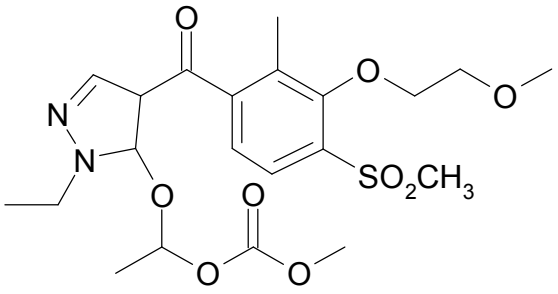
2. Materials

2.1 Analytical Standard 1 – 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:

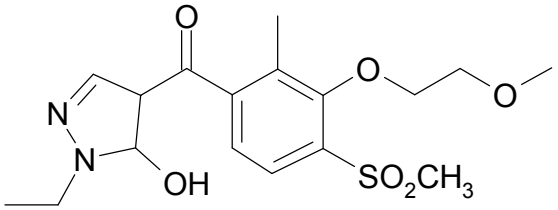


Purity:	99.9%
Expiry date:	31 May 2015
Batch number:	20120131
Physical state:	Solid
Appearance	Light yellow powder
Storage conditions:	Freezer (approximately -20ºC, in the dark)

2.2 Analytical Standard 2 – 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, in the dark)

Certificates of Analysis are presented in Appendix 1.

2.3 Control matrices

The control soil samples were supplied from Southern France and Germany. These samples were assigned unique identification numbers and stored at approximately 4°C prior to use as control samples in this study.

The soil samples had been previously classified as below:

Sample ID	Soil sample location	Classification
12/00/2683	Southern France	Silt loam
12/00/2691	Germany	Sandy loam

3. Methods

3.1 Validation

Sub-samples of each of the two soils were fortified with known concentrations of the two test substances and analysed according to the following regime:

2 sub-samples of untreated sample soil
5 sub-samples of untreated sample soil fortified at the LOQ (0.001 mg/kg)
5 sub-samples of untreated sample soil fortified at 0.05 mg/kg

These samples were then analysed using the analytical methodology, with each sample injected onto the chromatographic system once per analysis run.

3.2 Final extract stability

An experiment was set up to demonstrate the stability of the two analytes under the typical storage conditions of the final extracts if they are not quantified immediately after preparation. Processed control extracts, fortified with SL-573 and MT-2153 were stored at approximately -20ºC in the dark (i.e. in a freezer).

Aliquots of each of the control sample extracts were fortified with SL-573 and MT-2153 at a concentration of 5 ng analyte/mL of final extract. The concentration of analytes in the stored extracts was determined at day 0 and after 8 days. The concentration of the analytes in freshly fortified control extracts was also determined at that time.

3.3 Matrix effects

Any possible sample matrix effects were investigated by the comparison of the instrument response to the analytes in the fortified final extract samples with the response of the analytes in solvent based calibration standard solutions prepared at the same time.

3.4 Analytical method

Samples were extracted with a methanol/water/citric acid/ammonium formate/hydrochloric acid mixture, and cleaned-up with an Oasis HLB solid phase extraction (SPE) cartridge. 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 3.

3.5 Fortification/calibration solutions

Individual stock standard solutions of SL-573 and MT-2153 were prepared by dissolving an accurately weighed amount of each material in a suitable volume of acetonitrile, correcting for purity as appropriate. These stock solutions were further diluted with acetonitrile to produce mixed fortification solutions at 100 µg/mL, 10 µg/mL and 1 µg/mL concentrations.

The instrument calibration solutions, over the concentration range 0.025 ng/mL to 10 ng/mL, were prepared on each day of analysis by serial dilution of the mixed fortification solution in acetonitrile:water (40:60 v:v), as detailed below:

Standard solution used (ng/mL)	Volume taken (mL)	Final volume (mL)	Nominal concentration (ng/mL)
1000	0.1	10	10
1000	0.05	10	5
1000	0.025	10	2.5
10	1	10	1
10	0.5	10	0.5
10	0.25	10	0.25
1	1	10	0.1
1	0.5	10	0.05
1	0.25	10	0.025

3.6 Calculation of results for validation samples

Test samples were quantified using the following equation:

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

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

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

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

Example calculation of SL-573 detected in soil from Southern France fortified at 0.001 mg/kg (analytical identification 12/00/2683 F0.001 A, analysis batch 1). The primary data for this sample is presented in Table 14, Appendix 2.

