

Independent Laboratory Validation of the “Enforcement Method Validation of the Determination of Oxyfluorfen and its Isomers in Soil Matrices by Gas Chromatography with Mass Spectrometry”

INTRODUCTION

Scope

This method is applicable for the quantitative determination of residues of oxyfluorfen and its isomers, RH-50671 (X11488795), RH-34672, and RH-42382 in soil (loamy sand and clay). The method was independently validated over the concentration range of 0.01-0.10 ppm with a validated limit of quantitation of 0.01 ppm. Common names, chemical names, and molecular formulas for the analytes are given in Table 1.

This study was conducted to fulfill data requirements outlined in the EPA Residue Chemistry Test Guidelines, OCSPP 850.6100 (1). The validation also complies with the requirements of SANCO/825/00 rev.8.1 (2). The validation was conducted following EAG SOPs with exceptions noted in protocol amendments, protocol deviations and SOP deviations.

Method Principle

Residues of oxyfluorfen, RH-50671 (X11488795), RH-34672, and RH-42382 are extracted from samples by mixing with water and acetonitrile followed by vigorous shaking. The extract is shaken again with a citrate salt mixture followed by centrifugation. An aliquot of the upper acetonitrile layer is evaporated to dryness, reconstituted in toluene, vortexed and sonicated. The final sample extracts are filtered through a 0.2 µm PTFE syringe filter. The final samples are analyzed for oxyfluorfen, RH-50671 (X11488795), RH-34672, and RH-42382 by gas chromatography coupled with mass spectrometry (GC-MS).

Test Substances/Reference Compounds/Analytical Standards

| Test Substance | TSN | Percent Purity | Recertification Date | Reference |
|----------------------|-----------|----------------|----------------------|---------------|
| Oxyfluorfen | TSN102768 | 99.8% | 17-May-2018 | FAPC13-000008 |
| RH-50671 (X11488795) | TSN309143 | 99% | 15-May-2019 | FAPC17-000290 |
| RH-34672 | TSN102937 | 100% | 25-May-2025 | FAPC17-000311 |
| RH-42382 | TSN102955 | 98.7% | 12-May-2018 | FAPC14-000174 |

The Certificates of Analysis for the test substances can be found in Figure 1-Figure 4. The above standards may be obtained free of charge from Dow AgroSciences LLC, 9330 Zionsville Road, Building 304, Indianapolis, IN 46268-1054.

EXPERIMENTAL

Sample Origin, Numbering, Preparation, Storage, and Characterization

Untreated control samples were obtained from AGVISE Laboratories. All samples were tracked in the EAG Labs Information Management System (LIMS) database. Unique sample numbers were assigned to the samples to track them during receipt, storage, and analysis. Complete source documentation was included in the study file.

Soil samples were prepared at AGVISE Laboratories by passing through a 2 mm sieve.

During the course of the study, the samples were stored in temperature-monitored freezers at approximately -20 °C, except when removed for analysis. Physicochemical characteristics for the soils used are listed in Table 2. Complete documentation for the soil sources may be found in the raw study file.

Instrumentation

An Agilent 7890A GC-MS system was used in conjunction ChemStation mass spectrometer data system for analysis of the data.

| | |
|--------------------|---|
| Injector: | Gerstel MPS |
| Gas Chromatograph: | Agilent 7890A (G3440A) |
| Mass Spectrometer: | Agilent 5975C |
| Software: | ChemStation G1701EA E.02.02 |
| Column: | Restek Rtx-200, 60 m x 0.32 mm x 1.0 µm |

Typical Mass Spectrometer and General Instrument Conditions

| | |
|----------------------------|-----------------------------|
| Ionization Type: | Electron Ionization |
| Carrier Gas: | Helium |
| Mode: | Constant Flow of 3.5 mL/min |
| Transfer Line Temperature: | 250°C |

Typical Injector Conditions

| | |
|-----------------------|---|
| Injection Mode: | Pulsed Splitless, 50 psi for 0.5 minute |
| Injection Volume: | 3 µL |
| Injector Temperature: | 265°C |
| Purge Flow: | 40.0 mL/min for 1.50 minute |
| Liner: | 4 mm i.d. Agilent Low Pressure Drop Liner |
| Inlet Septa: | 11 mm |

