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Determination of Diclosulam in Water by Capillary Gas Chromatography with Mass Selective Detection

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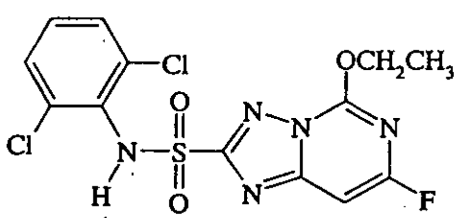
A. Scope

This method is applicable for the quantitation of residues of diclosulam in ground, surface, and tap water. The method was validated over the concentration range of 0.10 to 20 ng/mL with a limit of quantitation of 0.10 ng/mL.



Diclosulam
N-(2,6-Dichlorophenyl)-5-ethoxy-7-fluoro[1,2,4]triazolo[1,5-c]pyrimidine-2-sulfonamide
CAS 145701-21-9

B. Principle

Residues of diclosulam are partitioned from water onto an octadecyl (C_{18}) solid-phase extraction (SPE) column. The column is rinsed with a solution of 40% acetonitrile in 0.01 N hydrochloric acid and dried under vacuum. The column is eluted with acetonitrile and the eluate is evaporated to dryness. Residues of diclosulam are dissolved in acetone and derivatized at ambient temperature with iodoethane and triethylamine. The acetone solution is evaporated to dryness. *N*-ethyl-diclosulam residues are dissolved in a 5% sodium chloride solution and partitioned with toluene containing the internal standard (*N*-methyl-diclosulam). Residues of diclosulam as the *N*-ethyl-diclosulam derivative are determined by capillary gas chromatography with mass selective detection (GC/MSD).

C. Safety Precautions

1. Each analyst must be acquainted with the potential hazards of the reagents, products, and solvents used in this method before commencing laboratory work. SOURCES OF INFORMATION INCLUDE: MATERIAL SAFETY DATA SHEETS (MSDS), LITERATURE, AND OTHER RELATED DATA. Safety information on non-Dow AgroSciences products should be obtained from the container label or from the supplier. Disposal of reagents, reactants, and solvents must be in compliance with local, state, and federal laws and regulations.

2. Acetone, acetonitrile, ethyl acetate, toluene, and triethylamine are flammable and must be used in well-ventilated areas away from ignition sources.

3. Hydrochloric acid solutions are corrosive and can cause severe burns. It is imperative that proper eye and personal protection equipment be used when handling these reagents.

4. Iodoethane is corrosive and an alkylating agent. It is imperative that proper eye and personal protection equipment be used when handling this reagent.

5. Triethylamine is corrosive. It is imperative that proper eye and personal protection equipment be used when handling this reagent.

D. Equipment (Note L.1.)

1. Balance, analytical, Model AE-100, Mettler Instrument Corporation, Hightstown, NJ 08520.

2. Balance, toploading, Model P-1200, Mettler Instrument Corporation.

3. Centrifuge, with rotor to accommodate 12-mL screw-top culture tubes, Model Centra-8, International Equipment Company, Needham Heights, MA 02194.

