1 Summary

The objective of this study was to perform an independent laboratory validation of the methodology described in study report XG/17/007 (reference 1) 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).

For pyraflufen-ethyl, E-2 and E-3 the analytical method involved sequential extractions from water with ethyl acetate. After concentration and re-constitution in acetonitrile/water (10/90, v/v), final determination was by liquid chromatography with tandem mass spectrometry (LC-MS/MS), monitoring two ion mass transitions per analyte. The method is detailed in Section 4.

For E-1 residues were analysed by direct injection with final determination by LC-MS/MS, monitoring two ion mass transitions per analyte. The method is detailed in Section 5.

The method validation for each water type included a solvent blank (to assess carryover), a reagent blank, two control samples and six fortified samples at the limit of quantification (LOQ, 0.01 µg/L), ten times the LOQ and a hundred times the LOQ for each analyte.

The selectivity of the method was demonstrated as no matrix interferences or residues of pyraflufen-ethyl, E-1, E-2 or E-3 were observed at or above 30% of the LOQ in the reagent blank sample and the control samples. Sample carryover was less than 1%.

For E-1, E-2 and E-3 the LC-MS/MS response was shown to be linear over a concentration range equivalent to at least 30% of the LOQ up to at least 120% of the upper fortification level. For pyraflufen-ethyl the LC-MS/MS response was shown to be quadratic over a concentration range equivalent to at least 30% of the LOQ up to at least 120% of the upper fortification level.

Matrix effects were not deemed to be significant (< 20 %) for all analytes. Matrix matched standards were used for residue quantitation of pyraflufen-ethyl, E-2 and E-3 for consistency with the original method. Solvent calibration standards were used for residue quantitation of E-1. Results for matrix effects testing are presented in Tables 1 to 16.

The accuracy and precision of the method was successfully demonstrated for the primary and confirmatory ion mass transitions for each analyte, as the mean recovery values fell within the accepted range i.e. the mean recovery at 0.01 µg/L, 0.1 µg/L and 1 µg/L was 70 – 110 % with a relative standard deviation of ≤ 20%. Data is presented in Tables 17 to 32.

The limit of detection was confirmed to be less than 30 % of the LOQ as demonstrated by the response of the bottom calibration standard (equivalent to 30 % of the LOQ) which was greater than three times the signal to noise for each analyte and for each mass transition.

Extract stability was assessed for both water types by re-injection of the LOQ recoveries using freshly prepared calibration standards after 7 days refrigerated (2°C to 8°C) storage. The mean recovery values for pyraflufen-ethyl, E-1, E-2 and E-3 were in the acceptable range of 70 – 110% with an RSD of less than 20%. Final extracts were therefore shown to be stable for 7 days when stored refrigerated (2°C to 8°C). Data is presented in Tables 33 to 40.

Pyraflufen-ethyl, E-2 and E-3 mixed standard solutions prepared in water were shown to be stable for the duration of the experimental phase (21 days) when stored refrigerated (2°C to 8°C). The peak area of a freshly prepared standard (mean of three injections) was compared to the peak area of a stored standard of equivalent concentration (mean of three injections). The difference between the two was ≤ 10%.

E-1 standard solutions prepared in water were shown to be stable for the duration of the experimental phase (26 days) when stored refrigerated (2°C to 8°C). The peak area of a freshly prepared standard (mean of three injections) was compared to the peak area of a stored standard of equivalent concentration (mean of three injections). The difference between the two was ≤ 10%. This was done in duplicate as stability was initially assessed after 21 days refrigerated (2°C to

8°C) storage and the stored standard was shown to be > 10% (18.2%) of the new standard. The initial result was considered to be an anomaly. Standard stability data is presented in Tables 41 to 44.

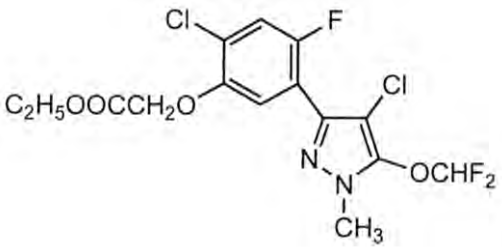
The methodology described in validation study report XG/17/007 was found to be valid for the determination of pyraflufen-ethyl, E-2 and E-3 residues in water at the second attempt with some minor modifications. After consultation with the sponsor, the validation batches were reconstituted using acetonitrile/water (10/90, v/v) in order to improve recovery levels. For pyraflufen-ethyl the detector response did not meet the acceptance criteria (r value was less than 0.995) using linear regression with 1/x weighting or linear regression with $1/x^2$ weighting which is described in method XG/17/007 (section 7). Quadratic regression with 1/x weighting was therefore used.

