

Category A

Analytical Method GAM-RM-001/86

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ICI AMERICAS INC.
AGRICULTURAL CHEMICALS DIVISION
RESEARCH AND DEVELOPMENT DEPARTMENT

REGULATORY METHOD

Compound: PP021 REFLEX™
Crop: Soybeans

The Determination of PP021 in Soybeans - A High
Performance Liquid Chromatographic Method

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Date: October 1, 1986

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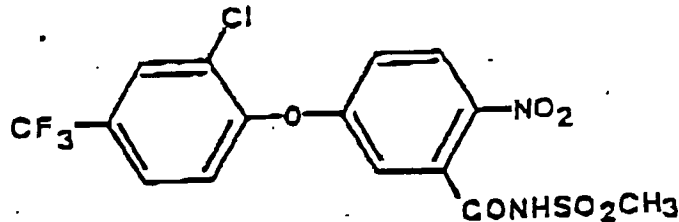
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PP021

Empirical formula: $C_{15}H_{10}O_6SClF_3$

Molecular weight: 438.7

Structural formula:



5-(2-chloro- α, α, α -trifluoro-4-tolyloxy)-N-methanesulphonyl-2-nitrobenzamide.

Scope

The Analytical procedures described are modifications of Plant Protection Division Residue Analytical Method Number 68. The modification is in the use of external standardization rather than using internal standardization. The analytical procedures described are suitable for the determination of residues of the selective herbicide fomesafen in soybean samples. The limit of determination has been set at 0.01-0.02 mg kg⁻¹.

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1. Summary

no test, 2008/04/28
after 3 months, not always, 2008/04/28
The finely ground soybean samples are extracted by shaking with acetonitrile:conc. HCL 98:2 v/v. After filtration, the extract is diluted with water, washed with hexane, and partitioned with dichloromethane. The dichloromethane layer is evaporated to dryness and the residue taken up in dichloromethane. To remove coextracted materials, the extract is subjected to ion-exchange chromatography on a NH₂ disposable Bond Elut column. The quantitative determination is by high performance liquid chromatography (HPLC) on a octadecyl bonded silica column (ODS) using UV detection at 290 nm.

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2. Procedure

2.1 Extraction

- a) Weigh a representative aliquot of finely ground soybeans (20 g) into a 250 ml round bottom flask. Add acetonitrile:concentrated hydrochloric acid, 98:2 v/v (50 ml) and shake on a wrist action shaker for 15 minutes. If shaking does not completely disperse the sample ultrasonication should be used to completely break up any coagulated material.
- b) Filter the extract through two Whatman (No. 1) filter papers under vacuum into a 250 ml graduated cylinder and wash the flask and residuum with further acetonitrile:concentrated hydrochloric acid 98:2 v/v (50 ml). Transfer an aliquot of the filtrate (= 10 g) to a 500 ml separating funnel. NB. Screw topped flasks with foil metal liners or homogenization apparatus (stainless steel) should not be used in the presence of acid as concentrated hydrochloric acid and metal have been found to react with fomesafen.

2.2 Wash

Dilute the extract with water (100 ml). Wash with hexane (2x 50 ml) and discard hexane.

2.3 Partition

- a) Check that the pH of the solution is <1. Adjust the pH if necessary by the addition of concentrated hydrochloric acid. Partition with dichloromethane (2x 50 ml).
- b) Combine the dichloromethane extracts, evaporate to dryness on a rotary evaporator and take up the residue in dichloromethane (2 ml).

3. Ion-Exchange Column Chromatographic Cleanup (see Appendix 6)

- 3.1 a) Ultrasonicate the extract from 2.3 b to ensure complete uptake of any material adhering to the flask.
- b) Place a disposable NH_2 column in the "Sepelco SPE Vacuum Manifold" assembly (see diagram Appendix 7). Wet the column with CH_2Cl_2 (2 ml). Transfer the extract onto the column. Wash the flask with 8 ml of dichloromethane and transfer to the column. Discard the dichloromethane eluate. Add 5 ml of 50:50 Acetonitrile: H_2O to the column and discard eluate. Elute the column with 10 ml of 1 Molar KNO_3 adjusted to pH = 9 with sodium hydroxide solution (1Molar) prior to using and collect the eluate.

