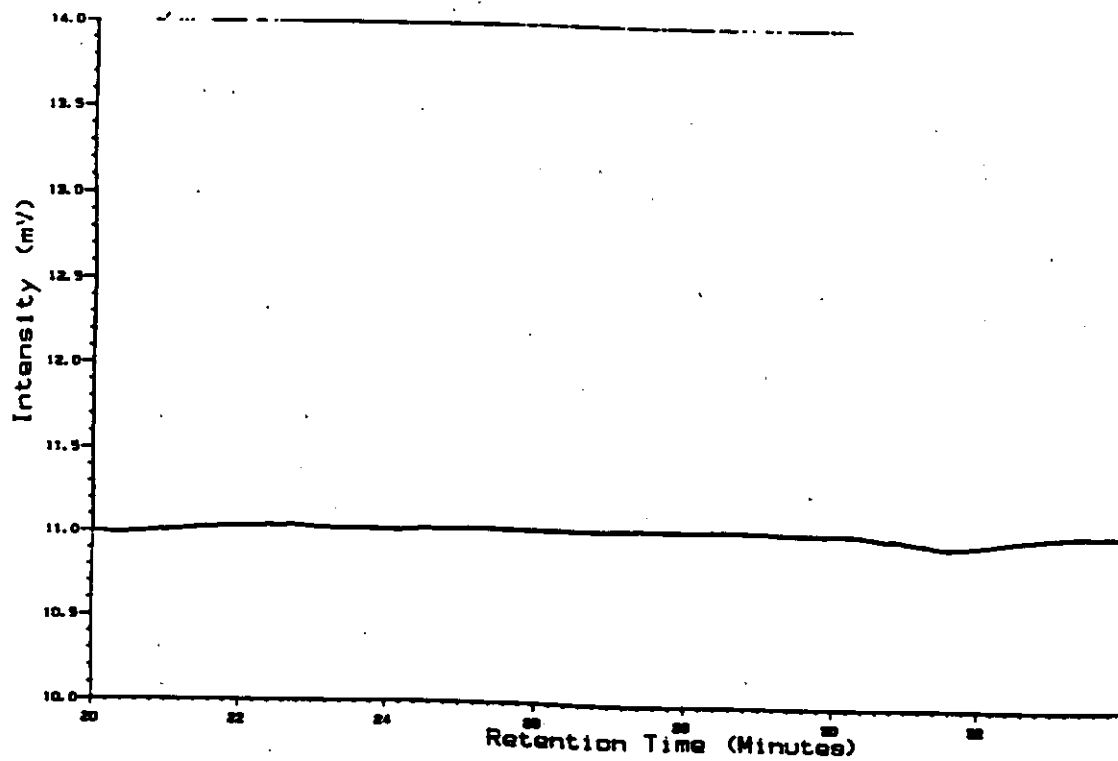