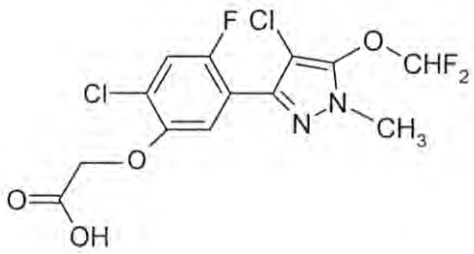
An LOQ of 0.01 µg/L was achieved for pyraflufen-ethyl, E-2 and E-3, monitoring two ion mass transitions per analyte. The independent laboratory validation met the criteria detailed in SANCO/825/00 rev. 8.1. (2010) and EPA OCSPP 850.6100 (2012) at the second attempt incorporating the minor modifications listed above.

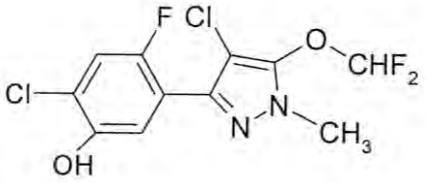
The methodology described in validation study report XG/17/007 was found to be valid for the determination of E-1 residues in water with an LOQ of 0.01 µg/L, monitoring two ion mass transitions. The independent laboratory validation met the criteria detailed in SANCO/825/00 rev. 8.1. (2010) and EPA OCSPP 850.6100 (2012) at the first attempt.

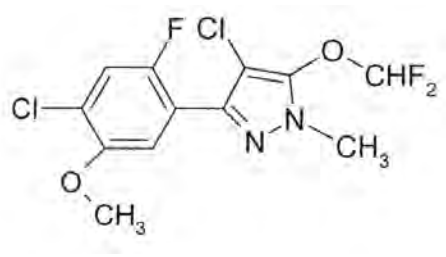
2 Reference Item Details

The following reference items were used in the study. The certificates of analysis are shown in Appendix A.

Common name	Pyraflufen-ethyl
IUPAC 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	413.2 g/mol
Structure	
ResChem Lot. No.	RAL 019/2016
Batch Identification	3AM0058P
Purity	98.6%
Expiry date	16 February 2020

Common name	Pyraflufen-ethyl metabolite E-1
IUPAC 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	385.1 g/mol
Structure	
ResChem Lot. No.	RAL 020/2016
Batch Identification	6AM4407S
Purity	98.6%
Expiry date	04 June 2022

Common name	Pyraflufen-ethyl metabolite E-2
IUPAC 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	327.1 g/mol
Structure	
ResChem Lot. No.	RAL 028/2017
Batch Identification	2AM0707S
Purity	99.8%
Expiry date	28 February 2021

Common name	Pyraflufen-ethyl metabolite E-3
IUPAC 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	341.1 g/mol
Structure	
ResChem Lot. No.	RAL 022/2016
Batch Identification	1AM0308S
Purity	99.4%
Expiry date	29 April 2019

3 Test System Details

3.1 Specimen Origin

Drinking water was sampled from a drinking water tap at the Derwent Business Centre, Clarke Street, Derby, UK, DE21 2BU. Grid reference SK358369. Surface water was sampled from a lake at Attenborough Nature Reserve, Nottingham, UK, NG9 6DY. Grid reference SK515339.

Water characterisation reports can be found in Appendix B.

4 Analytical Method – Pyraflufen-Ethyl, E-2 and E-3

The analytical method involved sequential extractions from water with ethyl acetate. After concentration and re-constitution in acetonitrile/water (10/90, v/v), final determination was by liquid chromatography with tandem mass spectrometry (LC-MS/MS), monitoring two ion mass transitions per analyte.

