

Analytical Procedure: CHE 608/26-02R
Test Article: ET-751,E1,E2,E3
Issue Date: 25 July 1995

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Determination of ET-751 and its Metabolites E1, E2 and E3 Residues in Soil

1 GENERAL PRINCIPLES

Soil is extracted by shaking with acidic acetonitrile. The extract is centrifuged and an aliquot is diluted with water and cleaned-up by C18 solid phase extraction cartridge. Samples are eluted from the cartridge with acetonitrile and diluted with water for analysis.

Determination is by multiple reaction monitoring high performance liquid chromatography with electrospray mass spectrometry (LC/MS-MS).

2 SAFETY AND HANDLING

All procedures in this method have handling and control codes which are detailed in Section 8.

Laboratory coats and safety glasses (categories 2a and 3a) must be worn at all times in the laboratory.

The test articles do not have any known hazards, or toxicity under normal laboratory usage. Operators should take the normal precaution of wearing gloves when handling it.

3 APPARATUS, MATERIALS, REAGENTS AND SOLUTIONS

3.1 Apparatus, glassware etc

6 oz plastic jars
100 mL measuring cylinders
10 mL volumetric flasks
Mechanical shaker
Centrifuge MSE 3000

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Vacuum manifold for solid phase extraction cartridges
Autosampler vials and caps
VG Quattro Triple Quadrapole Mass Spectrometer

3.2 Materials

The materials listed below should be labelled with a hazard symbol or other marking to indicate their potential hazards. The hazard should be listed below if this is not the case. If there is no marking or indication consult the responsible scientist.

Acetonitrile	- HPLC grade, Rathburns
Methanol	- HPLC grade, Rathburns
Water	- HPLC grade, BDH
Water	- Distilled
Hydrochloric acid	- Analar, specific gravity 1.18, BDH
Glacial Acetic acid	- GPR, 100%, BDH
C18 solid phase extraction cartridges	- Bond Elut (1 g/6 mL), Varian

3.3 Reagents and solutions

3.3.1 Test article stock solution (100 µg/mL)

For each test article accurately weigh the test article (approximately 5.0 mg). Dissolve the test article in acetone and make to 50 mL. Store in a freezer and prepare fresh every 3 months. [1a,4c]

3.3.2 Test article fortifying solutions

Dilute the stock solutions with acetone to give mixed fortifying solutions of 2.0, 10.0 and 20.0 µg/mL. Store in a freezer and prepare fresh every 3 months. [4a]

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3.3.3 Test article calibration solution

Dilute the stock solutions with acetonitrile to give mixed solutions of 10.0 µg/mL. Store in a freezer and prepare fresh every 3 months. [4a]

3.3.4 Preparation of calibration line

Dilute the solution prepared in Section 3.3.3 with acetonitrile and HPLC grade water (7:3 v/v) to give a 1.0 µg/mL mixed solution.

Dilute the 1.0 µg/mL in control matrix (see Section 4.2) to produce calibration standards of 2.5, 5, 10, 20, 35, 50 and 75 ng/mL. Prepare fresh calibration standards in control matrix with every sample batch and discard after use. [4a]

4 PROCEDURES

The recovery efficiency of the analytical procedure is determined with each analysis batch using fortified control sample. Fortification is carried out prior to extraction at concentrations applicable to those in study samples.

4.1 Extraction of soil

Weigh a sub-sample (20 g) of soil into a 6 oz plastic jar. Add a mixture of acetonitrile and 1N hydrochloric acid (4:1 v/v, 100 mL) and seal with a lid. Shake for 30 minutes on a mechanical shaker and then centrifuge at 2000 rpm for 5 minutes (Note 1). Transfer an aliquot (25 mL \equiv 5 g soil) to a 100 mL measuring cylinder, add distilled water (75 mL) and ensure sample extract is thoroughly mixed. [4a]

Note 1: If after extraction, separation between the organic and aqueous phases occurs. Decant the extraction solvent into a 200 mL measuring cylinder. Make to 200 mL with distilled water, ensure sample is thoroughly mixed. Transfer an

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aliquot (50 mL \equiv 5 g soil) to a 100 mL measuring cylinder, add distilled water (50 mL) and ensure sample extract is thoroughly mixed.

