3.6 LC-MS/MS analysis

LC-MS/MS conditions for Pyrifluquinazon, IV-01, IV-02, IV-15, IV-27 and IV-28

Instrument:	Micromass Quattro LC (using Mass Lynx 3.3 data management system)
Mode:	Electrospray positive (ESP+)
Ion monitoring details:	Pyrifluquinazon MRM *m/z* 465>107 and 465>92 IV-01 MRM *m/z* 423>107 and 423>92 IV-02 MRM *m/z* 421>105 and 421>107 IV-15 MRM *m/z* 437>92 and 437>107 IV-27 MRM *m/z* 453>107 and 453>105 IV-28 MRM *m/z* 437>105 and 437>148
Column:	Aqua C_{18} (50 mm x 2 mm id, 5 µm particle size, 125 Å pore size, Phenomenex)
Mobile phase A:	0.01M Ammonium formate in water:methanol:formic acid (90:10:0.1 v:v:v)
Mobile phase B:	Methanol:formic acid (100:0.1 v:v)

Gradient:

Time (min)	% A	% B
0	100	0
2	0	100
5	0	100
5.5	100	0
7.5	100	0

Cycle time:	8 min
Injection volume:	10 µL
Flow rate:	0.3 mL/min
Retention time:	Pyrifluquinazon Approximately 3 minutes IV-01 Approximately 3 minutes IV-02 Approximately 3 minutes IV-15 Approximately 3 minutes IV-27 Approximately 3 minutes IV-28 Approximately 3 minutes
Limit of quantitation:	0.1 µg/L in water
Limit of detection:	2.5 ng/mL (equivalent to 0.025 µg/L in water)

LC-MS/MS conditions for IV-203

Instrument:	Waters Acquity TQD (using Mass Lynx 4.1 data management system)
Mode:	Electrospray negative (ESP-)
Ion monitoring details:	IV-203 MRM *m/z* 329>309 and 329>289
Column:	Acquity UPLC BEH C_{18} (50 mm x 2.1 mm id, 1.7 µm particle size, 130 Å pore size) Column Temperature 45°C
Mobile phase C:	Water:acetonitrile:acetic acid (90:10:0.1 v:v:v)
Mobile phase D:	Acetonitrile:acetic acid (100:0.1 v:v)

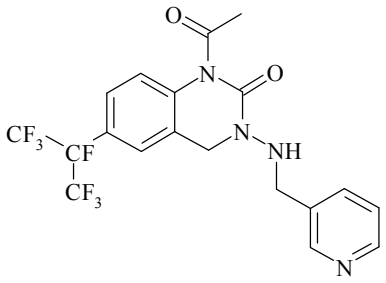
Gradient:

Time (min)	% A	% B
0	100	0
0.2	100	0
2	5	95
2.5	5	95
3	100	0
4	100	0

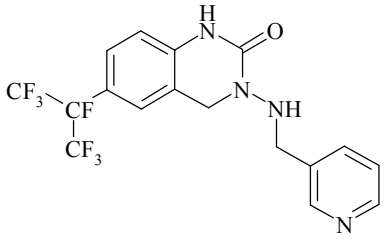
Cycle time:	4 min
Injection volume:	10 µL
Flow rate:	0.5 mL/min
Retention time:	Approximately 1.4 minutes
Limit of quantitation:	0.1 µg/L in water
Limit of detection:	2.5 ng/mL (equivalent to 0.025 µg/L in water)

2. Materials

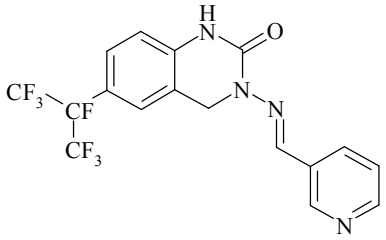
2.1 Analytical standard - Pyrifluquinazon

Identity	Pyrifluquinazon
Chemical name (IUPAC)	1-acetyl-1,2,3,4-tetrahydro-3-[(3-pyridylmethyl)amino]-6-[1,2,2,2-tetrafluoro-1-(trifluoromethyl)ethyl]quinazolin-2-one
Structure	
Storage conditions	Approximately +4°C in the dark
Batch number	4FZ0017P
CAS number	337458-27-2
Purity	99.9%
Supplier	Sponsor
Expiry date	3 April 2013

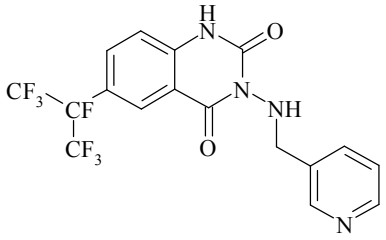
2.2 Analytical standard – Metabolite IV-01

