

**VALENT U.S.A. CORPORATION
VALENT TECHNICAL CENTER
DUBLIN, CALIFORNIA**

**DETERMINATION OF ETOXAZOLE,
R3, AND R13 METABOLITES IN SOIL
METHOD RM-37S -2**

DATE: January 29, 2001

INTRODUCTION

This method determines residues of Etoxazole, also known as V-1283, its R3, and its R13, metabolites in soil. This method is derived from soil methods obtained from Sumitomo Chemical Company, Ltd.

Briefly, etoxazole, R3, and R13 residues are extracted from soil using acetone and then partitioned into hexane. The hexane is evaporated and the sample is cleaned up with Florisil and Silica Gel SPE column chromatography. The etoxazole, R3, and R13 are quantitated by gas chromatography using a mass selective detector (MSD) with selective ion monitoring.

Method RM-37S was revised and reissued on June 19, 2000 as RM-37S-1 to include analysis of the metabolite R4. The extraction solvent was changed from acetonitrile to acetone and a partition step into hexane was added. Water bath temperatures were also reduced from 40°C to room temperature. This revision of RM-37S-1 was made to change sample weight used, to add a Silica Gel SPE column cleanup to the method, and to eliminate the analysis of the metabolite R4.

REAGENTS

Acetone - pesticide quality or equivalent.

Celite 545 - reagent grade or equivalent.

Dichloromethane - pesticide quality or equivalent.

Ethyl ether - pesticide quality or equivalent.

Florisil SPE columns - 6 mL, J.T. Baker Cat# 7213-07. (See Note 1).

Hexane - pesticide quality or equivalent.

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Silica Gel SPE columns – 12mL, Varian Cat# 12256018. (See Note 2). Sodium chloride – reagent grade or equivalent.

Triethylamine (TEA) – 99+%, available from Aldrich Chemical Co. (Cat# 23,962-3) or equivalent.

REAGENT SOLUTIONS

Ether:hexane, 1:1 (v/v) - Combine 1 part of ether with 1 part of hexane. For example, add 500 mL of ether and 500 mL of hexane sequentially to a reagent bottle. Store at room temperature.

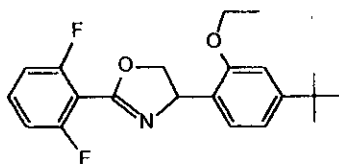
Ether:hexane, 7:3 (v/v) - Combine 7 parts of ether with 3 parts of hexane. For example, add 700 mL of ether and 300 mL of hexane sequentially to a reagent bottle. Store at room temperature.

Hexane:acetone, 4:1 (v/v) - Combine 4 parts of hexane with 1 part of acetone. For example, add 800 mL of hexane and 200 mL of acetone sequentially to a reagent bottle. Store at room temperature.

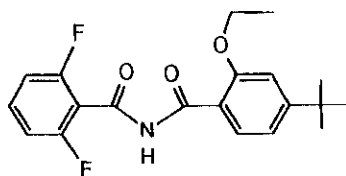
Triethylamine, 0.1% in hexane (v/v) - Combine 1 part of triethylamine with 999 parts of hexane. For example, add 1 mL of triethylamine and 999 mL of hexane sequentially to a reagent bottle. Store at room temperature.

REFERENCE STANDARDS

Etosazole, 5-*tert*-butyl-2-[2-(2,6-difluorophenyl)-4,5-dihydro-1,3-oxazol-4-yl]phenetole - analytical standard of a known purity. Prepare a stock solution containing 1.0 mg/mL in acetone. All solutions should be kept refrigerated when not in use.



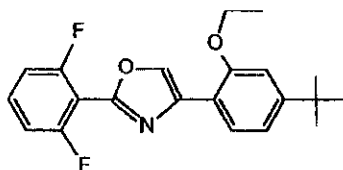
R3, [N-(2,6-difluorobenzoyl)-4-*tert*-butyl-2-ethoxy-benzamide] - analytical standard of a known purity. Prepare a stock solution containing 1.0 mg/mL in acetone. All solutions should be kept refrigerated when not in use.