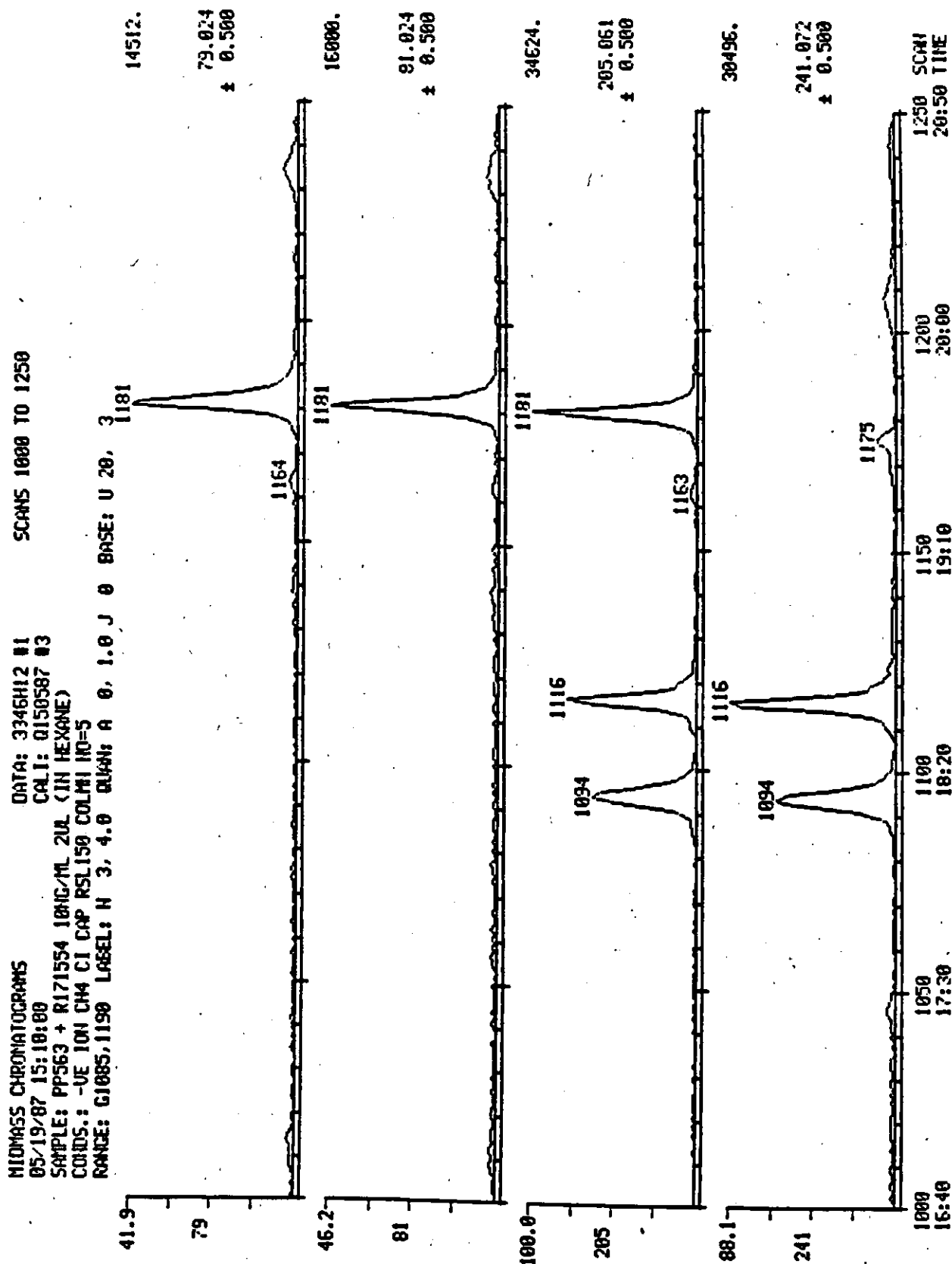