Identity	Metabolite IV-01
Chemical name	1,2,3,4-tetrahydro-3-[(3-pyridylmethyl)amino]-6-[1,2,2,2-tetrafluoro-1-(trifluoromethyl)ethyl]quinazolin-2-one
Structure	
Storage conditions	Approximately +4°C in the dark
Batch number	4FZ6404P
Purity	98.7%
Supplier	Sponsor
Expiry date	19 August 2017

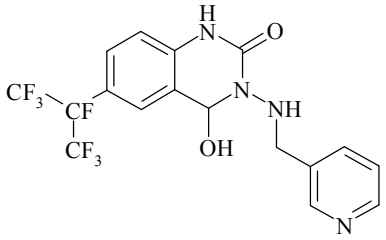
2.3 Analytical standard – Metabolite IV-02

Identity	Metabolite IV-02
Chemical name	1,2,3,4-tetrahydro-3-[(3-pyridylmethylene)amino]-6-[1,2,2,2-tetrafluoro-1-(trifluoromethyl)ethyl]quinazolin-2-one
Structure	
Storage conditions	Approximately +4°C in the dark
Batch number	4FZ6304P
Purity	99.3%
Supplier	Sponsor
Expiry date	13 October 2020

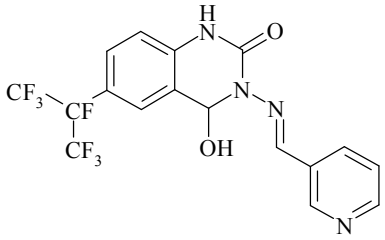
2.4 Analytical standard – Metabolite IV-15

Identity	Metabolite IV-15
Chemical name	1,2,3,4-tetrahydro-3-[(3-pyridylmethyl)amino]-6-[1,2,2,2-tetrafluoro-1-(trifluoromethyl)ethyl]quinazolin-2,4-dione
Structure	
Storage conditions	Approximately +4°C in the dark
Batch number	4FZ0301S
Purity	99.5%
Supplier	Sponsor
Expiry date	9 October 2020

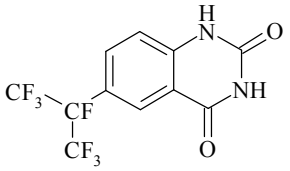
2.5 Analytical standard – Metabolite IV-27

Identity	Metabolite IV-27
Chemical name	1,2,3,4-tetrahydro-4-hydroxy-3-[(3-pyridylmethyl)amino]-6-[1,2,2,2-tetrafluoro-1-(trifluoromethyl)ethyl]quinazolin-2-one
Structure	
Storage conditions	Approximately +4°C in the dark
Batch number	4FZ0901S
Purity	91.5%
Supplier	Sponsor
Expiry date	22 October 2020

2.6 Analytical standard – Metabolite IV-28

Identity	Metabolite IV-28
Chemical name	4-hydroxy-3-[(pyridine-3-ylmethylene)amino]-6-[1,2,2,2-tetrafluoro-1-(trifluoromethyl)ethyl]-3,4-dihydro-1*H*-quinazolin-2-one
Structure	
Storage conditions	Approximately +4°C in the dark
Batch number	5FZ1301S
Purity	96.9%
Supplier	Sponsor
Expiry date	3 November 2013

2.7 Analytical standard – Metabolite IV-203

Identity	Metabolite IV-203
Chemical name	1,2,3,4-tetrahydro-6-[1,2,2,2-tetrafluoro-1-(trifluoromethyl)ethyl]quinazolin-2,4-dione
Structure	
Storage conditions	Approximately +4°C in the dark
Batch number	7FZ0602S
Purity	99.7%
Supplier	Sponsor
Expiry date	23 March 2015

Certificates of Analysis are presented in Appendix 1.

2.8 Control matrices

Surface water was obtained from Diss Mere, Norfolk, the ground water was obtained from a borehole in Sudbury and the drinking water was obtained from a tap in the Environmental Analysis Department. Upon receipt the water samples were allocated a unique Huntingdon Life Sciences, Environmental Analysis Department identification number.

