

The Miller method (2012, GLP) for the determination of SL-573 and MT-2153 in soil was based on an earlier non-GLP method (Furuo, 2012, "Validation of the residue analytical method for the determination of SL-573 and its metabolite MT-2153 in soil", laboratory report no. H-855) that is not included here. The Robaugh interlaboratory validation (2013, GLP) was for this earlier Furuo method.

Report	IIA 4.4/02 (OECD) / CA 4.1.2/06 (EU)	
Authors (year):	Miller, C. (2012)	
Title:	SL-573 and MT-2153: Validation of an analytical method for the determination of SL-573 and MT-2153 in soil	
PMRA No.	2522685	
MRID	49580116	PC: 573101
Laboratory report no. and date:	JSM0305, 27 September 2012	
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:	11 June 2012 to 23 June 2012	
Guideline(s) followed:	SANCO/3029/99 rev.4 (2000) 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

**Secondary review by:** Marianne Mannix, EPA  
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### Executive summary

A method was validated to determine SL-573 and MT-2153 in two soil types using LC-MS/MS. The LOQ for both analytes was 0.001 mg/kg. In control soils fortified at 0.001 and 0.05 mg/kg, mean recovery ranges for both analytes were 70 - 110% with relative standard deviations  $\leq 20\%$ , demonstrating acceptable accuracy and precision of the analytical method. The analytes were stable in the final extracts when stored at approximately  $-20^{\circ}\text{C}$  for eight days.

### Analyte / reference substance 1

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

### Analyte / reference substance 2

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

### Test matrices

1: Silt loam soil, from southern France  
2: Sandy loam soil, from Germany

### Principle of the method

Soil samples were extracted with methanol/water (80/20 v/v) containing ammonium formate (0.1 M), citric acid (0.05 M) and hydrochloric acid (0.5% v/v), and the extract cleaned up with an Oasis HLB solid phase extraction cartridge. Quantification was performed using LC-MS/MS. Ion transitions monitored were  $m/z$  485>383 (quantification) and  $m/z$  485>111 (confirmation) (SL-573) and  $m/z$  381>184 (quantification) and  $m/z$  381>307 (confirmation) (MT-2153).

For each soil type, two samples of untreated soil, five samples of soil fortified at 0.001 mg/kg and five samples of soil fortified at 0.05 mg/kg were analysed.

### Specificity

In untreated samples of each soil, 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. As the chosen detection principle (LC-MS/MS with two transitions for each analyte) was highly specific, no separate confirmatory method was required.

### Linearity

The response of the LC-MS/MS system to standard solutions of SL-573 and MT-2153 was linear over the range 0.025 to 10 ng/mL (equivalent to 0.00025 to 0.1 mg/kg in soil) for both the quantification and confirmatory transitions ( $r \geq 0.9995$ ).

### Accuracy

The method was validated at 0.001 and 0.05 mg/kg for the detection of both analytes in the two soil types. Results obtained were within guideline requirements (mean recoveries 70 - 110%).

### Precision (repeatability)

Results obtained were within guideline requirements (relative standard deviation  $\leq 20\%$ ).

**Table 2. Validation data for tolpyralate (SL-573) in soil**

Matrix <sup>a</sup>	Fortification level (mg/kg)	Number of tests	Mean recovery (%)	Coefficient of variation (%)
<b>Quantification transition (<math>m/z</math> 485&gt;383), retention time ~0.6 min</b>				
Soil from southern France	0.001	5	95	5.9
	0.05	5	89	1.8
		Total = 10	Overall mean = 92	Overall = 5.5
Soil from Germany	0.001	5	97	2.8
	0.05	5	87	2.7
		Total = 10	Overall mean = 92	Overall = 6.2
<b>Confirmatory transition (<math>m/z</math> 485&gt;111)</b>				
Soil from southern France	0.001	5	97	5.1
	0.05	5	88	1.7
		Total = 10	Overall mean = 93	Overall = 6.2
Soil from Germany	0.001	5	95	2.3
	0.05	5	87	2.5
		Total = 10	Overall mean = 91	Overall = 5.1

<sup>a</sup> Duplicate control samples of each soil type were analysed and no residues were detected

**Table 3. Validation data for MT-2153 in soil**

Matrix <sup>a</sup>	Fortification level (mg/kg)	Number of tests	Mean recovery (%)	Coefficient of variation (%)
<b>Quantification transition (<math>m/z</math> 381&gt;184), retention time ~1.2 min</b>				
Soil from southern France	0.001	5	100	4.8
	0.05	5	88	3.9
		Total = 10	Overall mean = 94	Overall = 7.8
Soil from Germany	0.001	5	88	12.3
	0.05	5	94	2.8
		Total = 10	Overall mean = 91	Overall = 9.0
<b>Confirmatory transition (<math>m/z</math> 381&gt;307)</b>				
	0.001	5	87	8.0

Soil from southern France	0.05	5 Total = 10	92 Overall mean = 90	3.0 Overall = 6.4
Soil from Germany	0.001 0.05	5 5 Total = 10	88 92 Overall mean = 90	14.4 1.2 Overall = 9.7

<sup>a</sup> Duplicate control samples of each soil type were analysed and no residues were detected

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

The LOQ, defined as the lowest fortification level at which acceptable recovery data were obtained, was 0.001 mg/kg for both SL-573 and MT-2153 in the two soil types tested.

The LOD of the method, defined as the concentration of the lowest calibration standard that gave rise to a measurable chromatographic response, was 0.025 ng/mL for both SL-573 and MT-2153 (equivalent to 0.00025 mg/kg in soil).

### Storage stability

A stability test showed that the analytes were stable in the final extracts when stored at approximately -20°C for eight days.

### Conclusion

An analytical method for the determination of SL-573 and MT-2153 in two soils was validated at 0.001 and 0.05 mg/kg in terms of specificity, linearity, accuracy and precision. Residues of SL-573 and MT-2153 were quantified using the LC-MS/MS ion transitions of  $m/z$  485>383 (quantification) and  $m/z$  485>111 (confirmation) (SL-573) and  $m/z$  381>184 (quantification) and  $m/z$  381>307 (confirmation) (MT-2153).

The LOQ was 0.001 mg/kg for both SL-573 and MT-2153 in the two soil types tested.

Study Classification:

EPA: Acceptable

**Summary written by:** Katherine Keppel-Jones, PMRA, on November 25, 2015

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**Secondary review by:** Marianne Mannix, Rochelle Bohaty EPA, on May 20, 2016