Linear regression $y = \text{m}\,x + \text{c}$

$$6.96155e3 = 73670.9x + 0.349781$$

Where
$y = 6.96155e3$
m = 73670.9
c = 0.349781

$$\text{Therefore, concentration of SL-573 } (x) = \frac{6.96155e3 - 0.349781}{73670.9} = 0.09449 \text{ ng/mL}$$

Matrix concentration = 0.1 g matrix/mL final extract
Dilution factor = 1

$$\text{SL-573 detected (mg/kg)} = \frac{0.09449 \text{ ng/mL} \times 1}{0.1 \text{ g/mL}} = 0.9449 \text{ ng/g} = 0.0009449 \text{ mg/kg}$$

$$\text{Recovery (\%)} = \frac{0.0009449 \text{ mg/kg} \times 100}{0.001 \text{ mg/kg}} = 94\%$$

Appendix 3 Analytical Method

DETERMINATION OF SL-573 AND MT-2153 IN SOIL

1. General principle

Samples are extracted with a methanol/water/citric acid/ammonium formate/hydrochloric acid mixture. An aliquot is cleaned up using HLB SPE cartridges. Quantitation is performed using liquid chromatography with tandem mass spectrometric detection (LC-MS/MS).

2. Apparatus, glassware etc.

Balances (various ranges)
Volumetric flasks (various sizes)
Syringes (various sizes)
Volumetric pipettes (various sizes)
Polypropylene tubes (15 and 50 mL)
Polyethylene bottles (250 mL)
Measuring cylinders (various sizes)
Vacuum manifold

1. 3. Materials	2. Typical Grade (or equivalent)
Ammonium formate	AR
Citric acid mono hydrate	AR
Ammonium acetate	AR
Hydrochloric acid (SG 1.18, approx. 36%)	AR
Acetic acid	AR
Methanol	HPLC
Acetonitrile	HPLC
Water	HPLC
Oasis HLB cartridges (60 mg, 3 mL)	

3. 4. Preparation of reagents

Preparation of "Extraction Solvent" – methanol:water (80:20 v:v) containing ammonium formate (0.1 M), citric acid (0.05 M) and hydrochloric acid (0.5% v/v) - methanol (1600 mL) is mixed thoroughly with water (400 mL) and ammonium formate (12.6 g), citric acid (21 g) and hydrochloric acid solution (SG 1.18, approx. 36%, 10 mL) are added. The bottle is capped and the contents mixed well.

Preparation of acetonitrile:water (40:60 v:v) - acetonitrile (400 mL) is mixed thoroughly with water (600 mL).

Preparation of acetonitrile:water (50:50 v:v) - acetonitrile (500 mL) is mixed thoroughly with water (500 mL).

Preparation of 0.2% acetic acid in water (v:v) – acetic acid (2 mL) is dissolved in water (1 L).

Preparation of mobile phase A, 0.01M ammonium acetate solution – ammonium acetate (0.77 g) is dissolved in water (1 L).

5. Analytical standard solutions

An appropriate amount of the test substances (corrected for purity) are accurately weighed and dissolved in acetonitrile to give the individual stock standard solutions. Appropriate dilutions of the stock standard solutions are made with acetonitrile to give mixed fortification standard solutions.

The mixed fortification solutions are progressively diluted with acetonitrile:water (40:60 v:v) to produce a series of instrument calibration solutions in the range 0.025 to 10 ng/mL.