2.9 Reagents

A list of all reagents used is presented below:

Materials	**Grade (or equivalent)**
Acetic acid	HPLC
Acetone	AR
Acetonitrile	HPLC
Ammonium formate	AR
Dichloromethane	HPLC
Formic acid	HPLC
Methanol	HPLC
Sodium sulphate, anhydrous	AR
Water	HPLC

3. Experimental procedures

3.1 Preparation of analytical standard solutions

3.1.1 Stock and fortification standard solutions

Weighed amounts (corrected for purity if required) of the analytical standards were dissolved in methanol (metabolite IV-02 was dissolved in an acetone:methanol mixture) to produce individual stock standard solutions. These stock standard solutions were progressively diluted with methanol to give a series of mixed fortification standard solutions as required.

3.1.2 Instrument calibration solutions

The fortification standard solutions were further diluted with methanol to produce a series of instrument calibration solutions in the range 2.5 to 250 ng/mL. For IV-203 in surface water only it was necessary to use matrix-matched calibration standards over the same range.

3.2 Preparation of reagents

Mobile phase A

Methanol (100 mL), ammonium formate (0.6 g) and formic acid (1 mL) were added to water (900 mL). The bottle was capped and shaken thoroughly to mix.

Mobile phase B

Formic acid (1 mL) was added to methanol (1000 mL), The bottle was capped and shaken thoroughly to mix.

Mobile phase C

Acetonitrile (100 mL) and acetic acid (1 mL) were added to water (900 mL). The bottle was capped and shaken thoroughly to mix.

Mobile phase D

Acetic acid (1 mL) was added to acetonitrile (1000 mL), The bottle was capped and shaken thoroughly to mix.

Note: variable quantities of the above may be prepared by adjusting the constituent quantities accordingly.

3.3 Validation

Sub-samples of each of the three water types were fortified at known concentrations of Pyrifluquinazon and metabolites IV-01, IV-02, IV-15, IV-27, IV-28 and IV-203 and analysed according to the following regime:

2 untreated sub samples
5 untreated sub samples fortified at the LOQ (0.1 µg/L)
5 untreated sub samples fortified at 1.0 µg/L

These samples were then processed using the analytical methodology described in Section 3.5.

3.4 Sample extract stability

Aliquots of the final extracts of untreated sub-samples of each water type were fortified with Pyrifluquinazon and metabolites IV-01, IV-02, IV-15, IV-27, IV-28 and IV-203 at a concentration of 100 ng/mL. The concentration of the analytes were quantified on day 0 and 5 days after storage at approximately –20°C in the dark in order to assess stability. A freshly fortified extract was also quantified at this time.

3.5 Procedure

1. Transfer water (200 mL) into a separating funnel (250 mL). Add fortification solutions at this stage, if required.
2. Immediately after fortification add aliquots of methanol (20 mL) and dichloromethane (10 mL) and shake vigorously for approximately 1 minute.
3. When the phases have separated transfer the lower dichloromethane extract through anhydrous sodium sulphate (~ 2 g, held in a SPE reservoir with glass wool) into a scintillation vial.
4. Add a further aliquot of dichloromethane (10 mL) and shake vigorously for approximately 1 minute.
5. When the phases have separated transfer the lower dichloromethane extract as before into the same scintillation vial combining the extracts.
6. Add a further aliquot of dichloromethane (5 mL) and shake vigorously for approximately 1 minute.
7. When the phases have separated transfer the lower dichloromethane extract as before into the same scintillation vial combining the extracts.
8. Remove the solvent under a stream of nitrogen in a water bath set at 25°C until just dry.
9. Reconstitute in methanol (2 mL) with the aid of ultrasonication and vortex mixing prior to quantitation using LC-MS/MS.

3.6 LC-MS/MS analysis

LC-MS/MS conditions for Pyrifluquinazon, IV-01, IV-02, IV-15, IV-27 and IV-28

Instrument:	Micromass Quattro LC (using Mass Lynx 3.3 data management system)
Mode:	Electrospray positive (ESP+)
Ion monitoring details:	Pyrifluquinazon MRM *m/z* 465>107 and 465>92 IV-01 MRM *m/z* 423>107 and 423>92 IV-02 MRM *m/z* 421>105 and 421>107 IV-15 MRM *m/z* 437>92 and 437>107 IV-27 MRM *m/z* 453>107 and 453>105 IV-28 MRM *m/z* 437>105 and 437>148
Column:	Aqua C_{18} (50 mm x 2 mm id, Phenomenex)
Mobile phase A:	0.01M Ammonium formate in water:methanol:formic acid (90:10:0.1 v:v:v)
Mobile phase B:	Methanol:formic acid (100:0.1 v:v)

Gradient:

