EXPERIMENTAL

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

Sample Origin, Preparation and Storage

The independent validation was carried out using fully characterised water samples, obtained from Battelle UK stocks of control samples. The water samples were characterised by Agvise, Northwood, ND, 58267, in a separate study and full characterisation details are given in Appendix 2. The water samples were stored in a freezer and defrosted prior to analysis. No homogenisation was necessary although samples were shaken and mixed well prior to analysis. Unique sample numbers were assigned to the samples to track them during storage, and analysis.

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 Q1/Q3 362/316 (quantitative)
Oxyfluorfen	m/z Q1/Q3 362/237 (confirmatory)
Oxyfluorfen IS	m/z Q1/Q3 367/237 (internal standard)

In order to generate a standard curve, plot the analyte concentration/internal standard concentration on the abscissa (x-axis) and the respective analyte peak area/internal standard peak area on the ordinate (y-axis) in Analyst. Using linear regression analysis with 1/x weighting, determine the equation for the curve with respect to the abscissa. Refer to Figure 3 and Figure 4 for example calibration plots and to Figure 5 for example calculations. Individual calibration results can be found in Table 2 through Table 5.

Confirmation of Residue Identity

The method is selective for the determination of oxyfluorfen by virtue of the chromatographic separation and MS/MS detection. When detection is by tandem mass spectrometry, confirmation of the presence of the analyte requires the observation of a precursor ion plus a structurally significant product ion observed at the same retention time [5]. Confirmation demonstrates the selectivity of the primary method for all representative sample matrices. It has to be confirmed that the primary method detects the correct analyte (analyte identity) and that the analyte signal of the primary method is quantitatively correct and not affected by any other compounds. Full scan mass spectra and product ion spectra are provided in Figure 6 and Figure 7 to justify the selection of ions used for determination of each analyte.
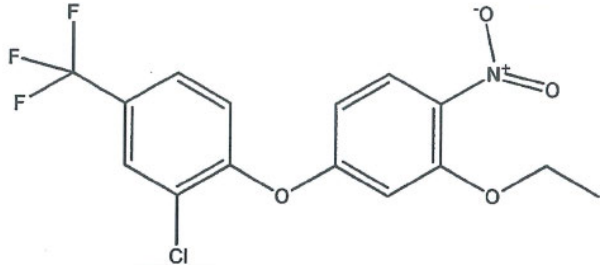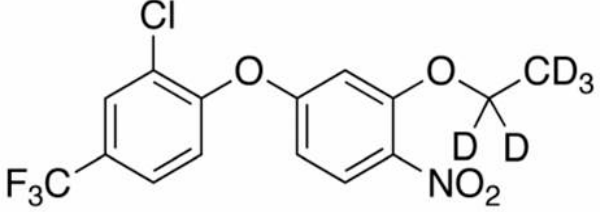
Statistical Treatment of Data

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

Recovery Levels and Precision

The independent laboratory validation study was conducted to determine the recovery levels and the precision of the method for the determination of oxyfluorfen in drinking water and surface water. The performance of the analytical method was determined with each set of samples by fortifying aliquots of appropriate control matrix with oxyfluorfen and analyzing the set following the procedures described in this report. Samples were fortified at the limit of detection (LOD) of 0.03 µg/L, the limit of quantitation (LOQ) of 0.1 µg/L, and at the higher fortification level of 1.0 µg/L (10 x LOQ). Samples fortified at the LOD were analyzed only to demonstrate that observable peaks at the LOD level could be distinguished from untreated control samples; the results were not included for average percent recovery calculations. Two unfortified control matrices and a reagent blank were also included in the analytical set.

Table 1   Identity and Structure of Oxyfluorfen and Internal Standard

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
Oxyfluorfen Stable Isotope Molecular Formula: $C_{15}H_6D_5ClF_3NO_4$ Molecular Weight: 366.73 CAS Number: Not Known	 2-chloro-1-(3-ethoxy-d5-4-nitrophenoxy)-4-(trifluoromethyl)benzene

APPENDIX 1   INSTRUMENTATION AND PARAMETERS

Independent Laboratory Validation of the Determination of Oxyfluorfen in Water

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

Laboratory Equipment

Balance, Sartorius Cubis MSU225S, Sartorius Ltd

Barnstead Smart2Pure 6UV Water Purification System, Thermo Scientific

Eppendorf Multipette Xstream®, Fisher Scientific

Pipettes, Gilson 'Microman', Anachem Ltd

Pipettes, Rainin Pos-D, Anachem Ltd

Vortex mixer, Fisher Scientific

Glassware and Materials

Glass amber scint vials, 12 mL and 20 mL, Fisher Scientific

Glass autosampler vials, 2 mL, Chromatography Direct

Chromatographic Systems

Autosampler, CTC Analytics HTS-xt PAL, Aquilant Scientific

Column, Acquity UPLC HSS T3, 50 × 2.1 mm, 1.8 µm, Part No: 186003538, Waters

Liquid chromatograph, Agilent HPLC 1290, binary pump and column oven, Agilent Technologies UK

Mass spectrometer, QTRAP 6500, electrospray ionization with TurboIonSpray probe, Applied BioSystems/MDS Sciex

Software, Analyst, version 1.6.2, Applied BioSystems/MDS Sciex

Reagents

Acetonitrile, HPLC grade, VWR

Formic Acid (≥95%), reagent grade, Sigma Aldrich

Methanol, HPLC grade, VWR

Ultrapure Water, Barnstead Smart2Pure 6UV Water Purification System, Thermo Scientific

Water, HPLC Grade, Rathburn

Preparation of Stock Solutions

1.   Approximately 10 mg (adjusted for purity) of oxyfluorfen was dissolved in approximately 10 mL of acetonitrile to obtain a 1000 µg/mL stock solution.

