Report	IIA 4.5/02 (OECD) / CA 4.1.2/10 (EU)	
Authors (year):	Matthews, J. (2015)	
Title:	SL-573 and MT-2153: Independent laboratory validation of a residue analytical method for the determination of SL-573 and metabolite MT-2153 in water	
PMRA no.	2522689	
MRID	49580132 PC: 573101	
Laboratory report no. and date:	JSM0737, 5 March 2015	
Owner:	Ishihara Sangyo Kaisha, Ltd., Japan	
Testing facility and address:	Huntingdon Life Sciences, Woolley Road, Alconbury, Huntingdon, Cambridgeshire, PE28 4HS, UK	
Dates of experimental work:	14 January 2015 to 11 February 2015	
Guideline(s) followed:	SANCO/825/00 rev. 8.1 (2010)	
Deviations from guidelines:	None	
GLP	Yes	UK Department of Health

Study Classification:
EPA: Acceptable

Summary written by: Katherine Keppel-Jones, PMRA, on November 25, 2015
Peer reviewed by: Kim Davis, PMRA, on January 26, 2016

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Executive summary

The analytical method (LC-MS/MS) described in laboratory report no. D96125 (Janusch, F., 2014a, "SL-573 and metabolite MT-2153: Validation of a residue analytical method for the determination of SL-573 and metabolite MT-2153 in drinking, ground and surface water") was successfully validated, in an independent laboratory, for the determination of SL-573 and MT-2153 in surface water and drinking water. Minor modifications were made to the original method to suit the available analytical instrumentation and to complete the validation for the determination of MT-2153 in drinking water.

Analyte / reference substance 1

ISO common name:	Tolpyralate
Code no.:	SL-573
CAS no.:	1101132-67-5
Lot/batch no.:	20120131
Purity:	99.9%

Analyte / reference substance 2

Code no.:	MT-2153
CAS no.:	Not available
Lot/batch no.:	20120125
Purity:	99.8%

Test matrices

1.	Drinking water (from testing facility)
2.	Surface water (from Diss Mere, Norfolk, UK)

Table 9. Characteristics of the surface water

Parameter	Surface water
pH	7.50

Dissolved organic carbon (mg/L)	14.903
Total organic carbon (mg/L)	15.016
Total hardness (mg/L as $CaCO_3$)	199
Conductivity (µS/cm)	695
Alkalinity (mg/L as $CaCO_3$)	217

Principle of the method

The analytical method involved passing the fortified matrix through a Strata X polymeric reversed phase solid phase extraction cartridge, eluting with acetonitrile and then methanol. The eluate was evaporated to dryness under N_2 and the residue re-dissolved in methanol/water (20/80, v/v) containing 5 mM ammonium acetate prior to analysis by LC-MS/MS. Ion transitions monitored were *m/z* 485>383 (quantification) and *m/z* 485>409 (confirmation) (SL-573) and *m/z* 383>111 (quantification) and *m/z* 383>325 (confirmation) (MT-2153).
Matrices were fortified at 0.01 and 0.1 µg/L (five replicates at each level).
Three minor changes were made to the method for the analysis of MT-2153 in drinking water:
1. 1% citric acid (w/v) 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 × 6 mL of Ultra Pure water and the cartridge was dried under vacuum for approximately two minutes.
3. The analyte was eluted from the cartridge with 2 × 3 mL of acetonitrile containing 1% citric acid (w/v) followed by 2 × 2 mL of methanol containing 1% citric acid (w/v).
Minor modifications were made to the method of quantification supplied by sponsor, in order to make the methodology compatible with the chromatography equipment available for use at the testing facility.

Results and Discussion

Specificity

In control (untreated) samples, there was no apparent response (i.e. <30% of the LOQ) in the regions of the chromatogram at the retention times of SL-573 and MT-2153.

Linearity

The response of the LC-MS/MS system to standard solutions of SL-573 and MT-2153 was linear over the range 0.2 ng/mL to 7.5 ng/mL (equivalent to 0.00333 to 0.125 µg/L in water, r ≥0.9991 for SL-573 and ≥0.9965 for MT-2153).

Accuracy

The method was validated at 0.01 µg/L and 0.10 µg/L. Results obtained were within guideline requirements (mean recoveries 70 - 110%).

Precision (repeatability)

Results obtained were within guideline requirements (relative standard deviations ≤20%).

Table 10. ILV validation data for tolpyralate (SL-573) in water

Fortification level (µg/L)	Matrix	Number of replicates	Mean recovery (%)	Relative standard deviation (%)
Quantification transition (*m/z* 485>383), retention time ~3.5 min				
0.01	Drinking water	5	80	7.8
0.10		5	72	7.4
		Total = 10	Overall mean = 76	Overall = 9.1
0.01	Surface water	5	70	9.1
0.10		5	78	9.7
		Total = 10	Overall mean = 74	Overall = 10.8
Confirmatory transition (*m/z* 485>409)				
0.01	Drinking water	5	82	11.3
0.10		5	72	7.4
		Total = 10	Overall mean = 77	Overall = 11.5
0.01	Surface water	5	77	9.0
0.10		5	78	9.0
		Total = 10	Overall mean = 77	Overall = 8.5

Duplicate control samples were analysed and no residues were detected

Table 11. ILV validation data for MT-2153 in water

Fortification level (µg/L)	Matrix	Number of replicates	Mean recovery (%)	Relative standard deviation (%)
Quantification transition (*m/z* 383>111), retention time ~1.3 min				
0.01 0.10	Drinking water	5 5 Total = 10	99 104 Overall mean = 102	4.7 4.5 Overall = 5.0
0.01 0.10	Surface water	5 5 Total = 10	93 99 Overall mean = 96	9.0 6.2 Overall = 7.9
Confirmatory transition (*m/z* 383>325)				
0.01 0.10	Drinking water	5 5 Total = 10	90 104 Overall mean = 97	4.9 5.7 Overall = 8.9
0.01 0.10	Surface water	5 5 Total = 10	88 102 Overall mean = 95	11.4 3.4 Overall = 10.7

Duplicate control samples were analysed and no residues were detected

Limit of quantification (LOQ) and limit of detection (LOD)

The limit of quantification is defined as the lowest fortification level with mean recoveries ranging from 70% to 120% at a relative standard deviation of ≤20%. The validation of the methodology demonstrated that SL-573 and MT-2153 can be accurately determined at a LOQ of 0.01 µg/L in both water types tested.
Based on the lowest calibration standard (0.2 ng/mL), the limit of detection was equivalent to 0.00333 µg/L.

Conclusion

The analytical method described in laboratory report no: D96125 (Janusch, F., 2014, "SL-573 and metabolite MT-2153: Validation of a residue analytical method for the determination of SL-573 and metabolite MT-2153 in drinking, ground and surface water") has been successfully validated in an independent laboratory for the determination of SL-573 and MT-2153 in surface water and drinking water. Minor modifications were made to the original method to suit the available analytical instrumentation and to complete the validation for the determination of MT-2153 in drinking water.

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