Typical Oven Operating Conditions

| | | | |
|------------------------------|-----------------------|---------------------|--------------------|
| Solvent Delay: | 3 minutes | | |
| Oven Gradient: | Gradient (°C/min) | Temperature (°C) | Hold Time (min) |
| | - | 215 | 1.0 |
| | 5 | 250 | 17.0 |
| Run Time: | 25 minutes | | |
| Approximate Retention Times: | Oxyfluorfen 17.6 mins | | |
| | RH-50671 15.7 mins | | |
| | RH-34672 18.0 mins | | |
| | RH-42382 16.3 mins | | |

Typical Mass Spectrometer Conditions

| | | | | |
|---------------------------|-----------------------------|----------------|----------------|----------------|
| MS Mode: | Single Ion Monitoring (SIM) | | | |
| MS Source Temperature: | 250 °C | | | |
| MS Quad Temperature: | 150 °C | | | |
| Ions Monitored: | Oxyfluorfen | RH-50671 | RH-34672 | RH-42382 |
| Quantitation: | <i>m/z</i> 361 | <i>m/z</i> 138 | <i>m/z</i> 166 | <i>m/z</i> 361 |
| Confirmation (primary): | <i>m/z</i> 317 | <i>m/z</i> 361 | <i>m/z</i> 138 | <i>m/z</i> 317 |
| Confirmation (secondary): | <i>m/z</i> 363 | <i>m/z</i> 363 | <i>m/z</i> 361 | <i>m/z</i> 363 |
| Dwell Time: | 150 | 150 | 150 | 150 |

Calculation of Standard Calibration Curve

Calculation of a standard curve begins with the injection of a series of calibration standards described in Appendix I and acquisition of peak areas for the following analytes.

Oxyfluorfen: *m/z* 361 (quantitative)
m/z 317 (confirmatory)
m/z 363 (confirmatory)

RH-50671
 (X11488795): *m/z* 138 (quantitative)
m/z 361 (confirmatory)
m/z 363 (confirmatory)

RH-34672: *m/z* 166 (quantitative)
m/z 138 (confirmatory)
m/z 361 (confirmatory)

RH-42382: *m/z* 361 (quantitative)
 m/z 317 (confirmatory)
 m/z 363 (confirmatory)

For each analyte, the linearity of detector response was evaluated using matrix-matched standard solutions. In order to generate a standard curve, the analyte concentration was plotted on the abscissa (x-axis), and the respective peak area was plotted on the ordinate (y-axis) in Excel. Using Excel function to perform 1/x regression analysis, the equation for the curve was determined. Refer to Figure 5-Figure 16 for example calibration curves and to Figure 17 for example calculations. Individual calibration results can be found in Table 3-Table 14.

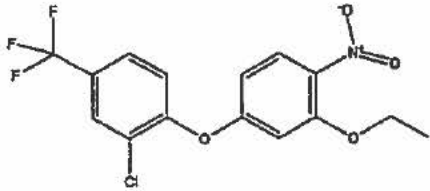
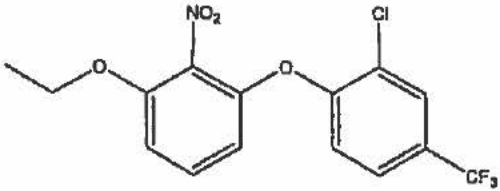
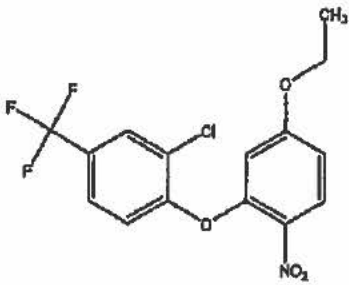
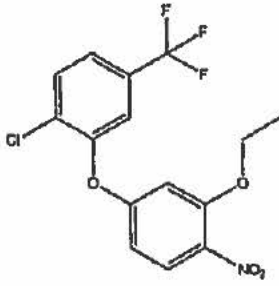
Confirmation of Residue Identity

The method is specific for the determination of oxyfluorfen, RH-50671 (X11488795), RH-34672, and RH-42382 by virtue of the chromatographic separation and selective detection system used. To demonstrate further confirmation, two additional MS ion transitions were monitored for each analyte.

Statistical Treatment of Data

Statistical treatment of data included but was not limited to the calculation of regression equations, correlation coefficients (*r*) for describing the linearity of calibration curves, means, standard deviations, and relative standard deviations of the results for the fortified recovery samples plus LOD and LOQ values.