Preparation of Fortification Solutions

1.   1.0 mL of the oxyfluorfen stock solution was pipetted into 9.0 mL of acetonitrile to obtain a 100 µg/mL solution.

2.   0.1 mL of the 100 µg/mL oxyfluorfen solution was pipetted into 9.9 mL of acetonitrile to obtain a 1.0 µg/mL solution.

3.   1.0 mL of the 1.0 µg/mL oxyfluorfen solution was pipetted into 9.0 mL of acetonitrile to obtain a 0.1 µg/mL solution.

4.   0.1 mL of the 1.0 µg/mL oxyfluorfen solution was pipetted into 9.9 mL of acetonitrile to obtain a 0.01 µg/mL solution.

Preparation of Stable Isotope Solutions

1.   2.5 mg (adjusted for purity) of oxyfluorfen IS was dissolved in 12.5 mL of acetonitrile to obtain a 200 µg/mL stock solution.

2.   1.0 mL of the oxyfluorfen IS stock solution was pipetted into 1.0 mL of acetonitrile to obtain a 100 µg/mL solution.

3.   0.05 mL of the 100 µg/mL oxyfluorfen IS solution was pipetted into 9.95 mL of acetonitrile to obtain a 0.5 µg/mL solution.

Preparation of Intermediate Calibration Solutions

1.   1.0 mL of the 1.0 µg/mL oxyfluorfen solution was pipetted into 9 mL of acetonitrile/water (50/50 v/v), to obtain a 100 ng/mL solution.

2.   0.5 mL of the 100 ng/mL oxyfluorfen solution was pipetted into 9.5 mL of acetonitrile/water (50/50 v/v), to obtain a 5.0 ng/mL solution.

Preparation of Calibration Solutions

1.   Solvent calibration standards were prepared by diluting the intermediate calibration solutions (100 and 5 ng/mL) and stable isotope solution (0.5 µg/mL) with acetonitrile/water (50/50 v/v) to obtain calibration solutions over the concentration range of 0.015 – 5 ng/mL as described in the following table:

Concentration of Intermediate Solution (ng/mL)	Aliquot (mL)	Volume of 0.5 µg/mL ISTD (mL)	Final Volume (mL)	Calibration Solution Final Conc. (ng/mL)	Equivalent Sample Conc. (µg/mL)[a]
100	1.0	0.04	20	5.0	0.01
	0.5	0.04	20	2.5	0.005
	0.2	0.04	20	1.0	0.002
5	2.0	0.04	20	0.5	0.001
	0.2	0.04	20	0.05	0.0001
	0.03	0.02	10	0.015	0.00003

[a]The equivalent sample concentrations are based on fortifying a 5.0 mL sample before diluting to a final volume of 10 mL.

Analytical Procedure

EXTRACTION

- For control and reagent blank samples pipette 5.0 mL of water sample into a 12 mL amber vial
- For the LOD fortified control sample pipette 4.985 mL of control water into a 12 mL amber vial
- For the LOQ and 10xLOQ fortified control samples pipette 4.95 mL of control water into a 12 mL amber vial
- Fortify if necessary following table below:

Fortification Solution (µg/mL)	Volume Taken (mL)	Sample volume (mL)	Fortification Level (µg/L)
0.01	0.015	5.0	0.03
0.01	0.05	5.0	0.1
0.1	0.05	5.0	1.0

- Add 20 µL of the 0.5 µg/mL internal standard solution, cap the vial and vortex to mix
- Add 5 mL of acetonitrile, cap the vial and vortex to mix

ANALYSIS

- Transfer an aliquot of the sample into an autosampler vial
- Analyse by LC-MS
- Solvent / matrix standards used

The LOQ of the validated method is: 0.1 µg/L

<u>Instrumental Conditions</u>

<u>Instrumentation</u>

Autosampler:	Eksigent CTC Analytics HTS-xt PAL
Liquid Chromatograph:	Agilent 1290, binary pump and column oven
Mass Spectrometer:	AB Sciex QTRAP 6500
Software:	AB Sciex Analyst, version 1.6.2

<u>Typical Liquid Chromatography Operating Conditions</u>

Column:	Waters HSS T3, 2.1 x 50 mm, 1.8 μm
Column Temperature:	40ºC
Injection Volume:	50 μL
Flow Rate:	500 μL/min
Mobile Phase A:	0.01% Formic Acid (aq)
Mobile Phase B:	0.01% Formic Acid in Methanol

Time – minutes	% Mobile Phase A	% Mobile Phase B
0.50	40	60
5.00	5	95
7.00	5	95
7.01	40	60
8.00	40	60

Flow Diverter		
	Flow to waste:	0.0 min → 2.5 min
	Flow to source:	2.5 min → 4.6 min
	Flow to waste:	4.6 min → end of run

Approximate Retention Time:	Oxyfluorfen 3.5 mins

<u>Typical Mass Spectrometry Operating Conditions</u>

Ion Source:	Turbo Spray IonDrive
Polarity:	Positive
Ion Spray Voltage (IS):	5500
Collision Gas (CAD):	High
Temperature (TEM):	600
Curtain Gas (CUR):	25

Ion Source Gas 1 (GS1):   50
Ion Source Gas 2 (GS2):   60
Entrance Potential (EP):   10

Analyte	Ion Mass Transitions (*m/z*)	Dwell Time (msec)	Declustering Potential (DP)	Collision Energy (CE)	Collision Cell Exit Potential (CXP)
Oxyfluorfen	362.1/316.9	50	50	19	10
	362.1/236.9	50	50	33	10
Oxyfluorfen IS	367.1/236.9	50	50	33	10