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R13, [5-tert-butyl-2-[2-(2,6-difluorophenyl)-1,3-oxazol-4-yl]phenetole] - analytical standard of a known purity. Prepare a stock solution containing 1.0 mg/mL in acetone. All solutions should be kept refrigerated when not in use.



STANDARD SOLUTIONS

Fortifying/Linearity Stock Solution - 10 $\mu\text{g/mL}$ - Transfer 1.0 mL of each 1.0 mg/mL stock solution to a single 100 mL volumetric flask and dilute to volume with acetone. All solutions should be kept refrigerated when not in use.

Fortifying/Calibrating Solution - 1.0 $\mu\text{g/mL}$ - Transfer 10 mL of the 10 $\mu\text{g/mL}$ fortifying solution to a 100 mL volumetric flask and dilute to volume with acetone. (See Note 3). All solutions should be kept refrigerated when not in use.

Linearity Standard Solutions - Prepare a minimum of four linearity standards by diluting the fortifying/linearity stock standard solution with acetone to concentrations ranging from 0.1 $\mu\text{g/mL}$ to 2.0 $\mu\text{g/mL}$ (See Note 4). The calibrating solution may be used as one of the four required linearity standards. All solutions should be kept refrigerated when not in use.

EQUIPMENT

Baker SPE-12G Column Processor (12-port vacuum manifold) - J.T. Baker Product # 7018-00 or equivalent system.

Büchner funnels - 9 cm diameter.

Connecting adapter - 24/40 clear-seal outer joint, 13-425, Aldrich cat # Z10632-1, equipped with connectors of screw-thread size of 13-425 to 24-400, Aldrich cat # Z10620-8 to fit screw-cap vials.

Filter flasks - 500 mL.

Filter paper - Whatman GF/A glass fiber or equivalent, 9 cm diameter.

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Gas Chromatograph - Hewlett-Packard Model 6890, equipped with temperature programable on column inlet, a mass selective detector, 7683 automatic sampler, and HP ChemStation or equivalent system.

Mason jars - 1 pint with plastic screw cap lids or equivalent.

Reciprocating shaker -- Eberbach or equivalent.

Rotary evaporator - Büchi or equivalent, equipped with a water bath.

Round-bottom flasks - 50 mL and 500 mL with 24/40 ground glass joints.

Separatory funnels -- 500 mL.

Ultrasonic cleaner -- Branson 3200 or equivalent.

Vials -- #4, with polyethylene-lined screw caps or equivalent.

ANALYTICAL PROCEDURE

1. Extraction

Weigh 25 grams (± 0.1 grams) of soil into a one pint Mason jar. At this point, if required by the testing facility, control samples for method recovery should be fortified with etoxazole, R3, and R13 (See Note 5). Add 100 mL of acetone to the sample and shake for 30 - 35 minutes.

Filter the sample into a 500 mL filter flask using a Büchner funnel containing a bed of celite (approximately 15g) spread evenly on Whatman GF/A glass fiber filter paper (premoistened with acetone). Rinse the Mason jar with two 20 mL portions of acetone and add each portion to the Büchner funnel.

2. Hexane/water Partition

Transfer the combined filtrates to a 500 mL separatory funnel. Rinse the filter flask with 100 mL of 5% sodium chloride solution followed by 100 mL of hexane and add each rinse to the separatory funnel. Shake the separatory funnel vigorously for one minute. Allow the phases to separate, then drain the lower aqueous layer into the filter flask. Pour the upper hexane layer through a filter funnel containing approximately 50 grams of sodium sulfate, suspended on a plug of glass wool, into a 500 mL round bottom flask.

Return the aqueous phase to the separatory funnel and re-extract twice with an additional 50 mL of hexane each time as described above. Drain the lower aqueous phase into the filter flask and discard after the third extraction. Pour the upper hexane layers through the filter funnel

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containing sodium sulfate into the 500 mL round bottom flask containing the first extract. Rinse the sodium sulfate with two 10 mL portions of hexane. Evaporate the extract to approximately 5 mL using a rotary-evaporator and a water bath at room temperature. Transfer the extract to a 100 mL round bottom flask with 2 x 10 mL of dichloromethane and concentrate to dryness using a rotary-evaporator and a water bath at room temperature.

