1. INTRODUCTION

The Bayer CropScience Residue Laboratory, Australia (BCRL), has developed a liquid chromatography (LC) method for the quantitative determination of KIH-485 and the metabolites M1 and M3 in soil by LC MS/MS.

The method is intended to be applied to residue studies required for product registration by the Australian Pesticides & Veterinary Medicines Authority, (APVMA), for Bayer CropScience.

2. SUMMARY

Residues of KIH-485 and M3 in test samples were extracted with 50:50 water:acetone. The extract was filtered using qualitative filter paper, reduced to dryness and reconstituted in 25:75 water:acetonitrile. Residues of M1 in test samples were extracted with water. The extract was filtered using qualitative filter paper, reduced to dryness and reconstituted in 25:75 water:acetonitrile. Chromatography was performed by high performance liquid chromatography coupled to a triple quadrupole mass spectrometer using MRM for detection. Quantitation was achieved using external analytical standards.

The Limit of Quantitation (LOQ) was 0.002 mg/kg for all soil types analysed, which corresponds to the lowest fortification level that was successfully recovered.

The untreated control samples were shown to contain no greater than 30% of the LOQ level.

An excellent linear relationship was observed over a five point calibration range from 0.1 µg/L to 4 µg/L.

Recovery of fortified samples of various soil types (coarse loamy sand, loam and clay) were performed at two levels 0.002 and 0.02 mg/kg.

The average recovery values of all analytes in all soil types at both the 0.002 mg/kg and 0.02 mg/kg levels were within 70-120% with a relative standard deviation of ≤ 20%. See appendix A for full details of recovery values.

The stability of the analytical standards has been previously determined to at least 50 days (2) when stored refrigerated at approximately 4°C in darkness.

3. REFERENCE ITEMS

KIH-485

Common Name:	Pyroxasulfone
Chemical Name:	3-[5-(difluoromethoxy)-1-methyl-3-(trifluoromethyl)pyrazole-4-ylmethylsulfonyl]-4,5-dihydro-5,5-dimethyl-1,2-oxalate
Code Name:	KIH-485
CAS Number:	447399-55-5
Empirical Formula:	$C_{12}H_{14}F_5N_3O_4S$
Molecular Mass:	391.32 g/mole

M1

Code Name:	M1
Chemical Name:	(5-difluoromethoxy-1-methyl-3-trifluoromethyl-1H-pyrazol-4-yl)-methanesulfonic acid
CAS Number:	Not available
Empirical Formula:	$C_7H_7F_5N_2O_4S$
Molecular Mass:	310.2

M3

Code Name:	M3
Chemical Name:	5-difluoromethoxy-1-methyl-3-trifluoromethyl-1H-pyrazole-4-carboxylic acid
CAS Number:	Not available
Empirical Formula:	$C_7H_5F_5N_2O_3$
Molecular Mass:	260.12

4. ANALYTICAL PROCEDURE

4.1 APPARATUS

General laboratory glassware

Balance:	A&D GR-200 or equivalent
Shaker:	Ratek platform mixer
Sonicator:	Transsonic T420
Centrifuge:	Hermle Z300
Ultra pure water system:	Milli-Q Plus
Rotary Evaporator:	Büchi
LC MS/MS:	Applied Biosystems – API 4000 triple quadrupole mass spectrometer Agilent 1100 binary pump Aglient 1100 autosampler or CTC-HTC PAL Agilent 1100 column oven Agilent 1100 degasser

4.2 MATERIALS, REAGENTS AND SOLUTIONS

LC column:	Agilent Eclipse XDB, C18, 5 µm, 150 x 4.6 mm ID
Filter:	Filtech 125mm qualitative filter paper No.1803
Acetone:	HPLC grade
Acetonitrile:	HPLC grade
Acetic acid:	AnalaR
Water:	Milli-Q ultra purified water

4.3. PREPARATION OF ANALYTICAL STANDARD SOLUTIONS

1. Weigh with accuracy 10.0 mg of primary reference grade KIH-485, M1 and M3 and transfer into separate 100 mL volumetric flasks using acetonitrile. Make up to the mark using acetonitrile ensuring that the KIH-485, M1 and M3 are fully dissolved (stock solutions A, B and C). These solutions contain 100.0 mg/L of each reference item.