4. Evaporator, TurboVap LV, Zymark Corporation, Hopkinton, MA 01748.

5. Gas chromatograph, Model 5890 Series II, Hewlett-Packard, Wilmington, DE 19808.

6. Mass selective detector, Model 5971A, Hewlett-Packard, Palo Alto, CA 94304.

7. Mass selective detector data system, Model G1034B, Hewlett-Packard.

8. Shaker, variable speed reciprocating with box carrier, Model 6000, Eberbach Corporation, Ann Arbor, MI 48106.

9. Ultrasonic bath, Model 1200, Branson Cleaning Equipment Company, Shelton, CT 06484.

10. Vacuum manifold, Model spe-21, Mallinckrodt Baker, Inc., Phillipsburg, NJ 08865.

11. Vial crimper, catalog number 8710-0979, Hewlett-Packard.

12. Vortex mixer, Model K-550-G, Scientific Industries, Inc., Bohemia, NY 11716.

E. Glassware and Materials (Note L.1.)

1. Bottle, 4-oz. (118-mL), with PTFE-lined cap, catalog number 03-321-1A, Fisher Scientific, Pittsburgh, PA 15219.

2. Bottle, amber, 4-oz. (125-mL), with PFTE-lined cap, catalog number 03-320-4B, Fisher Scientific.

3. Cap, for 16 x 100 mm screw-top culture tube, PTFE-lined, catalog number 14-930-15E, Fisher Scientific.

4. Column adapter, SPE, PTFE, catalog number 120-1100, Jones Chromatography, Inc., Lakewood, CO 80228.

5. Column, capillary gas chromatography, DB-5 liquid phase, 10 m x 0.18 mm i.d., 0.4-µm film thickness, catalog number 121-5013, J&W Scientific, Folsom, CA 95630.

6. Column inlet liner, deactivated, catalog number 5181-3315, Hewlett-Packard.

7. Column, octadecyl (C_{18}) SPE, catalog number 7020-07, Mallinckrodt Baker, Inc.

8. Column reservoir, 70-mL, catalog number 120-1008-F, Jones Chromatography, Inc.

9. Filters, charcoal, moisture, and oxygen, catalog numbers 7972, 7971, and 7970, Chrompack, Inc., Raritan, NJ 08869. (Note L.2.)

10. Pipetter, Eppendorf Repeater, catalog number 21-380-8, Fisher Scientific.

11. Pipetter tips, Eppendorf Combitip, 1.25-mL and 12.5-mL, catalog numbers 21-380-8E and 21-380-8C, Fisher Scientific.

12. Screw-top culture tube, 16 x 100 mm (12-mL), catalog number 14-957-86D, Fisher Scientific.

13. Vial, autosampler, 2-mL, catalog number C4011-1, National Scientific Co., Lawrenceville, GA 30243.

14. Vial, limited volume insert, 200-µL capacity, catalog number 03-375-3B, Fisher Scientific.

15. Vial seal, for 2-mL autosampler vial, catalog number C4011-1A, National Scientific Company.

16. Water purification system, Model Milli-Q UV Plus, Millipore Corporation, Milford, MA 01757.

F. Reagents and Prepared Solutions (Note L.1.)

1. Reagents

a. Acetone, Optima grade, catalog number A929-4, Fisher Scientific.

b. Acetonitrile, Optima grade, catalog number A996-4, Fisher Scientific.

c. Helium gas, 99.995% purity, Airco, Murray Hill, NJ 07974.

d. Hydrochloric acid, 1.0 N, certified concentration, catalog number SA48-1, Fisher Scientific.

e. Hydrochloric acid, 0.01 N, certified concentration, catalog number SA 62-1, Fisher Scientific.

f. Iodoethane, 99%, catalog number 1-778-0, Aldrich Chemical Company, Milwaukee, WI 53233.

g. Nitrogen gas, 99.99% purity, Airco.

h. Standards

Diclosulam: *N*-(2,6-dichlorophenyl)-5-ethoxy-7-fluoro[1,2,4]triazolo [1,5-c]pyrimidine-2-sulfonamide

N-ethyl-diclosulam: *N*-ethyl-*N*-(2,6-dichlorophenyl)-5-ethoxy-7-fluoro[1,2,4]triazolo[1,5-c]pyrimidine-2-sulfonamide

N-methyl-diclosulam: *N*-methyl-*N*-(2,6-dichlorophenyl)-5-ethoxy-7-fluoro[1,2,4]triazolo[1,5-c]pyrimidine-2-sulfonamide

Obtain from Test Substance Coordinator, Building 304, Dow AgroSciences, Indianapolis, IN 46268-1054.

i. Sodium Chloride, Certified ACS grade, catalog number S-271-1, Fisher Scientific.

j. Toluene, Optima grade, catalog number T291-4, Fisher Scientific.

k. Triethylamine, 99+% purity, catalog number 23,962-3, Aldrich Chemical Co.

2. Prepared Solutions

a. 40% acetonitrile in 0.01 N hydrochloric acid (v/v)

Transfer 400 mL of acetonitrile into a 1000-mL volumetric flask and dilute to volume with 0.01 N HCl.

b. 5% sodium chloride (w/v), aqueous

Transfer 50 g of sodium chloride to a 1000-mL volumetric flask. Dissolve the salt in 800 mL of deionized water (DI) water, and dilute to volume with DI water.

