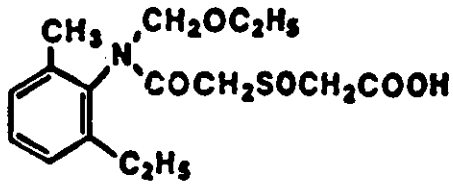
1. SCOPE

The analytical procedure described is suitable for the determination of [ethoxymethyl(6-ethyl-o-tolyl)carbamoylmethyl sulphinyl]acetic acid [thioacetic acid sulphoxide (I)] in soil using external standardisation. The limit of determination of the method is 0.01 mg kg^{-1}

(I)



2. METHOD SUMMARY

A prepared soil sample (20 g) is extracted by shaking with acetonitrile/ water (1:1 v/v) containing 40 mM ammonium acetate for 60 minutes at room temperature. The acetonitrile is then evaporated and the pH of the aqueous extract is adjusted to 1.9. The extract is then subjected to a C_{18} 'Mega Bond Elut' clean-up followed by derivatisation with MTBSTFA. The residue determination is by gas chromatography with mass selective detection.

3. PROCEDURE

3.1 Sample Preparation

Soil samples received in zero contamination tubes should be allowed to thaw for approximately half to one hour prior to preparation.

The soil from the plastic tubes is either bulked or divided into the various depths required for analysis. The bulked sample is weighed and put through a 2 mm sieve to remove any stones, roots etc. before the sample is reweighed. The soil is then thoroughly mixed before taking a representative sample for analysis.

A moisture content should be evaluated on the sample. (A known weight of soil (approximately 20g) is placed in a petri dish and dried to constant weight by placing in an oven at 120°C maximum).

3.2 Extraction

a) Weigh representative samples of soil in duplicate (20g) into screw cap plastic centrifuge tubes and spike at least two recovery samples with standard (dissolved in acetonitrile) as required. (See Section 6). One untreated control sample and one reagent blank should also be included. (See Section 5).

b) Add Acetonitrile : Water (1:1 v/v) containing 40 mM ammonium acetate (40 ml) to the centrifuge tube and cap it firmly. Shake the extracts vigorously on a mechanical shaker for 60 minutes, at room temperature.

c) Centrifuge the extracts at 2000 rpm for 15 minutes. Then transfer a 10 g aliquot (20 ml) to a 100 ml Round-bottomed flask.

d) Evaporate the 20 g aliquot to below 10 ml by rotary evaporation in a water bath of 40°C. Transfer each extract to a labelled scintillation vial, washing the round-bottomed flask with a small amount of water and combining the extracts. Add a magnetic stirrer to the vial and cap firmly.

e) Precisely adjust the pH of the extract to pH = 1.9 -2.0 using 1M HCl.

Note : (i) The precise pH setting is important. In case of too low pH, 1M KOH should be used to bring the pH back into range.

3.3 C_{18} Column Clean-up

3.3.1 Column Conditioning

a) Condition the C_{18} column with 2 x 2 ml of methanol followed by 2 x 2 ml of water.

Note : Do not let the column go dry before use.

3.3.2 Sample Application

The analyte is completely retained on the C_{18} column. Thus, all the application solution and the following washes should be discounted.

a) Load the extracts onto the column at a rate not exceeding 5 ml/mfm from a 15 ml reservoir on top of the cartridge.

b) Wash the column with 2 x 2.5 ml of water.

c) Remove the reservoir and wash the column with 1.2 ml of hexane. The column is then dried by applying full vacuum for about 5 mins.

d) The column is then washed with 2 x 1.2 ml of dichloromethane.

3.3.3 Elution of Analyte

a) Place a 7 ml Pierce Reactivial in the Supelco block and elute the column with 3 x 1 ml of ethyl acetate.

b) Further elute with 3 x 1 ml of 20% methanol in ethyl acetate.

c) Reduce the extracts to drynes. under vacuum.

d) The analyte is then taken up in 1.8 ml of Romil far U.V acetonitrile and ultrasonified.

3.4 Preparation of Derivative

At this stage, it is important that a standard is taken through the same procedure as the extracts for use in quantification at the M.S.D. stage.

Conditions for the standard should be similar to those of the extracts in order that a consistant derivatisation be achieved. Therefore the following procedure has been adopted eg. for the preparation of a 0.05 $\mu g\ ml^{-1}$ standard, place 0.25 ml of the 1.0 $\mu g\ ml^{-1}$ standard in a 7 ml Pierce reacti-vial and blow to dryness under a gentle stream of air. The standard is taken up in 1.8 ml of Romil far U.V Acetonitrile and should then be taken through procedures 3.4 (a) - (d).

