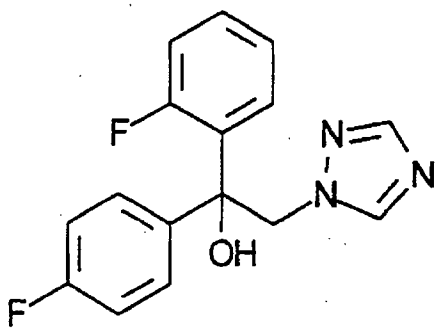
1 SCOPE

The analytical procedure described is suitable for the determination of residues of flutriafol in soil using external standardisation procedure. The limit of determination set in these laboratories is 0.01 mg kg^{-1}.



Flutriafol

(RS)-1-(2-fluorophenyl)-1-(4-fluorophenyl)-2-(1H-1,2,4-triazol-l-yl)ethanol.

2 SUMMARY

Residues of flutriafol are extracted by reflux with 70:30 acetonitrile:pH9 water for 1 hour. After filtration aliquots equivalent to 2 g of soil are taken and partitioned into dichloromethane. The samples are then subjected to adsorption chromatographic clean up to remove interfering endogenous materials. Final quantitative determination is by capillary column gas chromatography using thermionic nitrogen specific detection.

3 PROCEDURE

3.1 Sample Preparation

Soil samples should be prepared according to Zeneca Agrochemicals SOP 41/066.

3.2 Extraction

(a) Thoroughly mix the prepared soil sample and weigh a representative aliquot (20 g) into a round bottomed flask (250 ml).

(b) For external standardisation, at least two extra control aliquots should be weighed out and fortified with a suitable amount of flutriafol standard.

(c) Add 70:30 acetonitrile:water solution (adjusted to pH9 with ammonia solution) (100 ml) and reflux for 1 hour. Allow to cool.

(d) Filter the samples under vacuum through two Whatman No.5 filter papers into a round bottomed flask with further solvent (50 ml) and use this to wash the residue, combining both filtrates.

(e) Adjust the volume of the samples to 150 ml by addition of further 70:30 acetonitrile:pH9 water. 15 ml of extract is now equivalent to 2 g of soil.

3.3 **Partition**

(a) Transfer an aliquot equivalent to 2 g soil (15 ml) of sample from Section 3.2 (e) to a separating funnel (100 ml size).

(b) Add pH9 water (20 ml), partition with dichloromethane (20 ml) and allow the two layers to separate.

(c) Filter the lower organic layer through anhydrous sodium sulphate and wash with further dichloromethane (20 cm^3) collecting in a round bottomed flask (100 ml).

(d) Rotary evaporate the samples to dryness and redissolve the residual material in acetone (2 ml). Ultrasonicate the samples and then transfer the acetone to a graduated centrifuge tube (10 ml). Rinse the round bottomed flask with further acetone (2 ml) and combine the two acetone fractions.

(e) Evaporate the acetone to 300 µl using a stream of clean, dry compressed air and add diethyl ether (700 µl).

3.4 **Adsorption Chromatographic Clean Up**

(a) Place small glass wool plugs in the bottom of 1 cm diameter chromatography columns and add n-hexane. Slowly, with gentle tapping, add florisil (2 g). Allow the hexane to percolate through the columns.

(b) Transfer the extract from 3.3 (e) onto the columns and allow to percolate through.

(c) Wash the columns with 8% acetone: hexane solution (20 ml).

(d) Elute the flutriafol from the columns with ethyl acetate (50 ml). Collect the ethyl acetate eluate in 100 ml round bottomed flasks and rotary evaporate to dryness at 40°C.

(e) Add acetone (2 ml) to the round bottomed flasks, ultrasonicate and transfer the acetone to a graduated centrifuge tube (10 ml size). Rinse out the flask with further acetone (2 ml) and combine the two acetone fractions.

(f) Evaporate the samples down to 1.0 ml using a stream of clean, dry compressed air and transfer to GC vials ready for analysis. The sample concentration is 2.0 g ml^{-1}.

