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PLANT PROTECTION DIVISION RESIDUE

ANALYTICAL METHOD NO. 62/2

THE DETERMINATION OF RESIDUES OF TOTAL FLUAZIFOP

(FLUAZIFOP-BUTYL, FLUAZIFOP AND CONJUGATE ESTERS)

IN CROPS-an Internal Standard Procedure

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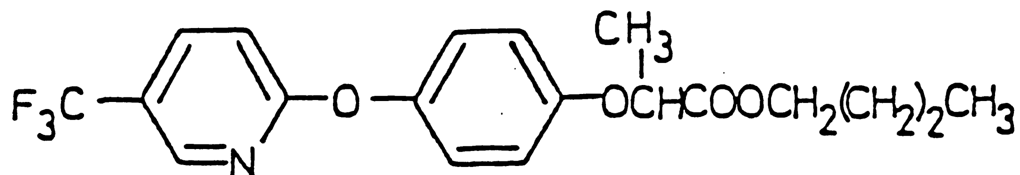
FLUAZIFOP-BUTYL - CHEMICAL PROPERTIES

Chemical Name : Butyl 2-[4-(5-trifluoromethyl-2-pyridyloxy) phenoxy] propionate
Code Number : PP009
Empirical Formula : $C_{19}H_{20}O_4NF_3$

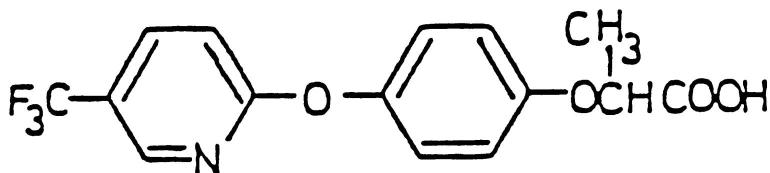
Molecular Weight : 383
Structural Formula :



FLUAZIFOP - CHEMICAL PROPERTIES

Chemical Name : 2-[4-(5-trifluoromethyl-2-pyridyloxy)phenoxy] propionic acid
Empirical Formula : $C_{15}H_{12}O_4NF_3$

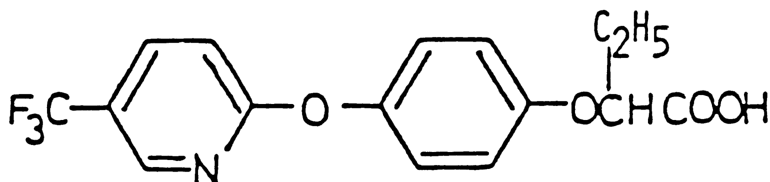
Molecular Weight : 327
Structural Formula :



INTERNAL STANDARD - CHEMICAL PROPERTIES

Chemical Name : 2-[4-(5-trifluoromethyl-2-pyridyloxy)phenoxy] butyric acid
Empirical Formula : $C_{16}H_{14}O_4NF_3$

Molecular Weight : 341
Structural Formula :



1. SCOPE

This method is suitable for the direct determination of fluazifop-butyl, fluazifop and its conjugates in various crops eg, sugarbeet, flax, oil seed rape, potatoes etc.

The limit of determination is 0.02-0.05 mg kg^{-1} according to the level of co-extractives in the different crops.

2. SUMMARY

Samples are soaked in water or 1M hydrochloric acid solution for a minimum of two hours, prior to fortification with internal standard and extraction of residues by homogenisation with acetonitrile : conc. hydrochloric acid solution (98:2) or acetonitrile respectively. The presoak is not necessary for "wet" vegetable crops such as potatoes and sugarbeet.

The extracts are evaporated to aqueous volume, and hydrolysed with 6M hydrochloric acid solution. Any ester or acid conjugates present are converted to fluazifop[1]. Samples are then diluted with water and partitioned into diethylether. A "coagulation procedure" is then carried out to remove some of the proteins, fats and oil, etc., after which the extracts are partitioned with dichloromethane and sodium bicarbonate solutions.

Remaining co-extractives are removed by adsorption chromatography using disposable silica columns. For some crops eg hops and cotton, a further normal phase High Performance Liquid Chromatography (HPLC) clean-up may be necessary.

Quantitative determination of total fluazifop is by HPLC using an Ultra Violet (UV) detector.

3. REAGENTS

All solvents and other reagents must be of high purity ie, glass distilled/HPLC grade solvents and analar grade reagents.

If the source of reagent supply has not previously been evaluated, then individual reagents should be examined for possible interferring impurities prior to analysis.

(a) Solvents : acetonitrile, diethylether, dichloromethane, n-hexane and methanol - Rathburn Chemicals Limited. (Walkerburn, Peebleshire) Scotland.

Particular care must be taken to avoid contact with materials, eg, plastic, which may contaminate the solvents.

(b) Acids :- Concentrated hydrochloric, ortho-phosphoric (88-93%), glacial acetic - Analar grade BDH.

(c) Ammonium chloride, sodium hydrogen carbonate - Analar grade BDH.

(d) Anti foam emulsion M30 - Hopkin and Williams

(e) BOND ELUT™ disposable extraction columns (2.8 cm^3) containing 500 mg unbonded silica.
Supplier :- Jones Chromatography Ltd UK, (item 601303).
Manufacturer :- Analytichem International Inc USA.

(f) Standards (Fluazifop-butyl, Fluazifop and Internal) of known purity which should be >98%.

(g) Celite 545 filter aid (Johns - Manville Corp. USA or Phase Separations Ltd UK).

3.1 Reagent Preparation

(a) Coagulation solution: Dissolve 6.25 g of ammonium chloride and 10ml of orthophosphoric acid in 1 litre of glass distilled water.

4. SAFETY COMMENTS

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 a monograph such as 'Hazards in the Chemical Laboratory', Ed G D Muir, The Chemical Society, London.

(a) Table 1 Solvent Hazards

Hazards	Acetonitrile	Diethyl ether	Methanol	n-hexane	Dichloromethane
	Solvents				
Harmful vapour	✓	✓	✓	✓	✓
Harmful if taken internally	✓	✓	✓	✓	✓
Highly flammable	✓	✓	✓	✓	-
Avoid breathing vapour	✓	✓	✓	✓	✓
Avoid contact with skin/eyes	✓	✓	✓	✓	✓
TLV ppm	40	400	200	100	100
TLV mg m^{-3}	70	1200	260	360	360

(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 to water.