Figure 4 : Mass chromatogram of PP563 and R171554.



2535.

MID MASS SPECTRUM

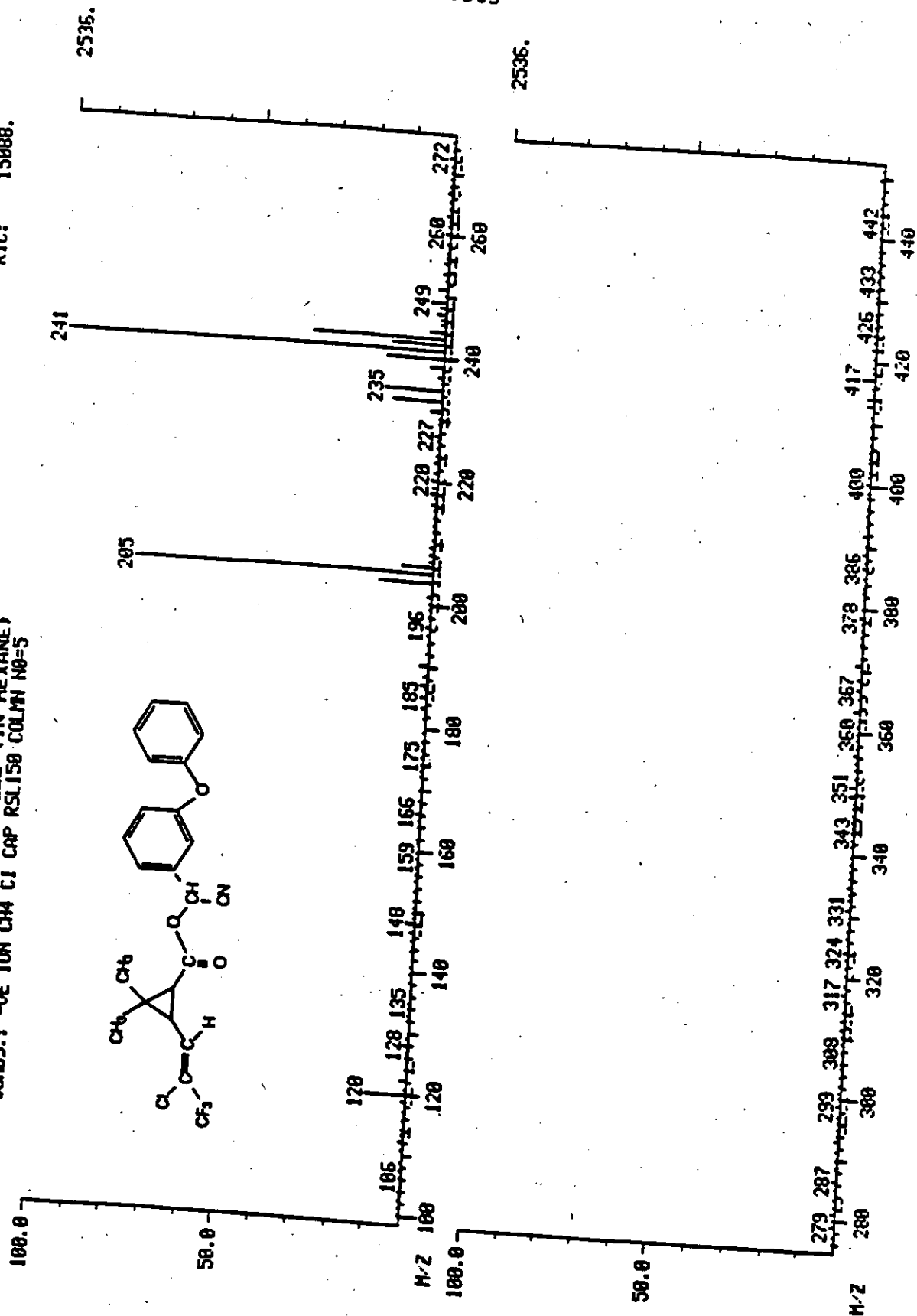
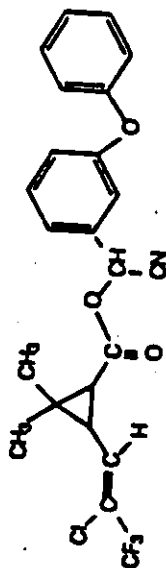
05/22/87 11:10:00 + 18:48

SAMPLE: PP563 + R171554 210

COND5.: -UE IUN CH4 CI CAP RSL150·COLUMN NO=5

DATA: 3346J1 1981

BASE M/Z: 241



2536.

