INTRODUCTION

Valent USA Corporation has developed a method of determining the residues of etoxazole and the R3 and R13 metabolites in soils treated with etoxazole. As a part of the registration package, an independent laboratory validation of this method, RM-37S-2, "Determination of Etoxazole, R3 and R13 Metabolites in Soil" is required.

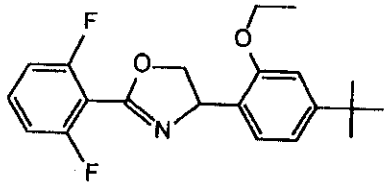
Valent USA Corporation conducted this independent laboratory validation using personnel and laboratories separate from those involved in the development and use of this method.

EXPERIMENTAL

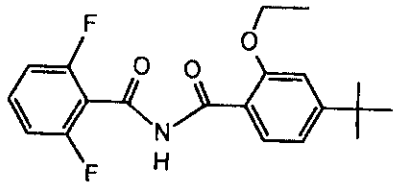
The protocol, with the analytical method RM-37S-2, can be found in Appendix I.

Test and Reference Substances: The test substance for this study is a laboratory dilution of standards of etoxazole and the R3 and R13 metabolites obtained from Valent USA Corporation. The chemical names, structures, lot numbers, purity and certification dates are shown below:

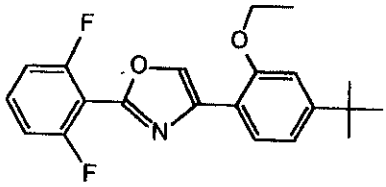
Etoxazole 5-*tert*-butyl-2-[2-(6,2-dilfuorophenyl)-4,5-dihydro-1,3-oxazol-4yl]phenetole
Lot Number: AS 1800b
Purity: 99.2%
Certification Date: June 29, 2000



R3 Metabolite: N-(2,6-difluorobenzoyl)-4-tert-2-ethoxy-benzamide
Lot Number: AS 1874b
Purity: 97.7%
Certification Date: September 25, 2000



R13 Metabolite: 5-tert-butyl-2-[2-(2,6,-difluorophenyl)-1,3-oxazol-4-yl]phenetole
Lot Number: AS 1860c
Purity: 99.1%
Certification Date: May 10, 2000



Certificates of Analysis for these technical standards can be found in Appendix II.

Individual solutions of these materials were made in acetone. The test substance solution was a solution containing all three analytes in acetone, made by diluting the individual analyte solutions. Analytical reference solutions were made by further diluting the test solution.

Test System: The test system for this study is soil. The soil used for the study was obtained from terrestrial field dissipation studies for etoxazole, conducted in California (V-20271, 1999-2000) and Oregon (V-22154, 2000-2001). Untreated soil samples, described below, were combined and homogenized to create a sample of adequate size.

Sample ID	Soil Depth (cm)	Amount Used (g)
V-20271-7U-1	0-7.5 cm	450 g
V-20271-7U-2	7.5-15 cm	250 g
V-20271-4U-1	0-7.5 cm	250 g
V-20271-4U-5	45-60 cm	250 g
V-20271-13U-3	15-45 cm	300 g
V-20271-15U-1	0-7.5 cm	550 g
V-20271-3U-1	0-7.5 cm	400 g
V-20271-3U-3	15-45 cm	500 g
V-20271-17U-1	0-7.5 cm	600 g
V-22154-7U-1	0-7.5 cm	600 g
V-22154-11U-1	0-7.5 cm	750 g

Each method trial consisted of a reagent blank, two control soil samples, five soil sample spiked at 0.02 ppm and five samples spiked at 0.20 ppm.

Reagents and Equipment: See Appendix I – Study Protocol and Analytical Method for a complete list of reagents and equipment used for this study. No substitutions were made to the reagents or equipment described.

Analytical Method: A complete description of the analytical method can be found in Appendix I - Study Protocol and Analytical Method.

