

INTRODUCTION

An independent laboratory validation of Dow AgroSciences LLC environmental chemistry residue analytical method GRM 98.04, "Determination of Residues of Fluroxypyr and Its Major Metabolites in Soil by Capillary Gas Chromatography with Mass Selective Detection" (Appendix A) was conducted with soil, the matrix on which residues of fluroxypyr, fluroxypyr-DCP, and fluroxypyr-MP must be determined to fulfill EPA requirements described in EPA *Subdivision N (Environmental Fate) of the Pesticide Assessment Guidelines*; Publication of Addenda for Data Reporting E, K, and N Requirements for Pesticide Assessment Guidelines; and Guideline OPPTS 850.7100(d)(1,2) "Public Draft"(1-3). This method is applicable for the quantitative determination of residues of fluroxypyr, (((4-amino-3,5-dichloro-6-fluoro-2-pyridinyl)oxy)acetic acid), also referenced as Dow AgroSciences LLC test substance inventory number, AGR222210; fluroxypyr-DCP, (4-amino-3,5-dichloro-6-fluoro-2-pyridinol), also referenced as Dow AgroSciences LLC test substance inventory number, TSN101651; and fluroxypyr-MP, (4-amino-3,5-dichloro-6-fluoro-2-methoxy pyridine), also referenced as Dow AgroSciences LLC test substance inventory number AGR250194. The method was validated in soil over the concentration range of 0.01 to 0.10 µg/g, with a validated lower limit of quantitation of 0.01 µg/g.

Residues of fluroxypyr, fluroxypyr-DCP, and fluroxypyr-MP were extracted from soil using 90% acetone/10% 0.1 N HCl. An aliquot of the extract was hydrolyzed with 1.0 N sodium hydroxide as it was concentrated under a stream of nitrogen. The hydrolyzed samples were acidified, diluted with deionized water, and purified using C₁₈ solid phase extraction (SPE). The fluroxypyr and fluroxypyr-DCP were eluted with a solution of 30% acetonitrile/69% water/1% 1.0 N HCl into a vial containing sodium chloride. The acetonitrile was evaporated, and the samples were partitioned into 1-chlorobutane. The C₁₈ solid SPE column was dried and the fluroxypyr-MP was eluted with 1-chlorobutane. The separate 1-chlorobutane fractions from each sample were combined and concentrated under a stream of nitrogen at 40° C. The samples and the calibration standards were derivatized for 1 hour at 60 °C with *N*-methyl-*N*-(*tert*-butyldimethylsilyl)-trifluoroacetamide (MTBSTFA) to form the *tert*-butyldimethylsilyl (TBDMS) derivatives of fluroxypyr and fluroxypyr-DCP. The fluroxypyr-MP was assayed underivatized. Fluroxypyr 1-butyl ester was added as an internal standard to each sample and each calibration

standard prior to analysis. The samples were analyzed by capillary gas chromatography with mass selective detection.

Environmental chemistry residue analytical method GRM 98.04 was developed and validated at Dow AgroSciences LLC. The independent laboratory, the Study Director, and analyst chosen to conduct the ILV were unfamiliar with the method, both in its development and subsequent use in analyzing field samples. The ILV analyst ordered and/or used all of his/her own equipment and supplies with the exception of the C₁₈ SPE columns, which were supplied by Dow AgroSciences LLC. Throughout the conduct of the study, any communications between Dow AgroSciences and the Study Director and/or the analyst were logged for reporting to the Agency. No one from Dow AgroSciences was allowed to visit at Enviro-Bio-Tech, Ltd. during the ILV trial to observe, offer help, or assist chemists or technicians. These steps successfully maintained the integrity of the ILV study.

The independent laboratory established the method prior to initiation of the first sample trial by determining the retention times of the analytes, computing the instrument detection limits using analytical standards, and verifying that matrix control samples contained negligible interferences ($\leq 15\%$ of the response of the analytes at the LOQ at the appropriate retention times and detector settings.)

