DESCRIPTION OF ANALYTICAL METHOD

Method Identification Number: DowElanco residue analytical method GRM 96.04

Title of Method: Determination of Residues of Cloransulam-methyl and Cloransulam in Water by Capillary Gas Chromatography with Mass Selective Detection

Scope of Method: This method is applicable for the quantitative determination of residues of cloransulam-methyl, [*N*-(2-carbomethoxy-6-chlorophenyl)-5-ethoxy-7-fluoro[1,2,4]triazolo-[1,5-c]-pyrimidine-2- sulfonamide] and cloransulam, [*N*-(2-carboxy-6-chlorophenyl)-5-ethoxy-7-fluoro-[1,2,4]triazolo[1,5-c]-pyrimidine-2- sulfonamide] in water over the concentration range of 0.10-2.0 ng/mL, with a validated limit of quantitation of 0.10 ng/mL.

Identification of analytical standard used: Name: cloransulam

AGR Number:	TSN100609	% Purity:	99%
Analytical Report No.:	FA&PC 940389	Report Date:	24 FEB 1995

Identification of analytical standard used: Name: cloransulam-methyl

TSN Number:	AGR293572	% Purity:	99.2%
Analytical Report No.:	FA&PC 963036	Report Date:	26 APR 1996

Identification of analytical standard used: Name: *N*-methyl-cloransulam-methyl

TSN Number:	TSN100070	% Purity:	>96%
Analytical Report No.:	FA&PC 945137	Report Date:	10 JUN 1994

Identification of analytical standard used: Name: *N*-ethyl-cloransulam-methyl

TSN Number:	TSN100102	% Purity:	>97%
Analytical Report No.:	FA&PC 945138	Report Date:	10 JUN 1994

Identification of analytical standard used: Name: *N*-ethyl-cloransulam-ethyl

TSN Number:	TSN100099	% Purity:	>99%
Analytical Report No.:	FA&PC 950119	Report Date:	16 MAY 1995

METHOD OUTLINE

RESIDUE METHOD: GRM 96.04

Independent Laboratory Validation of GRM 96.04 - Determination of Residues of Cloransulam-methyl and Cloransulam in Water by Capillary Gas Chromatography with Mass Selective Detection

Pipet 100 mL of water into a series of 4-oz glass bottles.

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Use unfortified samples as controls. For fortified samples, add 1.0 mL of the appropriate spiking solutions in acetone to obtain concentrations ranging from 0.10 to 2.0 ng/mL. A reagent blank, containing no water sample, is carried through the method with the samples.

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Add 1.0 mL of 2.0 N hydrochloric acid to each sample bottle and seal with a PTFE-lined cap. Shake the bottles briefly to mix.

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Concentrate and purify the samples using the following C_{18} SPE procedure

--Place a C_{18} SPE column on the vacuum manifold box.

--Rinse the SPE column with 5 mL of acetonitrile. (Do not allow the column bed to dry.)

--Condition the SPE column with 5 mL of 0.01 N hydrochloric acid solution. (Do not allow the column bed to dry.)

--Attach a 70-mL reservoir to the top of the column using an SPE column adapter. Fill the reservoir with the sample solution. With the aid of vacuum, pull the sample through the column at a flow rate of approximately 2 mL/min. Add the remaining sample solution to the reservoir when a sufficient volume of sample has passed through the column.

--After the entire sample has passed through the column, add 15 mL of the 20% acetonitrile in 0.01 N hydrochloric acid solution to the reservoir. With the aid of vacuum, pull the solution through the column at a flow rate of approximately 2 mL/min.

--Remove the reservoir and column adapter. Allow the column to dry under vacuum for 30 minutes.

METHOD OUTLINE (CONT.)

–Elute the cloransulam-methyl and cloransulam with 5.0 mL of acetonitrile, collecting the eluate in an 8-mL vial. Discard the SPE column.

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Evaporate the sample to dryness by placing the vial in an N-Evap evaporator, with a nitrogen flow of approximately 20 mL/min and a water bath temperature of 50 °C.

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Allow the vial to cool and add 1 mL of acetone.

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Add 25 µL of triethylamine (TEA) to the vial and seal with a PTFE-lined cap. Vortex the vial briefly to mix.

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Add 100 µL of the triethyloxonium tetrafluoroborate (TEOTFB) solution to the vial and seal with a PTFE-lined cap. Vortex the vial briefly, and shake the vial for 20 minutes on a reciprocating shaker at approximately 180 excursions/minute.

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Repeat. Add 25 µL of TEA to the vial and seal with a PTFE-lined cap. Vortex the vial briefly to mix, and add 200 µL of the TEOTFB solution to the vial and seal with a PTFE-lined cap. Vortex the vial briefly, and shake the vial for 20 minutes on a reciprocating shaker at approximately 180 excursions/minute.