Briefly, 25 g of soil was extracted with 100 mL of acetone by shaking for ca. 30 minutes, and the sample extract was suction filtered through a bed of Celite on a GF/A filter paper. The filtrate was transferred to a separatory funnel and 100 mL of a 5% aqueous sodium chloride solution was added, and the sample was extracted with 100 mL of hexane. The hexane portion was filtered through a bed of sodium sulfate, and the aqueous portion extracted twice more with 50 mL portions of hexane. The hexane portions were combined into a 500 mL round bottom flask, and concentrated using rotary evaporation to ca. 5 mL. The sample was transferred to a 100 mL round bottom flask using two 10 mL portions of methylene chloride to rinse the 500 mL flask, and concentrated to dryness using rotary evaporation.

The sample was redissolved in 5 mL of hexane, and eluted through a 6 mL Florisil solid phase extraction cartridge (preconditioned with acetone and hexane – see discussion in **Problems Encountered**). The hexane from the sample was discarded, and the analytes were eluted from the Florisil cartridge with 25 mL of hexane:acetone (4:1, v:v). The sample was concentrated to dryness using rotary evaporation, and redissolved in 5 mL of hexane:ethyl ether (1:1, v:v) for cleanup on a 12 mL Silica Gel solid phase extraction cartridge (preconditioned with ethyl ether and 0.01% triethylamine in hexane). Sample collection was started immediately after transferring the hexane:ethyl ether sample to the cartridge, and completed with an additional 15 mL of hexane:ethyl ether (1:1, v:v).

The sample was concentrated to dryness using rotary evaporation, and redissolved in acetone (2.5 mL for the Controls. Reagent Blanks and LOQ samples, or 5.0 mL for 10X LOQ samples) for analysis using a gas chromatograph equipped with a mass selective detector. Approximately 1 mL of extract was transferred to an autosampler vial for analysis. The remaining sample extract was placed in vials, the vials capped and stored under refrigeration for reanalysis as required.

Instrument Conditions: The following instruments and conditions were used for the sample analyses:

Instrument:	GC:	Agilent 6890 Series GC
	Inlet:	Temperature programmable, on column inlet
	Injector:	Agilent 7683 ALS
	Detector:	Agilent 5973 Mass Selective Detector
	Data System:	Agilent MSD Chemstation
GC Column:	Restek Rtx-200, 30 m x 0.320 mm x 0.5 μm film	
GC Conditions:	Inlet Temp.: Programmed:	98°C for 0 min., 200°C/min to 250°C for 10 min
	Oven Temp.: Programmed:	95°C for 2 min, 30°C/min to 200°C for 0 min, 20°C/min to 310°C for 5 min.
	Carrier Flow:	Helium at 2.0 mL/min

MSD Transfer: 280°C
Injection Vol: 0.1 µL

MS Conditions:
Ionization: EI
Quadrupole: 150°C
Source: 230°C
Solvent Delay: 8.0 minutes

Analyte	Ions Monitored	Dwell Time
Etoxazole	359.3, 330.2	80 msec each
R3	361.0, 346.0	80 msec each
R13	342.0	80 msec

Using these conditions, etoxazole eluted at ca. 9.7 minutes, R13 eluted at ca. 10.0 minutes, and R3 eluted at ca. 11.0 minutes.

Analysis Procedure: The GC system was conditioned by injecting sample extracts prior to the initial standard analyses. The linearity of the instrument was validated by analyzing four different concentrations of standards at the start of the analytical sequence. The instrument reproducibility was verified by injecting a calibration standard before the first sample, after every two or three samples and at the end of the sequence. Response factors (integration counts / µg/mL standard concentration) were determined for each standard injection. For the analytical run to be acceptable, the coefficient of variation (%CV) for the linearity standards and the calibration standards had to be 15% or less.

