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Acetochlor Registration Partnership
c/o ZENECA Ag Products
P.O. Box 751
Wilmington, DE 19850-5458

VOLUME 2

Study Title

The Determination of Acetochlor and R-25788 (Dichloromid) in Maize Grain,
Forage and Fodder; Soybean Seed and Hay

Data Requirement

Guideline Reference: 171-4 (c)

Author(s)

S. J. Crook

Study Completed On

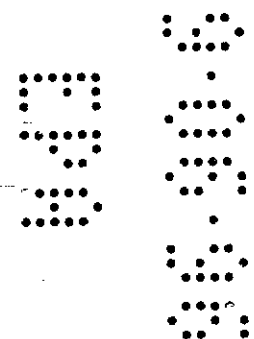
August 26, 1994

Performing Laboratory

Zeneca Agrochemicals
Jealott's Hill Research Station
Bracknell, Berkshire
U.K.

Laboratory Project ID

RAM244/02

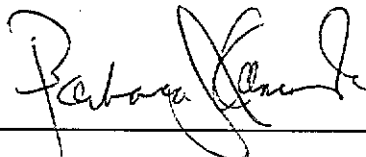


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Company: Acetochlor Registration Partnership

Company Agent: Barbara J. Kaminski

Signature: 

Title: Regulatory Manager

Date: 8/20/96

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This study has been conducted in compliance with the Principles of Good Laboratory Practice (GLP) laid down in the United Kingdom Department of Health Compliance Programme (1989). These Principles are consistent with the Organisation of Economic Co-operation and Development Principles of Good Laboratory Practice OCDE/GD(92)32.

This study is therefore considered to satisfy the requirement that it be conducted in accordance with 40 CFR Part 160.

This study is valid for the purposes for which it was conducted and is a true reflection of the raw data generated.

Study Director:

R. Elliott

R Elliott
Section Manager

Sponsor:

Barbara Kaminski
Barbara J. Kaminski
Acetochlor Registration Partnership

Date:

8/20/96

Submitter:

Barbara Kaminski
Barbara J. Kaminski
Acetochlor Registration Partnership

Date:

8/20/96

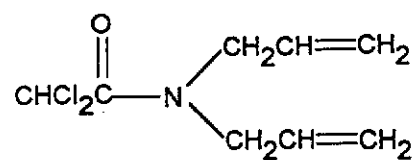
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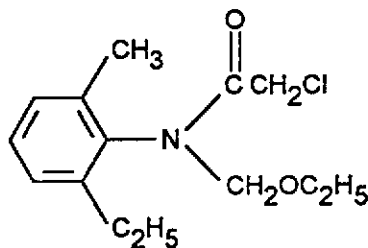
SCOPE

The analytical method described is suitable for the determination of acetochlor and the safener R-25788 (dichlormid) in maize grain, forage and fodder; soybean seed and hay using external standardisation. The limit of determination for this method is 0.01 mg kg⁻¹.

Structure of R-25788
N,N diallyl dichloroacetamide



Structure of Acetochlor
2-chloro-N-(ethoxymethyl)-N-(2-ethyl-6-methylphenyl)acetamide



METHOD

A prepared crop sample is extracted by maceration with methanol at room temperature. After extraction, each sample is filtered into a round bottomed flask and reduced to a volume not less than 10ml by rotary evaporation. The remaining volume of methanol is transferred to a plastic centrifuge tube, diluted with sodium chloride solution and partitioned into toluene. The organic fraction is collected and adjusted to a known volume. A 2g aliquot is cleaned up using solid phase amino (NH₂) and silica (Si) columns. The samples are eluted using ethyl acetate : hexane (40:60 v/v) and adjusted to a known volume. Residues are determined by gas liquid chromatography using a nitrogen selective thermionic specific detector. Quadrupole mass selective detection may be employed for confirmatory purposes.

PROCEDURE**Sample Preparation**

Maize and soybean samples should be prepared as described in Standard Operating Procedure 41/065/ - - ; Preparation of crop samples for residue analysis.

Extraction and Filtration

- (a) Weigh representative samples of crop (5g), in duplicate, into storage jars (250ml). Fortify at least two untreated samples by the addition of a suitable amount of acetochlor and R-25788 standard in methanol, using either a syringe or pipette. At least one recovery should be fortified at 0.01mg kg⁻¹ to prove that the limit of determination of the method can be achieved. An untreated control sample should also be run.
- (b) For each sample add methanol (50ml) to the storage jar and macerate gently using a Polytron macerator until well homogenised. It is advised that during maceration the jar is contained in an ice bath as the maceration process involves the generation of heat and loss of R-25788 may result. (NB. Volume of solvent added may vary depending on crop type, eg. dry crops may need more solvent to enable extraction. This will be recorded in method notes).
- (c) Filter the extracts under vacuum through two Whatman filter papers (either no.1, no.5 or glass fibre), into round bottomed flasks (250ml). Wash each jar with two further aliquots of methanol (10-20ml) and filter.

