1. EXECUTIVE SUMMARY

The objective of this study was to validate an analytical method for the determination of residues of pyraflufen-ethyl and metabolites E-1, E-2 and E-3 in two types of water (drinking and surface water) to fulfil the requirements of guidance documents OECD ENV/JM/MONO (2007)17, SANCO/825/00 rev.8.1 (2010), SANCO/3029/99 rev.4 (2000) and EPA OCSPP 850.6100 (2012).

Residues of pyraflufen-ethyl, E-2 and E-3 were extracted from water by sequential extractions with ethyl acetate. After concentration and re-constitution in water, final determination was by liquid chromatography with tandem mass spectrometry (LC-MS/MS), monitoring two ion mass transitions per analyte.

Residues of E-1 in water were analysed by direct injection with final determination by liquid chromatography with tandem mass spectrometry (LC-MS/MS), monitoring two ion mass transitions.

The method was validated in terms of linearity, specificity, LOQ, accuracy, precision, matrix effects and stability.

Matrix effects were deemed significant (>20 % suppression or enhancement) for pyraflufen-ethyl and E-3 but were insignificant for E-2 as per extraction method 1. As pyraflufen-ethyl, E-2 and E-3 were all analysed using mixed solutions containing all three analytes, matrix matched standards were used for the analyses.

Matrix effects were not deemed significant (>20 % suppression or enhancement) for E-1 as per extraction method 2. As such, solvent standards were used for the analysis of E-1 in both drinking and surface water.

Calibration curves were obtained from at least six calibration solutions. Calibration solutions covered a range from at least 30 % of the LOQ to at least 20 % above the highest concentration level, with correlation coefficients (r) all greater than 0.99, demonstrating satisfactory linearity.

Interfering peaks in control samples that eluted at the same retention time as pyraflufen-ethyl and metabolites were either non-detectable or less than 30 % of the limit of quantification (LOQ) demonstrating acceptable specificity.

2. OBJECTIVE

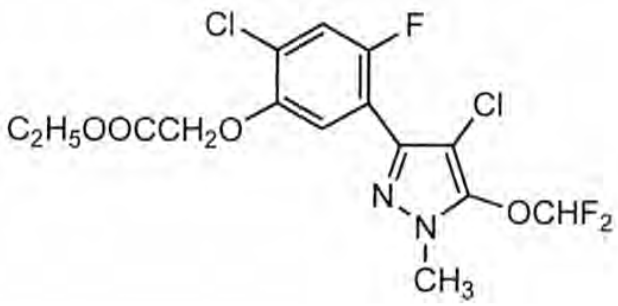
The objective of this study was to validate an analytical method for the determination of residues of pyraflufen-ethyl and metabolites E-1, E-2 and E-3 in water (drinking and surface water) to fulfil the requirements as per guidance documents OECD ENV/JM/MONO (2007)17, SANCO/825/00 rev.8.1 (2010), SANCO/3029/99 rev.4 (2000) and EPA OCSPP 850.6100 (2012).

3. TEST ITEMS

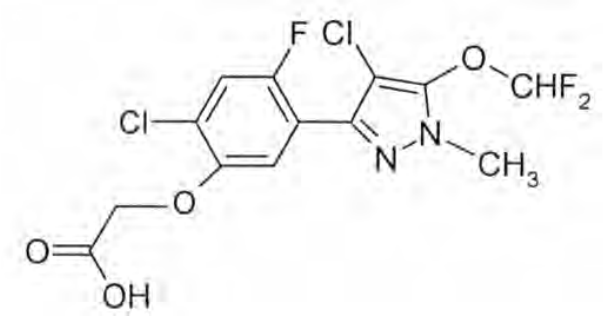
The certificates of analysis for pyraflufen-ethyl, E-1, E-2 and E-3 are presented in Appendix 5 to Appendix 8. In addition, full Q1 mass spectral and product ion scans to illustrate the ion mass transitions used for final determination are presented in Appendix 11 to Appendix 14.