Calculations: The residues of etoxazole, R3 and R13, in ppm, was calculated using the formula:

$$ppm = \frac{B * C * V * DF}{RF * W}$$

Where:

B = integration counts for the analyte in the sample
C = concentration of analyte in the calibration standard (1.0 µg/mL)
V = final volume of the sample extract (2.5 mL)
DF = dilution factor, used if sample extracted was diluted prior to analysis
RF = average response factor for the calibration standards
W = initial sample weight (25 g)

The percent recovery for the samples was calculated using the formula:

$$\% \, recovery = \frac{ppm \, in \, sample}{ppm \, fortified} * 100\%$$

An example of these calculations is presented in Appendix III, Analytical Data.

RESULTS AND DISCUSSION

Results: The method was successfully validated on the second trial. The first trial failed due to unacceptable and variable recoveries. All recoveries for the second trial were acceptable

During the first method trial, several samples were observed to "bump" and flash into the rotary evaporator setups during the concentration step after the Florisil cleanup, which may have lead to the variable recoveries.

No changes were made to the procedure before the second trial. Additional care was taken during the rotary evaporation steps to reduce the "bumping" of samples and to avoid any flashing of the sample. In one of the two control samples, residues of each analyte were detected at the method Limit of Detection (0.01 ppm). A fresh aliquot of this control sample was taken and reanalyzed, and found to contain no residues. The residues detected in the first analysis were therefore considered to be due to sample contamination that occurred in the laboratory while aliquoting the final extract into the autosampler vial for analysis because of the presence of all three analytes at the same concentration, 0.01 ppm.

Problems Encountered: Prior to starting the analyses, the Florisil and Silica Gel SPE cartridges were checked for recovery as described within the method (Method Notes 1 and 2). The Florisil elution procedure described in the method used a 5 mL hexane conditioning, followed by adding the sample to the cartridge and rinsing the cartridge with 5 mL of hexane, which was discarded. The analytes were then eluted from the Florisil with 25 mL of

hexane:acetone (4:1, v:v). Using this profile, recoveries for R13 were low and R3 recoveries were high

The profile was modified by adding a one column volume (ca. 5 mL) acetone wash followed by two 5 mL hexane washes to condition the cartridge and eliminating the hexane rinse after adding the sample to the cartridge. A detailed description of the Florisil column procedure can be found in Appendix I.

Description of Contact:

No contact between the method author or personnel having experience with the method, and the Study Director was made during the conduct of this study. The method allows for modification of the elution profiles for the cleanup cartridges based on the initial column profile, so no contact was necessary for changes in the Florisil column elution profile. The variable and high recoveries were considered to be sample handling issues and not problems with the method per se, no discussions regarding the recoveries occurred.

Time Requirement:

The time required to complete the workup of samples from extraction to the final volume adjustment was approximately 12 hours. The sample workup was conducted over a three day period. The samples were stored overnight, at room temperature, after the initial extraction and after the Florisil SPE cleanup steps. The sample set required approximately ten hours to analyze, and was automated using the capabilities of the instrument.

CONCLUSIONS

The analytical method RM-37S-2, "Determination of Etoxazole, R3 and R13 Metabolites in Soil" was successfully validated within the guidelines of EPA's Ecological Effects Test Guidelines, OPPTS 850.7100, Data Reporting for Environmental Chemistry Methods.

Notes for ILV Study of Valent Method RM-37S-2

The Florisil column calibration performed prior to conducting the analyses indicated that a different conditioning/elution profile was required for adequate recoveries. The Florisil column cleanup will be done as follows:

Condition each column with 1 column volume of acetone, and allow to drain completely. Continue the conditioning with 2 x 5 mL of hexane, allowing the solvent to drain just to the top of the packing.

Transfer the sample (in 5 mL of hexane) to the column, and allow to drain to the top of the packing. Discard the hexane. **Do not include the 5 mL hexane wash described in the method.**

Elute the column with 10 mL 4:1 hexane:acetone (v:v), followed by 15 mL of 4:1 hexane: acetone (v:v) as described in the method.

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