3.3

Concentration of Sample

- (a) Rotary evaporate extracts to a low volume (10-20ml) in a cold water bath not exceeding 25°C. **NB.** Evaporation to dryness will result in the loss of R-25788.
- (b) Transfer extracts to 50ml plastic centrifuge tubes.
- (c) Rinse the round bottomed flasks with a 100g l⁻¹ sodium chloride solution (~20ml) and transfer to the plastic centrifuge tubes. Adjust volume to 40ml with 100g l⁻¹ sodium chloride solution.

3.4

Partition

- (a) Add toluene (5ml) to the plastic centrifuge tubes, cap tightly and shake by hand for approx. 30 seconds.
- (b) Centrifuge the tubes at 2000rpm for 1 minute to aid separation of the layers.
- (c) Using a Pasteur pipette, transfer the upper organic layer to a 10ml graduated centrifuge tube and adjust the volume to 5ml by the addition of toluene.

Before this method is run routinely, the graduated tubes to be used should be calibrated to ensure accurate volumes are achieved.

(**NB.** Extra care should be taken when adjusting volumes in 10ml graduated tubes, ie. in both this step and 3.5 (g). Ensure that the lowest meniscus of the solvent coincides with the graduation required. The dark colour of certain matrix types makes the viewing of the meniscus difficult.)

3.5

Solid Phase Clean-up

- (a) Take a 3cc Si and a 3cc NH₂ Bond-Elut™ column and stack the NH₂ column on top of the Si column, using appropriate connectors. Insert the columns into the Bond-Elut™ apparatus.
- (b) Pre-wet the columns by adding toluene (5ml) to the top of the NH₂ column and drawing the solvent through both columns at a rate of ~2ml min⁻¹ under vacuum. All solutions, throughout the clean-up procedure, should be drawn through to the level of the frit in the uppermost column in order to prevent the columns going dry.
- (c) Load a 2ml (2g) aliquot of each extract from 3.4 (c) to the top of the NH₂ column and draw through under vacuum.
- (d) Add toluene (2ml) to the top of the NH₂ column and draw through under vacuum. Discard the NH₂ column.

- (e) Add toluene (2ml) to the top of the Si column and draw through under vacuum.

NB. All solutions drawn through the columns to this stage can be run to waste.

- (f) Place a 10ml graduated centrifuge tube in the Bond-Elut™ apparatus and elute the Si column with a ethyl acetate : hexane (40 : 60 v/v) solution (2ml).
- (g) Dilute the collected eluent to the nearest 0.5ml (usually 2.0 or 2.5ml) with ethyl acetate : hexane (40 : 60 v/v) solution.
- (h) Transfer an aliquot to a GC autosampler vial in preparation for analysis.

NB. A standard solution must be injected after a maximum of four sample injections. If particularly dirty traces are obtained with certain crop types, a polar washing solvent (eg. methanol) may be injected at intervals throughout a GC run.

3.6 Gas Liquid Chromatography

Analysis should be carried out initially by GLC using nitrogen specific TSD detection (see 3.6.1). If however, particularly dirty chromatograms or unexpected residues are obtained, then GLC with quadrupole mass selective detection may be used for confirmatory purposes only (see 3.6.2).

The conditions for the analysis by GLC will depend upon the equipment available. The operating manuals for the instruments should always be consulted to ensure safe, optimum use. The following conditions have found to be satisfactory using the instruments detailed below.

3.6.1 Gas Chromatograph (Nitrogen Specific TSD)

- Gas chromatograph : HP5890 series I
 Detector : Nitrogen specific thermionic selective detector.
 Autosampler : HP7673A
 Injection technique : Splitless with a 4 mm i.d. silanised glass liner packed with a silanised glass wool plug.
 Injection Volume : 1 or 2 μ l

Column BP _x 5			
Phase	Polarity	Dimensions	Temperature Limits
5% diphenyl 95% dimethyl polysiloxane	Non Polar	Length :25m ID :0.32mm df :0.25 μ m	-80°C - 360°C

Carrier gas	Helium	Flow rate : 4 ml min ⁻¹
Make up gas	Helium	Flow rate : 30 ml min ⁻¹
Detector gasses	Hydrogen Air	Flow rate : 3.6 ml min ⁻¹ Flow rate : 102 ml min ⁻¹

Injector Temperature : 220 °C
 Detector Temperature : 300 °C
 Oven Program :

50°C _____ 130°C _____ 210°C _____ 270°C
 1 min 5°C/min 3 mins 20°C/min 3 mins 40°C/min 2 mins

This temperature program is only advisory and may be altered at the discretion of the scientist.