Table 1. Identities and Structures of Oxyfluorfen and its Isomers

| Common Name | Structural Formula and Chemical Name |
|---|---|
| <p>Oxyfluorfen</p> <p>Molecular Formula: C₁₅H₁₁ClF₃NO₄</p> <p>CAS Number: 42874-03-3</p> |  <p>2-Chloro-1-(3-ethoxy-4-nitrophenoxy)-4-(trifluoromethyl)benzene</p> |
| <p>RH-50671 (X11488795)</p> <p>Molecular Formula: C₁₅H₁₁ClF₃NO₄</p> <p>CAS Number: NA</p> |  <p>1-(2-Chloro-4(trifluoromethyl)phenoxy)-3-ethoxy-2-nitrobenzene</p> |
| <p>RH-34672</p> <p>Molecular Formula: C₁₅H₁₁ClF₃NO₄</p> <p>CAS Number: NA</p> |  <p>2-Chloro-5'-ethoxy-2'-nitro-4-trifluoromethyl diphenyl ether</p> |
| <p>RH-42382</p> <p>Molecular Formula: C₁₅H₁₁ClF₃NO₄</p> <p>CAS Number: NA</p> |  <p>2-Chloro-1-(3-ethoxy-4-nitrophenoxy)-5-(trifluoromethyl)benzene</p> |

APPENDIX I ANALYTICAL METHOD

Enforcement Method Validation of the Determination of Oxyfluorfen and its Isomers in Soil Matrices by Gas Chromatography with Mass Spectrometry

The following provides an explanation of the actual materials and instrumentation used for this validation.

Equipment, Glassware and Materials

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 glassware and supplies are assumed to be readily available.

Laboratory Equipment

Balance, analytical, Model XP205DR, Mettler-Toledo, Inc.

Balance, top-loader, Model ML 3002E, Mettler-Toledo, Inc.

Centrifuge, with rotor to accommodate 50 mL polypropylene centrifuge tubes, Sorvall Model Legend XTR, Thermo Scientific

Evaporator, N-Evap, Model NI 16, Organomation Associates, Inc.

Sonicator, Branson 8800 Bransonic series, Model CPX8800H

Vortex Mixer, Model M16715, Thermo Scientific

Pipet, positive-displacement, 3-25 μ L capacity, Model M25, Gilson Inc.

Pipet, positive-displacement, 10-100 μ L capacity, Model M100, Gilson Inc.

Pipet, positive-displacement, 50-250 μ L capacity, Model M250, Gilson Inc.

Pipet, positive-displacement, 100-1000 μ L capacity, Model M1000, Gilson Inc.

Repeater Xstream, positive-displacement, Eppendorf

Glassware and Materials

Falcon™ 50-mL polypropylene centrifuge tubes, part number 14-959-49A, Fisher Scientific

Glass Tubes, disposable borosilicate, 16 x 125 mm, Fisherbrand, part number 14-959-35A, Fisher Scientific

Cap, Teflon-lined screw cap to fit 16 x 125 mm glass tube, part number 02-993-413, Fisher Scientific

PTFE Syringe Filters, Whatman puradisc 13 mm, 0.2 μ m, part number 09-911-6, Fisher Scientific

Disposable syringes with Luer-Lok™ tips, 3-mL, part number 14-823-435, Fisher Scientific

Vial, 4-dram, amber glass, catalog number 03-339-23D, Fisher Scientific

Cap, Teflon-lined screw-cap to fit 4-dram vial, part number 02-913-111, Fisher Scientific

Vial, 8-dram, amber glass, catalog number 03-339-23E, Fisher Scientific

Cap, Teflon-lined screw-cap to fit 8-dram vial, part number 02-993-396, Fisher Scientific

Vial, autosampler, 2 mL, 11 mm amber glass crimp/snap top, catalog number C4011-6W, Thermo Scientific™

Autosampler vial inserts, 9 mm, 500 µL, catalog number 03-375-3B, Fisher Scientific

Autosampler vial inserts, ~350 µL flat bottom, part number 24517, Restek

Pipettes, glass class A, volumetric, various sizes

Pipettes, glass serological, 10 mL

Combitips 50 mL, Eppendorf

Reagents

Acetonitrile, OPTIMA, catalog number A996, Fisher Scientific

QuEChERS EN method extraction salts (4 g MgSO₄, 1 g NaCl, 1 g NaCitrate, 0.5 g disodium citrate sesquihydrate), catalog number 26236, Restek