3.1. Reference Items

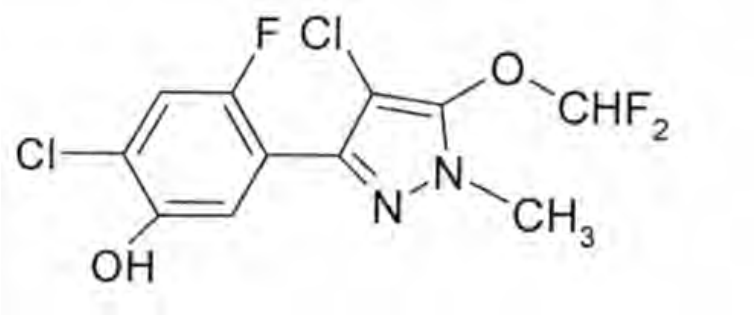
3.1.1. Pyraflufen-ethyl

Product Name:	Pyraflufen-ethyl standard
Common Name:	Pyraflufen-ethyl
Chemical Name:	Ethyl 2-chloro-5-(4-chloro-5-difluoromethoxy-1-methylpyrazol-3-yl)-4-fluorophenoxyacetate
Empirical formula	$C_{15}H_{13}Cl_2F_3N_2O_4$
Molar mass	413
Structure:	
Batch Identification:	3AM0058P
Purity:	98.6 %
Storage Conditions:	Refrigerator in dark conditions
Expiry Date:	16 February 2020

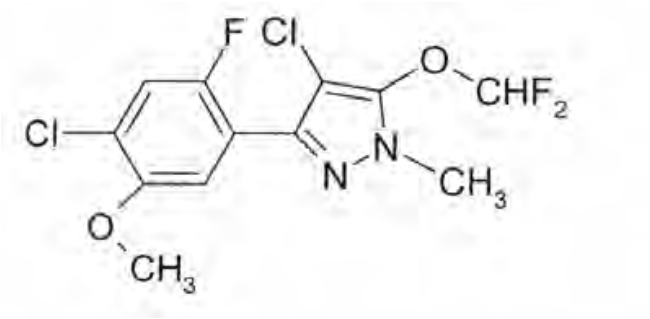
3.1.2. E-1

Product name	E-1 Standard
Chemical Name	2-chloro-5-(4-chloro-5-difluoromethoxy-1-methylpyrazol-3-yl)-4-fluorophenoxyacetic acid
Empirical formula	$C_{13}H_9Cl_2F_3N_2O_4$
Molar mass	385
Structure:	
Batch Identification:	6AM4407S
Purity:	98.6 %
Storage Conditions:	Room temperature in dark conditions
Expiry Date:	4 June 2022

3.1.3. E-2

Product name	E-2 Standard
Chemical Name	2-chloro-5-(4-chloro-5-difluoromethoxy-1-methylpyrazol-3-yl)-4-fluorophenol
Empirical formula	$C_{11}H_7Cl_2F_3N_2O_2$
Molar mass	327
Structure:	
Batch Identification:	2AM0707S
Purity:	99.8 %
Storage Conditions:	Refrigerator in dark conditions
Expiry Date:	28 February 2021

3.1.4. E-3

Product name	E-3 Standard
Chemical Name	4-chloro-3-(4-chloro-2-fluoro-5-methoxyphenyl)-5-difluoromethoxy-1-methylpyrazole
Empirical formula	$C_{12}H_9Cl_2F_3N_2O_2$
Molar mass	341
Structure:	
Batch Identification:	1AM0308S
Purity:	99.4 %
Storage Conditions:	Refrigerator and in dark conditions
Expiry Date:	29 Apr 2019

4. TEST SYSTEMS

Drinking and surface water were taken from Battelle's validation control stock samples. The drinking water was sampled from a drinking water tap at the Battelle UK Test Facility, Chelmsford, Essex, UK, CM2 5LB and was sampled on 14/01/15. The surface water was sampled from Carsington Lake – Millfields. Grid reference SK 24813 49995 and was sampled on 3/11/16.