4.1 Reagents and Materials Used

Reagent	Description	Supplier
Acetonitrile	HPLC Grade	Rathburns
Deionised Water	HPLC Grade	Rathburns
Ethyl Acetate	Pesticide Grade	Rathburns
Formic Acid	LC-MS Grade	Fisher Scientific
Deionised Water	LC-MS Grade	Romil
Acetonitrile	LC-MS Grade	Romil

4.2 Equipment and Apparatus used

Item	Description
Laboratory Balance's	A&D GR-202, UWE HGS-300
Ultrasonic Bath	GT Sonic, 10 L Capacity
Vortex mixer	Fisions, Whirlimixer
Adjustable Pipettes	Gilson P100, P200, P1000, P10mL
Sample Concentrator	Techne Dri-Block® DB-3A
General Laboratory Supplies	Volumetric Flasks, Pipette's, Beakers, Autosampler Vials, Centrifuge tubes, Measuring Cylinders, HDPE bottles (150 mL) etc.
LC-MS/MS	AB Sciex 5500 Mass Spectrometer with an Agilent 1260 Binary HPLC Pump, Agilent 1260 Degasser, CTC Analytics HTC PAL Autosampler, Agilent 1260 Column Oven and a Peak Scientific ABN2ZA Gas Generator
HPLC Column	Zorbax SB-C3, 150 x 3.0 mm, 5.0 µm Particle Size, Agilent

4.3 Standard Preparation

Individual pyraflufen-ethyl, E-2 and E-3 stock solutions were prepared in acetonitrile with the aid of an ultrasonic bath, by dissolving 10 mg in 10 mL of solvent. The standards were allocated unique reference numbers i.e. PE1-3/7/17, E2-3/7/17 and E3-3/7/17.

The stock solutions were combined and further diluted for use as fortification standards in the procedural recovery process and for calibration standards.

4.3.1 Preparation of Fortification Solutions

Fortification standard solutions were prepared by serial dilution of the stock solutions using acetonitrile/water (50/50, v/v) as listed below.

Standard Ref.	Standard Conc. (µg/mL)	Volume Used (mL)	Final Vol. (mL)	Final Conc. (µg/mL)	Standard Ref.
PE1-3/7/17 E2-3/7/17 E3-3/7/17	1000 1000 1000	0.075 0.075 0.075	50	1.5	MIX1-13/7/17
MIX1-13/7/17	1.5	0.2	20	0.015	MIX2-13/7/17
MIX2-13/7/17	0.015	2.0	20	0.0015	MIX3-13/7/17
MIX3-13/7/17	0.0015	2.0	20	0.00015	MIX4-13/7/17

4.3.2 Preparation of Solvent Calibration Standard Solutions

Dilutions were performed using acetonitrile/water (10/90, v/v) as listed below;

Standard Ref.	Standard Conc. (ng/mL)	Volume Used (mL)	Final Vol. (mL)	Final Conc. (ng/mL)	Standard Ref.
MIX1-13/7/17	1500	0.5	6.0	125	MIX5-13/7/17
MIX5-13/7/17	125	1.0	10	12.5	MIX6-13/7/17
MIX5-13/7/17	125	0.8	10	10	MIX7-13/7/17
MIX5-13/7/17	125	0.6	10	7.5	MIX8-13/7/17
MIX5-13/7/17	125	0.3	10	3.75	MIX9-13/7/17
MIX8-13/7/17	7.5	1.0	10	0.75	MIX10-13/7/17
MIX10-13/7/17	0.75	1.0	10	0.075	MIX11-13/7/17
MIX10-13/7/17	0.75	0.3	10	0.0225	MIX12-13/7/17

4.3.3 Preparation of Matrix-Matched Calibration Standard Solutions

Seven additional control samples of each type of water were taken through the method, after the concentration step they were re-constituted in 2 mL of the appropriate standard as listed below:

Drinking Water

Standard Ref.	Standard Conc. (ng/mL)	Volume Used (mL)	Final Vol. (mL)	Final Conc. (ng/mL)	Standard Ref.
MIX12-13/7/17	0.0225	2	2	0.0225	DMTX1-14/7/17
MIX11-13/7/17	0.075	2	2	0.075	DMTX2-14/7/17
MIX10-13/7/17	0.75	2	2	0.75	DMTX3-14/7/17
MIX9-13/7/17	3.75	2	2	3.75	DMTX4-14/7/17
MIX8-13/7/17	7.5	2	2	7.5	DMTX5-14/7/17
MIX7-13/7/17	10	2	2	10	DMTX6-14/7/17
MIX6-13/7/17	12.5	2	2	12.5	DMTX7-14/7/17