Toluene, HPLC Grade, A.C.S, catalog number T290, Fisher Scientific

Toluene, OPTIMA, catalog number T921, Fisher Scientific

Water, HPLC Grade, catalog number W5, Fisher Scientific

Preparation of Fortification Solutions

1. 10 mg (adjusted for purity) of oxyfluorfen, RH-50671, RH-34672 and RH-42382 were separately dissolved in approximately 10 mL of acetonitrile to obtain a 1000 µg/mL fortification stock solution of each analyte.
2. 0.0500 mL of the oxyfluorfen, RH-50671, RH-34672 and RH-42382 fortification stock solutions were pipetted into 9.8 mL of acetonitrile to obtain a 5.0 µg/mL mixed solution.
3. 1.00 mL of the 5.0 µg/mL mixed oxyfluorfen, RH-50671, RH-34672 and RH-42382 solution was pipetted into 9 mL of acetonitrile to obtain a 0.50 µg/mL mixed solution.

Preparation of Calibration Solutions

1. 10 mg (adjusted for purity) of oxyfluorfen, RH-50671, RH-34672 and RH-42382 were separately dissolved in approximately 10 mL of toluene to obtain a 1000 µg/mL calibration stock solution of each analyte.

2. 2.00 mL of the oxyfluorfen, RH-50671, RH-34672 and RH-42382 calibration stock solutions were pipetted into 12 mL of toluene to obtain a 100 µg/mL mixed solution.
3. Intermediate calibration standards were prepared by diluting the appropriate amount of the mixed oxyfluorfen, RH-50671, RH-34672 and RH-42382 100 µg/mL solution with toluene to obtain intermediate calibration solutions as described in the following table:

| Concentration of Original Solution (µg/mL) | Aliquot (mL) | Final Volume (mL) ^a | Intermediate Calibration Solution Final Conc. (µg/mL) |
|--|--------------|--------------------------------|---|
| 100 | 4.00 | 10.0 | 40 |
| 100 | 3.00 | 10.0 | 30 |
| 100 | 1.50 | 10.0 | 15 |
| 100 | 0.600 | 10.0 | 6.0 |
| 100 | 0.300 | 10.0 | 3.0 |
| 100 | 0.090 | 10.0 | 0.90 |

^a mL aliquots added to a 4-dram amber vial already containing the appropriate volume of toluene so that the total volume was 10.0 mL

4. Matrix matched calibration standards were prepared by extracting an extra six control samples per matrix and taking them through the analytical procedure. After the evaporation step, the control samples were reconstituted in 0.475 mL of toluene and fortified with the appropriate intermediate calibration solution (0.025 mL) before being filtered through a 0.2 µm x 13 PTFE syringe filter to obtain calibration solutions over the concentration range of 0.045-2.0 µg/mL as described in the following table:

| Concentration of Intermediate Calibration Solution (µg/mL) | Aliquot (mL) | Volume of Toluene (mL) | Final Volume (mL) | Calibration Solution Final Conc. (µg/mL) | Equivalent Sample Conc. (ppm) ^a |
|--|--------------|------------------------|-------------------|--|--|
| 40 | 0.025 | 0.475 | 0.5 | 2.0 | 0.13 |
| 30 | 0.025 | 0.475 | 0.5 | 1.5 | 0.10 |
| 15 | 0.025 | 0.475 | 0.5 | 0.75 | 0.05 |
| 6.0 | 0.025 | 0.475 | 0.5 | 0.30 | 0.02 |
| 3.0 | 0.025 | 0.475 | 0.5 | 0.15 | 0.01 |
| 0.90 | 0.025 | 0.475 | 0.5 | 0.045 | 0.003 |

^aThe equivalent sample concentrations are based on taking an initial sample mass of 10.0 g and extracting with 10 mL of acetonitrile. After shaking and the addition of extraction salts, a 7.5 mL aliquot is evaporated to dryness and reconstituted in toluene to give a final volume of 0.5 mL.

All standard solutions and sample extracts are stored in a freezer at approximately -20 °C in either a 4- or 8-dram amber vial.