See Appendix 9 and Appendix 10 for the characterisation reports.

5. METHOD VALIDATION

The method was validated in terms of linearity, specificity, LOQ, accuracy and precision, matrix effects and stability.

5.1. Linearity

5.1.1. Pyraflufen-ethyl, E-2 and E-3

Matrix-matched calibration solutions containing pyraflufen-ethyl, E-2 and E-3 were prepared in each water type covering a nominal range from 0.0150 to 12.5 ng/mL and calibration curves constructed after injection of the solutions.

5.1.2. E-1

Solvent calibration solutions containing E-1 were prepared in HPLC water covering a nominal range from 0.00200 to 1.50 ng/mL and calibration curves constructed after injection of the solutions.

5.2. Specificity

Specificity was determined by assessing the method's ability to differentiate between chromatographic peaks that are a result of the presence of each analyte from those chromatographic signals that generated co-extractive peaks in untreated samples that eluted at the same retention time as each analyte. A reagent blank and duplicate control samples for each water type were assessed.

5.3. Accuracy

Accuracy was assessed by conducting a series of recovery efficiency tests at the LOQ, 10xLOQ and 100 x LOQ.

5.4. Precision

The precision of the method was determined by measuring the relative standard deviation of the recovery efficiency data at each fortification level.

5.5. Matrix Effects

Matrix effects were investigated at the LOQ, 10 x LOQ and 100 x LOQ levels, by comparing peak areas of solvent standard solutions to peak areas of matrix-matched standard solutions for each water type at equivalent concentrations. Experiments assessed whether matrix effects were significant (i.e. > 20 % enhancement or suppression).

5.6. Extract Stability

All samples from the validation were stored under refrigerated conditions at a nominal temperature of 4 °C. After a minimum of 14 days storage, an LOQ recovery sample from each water type was re-vialled and injected within batches containing freshly prepared calibration standards.

5.7. Solvent Standard Stability

The stability of the solvent stock solution was assessed after storage under refrigerated conditions at a nominal temperature of 4°C. A solvent standard at 1.00 ng/mL was prepared in water from the original stock solutions after storage of 40 days. A freshly prepared solvent standard was also prepared at 1.00 ng/mL in water from newly weighed stocks. Both stored and fresh standards were injected and the areas compared.

5.8. Limit of Detection

The limit of detection (LOD) for each analyte in each standard type was defined as the lowest quantifiable calibration standard.

6. EXPERIMENTAL

6.1. Principle of the Method

6.1.1. Pyraflufen-ethyl, E-2 and E-3 (Method 1)

The method consisted of sequential extractions from water with ethyl acetate followed by concentration and re-constitution in water. Final residue determination was by LC-MS/MS monitoring two ion mass transitions per analyte.

The extraction flow chart is presented in Appendix 1.

6.1.2. E-1 (Method 2)

The method consisted of direct injection of water samples. Final residue determination was by LC-MS/MS monitoring two ion mass transitions.

The extraction flow chart is presented in Appendix 2.

6.2. Reagents and Equipment

Full details of all equipment and reagents are presented in Appendix 3 and Appendix 4.