Figure 5 : Mass spectrum of R171554

MID MASS SPECTRUM

05/22/87 11:10:00 + 19:54

SAMPLE: PP563 + R171554 ZUL (IN HEXANE)

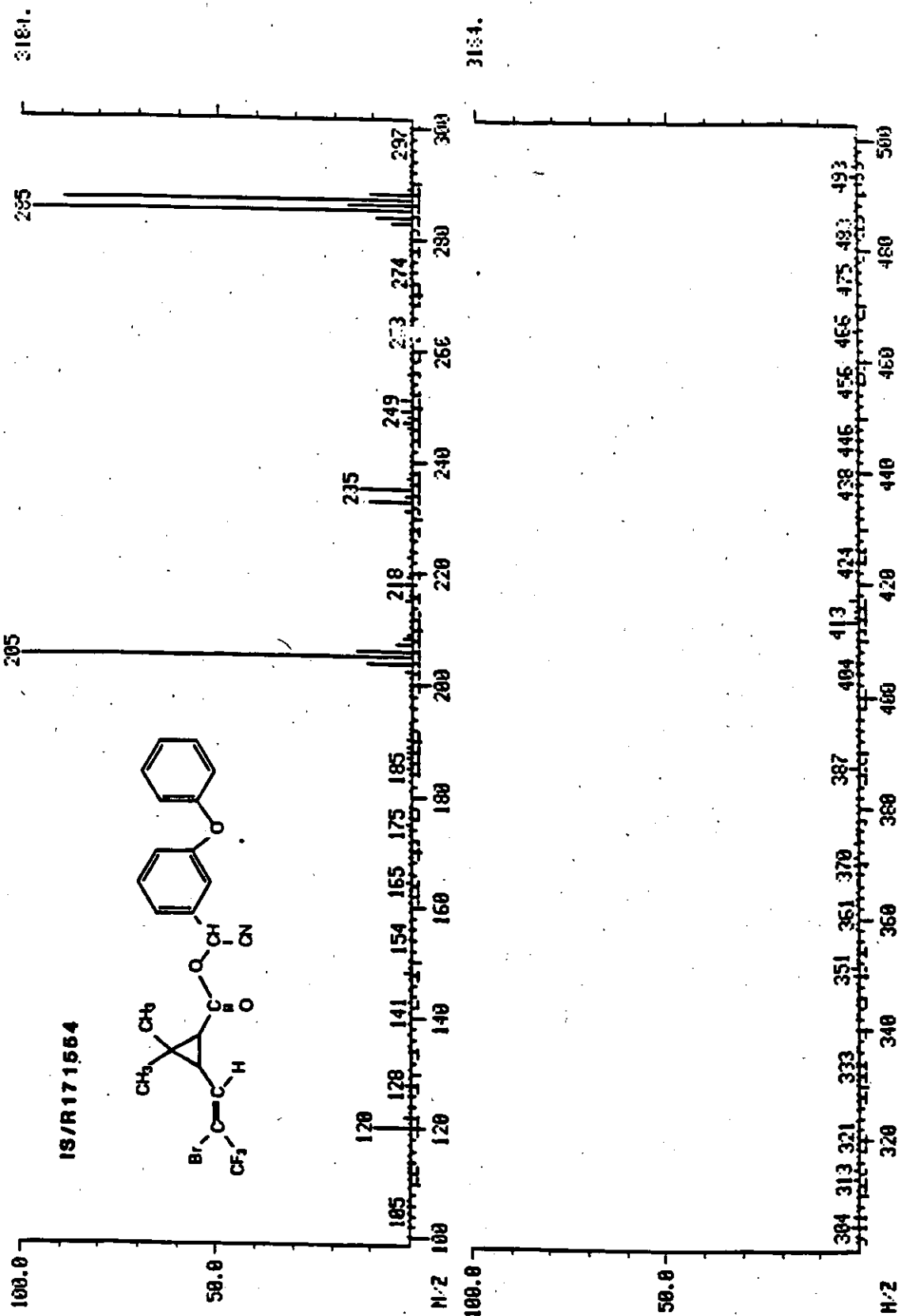
COND.S.: -VE ION CH4 CI CAP RSL150 COLUMN NO=5

DATA: 334EJ1 #1038

CALL: Q150587 #3

BASE M/Z: 205

RIC: 13008.



