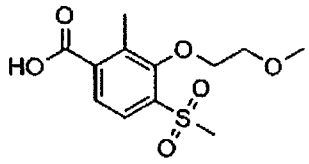
2. Materials

2.1 Analytical Standard

Name:	MMTA
Chemical name:	3-(2-methoxyethoxy)-2-methyl-4-(methylsulfonyl)benzoic acid
Structural formula:	
Storage conditions:	Frozen
Physical State:	White powder
Lot Number:	20140226
Purity:	100%

Certificate of Analysis is presented in Appendix 1.

2.2 Control matrices

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

Soil sample (analytical ID 14/00/77) had been previously classified as a clay soil. Characterisation for this sample is presented below below:

Parameter	Unit	Found value
pH water [1:2.5]		7.6
Sand 2.00-0.063mm	% w/w	22
Silt 0.063-0.002mm	% w/w	37
Clay <0.002mm	% w/w	41
Total Nitrogen	% w/w	0.45
pH $CaCl_2$		7.3
Cation Exchange Capacity	meq/100g	35.7
Organic Carbon by Wet Oxidation	% w/w	4.3

Soil sample (analytical ID 14/00/79) had been previously classified as a sandy loam soil. Characterisation for this sample is presented below below:

Parameter	Unit	Found value
pH water [1:2.5]		6.3
Sand 2.00-0.063mm	% w/w	69
Silt 0.063-0.002mm	% w/w	17
Clay <0.002mm	% w/w	14
Total Nitrogen	% w/w	0.10
pH $CaCl_2$		5.6
Cation Exchange Capacity	meq/100g	10.3
Organic Carbon by Wet Oxidation	% w/w	1.8

3. Methods

3.1 Validation

Sub-samples of each of the two soils were fortified with known concentrations of MMTA then 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 chromatograph once.

3.2 Final extract stability

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

Aliquots of each of the control sample extracts were fortified with MMTA at a concentration of 1 ng analyte/mL of final extract. The concentration of analyte in the stored extracts was determined at day 0 and after 7 days. The concentration of the analyte 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 analyte in the fortified final extract samples with the response of the analyte 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. An aliquot was cleaned up using HLB 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.

3.5 Fortification/calibration solutions

A stock standard solution of MMTA was prepared by dissolving an accurately weighed amount of the test material in a suitable volume of acetonitrile. This stock solution was further diluted with acetonitrile to produce fortification solutions at 10 and 1 µg/mL concentrations.

The instrument calibration solutions for MMTA, over the concentration range 0.025 ng/mL to 10 ng/mL, were prepared on each day of analysis by serial dilution of the fortification solutions in acetonitrile:0.2% acetic acid(10:90 v:v) (details of reagent preparation is detailed in the analytical methodology in Appendix 2):

Standard solution used (ng/mL)	Volume taken (mL)	Final volume (mL)	Nominal concentration (ng/mL)
1000	1	10	100
100	1	10	10
100	0.5	10	5
100	0.25	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 MMTA detected clay type soil at 0.001 mg/kg (analytical identification 14/00/77 F0.001 A, analysis batch 1).

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

$$2.00102\text{e}3 = 23593.5x + 0.178724$$

Where
$y = 2.00102\text{e}3$
$\text{m} = 23593.5$
$\text{c} = 0.178724$

$$\text{Therefore, concentration of MMTA } (x) = \frac{2.00102e3 - 0.178724}{23593.5} = 0.0848 \text{ ng/mL}$$

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

$$\text{MMTA detected (mg/kg)} = \frac{0.0848 \text{ ng/mL} \times 1}{0.1 \text{ g/mL}} = 0.848 \text{ ng/g} = 0.000848 \text{ mg/kg}$$

$$\text{Recovery (\%)} = \frac{0.000848 \text{ mg/kg} \times 100}{0.001 \text{ mg/kg}} = 85\%$$

Appendix 2 Analytical Method

DETERMINATION OF MMTA 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

3. Materials	**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)	

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

Preparation of acetonitrile:0.2% acetic acid (10:90 v:v) - acetonitrile (100 mL) is mixed thoroughly with 0.2% acetic acid in water (900 mL).

Preparation of acetonitrile: 0.2% acetic acid (50:50 v:v) - acetonitrile (500 mL) is mixed thoroughly with 0.2% acetic acid in water (500 mL).

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 substance (corrected for purity if necessary) is accurately weighed and dissolved in acetonitrile to give the stock standard solution. Appropriate dilutions of the stock standard solution are made with acetonitrile to give the fortification standard solutions.

The fortification solutions are progressively diluted with acetonitrile:0.2% acetic acid (10:90 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 cleanup

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: 0.2% acetic acid (50:50 v:v), collecting in a 15 mL graduated polypropylene tube.
6.15 Reduce the extract volume to < 4mL under a steady stream of nitrogen at approximately 40°C.
6.16 Add an aliquot (1 mL) of acetonitrile to the sample extract
6.17 Make to volume (10 mL) using 0.2% acetic acid in water
6.18 Any further dilutions (if required) are made using acetonitrile: 0.2% acetic acid (10:90 v:v).
6.19 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 0.2% acetic acid

↓

Clean-up by OASIS HLB

↓

Reduce volume to < 4 mL

↓

Add an aliquot (1 mL) of acetontrile

↓

Dilute to volume (10 mL) using 0.2% acetic acid

↓

Quantitative analysis by LC-MS/MS

8. LC-MS/MS conditions

Instrument:	AB Sciex API 4000 (Analyst 1.4.2 software) coupled to Waters Acquity UPLC system
Mode:	Ionspray negative
Ion monitoring details:	MMTA: *m/z* 287>184 (Quantitation analysis) MMTA: *m/z* 287>243 (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	90	10
0.2	90	10
2.0	5	95
2.5	5	95
3	90	10
4	90	10

Cycle time:	4 min
Injection volume:	10 µL
Flow rate:	0.5 mL/min
Retention time:	MMTA - approximately 0.9 minutes
LOQ:	0.001 mg/kg
LOD:	0.025 ng/mL (≡ 0.00025 mg/kg in sample matrix)