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Evaporate the sample to dryness by placing the vial in an N-Evap evaporator, with a nitrogen flow of approximately 20 mL/min and a water bath temperature of 50 °C.

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Allow the vial to cool and add 2.5 mL of 20% MTBE in hexane and 3 mL of 0.1 M potassium bicarbonate solution. Seal the vial with a PTFE-lined cap.

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Shake the vial for 3 minutes on a reciprocating shaker at approximately 180 excursions/minute.

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Centrifuge the vial at 2500 rpm for 2 minutes.

METHOD OUTLINE (CONT.)

Using a disposable Pasteur pipet, transfer the top organic layer to a clean 8-mL vial.

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Extract the aqueous solution with a second 2.5 mL of 20% MTBE in hexane, shake the vial for 3 minutes on a reciprocating shaker at approximately 180 excursions/minute, centrifuge the vial at 2500 rpm for 2 minutes and using a disposable Pasteur pipet, transfer the top organic layer to the appropriate 8-mL vial.

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Purify the samples using the following silica gel SPE:
--Place a silica gel SPE column on the vacuum manifold box.
--Rinse the SPE column with 5 mL of toluene.
--Condition the reservoir and SPE column with 5 mL of hexane. (Do not allow the column bed to dry.)
--Transfer the sample solution to the SPE column. With the aid of vacuum, pull the sample through the column at a flow rate of approximately 2 mL/min.
--Rinse the sample vial with 2.5 mL of 20% MTBE in hexane and transfer the rinse to the SPE column. With the aid of vacuum, pull the rinse through the column at a flow rate of approximately 2 mL/min.
--Elute the SPE column with 10 mL of 5% acetone in toluene. Collect the eluate in a 12-mL vial.

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Evaporate the sample to dryness by placing the vial in an N-Evap evaporator, with a nitrogen flow of approximately 20 mL/min and a water bath temperature of 50 °C.

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Allow the vial to cool and add 0.5 mL of toluene containing the internal standard.

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Vortex and sonicate the vial briefly, and centrifuge at 2500 rpm for 5 minutes.

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Transfer the sample to a 2-mL autosampler vial and seal with a cap and crimper.

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Analyze the samples by capillary gas chromatography with mass selective detection.

ANALYTICAL

Calculations

The calibration standards were injected and the peak areas were determined for the *m/z* 212 and *m/z* 180 ions for the *N*-ethyl-cloransulam-methyl, *m/z* 226 and *m/z* 180 ions for the *N*-ethyl-cloransulam-ethyl and for the *m/z* 198 ion for the *N*-methyl-cloransulam-methyl internal standard.

For each calibration standard, a confirmation ratio was calculated. The average confirmation ratio for the cloransulam-methyl and cloransulam calibration standards was used to verify the presence of cloransulam-methyl and cloransulam in water samples.

$$\text{Confirmation Ratio} = \frac{\text{peak area of confirmation ion}}{\text{peak area of quantitation ion}}$$

$$\text{Cloransulam-methyl Confirmation Ratio} = \frac{\text{peak area at } m/z\ 212}{\text{peak area at } m/z\ 180}$$

$$\text{Cloransulam Confirmation Ratio} = \frac{\text{peak area at } m/z\ 226}{\text{peak area at } m/z\ 180}$$

For example, using the data for cloransulam-methyl from Figure 5:

$$\text{Cloransulam-methyl Confirmation Ratio} = \frac{53}{124}$$

$$\text{Cloransulam-methyl Confirmation Ratio} = 0.427$$

Positive confirmation of cloransulam-methyl and cloransulam was indicated when the confirmation ratio for the samples was in the range of ±20% of the average found for the standards.

For each standard, the cloransulam-methyl and cloransulam quantitation ratios were calculated.

$$\text{Quantitation Ratio} = \frac{\text{peak area of quantitation ion}}{\text{peak area of internal standard ion}}$$

$$\text{Cloransulam-methyl Quantitation Ratio} = \frac{\text{peak area at } m/z \text{ 212}}{\text{peak area at } m/z \text{ 198}}$$

$$\text{Cloransulam Quantitation Ratio} = \frac{\text{peak area at } m/z \text{ 226}}{\text{peak area at } m/z \text{ 198}}$$