2. Make dilutions of stock solutions A, B and C in 50:50 acetonitrile:water, to obtain a 1.0 mg/L solution of KIH-485, M1 and M3 (working solution E). Make a dilution of working solution E in 50:50 acetonitrile:water to obtain a 20 µg/L working solution (working solution F).

3. For the analysis of KIH-485, M1 and M3 make dilutions of working solution F in 1:3 water:acetonitrile to obtain working calibration solutions ranging between 0.1 µg/L and 4.0 µg/L. Note: higher concentration calibration solutions may be required.

Stock and working solutions have been determined to be stable for a period of at least 50 days.

4.4 EXTRACTION OF KIH-485 and M3

1. Weigh 50 g of test sample into a 500 mL flat bottom flask.

2. Pipette 100 mL of 50:50 water:acetone into flask.

3. Place flask on a shaker for 30 minutes at 200 rpm.

4. Transfer solution to a tall beaker and sonicate for 15 minutes.

5. Decant an amount of solution into a Teflon centrifuge tube and centrifuge at 6000 rpm for 15 minutes.

6. Pipette 20 mL of solution from centrifuge tube and filter through 125 mm qualitative filter paper into a 500 mL flat bottom flask.

7. Reduce to dryness using a rotary evaporator at approximately 60°C.

8. Reconstitute the residue in 20 mL of 25:75 water:acetonitrile and transfer 0.4 mL to a 2 mL glass autosampler vial and add 1.6 mL of 25:75 water:acetonitrile.

4.5 EXTRACTION OF M1

1. Weigh 50 g of test sample into a 500 mL flat bottom flask.

2. Pipette 100 mL of water into flask.

3. Place flask on a shaker for 30 minutes at 200 rpm

4. Transfer solution to a tall beaker and sonicate for 15 minutes.

5. Decant an amount of solution into a Teflon centrifuge tube and centrifuge at 6000 rpm for 15 minutes.

6. Pipette 20 mL of solution from centrifuge tube and filter through 125 mm qualitative filter paper into a 500 mL flat bottom flask.

7. Reduce to dryness using a rotary evaporator at approximately 60°C.

8. Reconstitute the residue in 20 mL of 25:75 water:acetonitrile and transfer 0.4 mL to a 2 mL glass autosampler vial and add 1.6 mL of 25:75 water:acetonitrile.

Note: If the test item in the test sample is found to be above the calibration range of any reference item, the calibration range can be expanded by the inclusion of additional higher concentrations to encompass the concentration of the test item found in the test sample, provided acceptable linearity is maintained. If this is not possible the samples must be diluted to a level that will fall within the calibration range.

4.6 FORTIFICATION OF TEST SAMPLES

Formula

Fort = (VxC)/W

Where:

Fort = Fortification concentration (mg/kg)
W = Weight of test sample (g)
V = Aliquot volume of reference item solution (mL)
C = Concentration of reference item(s) (mg/L)

Recovery of Test Item

Untreated control test samples with a demonstrated specificity of no interference substances exceeding 30% of the LOQ (0.002 mg/kg) were fortified with the appropriate working solution.

Three replicated recoveries were performed at the fortification levels of 0.002 mg/kg (LOQ) and 0.02 mg/kg for each soil type identified in appendix A.

4.7 INSTRUMENT CONDITIONS

The final test sample extract was analysed by LC MS/MS using the conditions outlined below.

