

Report	IIA 4.4/03 (OECD) / CA 4.1.2/07 (EU)	
Authors (year):	Miller, C. (2014)	
Title:	MMTA: Validation of methodology for the determination of residues in two soil types	
PMRA No.	2522686	
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Laboratory report no. and date:	JSM0757, 11 December 2014	
Owner:	Ishihara Sangyo Kaisha, Ltd., Japan	
Testing facility and address:	Huntingdon Life Sciences, Woolley Road, Alconbury, Huntingdon, Cambridgeshire, PE28 4HS, UK	
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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

**Secondary review by:** Marianne Mannix, EPA  
Rochelle Bohaty, EPA

*Marianne A. Mannix*

Digitally signed by MARIANNE MANNIX  
Date: 2017.10.04 15:26:51 -04'00'

*Rochelle Bohaty*

2018.03.19 13:43:34  
-04'00'

### Executive summary

A method was validated to determine MMTA in two soil types using LC-MS/MS. The LOQ was 0.001 mg/kg. In control soils fortified at 0.001 and 0.05 mg/kg, mean recovery ranges were 70 - 120% with relative standard deviations  $\leq 20\%$ , demonstrating acceptable accuracy and precision of the analytical method. The analyte was stable in the final extracts when stored at approximately  $-20^{\circ}\text{C}$  for seven days.

### Analyte / reference substance

Code no.: MMTA  
CAS no.: Not available  
Lot/batch no.: 20140226  
Purity: 100%  
Description: White powder

### Test matrices

1: Clay soil ( $\text{pH}_{\text{CaCl}_2}$ : 7.3, organic carbon: 4.3%)  
2: Sandy loam soil ( $\text{pH}_{\text{CaCl}_2}$ : 5.6, organic carbon: 1.8%)

### 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  $m/z$  287>184 (quantification) and  $m/z$  287>243 (confirmation).

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 time of MMTA. As the chosen detection principle (LC-MS/MS with two transitions) was highly specific, no separate confirmatory method was required.

### Linearity

The response of the LC-MS/MS system to standard solutions of MMTA 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 ( $r \geq 0.9982$ ).

### Accuracy

The method was validated at 0.001 and 0.05 mg/kg for the detection of MMTA in the two soil types. Results obtained were within guideline requirements (mean recoveries 70 - 120%).

### Precision (repeatability)

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

**Table 4. Validation data for MMTA 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> 287&gt;184), retention time ~0.9 min</b>				
Clay soil	0.001	5	92	6.5
	0.05	5	96	3.0
	Total = 10	Overall mean = 94	Overall = 5.2	
Sandy loam soil	0.001	5	89	4.7
	0.05	5	89	3.4
	Total = 10	Overall mean = 89	Overall = 3.9	
<b>Confirmatory transition (<math>m/z</math> 287&gt;243)</b>				
Clay soil	0.001	5	90	8.8
	0.05	5	95	2.8
	Total = 10	Overall mean = 93	Overall = 6.6	
Sandy loam soil	0.001	5	98	7.9
	0.05	5	89	2.7
	Total = 10	Overall mean = 93	Overall = 7.8	

<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 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 (equivalent to 0.00025 mg/kg in the sample matrix).

### Storage stability

A stability test showed that MMTA was stable in the final extracts when stored at approximately -20°C for seven days.

### Conclusion

An analytical method for the determination of MMTA in two soils was validated at 0.001 and 0.05 mg/kg in terms of specificity, linearity, accuracy and precision. Residues of MMTA were quantified using the LC-MS/MS ion transitions of  $m/z$  287>184 (quantification) and  $m/z$  287>243 (confirmation).

The LOQ was 0.001 mg/kg in the two soil types tested.

Note that an independent laboratory validation (ILV, Ref. #4) was also carried out for MMTA in one soil type which demonstrated similar accuracy and precision to the original results: LOQ of 0.001 mg/kg, recoveries of 91.7 – 93.4%, and RSDs of 1.91 – 8.94%.

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, Rochelle Bohaty EPA, on May 20, 2016