Under these conditions the retention time of R-25788 is 14.9 minutes and the retention time for acetochlor is 24.5 minutes.

3.6.2 Gas Chromatography (Mass Selective Detection)

Gas chromatograph : HP5890 series I
 Detector : Quadrapole Mass Selective Detector.
 Autosampler : HP7673A
 Injection technique : Splitless with a 4 mm i.d. silanised glass liner packed with a silanised glass wool plug.
 Injection Volume : 1 or 2 µl

Column DB 17			
Phase	Polarity	Dimensions	Operating Temperature Range
50% phenyl methylpolysiloxane	Mid Polar	Length : 10m ID : 0.18mm df : 0.3µm	40°C - 290°C

Carrier gas	Helium	Head pressure 5 psi
-------------	--------	---------------------

Injector Temperature : 250 °C
 Transfer Line Temperature : 275 °C
 Oven Program : 60°C _____ 280°C
 1 min 20°C/min 3 mins

MSD CONDITIONS

Electron Multiplier : 1700 volts
 Electron Energy : 70 eV
 System Calibration : Manual tunes carried out weekly.
 (Set up using PFTBA ions 169,219,264)
 Acquisition Mode : Selected Ion Monitoring
 R-25788 Target Ion : 172 m/z
 Dwell time per ion : 175 msec
 Acetochlor Target Ion : 162 m/z
 Dwell time per ion : 175 msec
 (Using low mass resolution for both ions)
 Solvent Delay : minutes

Under these conditions the retention time of R-25788 is 8.8 minutes and the retention time of acetochlor is 9.83 minutes.

3.7

Calculation of Results

Residues of both compounds may be calculated in mg kg⁻¹ for each sample extract using a mean response signal from the standard injections bracketing that sample as follows:

$$Residue = \frac{Res(SA)}{Res(STD)} \times \frac{Conc(STD)}{Conc(SA)} \times \frac{Inj(STD)}{Inj(SA)}$$

Res(SA) = Peak height / area for sample
 Res(STD) = Average peak height / area for bracketing standards
 Conc(STD) = Concentration of compound in standard (µg ml⁻¹)
 Conc(SA) = Concentration of crop in final sample (g ml⁻¹)
 Inj(STD) = Standard injection volume (µl)
 Inj(SA) = Sample injection volume (µl)

These sample residues should be further corrected using the average percentage recovery. i.e. calculate each recovery as above and express as a percentage of the fortification level. Then average all the recovery percentages for use in the calculation below.

$$\text{Corrected Residue} = \frac{\text{Residue}}{\text{APR}} \times 100 \text{ mg/kg}$$

APR = Average Percentage Recovery

4 RECOVERIES

A minimum of two external recoveries must be analysed alongside each set of samples analysed.

Fortification levels should be based on the expected residue levels, but at least one of the recoveries should be fortified at twice the limit of determination.

Recovery values obtained must be between 70 - 120 % with a %CV within a run of not greater than 15, for that run to be acceptable. Variations outside these parameters will only be accepted at the Study Director's discretion.

5 LIMIT OF DETERMINATION

A true assessment of the limit of determination of the method may be determined by fortification of untreated samples at 0.01 mg kg⁻¹ with both compounds and subjecting them to the full analytical procedure. The chromatographic response obtained for each compound should exceed the baseline noise by a factor of four.

In these laboratories the limit of determination for both compounds has been set at 0.01mgkg

6 REAGENT BLANKS/CONTROLS

At least one untreated sample will be analysed alongside each set of samples. In order to confirm the presence of contamination within an analytical run, reagent blanks will be taken through the analytical procedure as necessary. This will confirm whether a contaminant is present in either the reagents or equipment.

In-House Method Validation

In these laboratories to date, the method described above has been applied to the analysis of maize grain, forage and fodder; soybean seed and hay. A range of accurately fortified untreated samples of each commodity were taken through the analytical procedure and calculated against external standards. The range of recovery values obtained are presented in Tables 1 and 2.

TABLE 1 : Recovery Data for R-25788 in Maize and Soybean Fractions.

Commodity Analysed	Fortification Level / mg kg ⁻¹				MEAN	CV
	0.01	0.05	0.10	0.20		
MAIZE GRAIN	96,79,85,99	76	89	108, 92	91%	11.7%
MAIZE FORAGE	105,117, 88, 89	103	91	96, 107	100%	10.3%
MAIZE FODDER	74, 91, 105, 78	84, 87	86, 71	78, 82	84%	11.6%
SOYBEAN SEED	73,83, 80, 90	66, 88	77	71	79%	10.7%
SOYBEAN HAY	77,80, 91, 86	74, 70	89, 92	88, 94	84%	9.9%

TABLE 2 : Recovery Data for Acetochlor in Maize and Soybean Fractions.