APPENDIX 3

1.

APPARATUS

- a) Vacuum manifold system (Supelco).
- b) Collection vessels (eg. measuring cylinders).
- c) Graduated tubes of 10cm³ calibrated down to 1.0cm³ in 0.1cm³ units.
- d) Capillary gas chromatograph fitted with an electron capture detector which will meet the sensitivity requirements.

N.B. An autosampler may be used with the gas chromatograph providing:-

- 1) That it is ensured it is not a source of cross-contamination to samples.
 - 2) Suitably precise injections can be achieved with a reproducibility better than $\pm 5\%$.
- e) Reservoirs (polypropylene) 75 cm³ capacity, porous polyethylene frits (20 μ m). (Supplier: Jones Chromatography).

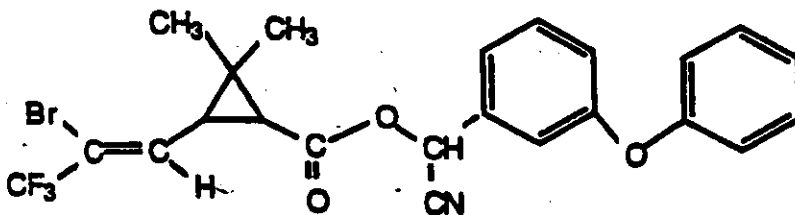
2.

REAGENTS

- a) Solvents: Re-distilled methanol, acetonitrile, diethyl ether, n-hexane, and ultrapure water.
- b) C₈ octyl sepralyte sorbent (Supplier:- Jones Chromatography).
- c) Clark and Lubs buffer solution (pH1). Mix 0.2 M KCl (25 cm³) + 0.2 M HCl (67 cm³) make to 100 cm³ with water.
- d) A sample of PP321 or cyhalothrin of known purity.
- e) A sample of R171554 for use as an internal standard.

The internal standard used is the resolved cis isomer assumed by analogy to the chromatographic GLC characteristics of PP563 and other synthetic pyrethroids to be:

(RS)- α -cyano-3-phenoxybenzyl-(1RS)-cis-3-(Z-2-bromo-3, 3, 3-trifluoroprop-1-enyl)-2,2-dimethylcyclopropanecarboxylate.



3. PREVENTION OF CONTAMINATION

Great care must be taken when working at low levels to minimise the risk of contamination.

- a) To achieve the set limits of determination, whenever possible all analysis should be carried out in a designated 'low level' area.
- b) All glassware must be segregated and used solely for the low level analysis. If possible a washing machine should be designated for such glassware.
- c) All glassware should be thoroughly soaked and rinsed with appropriate solvents before use.
- d) Plastic and glassware for the analysis should be minimised.
- e) All new 'batches' of solvent and sorbent should be analysed (for interfering contaminants in ECD analysis) before use.
- f) Solvents should be freshly dispensed into glass vessels for each analysis and any solution which is required, should be freshly prepared.
- g) Staff must not come into contact with high residues or work in areas exposed to these.

4. HAZARDS

The following information is included as an indication to the analyst of the nature and hazards of the reagents used in this procedure. If in doubt, consult the appropriate safety manual (eg, ICI Laboratory Safety Manual) containing recommendations and procedures for handling chemicals, and monograph such as 'Hazards in the Chemical Laboratory', Ed G D Muir, The Chemical Society, London.

a) Solvent Hazards

	Acetone	Acetonitrile	Diethyl ether	Hexane	Methanol
Harmful vapour	Yes	Yes	Yes	Yes	Yes
Highly flammable	Yes	Yes	Yes	Yes	Yes
Harmful by skin absorption	-	Yes	-	-	Yes
TLV mg m ⁻³	2400	70	1200	180	260

In all cases avoid breathing vapour. Avoid contact with eyes/skin.