Time (min)	% A	% B
0	100	0
2	0	100
5	0	100
5.5	100	0
7.5	100	0

Cycle time:	8 min
Injection volume:	10 µL
Flow rate:	0.3 mL/min
Retention time:	Pyrifluquinazon Approximately 3 minutes IV-01 Approximately 3 minutes IV-02 Approximately 3 minutes IV-15 Approximately 3 minutes IV-27 Approximately 3 minutes IV-28 Approximately 3 minutes
Limit of quantitation:	0.1 µg/L in water
Limit of detection:	2.5 ng/mL (equivalent to 0.025 µg/L in water)

LC-MS/MS conditions for IV-203

Instrument:	Waters Acquity TQD (using Mass Lynx 4.1 data management system)
Mode:	Electrospray negative (ESP-)
Ion monitoring details:	IV-203 MRM *m/z* 329>309 and 329>289
Column:	Acquity UPLC BEH C_{18} (50 mm x 2.1 mm id) Column Temperature 45°C
Mobile phase C:	Water:acetonitrile:acetic acid (90:10:0.1 v:v:v)
Mobile phase D:	Acetonitrile:acetic acid (100:0.1 v:v)

Gradient:

Time (min)	% A	% B
0	100	0
0.2	100	0
2	5	95
2.5	5	95
3	100	0
4	100	0

Cycle time:	4 min
Injection volume:	10 µL
Flow rate:	0.5 mL/min
Retention time:	Approximately 1.4 minutes
Limit of quantitation:	0.1 µg/L in water
Limit of detection:	2.5 ng/mL (equivalent to 0.025 µg/L in water)

4. Calculation of results

For Pyrifluquinazon and metabolites IV-01, IV-02, IV-15, IV-27 and IV-28 validation samples were quantified using the following equation:

$$\text{Residue found } (\mu g/L) = x \times \frac{1}{M} \times D$$

Where x (residue concentration in final solution) was calculated using the linear regression

$$y = m x + c \qquad \text{where } x \text{ (concentration in ng/mL)} = \frac{y - c}{m}$$

y = peak area
m = slope
c = intercept
M = matrix concentration (mL matrix/mL final extract)
D = dilution factor

Example calculation of Pyrifluquinazon detected in drinking water fortified at 1.0 µg/L; quantitation ion transition (sample identification 12/00/9758 F1.0 A, Batch 1A):

Linear regression $y = m x + c$

$3752.372 = 36.6203 x + 29.0825$

where $y = 3752.372$
m = 36.6203
c = 29.0825

$$\text{Therefore, concentration } (x) = \frac{3752.372 - 29.0825}{36.6203} = 101.7 \text{ ng/mL}$$

Matrix concentration = 100 mL matrix / mL final extract
Dilution factor = 1

$$\text{Pyrifluquinazon detected} = \frac{101.7 \text{ ng/mL} \times 1}{100 \text{ mL/mL}} = 1.017 \text{ ng/mL} = 1.02 \text{ µg/L}$$

$$\text{Recovery (\%)} = \frac{1.02 \text{ µg/L} \times 100\%}{1.0 \text{ µg/L}} = 102\%$$

For metabolite IV-203 validation samples were quantified using the following equation:

Residue found (µg/L) = x x $\frac{1}{M}$ x D

Where x (residue concentration in final solution) was calculated using the quadratic regression

$$x = \frac{-b + \sqrt{(b^2 - 4a(c-y))}}{2a}$$

y	=	peak area of sample
a, b, c	=	coefficients from quadratic regression analysis
M	=	matrix concentration (mL matrix/mL final extract)
D	=	dilution factor

Example calculation of IV-203 detected in drinking water fortified at 1.0 µg/L; quantitation ion transition (sample identification 12/00/9758 F1.0 A, Batch 1E):

Quadratic regression $y = ax^2 + bx + c$

$$1319.172 = -0.0262933x^2 + 17.9975x + 15.7437$$

where
$y = 1319.172$
$a = -0.0262933$
$b = 17.9975$
$c = 15.7437$

Therefore, concentration (x)

$$= \frac{-17.9975 + \sqrt{(17.9975^2 - 4 \times -0.0262933\,(15.7437 - 1319.172))}}{2 \times -0.0262933} = 82.3 \text{ ng/mL}$$

Matrix concentration = 100 mL matrix / mL final extract
Dilution factor = 1

IV-203 detected = $\frac{82.3\,\text{ng/mL} \times 1}{100\ \text{mL/mL}}$ = 0.823 ng/mL = 0.823 µg/L

Recovery (%) = $\frac{0.823\,\mu\text{g/L} \times 100\%}{1.0\,\mu\text{g/L}}$ = 82%