Commodity Analysed	Fortification Level / mg kg ⁻¹				MEAN	CV
	0.01	0.05	0.10	0.20		
MAIZE GRAIN	93,95,90, 91	87	90	102, 104	93%	6.6%
MAIZE FORAGE	102, 98, 111, 111	103,	96	103, 112	105%	5.9%
MAIZE FODDER	98, 108, 109, 112	97, 92	88, 74	83, 82	94%	13.6%
SOYBEAN SEED	114, 123, 111,113	94, 94	94,	83,	103%	13.3%
SOYBEAN HAY	77, 107, 95, 98	77, 77	92, 83	87, 82	88%	11.7%

7.2

Detector Linearity

For accurate determination of residue concentration, all analyses should be carried out within the linear range of the detector used. If a residue lies outside this range the sample must be accurately diluted until it falls within this range.

The linearity range for both acetochlor and R-25788 using nitrogen selective TSD is 0.01 -1.0 mgkg⁻¹ in this laboratory (see appendix 1).

APPENDIX 1
Detector Linearity Graphs

FIGURE 1. Linearity Graph of R-25788 Using Nitrogen Selective Detection.

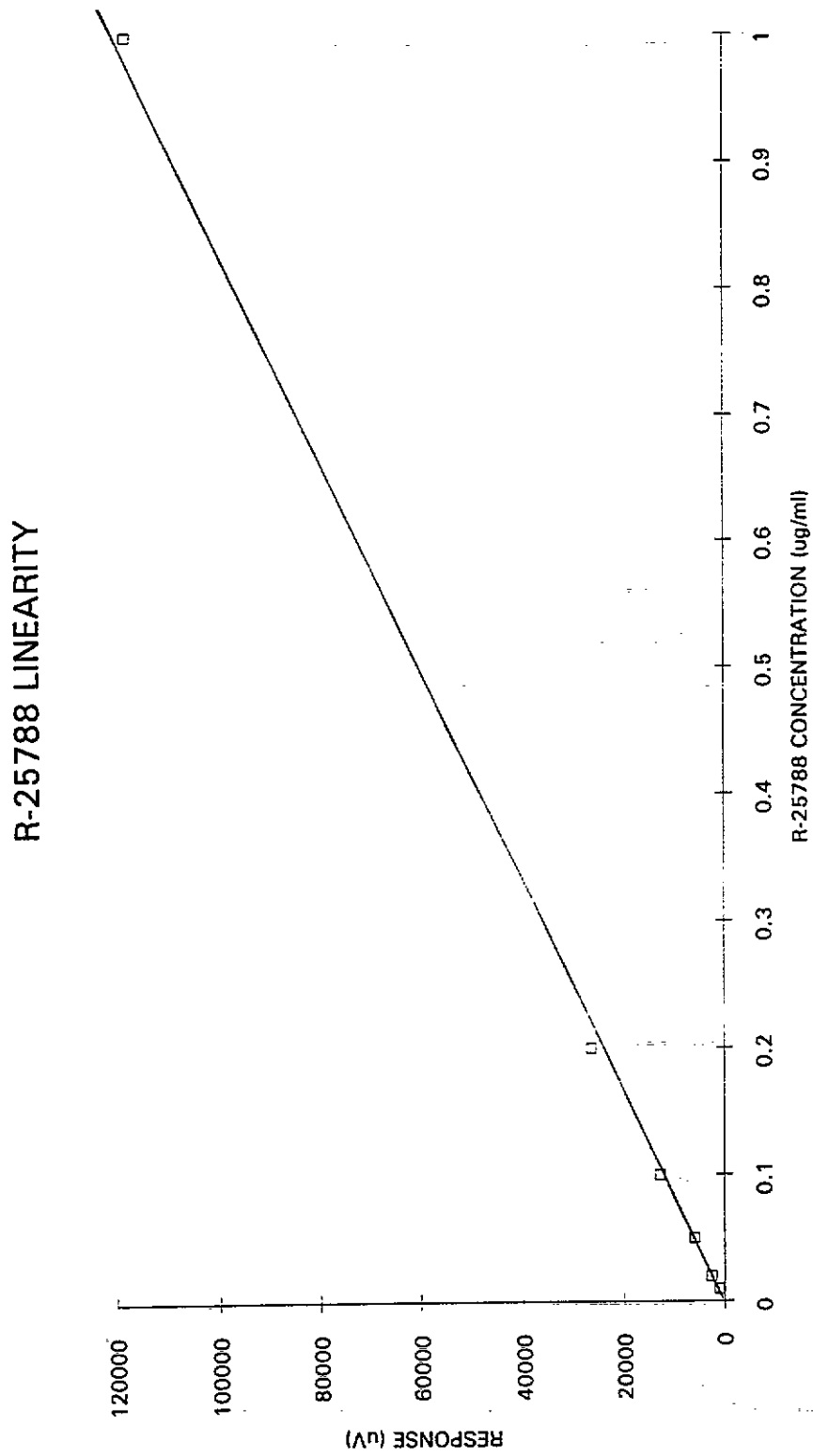
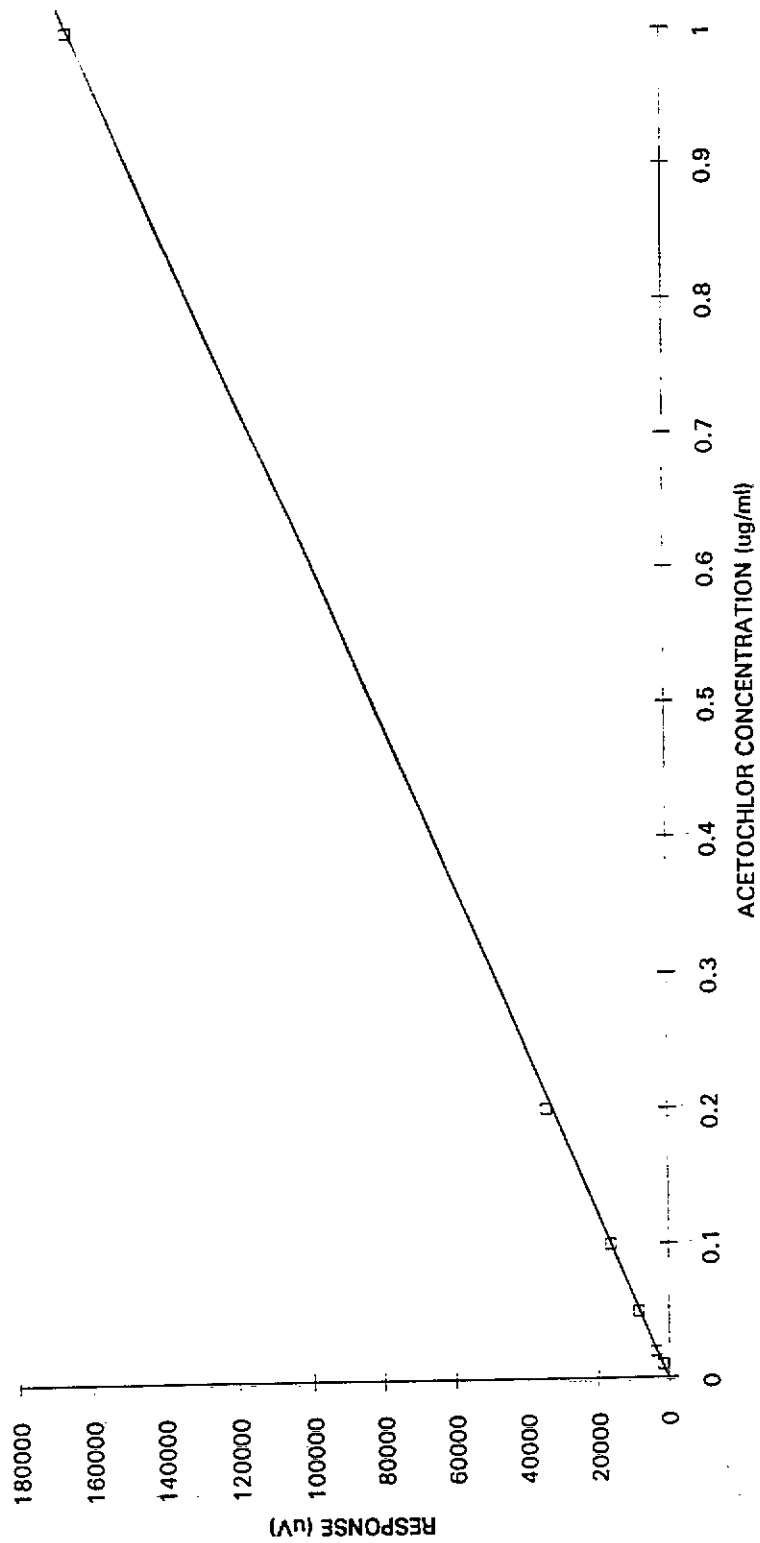


FIGURE 2. Linearity Graph of Acetochlor Using Nitrogen Selective Detection.