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3.2 Partition

- a) Transfer the eluate into a 125 ml separating funnel. Wash the test tube with 5 ml of potassium nitrate (1 Molar) and add to the funnel. Acidify the solution with concentrated hydrochloric acid to pH <1. Partition with dichloromethane (2x 10 ml).
- b) Combine the dichloromethane extracts, evaporate down to a small volume (2-3 ml) on a rotary evaporator and transfer quantitatively to a graduated centrifuge tube.
- c) Blow down to dryness using clean dry air and take up the residue in acetonitrile:water pH = 3.0 (water adjusted to pH 3.0 with orthophosphoric acid) 35:65 (2 ml, 1 ml = 5 g of crop). Ultrasonicate to ensure complete dissolution of the extract and filter if necessary to remove any particulate matter. Transfer the solution to a vial for analysis by HPLC.

4. High Pressure Liquid Chromatography (HPLC) with UV Detection for the Analysis of PP021

Column: Spherex 3ODS - 3 micron octadecyl bonded silica packed in a stainless steel column 5 cm x 4.6 mm.

Mobile Phase: acetonitrile:water pH 3.0 35:65 (adjusted to pH 3.0 by addition of concentrated orthophosphoric acid to water)

Flow Rate: 1.5 ml/min

Detector: UV 290 nm 0.01 AUFS

Under these conditions PP021 gives a single peak at a retention time of 8.5 min. (See Appendix 9)

Alternate octadecyl bonded silica packed columns can be used, such as Spherisob ODS 2 - 5 micron, in 5 to 15 cm lengths. When using different columns, modifications in mobile phase may be necessary. (See Appendix 8)

5. Quantitative Determination of PP021 Residues

- a) Make injections (50 µl) of an analytical standard solution of PP021 into an HPLC operated under the conditions given in 4. When a consistent response is obtained, measure the peak height (or peak area when electronic integration facility is available) recorded for the standard solution.
- b) Inject 50 µl of the sample solution obtained from 3.2c and similarly measure the response at the retention time of PP021.

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- c) Calculate the measured residue, expressed in mg kg^{-1} using a simple proportion calculation, i.e.,

$$\frac{RS}{RA} \times \frac{VA}{VS} \times \frac{CA}{CS} = \text{measured residue (mg kg}^{-1}\text{)}$$

when:

RS = Response (peak height or area) of sample.

RA = Response (peak height or area) of analytical standard solution.

VS = Volume (μl) of sample injected.

VA = Volume (μl) of analytical standard injected.

CS = Concentration (g/ml) of final sample solution.

CA = Concentration ($\mu\text{g/ml}$) of analytical standard solution.

6. Control and Recovery Experiments

- a) Untreated control samples must be analyzed using the procedures described in order to demonstrate the endogenous substances present in the samples do not interfere with the final determination of PP021.
- b) Recovery experiments in which untreated control samples are accurately fortified with known amounts of PP021 must be analyzed alongside any batch of samples in order to determine the percentage efficiency of recovery through the procedures used.

7. Limit of Determination

The limit of determination of residues of PP021 can be assessed by carrying out recovery experiments at low levels of fortification ($0.005 - 0.02 \text{ mg kg}^{-1}$). The true limit of determination will give a final chromatographic response of at least 4x the background noise at the relevant retention time.

In these laboratories, the limit of determination has been set at $0.01 - 0.02 \text{ mg kg}^{-1}$.

8. Selectivity

Other common herbicides used on soybeans were injected on to the HPLC column under the conditions for analysis of PP021. The relative retention of these herbicides against PP021 was determined (see Table 2). None of these herbicides would interfere in the analysis of PP021.

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Table 2

Herbicide

Relative Retention of PP021

Atrazine	0.66
Fluralin	*
Chloroform	0.22
Cyanazine	0.26
Dinitramine	1.95
Diuron	0.38
Fluchloralin	4.13
Linuron	0.66
Metribuzin	0.31
Profluralin	*
Simazine	0.30

* Not detected.