3. Florisil SPE Column Cleanup

Attach a Florisil Solid Phase Extraction column to the Baker-SPE vacuum manifold (See Note 1). Pre-condition the column with 5 mL hexane. Do not exceed a flow rate of 5 mL/minute. Do not allow the column to dry before the sample is applied. Discard the conditioning solvent. Re-dissolve the extract (from Step 2) in 5 mL of hexane. Sonicate the round-bottom flask for approximately 15 seconds and transfer the sample to the column. Drain the solvent to the top of the column. Rinse the round-bottom flask with 5 mL of hexane and transfer to the column. Drain the solvent to the top of the column. Discard the rinse solvent.

Place an #4 screw-cap vial or equivalent under the column. Elute the analytes of interest with 10 mL followed by 15 mL portions of hexane:acetone (4:1,v/v) using each portion to rinse the round-bottom flask before transferring to the column.

Attach connecting adapter to vial and evaporate to dryness using a rotary-evaporator and water bath at room temperature.

4. Silica Gel SPE Column Cleanup

Attach a Silica Gel Solid Phase Extraction column to the Baker-SPE vacuum manifold (See Note 2). Pre-condition the column with 10 mL ether followed by 10 mL 0.1% TEA in hexane. Do not exceed a flow rate of 5 mL/minute. Do not allow the column to dry before the sample is applied. Discard the conditioning solvent.

Place a #4 screw-cap vial or equivalent under the column. Re-dissolve the extract (from Step 3) in 5 mL of ether:hexane (1:1, v/v). Sonicate the round-bottom flask for approximately 15 seconds and transfer the sample to the column. Drain the solvent to the top of the column and collect in vial below. Rinse the round-bottom flask with 5 mL followed by 10 mL portions of ether:hexane (1:1,v/v) and transfer each rinse to the column. Drain the solvent to the top of the column after each rinse.

Attach connecting adapter to the vial and evaporate to dryness using a rotary-evaporator and water bath at room temperature. Re-dissolve the extract in 2.5 mL of acetone, sonicate for approximately 15 seconds, then cap the vial for storage. Store at $\leq 0^{\circ}\text{C}$ until GC analysis.

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5. Gas Chromatography Measurement

Condition the instrument with at least three injections of sample extract. Analyze a range of linearity standards with the analytical sequence. The highly recommended sequence of samples and standards for analysis is: three conditioning samples of sample matrix, linearity standards, three conditioning samples of sample matrix, calibrating standard, sample, sample, sample, calibrating standard, etc. This sequence may be modified if the reproducibility requirement is met. (See Note 4). Each sequence must begin and end with a calibrating standard.

Transfer a portion of the sample extract to an autosampler vial and analyze, along with the calibrating and linearity standard solutions, using the following operating conditions:

Column: (30 M x 320 μ m) capillary
Phase: Rtx[®] - 200 (.5 μ m film thickness)
Restek Cat # 15023 or equivalent

Injection:
Type: on-column
Volume: 0.1 μ L
Viscosity: 0
Plunger Speed: Fast

Injector Temperature Program:
Initial Temp: 98°C
Hold time: 0 minutes
Program Rate: 200°C/minute
Final Temp: 250°C

Oven Temperature Program:
Initial Temp: 95°C
Hold time: 2 minutes
Program Rate: 30°C/minute
Final Temp: 200°C
Hold Time: 0 minutes
Program Rate: 20°C/minute
Final Temp: 310°C
Hold Time: 5 minutes

Carrier Gas: Helium
Flow Rate: 2 mL/minute

Detector:
Type: MS

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Ionization: EI
Transfer Line Temp.: 280°C
Quadrupole Temp.: 150°C
Source Temp.: 230°C

Acquisition mode: Selective Ion Monitoring (SIM)

Etoxazole

Retention Time: 9.75 minutes
Acquisition Interval: 9 – 9.95 minutes
Quantitation Ion: 359.3
Qualifier Ion: 330.2
Dwell Time: 80 msec. (each ion)

R13

Retention Time: 10.12 minutes
Acquisition Interval: 9.95 – 10.8 minutes
Quantitation Ion: 342.0
Qualifier Ion: 357.0
Dwell Time: 80 msec (each ion)

R3

Retention Time: 11.04 minutes
Acquisition Interval: 10.8 – 12.5 minutes
Quantitation Ion: 361.0
Qualifier Ion: 346.0
Dwell Time: 80 msec (each ion)

The instrument parameters shown above are given only as a guide. They may be modified as needed to optimize the chromatography or to resolve matrix interferences. Each set of chromatograms must be clearly labeled with the GC parameters used.