ACETOCHLOR LINEARITY



APPENDIX 2

Typical gas chromatograms for R-25788 and Acetochlor

A. Nitrogen Specific TSD

A. Nitrogen Specific TSD

FIGURE 1: Standard R-25788 and Acetochlor; $0.1\mu\text{g ml}^{-1}$ in Ethyl Acetate : Hexane (40: 60 v/v)

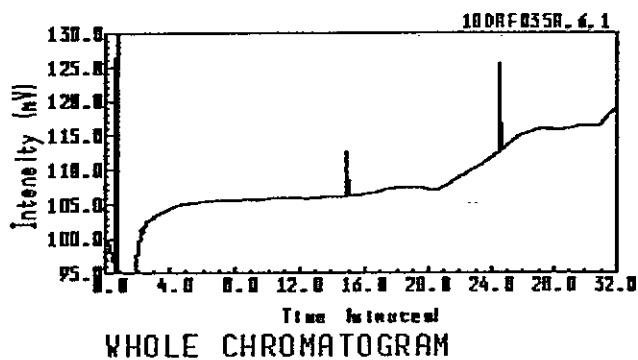
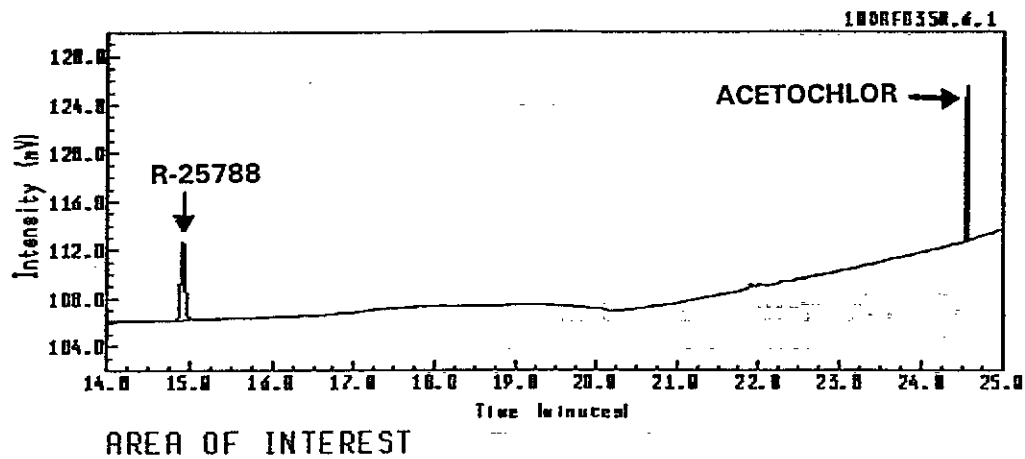
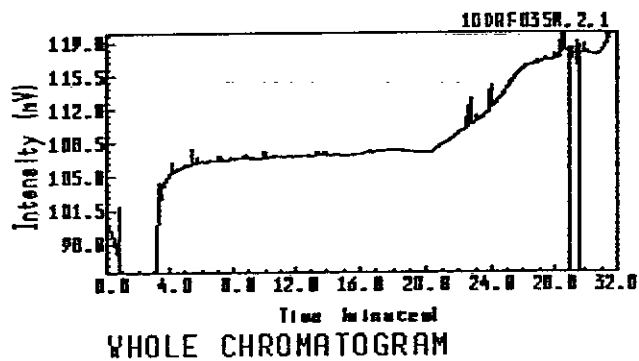
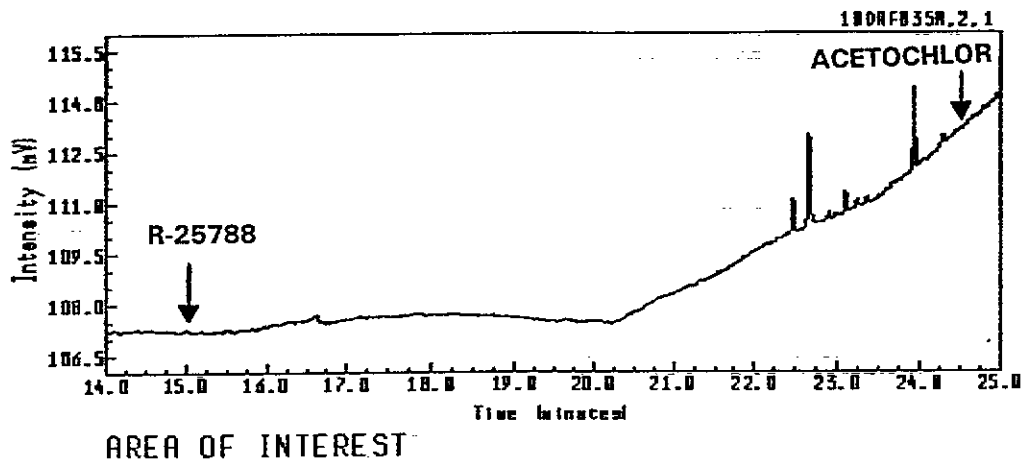


FIGURE 2: Control Maize Fodder; 1.0g ml⁻¹ in Ethyl Acetate : Hexane (40 : 60 v/v)



**FIGURE 3: Maize Fodder Fortified at $0.01\mu\text{g g}^{-1}$; 0.8g ml^{-1} in Ethyl Acetate : Hexane (40 : 60 v/v)
105% R-25788 Recovery 109% Acetochlor Recovery**

