1 SCOPE

The analytical procedures described are suitable for the determination of residues of the herbicide tralkoxydim (Figure 1) in soil.

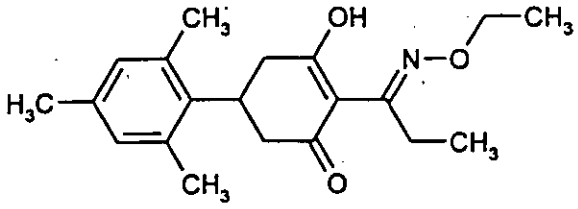

Figure 1 : 2-[1-(ethoxyimino)propyl]-3-hydroxy-5-mesitylcyclohex-2-enone

To date, in these laboratories, the method has been applied to a variety of soil samples and the limit of quantification (LOQ) of the method is 0.005 mg kg^{-1}.

2 SUMMARY

Tralkoxydim is extracted from samples, which have been accurately fortified with an internal standard, by shaking with acetonitrile. A 5 g aliquot of the extract is then evaporated to dryness under reduced pressure.

The residuum is then subjected to a combined diol and aminopropyl solid phase extraction (SPE) column clean up to remove interfering co-extractives. Acetone eluates are blown to dryness and resuspended in 60:40 (v/v) acetonitrile:water for final quantitative determination by high performance liquid chromatography (HPLC) using UV detection.

3 ANALYTICAL PROCEDURE

3.1 Sample Preparation

Samples should be prepared using an approved method of sample preparation for residue analysis, such as ZENECA Agrochemicals standard operating procedure 41/065/–.

3.2 Extraction

a) Prior to analysis, samples should be removed from the deep freeze and allowed to thaw at room temperature for the minimum period required to allow weighing out.

b) Weigh representative amounts of soil (20 g) into plastic centrifuge bottles (250 ml size). At least one untreated control and two control samples fortified with known amounts of tralkoxydim in acetone should be analysed with each batch of samples to enable verification of the method and recovery corrections to be made.

c) Fortify all samples except the control samples with an accurately known amount of internal standard (R162684 in acetone) using a pipette or a syringe.

d) Add acetonitrile (80 ml) and shake on a mechanical shaker for 45 minutes at a speed which visibly agitates the samples (130 +/- 20 rpm).[2]

e) Filter the samples under vacuum through two Whatman number 1 filter papers into round bottomed flasks (250 ml), rinse the centrifuge bottles used for extraction with acetonitrile (e.g. 2 x 25 ml) and use this to wash the filter cakes. Combine the washings with the main extracts and adjust the volumes of the extracts to a known volume e.g. 160 ml with acetonitrile. 40 ml aliquots of the extracts are now equivalent to 5 g soil.

3.3 Solid Phase Extraction (SPE) Column Clean-up

a) Transfer an aliquot (equivalent to 5 g of soil) to another round bottomed flask (250 cm^3) and evaporate to dryness on a rotary evaporator under reduced pressure at ≤40°C.

Note : Samples should be carefully monitored and removed from the rotary evaporator immediately upon reaching dryness. Breakdown of tralkoxydim may occur if samples are left dry on the rotary evaporator. See section 7 for further detail.

b) Add hexane:ether 90:10 v/v (4 cm^3) to the flasks and ultrasonicate thoroughly to ensure that the samples are fully resuspended.

Note : It is recommended that fresh diethyl ether is used for each analysis. Breakdown of tralkoxydim has been observed when old diethyl ether has been used. See section 7 for further detail.

c) Take a Varian aminopropyl (NH2) (500 mg, 3 ml) and a Varian diol (2OH) (500 mg 3 ml) solid phase extraction column for each sample to be analysed and place on a suitable vacuum manifold (e.g. IST Vacmaster). Add hexane (3 ml) and draw through under vacuum to the level of the top frit at a rate of approximately 2 ml min^{-1}, discarding the column eluate. Add a further volume of hexane (3 ml) to the top of each cartridge and draw through under vacuum to the level of the top frit at the same rate, again discarding the column eluents. Do not allow cartridges to become dry.