6. Procedure

Analysis of soil samples

6.1 Weigh a sub-sample (20 g) of soil into a 250 mL polypropylene bottle.
6.2 Add fortification solution at this stage if required.
6.3 Add extraction solvent (100 mL).
6.4 Securely cap the sample bottle and place onto a mechanical shaker for approximately 30 minutes at approximately 200 rpm.
6.5 Centrifuge the sample at approximately 3500 rpm for approximately 3 minutes.
6.6 Decant the supernatant into a 250 mL polypropylene bottle.
6.7 Re-extract the solid residue with extraction solvent (80 mL), as steps 6.4 to 6.6, combining the extracts in the 250 mL polypropylene bottle.
6.8 Dilute the extract to volume (200 mL) with extraction solvent.

SPE clean-up

6.9 Condition the Oasis HLB SPE cartridge with methanol (3 mL) and 0.2% acetic acid in water (3 mL), discarding the eluate.
6.10 Transfer an aliquot of sample extract (10 mL ≡ 1 g) to a 50 mL polypropylene tube.
6.11 Add 0.2% acetic acid in water (20 mL) and mix well.
6.12 Load the extract onto the SPE cartridge, discarding the eluate.
6.13 Wash the cartridge with an aliquot (2 mL) of water, discarding the eluate.
6.14 Elute the SPE cartridge with an aliquot (8 mL) of acetonitrile:water (50:50 v:v), collecting in a 15 mL graduated polypropylene tube.
6.15 Dilute the extract to volume (10 mL), using water.
6.16 Perform any further dilutions using acetonitrile:water (40:60 v:v), as required.
6.17 Quantify the samples by the use of LC-MS/MS.

7. Flow chart of analytical procedure

Weigh 20 g sub-sample into polyethylene bottle
↓
Fortification with analytical standards (if required)
↓
Extraction with “extraction solvent” by mechanical shaker
↓
Further extraction with “extraction solvent” by mechanical shaker
↓
Dilute the combined extracts to volume (200 mL) with “extraction solvent”
↓
Transfer aliquot (10 mL) to tube and add water
↓
Clean-up by OASIS HLB
↓
Dilute to volume with water
↓
Quantitative analysis by LC-MS/MS

8. LC-MS/MS conditions – SL-573

Instrument:	AB Sciex API 4000 (Analyst 1.4.2 software) coupled to Waters Acquity UPLC system
Mode:	Ionspray positive
Ion monitoring details:	SL-573: *m/z* 485>383 (Quantitation analysis) SL-573: *m/z* 485>111 (Confirmation analysis)
Column:	Acquity UPLC® BEH C_{18} (2.1 cm x 50 mm, 1.7 µm), column temperature 45°C
Mobile phase A:	0.01M ammonium acetate
Mobile phase B:	Methanol
Gradient:	Isocratic 35% A, 65% B
Cycle time:	4 min
Injection volume:	10 µL
Flow rate:	0.5 mL/min
Retention time:	SL-573 - approximately 0.6 minutes
LOQ:	0.001 mg/kg
LOD:	0.025 ng/mL (≡ 0.00025 mg/kg in sample matrix)

9. LC-MS/MS conditions – MT-2153

Instrument:	AB Sciex API 4000 (Analyst 1.4.2 software) coupled to Waters Acquity UPLC system
Mode:	Ionspray negative
Ion monitoring details:	MT-2153: *m/z* 381>184 (Quantitation analysis) MT-2153: *m/z* 381>307 (Confirmation analysis)
Column:	Acquity UPLC® BEH C_{18} (2.1 cm x 50 mm, 1.7 µm), column temperature 45°C
Mobile phase A:	0.01M ammonium acetate
Mobile phase B:	Methanol

Gradient:

Time	%A	%B
0	95	5
0.2	95	5
2.0	5	95
2.5	5	95
3	95	5
4	95	5

Cycle time:	4 min
Injection volume:	8 µL
Flow rate:	0.5 mL/min
Retention time:	MT-2153 - approximately 1.2 minutes
LOQ:	0.001 mg/kg
LOD:	0.025 ng/mL (≡ 0.00025 mg/kg in sample matrix)

NOTE – alternative instruments may also be used, operated under conditions that are considered to be equivalent to those described above. However, some differences may be observed in the resulting data, such as slight differences in analyte retention times, or the observed sensitivity of the ion transitions monitored.