- b) PP321, PP563 and R171554 are synthetic pyrethroid insecticides with a mammalian toxicity (acute oral LD₅₀) in the rat in the order of 50-60 mg kg⁻¹ (PP321).

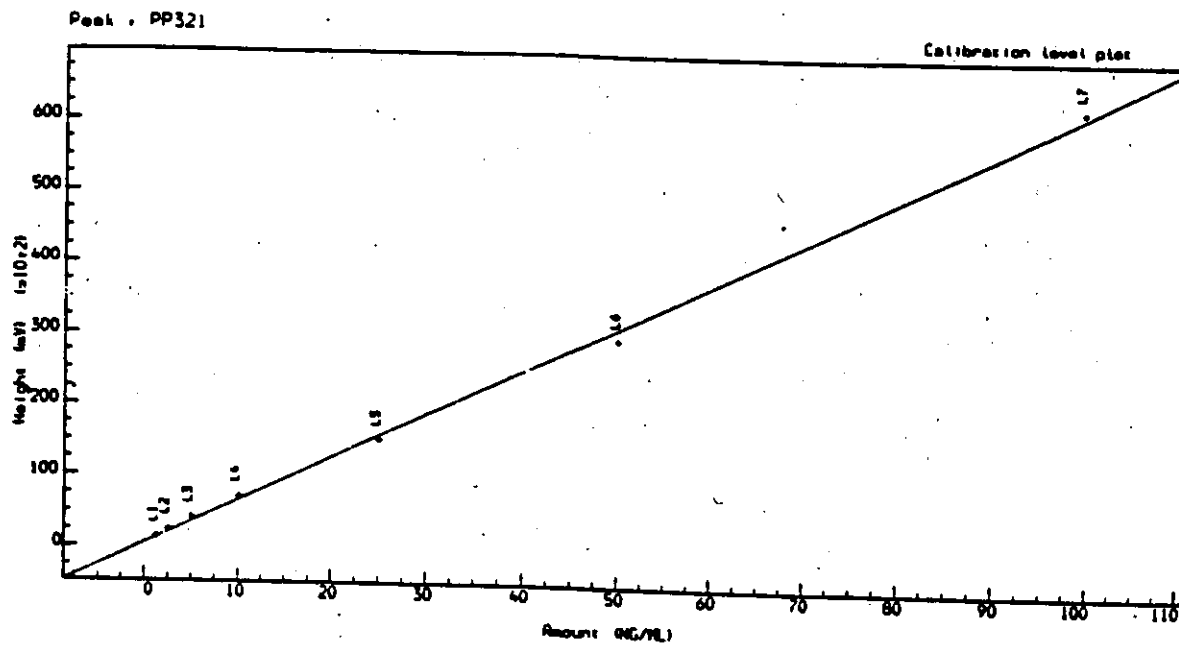
5.

PREPARATION OF ANALYTICAL STANDARDS

Weigh out accurately, using a 'five-figure' balance, sufficient PP321, PP563 or R171554 to allow dilution in acetone to give a 1000 µg/cm³ stock solution in a volumetric flask. Make serial dilutions of this stock to give 100 µg/cm³, 10 µg/cm³ and 1.0 µg/cm³ standard solutions in hexane. Prepare serial dilutions of mixed standards of PP321 and internal standard in hexane (for use as GLC reference standards) down to 2.5 ng/cm³.