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9. Confirmation of Residues

Residues of PP021 can be confirmed by gas-liquid chromatography (GLC) after methylation with diazomethane. Methylation of PP021 results in two products the N- and O-methylated derivatives.

NB. All Operations Involving Diazomethane Should Be Carried Out in a Fumecupboard

- a) Take the extract in the HPLC mobile phase and evaporate down to dryness in a stream of dry air. Take up the residuum in diazomethane (2 cm³) and allow the extract to react for 15 minutes. Add 1-2 drops of boron trifluoride in ether and leave for a further 5 minutes.
- b) Evaporate off excess reagent and take down to dryness in a stream of dry air. Take up the residuum in hexane (1 ml). Inject aliquots into a GC under the following conditions:

column: 3% OV17 on chromasorb WHP 100-120# 2m x 2mm i d gla

Oven temperature: 260°C

Injector temperature: 250°C

Detector: Electron capture detector

Detector temperature: 300°C

Carrier gas: Argon:methane 95:5

Flow rate: 60 ml/min

Methylated PP021 gives two peaks at retention times of 5.2 and 6.0 minutes under these conditions.

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Appendix 1

Safety Comments

The following information is included as an indication to the analyst of the nature and hazards of the reagents used in the procedure. If in doubt, consult the appropriate safety manual e.g., ICI Laboratory Safety Manual containing recommendation and procedures for handling chemicals and/or monographs such as "Hazards in the chemical laboratory" ED G D Muir Royal Society of Chemistry, London.

a) Solvent Hazards

	<u>Acetonitrile</u>	<u>Dichloromethane</u>
Harmful vapor	x	x
Harmful by skin absorption	x	x
Highly flammable	x	-
Vapor-air mixtures explosive	-	-
TLV PPM	40	100
TLV mg cm ⁻³	70	360

In all cases, avoid breathing vapor and contact with skin and eyes.

b) Acids (hydrochloric orthophosphoric)

These are highly corrosive and the analyst must always wear adequate protective clothing when handling the concentrate. Acids must not be diluted by the addition of water, but always by adding the acid to water.

c) Sodium Hydroxide

Sodium hydroxide is highly corrosive and contact with skin and eyes must be avoided.

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Appendix 2

Sample Preparation

Soybeans can be prepared directly after removal from the deep freeze. Thoroughly mix the whole sample and by quartering, take approximately a 200g representative sub sample. The soybeans should then be ground to produce a homogenous sample using an ultra centrifugal mill or similar equipment.

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Appendix 3

Preparation of Analytical Standards

Weigh out accurately, using a five figure balance sufficient PP021 to allow dilution in acetonitrile to give 1000 µg/ml stock solution in a volumetric flask. Make serial dilutions of this stock solution to give 100 µg/ml, 10 µg/ml and 1.0 µg/ml standard solutions in acetonitrile. These standards should be used for the fortification of recovery samples.

Also a 1.0 µg ml standard should be prepared in the mobile phase used to determine the samples.

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

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Appendix 4

Reagents

- a) Solvents: Acetonitrile HPLC Grade, Dichloromethane
HPLC Grade, Fisher Scientific, New Jersey
- b) Acids: Concentrated hydrochloric, Baker Analyzed Reagents,
New Jersey
Phosphoric Acid 85% HPLC Grade, Fisher Scientific,
New Jersey
- c) Potasium Nitrate, Fisher Scientific, New Jersey
- d) NH₂ disposable Bond Elut column, Analytichem International,
California VWR Pg 548 JT7088-3
- e) A sample of PP021 of known purity.