Surface Water

Standard Ref.	Standard Conc. (ng/mL)	Volume Used (mL)	Final Vol. (mL)	Final Conc. (ng/mL)	Standard Ref.
MIX12-13/7/17	0.0225	2	2	0.0225	SMTX1-14/7/17
MIX11-13/7/17	0.075	2	2	0.075	SMTX2-14/7/17
MIX10-13/7/17	0.75	2	2	0.75	SMTX3-14/7/17
MIX9-13/7/17	3.75	2	2	3.75	SMTX4-14/7/17
MIX8-13/7/17	7.5	2	2	7.5	SMTX5-14/7/17
MIX7-13/7/17	10	2	2	10	SMTX6-14/7/17
MIX6-13/7/17	12.5	2	2	12.5	SMTX7-14/7/17

For extract stability and standard stability testing, solvent calibration standards and matrix matched calibration standards were freshly prepared on the day of testing using a similar dilution scheme as above.

4.4 Extraction

1. An aliquot of water (15 mL) was dispensed into a glass vial (28 mL capacity).
2. Procedural recoveries were prepared by fortifying aliquots of untreated water with the appropriate mixed analyte fortification solution as detailed in the table below.

Sample Volume (mL)	Standard Reference	Standard Conc. (µg/mL)	Volume added (mL)	Fortification Level (µg/L)
15.0	MIX4-13/7/17	0.00015	1.0	0.01
15.0	MIX3-13/7/17	0.0015	1.0	0.1
15.0	MIX2-13/7/17	0.015	1.0	1.0

3. Ethyl acetate (5 mL) was added and the sample shaken vigorously for 30 seconds.
4. After the layers partitioned, the top ethyl acetate layer was removed and placed into a separate glass vial (28 mL capacity).
5. The partition was repeated two further times and all ethyl acetate layers were combined.
6. The ethyl acetate extract was dried under a stream of air using a sample concentrator with the heated block set to 40 °C.
7. After drying, the samples were reconstituted in acetonitrile/water (10/90, v/v) (2 mL) with the aid of an ultrasonic bath and vortex mixer.
8. An aliquot was transferred to an HPLC vial prior to quantitation of pyraflufen-ethyl, E-2 and E-3 residues by LC-MS/MS.

4.5 LC-MS/MS Conditions

An AB Sciex 5500 Mass Spectrometer with an Agilent 1260 Binary HPLC Pump, Agilent 1260 Degasser, CTC Analytics HTC PAL Autosampler, Agilent 1260 Column Oven and a Peak Scientific ABN2ZA Gas Generator was used for quantitation.

4.5.1 Chromatography Parameters for Pyraflufen-ethyl, E-2 and E-3

Parameter	Description		
HPLC Column	Zorbax SB-C3, 150 x 3.0 mm, 5.0 µm Particle Size		
Column Temperature	50 °C		
Injection Volume	60 µL		
Retention Times (approximate)	Pyraflufen-ethyl - 3.7 minutes E-2 – 3.2 minutes E-3 – 3.6 minutes		
Mobile Phase	A: 0.1% Formic Acid in Water B: 0.1% Formic Acid in Acetonitrile		
Flow Rate	1.0 mL/min		
Gradient	Time (minutes)	A (%)	B (%)
	0.0	80	20
	4.0	5	95
	5.0	5	95
	5.1	80	20
	6.5	80	20

4.5.2 Mass Spectrometry Parameters for Pyraflufen-ethyl, E-2 and E-3

Parameter	Description				
Ionisation Mode	Turbospray (Electrospray)				
Probe Position	5 Horizontal, 5 Vertical				
Polarity	Positive				
Curtain Gas	45				
CAD Gas	8				
Gas 1	45				
Gas 2	50				
Source Temperature	700 °C				
Spray Voltage	5500				
Entrance Potential	10				
Mass Transitions	Ions monitored (*m/z*)	Declustering Potential	Collision Energy	Cell Exit Potential	Primary / Confirmatory
Pyraflufen-ethyl	413 → 339	150	27	14	Primary
	413 → 289	150	40	14	Confirmatory
E-2	327 → 277	70	33	10	Primary
	329 → 279	70	33	10	Confirmatory
E-3	341 → 291	100	34	10	Primary
	341 → 276	100	45	10	Confirmatory

4.6 Quantitation

The quantitative determination of all analytical samples was carried out by external standardisation using calibration standards in matrix. Injections of calibration standards were interspersed throughout the sequence between injections of samples (not greater than 4).