6.3. Standards and Fortifications

6.3.1. Stock Solutions

Stock solutions were prepared by dissolving a known weight (mg) of each analyte, correcting for purity and dissolving in acetonitrile to produce a final concentration of 1000 µg/mL. Full stock solution preparation and final concentrations are detailed in the following table:

Analyte	Battelle (BUKL) Stock ID	Purity (%)	Actual Amount Weighed (mg)	Actual Volume Added (mL)	Concentration (µg/mL)
Pyraflufen-ethyl	7427	98.6 %	10.14	9.998	1000
	7443		5.15	5.077	1000
	7444		5.20	5.127	1000
	7488		5.09	5.018	1000
E-1	7428	98.6 %	10.15	10.010	1000
	7445		5.07	4.999	1000
	7446		5.20	5.127	1000
	7489		5.29	5.215	1000

Analyte	Battelle (BUKL) Stock ID	Purity (%)	Actual Amount Weighed (mg)	Actual Volume Added (mL)	Concentration (µg/mL)
E-2	7429	99.8 %	10.27	10.249	1000
	7447		5.17	5.159	1000
	7448		5.20	5.189	1000
	7490		5.32	5.309	1000
E-3	7430	99.4 %	10.32	10.258	1000
	7449		5.20	5.168	1000
	7450		5.02	4.989	1000
	7491		5.19	5.158	1000

One stock solution (per analyte) was used for matrix tests, one used for calibration standard preparation, one was used for recovery fortification preparation and one was used for stability testing.

6.3.2. Fortification Solutions

6.3.2.1. Pyraflufen-ethyl, E-2 and E-3

An intermediate solution at a concentration of 1500 ng/mL was prepared by diluting appropriate amounts of the stock solutions with acetonitrile: water (1:1 v: v). Three fortification solutions, containing pyraflufen-ethyl, E-2 and E-3 at concentrations of 0.150, 1.50 and 15.0 ng/mL, were then prepared by serial dilution of the intermediate solution with water.

All standard solutions were stored in the refrigerator when not in use.

Recovery efficiency samples were fortified as per the following tables:

Matrix	Reagent Blank Replicates	Untreated Control Replicates	Replicates at LOQ Fortification Level	Replicates at LOQ × 10 Fortification Level	Replicates at LOQ × 100 Fortification Level
Drinking Water	1	2	6 at 0.01 µg/L	6 at 0.1 µg/L	6 at 1.0 µg/L
Surface Water	1	2	6 at 0.01 µg/L	6 at 0.1 µg/L	6 at 1.0 µg/L

Matrix	Sample Volume (mL)	Fortification Standard Concentration (ng/mL)	Fortification Volume (mL)	Fortification Level (µg/L)
Drinking Water	15.0	0.150	1.0	0.01
	15.0	1.50	1.0	0.10
	15.0	15.0	1.0	1.0

Matrix	Sample Volume (mL)	Fortification Standard Concentration (ng/mL)	Fortification Volume (mL)	Fortification Level (µg/L)
Surface Water	15.0	0.150	1.0	0.01
	15.0	1.50	1.0	0.10
	15.0	15.0	1.0	1.0

6.3.2.2. E-1

An intermediate solution at a concentration of 10000 ng/mL was prepared by diluting an appropriate amount of the stock solution with acetonitrile: water (1:1 v: v). Three fortification solutions, containing E-1 at concentrations of 2.00, 20.0 and 200 ng/mL, were then prepared by serial dilution of the intermediate solution with water.

All standard solutions were stored in the refrigerator when not in use.

Recovery efficiency samples were fortified as per the following tables:

Matrix	Reagent Blank Replicates	Untreated Control Replicates	Replicates at LOQ Fortification Level	Replicates at LOQ × 10 Fortification Level	Replicates at LOQ × 100 Fortification Level
Drinking Water	1	2	6 at 0.01 µg/L	6 at 0.1 µg/L	6 at 1.0 µg/L
Surface Water	1	2	6 at 0.01 µg/L	6 at 0.1 µg/L	6 at 1.0 µg/L

Matrix	Sample Volume (mL)	Fortification Standard Concentration (ng/mL)	Fortification Volume (mL)	Fortification Level (µg/L)
Drinking Water	10.0	2.00	0.05	0.01
	10.0	20.0	0.05	0.10
	10.0	200	0.05	1.0
Surface Water	10.0	2.00	0.05	0.01
	10.0	20.0	0.05	0.10
	10.0	200	0.05	1.0

6.3.3. Calibration Solutions

6.3.3.1. Pyraflufen-ethyl, E-2 and E-3 (Matrix matched standards)

An intermediate solution containing pyraflufen-ethyl, E-2 and E-3 at a concentration of 1250 ng/mL was prepared by diluting appropriate amounts of the stock solutions with acetonitrile: water (1:1 v: v).