NB. See matrix - matched standards (Section 6).

a) In a fume cupboard, add 200 µl of MTBSTFA to the extracts, cap tightly and shake.

b) Place in a heating block at 110°C for 2 hours.

c) Allow to cool and dilute the derivatising agent by adding a further 3 ml of Romil far U.V Acetonitrile.

d) Transfer on aliquot of the sample to a G.C vial for analysis.

3.5 **Gas Liquid Chromatography**

The following conditions gave satisfactory results using a Hewlett Packard HP5890A gas liquid chromatograph fitted with a mass selective detector, model HP5970.

3.5.1 Chromatographic Conditions

Columns and column packing : DB17 Capillary Column
10 m x 0.18 mm i.d
0.3 µm film thickness

G.C Conditions

Carrier Gas : Helium at 5.0 psi
Injection Volume : 1 µl
Injector Temperature : 250°C
Temperature Program : 60°C/0 min
20°C min^{-1} 280°C 0 min

MSD Conditions

Electron Multiplier : 3 x 10^2 Volts Relative

Electron energy : 70 eV

System Calibration : Autotune

Acquisition Mode : Selective Ion Monitoring, low resolution for ionm/z = 348
A counter ion m/z = 302 can also be monitored.

The MTBSTFA derivative of the thioacetic acid sulphoxide metabolite has a retention time of ~12 minutes under these conditions in the laboratory.

Typical chromatograms are shown in Appendix 5.

3.6 Calculation of Results

The residues may be calculated in mg kg^{-1} for each sample extract using a mean standard response from injections bracketing the sample as follows:

$$\text{Residue} = \frac{\text{Pk area (Sample)}}{\text{Pk area (Std)}} \times \frac{\text{W (Std)}}{\text{I (Sample)}} \times \frac{\text{V(s)}}{\text{W(s)}} \quad \text{mg kg}^{-1}.$$

Where Pk area (Sample) = Peak area for sample (μVs)
Pk area (Std) = Average peak height area for bracketing standards (μVs)
W (Std) = Weight of analyte in reference standard (μg)
I (Sample) = Sample solution injection volume (ml)
V (s) = Solvent volume in sample extract (ml)
W (s) = Sample weight equivalent to sample extract (g)

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).

$$\text{Corrected Residue (mg kg}^{-1}) = \frac{\text{Residue}}{\text{Average percentage recovery}} \times 100$$

Results should finally be corrected for the soil moisture content using the following equation:

$$\text{Dry weight residue (mg kg}^{-1}) = \frac{\text{Wet Weight Residue} \times 100}{100 - \% \text{ Soil Moisure Content}}$$

Results should be corrected to two significant figures (S.F.) or one S.F. if the residue is >0.1 mg kg^{-1}

4. LIMIT OF DETERMINATION

A true assessment of the limit of determination of the method may be determined by fortification of untreated samples at the limit of determination levels with standards and subjecting them to the complete analytical procedure. The chromatographic response obtained for these samples at a retention time of ~12 minutes should exceed the background signal noise by a factor of at least four to be considered an acceptable quantitative limit of determination.

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

5 CONTROLS/REAGENT BLANKS

The analyst must verify that the sample chromatograms are free from interferences orginating from either untreated soil samples or from the reagents, and that sample contamination with analytes has not occurred before or during the analysis.

Therefore at least one control sample and one reagent blank (whole procedure followed in absence of soil) should be analysed alongside each set of samples.

6 MATRIX - MATCHED STANDARDS

If high recoveries are regularly being obtained on running the method, this may be due to adsorption of the active in the standard solution onto the liner and column active sites.

To prevent this effect, the standards are run in the presence of 'matrix', which involves taking on extra control extract through the method to Section 3.3.3c). The procedure described for standard solutions is then followed, with the standard being added to the extra control extract.

7 RECOVERIES

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 analytes prior to extraction). In this laboratory these external recoveries are generally run alongside each set of samples.

For external standard recoveries, fortification levels should be based on the expected soil residue levels. When no residues are expected the recoveries should be fortified at low levels typically 0.1 mg kg^{-1} and include at least one fortified at the limit of determination. Provided the recovery values obtained are acceptable, they may be used to correct the determined residues, as in Section 3.8.

8 METHOD VALIDATION

a) Standard Calibration Graph

A series of standard solutions were made up in acetonitrile in the range 0.01-5.0 mg kg^{-1}. These were run on the G.C. using the conditions described. A calibration graph was constructed of peak area/vs analyte concentration/ $\mu g\ ml^{-1}$. (see Appendix 6).