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6. Calculations

The amount of etoxazole, R3, and R13 in each sample is calculated using the following formula:

$$ppm = \frac{B \times C \times V \times DF}{A \times W}$$

where:

- B = integration counts for analyte in the sample.
- C = concentration of analyte in the calibrating standard, (1.0 ug/mL).
- V = final volume of the sample extract, (2.5 mL).
- DF = dilution factor, used if the sample extract is diluted prior to analysis.
- A = mean integration counts for the analyte in the calibrating standards.
- W = sample weight analyzed, (25.0 g).

LIMITS OF DETECTION AND QUANTITATION

The validated limit of quantitation (LOQ) of etoxazole, R3, and R13 in soil analyzed by this method is 0.02 ppm. The estimated limit of detection (LOD) is 0.01-ppm.

ANALYSIS TIME

A trained analyst, familiar with this method, can complete the analysis of a set of twelve samples for etoxazole, R3, and R13 in approximately 12 hours. The results are available within 24 hours of initiating the analysis.

NOTES

1. Each lot of Florisil SPE cartridges must be checked for recovery of analytes as follows: Transfer 1.0 mL of the calibrating standard solution to a 50 mL round-bottom flask and evaporate using a rotary-evaporator with a water temperature bath at room temperature. Re-dissolve in 5 mL of hexane and transfer to a florisil cartridge and elute the analytes as described under Step 3, *Florisil SPE Column Cleanup*.

Evaporate the eluate **just to dryness** using a rotary-evaporator and water bath at room temperature. Re-dissolve the sample in 1.0 mL of acetone and analyze with the calibrating standard as described under Step 5, *Gas Chromatography Measurement*. If the recovery of

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any individual analyte is less than 90%, the elution profile should be modified to obtain 90% recovery of each analyte.

2. Each lot of Silica Gel SPE cartridges must be checked for recovery of analytes as follows: Transfer 1.0 mL of the calibrating standard solution to a 50 mL round-bottom flask and evaporate using a rotary-evaporator with a water temperature bath at room temperature. Re-dissolve in 5 mL of ether:hexane (1:1, v/v) and transfer to a silica gel cartridge and elute the analytes as described under Step 4, *Silica Gel SPE Column Cleanup*.

Evaporate the eluate **just to dryness** using a rotary-evaporator and water bath at room temperature. Re-dissolve the sample in 1.0 mL of acetone and analyze with the calibrating standard as described under Step 5, *Gas Chromatography Measurement*. If the recovery of any individual analyte is less than 90%, the elution profile should be modified to obtain 90% recovery of each analyte.

3. At Valent, reproducibility of an analytical run is determined by calculating the coefficient of variation (CV) from the peak units obtained for the calibrating standards analyzed during the run. For a run to be acceptable, these CV's must be 15% or less unless approved by the chemist responsible for the analysis.
4. At Valent, linearity of the gas chromatograph must be determined each day that samples are analyzed. Linearity is determined by analyzing a series of linearity standards. The response for each standard is normalized to response per 1.0 µg/mL by dividing the response of each standard by its concentration. The coefficient of variation (CV) of these responses must be 15% or less. Sample extracts must be diluted to bring the concentration of analytes within the range of linearity established.
5. At Valent, a standard operating procedure requires that a fortified control sample be analyzed with each set of samples. If the testing facility does not require concurrent analysis of fortified control samples, or if a UTC sample is not available, this method requirement may be waived.

The level of fortification is generally at the LOQ of the method and/or five times the LOQ. Method recoveries must be 70% to 120% to be acceptable unless approved by the chemist responsible for the analysis.