Solvent standards were prepared at concentrations of 3.75, 7.50, 10.0 and 12.5 ng/mL by dilution of the intermediate solution with water. The 7.50 ng/mL standard was then used to prepare solvent standards at concentrations of 0.750, 0.0750, 0.0225 and 0.0150 ng/mL by dilution with water.

To prepare matrix matched standards, one control sample per standard was extracted as per the extraction method up until the point of re-constitution. Samples were re-constituted in 2 mL of each of the eight solvent standards (0.0150 – 12.5 ng/mL) instead of pure water.

All standard solutions were stored in the refrigerator when not in use.

6.3.3.2. E-1 (Solvent standards)

An intermediate solution containing E-1 at a concentration of 1000 ng/mL was prepared by diluting an appropriate amount of the stock solution with acetonitrile: water (1:1 v: v).

A secondary intermediate solution was prepared at 10.0 ng/mL by dilution of the 1000 ng/mL solution with water.

Solvent standards were prepared in the range of 0.0500 ng/mL to 1.50 ng/mL by dilution of the 10.0 ng/mL solution with water. A 1.00 ng/mL solvent standard prepared as part of the calibration range was then used to prepare standards in the range of 0.00200 ng/mL to 0.0100 ng/mL by dilution with water.

All standard solutions were stored in the refrigerator when not in use.

6.4. Extraction Procedure

6.4.1. Pyraflufen-ethyl, E-2 and E-3

Aliquots of 15 mL of water were placed into glass vials. For recovery efficiency tests, the control matrices were fortified with the appropriate spiking solutions at the LOQ, 10 x LOQ and 100 x LOQ levels.

For all samples, 5 mL of ethyl acetate was added and the samples shaken vigorously for 30 seconds. After the layers partitioned, the top ethyl acetate layer was removed and placed into a separate glass vial. The partition was repeated two further times and all ethyl acetate layers were combined. The ethyl acetate was then dried under a stream of nitrogen with the samples in a heating block set to 40 °C. After drying, the samples were reconstituted in 2 mL water with the aid of the sonicator and vortex mixer.

An aliquot was transferred to an HPLC vial and final residue levels were determined by LC-MS/MS.

The extraction method is presented in the form of a flow chart in Appendix 1.

6.4.2. E-1

Aliquots of 10 mL of water were placed into glass vials. For recovery efficiency tests, the control matrices were fortified with the appropriate spiking solutions at the LOQ, 10 x LOQ and 100 x LOQ levels.

All samples were shaken to mix and an aliquot transferred to an HPLC vial. Final residue levels were determined by LC-MS/MS

6.5. LC-MS/MS Analysis

All samples were analysed by liquid chromatography coupled with a tandem mass spectrometer (LC-MS/MS), monitoring two ion mass transitions for each analyte.

A summary of these conditions is presented below:

6.5.1. LC-MS/MS Conditions (XG17007 – All Analytes)

HPLC Conditions			
Columns	Zorbax SB-C3, 150 x 3.0 mm, 5.0 µm particle size		
Column Oven Temperature	20 °C		
Mobile Phase A	0.1 % Formic acid in water		
Mobile Phase B	0.1 % Formic acid in acetonitrile		
Method	Time	% A	% B
	0.0	90	10
	4.0	5	95
	5.0	5	95
	5.1	90	10
	6.5	90	10
Flow Rate	1.0 mL/min		
Injection Volume	95 µL		
Mass Spectrometer and General Instrument Conditions			
Instruments	API 6500 Triple Quadrupole Mass Spectrometer fitted with Turbo ion spray ion source		
Ion Source	Positive Mode (Pyraflufen-ethyl, E-2 and E-3) Negative Mode (E-1)		
Run Time	6.5 minutes Approximate retention times: Pyraflufen-ethyl = 3.9 – 4.1 minutes E-1 = 3.4 – 3.6 minutes E-2 = 3.5 – 3.6 minutes E-3 = 3.7 – 4.0 minutes		