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Appendix 5

Apparatus

- a) Equipment for the initial preparation of samples, e.g. ultra centrifugal mill, Retsch ZMI, fitted with a 3mm screen. Available from Glen Creston, Stanmore, UK.
- b) High speed macerator, e.g. sorval Omni-mixer homogenizer, available from DuPont UK Ltd.
- c) Graduated glass centrifuge tubes of 10 cm³ capacity calibrated down to 1.0 cm³ in 0.1 cm³ units, with an accuracy of a least $\pm 1\%$ measured at 10 cm³.
- d) High performance liquid chromatograph with an ultra-violet detector e.g. Water M6000A pump, Waters U6K valve injector and a Pye LC-UV detector, or equivalent instrument.
- e) Syringe for high performance liquid chromatography, e.g. Hamilton 25 μ l.

Note: The use of an autosampler apparatus with HPLC, e.g., Waters WISP is satisfactory provided:

- a) Suitable precise injections are achieved, i.e. reproducibility better than $\pm 5\%$;
- b) No cross contamination from consecutive injections is observed and that no contamination arises in the final sample, due to autosample vials or vial caps.
- c) Potentiometer pen recorder (10 mv), e.g. Perkin Elmer 56 or equivalent instrument.

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Appendix 6

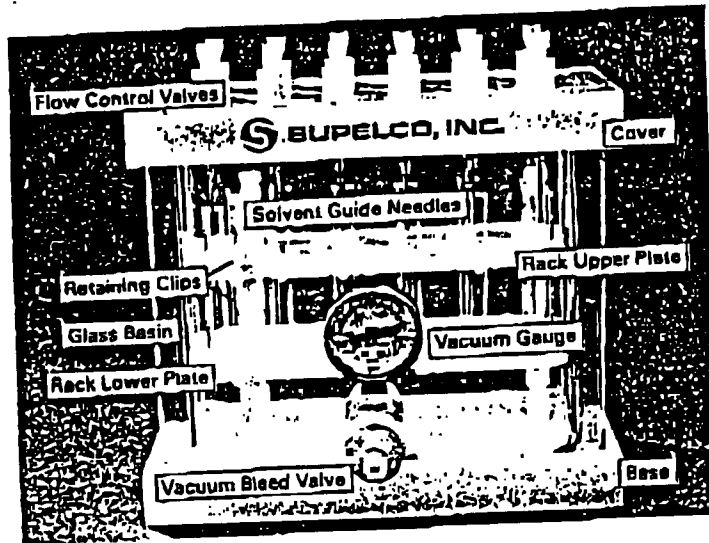
Calibration of Ion-Exchange Column Chromatographic Cleanup

Prior to use, each new batch of disposable NH_2 columns must be calibrated for the substrate to be analyzed as follows. Fortify a control sample that has been extracted according to the method through step 2.3b with 1 ml of a 10 $\mu\text{g}/\text{ml}$ PP021 standard solution in dichloromethane. Load onto the column and allow the vacuum to pull the solution down to the frit. Wash with an additional 8 mls of dichloromethane, followed by 5 ml of 50:50 acetonitrile: water. Elute the column with 1 Molar KNO_3 and collect ten fractions (2 mls each) of the eluate. Partition the fractions as described in 3.2 of the method and analyze for PP021 by HPLC to determine the elution pattern. In these laboratories, PP021 was found normally to be eluted in the first 10 mls of the 1 Molar KNO_3 fraction.

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Instructions For Vacuum Manifold

Catalog No. 5-7030



The vacuum manifold enables analysts using Supelclean solid phase extraction tubes to simultaneously prepare up to 12 samples. The manifold consists of: chemical resistant cover and gasket, glass basin, base, vacuum gauge, vacuum bleed valve, 12 replaceable solvent guide needles, and rack for sample collection vessels (base, 5 support plates, 12 retaining clips).