4.2 Solid phase clean-up

Condition the C18 solid phase extraction cartridge with methanol (10 mL) followed by distilled water (10 mL). Do not allow the cartridge to go dry. Apply the diluted sample extract (100 mL) to the cartridge and discard the eluate. Wash out the measuring cylinder with distilled water (10 mL) followed by a mixture of acetonitrile and distilled water (3:7 v/v, 10 mL). Apply each wash to the cartridge and discard the eluate. Elute the cartridge with acetonitrile (7 mL) and collect in a 10 mL volumetric flask allowing the cartridge to go dry. Add HPLC grade water to make the volume up to 10 mL and analyse by LC/MS-MS. Extra aliquots of control sample will be prepared to provide control matrix for preparation of calibration solutions (see Section 3.3.4). [4b]

Note 2: Due to the alkalinity of some soil types it may be necessary to alter the acidity of the sample extract before applying it to the solid phase extraction cartridge. This can be done by the dropwise addition of 1N hydrochloric acid until acidic < pH 4 is achieved.

Note 3: The elution profile of the cartridge is given only as a guideline and may vary between batches.

4.3 Liquid chromatographic conditions

Column: Inert Pack Phenyl
(5 μ m, 25 cm x 4.6 mm id)

Mobile phase: Acetonitrile + Water + Acetic acid
(70:30:1 v/v/v) at 1.0 mL/min

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Injection volume:	100 μ l
Detector:	VG Quattro Triple Quadrapole Mass Spectrometer
Quantifying ions	413 > 339 - ET751 385 > 276 - E1 327 > 277 - E2 341 > 291 - E3
Retention times	ET-751 - 6.6 minutes E1 - 4.8 minutes E2 - 4.8 minutes E3 - 6.5 minutes

5 CALCULATIONS

ET-751 and its metabolite residues are determined on the basis of peak area using a calibration line. All data acquisition, peak measurements and calculations are performed on the Fisons Mass Lynx PC based system.

The analytical specifications are as follows:

Number of calibration standards to be used ≥ 5

Standard working range $C_{\min} \leq$ limit of determination

$C_{\max} >$ highest concentration found

Correlation coefficient of the calibration ≥ 0.99

Recovery values accepted in the range 70 to 110%

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The response from calibration solutions (R) is plotted against the amount of test article injected (A) to generate a straight line graph ($R = BO + B1A$) where B1 is the gradient and BO is the intercept.

Concentrations of test article (A) in samples are calculated from their response using the equation:

$$A (\mu\text{g/mL}) = \frac{(R - BO)}{B1}$$

Recoveries are calculated as follows:

$$\text{Residue (mg/kg)} = \frac{\text{extract concentration } (\mu\text{g/mL}) \times \text{final volume (mL)}}{\text{sample weight (g)}}$$

where the final volume includes dilution steps if applicable.

Recovery data from fortified samples are calculated using the following equation:

$$\text{Recovery (\%)} = \frac{A - C}{S} \times 100$$

Where

A = concentration found in fortified control sample (mg/kg)

C = concentration found in control sample (mg/kg)

S = concentration added to the fortified control sample (mg/kg)

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6 DEVIATIONS FROM THIS METHOD

Any minor practical changes to this written procedure which may be necessary are recorded, along with results, calibration and chromatogram data.

7 COSHH ASSESSMENT OF THIS METHOD

The hazards and risks of the substances hazardous to health used in this method have been considered. Provided that the method is accurately followed and the control measures specified in the method are correctly used, there should be no foreseeable hazards to health.

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8 GENERAL HANDLING CONTROL CATEGORIES

CATEGORY		CONTROL
Main	Division	Name and Specification
1	a b c d	GLOVES Disposable latex Disposable Nitrile Rubber gloves Specific type for job (see assessment giving details)
2	a b c d e	PROTECTIVE CLOTHING Laboratory coat or equivalent Disposable overalls Oversleeves Overshoes Plastic apron
3	a b c	EYE/FACE PROTECTION Safety glasses to BS 2092/2 or better Face shield to BS 2092/2 C or better Safety Goggles to BS 2092/2 C or better
4	a b c d e f g	ENGINEERING CONTROLS Open bench in ventilated area Fume cupboard to BS 7258 Laminar flow cabinet to BS 5295 Class 1 Re-circulating fume chamber Radioisotope lab Biohazard lab Glove Box
5	a b c	RESPIRATORY PROTECTIVE EQUIPMENT Disposable filtering facemask (HSE approved), i - organic vapour ii - dust iii - combination organic vapour/dust MUST SPECIFY TYPE Powered respirators/helmets with safety visor to BS 2092/2 C or better (HSE approved) Respirator with specified canister (HSE approved)
6		SPECIFIC IMMUNISATION REQUIRED (GIVE DETAILS)
7		ALLERGIC PERSONS PROHIBITED (SPECIFY ALLERGY)
8		REFER TO MATERIAL SAFETY DATA SHEET
9		KNOWN OR SUSPECTED REPRODUCTIVE HAZARD TO EITHER SEX (must specify details)
10		POISON - ensure antidote is available and is within its expiry date (must specify details)