d) Connect the diol column on top of the amino propyl column by using a column connection adaptor.

e) Load the solution produced in Section 3.3 (b) onto the diol column and draw through under vacuum to the level of the top frit at a rate of approximately 2 ml min^{-1}, discarding the column eluate.

f) Rinse the sample flask with further portions of hexane:ether 90:10 v/v (2 x 3 ml) and load these on to the diol column, discarding the column eluate.

g) Wash the diol column with hexane:dichloromethane 50:50 (2 x 3 ml), discarding the column eluate . The tralkoxydim and R162684 are not retained on the diol column but elute on to the aminopropyl column where they are retained. Discard the diol column.

h) Add acetone (1 ml) to the top of the amino column. Draw through the column under vacuum, to the level of the top frit at a rate of approximately 2 ml min^{-1}. Discard the column eluate.

i) Place collection tubes (10 ml) under each port in the manifold rack as required. Add acetone (4 ml) to the top of each column and draw through under vacuum at a rate of approximately 2 ml min^{-1} to elute the tralkoxydim and internal standard, finally taking the cartridge to dryness.

Note :

The above SPE procedure has been developed using columns from the stated manufacturer, however, it is possible to carry out the procedure using similar columns from another manufacturer. In all cases, it is strongly recommended that the elution profile of the chosen batch of columns is checked prior to commencing analysis to rule out any variation between manufacturers products and between batches.

j) Evaporate the eluate to dryness under a stream of clean, dry air and resuspend in 1 ml water:acetonitrile (60:40) prior to analysis.

Note : Samples should be removed upon reaching dryness. Breakdown of tralkoxydim may occur if samples are left dry. See section 7 for further detail.

k) Transfer to HPLC vials for analysis by high performance liquid chromatography with UV detection.

l) For HPLC analysis a standard solution in acetonitrile:water 60:40 v/v should be prepared. For example, to prepare a 0.1 µg ml^{-1} standard take 100 µl of a 1.0 µg ml^{-1} standard and transfer to a suitable test tube. Blow to dryness under a stream of dry air. Elevated temperatures of up to 40°C may be used to aid this process. Resuspend in acetonitrile: water 60:40 v/v (1 ml) to give a 0.1 µg ml^{-1} standard.

Note : Sample extracts should be analysed by HPLC on the same day that they are prepared. Breakdown of tralkoxydim has been observed when samples have been stored in acetonitrile solutions.

4 HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)

The conditions for the analysis of tralkoxydim and R162684 will depend upon the equipment available. The instrument operating manuals should be consulted at all times to ensure safe and optimum use. The following conditions have been found satisfactory in this laboratory, using a Hewlett Packard 1100 series instrument fitted with an ultra violet detector (UV).

4.1 Equipment

Pump	:	Hewlett Packard 1100 Quaternary Pump
Degasser	:	Hewlett Packard 1100 Degasser
Autosampler	:	Hewlett Packard 1100 Automatic Liquid Sampler
Detector	:	Hewlett Packard 1100 Variable Wavelength detector
Column oven	:	Hewlett Packard 1100 Column Compartment

4.2 Instrument Conditions

Column	:	Kromasil C18 25 cm x 3.2 mm i.d.
Column temperature	:	30 °C
Mobile phase	:	Acetonitrile:water 65 : 35 + 0.4% glacial acetic acid

Prior to use all solvents should be filtered through a 0.45 µm (or smaller) filter under vacuum in order to remove particulates.

Flow rate	:	1 ml min^{-1}
Injection volume	:	75 µl
Injection protocol	:	Analyse calibration standard after 3 to 4 sample extracts
Retention time	:	~ 5 minutes (tralkoxydim) and ~7.5 minutes (R162684)
Detector wavelength	:	255 nm

5 CALCULATION OF TRALKOXYDIM RESIDUE RESULTS

Note : The internal standardisation procedure determines the concentration of the tralkoxydim residue in the final extract relative to that of a known concentration of R162684 which is added by accurate fortification of the sample prior to extraction. Correction for percentage recovery throughout the procedure is thereby inherent for each individual sample.