Analytical Procedure

- 1) Weigh 10.0 g of soil into a 50 mL polypropylene centrifuge tube.
- 2) For the fortified samples, an appropriate aliquot of fortification solution is added to encompass the necessary concentration range. See table below:

| Concentration of Fortification Solution (µg/mL) | Volume of Fortification Solution (mL) | Sample Weight (g) | Concentration of Fortified Sample (ppm) |
|---|---------------------------------------|-------------------|---|
| 0.50 | 0.060 | 10.0 | 0.003 |
| 0.50 | 0.20 | 10.0 | 0.01 |
| 5.0 | 0.20 | 10.0 | 0.1 |

- 3) Add 5 mL of HPLC grade water.
- 4) Add 10 mL of acetonitrile.
- 5) Shake vigorously by hand for two minutes.
- 6) Add citrate salt mixture (QuEChERS EN: 4 g MgSO₄, 1 g NaCl, 1 g NaCitrate, 0.5 g disodium citrate sesquihydrate).
- 7) Shake vigorously by hand for two minutes.
- 8) Centrifuge for five minutes at 4000 rpm.
- 9) Transfer a 7.5 mL aliquot of the upper acetonitrile layer into a clean 16 x 125 mm glass tube and evaporate to dryness under a gentle stream of nitrogen on an N-Evap with the water bath at 40 °C.
- 10) Reconstitute samples by adding 0.5 mL toluene, vortex mix and sonicate samples approximately 5 minutes to dissolve residues.
- 11) Filter final sample extract (from Step 10) through a 0.2 µm x 13 mm PTFE syringe filter back into the original tube.
- 12) Transfer an aliquot of the sample into an autosampler vial containing a vial insert.
- 13) Analyze by GC-MS.

Instrumental Conditions

Instrumentation

Injector: Gerstel MPS
 Gas Chromatograph: Agilent 7890A (G3440A)
 Mass Spectrometer: Agilent 5975C
 Software: ChemStation G1701EA E.02.02
 Column: Restek Rtx-200, 60 m x 0.32 mm x 1.0 µm

Typical Mass Spectrometer and General Instrument Conditions

Ionization Type: Electron Ionization
 Carrier Gas: Helium
 Mode: Constant Flow of 3.5 mL/min
 Transfer Line Temperature: 250°C

Typical Injector Conditions

Injection Mode: Pulsed Splitless, 50 psi for 0.5 minute
 Injection Volume: 3 µL
 Injector Temperature: 265°C
 Purge Flow: 40.0 mL/min for 0.5 minute
 Liner and O-ring*: 4 mm i.d. Agilent Low Pressure Drop Liner (part number 5190-2295)
 Inlet Septa*: 11 mm, Agilent Advanced Green, non-stick (part number 5183-4759-100)

* The liner with its accompanying O-ring and inlet septa were changed between each analytical set to prevent particulates from accumulating at the front of the GC column. If these are not changed between sets, particulates from both the sample injections and the matrix-matched standards will lead to a buildup of particulates at the front of the column which can lead to a sudden loss of sensitivity. The remedy would be to remove a portion from the front of the column.

Typical Oven Operating Conditions

| | | | |
|----------------|----------------------|---------------------|--------------------|
| Solvent Delay: | 3 minutes | | |
| Oven Gradient: | Gradient (°C/min) | Temperature (°C) | Hold Time (min) |
| | - | 215 | 1.0 |
| | 5 | 250 | 17.0 |
| Run Time: | 25 minutes | | |

Approximate Retention Times: Oxyfluorfen 17.6 mins
RH-50671 15.7 mins
RH-34672 18.0 mins
RH-42382 16.3 mins

Typical Mass Spectrometer Conditions

| | | | | |
|---------------------------|-----------------------------|----------------|----------------|----------------|
| MS Mode: | Single Ion Monitoring (SIM) | | | |
| MS Source Temperature: | 250 °C | | | |
| MS Quad Temperature: | 150 °C | | | |
| Ions Monitored: | Oxyfluorfen | RH-50671 | RH-34672 | RH-42382 |
| Quantitation: | <i>m/z</i> 361 | <i>m/z</i> 138 | <i>m/z</i> 166 | <i>m/z</i> 361 |
| Confirmation (primary): | <i>m/z</i> 317 | <i>m/z</i> 361 | <i>m/z</i> 138 | <i>m/z</i> 317 |
| Confirmation (secondary): | <i>m/z</i> 363 | <i>m/z</i> 363 | <i>m/z</i> 361 | <i>m/z</i> 363 |
| Dwell Time: | 150 | 150 | 150 | 150 |