(c) Fluazifop-butyl, fluazifop and internal standard

It is recommended that the following handling precautions should be taken when weighing the analytical standard material.

1. Ensure good ventilation.
2. Wear gloves and laboratory coat.
3. Prevent inhalation and contact with mouth.
4. Wash any contaminated area immediately.

5. APPARATUS

(a) Equipment for the initial preparation of samples eg,

1. Hobart mincer. Available from Glen Creston, Stanmore, UK.

2. An ultra centrifugal mill, Retsch ZM1, fitted with a 3 mm screen - (Glen Creston).

(b) High speed macerator, eg, laboratory mixer emulsifier available from Silverson Machines Ltd UK, or Sorvall Omni-mixer homogeniser, available from Du Pont UK Ltd.

(c) Graduated glass centrifuge tubes of 10 cm^3 capacity calibrated down to 1.0 cm^3 in 0.1 cm^3 units, with an accuracy of at least ± 1% measured at 10 cm^3.

(d) A thermostatically controlled water bath.

(e) Complete VAC ELUT™ ten place vacuum manifold assembly with sample collector rack (for use with BOND ELUT™ disposable extraction columns). Jones Chromatography Ltd, UK.

(f) 'Whirlimixer' - Fisons Scientific Apparatus, UK.

(g) Toughened glass centrifuge bottles, 'nominal' size 100 cm^3 (capacity 122 cm^3) - MSE.

(h) High performance liquid chromatograph (HPLC) fitted with UV detector eg, Water 6000A pump coupled to a U6-K manual injector or 710A Waters Intelligent Sample Processor (WISP) , with a Pye Unicam LC3 UV detector, or equivalent system.

(i) Potentiometric pen recorder (10mv), eg, Perkin Elmer 56 or equivalent.

6. PROCEDURE

6.1 Preparation of Analytical Standards

(Fluazifop-butyl, Fluazifop and Internal standard).

Weigh out accurately, using a five figure balance sufficient of the standard to allow dilution in acetonitrile to give a 1000 $\mu g\ cm^{-3}$ stock solution, in a volumetric flask. Make serial dilutions of this stock to give 100 $\mu g\ cm^{-3}$, 10 $\mu g\ cm^{-3}$, and 1.0 $\mu g\ cm^{-3}$ standard solutions in acetonitrile.

These standards should be used either to provide external standard recoveries by fortification of untreated controls, or to provide internal standard recoveries by fortification of all samples (excluding controls).

Also a mixed 1.0 µg cm^{-3} standard of fluazifop and internal standard should be prepared in acetonitrile : water (50:50) for HPLC use.

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 four months of use.

6.2 Sample Preparation

(a) Dry Crops (eg, grain or seed crops)

These may be prepared directly after removal from the deep freeze. Loose chaff/pods should initially be removed. Thoroughly mix the whole sample and by quartering, take approximately a 200 g representative subsample. Samples should then be ground/powdered using an ultra centrifugal mill or similar equipment.

(b) Wet Crops (eg, root/tuber/vegetable/fruit)

Samples should be allowed to thaw for one to two hours prior to preparation. During this time adhering soil should be removed be rinsing briefly in running cold water or by gentle brushing of the dry commodity.

Roots and tops should be separated for individual analysis where applicable. Chop and mix the whole sample thoroughly using a laboratory mincer eg, Hobart.

6.3 Reagent Blanks/Controls

While all the reagents and apparatus may have been individually checked for purity, it is necessary to analyse reagent blank samples, ie, where the complete procedure is carried out in the absence of crop.

This will enable the analyst to verify that the analytical procedure produces an HPLC trace which is free of interference at the retention times of fluazifop and internal standard and thus ensures that no contamination of samples occurred prior to or during the analysis.

Routinely at least one untreated sample and a reagent blank should be analysed alongside each set of samples analysed. They should not be fortified with internal standard.

6.4 Recoveries and Internal Standard

Either a minimum of two external recovery experiments should be run alongside each set of samples analysed; that is untreated samples accurately fortified with a known amount of fluazifop-butyl or fluazifop prior to extraction. Or all samples, excluding controls, should be fortified with internal standard prior to extraction.

For external standard recoveries fortification levels should be based on the expected crop residue levels. When no residues are expected eg, long harvest interval samples, then recoveries should be fortified at low levels typically 0.1 mg kg^{-1} and include at least one fortified at the limit of determination.

For internal standard recoveries fortification should be at a level to provide a similar peak height to that of the standard on final determination. Typically 0.5-1.0 mg kg^{-1} with a final crop to solvent ratio of 2g cm^{-3}.

6.5 Sample Pretreatment

To ensure efficient extraction of fluazifop and fluazifop-butyl, it is necessary to soak dry/seed crops for a minimum of two hours (preferably overnight) with 1M hydrochloric acid solution prior to solvent extraction. Wet crops do not require any sample pretreatment.

6.6 Extraction

6.6.1 Dry/Seed Crops

Weigh a representative sample of powdered crop (10 g) into a glass centrifuge bottle (100cm^3 size) and add 1M hydrochloric acid solution (20 cm^3). Allow samples to stand overnight at room temperature (or for a minimum of two hours). Add acetonitrile (50 cm^3) and homogenise at high speed for five minutes.

6.6.2 Wet Crops

Weigh a representative sample (10g) into a glass centrifuge bottle (100 cm^3 size) and add freshly made 98:2 v/v acetonitrile : conc HCl solution (50 cm^3). Homogenise at high speed for five minutes.

6.7 Filtration, Hydrolysis and Partition Clean-up

(a) Filter the homogenate from either 6.6.1 or 6.6.2 through two Whatman No 5 (9 cm) papers into a 250 cm^3 round bottomed flask. Wash the macerating jar and solid residuum with further solvent (50 cm^3).

(b) Concentrate the extract by rotary evaporation until all the acetonitrile has evaporated and only the aqueous solution remains.

Notes (i) When rotary evaporation stages are employed water bath temperature should not exceed 40°C.

(ii) Prior to evaporation at this stage some crop samples may require the addition of a small amount of antifoam emulsion reagent (1-2 drops).

(c) Hydrolyse any fluazifop-butyl or conjugates by the addition of 6M hydrochloric acid solution (20 cm^3). Loosely stopper the flask and place in a heated water bath at 60°C. Allow the flask contents time to attain water bath temperature, then leave for one hour with occassional agitation. Remove from water bath and cool.