Analyte	Dwell Time (msec)	Transition (m/z)	Declustering Potential	Collision Energy	Cell Exit Potential
Pyraflufen-ethyl	25	413/339	16	25	34
	25	413/289	16	37	32
E-1	100	383/274	-60	-40	-5
	100	385/276	-30	-50	-11
E-2	25	327/277	40	30	15
	25	329/279	40	30	20
E-3	25	341/291	101	31	34
	25	341/276	101	45	18
Curtain Gas	20				
CAD Gas	-1				
Gas 1	40				
Gas 2	Positive Mode: 40 Negative Mode: 50				
Spray Voltage	Positive Mode: 4000 V Negative Mode: -4500 V				
Source Temperature	700 °C				
Entrance Potential	Positive mode: 10 Negative Mode: -10				
Notes:	Method used to generate all data for pyraflufen-ethyl, E-2 and E-3 Method also used to generate stock stability data for E-1				

6.5.2. LC-MS/MS Conditions (E-1 only)

HPLC Conditions					
Columns	Zorbax SB-C3, 150 x 3.0 mm, 5.0 µm particle size				
Column Oven Temperature	20 °C				
Mobile Phase A	0.1 % Formic acid in water				
Mobile Phase B	0.1 % Formic acid in acetonitrile				
Method	Time	% A	% B		
	0.0	90	10		
	4.0	5	95		
	5.0	5	95		
	5.1	90	10		
	6.5	90	10		
Flow Rate	1.0 mL/min				
Injection Volume	95 µL				
Mass Spectrometer and General Instrument Conditions					
Instruments	API 6500 Triple Quadrupole Mass Spectrometer fitted with Turbo ion spray ion source				
Ion Source	Negative Mode				
Run Time	6.5 minutes Approximate retention time: E-1 = 3.4 – 3.6 minutes				
Analyte	Dwell Time (msec)	Transition (m/z)	Declustering Potential	Collision Energy	Cell Exit Potential
E-1	100	383/274	-60	-40	-5
	100	385/276	-30	-50	-11
Curtain Gas	20				
CAD Gas	-1				
Gas 1	40				
Gas 2	50				
Spray Voltage	-4500 V				
Source Temperature	700 °C				
Entrance Potential	-10				
Notes:	Method used to generate all data for E-1 with the exception of stock stability data				

6.6. Time Management

6.6.1. Pyraflufen-ethyl, E-2 and E-3

Pyraflufen-ethyl, E-2 and E-3 can be extracted and analysed together. One batch consisting of 21 samples (18 recoveries, 1 reagent blank and 2 control samples) and 8 calibration standards along with analysis on the LC-MS/MS (3.5 hours) and processing of data can be carried out in 11 hours.

6.6.2. E-1

E-1 is extracted by a separate extraction method. One batch consisting of 21 samples (18 recoveries, 1 reagent blank and 2 control samples) and 8 calibration standards along with analysis on the LC-MS/MS (3.5 hours) and processing of data can be carried out in 7.5 hours.

7. CALIBRATION AND CALCULATION

Matrix matched calibration solutions containing pyraflufen-ethyl, E-2 and E-3 were prepared in the nominal concentration range of 0.0150 – 12.5ng/mL.

Solvent calibration solutions containing E-1 were prepared in the nominal concentration range of 0.00200 – 1.50 ng/mL.