Detector linearity was assessed by constructing a calibration curve of peak area versus analyte concentration. Quadratic regression with 1/x weighting at 7 different concentrations ranging from 0.0225 ng/mL to 12.5 ng/mL was used for pyraflufen-ethyl. Linear regression with 1/x weighting at 7 different concentrations ranging from 0.0225 ng/mL to 12.5 ng/mL was used for E-2 and E-3. Correlation co-efficients (r) above 0.995 were obtained which meets the criteria of ResChem Analytical standard operating procedures.

Residues (R) in µg/L were calculated according to the following equation:

$R = (C_{END} \times FV) / IV$

where:

R = Analyte residue, µg/L

C_{END} = Final concentration of analyte in sample extract in ng/mL (calculated by Analyst software version 1.6.2)

FV = Final extract volume (2 mL)

IV = Initial extract volume (15 mL)

Percent recovery from fortified samples was calculated as described below:

$$\text{Recovery (\%)} = \frac{(R_{fortified})}{F} \times 100$$

where:

$R_{fortified}$ = Residue determined in fortified sample (µg/L)

F = Fortification rate (µg/L)

4.6.1 Example Calculation

For a 0.01 µg/L fortified drinking water sample (sample no. 4748) the concentration of pyraflufen-ethyl found was calculated as follows:

R = (0.0725403 ng/mL x 2 mL) / 15 mL
= 0.0096720 µg/L

The percent recovery found was calculated as follows:

Recovery (%) = (0.0096720 µg/L / 0.01 µg/L) x 100
= 96.7 %

4.7 Method Flow Chart

Transfer an aliquot of water (15 mL) to a glass vial (28 mL capacity).

↓

Accurately fortify if necessary.

↓

Add ethyl acetate (5 mL), cap and shake vigorously for 30 seconds

↓

Transfer the ethyl acetate layer to a separate glass vial (28 mL capacity).

↓

Repeat the partition two more times and combine the ethyl acetate extracts

↓

Dry the ethyl acetate extract under a stream of air using a sample concentrator with the heated block set to 40 °C

↓

Reconstitute the dried extract in acetonitrile/water (10/90, v/v) (2 mL) with the aid of an ultrasonic bath and vortex mixer.

↓

Transfer an aliquot to an HPLC vial prior to quantitation by LC-MS/MS

4.8 Time Management

Preparation of standards and extraction of two sample sets can be conducted in 1 working day (7.5 hours) by two analysts. Analysis of samples by LC-MS/MS can be conducted over approximately 12 hours.

Therefore, two sample sets, each consisting of 21 samples (including 18 recoveries, 1 reagent blank, 2 control samples) and 8 calibration standards, can be prepared, extracted and analysed within approximately 1 day by two analysts i.e. extraction starts at beginning of day 1, submitted for quantitation at end of day 1 and quantitation completed by start of day 2.

5 Analytical Method – E-1

E-1 residues were analysed by direct injection with final determination by LC-MS/MS, monitoring two ion mass transitions per analyte.

5.1 Reagents and Materials Used

Reagent	Description	Supplier
Acetonitrile	HPLC Grade	Rathburns
Deionised Water	HPLC Grade	Rathburns
Formic Acid	LC-MS Grade	Fisher Scientific
Deionised Water	LC-MS Grade	Romil
Acetonitrile	LC-MS Grade	Romil

5.2 Equipment and Apparatus used

Item	Description
Laboratory Balance's	A&D GR-202, UWE HGS-300
Ultrasonic Bath	GT Sonic, 10 L Capacity
Adjustable Pipettes	Gilson P100, P200, P1000, P10mL
General Laboratory Supplies	Volumetric Flasks, Pipette's, Beakers, Autosampler Vials, Centrifuge tubes, Measuring Cylinders, HDPE bottles (150 mL) etc.
LC-MS/MS	AB Sciex 5500 Mass Spectrometer with an Agilent 1260 Binary HPLC Pump, Agilent 1260 Degasser, CTC Analytics HTC PAL Autosampler, Agilent 1260 Column Oven and a Peak Scientific ABN2ZA Gas Generator
HPLC Column	Zorbax SB-C3, 150 x 3.0 mm, 5.0 µm Particle Size, Agilent

5.3 Standard Preparation

An E-1 stock solution was prepared in acetonitrile with the aid of an ultrasonic bath, by dissolving 10 mg in 10 mL of solvent. The standard was allocated a unique reference numbers i.e. E1-3/7/17.