Using the Manifold

1. Remove cover and place a luer hub solvent guide needle on the male luer fitting of each flow control valve.
2. Remove collection vessel rack and replace cover.
3. Open vacuum bleed valve by aligning the two holes.
4. Close all flow control valves on the manifold cover by rotating clockwise until they turn no further. *Do not overtighten.*
5. Turn vacuum on at the source.
6. Place desired number of Supelclean tubes into the female luer receptacles on the cover.
7. Add conditioning solvent to each tube.
8. Grasp top of each tube and rotate 1/4 turn counter-clockwise to partially open the flow control valve.
9. Slowly close bleed valve until the gauge indicates a vacuum of 10" Hg. As solvent level in each tube nears the packing bed, rotate flow control valve clockwise to slow solvent flow. When solvent in a tube reaches the packing bed, turn the flow control valve fully off.
10. Open the vacuum bleed valve.
 11. If required by the method in use, add additional solvent and/or attach a filtration tube or reservoir to the extraction tube.
 12. Transfer samples to the extraction tubes, reservoirs, or filtration columns.
 13. Close vacuum bleed valve and open flow control valves 1/4 turn. Using the bleed valve, adjust vacuum to aspirate liquid through the tube at the desired flow rate. A dropwise flow is best, but flow rates up to 5ml/min usually are acceptable. *Do not exceed a vacuum of 25" Hg.*
 14. Open the vacuum bleed valve.
 15. Add wash solution to the tubes. Partially close bleed valve to aspirate the liquid through the tubes.
 16. Open the vacuum bleed valve. Repeat Step 15, if required.
 17. Remove cover and place rack containing collection vessels in the manifold base. (Rack assembly instructions follow.)
 18. Replace cover. Be sure each solvent guide needle extends approximately 1cm into the collection vessel.
 19. Close the flow control valves.
 20. Add elution solvent. Close bleed valve and open each flow control valve just enough to allow eluant to flow dropwise through the tubes.
 21. Open the vacuum bleed valve. Repeat Steps 19 and 20, required. Do not remove tubes from the cover before vacuum is fully released, or eluate will splash onto the manifold interior.
 22. Remove the collection rack. Dilute, evaporate, or analyze samples as required.

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Appendix 8

Alternative Mobile Phases for HPLC Determination

If interfering peaks occur at the retention time of PP021, these can often be avoided by varying the ratio of acetonitrile to water pH 2.5 in the mobile phase or by the addition of either tetrahydrofuran or methanol in replacement of part or all of the acetonitrile i.e. tetrahydrofuran: acetonitrile: water pH 2.5 30:20:50.

If this is not sufficient, additional cleanup can be achieved by HPLC on a cyanopropyl bonded silica column.

Column: 25 cm x 8 mm i.e. stainless steel column packed with Spherisorb 5 nitrile (Spherisorb CN)

Mobile Phase: dichloromethane: glacial acetic acid (99.8 : 0.2)

Flow Rate: 4 ml/min

Detector: UV wavelength 290nm 1.28 AufS

Under the above conditions, PP021 gives a single peak at retention time 8.7 mins. The fraction is collected from a peak width each side of the peak.

Procedure for column cleanup by HPLC.

- a) After cleanup on the Fractosil 200 column (2g), evaporate the methanol chloroform eluate to dryness. Take up the residue in dichloromethane (5-6 ml) and transfer to a graduated centrifuge tube.
- b) Reduce the volume to exactly (1 cm³). Filter the solution to remove any particulate matter, inject 0.5 cm³ of the extract (0.5 ml = 5 g) onto the HPLC column. Elute the PP021 under the above conditions and collect the fractions at the appropriate time.
- c) Evaporate the collected fraction just to dryness and take up the residue in acetonitrile: water 50:50 (0.5 ml = 10 g/ml). Analyze by HPLC (see 5).

