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-34672, RH-42382 and RH-50671 in soil. The method was validated over the concentration range of 0.01 – 0.1 mg/kg with a validated limit of quantitation of 0.01 mg/kg.

The chemical names, molecular structures, molecular formulae and molecular weights for the analytes are given in Table 1.

This study was conducted to fulfil data requirements outlined in Commission Regulation (EU) No 283/2013 setting out the data requirements, in accordance with Regulation (EC) No 1107/2009 [1], EPA Guideline OCSPP 850.6100 [2] and Guidance Document SANCO/825/00 rev.8.1 [3] as well as PMRA Residue Chemistry Guidelines as Regulatory Directive Dir98-02 [4].

Method Principle

Residues of oxyfluorfen, RH-34672, RH-42382 and RH-50671 were extracted from soil samples by shaking with water and acetonitrile. The extracts were then shaken with the contents of an EN 15662 QuEChERS salt sachet and centrifuged. After centrifugation, an aliquot of the acetonitrile layer was evaporated to dryness and reconstituted in toluene. The final sample extracts were filtered through a 0.2 µm PTFE syringe filter. The samples were analysed by gas chromatography with mass spectrometry (GC-MS).

Test Substances/Reference Compounds/Analytical Standards

Analytical Standard[a]	TSN	Percent Purity	Re-Certification Date	Reference
Oxyfluorfen	TSN104406	99.6	17 May 2018	FAPC12-000718
RH-34672	TSN102937	100	25 May 2025	FAPC17-000311
RH-42382	TSN102955	98.7	12 May 2018	FAPC14-000174
RH-50671	TSN309143	99	15 May 2019	FAPC17-000290

[a]The molecular formulae and structures are given in Table 1. The certificates of analysis are given in Figure 1 to Figure 4.

EXPERIMENTAL

Full details of the instrumental conditions used during this validation are given in Appendix 1.

Sample Origin, Preparation and Storage

The validation was carried out using characterised soil samples, obtained from Battelle UK stocks of control samples. The two types of soil used in the validation were a sandy loam soil (Lufa Speyer 2.2) and a clay soil (Lufa Speyer 6S). The soil samples were characterised by Agvise, Northwood, ND, 58267, in a separate study and full characterisation details are given in Appendix 2. Unique sample numbers were assigned to the samples to track them during receipt, storage and analysis. No sample preparation was necessary. The soil samples were stored in a temperature monitored refrigerator throughout the course of the study.

Calculation of Standard Calibration Curve

Calculation of a standard curve begins with the injection of a series of calibration standards described in Appendix 1 and acquisition of peak areas for the following analytes:

Oxyfluorfen	*m/z* 361 (quantitative)
Oxyfluorfen	*m/z* 317 (confirmatory)
Oxyfluorfen	*m/z* 363 (confirmatory)
RH-34672	*m/z* 166 (quantitative)
RH-34672	*m/z* 138 (confirmatory)
RH-34672	*m/z* 361 (confirmatory)
RH-42382	*m/z* 361 (quantitative)
RH-42382	*m/z* 317 (confirmatory)
RH-42382	*m/z* 363 (confirmatory)
RH-50671	*m/z* 138 (quantitative)
RH-50671	*m/z* 361 (confirmatory)
RH-50671	*m/z* 363 (confirmatory)

In order to generate a standard curve, plot the analyte concentration on the abscissa (x-axis) and the respective analyte peak area on the ordinate (y-axis) in OpenLab. Using linear regression analysis with 1/x weighting, determine the equation for the curve with respect to the abscissa. Refer to Figure 5 through Figure 28 for example calibration plots and to Figure 29 for example calculations. Individual calibration results can be found in Table 2 to Table 13.

