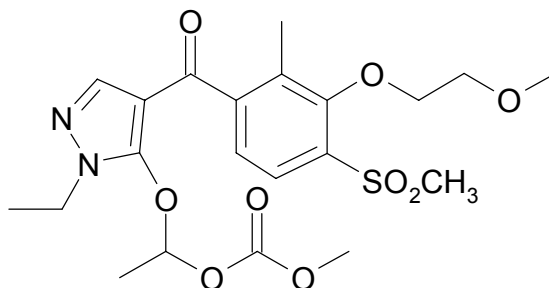# 2. Materials

2.1 Analytical Standards

Identity   SL-573

Chemical name: 1-[[1-ethyl-4-[3-(2-methoxyethoxy)-2-methyl-4-(methylsulfonyl)benzoyl]-1*H*-pyrazol-5-yl]oxy]ethyl methyl carbonate
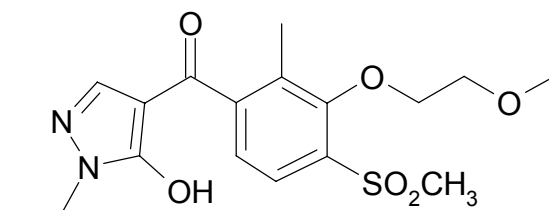
Structural formula:

Purity: 99.9%

Expiry date: 31 May 2015

Batch number: 20120131

Physical state: Solid

Appearance: Light yellow powder

Storage conditions: Freezer (approximately -20ºC)

Identity   MT-2153

Chemical name: 5-Hydroxy-1-ethylpyrazol-4-yl)(3-(2-methoxyethoxy)-2-methyl-4-(methylsulfonyl)phenyl)methanone

Structural formula:

Purity: 99.8%

Expiry date: 30 May 2015

Batch number: 20120125

Physical state: Solid

Appearance: White powder

Storage conditions: Freezer (approximately -20ºC)

Certificates of Analysis are presented in Appendix 1.

2.2   Control matrices

The control water samples were assigned unique identification numbers and stored at approximately 4°C prior to use as control samples in this study.

The control drinking water was obtained from within the Environmental Analysis building.

The control surface water was obtained from Diss Mere, Norfolk, England; the characterisation details are as follows:

Parameter	Found Value
pH	7.50
Conductivity	695 μS/cm°
Alkalinity	217 mg/L as $CaCO_3$
Total Hardness	199 mg1L as $CaCO_3$
Total Organic Carbon	15.016 mgC/L
Dissolved Organic Carbon	14.903 mgC/L

3. Methods

3.1   Validation

Sub-samples of each of the two water types were fortified with known concentrations of SL-573 and MT-2153 then analysed according to the following regime:

2 sub-samples of untreated water
5 sub-samples of untreated water fortified at the LOQ (0.01 µg/L)
5 sub-samples of untreated water fortified at 0.1 µg/L

These samples were then analysed using the analytical methodology, with each sample injected onto the chromatograph once.

3.2 Analytical method

Samples were cleaned up using Strata X (Polymeric Reversed phase) SPE cartridges. Quantitation was performed using liquid chromatography with tandem mass spectrometric detection (LC-MS/MS).

The analytical method used in the laboratory is presented in Appendix 2.

3 minor changes were made to the method for the analysis of MT-2153 in drinking water:

1) 1% citric acid (w:v) (1.8 g)was added to the drinking water with the aid of ultrasonication after fortification but prior to loading onto the SPE cartridge.

2) After the sample was loaded onto the cartridge, the cartridge was washed with 2 x 6 mL of Ultra Pure water and the cartridge was dried under vacuum for approximately 2 minutes.

3) The analyte was eluted from the cartridge with 2 x 3 mL of acetonitrile containing 1% citric acid (w:v) followed by 2 x 2 mL of methanol containing 1% citric acid (w:v).

Minor modifications were made to the method of quantitation supplied by the Sponsor, in order to make the methodology compatible with the chromatography equipment available for use in this laboratory. They were as follows:

LC-MS/MS conditions – SL-573

Instrument:	AB Sciex API 4000 (Analyst 1.4.2 software) coupled to Waters Acquity UPLC system
Column:	Acquity UPLC® BEH C_{18} (2.1 cm x 50 mm, 1.7 μm), column temperature 45°C
Injection volume:	20 μL
Retention time:	Approximately 3.5 minutes

LC-MS/MS conditions – MT-2153

Instrument:	AB Sciex API 4000 (Analyst 1.4.2 software) coupled to Waters Acquity UPLC system
Column:	Acquity UPLC® BEH C_{18} (2.1 cm x 50 mm, 1.7 μm), column temperature 45°C
Injection volume:	20 μL
Retention time:	Approximately 1.3 minutes

Instrument conditions not mentioned above are as stated in the provided analytical method.

These modifications were approved by the study monitor during the course of the study.

3.3   Calculation of results for validation samples

Test samples were quantified using the following equation:

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

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

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

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

Example calculation of SL-573 in surface water at 0.1 µg/L (analytical identification 4/00/17 F0.1 A, analysis batch 2, quantitation ion transition).

Linear regression $y = m\,x + c$

$$6.89934e5 = 1.61e5x + 3.87e3$$

Where $y = 6.89934e5$
            $m = 1.61e5$
            $c = 3.87e3$

$$\text{Therefore, concentration of SL-573 } (x) = \frac{6.89934e5 - 3.87e3}{1.61e5} = 4.26 \text{ ng/mL}$$

Matrix concentration = 60 mL matrix/mL final extract

$$\text{SL-573 detected (µg/L)} = \frac{4.26 \text{ ng/mL} \times 1}{60 \text{ mL/mL}} = 0.071 \text{ µg/L}$$

$$\text{Recovery (\%)} = \frac{0.071\,\text{µg/L} \times 100}{0.1\,\text{µg/L}} = 71\%$$