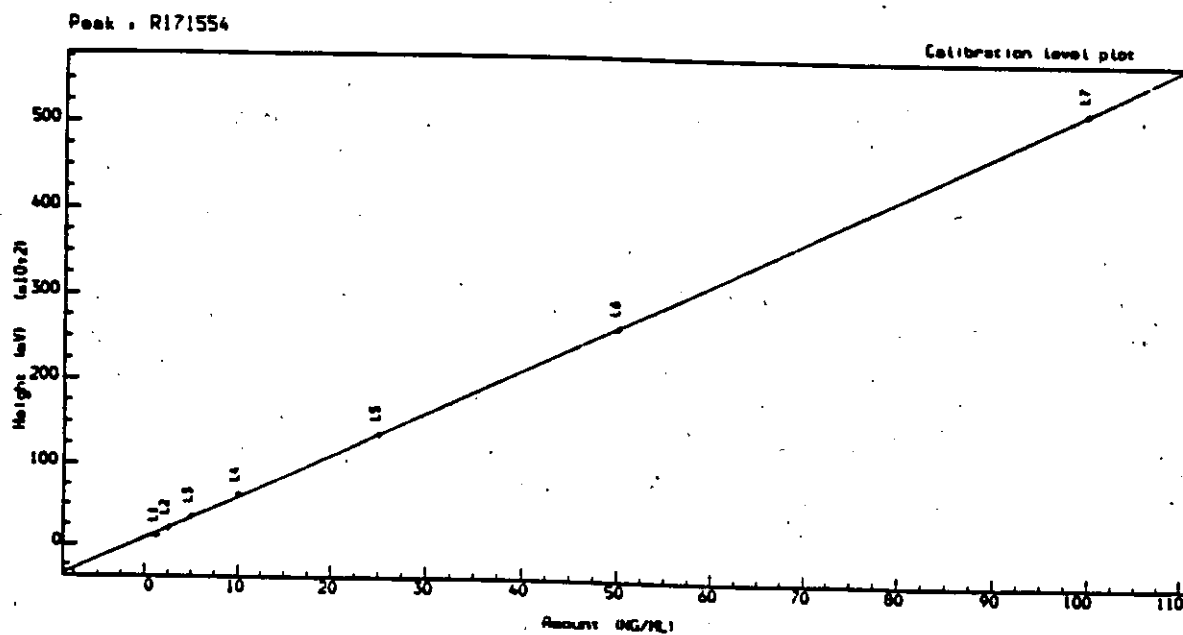
When not in use, always store the standard solutions in a refrigerator at <4°C to prevent decomposition/evaporation/concentration of the standard strength. Analytical standards should be replaced with freshly prepared standards after 3 months of use.

APPENDIX 4 - Linearity Graphs



Constant : 5.00576E+2
 1-sigma : 6.15341E+2

Curve fit : Linear
 Correlation coefficient : 0.99926



Constant : 8.38244E+2
 1-sigma : 6.14677E+2

Curve fit : Linear
 Correlation coefficient : 0.99996

APPENDIX 5 - Flow Chart Summary of Method

C6+SAX Cartridge Conditioning

- i) Methanol (25 cm³)
- ii) Water (5 cm³)

Cg+SAX Cartridge Sampling

Load at 50 cm³ min (up to 350 cm³ water).

Sample Storage

Freeze cartridges at <-18°C until required for analysis

Sample Analysis

Elution : Acetonitrile (3 x 5 cm³) - Collect

Remove excess with vacuum - Collect
Clark + Lubs buffer (1 x 2 cm³) - Discard
Remove excess with vacuum - Discard
Diethyl ether:hexane 70:30 (5 cm³) - Collect
Acetonitrile (1 x 5 cm³) - Collect

Combine

- i) Rotary evaporate to <10 cm³
- ii) Make to 15 cm³ with acetonitrile

Cg Clean Up

- i) Add ultra pure water (50 cm³)
- ii) Load into 1 g Cg cartridge at 1 cm³/min
- iii) Wash with water (5 cm³) followed by 3)
acetonitrile:water 50:50 (2 x 2.5 cm³)
Discard
- iv) Dry under vacuum for one half hour
- v) Elute with hexane (4 x 2.5 cm³)
- vi) Make to an appropriate volume for gas-liquid
chromatographic determination.