(d) Carefully transfer the total extract to a 250 cm^3 separating funnel, washing the round bottom flask with glass distilled water (80 cm^3). Check the pH of the solution is <1, add diethylether (100 cm^3) and extract the fluazifop by shaking for 2 minutes. Discard the aqueous layer.

(e) Transfer the diethylether to a 250 cm^3 round bottom flask, wash the separating funnel with diethylether (20 cm^3) and transfer the washing to the round bottom flask. Concentrate the extract to dryness on a rotary evaporator.

(f) Dissolve the residuum in acetone (10 cm^3) and add freshly prepared coagulation solution (50 cm^3) and celite (2g). Shake the flask for thirty seconds and allow to stand for ten minutes. Filter the solutions under gravity through a Whatman No. 1 (15 cm) filter paper into a 250 cm^3 separating funnel. Wash the flask and residuum with further coagulation solution (100 cm^3) containing 10% acetone.

(g) Adjust the pH of the solution to <1 by adding a few drops of conc. HCl solution. Extract the fluazifop by shaking with dichloromethane (50 cm^3) for two minutes. Discard the aqueous layer.

(h) Shake the dichloromethane with 1% sodium bicarbonate solution (100 cm^3) for two minutes, discard the dichloromethane.

(i) Acidify the aqueous fraction using concentrated hydrochloric acid solution. Check that the pH is ≤ 1. Extract the fluazifop by shaking with dichloromethane (50 cm^3) for two minutes. Discard the aqueous layer.

Note :

Care should be taken when shaking since carbon dioxide is liberated from the sodium bicarbonate on acidification.

(j) Transfer the dichloromethane extract to a 250 cm^3 round bottom flask. Evaporate down to dryness on a rotary evaporator.

Note :

It is not necessary to dry the extracts over anhydrous sodium sulphate provided that care is taken to exclude any of the aqueous solution from the round bottom flask.

The sample should be completely dry prior to column chromatographic clean-up.

6.8 Adsorption Column Chromatographic Clean-up

Table 2 Composition of Solvent Solutions Required for Adsorption Column Clean-up

Solution	Dichloromethane	n-hexane	acetic acid	methanol
A	100	-	0.5	1.5
B	40	60	0.5	1.5

(a) Dissolve the residuum from section 6.7 (j) in acetone (10 cm^3) and ensure complete uptake of any material adhering to the flask.

(b) Remove an aliquot (5 cm^3) of the sample extract and transfer to a test tube. Concentrate the aliquot to dryness under a gentle stream of clean dry air and take up the residuum in solution A (1 cm^3).

(c) Place a disposable silica column in the 'Vac Elut™' assembly (see diagram appendix I). Add Solution B (2.5 cm^3) to the column; turn on the vacuum supply and draw the solution through to the level of the column frit (approx flow 2.5 cm min^{-1} or less). Turn off vacuum.

(d) Transfer the sample extract onto the column, turn the vacuum on and draw the sample solution through the column. Turn off vacuum.

(e) Add 2cm^3 of solution B to the column, turn on the vacuum, and draw solution through. Turn off vacuum.

Note :

All column solutions above may be discarded.

(f) Insert a rack containing labelled tubes (5 cm^3 capacity) into the Vac Elut assembly. Elute the column with a further (5 cm^3) of solution B drawing solution through under vacuum into the collecting tube.

(g) Evaporate the sample eluate to dryness and dissolve the residuum in an appropriate volume of 50% acetonitrile in water eg, 2.5 cm^3 giving ≡ of 2g ml^{-1} prior to HPLC determination.

Note:

Prior to running samples it is recommended to check the elution pattern of fluazifop and the internal standard in the presence of the crop extract. The elution pattern may be checked as follows:-

The untreated crop extract (5g) in acetone is fortified with 10µg of both fluazifop and internal standard. This solution is evaporated to dryness and the residuum taken up in solution A (1 cm^3).

Load the fortified extract onto the top of a prewetted silica column as (c), (d),and draw the solution through under gentle vacuum.

Elute the column with total of 10 cm^3 of solution B collecting the eluate in 1 cm^3 fractions. Analyse each by HPLC to enable the elution pattern of both fluazifop and internal standard to be determined.

Fluazifop and internal standard are normally eluted within the 2 to 7 cm^3 fraction of solution B.

Some samples eg, oil seed rape, cotton may require an additional clean-up procedure as follows.

6.9 High Performance Liquid Chromatography Clean-up

Where samples require an additional clean-up procedure the following conditions have been found to be satisfactory.

Silica column	:	25cm x 4.9 mm i.d. stainless steel
Packing	:	Silica 'Zorbax Sil' (Du Pont)
Particle characteristics	:	Surface area (m^2g^{-1}) - 300 Particle size (µm) - 7.5

Mobile phase	: Dichloromethane : Hexane : Acetic acid : Methanol 40 : 60 : 0.5 : 0.7
Flow rate	: 3 cm^3 min^{-1}
Wavelength	: 270 nm

For typical trace see Appendix 2

Notes : (i) The use of a suitable guard column is recommended
(ii) The mobile phase should be degassed by filtration and then purged with helium for a few minutes prior to use
(iii) When using automatic injection systems (eg, WISP 710A) slow syringe speeds should be used and a volume verification check carried out
(iv) Other columns have been evaluated and may be satisfactory, these are summarised in Appendix 2.

(a) Take up the sample residuum from section 6.8 (g) in 0.5 cm^3 of Dichloromethane 100 : acetic acid 0.5: methanol 0.7 (≡ 10g cm^{-3}).

(b) Also prepare a mixed internal and fluazifop standard (10 µg cm^{-3}) in a similar solution Dichloromethane 100: acetic acid 0.5 : methanol 0.7.

Note : The solution used for taking up samples and standards does not contain any hexane.

(c) Inject 100 µl of the mixed internal and fluazifop standard (10 µg cm^{-3}) into the HPLC under the conditions described above. Determine the elution time for the start (TS) of the internal standard peak and finish (TF) of the fluazifop peak. Repeat injections until a consistent time is obtained.

(d) Inject the sample extract (100 µl) from (a) ≡ 1g crop and collect the appropriate fraction (ie, TS to TF plus 15 to 30 seconds either side of retention window) in a suitable vial. Re check the elution time of the standard peaks every 2-3 sample injections.