G. Preparation of Standards

1. Preparation of Diclosulam Stock Solution

Weigh 0.0100 g of the diclosulam analytical standard and quantitatively transfer into a 100-mL volumetric flask. Dilute to volume with acetone to obtain a 100-µg/mL stock solution.

2. Preparation of Diclosulam Spiking Solutions

a. Pipet 0.050 mL of the stock solution from Section G.1. into a 100-mL volumetric flask. Dilute to volume with acetone to obtain a 50-ng/mL solution of diclosulam.

b. Pipet 0.50 mL of the stock solution from Section G.1. into a 100-mL volumetric flask. Dilute to volume with acetone to obtain a 500-ng/mL solution of diclosulam.

c. Pipet 1.0 mL of the stock solution from Section G.1. into a 100-mL volumetric flask. Dilute to volume with acetone to obtain a 1000-ng/mL solution of diclosulam.

d. Fortify 75 mL of control water with the indicated amounts of the appropriate solutions to obtain the range of equivalent sample concentration, as follows:

Spiking Soln. Conc. ng/mL	Volume of Spiking Soln. mL	Equiv. Sample Conc.[a] ng/mL
50.0	0.050	0.03[b]
50.0	0.15	0.10
50.0	0.30	0.20
500.	0.075	0.50
500.	0.75	5.0
1000	1.5	20.

[a] The equivalent sample concentration is based on fortifying a 75-mL water sample with the indicated amount of the appropriate solution.

[b] The 0.03 ng/mL equivalent sample concentration standard is for confirmation of the detection limit.

3. Preparation of *N*-Methyl-Diclosulam Internal Standard Solutions

   a. Weigh 0.0100 g of the *N*-methyl-diclosulam analytical standard and quantitatively transfer to a 100-mL volumetric flask. Dilute to volume with acetone to obtain a 100-µg/mL stock solution for use as an internal standard.

   b. Pipet 4.0 mL of the 100-µg/mL stock solution from Section G.3.a. into a 2000-mL volumetric flask. Dilute to volume with toluene to obtain a 200-ng/mL solution of *N*-methyl-diclosulam.

4. Preparation of *N*-Ethyl-Diclosulam Calibration Standard Solutions

   a. Weigh 0.0107 g of the *N*-ethyl-diclosulam analytical standard and quantitatively transfer to a 100-mL volumetric flask. Dilute to volume with acetone to obtain a 107-µg/mL stock solution (equivalent to 100 µg/mL diclosulam).

   b. Add the appropriate aliquot of the *N*-ethyl-diclosulam solution from Section G.4.a. and dilute to 100-mL with the 200-ng/mL *N*-methyl-diclosulam (Section G.3.b) as follows:

Aliquot of Soln. G.4.a. mL	Final Conc.[a] ng/mL	Equivalent Sample Conc.[b] ng/mL
0.010	10.0	0.06667
0.020	20.0	0.1333
0.10	100.	0.6667
0.20	200.	1.333
0.50	500.	3.333

[a] The final concentration is expressed as the diclosulam equivalence of the standard. Each standard contains 200 ng/mL of the internal standard.
[b] The diclosulam equivalent sample concentration is based on taking the 75-mL water sample extract to a final volume of 0.50 mL.

H. Gas Chromatography/Mass Spectrometry

1. Column

   Install the splitless column insert liner (Section E.6.) and the capillary column (Section E.5.) in the split/splitless injection port of the GC/MSD following the manufacturer's recommended procedure.

2. Typical Operating Conditions

Instrumentation:	Hewlett-Packard Model 5890 Series II gas chromatograph Hewlett-Packard Model 7673 autoinjector Hewlett-Packard Model 5971A mass selective detector Hewlett-Packard Model G1034B data system