Linear relationships seemed dependant on the performance of the detector. If a non-linear relationship is being obtained, it is imperative to bracket the samples during a run with standards of approximately the same strength as the expected residues.

b) A series of recoveries were fortified at levels between 0.11-1.0 mg kg^{-1}. These were then taken through the method.

APPENDIX 1 : Apparatus

a) Equipment for the initial preparation of samples e.g. a Hobart Band Saw for cutting soil cores.

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

c) Complete Visiprep™ SPE twelve place vacuum manifold assembly with sample collector rack (for use with BOND ELUT™ disposable extraction columns). Supelco SA, Switzerland.

d) Gas liquid chromatograph fitted with a mass selective detector, eg. Hewlett Packard HP5890A fitted with an HP7673A autosampler and an HP5970 MSD.

e) Analytical GC Capillary column: 10 m x 0.18 mm i.d; 0.3 µm film thicknes DB17 available from Thames Chromatography, Maidenhead, Berkshire.

f) Laboratory centrifuge eg WIFUG Model 2000E, WIFUG (A Division of Eltex of Sweden Ltd, Bradford, UK).

g) 7 ml Pierce™ reacti-vials, caps and septa.

h) Disposable sterile centrifuge tubes (250 ml), Scientific furnishings (Corning) U.K.

i) Reacti-therm heating module. (Available from Pierce)

j) pH Meter. Available from Corning.

k) Flask shaker. Available from Stuart Scientific U.K.

f) 6cc C_{18} 'Mega Bond Elut' columns, available from Jones Chromatography, Mid-Glamorgan.

APPENDIX 2 : Reagents

a) Solvents: glass distilled and Romil far UV grade acetonitrile. Glass distilled grade hexane, dichloromethane, ethylacetate and methanol, Romil Chemical UK.

b) Ultra pure water.

c) C_{18} MEGA BOND ELUT™ disposable extraction columns (6 cc). Supplier:- Jones Chromatography Ltd, UK.

d) Ammonium Acetate, Baker Analysed HPLC Reagent.

e) HCl, FSA, UK.

f) Thioacetic Acid Sulphoxide Standard (ICIA5676/48)-[Ethoxymethyl (6-ethyl-o-tolyl)carbamoylmethyl sulphinyl]acetic acid. (known purity 98%).

g) N-methyl-N-(tert-butyldimethylsilyl)trifluoroacetamide.

APPENDIX 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 doubt, consult the appropriate safety manual (e.g. ICI Laboratory Safety Manual) containing recommendations and procedures for handling chemicals, and a monograph such as 'Hazards in the Chemical Laboratory' edited by L Bretherick, The Royal Society of Chemistry, London.

a) ACETONITRILE

Toxic by inhalation or contact with skin
Highly flammable
Do not breathe vapour
Avoid contact with eyes and skin
(RL 70 mg m^{-3})

b) HEXANE

Highly flammable
Harmful by inhalation and in contact with skin.
Possible risk of irreversible effects.
Avoid breathing vapour. (RL 360 mg m^{-3})

c) DICHLOROMETHANE

Harmful by inhalation
Avoid breathing vapour
Avoid contact with skin of eyes
(RL 350 mg m^{-3})

N.B This compound is now Class 2.
If in doubt use in a fume cupboard

d) ETHYL ACETATE

Highly flammable
Avoid breathing vapour
Avoid contact with eyes
(RL 1400 mg m^{-3})

e) METHANOL

Highly flammable
Serious risk of poisoning by inhalation or swallowing
Avoid contact with skin and eyes
(RL 260 mg m^{-3})

f) [Ethoxymethyl(6-ethyl-o-tolyl)carbamoylmethyl sulphinyl] acetic acid (thioacetic acid sulphoxide, ICIA5676/48)

Avoid contact with eyes, skin and clothing.
Avoid breathing vapours

g) HYDROCHLORIC ACID

Harmful Vapour
Causes burns, Irritating to respiratory system
Avoid breathing vapour
Prevent contact with eyes and skin
RL (as HCl) 7 mg m^{-3}

APPENDIX 4 : Preparation of Analytical Standards

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.

Weigh out accurately, using a five figure balance, sufficient thioacetic acid sulphoxide standard to allow dilution in acetonitrile to give a 1000 $\mu g\ ml^{-1}$ stock solution in a volumetric flask (100 ml). Make serial dilutions of this stock solution to give 100 $\mu g\ ml^{-1}$, 10 $\mu g\ ml^{-1}$ and 1.0 $\mu g\ ml^{-1}$ standard solutions in acetonitrile. These solutions should be used for the fortification of recovery samples.

When not in use, standard solutions should always be stored in a refrigerator at 4°C to prevent evaporation and concentration. It is recommended that analytical standards should be replaced with freshly prepared standards after four months of use.