(e) Evaporate the eluate to dryness and take up the residuum in 50% acetonitrile in water (0.5 cm^3) ≡ 2g crop cm^{-3}, prior to determination by reverse phase HPLC.

6.10 Determination by HPLC

The following conditions were found to be satisfactory using a Waters 6000A pump coupled to 710A Waters Intelligent Sample Processor (WISP) or manual injections using a Waters U6-K, with a Pye Unicam LC3 UV detector.

(i)	Column	: 15 cm x 4.6 mm i.d. stainless steel
(ii)	Column packing	: 'Altex' ultrasphere ODS 5 μm
(iii)	Mobile phase	: Acetonitrile : water (50:50) containing 0.4% acetic acid.
(iv)	Flow rate	: 1.5 to 2 cm^3 min^{-1}
(v)	Wavelength	: 270 nm
(vi)	Chart speed	: 5 mm min^{-1}

Using these conditions the retention times of fluazifop and internal standard are approximately 3.3 and 4.8 minutes respectively. Sensitivity is such that 5 x 10^{-8}g of fluazifop injected on column gives a response of approximately 30% f.s.d with electrometer absorption unit at 0.01 and potentiometric recorder set on 10 mv range. A similar response is also obtained for the internal standard. A typical trace is included appendix 3.

(a) Make repeated 50 µl injections of a mixed internal and fluazifop standard solution in acetonitrile : water (50:50) 1 µg cm^{-3} onto the HPLC operated under the conditions described above. Measure the peak heights/areas obtained for the standard solution.

(b) Make an injection (50 µl) of the sample extract solution and measure the recorded heights/areas at the retention times of fluazifop and or internal standard.

Note : This method of quantitative estimation is most accurate when peak heights/areas compared are of similar size.

If contamination peaks due to crop co-extractives occur at the retention times of either fluazifop or the internal standard and these interfere with the determination; or to confirm any sample residue, the following mobile phase should be used.

Alternate mobile phase : methanol : acetonitrile : water (20:30:50) containing 0.4% acetic acid.

A typical trace for this system is included in appendix 3. The methanol content of this phase may be altered to give the required separation.

7. CALCULATION OF RESULTS

Calculate the measured fluazifop residue in the sample extracts in $mg\ kg^{-1}$, by proportionation of the sample response to the mean standard response from the injections bracketing the sample ie,

$$\text{Total fluazifop residue} = \frac{PK(S)}{PK\ (Std)} \times \frac{W\ (Std)}{I(S)} \times \frac{V(S)}{W(S)} \quad (mg\ kg^{-1})$$

Where

PK(S) = Peak height or area recorded in sample (cm)
PK(Std) = Peak height or area recorded in reference standard (cm)
W(Std) = Weight of fluazifop in reference standard (nanograms).
I(S) = Volume injected of sample solution (microlitre)
V(S) = Volume of solvent in sample solution (millilitres)
W(S) = Weight or volume of sample equivalent in sample solution (grams of millilitres)

Similarly calculate the internal standard residue figure for each sample. Express each result in terms of percent recovery ie.

$$\frac{\text{Internal standard residue determined } (mg\ kg^{-1}) \times 100}{\text{Internal standard fortification level } (mg\ kg^{-1})}$$

8. CONTROL AND RECOVERY EXPERIMENTS

Control and internal/external recovery experiments should be completed as sections 6.3 and 6.4 for each set of samples analysed. Provided the recovery values obtained are acceptable they may be used to correct the fluazifop residue levels calculated in section 7.

Results should be corrected to two significant figures or one significant figure if residue <0.1 $mg\ kg^{-1}$.

Recovery data is generally considered acceptable when the mean value is about 85% with confidence limit <±15%.

Note : For external standard recoveries fortified with fluazifop-butyl, the final calculated residue should be multiplied by 1.17 (molecular weight factor).

9. 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 with either fluazifop-butyl or fluazifop and subjecting them to the complete analytical procedure. The chromatographic response obtained for these recoveries at the retention time of fluazifop 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 ± 15%.

In these laboratories the limit of determination has been set between 0.02-0.05 mg kg^{-1}.

10. EXTRACTABILITY AND STORAGE STABILITY STUDIES

10.1 Extractability

Fluazifop-butyl when applied under field conditions degrades rapidly[2] and therefore no residues of fluazifop-butyl are likely to be present in the harvested crops. A limited extractability study was carried out on a weathered sample of oil seed rape. Of the solvent systems tried acetonitrile: water (90:10) was found to be the most suitable since it removed fewer co-extractive materials than other solvents eg, methanol : dichloromethane.

A detailed extractability was carried out for fluazifop in soybean[3]. It was found that sample pre-treatment prior to extraction of fluazifop was essential. Only a cold extraction procedure is necessary after sample pre-treatment.

The following systems were found to be the most effective.

(i) Soak with 1M hydrochloric acid overnight or for a minimum of two hours prior to extraction with acetonitrile.

(ii) Soak with water overnight prior to solvent extraction with freshly prepared acetonitrile : conc. hydrochloric acid (98 : 2).

10.2 Storage Stability

Storage stability data indicates that residues of total fluazifop in sugarbeet [4], cauliflower, green beans, oil seed rape, strawberries [7] and soybean [5] are stable for at least nine to twelve months when stored at -20°C ± 2°C.

11 CONFIRMATION OF RESIDUES

11.1 Gas Chromatography - Mass Spectrometry (GC-MS)

Combined gas chromatography - mass spectrometry (GC-MS) operated in the Selected Ion Monitoring (SIM) mode may be used for the qualitative and quantitative confirmation of residues of derivatised fluazifop* down to levels at the limit of determination ie, 0.01 mg kg^{-1}. Samples obtained from the residue analytical method for total fluazifop are examined by SIM ie, two or more of the most abundant m/z values present in the mass spectrum are continuously monitored throughout the gas chromatographic run and recorded using a multi-channel pen recorder. 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 addition the ratios between the peak height, or area, reponses given for each m/z value should be identical to that given by an analytical standard of derivatised fluazifop.

Quantitative confirmation of derivatised fluazifop residues is carried out by comparison of the peak height, or area, measured for the most abundant m/z value recorded, against an external standard of derivatised fluazifop.

The selectivity of the technique is such that high crop to solvent ratios eg, 20g cm^{-3} may be injected (5 µl) into the instrument.

A typical trace is included in appendix 4.

Note :

DIAZOMETHANE [6] (ethereal solution)

Diazomethane is carcinogenic and should be treated with great care. Solutions should be stored in a closed glass vessel at -10°C or below, (Ground glass stoppers should not be used).