ANALYTICAL

Sample Numbering, Preparation, and Storage

Control soil was provided by the Dow AgroSciences Sample Management Group with the following unique sample number: SN 27292201, also referred to as M487 in the Dow AgroSciences LLC soil data base. The sample was prepared for analysis by the Dow AgroSciences Sample Management Group. The control sample was stored frozen in a temperature-monitored freezer upon receipt 7-April-1999 except when removed for analysis on 19-May-1999. The frozen control sample was aliquotted for analysis without thawing. The sample was returned instead to a refrigerator on 19-May-1999 as stated in the protocol (section F. Sample History and Identification).

Preparation of Solutions and Standards

The following analytical reference standards/test substances were utilized during the independent laboratory method validation:

Test Substance/ Analytical Standard	AGR/TSN No.	Percent Purity	Certification Date	Reference
Fluroxypyr ^a	AGR222210	99.6	28-Aug-1997	GHE-P-6545
Fluroxypyr-DCP ^a	TSN101651	>99	18-Mar-1999	FA&PC 993038
Fluroxypyr-MP ^a	AGR250194	99.9	18-May-1998	FA&PC 983079
Fluroxypyr 1-butyl ester ^b	AGR279775	99.8	15-Apr-1998	GHE-P-7114

^a Test Substance/Analytical Standard

^b Analytical Standard

Standard solutions were prepared as described in Section 7. of environmental chemistry residue analytical method GRM 98.04.

Fortification of Recovery Samples

One ILV trial of the method was run for soil. The sample set consisted of the following:

2 (two) unfortified control samples

5 (five) control samples fortified at the LOQ of 0.01 µg/g with fluroxypyr, fluroxypyr-DCP and fluroxypyr-MP

5 (five) control samples fortified at 10X the LOQ of 0.1 µg/g with fluroxypyr, fluroxypyr-DCP and fluroxypyr-MP

1 (one) reagent blank

Fortification solutions were prepared as described in Section 7. of environmental chemistry residue analytical method GRM 98.04.

Sample Extraction, Purification, and Analysis

Analyses were conducted as described in Section 9.3. of Dow AgroSciences environmental chemistry residue analytical method GRM 98.04, with no changes.

Analytical Instrumentation and Equipment

The following instruments and equipment were utilized in the conduct of the independent laboratory validation of the residue analytical method:

Instrumentation:

Capillary Gas Chromatograph/Mass Selective Detector

Hewlett Packard Model 5972 consisting of the following components:

Detector - Model 5972, S/N 3524A03010

Gas Chromatograph - Model 5890C series II Plus, S/N 3336A60351

Injector, automatic, Model 7673A, tower, S/N 2704A07582

Injector, automatic, Model 7673A, controller, S/N 2702A06341

Injector, automatic, Model 7673A, tray, S/N 2718A06186

Chem Station - Model G1030A, S/N 3344A69232

Vectra VL Series 3 Computer - S/N US53850686

HP Ultra VGA 1280 Monitor - S/N JP53260084

Printer -HP Laserjet 4 Plus, S/N USFB195626

Capillary GC Column - J & W Scientific DB-1701 column, 10 m x 0.18 mm;
film thickness 0.4 μ m, S/N 8987711A

Operating Parameters:

Capillary Gas Chromatograph/Mass Selective Detector

Temperatures:

Column 90 °C for 1.0 min
90 °C to 255 °C at 10 °C/min
255 °C to 280 °C at 20 °C/min
280 °C for 2.00 min

Injector 260 °C
Interface 280 °C

Carrier Gas: helium

Head Pressure 16 kPa
Linear Velocity approximately 39.6 cm/sec

Injection Mode: splitless

Purge delay 1.00 min
Splitter Flow 37 mL/min
Septum Purge 1.0 mL/min

Injection Volume: 3 µL

Detector: electron impact selected ion monitoring

Calibration Program maximum sensitivity autotune
Electron Multiplier 2000 volts (about 300 volts above autotune)