The calculation used for the determination of tralkoxydim residues by internal standardisation using R162684 may be performed using a 'single point ratio calibration'.[1] It should be noted that such calibrations are only feasible when the internal standard chosen meets certain criteria

(a) Make repeated injections (75 µl) of an analytical standard solution containing a mixture of tralkoxydim and Internal Standard (R162684) into an HPLC operated under the conditions described in Section 5. When a consistent response is obtained measure the peak height/area for tralkoxydim and R162684.

(b) Inject 75 µl of sample solution and measure the peak height/area of the peaks corresponding to tralkoxydim and R162684.

(c) Re-inject the standard solution after a maximum of 4 injections of sample solutions

(d) Calculate the tralkoxydim residue in the sample, expressed as mg kg^{-1}, by proportionation of the tralkoxydim/R162684 peak height or peak area ratio measured for the sample against that for the analytical standard solution.

$$\text{Residue tralkoxydim} = \frac{\text{Response ratio (SA)}}{\text{Response ratio (STD)}} \times \text{CA} \times \frac{\text{ISTD Level}}{\text{CIS}}$$

Response ratio (SA)	=	Peak response ratio for sample
Response ratio (STD)	=	Average peak response for bracketing standards
CA	=	Concentration of tralkoxydim standard (µg ml^{-1})
CIS	=	Concentration of R162684 standard (µg ml^{-1})
ISTD Level	=	Internal standard fortification level (µg g^{-1})

Note : In the case where laboratory data systems/computing integrators are used the computer algorithm may adopt a slightly different method for calculation of results. The final calculated results are, of course, the same as the above manual calculation.

6 CONTROL AND RECOVERY EXPERIMENTS

A minimum of one control and two recovery experiments should be run alongside each set of samples analysed (that is untreated samples accurately fortified with a known amount of tralkoxydim and R162684 prior to extraction).When no residues are expected, the recoveries should be fortified at low levels, typically 0.005- 0.01 mg kg^{-1}

Recovery data is generally considered acceptable when the mean values of both internal and external recoveries are between 70% and 110% and with a coefficient of variation of <20%.

Reagent blanks may also be analysed to ensure that no contamination occurs during analysis due to solvents or materials used.

7 MODIFICATIONS AND POTENTIAL PROBLEMS

a) Tralkoxydim is known to be unstable under certain conditions. Specifically, it is prone to isomerisation and is susceptible to free radical attack. It is strongly recommended that the analytical procedure is completed within a single day.

Areas of the method in which particular care should be taken are listed in (b) to (e).

b) It is recommended that fresh diethyl ether is used for each analysis. Breakdown of tralkoxydim has been observed when old diethyl ether has been used.

c) Sample extracts should be analysed by HPLC on the same day that they are prepared. Breakdown of tralkoxydim has been observed when stored in acetonitrile solutions.

d) Care should be taken during the evaporation of solutions containing tralkoxydim. Breakdown of tralkoxydim has been observed when samples are left dry for any period of time.

e) Samples and standards must be injected from each vial only once and therefore standards must be placed in individual vials for each injection required throughout the analytical run.

8 LIMITS OF QUANTIFICATION AND DETECTION

Limit of Quantification (LOQ)

The limit of quantification of the method is defined as the lowest analyte concentration in a sample at which the methodology has been validated and a mean recovery of 70 - 110% with a cv of ≤ 20% has been obtained.

Generally, for accurate quantification, the response for an analyte peak should be no lower than four times the mean amplitude of the background noise in an untreated sample at the corresponding retention time.

The limit of quantification has been set at 0.005 mg kg^{-1} for HPLC-UV determination.