For example, using the data for cloransulam-methyl from Figure 5:

$$\text{Cloransulam-methyl Quantitation Ratio} = \frac{53}{1207}$$

$$\text{Cloransulam-methyl Quantitation Ratio} = 0.044$$

Separate standard curves for cloransulam-methyl and cloransulam were prepared by plotting the equivalent concentration on the abscissa (x-axis) and the respective quantitation ratio on the ordinate (y-axis) as shown in Figures 1 and 2. Using power regression analysis (2), the equation for the curve with respect to the abscissa was determined. The least squares coefficient of determination (r^2 value) of each power regression equation was 0.995 or greater. Concentrations of the analytes in the final solutions were determined by substituting the peak area responses into the power regression equation as shown below:

$$Y = (\text{constant})X^{\text{exponent}}$$

$$X = \left[\frac{Y}{\text{constant}}\right]^{1/\text{exponent}}$$

For example, using the cloransulam-methyl data from Figure 5:

$$\text{Cloransulam-methyl Conc. (ng/mL)} = \left[\frac{\text{Cloransulam-methyl Quantitation ratio}}{\text{constant}}\right]^{1/\text{exponent}}$$

$$\text{Cloransulam-methyl Conc. (ng/mL)} = \left[\frac{\text{Cloransulam-methyl Quantitation ratio}}{0.6953}\right]^{1/1.2034}$$

The net concentration of cloransulam-methyl and cloransulam in each recovery sample was determined by first subtracting the average quantitation ratios in the control sample from the respective ratios of each recovery sample. The net quantitation ratio obtained was substituted into the appropriate equation above to determine the concentration.

For example, using the cloransulam-methyl data from Figures 1, 4, and 5:

$$\text{Cloransulam-methyl Conc. (ng/mL)} = \left[\frac{\text{Cloransulam-methyl Quantitation ratio}}{0.6953}\right]^{1/1.2034}$$

$$\text{Cloransulam-methyl Conc. (ng/mL)} = \left[\frac{0.044 - 0.000}{0.6953}\right]^{1/1.2034}$$

$$\text{Cloransulam-methyl} = 0.101 \text{ ng/mL}$$

The percent recovery was determined by dividing the net concentration of each recovery sample by the theoretical concentration added.

$$\text{Recovery} = \frac{\text{Concentration Found}}{\text{Concentration Added}} \times 100\%$$

For example, using the cloransulam-methyl data from Figure 5:

$$\text{Recovery} = \frac{0.101 \text{ ng/mL}}{0.100 \text{ ng/mL}} \times 100\%$$

$$\text{Recovery} = 101\%$$

Statistical Treatment of Data

Statistical treatment of data included the calculation of the means, standard deviations, and least squares correlation coefficients.

Summary of Key Dates

Sample Identification	Sample Description	Extracted	Analyzed
BB	Reagent Blank	01-Oct-96	01-Oct-96
18848401-C1	Pond Water Control	01-Oct-96	01-Oct-96
18848401-C2	Pond Water Control	01-Oct-96	01-Oct-96
18848401-FC1	Fortified Pond Water Control	01-Oct-96	01-Oct-96
18848401-FC2	Fortified Pond Water Control	01-Oct-96	01-Oct-96
18848401-FC3	Fortified Pond Water Control	01-Oct-96	01-Oct-96
18848401-FC4	Fortified Pond Water Control	01-Oct-96	01-Oct-96

4. FULL DESCRIPTION OF ANALYTICAL INSTRUMENTATION USED

Instrumentation	Hewlett-Packard Model 5890 Series II Gas Chromatograph/Model 5972 Mass Selective Detector, Autosampler Model HP 7673 GC/SFC Injector, HP ChemStation G1034B Ver B.02.03

Column	DB-5, 0.18 mm id x 10 m, 0.4-µm film thickness, J & W Scientific, Serial No. 5868915A
Oven Temperature	Hold at 120 °C for 1.0 min, then 120 °C to 325 °C at 15 C/min, hold for 1 min
Injector Temperature	270 °C
Transfer Line Temperature	300 °C
Carrier Gas	helium
Carrier Gas Linear Velocity	approximately 40 cm/sec
Head Pressure	50 kPa
Injection Mode	splitless
Injection Liner	deactivated, double taper
Injector Purge Delay	0.7 min
Split Flow	60 mL/min
Septum Purge	1.0 mL/min
Injection Volume	3 µL
Detector Mode	Electron impact, selected ion monitoring
Calibration Program	Maximum sensitivity autotune
Electron Multiplier Voltage	1541 volts (tune voltage plus 200)
Ions Monitored	
N-Methyl-cloransulam-methyl	*m/z* 198 (internal standard)
N-Ethyl-cloransulam-methyl	*m/z* 212 (quantitation), *m/z* 180 (confirmation)
N-Ethyl-cloransulam-ethyl	*m/z* 226 (quantitation), *m/z* 180 (confirmation)
Dwell Time	100 msec

5. DESCRIPTION OF ANY PROBLEMS ENCOUNTERED IN CONFIRMING THIS METHOD

Section/Step/Operation:

(1): No problems were encountered while validating this method.

6. IDENTIFICATION OF CRITICAL STEPS i.e., STEPS WHERE LITTLE VARIATION IS ALLOWED OR DIRECTIONS MUST BE FOLLOWED PRECISELY

(1): No critical steps were identified while validating this method.