Ions Monitored:

Fluroxypyr *m/z* 311 (quantitation)
m/z 253 (confirmation)

Fluroxypyr-DCP *m/z* 253 (quantitation)
m/z 257 (confirmation)

Fluroxypyr-MP *m/z* 210 (quantitation)
m/z 181 (confirmation)

Fluroxypyr 1-butyl ester *m/z* 310 (internal standard)

Dwell Time: 75 msec

Equipment:

Analytical Balance –Cahn TA 4100, S/N 40609
Analytical Balance – Fisher Scientific, Model 7240 DA, S/N 17633
Bath, ultrasonic, Bransonic 220
Centrifuge, Universal Model UV
Centrifuge, Metpath Model 0151
Evaporator, Meyer N-Evap, Model 112
Mixer, vortex, SP, Model S8223-1
Shaker, Platform, in-house construction
Water Purified, Barnstead NanoPure, S/N M-81-16386
Vacuum manifold, Supelco Visiprep D-L, catalog number 5-7044
Bottles, vials and C₁₈ SPE columns as described in analytical method GRM 98.04

Calculations

Calculation of percent recovery of fluroxypyr, fluroxypyr-DCP, and fluroxypyr-MP from soil was as described in environmental chemistry residue analytical method GRM 98.04, Section 9.4. Calibration standards (0.005, 0.01, 0.05, 0.10, 0.20, and 0.50 µg/mL) were analyzed with the sample set. Confirmation ratios were calculated by dividing the peak area of the confirmation ion (*m/z* 253 for fluroxypyr, *m/z* 257 for fluroxypyr-DCP, and *m/z* 181 for fluroxypyr-MP) by the peak area of the quantitation ion (*m/z* 311 for fluroxypyr, *m/z* 253 for fluroxypyr-DCP, and *m/z* 210 for fluroxypyr-MP). Quantitation ratios were calculated by dividing the peak area of the quantitation ion by the peak area of the internal standard ion (*m/z* 310 for fluroxypyr 1-butyl ester). Power regression equations were generated for fluroxypyr, fluroxypyr-DCP, and fluroxypyr-MP using the natural log (ln) of the concentrations of the calibration standards versus the ln of the respective peak area responses. Concentrations of the analytes in the final solutions were determined by substituting the peak area responses into the applicable power regression equation as shown below:

$$\text{Analyte Conc. (gross } \mu\text{g/g)} = ((\text{Analyte quantitation ratio})/(\text{constant}))^{1/\text{exponent}}$$

For example, using the fluroxypyr data from Figure 1 and Figure 10:

$$\begin{array}{l} \text{Fluroxypyr-TBDMS Conc.} \\ \text{(gross } \mu\text{g/g)} \end{array} = ((934/18340)/(5.4496))^{(1/1.0102)}$$

$$\begin{array}{l} \text{Fluroxypyr-TBDMS Conc.} \\ \text{(gross)} \end{array} = 0.0098 \mu\text{g/g}$$

The fluroxypyr values were calculated using a computerized spreadsheet which used more decimal places than those displayed. As a result, the calculated values shown in this example may vary slightly if the values are recalculated using the displayed parameters.

Percent recovery was calculated using the equation shown below:

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

$$\text{Recovery} = \frac{0.0098}{0.010} \times 100$$

$$\text{Recovery} = 98\%$$

Statistical Treatment of Data

The mean recovery for a sample matrix was calculated using the "AVERAGE" function of the Microsoft Excel spreadsheet computer program which divides the sum of the selected cells by the number of determinations. The standard deviation of the recovery for a matrix was calculated using the "STDEV" function of the same spreadsheet program which sums the squares of the individual deviations from the mean, divides by the number of degrees of freedom, and extracts the square root of the quotient. Percent relative standard deviation, % RSD, is calculated by dividing the standard deviation by the mean, then multiplying by 100.