LC conditions for KIH-485 and M3

LC column:	Agilent Eclipse XDB, C18, 5 µm, 150 mm x 4.6 mm
Injection volume:	10 µL
Pump A:	0.1% v/v acetic acid in water
Pump B:	0.1% v/v acetic acid in acetonitrile
LC run time:	9.0 minutes
LC column heater:	40°C

LC gradient

Gradient time (minutes)	Flow rate (mL/min)	Gradient ratio (A:B)	Gradient curve
0.00	0.5	50:50	
1.00	0.5	50:50	Linear
2.00	0.5	10:90	
7.00	0.5	10:90	
9.00	0.5	50:50	

MS conditions for KIH-485 and M3

Ion source:	Turbo spray
Ionisation mode:	Negative
Scan type:	MRM
Resolution Q1:	Unit
Resolution Q3:	Unit
Source conditions:	
CUR:	10 psi
GS1:	50 psi
GS2:	50 psi
IS:	-4500 V
TEM:	300°C
ihe:	On
CAD:	10 psi

Compound Conditions:

Compound	Q1 Mass (m/z)	Q3 Mass (m/z)	Dwell (msec)	DP (volts)	CE (volts)	EP (volts)	CXP (volts)
KIH-485	390	340	800	-65	-22	-10	-15
M3	259	165	800	-24	-23	-10	-15

Where:
DP = Declustering potential
CE = Collision energy
EP = Entrance potential
CXP = Exit potential

Retention Time

The expected retention times for the test/reference items under the above conditions are approximately:

Test/reference Items	Retention Time (minutes)
KIH-485	5.95
M3	4.40

The above retention times might vary slightly due to variation in the mobile phase.

LC conditions for M1

LC column:	Agilent Eclipse XDB, C18, 5 µm, 150 mm x 4.6 mm
Injection volume:	10 µL
Pump A:	0.1% v/v acetic acid in water
Pump B:	0.1% v/v acetic acid in acetonitrile
LC run time:	6.0 minutes
LC column heater:	40°C

LC gradient

Gradient time (minutes)	Flow rate (mL/min)	Gradient ratio (A:B)	Gradient curve
0.00	0.5	50:50	Linear
6.00	0.5	50:50	

MS conditions for M1

Ion source:	Turbo spray
Ionisation mode:	Negative
Scan type:	MRM
Resolution Q1:	Unit
Resolution Q3:	Unit

Source conditions:

CUR:	10 psi
GS1:	50 psi
GS2:	50 psi
IS:	-4500 V
TEM:	300°C
ihe:	On
CAD:	10 psi

Compound Conditions:

Compound	Q1 Mass (m/z)	Q3 Mass (m/z)	Dwell (msec)	DP (volts)	CE (volts)	EP (volts)	CXP (volts)
M1	309	259	800	-56	-14	-10	-15

Where:
DP = Declustering potential
CE = Collision energy
EP = Entrance potential
CXP = Exit potential

Retention Time for M1

The expected retention time for the test/reference item under the above conditions is approximately:

Test/reference Item	Retention Time (minutes)
M1	3.77

The above retention time might vary slightly due to variation in the mobile phase

Integration Method

The analyte peaks are integrated using the "Analyst" software supplied by Applied Biosystems.

5. CONVERSION FACTORS

Dilution Formula

$$Dil = (VxF_{inal})/(WxV_1)$$

Where:

Dil = Dilution factor
W = Weight of test sample (g)
V_1 = Aliquot volume of test sample (mL)
V = Total extraction volume (mL)
F_{inal} = Final extract volume (mL)

Molecular Mass Conversion Formula

$Conc_A = Conc_{metabolite} \times (MW_{parent} / MW_{metabolite})$

Where:

$Conc_A$ = Concentration of parent test item (mg/kg)
MW_{parent} = Molecular weight of parent test (g/mole)
$Conc_{metabolite}$ = Concentration of metabolite test item (mg/kg).
$MW_{metabolite}$ = Molecular weight of metabolite test item (g/mole)

Conversion of M1 to KIH-485

$Conc_{KIH-485} = Conc_{M1} \times (391.3 / 310.20)$

Conversion of M3 to KIH-485

$Conc_{KIH-485} = Conc_{M3} \times (391.3 / 260.12)$