Column:	J&W Scientific fused silica capillary DB-5 liquid phase 10 m x 0.18 mm i.d. 0.4-µm film thickness
Temperatures:	
Column	120 °C for 1.10 minute 120 °C to 220 °C at 30 °C/min. 220 °C to 325 °C at 10 °C/min. 325 °C to 120 °C at 70 °C/min.
Injector	300 °C
Interface	310 °C
Carrier Gas:	
Head Pressure	50 kPa
Linear Velocity	approximately 40 cm/sec at an oven temperature of 310 °C
Injection Mode:	splitless
Purge Delay	1.0 minute
Splitter Flow	60 mL/min.
Septum Purge	1.0 mL/min.
Injection Volume:	3 µL
Detector Mode:	electron impact ionization with selected ion monitoring
Calibration Program	maximum sensitivity autotune
Electron Multiplier	1741 volts (tune voltage)
Ions Monitored:	
N-Methyl-diclosulam	*m/z* 174 (internal standard)
N-Ethyl-diclosulam	*m/z* 188 (diclosulam quantitation) *m/z* 190 (diclosulam primary confirmation) *m/z* 138 (diclosulam secondary confirmation)
Dwell Time	75 msec

Typical mass spectra of *N*-methyl-diclosulam and *N*-ethyl-diclosulam are shown in Figure 1.

3. Calibration Curve

Demonstrate that the calibration curve fits a least squares power regression equation (1) over the concentration range of 10.0 to 500 ng/mL (equivalent sample concentration range of 0.067 to 3.3 ng/mL). The least squares coefficient of determination (r^2) must be equal to or greater than 0.995. A representative calibration curve for diclosulam is shown in Figure 2.

4. <u>Typical Chromatograms</u>

Typical chromatograms of a 20-ng/mL standard (equivalent to a sample concentration of 0.13 ng/mL), a control surface water sample, and a control surface water sample fortified at 0.10 ng/mL with diclosulam are shown in Figures 3-5.

I. <u>Determination of Recovery of Diclosulam from Water</u>

1. <u>Preparation of Recovery Samples for Water</u>

a. Transfer 75 mL of water sample into each of a series of 4-oz bottles. For preparing fortified samples, use some of the samples as controls and fortify the remaining samples by adding the appropriate volumes of spiking solutions (Section G.2.d.) to obtain concentrations ranging from 0.03 to 20 ng/mL. A reagent blank, containing no water sample, is carried through the method with the samples.

b. Add 1 mL of 1.0 N hydrochloric acid. Seal the bottle with a PTFE-lined cap and swirl briefly to mix.

c. Concentrate and purify the sample on a C_{18} SPE column utilizing the following procedures.

(1) Place a C_{18} SPE column on the vacuum manifold box.

(2) Attach a 70-mL reservoir to the top of the column using a SPE column adapter.

(3) Rinse the SPE column with 5 mL of acetonitrile.

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

(5) Transfer the sample solution from Step I.1.b. to the reservoir. With the aid of vacuum, pull the sample through the column at a flow rate of ~4 mL/min.

(6) Rinse the sample bottle with 5 mL of the 40% acetonitrile in 0.01 N hydrochloric acid solution. After the entire sample has passed through the column, add the sample rinse to the reservoir. With the aid of vacuum, pull the rinse through the column at a flow rate of ~4 mL/min.

(7) After the rinse has passed through the column, increase the vacuum to the maximum that is safely recommended by the vacuum manifold manufacturer. Maintain the vacuum for a minimum of 10 minutes to facilitate air drying of the column.

(8) Elute the diclosulam with 6 mL of acetonitrile, collecting the eluate in a 12-mL screw-cap culture tube.

d. Evaporate the solvent in the tube from Step I.1.c.(8) to dryness using a TurboVap evaporator at ~60 °C.

e. Add 1 mL of acetone.

f. Using an Eppendorf pipetter with a 1.25-mL combitip, add 25 µL of triethylamine, and 25 µL of iodoethane. Seal the tube with a PTFE-lined cap.

g. Sonicate the vial for ~10 seconds, and vortex briefly to mix. Allow the derivatization reaction to proceed at room temperature for at least 30 minutes.

h. Evaporate the solvent to dryness using a TurboVap evaporator at ~40 °C.

i. Add 1 mL of the 5% sodium chloride solution and 0.50 mL of toluene containing 200-ng/mL of *N*-methyl-diclosulam (Section G.3.b) as an internal standard. Seal the tube with a PTFE-lined cap.

j. Sonicate the tube for approximately 10 seconds and pulse vortex for at least 15 seconds.