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Appendix 9

Example Chromatograms

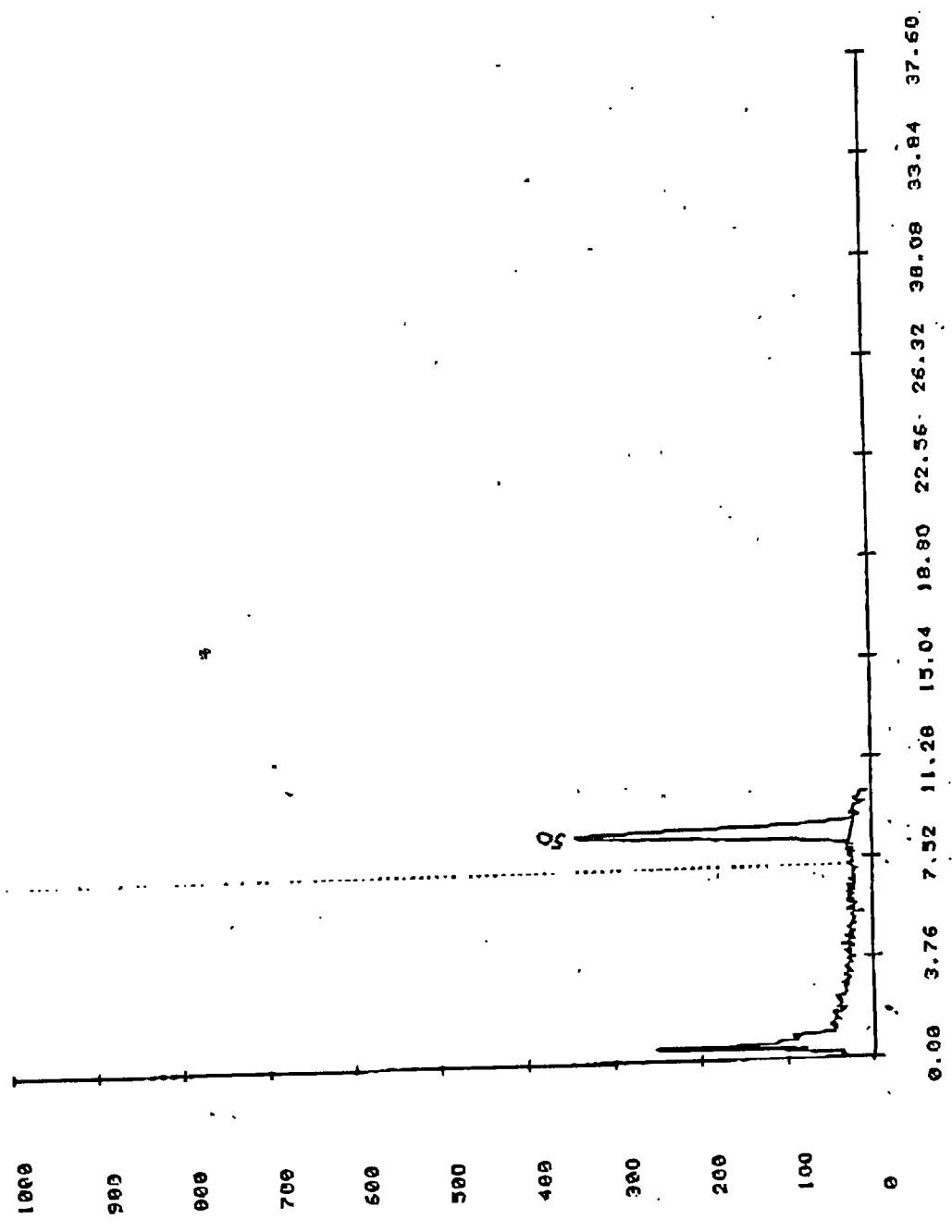
Column: Spherex 30DS 5 cm x 4.6 mm
Mobile Phase: Acetonitrile: H₂O pH = 3.0 35:65
UV: 290 nm 0.01 AUFS
Flow Rate: 1.5 ml/min

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STD

SAMPLE NO.: 19-01 .01
 TEST NO.:
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 INSTRUMENT: 05
 DATE TIME: 09/23/86 12109112
 CHART SPEED: 0.5 BUNCH: 0