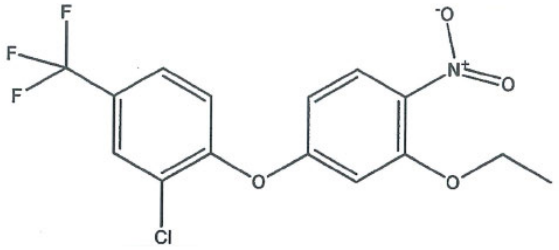
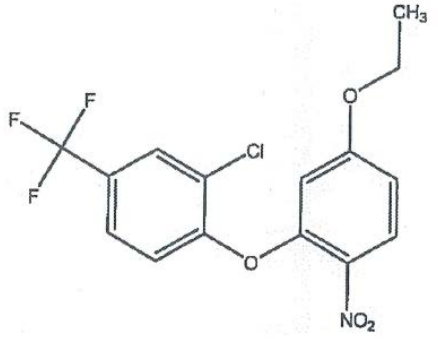
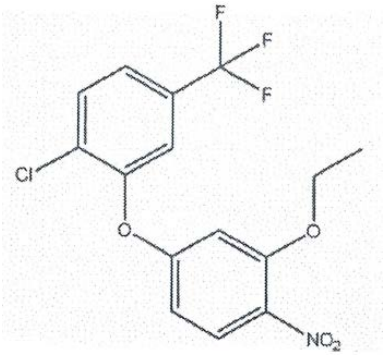
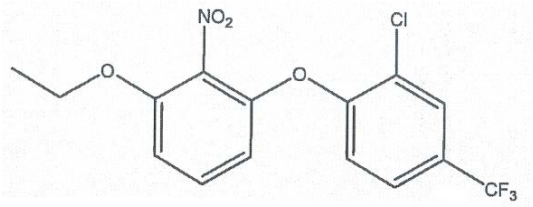
Full-Scan Mass Spectra

Full-scan mass spectra of oxyfluorfen, RH-34672, RH-42382 and RH-50671 are illustrated in Figure 30 to Figure 33.

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, and means, standard deviations, and relative standard deviations of the results for the fortified recovery samples.

Table 1 Identity and Structure of Oxyfluorfen, RH-34672, RH-42382 and RH-50671

Common Name	Structural Formula and Chemical Name
Oxyfluorfen Molecular Formula: $C_{15}H_{11}ClF_3NO_4$ Molecular Weight: 361.71 CAS Number: 42874-03-3	 2-chloro-1-(3-ethoxy-4-nitrophenoxy)-4-(trifluoromethyl)benzene
RH-34672 Molecular Formula: $C_{15}H_{11}ClF_3NO_4$ Molecular Weight: 361.70 CAS Number: Not Known	 2-chloro-5'-ethoxy-2'-nitro-4-trifluoro-methyldiphenyl ether
RH-42382 Molecular Formula: $C_{15}H_{11}ClF_3NO_4$ Molecular Weight: 361.70 CAS Number: Not Known	 2-chloro-1-(3-ethoxy-4-nitrophenoxy)-5-(trifluoromethyl)benzene
RH-50671 Molecular Formula: $C_{15}H_{11}ClF_3NO_4$ Molecular Weight: 361.70 CAS Number: Not Known	 1-(2-chloro-4(trifluoromethyl)phenoxy)-3-ethoxy-2-nitrobenzene

APPENDIX 1 INSTRUMENTATION AND PARAMETERS

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, A&D GX1000, European Instruments

Balance, Sartorius U4100, Sartorius Ltd

Balance, Sartorius Cubis MSU225S, Sartorius Ltd

Barnstead Smart2Pure 6UV Water Purification System, Thermo Scientific

Centrifuge, Varifuge 3.0R, Heraeus Instruments Ltd

Centrifuge, Hettich Rotanta 460R, Hettich Lab

Eppendorf Multipette Xstream®, Fisher Scientific

Techne Sample Concentrator and Dri-Block® DB-3D, Bibby Scientific Ltd

Ultrasonic Bath, VWR

Vortex mixer, Fisher Scientific

Glassware and Materials

2 mL disposable syringes, BD Plastipak

50 mL plastic centrifuge tubes, Fisher Scientific

Glass tubes, disposable borosilicate, 16 x 125 mm, Corning

PTFE syringe filters, 13 mm, 0.2 µm, 5190-5265, Agilent Technologies UK

Reagents

Acetonitrile, HPLC grade, Fisher Scientific

QuEChERS EN method extraction salts, 5982-6650, Agilent

Toluene, Pesticide residue analysis grade, Sigma Aldrich

Ultrapure Water, Barnstead Smart2Pure 6UV Water Purification System, Thermo 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. 0.05 mL of each 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. 1.0 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 0.5 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. 2.0 mL of each 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)	Intermediate Solution Final Conc. (µg/mL)
100	4.0	10	40
100	3.0	10	30
100	1.5	10	15
100	0.6	10	6.0
100	0.3	10	3.0
100	0.09	10	0.9

4. Matrix matched calibration standards were prepared by extracting an extra six control aliquots per matrix and taking them through the analytical procedure. After the evaporation step, the control samples were reconstituted in toluene and fortified with the appropriate intermediate calibration solution before being filtered through a 0.2 µm 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 Solution (µg/mL)	Aliquot (mL)	Volume of Toluene (mL)	Final Volume (mL)	Calibration Solution Final Conc. (µg/mL)	Equivalent Sample Conc. (mg/kg)[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
0.9	0.025	0.475	0.5	0.045	0.003

[a]The 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 were stored in a freezer. Due to the volatile nature of the final solvent, it is recommended that matrix matched standards are prepared on the same day as the sample extracts to compensate for any solvent evaporation occurring.