The stock solution was further diluted for use as fortification standards in the procedural recovery process and for the preparation of calibration standards.

5.3.1 Preparation of Fortification Solutions

Fortification standard solutions were prepared by serial dilution of the stock solution using acetonitrile/water (50/50, v/v) as listed below.

Standard Ref.	Standard Conc. (µg/mL)	Volume Used (mL)	Final Vol. (mL)	Final Conc. (µg/mL)	Standard Ref.
E1-3/7/17	1000	0.1	10	10	E1A-3/7/17
E1A-3/7/17	10	0.2	10	0.2	E1B-3/7/17
E1B-3/7/17	0.2	1.0	10	0.02	E1C-3/7/17
E1C-3/7/17	0.02	1.0	10	0.002	E1D-3/7/17

5.3.2 Preparation of Solvent Calibration Standard Solutions

Dilutions were performed using deionised water as listed below:

Standard Ref.	Standard Conc. (ng/mL)	Volume Used (mL)	Final Vol. (mL)	Final Conc. (ng/mL)	Standard Ref.
E1B-3/7/17	200	0.075	10	1.5	E1E-3/7/17
E1B-3/7/17	200	0.0625	10	1.25	E1F-3/7/17
E1B-3/7/17	200	0.05	10	1.0	E1G-3/7/17
E1E-3/7/17	1.5	2.0	10	0.3	E1H-3/7/17
E1G-3/7/17	1.0	1.0	10	0.1	E1I-3/7/17
E1G-3/7/17	1.0	0.5	10	0.05	E1J-3/7/17
E1I-3/7/17	0.1	1.0	10	0.01	E1K-3/7/17
E1K-3/7/17	0.01	3.0	10	0.003	E1L-3/7/17

For extract stability and standard stability testing, solvent calibration standards were freshly prepared on the day of testing using a similar dilution scheme as above.

5.3.3 Preparation of Solvent Standard Solutions equivalent to the LOQ

A standard solution was prepared for assessment of matrix effects using drinking water as the dilution solvent as listed below:

Standard Ref.	Standard Conc. (ng/mL)	Volume Used (mL)	Final Vol. (mL)	Final Conc. (ng/mL)	Standard Ref.
E1D-3/7/17	2.0	0.05	10.0	0.01	DE1MTX-3/7/17

A standard solution was prepared for assessment of matrix effects using surface water as the dilution solvent as listed below:

Standard Ref.	Standard Conc. (ng/mL)	Volume Used (mL)	Final Vol. (mL)	Final Conc. (ng/mL)	Standard Ref.
E1D-3/7/17	2.0	0.05	10.0	0.01	SE1MTX-3/7/17

5.4 Extraction

1. An aliquot of water (10 mL) was dispensed into a glass vial (28 mL capacity).
2. Procedural recoveries were prepared by fortifying aliquots of untreated water with the appropriate fortification solution as detailed in the table below.

Sample Volume (mL)	Standard Reference	Standard Conc. (µg/mL)	Volume added (mL)	Fortification Level (µg/L)
10.0	E1D-3/7/17	0.002	0.05	0.01
10.0	E1C-3/7/17	0.02	0.05	0.1
10.0	E1B-3/7/17	0.2	0.05	1.0

3. Samples were shaken to mix and an aliquot was transferred to an HPLC vial prior to quantitation of E-1 residues by LC-MS/MS.

5.5 LC-MS/MS Conditions

An AB Sciex 5500 Mass Spectrometer with an Agilent 1260 Binary HPLC Pump, Agilent 1260 Degasser, CTC Analytics HTC PAL Autosampler, Agilent 1260 Column Oven and a Peak Scientific ABN2ZA Gas Generator was used for quantitation.