There is an extreme risk of explosion by shock, friction, fire or other sources of ignition. It is toxic by inhalation (TLV 0.2 ppm/0.4 mg m^{-3}) and MUST only be used behind a safety screen in a well ventilated fume cupboard.

*The derivatisation of fluazifop and internal standard is by methylation with diazomethane. The sample eluent from section 6.8 g or 6.9 e is blown to dryness and diazomethane (2 cm^3) added. React for a minimum of 30 minutes. Evaporate the solution to dryness and dissolve the residuum in hexane for GC-MS determination.

11.1.1 Gas Chromatography

a) Column 120 cm x 0.2 cm id, 3% OV17 on Chromosorb WHP (80-100#), carrier gas (helium) flow rate 30 cm^3 min^{-1}.

Oven temperature 220°C; injection port temperature 250°C. The mass spectrometer jet separator interface should be maintained at 220°C. Using the above GLC conditions the methyl ester of fluazifop gives a single peak at a retention time of 4.4 minutes.

11.1.2 Mass Spectrometry

Electron impact mode; source pressure 1 x 10^{-6} Torr, source temperature 240°C, energy 70eV, filament current 180µA (trap stabilised) accelerating voltage 4 kV.

Resolution for SIM set at 500 (10% valley definition) with flat topped peaks.

The full mass spectrum of the methyl ester of fluazifop is shown in Figure 1.

The Selected Ion Monitoring (SIM) unit is programmed to initially monitor the following m/z values in the spectrum of the methyl ester of fluazifop.

Fluazifop (methyl ester)

m/z monitored	ion species	% relative abundance
341	M^+	100%
322	$M-F^+$	5%
282	$M-CO_2CH_3{}^+$	98%
254	$M-CHCO_2CH_3$	80%

Note - For quantitative determination usually only one ion (that giving the highest signal/noise ratio) is used.

Figure 1

12. REFERENCES

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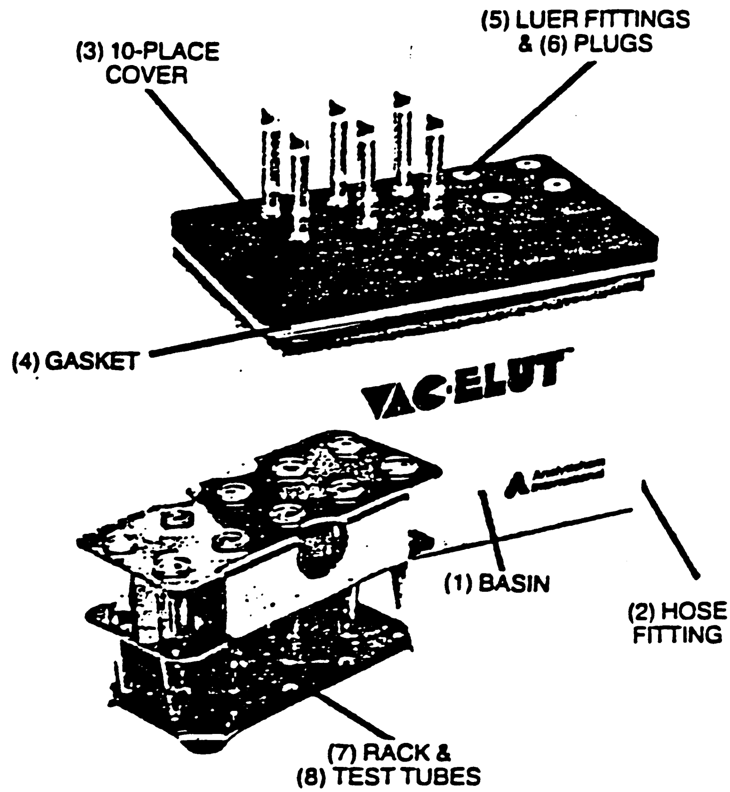
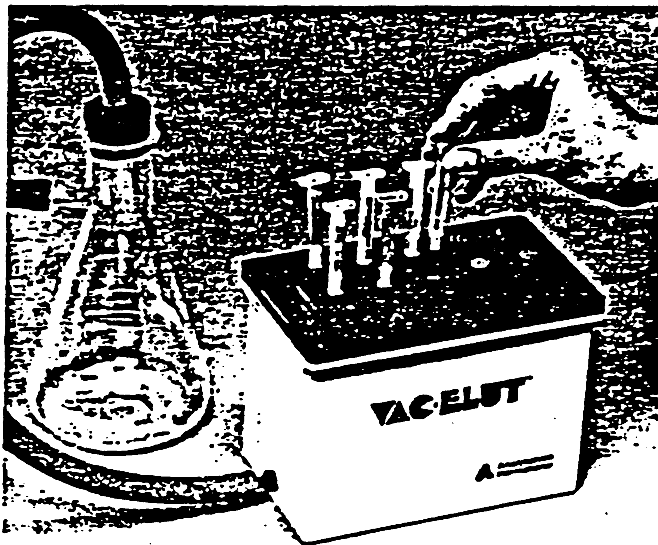
Disc No : 61 PPRAM 62/1
Date : Dec 82
Ref No : NCA/HPD/BU/AM

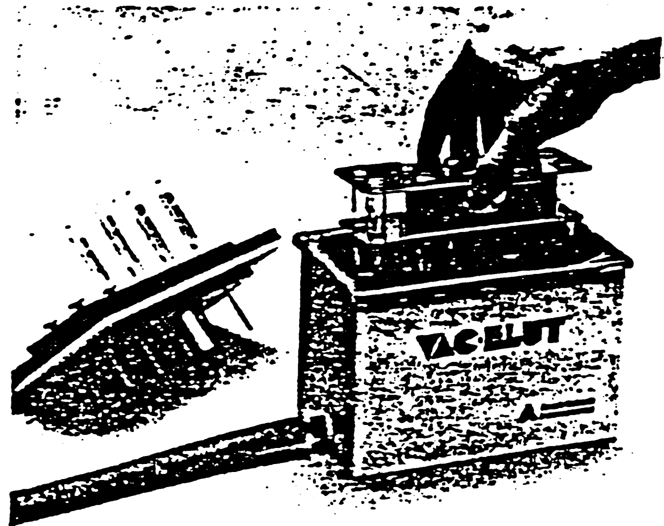
APPENDIX 1

VAC-ELUT is a ten place vacuum manifold which will process up to ten samples simultaneously, through the wash, sample extraction and elution steps.