Analytical Procedure

1) Weigh 10.0 g of soil into a 50 mL centrifuge tube

2) For the fortified samples, an appropriate aliquot of spiking 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 (mg/kg)
0.5	0.06	10.0	0.003
0.5	0.2	10.0	0.01
5.0	0.2	10.0	0.1

3) Add 5 mL of water

4) Add 10 mL of acetonitrile

5) Shake vigorously by hand for two minutes

6) Addition of citrate salt mixture*

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 phase into a clean tube and evaporate to dryness under a gentle stream of nitrogen (40°C)

10) Reconstitute samples by adding 0.5 mL toluene, vortex mix and sonicate samples to dissolve residues

11) Filter final sample extract through a 0.2 µm PTFE syringe filter

12) Transfer an aliquot of the sample into an autosampler vial

13) Analyse by GC-MS

*4 g $MgSO_4$, 1 g NaCl, 1 g NaCitrate, 0.5 g disodium citrate sesquihydrate

Instrumental Conditions

Instrumentation

Injector:	Agilent 7683B Series
Gas Chromatograph:	Agilent HP6890N
Mass Spectrometer:	Agilent 5975
Software:	Agilent OpenLAB CDS 2.1
Column:	Restek Rtx-200, 60 m x 0.32 mm x 1.0 µm

Typical Mass Spectrometer and General Instrument Conditions

Ionisation Type:	Electron Ionisation
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:	Restek Sky Inlet Liner (catalogue no. 23467.5) with Supelco Therm-O-Ring™ Inlet Liner O-Ring (catalogue no. 21004-U)	
Inlet Septa:	Agilent Advanced Green Septa (part no. 5183-4759)	
	Pre-Injection	Post Injection
Sample Washes:	0	-
Solvent A Washes:	2	4
Solvent B Washes:	2	4
Sample Pumps:	2	-
Solvent A:	Toluene	
Solvent B:	Toluene	

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[a]: RH-50671 15.1 mins (batches 1 + 5), 14.8 mins (batches 9 + 10)
RH-42382 15.6 mins (batches 1 + 5), 15.3 mins (batches 9 + 10)
Oxyfluorfen 16.8 mins (batches 1 + 5), 16.5 mins (batches 9 + 10)
RH-34672 17.3 mins (batches 1 + 5), 16.9 mins (batches 9 + 10)

[a]Due to a loss of sensitivity, a segment of the column was removed before the analysis of batches 9 + 10. Therefore, the retention times have shifted earlier. This does not negatively impact the data as the retention times are consistent throughout each analytical run.

Typical Mass Spectrometer Conditions

MS Mode: Single Ion Monitoring (SIM)
MS Source Temperature: 250°C
MS Quad Temperature: 150°C

Ions Monitored:	Oxyfluorfen	RH-34672	RH-42382	RH-50671
Quantitation	*m/z* 361	*m/z* 166	*m/z* 361	*m/z* 138
Confirmation (primary)	*m/z* 317	*m/z* 138	*m/z* 317	*m/z* 361
Confirmation (secondary)	*m/z* 363	*m/z* 361	*m/z* 363	*m/z* 363

SIM Time Segment 1 – Time 3 minutes (all batches)

Compound	Ion	Dwell Time	Resolution
RH-50671	138	150	High
	361	150	High
	363	150	High

SIM Time Segment 2 – Time 15.3 minutes (batches 1 + 5), 15.05 minutes (batches 9 + 10)

Compound	Ion	Dwell Time	Resolution
RH-42382	317	150	High
	361	150	High
	363	150	High

SIM Time Segment 3 – Time 16.5 minutes (batches 1 + 5), 16.25 minutes (batches 9 + 10)

Compound	Ion[a]	Dwell Time	Resolution
Oxyfluorfen	138	150	High
	166	150	High
	317	150	High
	361	150	High
	363	150	High

SIM Time Segment 4 – Time 17.05 minutes (batches 1 + 5), 16.75 minutes (batches 9 + 10)

Compound	Ion[a]	Dwell Time	Resolution
RH-34672	138	150	High
	166	150	High
	317	150	High
	361	150	High
	363	150	High

[a]For oxyfluorfen and RH-34672, the same five ions were monitored for both analytes, although only three per analyte were quantified. Due to how closely the two analytes elute, this was done to ensure that each peak was fully detected before the SIM time segment changed.