5.5.1 Chromatography Parameters for E-1

Parameter	Description		
HPLC Column	Zorbax SB-C3, 150 x 3.0 mm, 5.0 µm Particle Size		
Column Temperature	50 °C		
Injection Volume	60 µL		
Retention Time	E-1 - 3.1 minutes		
Mobile Phase	A: 0.1% Formic Acid in Water B: 0.1% Formic Acid in Acetonitrile		
Flow Rate	1.0 mL/min		
Gradient	Time (minutes)	A (%)	B (%)
	0.0	80	20
	4.0	5	95
	5.0	5	95
	5.1	80	20
	6.5	80	20

5.5.2 Mass Spectrometry Parameters for E-1

Parameter	Description				
Ionisation Mode	Turbospray (Electrospray)				
Probe Position	5 Horizontal, 5 Vertical				
Polarity	Negative				
Curtain Gas	45				
CAD Gas	12				
Gas 1	45				
Gas 2	50				
Source Temperature	750 °C				
Spray Voltage	-4500				
Entrance Potential	-10				
Mass Transitions	Ions monitored (*m/z*)	Declustering Potential	Collision Energy	Cell Exit Potential	Primary / Confirmatory
E-1	383 → 274	-90	-43	-12	Primary
	385 → 276	-90	-43	-12	Confirmatory

5.6 Quantitation

The quantitative determination of all analytical samples was carried out by external standardisation using calibration standards in solvent. Injections of calibration standards were interspersed throughout the sequence between injections of samples (not greater than 4).

Detector linearity was assessed by constructing a calibration curve of peak area versus analyte concentration. Linear regression with 1/x weighting at 8 different concentrations ranging from 0.003 ng/mL to 1.5 ng/mL was used. Correlation co-efficients (r) above 0.995 were obtained which meets the criteria of ResChem Analytical standard operating procedures.

Residues (R) in µg/L (or ng/mL) were calculated using Analyst software version 1.6.2.

Percent recovery from fortified samples was calculated as described below:

$$\text{Recovery (\%)} = \frac{(R_{fortified})}{F} \times 100$$

where:

$R_{fortified}$ = Residue determined in fortified sample (µg/L)

F = Fortification rate (µg/L)

5.6.1 Example Calculation

For a 0.01 µg/L fortified drinking water sample (sample no. 4748) the concentration of E-1 found was calculated as follows:

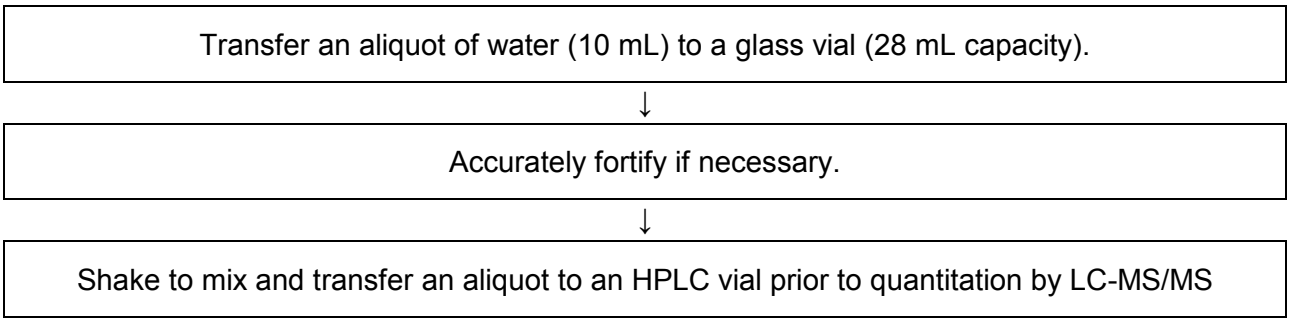
R = 0.0096563 µg/L

The percent recovery found was calculated as follows:

Recovery (%) = (0.0096563 µg/L / 0.01 µg/L) x 100

= 96.6 %

5.7 Method Flow Chart



5.8 Time Management

Preparation of standards and extraction of two sample sets can be conducted in 1 working day (7.5 hours) by one analyst. Analysis of samples by LC-MS/MS can be conducted over approximately 10 hours.

Therefore, two sample sets, each consisting of 21 samples (including 18 recoveries, 1 reagent blank, 2 control samples) and 8 calibration standards, can be prepared, extracted and analysed within approximately 1 day by one analyst i.e. extraction starts at beginning of day 1, submitted for quantitation at end of day 1 and quantitation completed by start of day 2.