Washes are collected in a trap attached to the manifold. Sample eluants are collected in tubes positioned in a removable rack placed inside the unit.





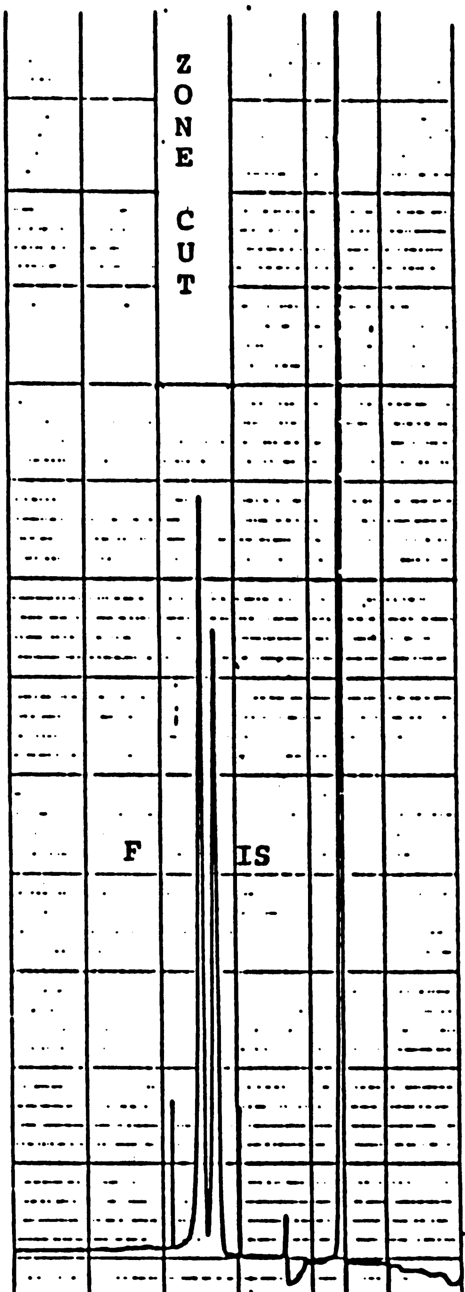


APPENDIX 2

HPLC CLEAN-UP TRACE

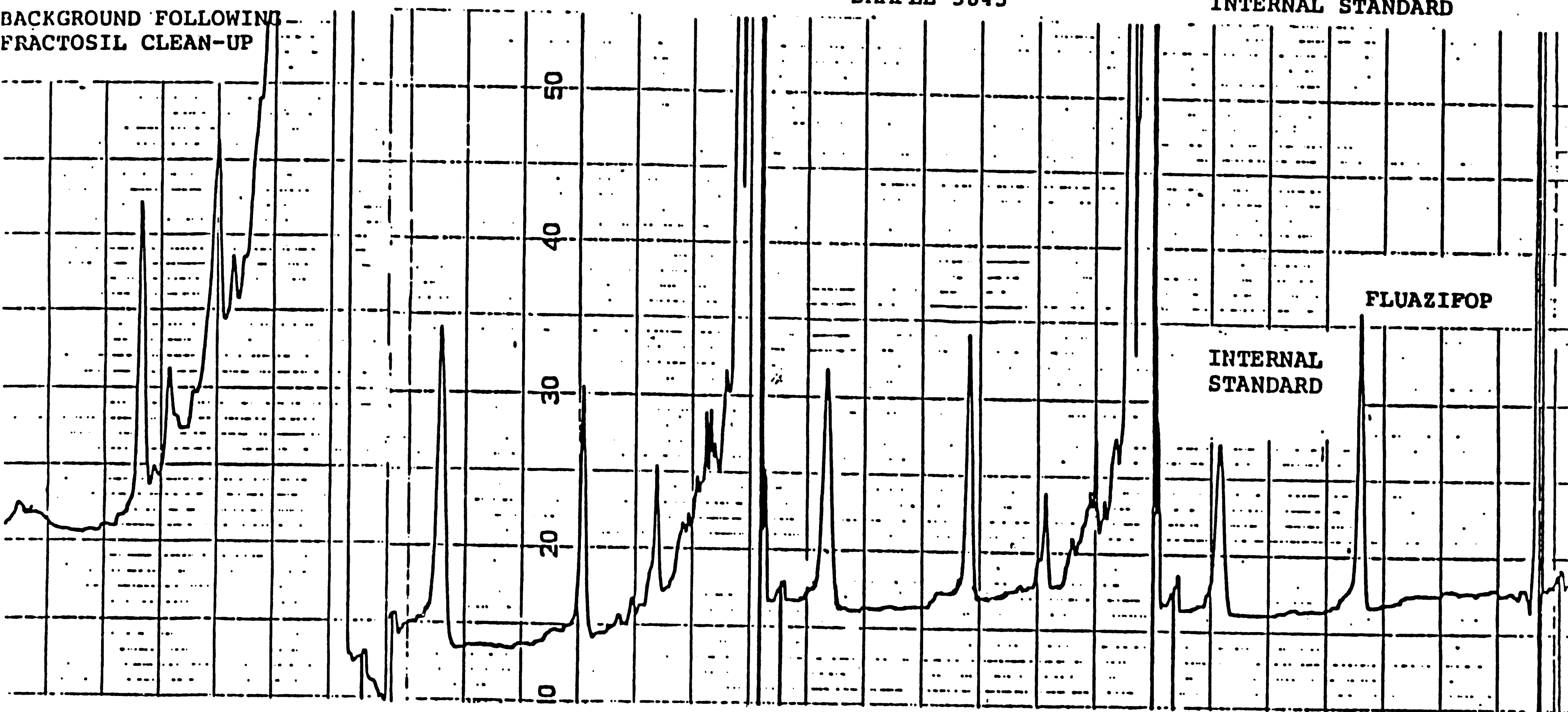
OIL SEED RAPE SAMPLE 5845 ($10\ G\ ML^{-1}$) $100\ \mu\ell$ INJECTION ≡ 1G