A multi-point calibration curve was obtained from injections of calibration solutions by plotting peak areas versus the concentration in ng/mL. The curve was calculated by the method of least squares linear regression. A weighting factor of 1/x or $1/x^2$ was applied to each curve to improve the accuracy. Correlation coefficients (r) were all greater than 0.99.

The quantification of the analyte in the samples was made by comparison to the calibration curve of the form $y = mx + c$. The amount of analyte in a given sample was calculated as follows:

Pyraflufen-ethyl, E-2 and E-3

$$\text{Compound [µg/L]} = \frac{(A - C) \times V_2}{M \times V_1}$$

Where:

A	=	Area of analyte peak
M	=	slope of the calibration curve
C	=	intercept of the calibration curve
V_1	=	initial volume of sample (mL)
V_2	=	final volume of sample (mL)

E-1

$$\text{Compound [µg/L]} = \frac{(A - C) \times V_2}{M \times V_1}$$

Where:

A	=	Area of analyte peak
M	=	slope of the calibration curve
C	=	intercept of the calibration curve
V_1	=	initial volume of sample (mL)
V_2	=	sample volume after fortification (mL)

The recovery efficiency in the fortified samples was calculated as follows:

$$\text{Recovery efficiency [\%]} = \frac{\text{Amount found (µg/L)}}{\text{Amount spiked (µg/L)}} \times 100$$

Example LC-MS/MS chromatograms of calibration solutions, control and fortified samples are presented in Figure 1 to Figure 16 with example calibration curves presented in Figure 17 to Figure 24. Response factors for each analyte, each water type and for solvent standards are presented in Figure 25 to Figure 31.

Table 6: Mass Fractions

Calibration Standards Corresponding Mass Fraction in Sample (Drinking and Surface Water) for Pyraflufen-ethyl, E-2 and E-3

Nominal Calibration Standard Concentration [ng/mL]	Nominal Corresponding Mass Fraction in Original Sample [µg/L]
12.5	1.67
10.0	1.33
7.50	1.00
3.75	0.500
0.750	0.100
0.0750	0.0100
0.0225	0.00300
0.0150	0.00200

$$\text{Mass fraction } (\mu g/L) = (C \times V_2) / V_1$$

C = concentration (ng/mL)

V_1 = initial volume of sample (mL) 15

V_2 = final volume of sample (mL) 2

Table 6: Mass Fractions (continued)

Calibration Standards Corresponding Mass Fraction in Sample (Solvent Standards in Water) for E-1

Nominal Calibration Standard Concentration [ng/mL]	Nominal Corresponding Mass Fraction in Original Sample [µg/L]
1.50	1.51
1.20	1.21
1.00	1.01
0.100	0.101
0.0500	0.0503
0.0100	0.0101
0.00300	0.00302
0.00200	0.00201

* 10.05 mL for recovery samples, 10.0 mL for control/blank samples. The mass fractions displayed above will be based on 10.05 mL for recovery samples

$$\text{Mass fraction } (\mu g/L) = (C \times V_2) / V_1$$

C = concentration (ng/mL)

V_1 = initial volume of sample (mL) 10.00

V_2 = volume of sample after fortification (mL) 10.05*

13. APPENDICES

Appendix 1: Method 1 – Pyraflufen-ethyl, E-2 and E-3 in Drinking and Surface Water

EXTRACTION

- Aliquot 15 mL sample into a glass vial
- Fortify if necessary
- Add 5 mL ethyl acetate and shake vigorously for 30 seconds
- Leave sample to partition into two layers
- Remove the top ethyl acetate layer and place in a separate glass vial
- Repeat partitioning twice more with another 5 mL ethyl acetate, combining all ethyl acetate layers together
- Dry ethyl acetate under a stream of nitrogen in a heating block set to 40 °C
- Re-constitute sample in 2 mL water with the aid of the sonicator and vortex mixer

ANALYSIS

- Transfer an aliquot of the sample into a clean clear screw cap vial
- Analyse by LC-MS/MS
- Matrix Standards Used

The LOQ of the validated method was: 0.01 µg/L

Note: Surface water sampled from Carsington Lake – Millfields. Grid reference SK 24813 49995. Sampled on 3/11/16.