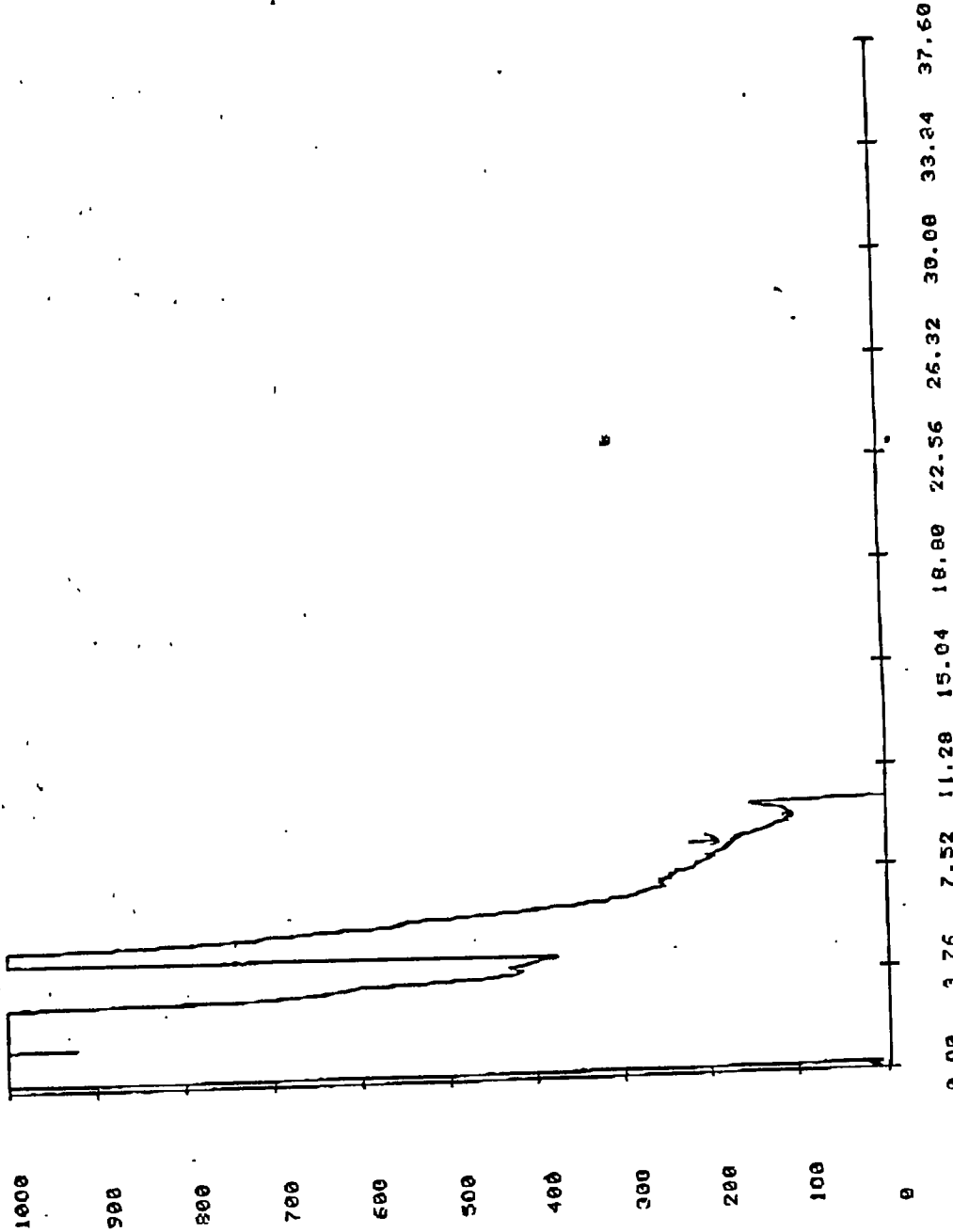
RETENTION TIME (MINUTES)

Y MAXIMUM: 1000.
 Y MINIMUM: 0.
 START TIME: 0.00
 END TIME: 10.00

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CONT HEXANE

SAMPLE NO.: 13/02 .01
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 DATE TIME: 09/23/06 12:20:18
 METHOD NO.: 111515
 CHART SPEED: 9.5 RUNCHE: 0



RETENTION TIME (MINUTES)