4 FINAL DETERMINATION BY GAS CHROMATOGRAPHY

The conditions for analysis by GC will depend upon the equipment available. The operating manuals for the instruments should always be consulted to ensure safe and optimum use. The following conditions have been found to be satisfactory using a Varian 3400 GC fitted with a septum equipped programmable injector (SPI) and a thermionic nitrogen specific detector.

i)	Column	:	BPX 35 25 m x 0.32 mm 0.25 µm film thickness.
ii)	Carrier Gas	:	Helium at 4 ml min^{-1}.
iii)	Temperature Program	:	70°C hold for 1 minute then rise at 15°C per minute to 300°C and hold for 3 minutes.
iv)	Injection Mode	:	SPI injection with a SPI with buffer injection liner packed with glass wool.
v)	Injector Program	:	40°C hold for 0.1 minutes then rise at 150°C per minute to 250°C and hold for 15 minutes.
vi)	Detector Temperature	:	300°C
vii)	Bead Current	:	3.25A
viii)	Hydrogen flow rate	:	4.5 ml min^{-1}.
ix)	Air flow rate	:	175 ml min^{-1}.
x)	Make up flow rate	:	helium at 26 ml min^{-1}.
xi)	injection volume	:	2 µl

Under these conditions the retention time of the flutriafol is approximately 13 minutes.

5 CALCULATION OF RESULTS

Calculate the 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, as follows:.

$$Residue = \frac{Pk\ Area\ (sample)}{Pk\ Area\ (standard)} \times \frac{Standard\ Conc.}{Sample\ Conc.}$$

Pk Area (sample)	=	Peak area of sample
Pk Area (standard)	=	Average peak area for bracketing standards
Standard Conc.	=	The concentration of standard reference material (µg ml^{-1})
Sample Conc.	=	The concentration of the final sample solution (g ml^{-1})

Similarly calculate the results of each recovery sample and express as a percentage of the fortification level. Take the mean percentage recovery and express the residue for each sample in terms of the figure obtained:-

$$Corrected\ Residue = \frac{Residue \times 100}{Mean\ Percentage\ Recovery}\ mg\ kg^{-1}$$

Results should be corrected to two significant figures.

6 LIMIT OF DETERMINATION

The limit of determination in these laboratories has been set at 0.01 mg kg^{-1}. The chromatographic response for recoveries at this level should exceed the background signal noise by a factor of at least four to be considered an acceptable quantitative limit of determination.

7 CONTROL AND RECOVERY EXPERIMENTS

Control and external recovery experiments should be completed as Section 3 for each set of samples analyses. Provided the recovery values are acceptable they may be used to correct any flutriafol residue found.

The levels of external recoveries should be decided by the residue levels expected. A minimum of one control and two external recovery experiments should be run alongside each set of flutriafol samples analyses (that is untreated samples accurately fortified with a known amount of flutriafol prior to extraction) in the range of 0.01 mg kg^{-1} to 0.5 mg kg^{-1}.

Recovery data is generally considered acceptable when the mean recovery values are between 70 and 110% with a coefficient of variation of ≤20%.

Apparatus used during the Analysis of the soil samples.

(a) Equipment for the initial preparation of soil e.g. Hobart Bowl Chopper, available from Glenn Creston, Stanmore, UK.

(b) Vacuum rotary evaporator with thermostatically controlled water bath, available from Buchi, Switzerland.

(c) Heating mantle (to take 250 ml round bottomed flasks) available from Electrothermal Ltd, Southend On Sea, SS2 5PH, UK.

(d) Round bottomed flasks with ground glass joints (250 ml and 100 ml size).

(e) Glass chromatographic columns, 10 mm id, 300 mm long with solvent reservoir.

(f) Glass separating funnels (100 ml size).

(g) An ultrasonic bath, available from Sonicor Instrument company, Copiague, New York, USA.