Appendix 2: Method 2 – E-1 in Drinking and Surface Water

EXTRACTION

- Aliquot 10 mL sample into a glass vial
- Fortify if necessary
- Shake sample to mix

ANALYSIS

- Transfer an aliquot of the sample into a clean clear screw cap vial
- Analyse by LC-MS/MS
- Solvent Standards Used

The LOQ of the validated method was: 0.01 µg/L

Note: Surface water sampled from Carsington Lake – Millfields. Grid reference SK 24813 49995. Sampled on 3/11/16.

Appendix 3: Equipment List

EQUIPMENT	MANUFACTURER / SUPPLIER
Sample Extraction	
VWR Ultrasonic Cleaner	VWR international Ltd (BDH, MERCK Eurolab) Hunter Boulevard Magna Park Lutterworth, LEICS, LE17 4XN, UK
General Laboratory Equipment	
Dispensers: - Eppendorf Multipette Xstream®	Fisher Scientific UK Ltd., Bishop Meadow Road, Loughborough, Leicestershire, LE11 5RG
Balances: - Sartorius MSU225S Cubis 220-5 (Balance 18)	Sartorius Ltd. Longmead Business Centre, Blenheim Road, Epsom, Surrey, UK
VWR VV3 Vortex mixer	VWR international Ltd (BDH, MERCK Eurolab) Hunter Boulevard Magna Park Lutterworth, LEICS, LE17 4XN, UK
Barnstead™ Smart2Pure™ 6UV Water Purification System	Thermo Scientific Supplied by Fisher Scientific UK Ltd. Bishop Meadow Road, Loughborough, Leicestershire, UK
Sample Concentration	
Techne Sample Concentrator Dri-Block® DB-3D	Bibby Scientific Ltd. Stone, Staffordshire, ST15 0SA, UK
LC-MS/MS	
MDS Sciex API 6500 Analyst 1.6.2 Software Agilent HPLC 1290 Series Eksigent (Presearch) HTS-xt Autosampler	Applied Biosystems Lingley House 120 Birchwood Boulevard, Warrington, WA3 7QH, UK Agilent Technologies UK Ltd, Lakeside, Cheadle Royal Business Park, Stockport, Cheshire, SK8 3GR, UK Presearch Ltd., System House, 59-61 Knowl Piece, Hitchin, Herts, SG4 OTY, UK
Nitrogen Generator	Peak Scientific Instruments Ltd. Fountain Crescent Inchinnan Business Park Renfrewshire, PA4 9RE, UK
HPC Air Generator	CPC Ltd. Chelmer House, Bellcroft Eastways Industrial Estate, Witham, CM8 3YU, UK

EQUIPMENT	MANUFACTURER / SUPPLIER
LC Columns	
Agilent (Zorbax)	Agilent Technologies UK Ltd, Lakeside, Cheadle Royal Business Park, Stockport, Cheshire, SK8 3GR, UK

Appendix 4: Reagents

Chemical	Supplier
Acetonitrile HPLC grade	Fisher Scientific (Acros Organics, Fisons) Fisher Scientific UK Ltd Bishop Meadow Road Loughborough Leicestershire LE11 5RG
Ethyl Acetate AnalaR NORMAPUR Formic acid 99-100%	VWR international Ltd (BDH, MERCK Eurolab) Hunter Boulevard Magna Park Lutterworth LEICS LE17 4XN
HPLC grade water	Rathburn Chemicals Ltd. Walkerburn, Scotland
Smart2pure Water	Using Barnstead™ Smart2Pure™ 6UV Water Purification System at Battelle UK Ltd.