STANDARD $10\ \mu G\ ML^{-1}$
FLUAZIFOP - INTERNAL STD



DIRECT HPLC DETERMINATION

CROP:- OIL SEED RAPE, (2 G ML^{-1}) FOLLOWING HPLC CLEAN-UP

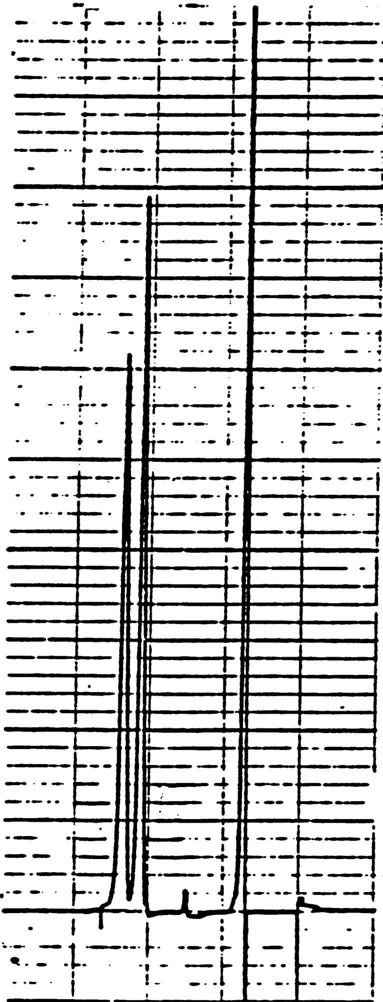


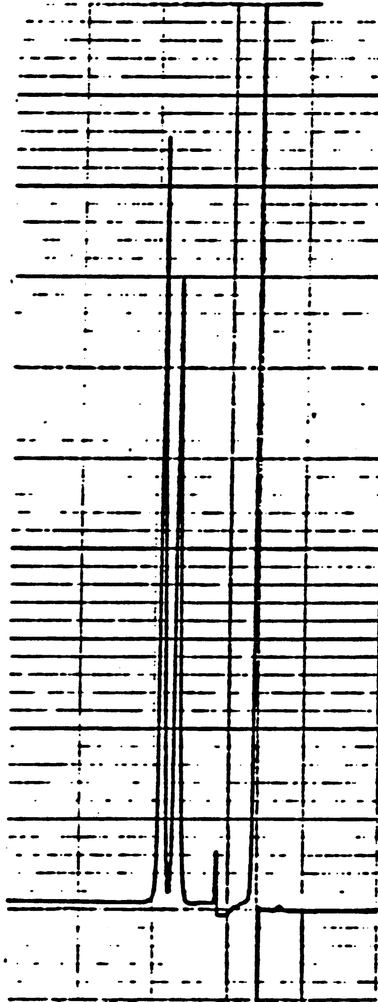
Alternative HPLC clean-up columns

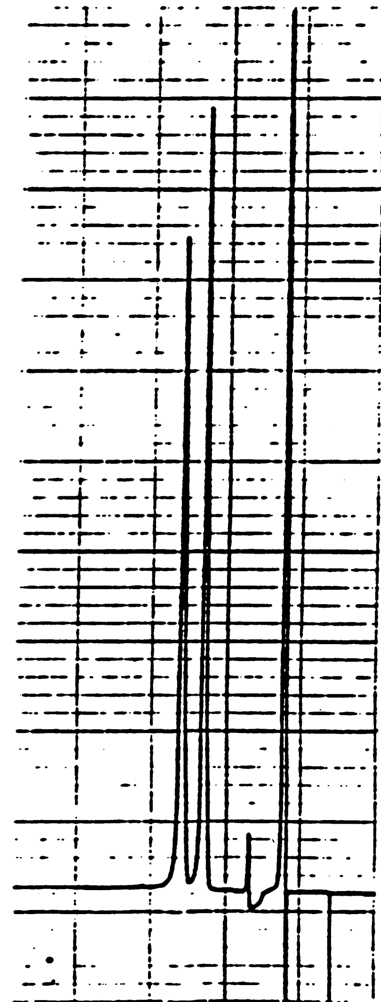
Column : 25 cm x 4.9 mm i.d. stainless steel
Flow rate : 3 cm^3 min^{-1}
Wavelength : 270 nm Absorbance : 0.08
Chart : 5 mm min^{-1}
Injections : 100 μl of 10 μg cm^{-3} fluazifop and internal standard

Mobile phase

Dichloromethane	:- 40	40	40
Hexane	:- 60	60	60
Acetic acid	:- 0.5	0.5	0.5
Methanol	:- 0.7	0.7	0.2







Packing:

LiChrosorb Diol

Spherisorb 5 silica (S5W)

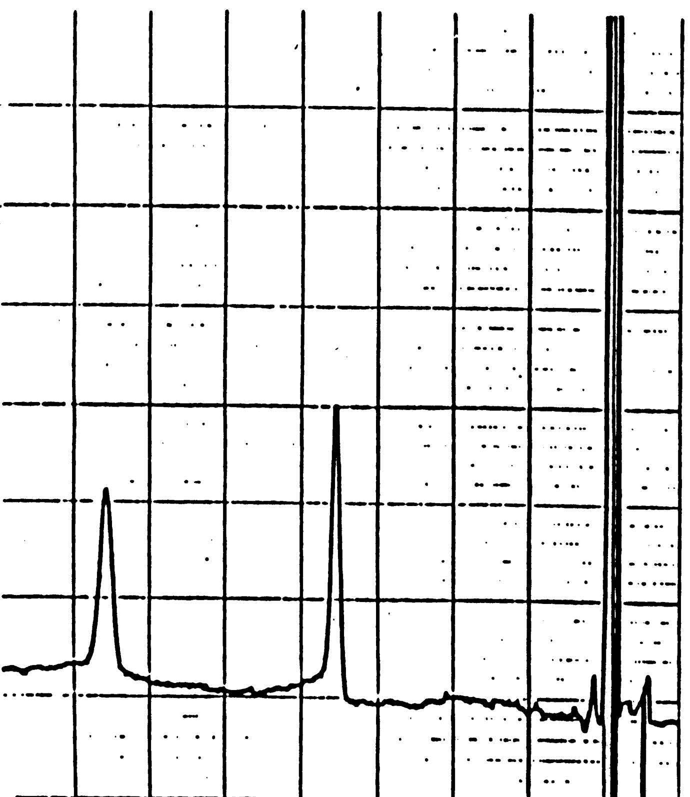
Spherisorb 5 nitrile (S5CN)