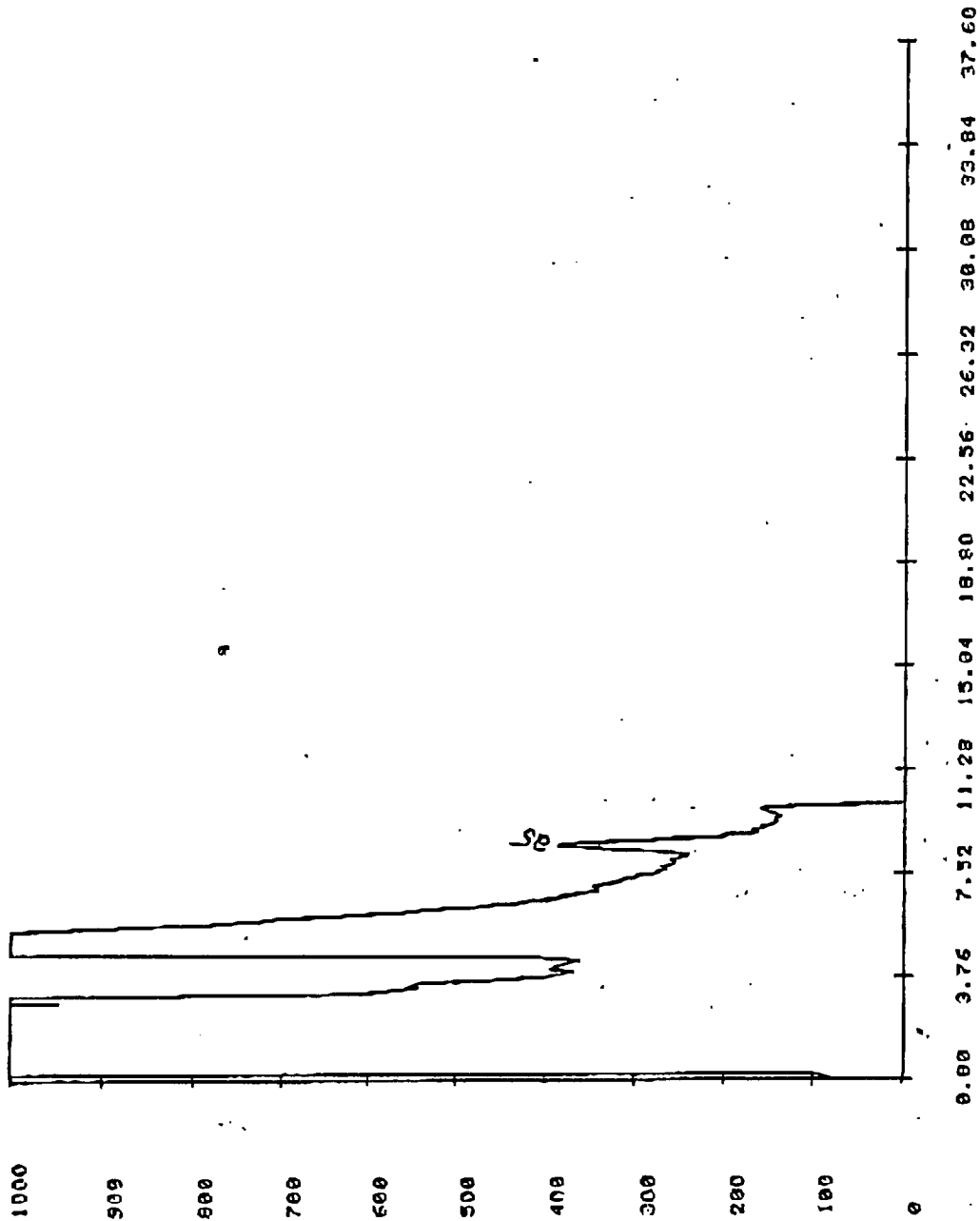
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 END TIME: 10.00

Y MAXIMUM: 1000.

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0.1PPM HEXANE #1

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 METHOD NO.: 11813 CHART SPEED: 0.5 RICH: 0



RETENTION TIME (MINUTES)

Y MAXIMUM: 1000 START TIME: 0.00
 Y MINIMUM: 0 END TIME: 37.60