(h) Graduated glass centrifuge tubes of 10 ml capacity calibrated down to 1.0 ml in 0.1 ml units, with an accuracy of at least $\pm$ 1% measured at 10 mls.

(j) Gas-liquid chromatograph fitted with a SPI injector and a thermionic nitrogen specific detector and an automatic liquid sampler eg Varian 3400 fitted with an 8100 autosampler.

(k) BPX 35 25 mm x 0.32 mm id 0.25 µm film thickness capillary GC column available from SGE UK LTD. Tel (0908) 568844.

(l) SPI with buffer injection liners available from Restek Corporation, 110 Benner Circle, Bellefonte, PA 16823-8812, USA. Part No. 20850.

(m) Potentiometric Pen chart recorder (1 mv) e.g. Kipp and Zonen BD40 or equivalent.

All solvents and other reagents must be of high purity i.e. HPLC grade solvents and analar grade reagents. If a source of reagent/solvent has not previously been evaluated, then individual reagents/solvents should be examined for possible interfering impurities prior to analysis.

(a) Acetone, glass distilled grade.

(b) Acetonitrile, HPLC grade.

(c) Dichloromethane, HPLC grade.

(d) Diethyl ether, glass distilled grade

(e) Ethyl acetate, glass distilled grade.

(f) Granular anhydrous sodium sulphate. Heat in oven at 140°C for 24 hours to remove volatile contaminants.

(g) Glass wool.

(h) Presilanised glass wool for packing GC injection liners - obtainable from chromatographic suppliers.

(i) Florisil (100-120 US mesh size) for chromatographic use available from BDH Ltd, Poole, UK.

(J) Flutriafol characterised analytical standard of >98% purity.

(k) Hexane - HPLC grade.

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, Zeneca Laboratory Safety Manual) containing recommendations and procedures for handling chemicals, and a monograph such as 'Hazards in the Chemical Laboratory', S G Luxon, Royal Society of Chemistry, Cambridge, 1992.

(a) **Acetone**

Highly inflammable
Vapour - air mixture explosive
Avoid breathing vapour.
Avoid contact with eyes.
OES short term 3560 mg m^{-3}
OES long term 1780 mg m^{-3}

(b) **Acetonitrile**

Harmful vapour
Harmful by skin adsorption
Highly inflammable
Avoid breathing vapour.
Avoid contact with skin and eyes.
OES short term 105 mg m^{-3}
OES long term 70 mg m^{-3}

(c) **Diethyl ether**

Harmful vapour
Highly inflammable
Avoid breathing vapour
OES short term 1500 mg m^{-3}
OES long term 1200 mg m^{-3}

(d) **Ethyl acetate**

Extremely Flammable
Avoid breathing vapour.
Avoid contact with eyes.
OES long term 1400 mg m^{-3}

(e) **Dichloromethane**

Harmful vapour
Irritating to eyes
Avoid breathing vapour.
Avoid contact with skin and eyes.
MEL short term 870 mg m^{-3}
MEL long term 350 mg m^{-3}

(f) **Hexane**

Highly inflammable
Harmful by inhalation and in contact with skin
Possible risk of irreversible effects
Avoid breathing vapour
MEL short term 3600 mg m^{-3}
MEL long term 1800 mg m^{-3}

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

1 Ensure good ventilation

2 Wear gloves and laboratory coat

3 Prevent inhalation and contact with mouth

4 Wash any contaminated area immediately

Weigh out accurately, using a five figure balance, sufficient flutriafol standard to allow dilution in acetone to give a 1000 µg ml^{-1} stock solution in a volumetric flask. Make serial dilutions of this stock solution to give a 100 µg ml^{-1}, 10 µg ml^{-1} and 1.0 µg ml^{-1} standard solutions in acetone.

When not in use, always store the standard solutions in a refrigerator at <7°C to prevent decomposition or concentration of the standards. Analytical standards should be replaced with freshly prepared standards after 4 months of use.