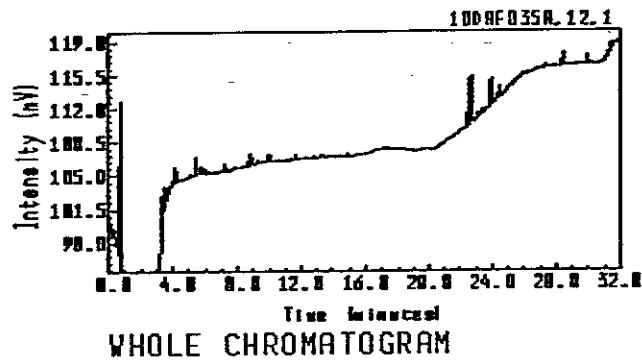
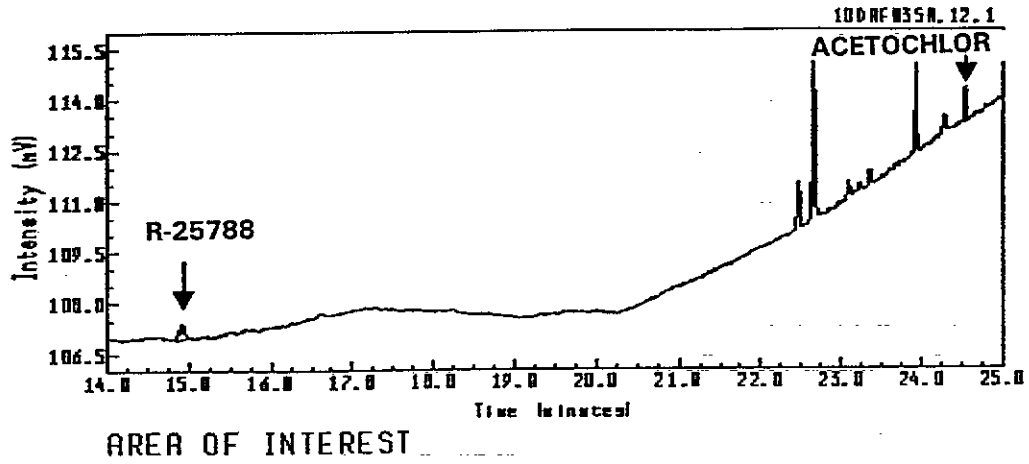
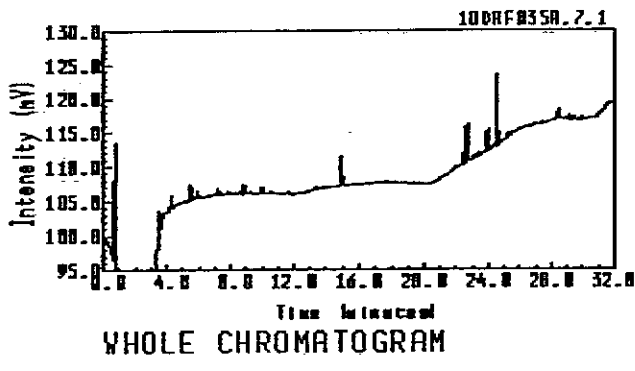
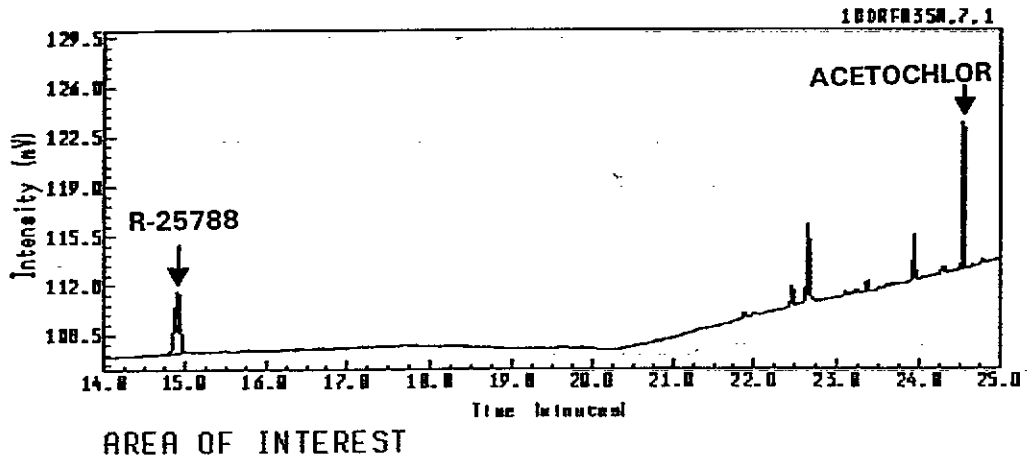


FIGURE 4: Maize Fodder Fortified at $0.1 \mu\text{g g}^{-1}$; 1.0g ml^{-1} in Ethyl Acetate : Hexane (40 : 60 v/v)
86% R-25788 Recovery 88% Acetochlor Recovery



APPENDIX 3

- 1. Apparatus**
- 2. Reagents**
- 3. Hazards**
- 4. Preparation of Analytical Standards**

1.