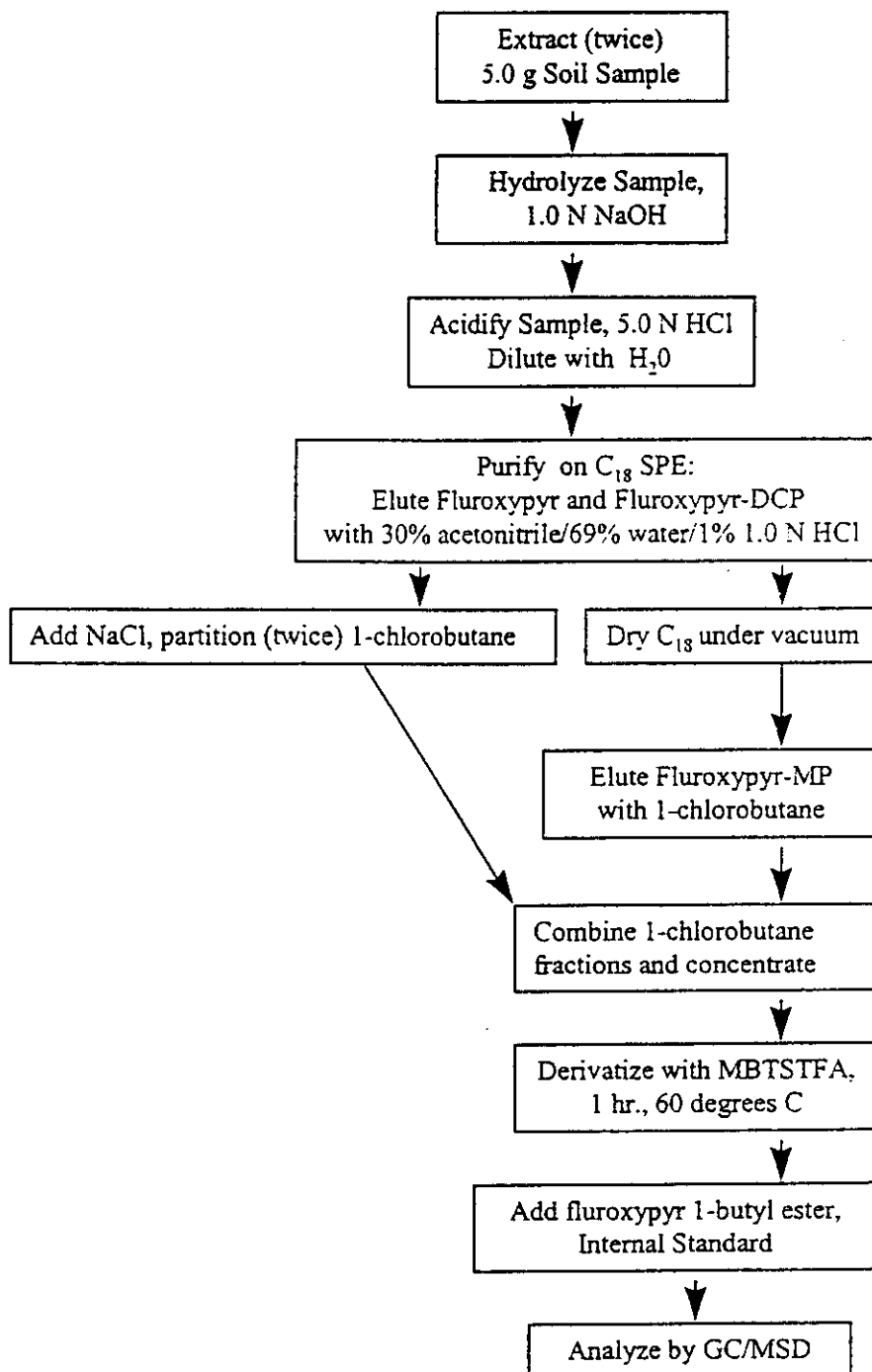


Figure 1. Flow Chart for the Determination of Fluroxypyr, Fluroxypyr-DCP, and Fluroxypyr-MP in Soil