Apparatus (UK Suppliers)

a) Equipment for the initial sample preparation: Tecator Homogeniser, available from Philip Harris Scientific, 618 Western Avenue, Park Royal, London W3 0TE, UK. Part number M48-525.

b) Nalgene™ polypropylene centrifuge bottles 250 ml size, available from Fisher Scientific UK, Bishop Meadow Road, Loughborough, Leicestershire LE11 5RG, UK. Part number CFT-892-S.

c) Whatman No. 1 filter Paper, available from Whatman International Ltd, St. Leonard's Road, 20/20 Maidstone, Kent, ME16 OLS

d) Mechanical shaker, available from available from Fisher Scientific UK, Bishop Meadow Road, Loughborough, Leicestershire LE11 5RG, UK. Part number SGM-200-010F.

e) Vacuum rotary evaporator e.g. Buchi Rotavapor R114, available from Fisher Scientific UK, Bishop Meadow Road, Loughborough, Leicestershire LE11 5RG, UK. Part number EVF-910-011N.

f) Ultrasonic bath e.g. Ultrawave U300/D, available from Fisher Scientific UK, Bishop Meadow Road, Loughborough, Leicestershire LE11 5RG, UK. Part number BMA-100-020P.

g) Laboratory glassware, available from Fisher Scientific UK, Bishop Meadow Road, Loughborough, Leicestershire LE11 5RG, UK.

h) Isolute Vacmaster-20™ sample processing station, available from Jones Chromatography Ltd., Tir-y-Berth Industrial Estate, New Road, Hengoed, Mid Glamorgan CF8 8AU, UK. Part number 121-2016.

i) NH_2 Aminopropyl solid phase extraction columns (500 mg, 3 ml capacity) available from Varian Sample Preparation Products, Varian Ltd., 28 Manor Road, Walton-on-Thames, Surrey, KT12 2QF, UK.

j) 2OH Diol solid phase extraction columns (500 mg, 3 ml capacity) available from Varian Sample Preparation Products, Varian Ltd., 28 Manor Road, Walton-on-Thames, Surrey, KT12 2QF, UK.

k) Column connection adapters, available from Jones Chromatography Ltd., Tir-y-Berth Industrial Estate, New Road, Hengoed, Mid Glamorgan CF8 8AU, UK. Part number 120-1101.

l) Hewlett Packard 1100 series high performance liquid chromatograph or equivalent available from Hewlett Packard Co., Cain Road, Bracknell, Berkshire RG12 1HN, UK.

m) Reversed phase HPLC column with KR100-5C18 packing, 25 cm x 3.2 mm i.d. Available from Hichrom Ltd., 1 The Markham Centre, Station Road, Theale, Reading, Berkshire RG7 4PE.

n) Crimp cap auto sampler vials and caps available from Hewlett Packard Co., Cain Road, Bracknell, Berkshire RG12 1HN, UK.

Apparatus (US Suppliers)

a) Equipment for the initial preparation of samples eg Tecator homogeniser available from Perstorp Analytical inc., 12101 Tech Road, Silver Spring, Maryland 20904.

b) Nalgene™ polypropylene centrifuge bottles 250 ml size, Available from Nalgene Company, 75 Panorama Creek Drive, PO Box 20365, Rochester, NY 14602-0365. Part number 3120-0250.

c) Mechanical shaker, available from available from Fisher Scientific, Liberty Lane, Hampton, NH 03842, USA. Part number 14-260-10.

d) Laboratory glassware is available from Fisher Scientific, Liberty Lane, Hampton, NH 03842, USA.

e) Whatman Number 1 filter papers available from Bryant Laboratories Inc., 1101 Fifth Street, Berkeley, CA 94710..

f) Vacuum rotary evaporator with thermostatically controlled water bath, eg Buchi model R114 available from Fisher Scientific, Liberty Lane, Hampton, NH 03842, USA.

g) Ultrasonic bath available from Fisher Scientific, Liberty Lane, Hampton, NH 03842, USA.