k. Centrifuge the tube at 2000 rpm for 5 minutes.

l. Place a limited volume insert in an autosampler vial. Using a disposable Pasteur pipet, transfer an aliquot of the top toluene layer to the limited volume insert and seal the autosampler vial with a cap and crimper.

m. Analyze the samples and calibration standards from Step G.4.b. by GC/MSD as described in Section H. Determine the suitability of the chromatographic system using the following performance criteria:

(1) Standard curve linearity: Determine that the coefficient of determination (r^2) for the least squares power regression equation which describes the detector response as a function of the concentration of calibration standards is equal to or greater than 0.995.

(2) Peak resolution: Visually determine that sufficient resolution has been achieved for the *N*-ethyl-diclosulam and *N*-methyl-diclosulam (internal standard) peaks relative to background interferences.

(3) Detector sensitivity: Visually determine that a minimum signal-to-noise ratio of 5:1 has been attained for the *m/z* 188 diclosulam quantitation ion peak of the 10-ng/mL calibration standard.

2. <u>Calculation of Percent Recovery</u>

a. Using the data for the series of calibration standards analyzed in Section I.1.l., determine the peak areas for *N*-ethyl-diclosulam (*m/z* 188, 190, and 138) and *N*-methyl-diclosulam (*m/z* 174).

b. For each standard, calculate the quantitation ratio.

For example, using the data for diclosulam from Figure 3:

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

$$\text{Quantitation Ratio} = \frac{\text{peak area at } m/z \text{ 188}}{\text{peak area at } m/z \text{ 174}}$$

$$\text{Quantitation Ratio} = \frac{427}{5212}$$

$$\text{Quantitation Ratio} = 0.08193$$

c. Prepare a standard curve by plotting the equivalent diclosulam concentration on the abscissa (x-axis) and the respective quantitation ratio on the ordinate (y-axis) as shown in Figure 2. Using regression analysis, determine the equation for the curve with respect to the abscissa.

For example, using power regression with the diclosulam data from Figure 2:

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

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

$$\text{Diclosulam Conc. (ng/mL)} = \left[\frac{\text{diclosulam quantitation ratio}}{\text{constant}}\right]^{1/\text{exponent}}$$

$$\text{Diclosulam Conc. (ng/mL)} = \left[\frac{\text{diclosulam quantitation ratio}}{0.8199}\right]^{1/1.098}$$

d. Determine the gross concentration in each recovery sample by substituting the quantitation ratio obtained into the above equation and solving for the concentration.

For example, using the diclosulam data from Figure 5:

$$\begin{array}{c}\text{Diclosulam Conc.}\\ \text{(gross ng/mL)}\end{array} = \left[\frac{\text{diclosulam quantitation ratio}}{0.8199}\right]^{1/1.098}$$

$$\text{Diclosulam Conc. (gross ng/mL)} = \left[\frac{0.05956}{0.8199}\right]^{1/1.098}$$

$$\text{Diclosulam Conc. (gross)} = 0.0918 \text{ ng/mL}$$

e. Determine the net concentration in each recovery sample by subtracting the diclosulam concentration in the control sample from that of the gross diclosulam concentration in the recovery sample.

For example, using the diclosulam data from Table I and Figures 4 and 5:

$$\text{Diclosulam Conc. (net ng/mL)} = \text{Diclosulam Conc. (gross ng/mL)} - \text{Diclosulam Conc. (control ng/mL)}$$

$$\text{Diclosulam Conc. (net ng/mL)} = 0.0918 \text{ ng/mL} - 0.00 \text{ ng/mL}$$

$$\text{Diclosulam Conc. (net)} = 0.0918 \text{ ng/mL}$$

f. Determine the percent recovery by dividing the net concentration of each recovery sample by the theoretical concentration added.

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

$$\text{Recovery} = \frac{0.0918 \text{ ng/mL}}{0.10 \text{ ng/mL}} \times 100\%$$

$$\text{Recovery} = 92\%$$

J. Determination of Diclosulam in Water

1. Prepare reagent blank, control, recovery, and treated samples as described in Section I.1.

2. Prepare a standard calibration curve for diclosulam and determine the power regression equation that fits the curve as described in Section I.2.