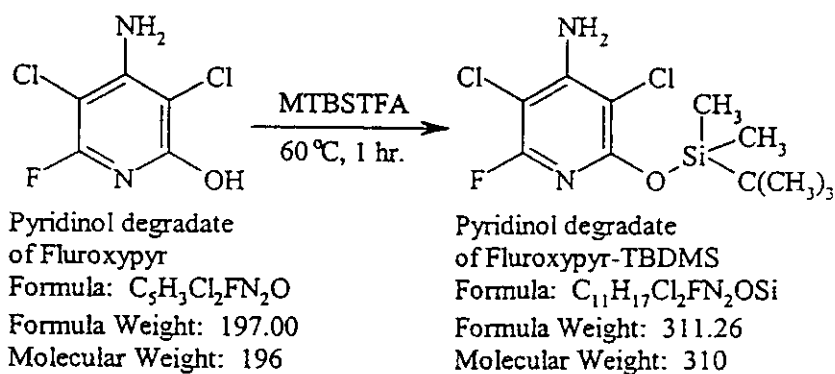
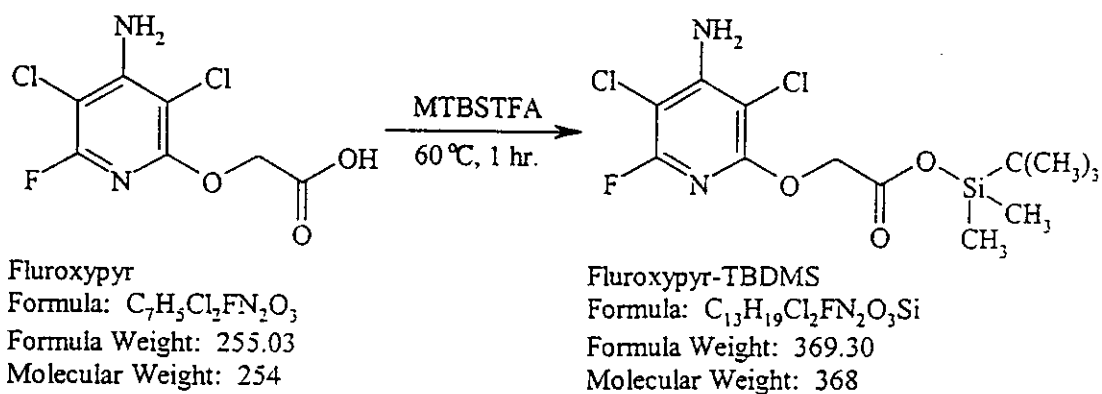


Figure 2. Chemical Structures of Fluroxypyr and Fluroxypyr-DCP and their TBDMS Derivatives

COMMUNICATIONS

The following is documentation of all contacts between the confirmatory laboratory and the method developers or others familiar with the method. Included are the reasons for the contact, any changes in the method that resulted, and the time of this communication with respect to the progress of the confirmatory trial (i.e., after the first set, during the second set, etc.):

- (1) 1-June-1999 – A telephone conversation between the Study Director and the Study Monitor covered the following topics: 1. Background interference in the control soil sample seen during Trial 1: In spite of the higher background levels than specified in the protocol, recovery results for Trial 1 were deemed acceptable after background subtraction. 2. The stopping of the Method during Trial 1 at a point not specified as an acceptable overnight stopping point: This will be handled as a protocol deviation, with no negative impact on Trial 1 results. 3. Calculation and reporting of results: The number of significant figures to report was discussed, as well as the route of calculation to obtain the final percent recovery. 4. Placement of soil sample in freezer at EBT following receipt of sample at EBT: The Protocol states to store the soil in a refrigerator. The Study Monitor suggested that a note to the study file would be sufficient to address this. In addition, the Study Monitor agreed that the results from Trial 1 (a copy was sent by the Study Director 5-May-1999) were acceptable, and that the report should be written.