h) Isolute Vacmaster-20™ sample processing station available from Jones Chromatography USA Ltd., PO Box 280 329, Lakewood, Colorado, 8022-0329

i) NH_2 Aminopropyl solid phase extraction columns (500 mg, 2.8 ml capacity) available from Varian Sample Preparation Products, Varian Ltd., 24201 Frampton Avenue, Harbor City, CA 90710, USA.

j) 2OH Diol solid phase extraction columns (500 mg, 2.8 ml capacity) available from Varian Sample Preparation Products, Varian Ltd., 24201 Frampton Avenue, Harbor City, CA 90710, USA.

k) Column connection adapters, available from Jones Chromatography USA Ltd., PO Box 280 329, Lakewood, Colorado, 8022-0329

l) Crimp cap autosampler vials and caps available from Hewlett Packard Co., PO Box 1000, Avondale, PA 19311-1000 (Tel: 800-223-9700).

m) Reversed phase HPLC column with KR100-5C18 packing, 5 cm x 3.2 mm id. Available from Phase Separations Inc., 20 Liberty Way, Franklin, MA 0238, USA.

n) Hewlett Packard 1100 series high performance liquid chromatograph or equivalent available from Hewlett Packard Co., PO Box 1000, Avondale, PA 19311-1000, USA

Reagents (UK Suppliers)

a) Acetonitrile, hexane, diethyl ether, dichloromethane and acetone super purity grade, available from Romil Ltd., The Source, Convent Drive, Waterbeach, Cambridge CB5 9QT, UK.

b) Ultra pure water from a laboratory water purification system eg Elga Maxima available from Elga Ltd., High Street, Lane End, High Wycombe, Bucks HP14 3JH, UK.

c) Glacial acetic acid, available from Fisher Scientific UK, Bishop Meadow Road, Loughborough, Leicestershire LE11 5RG, UK.

d) Sample of tralkoxydim of known purity.

e) A sample of R162684 of known purity for use as internal standard.

Reagents (US) Suppliers

a) Acetonitrile, hexane, diethyl ether, dichloromethane and acetone available from B & J Brand Solvents, from Scientific Products Division of Baxter Healthcare Corporation, USA (Tel: 312-689-8410).

b) Ultra-pure water from a laboratory water purification system available from Waters Corporation, Milford, MA, USA

c) Glacial acetic acid, available from Fisher Scientific, Liberty Lane, Hampton, NH 03842, USA.

d) Sample of tralkoxydim of known purity.

e) A sample of R162684 of known purity for use as internal standard.

Hazards and Safety

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 (e.g. ZENECA 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

	Acetic Acid	Dichloromethane	Acetone	Hexane	Diethyl Ether
Harmful Vapour	✓	✓	✓	✓	✓
Highly flammable	✓	×	✓	✓	✓
Harmful by skin absorption	✓	✓	×	✓	×
Zeneca Divisional Toxicity Class	3	2	4	3	4
RL mg m^{-3}	25	350(CL)	2400	360	1200

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

b) Tralkoxydim has a mammalian toxicity (acute oral LD_{50}) in the rat greater than 900 mg kg^{-1}

c) Tralkoxydim has a ZENECA Divisional Toxicity class of 3

d) R162684 has a ZENECA Divisional Toxicity class of 3

Preparation of Standards

Weigh out accurately, using a five figure balance, sufficient tralkoxydim and R162684 solid standards to allow dilution in acetone to give a 1000 µg ml^{-1} stock solution in volumetric flask. Make serial dilutions of this stock solutions to give 100, 10, 1 and 0.1 µg ml^{-1} standard solutions in acetone. These solutions of tralkoxydim and R162684 are to be used for fortification of recovery samples.

Also mixed standards of an appropriate concentration should be prepared in water:acetonitrile (60:40) for HPLC use just prior to analysis, as described in section 3.3 l).

Standard solutions of tralkoxydim and R162684 in acetone have been shown to be stable for four months when stored at <7°C. Analytical standards should be freshly prepared from the solid material after four months of use.