3. Determine the gross concentration of diclosulam in each treated sample by substituting the quantitation ratio obtained into the equation for the standard calibration curve, and calculating the uncorrected residue result as described in Section I.2.d.

4. For those analyses that require correction for method recovery, use the average recovery of all the recovery samples from a given sample set to correct for method efficiency.

For example, using the diclosulam data from Figure 5 and Table I for the samples analyzed on December 22, 1997:

a. Determine the gross analyte concentration in the water sample as described in Section I.2.d.

b. Determine the corrected analyte concentration in the water sample as follows:

$$\begin{array}{c}\text{Diclosulam Conc.}\\ \text{(corrected ng/mL)}\end{array} = \begin{array}{c}\text{Diclosulam Conc.}\\ \text{(gross ng/mL)}\end{array} \times \left[\frac{100}{\begin{array}{c}\text{Ave. Percent Recovery}\\ \text{of Sample Set}\end{array}}\right]$$

$$\begin{array}{c}\text{Diclosulam Conc.}\\ \text{(corrected ng/mL)}\end{array} = 0.0918 \text{ ng/mL} \times \frac{100}{86}$$

$$\begin{array}{c}\text{Diclosulam Conc.}\\ \text{(corrected)}\end{array} = 0.107 \text{ ng/mL}$$

4. Assay Time

A typical analytical run would consist of a minimum of four standards encompassing the linear range of sample concentrations, a reagent blank, a control (a non-fortified sample), a minimum of two fortified controls (one of which must be at the LOQ), and ten samples. This typical analytical run could be completed in less than two working days.

5. Standardization of C_{18} SPE Cartridge Elution Profile

Variation in the C_{18} SPE cartridges can influence the elution profile of diclosulam. It is necessary to obtain an elution profile for each lot of SPE cartridges used to ensure optimum recovery and clean-up efficiency. The following procedure may be used.

a. Prepare a C_{18} SPE cartridge as described in Steps I.1.c.(1-4).

b. Pipet 0.2 mL of the 1000-ng/mL fortification solution from Section G.2.c. into a 4-oz bottle containing 75 mL of DI water. Add 1 mL of 1.0 N hydrochloric acid, seal the bottle with a PTFE-lined cap and swirl briefly to mix.

c. Transfer the sample solution from Step K.5.b. to the reservoir. With the aid of vacuum, pull the sample through the column at a flow rate of ~4 mL/min.

d. Proceed as described in Steps I.1.c.(6-7) collecting the rinse in a 12-mL screw-cap culture tube.

e. Add ~0.5 g of sodium chloride and 2 mL of ethyl acetate to the screw-cap tube. Seal the tube with a PTFE-lined cap and shake by hand for ~30 seconds. Transfer the top ethyl acetate layer to a clean tube and save for Step K.5.g.

f. Elute the diclosulam with 8 mL of acetonitrile, sequentially collecting ~1 mL aliquots (fractions) of the eluate in a series of eight 12-mL screw-cap culture tubes.

g. Evaporate the solvent in each tube to dryness using a Turbo Vap evaporator set at 60 °C.

h. Proceed as described in Steps I.1.e. through I.1.m.

i. Based upon a 2.667 ng/mL equivalent water sample load, calculate separate percent recoveries of diclosulam for the rinse and each fraction as described in Section I.2.

j. A typical diclosulam elution profile from a C_{18} SPE cartridge is presented in Figure 6.

L. Notes

1. Equipment, glassware, materials, reagents, and chemicals considered to be equivalent to those specified may be substituted with the understanding that their performance must be confirmed by appropriate tests. Common laboratory supplies are assumed to be readily available and are, therefore, not listed.

2. The filters are used in the carrier gas supply lines to purify the helium entering the gas chromatograph.

M. References

1. Freund, J. E.; Williams, F. J. *Dictionary/Outline of Basic Statistics*, Dover Publications: Mineola, NY, 1991; p 170, eq I.3a.

2. Keith, L. H.; Crummett, W.; Deegan, J.; Libby, R. A.; Taylor, J. K.; Wentler, G., *Anal. Chem.*, 1983, 55, 2210-2218.