APPENDIX 3

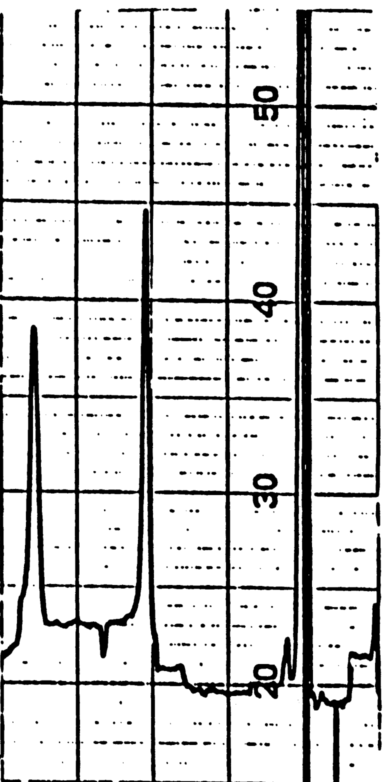
Comparison of Retention Times of Fluazifop and Internal Standard using Different Mobile Phases on an Ultrasphere ODS column

Injected - 50 µl of 1 µg cm^{-3}. Abs 0.01. All phases contained 0.4% Acetic acid.

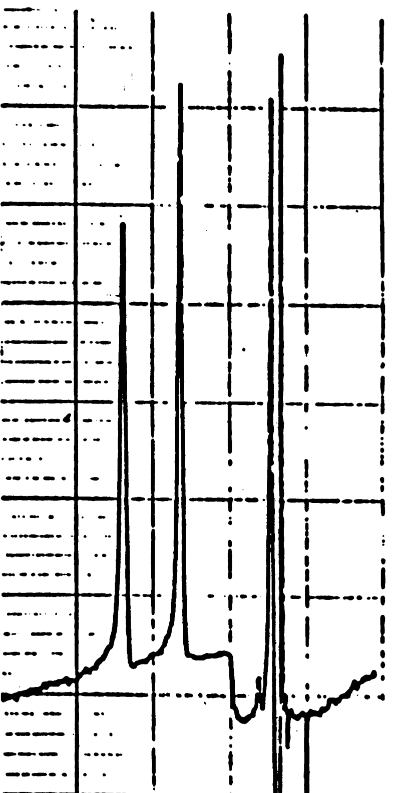
Methanol : Acetonitrile : Water
20 : 30 : 50



Methanol : Acetonitrile : Water
10 : 40 : 50

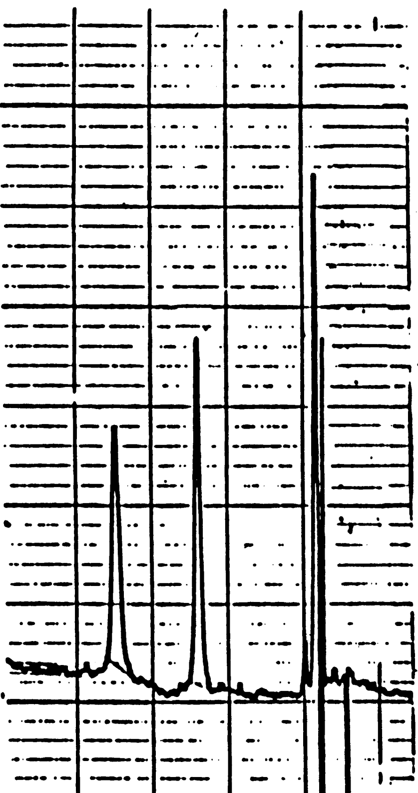


Acetonitrile : Water
50 : 50

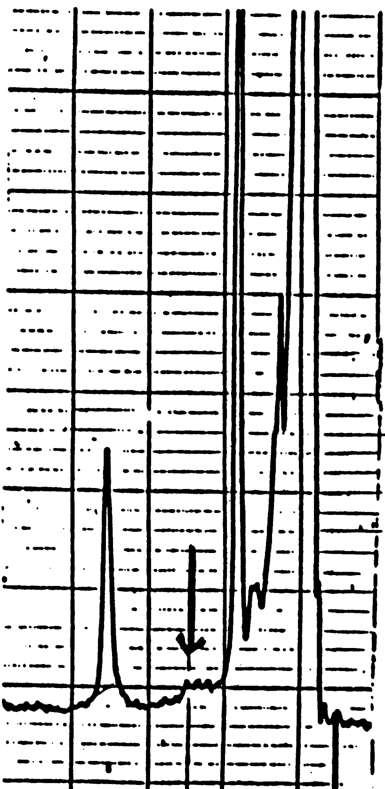


Typical Chromatograms of Pear Samples

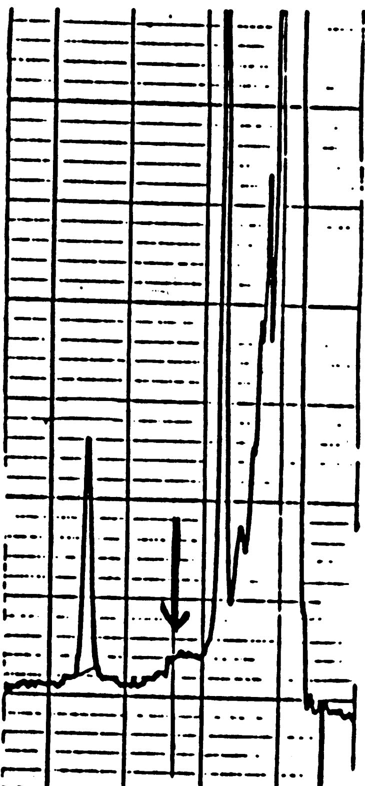
STANDARD 1 $\mu g\ cm^{-3}$
Fluazifop and Internal
Standards



CONTROL 2.5 $g\ cm^{-3}$
(Internal standard added)



SAMPLE 2.5 $g\ cm^{-3}$
Internal standard added
at 0.5 $mg\ kg^{-1}$



APPENDIX 4

FLUAZIFOP RESIDUES (MG KG^{-1}) IN OIL SEED RAPE DETERMINED AS METHYL ESTER BY GCMS

A:- WHOLE SEED

S[illegible]LE C	SAMPLE B	SAMPLE A	RECOVERY	CONTROL	FLUAZIFOP
0.49	0.15	0.02	78%	<0.01	5 NG

R [illegible]CTIVE CAKE

[illegible].4[illegible]	0.12	0.02	96%	<0.01	5 NG