APPARATUS

- a) Macerator: Polytron CH-6010 (Head Size PTA205) or Tekmar (3412-B25), available from Tekmar Co. PO Box 371856, Cincinnati, OH 45222-1856 (Tel: (800) 543 4461).
- b) Vacuum Rotary Evaporator with thermostatically controlled water bath (RE-121A), available from Buchi/Brinkmann via VWR Scientific, Cat No. 27558-354, PO Box 7900, San Francisco, CA 94120 (Tel: (415) 467 6202).
- c) Centrifuge tubes, eg. 50ml polypropylene centrifuge tubes with plug seal caps available from CORNING.
- d) Laboratory centrifuge eg WIFUG model 2000e, Wifug (a division of Eltrex of Sweden), Bradford, UK.
- e) Centrifuge tubes, eg. 10ml graduated centrifuge tubes available from Volac.
- f) 3cc Si and NH₂ Analytichem Bond Elut columns. Available from Varian Sample Preparation Products, 24201 Frampton Avenue, Harbor City, CA 90710 (Tel: (310) 539 6490).
- g) Visiprep 12 Station Vacuum Manifold, available from Supelco Incorporated, Supelco Park, Bellefonte, PA 16823-0048 (Tel: (800) 247 6628).
- h) Gas chromatograph with either a TSD (Nitrogen Selective) or a Mass Selective Detector, eg. HP5890 and autosampler HP7673A plus detector (HP5970 MSD). Available from Hewlett Packard Co., PO Box 1000, Avondale, PA 19311-1000 (Tel: (800) 223 9700).
- i) Chart recorder or integrator eg. HP3396A from Hewlett Packard Co., PO Box 1000, Avondale, PA 19311-1000 (Tel: (800) 223 9700).
- j) Analytical gas chromatography capillary columns :
 - 1) 25m x 0.32mm id with a 0.25 μ m df: BP*5, from SGE Incorporated, 2007 Kramer Lane, Austin, Texas 78758 (Tel: (512) 837 7190).
 - 2) 10m x 0.18mm id with a 0.3 μ m df: DB 17, from J & W Scientific, 91 Blue Ravine Road, Folsom, CA 95630 (Tel: (800) 223 3424)

2. REAGENTS

- a) Sodium chloride (AnalaR grade). BDH Chemicals Ltd, Poole, UK.
- b) Solvents: methanol, toluene, ethyl acetate and hexane (glass distilled).
- c) Ultra pure water.

3. 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 any doubt, consult the appropriate safety manual (eg. ICI Laboratory Safety Manual) which contains recommendations and procedures for handling chemicals or a monograph such as 'Hazards in the Chemical Laboratory', Edited by L Bretherick, The Chemical Society, London.

- a) Solvent Hazards.

	Methanol	Toluene	Ethyl Acetate	Hexane
Harmful Vapour	Y	Y	N	Y
Highly Flammable	Y	Y	Y	Y
Risk of Irreversible Effects	N	N	N	Y
Recommended Limit (RL) /ppm	200	100	400	100

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

- b) Zeneca Agrochemicals Toxicity Classifications.

Acetochlor has a divisional toxicity class of 2 (highly toxic).

R-29148 has a divisional toxicity class of 3 (toxic).

PREPARATION OF ANALYTICAL STANDARDS

Weigh out accurately using a five figure balance, sufficient of each analyte to allow dilution in methanol to give a mixed $1000 \mu\text{g ml}^{-1}$ stock solution in a volumetric flask. Make serial dilutions of this standard to give $100\mu\text{g ml}^{-1}$, $10 \mu\text{g ml}^{-1}$ and $1.0 \mu\text{g ml}^{-1}$ in methanol. These standards can then be used to fortify samples.

Further serial dilutions of the $100\mu\text{g ml}^{-1}$ standard with ethyl acetate : hexane (40 : 60 v/v) are made to give a $10\mu\text{g ml}^{-1}$, $1\mu\text{g ml}^{-1}$ and $0.1 \mu\text{g ml}^{-1}$ standard solutions in this solvent. These standards are used as bracketing standards in analytical runs.

When not in use, always store the standards solutions in a refrigerator at $<5^{\circ}\text{C}$ to prevent decomposition and/or concentration of solvent strength. Analytical standards are valid for 4 months from the date of the manufacture of the initial stock solution.

After this period, a new set of standard solutions must be prepared. These should then be checked according to Standard Operating Procedure 41/083/--.