1. INTRODUCTION

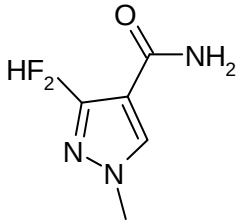
1.1 Scope and Chemical Structures

Analytical method GRM023.04A is suitable for the determination of CSCC210616 in soil. The limit of quantification (LOQ) of the method has been established at 0.0001 mg/kg for CSCC210616.

This method satisfies OECD guidance document ENV/JM/MON)(2007)17, US EPA guidelines OPPTS 860.1340 and OPPTS 850.7100 and EU guidelines SANCO/3029/99 rev. 4 and SANCO/825/00 rev. 7.

Figure 1

Compound Code Number	:	CSCC210616
Alternative Compound Code Number	:	SYN508272
CAS Number	:	Not in registry
IUPAC Name	:	3-difluoromethyl-1-methyl-1H-pyrazole-4-carboxylic acid amide
Molecular Formula	:	$C_6H_7F_2N_3O$
Molecular Weight	:	175.14



1.2 Method Summary

10 g soil samples are extracted by shaking with acetonitrile/water 80/20 v/v. 10 mL aliquots are evaporated to eliminate the acetonitrile then diluted with ultra pure water. Sample clean up and concentration is by a solid phase extraction (SPE) procedure using Oasis HLB SPE cartridges. CSCC210616 is eluted from the cartridge with acetonitrile. Samples are evaporated to dryness and dissolved in ultra pure water. Final determination is by high performance liquid chromatography with triple quadrupole mass spectrometric detection (LC-MS/MS). The limit of quantification of the method is 0.0001 mg/kg.

2. MATERIALS AND APPARATUS

2.1 Apparatus

The recommended equipment and apparatus are listed in Appendix 1. Equipment with equivalent performance specifications may be substituted.

2.2 Reagents

All solvents and other reagents must be of high purity, e.g. glass distilled/HPLC grade solvents and analytical grade reagents. Particular care must be taken to avoid contamination of the reagents used. Reagents of comparable purity may be substituted as long as acceptable performance is demonstrated. A list of reagents used in this method along with details of preparation of solutions is included in Appendix 2.

2.3 Preparation of Analytical Standard Solutions

It is recommended that the following precautions should be taken when weighing the analytical materials.

1. Ensure good ventilation.
2. Wear gloves and laboratory coat.
3. Prevent inhalation and contact with mouth.
4. Wash any contaminated area immediately.

2.3.1 Stock Solutions

Prepare 200 μg/mL stock solution for CSCC210616 by one of the following methods.

Weigh out accurately, using a five figure balance, sufficient CSCC210616 analytical standard and carefully transfer into a “Class A” volumetric flasks (50 mL). Dilute to the 50 mL mark with acetonitrile to give 200 μg/mL stock solutions of CSCC210616.

Alternatively, the appropriate volume of acetonitrile to add to a known amount of standard material may be determined using the equation below. The standard concentration is corrected for its chemical purity.

$$V = \frac{W \times P}{C} \times 1000$$

P = Standard purity in decimal form (P(%)/100)
V = Volume of acetonitrile required
W = Weight, in mg, of the solid analytical standard
C = Desired concentration of the final solution, (μg/mL)
1000 = Unit conversion factor

In this case, the standard material is weighed directly into an appropriate storage vessel.

2.3.2 Fortification Solutions

Sample fortification solutions should be prepared in acetonitrile from the primary stock solution in "Class A" volumetric flasks. It is recommended that, as a minimum, the following solutions are prepared by serial dilution: 10 μg/mL, 1.0 μg/mL, 0.1 μg/mL and 0.01 μg/mL. The preparation of LC-MS/MS calibration standards is discussed in Section 3.8.

2.3.3 Standard Solution Storage and Expiration

All stock solutions should be stored in a refrigerator or freezer when not in use to prevent decomposition and/or concentration of the standard. Standard solutions should be allowed to equilibrate to room temperature prior to use.

An expiration date of six months is recommended unless additional data are generated to support a longer expiration date.

2.4 Safety Precautions and Hazards

The following information is included as an indication to the analyst of the nature and hazards of the reagents used in this procedure. If in any doubt, consult the appropriate MSDS or a monograph such as 'Hazards in the Chemical Laboratory', edited by S G Luxon, The Chemical Society, London (Reference 1).

Solvent and Reagent Hazards

	Acetonitrile	Methanol	Acetic acid
Harmful Vapour	✓	✓	✓
Highly Flammable	✓	✓	✖
Harmful by Skin Absorption	✓	✓	✓
Irritant to respiratory system and eyes	✓	✓	✓
Causes severe burns	✖	✖	✓
Syngenta Hazard category	SHC-C, S	SHC-C, S	SHC-C, S
OES Short Term (mg/m^3)	105	310	37
OES Long Term (mg/m^3)	70	260	25

N/A – not known

In all cases avoid breathing vapour. Avoid contact with eyes and skin.

At present there are insufficient data available to assign a Syngenta Hazard Category for CSCC210616. It should be treated as a category SHC-D compound until further information indicates otherwise. The Syngenta Hazard Category scale rates highly toxic chemicals as category SHC-E and non toxic chemicals as category SHC-A. An additional S designation indicates a skin irritant.

3. ANALYTICAL PROCEDURE

The method is summarized in flow chart form in Appendix 8.

3.1 Modifications and Potential Problems

a) Bottled HPLC grade water is used to prepare aqueous mobile phase as this gives a reduced MS/MS background signal when compared to water from a laboratory water purification system.

b) To prevent contamination of the instrument and to minimise possible carry-over issues, it is recommended that high level recoveries (>0.1 mg/kg) and samples with expected residues greater than 0.1 mg/kg should be diluted so that the final analyte concentration does not exceed 0.005 µg/mL. It may also be useful to include blank injections of ultra pure water after high level samples to clear any observed carry-over greater than 10% of the LOQ.

c) During development it has been observed that the sensitivity of the method is affected by the presence of acetonitrile in the HPLC system. It is therefore important that the system is purged with the specified mobile phase prior to analysis. See Section 4.2. It is also recommended that a minimum of 20 priming injections are included at the start of an analysis batch.

d) During development it has been observed that response of CSCC210616 is variable on the Applied Biosystems API 4000 triple quadrupole mass spectrometer dependent on the instrument condition. Should poor sensitivity be observed during analysis it is recommended that the front plate in the ionisation source is wiped clean with tissue moistened with water and methanol.

3.2 Sample Preparation

All samples should be prepared using an approved method of preparation to obtain a homogeneous sample prior to analysis.

3.3 Sample Fortification

In order to verify method performance and allow recovery corrections to be made (if appropriate), fortified control samples should be included with each sample set. To each pre-weighed control soil sample, add the appropriate amount of standard solution containing CSCC210616 in acetonitrile. Let each sample stand for at least five minutes after fortification to allow the spiking solution to soak into the matrix before proceeding with the extraction procedure. At least one untreated control and two fortified control samples should be analysed with each sample set.

3.4 Extraction

a) Weigh a representative amount of soil (10 g) into a 250 mL plastic bottle and add 50 mL acetonitrile / ultra pure water 80/20 v/v. Secure the lid and shake sample on a flat bed shaker for 1 hour at a speed which visibly agitates the contents of the bottle.

b) Centrifuge the samples at a speed which separates the supernatant from the soil e.g. 4500 rpm for 5 minutes. The sample concentration is 0.2 g/mL.

c) Transfer 10 mL aliquots (equivalent to 2 g soil) into clean, plastic graduated centrifuge tubes (15 mL size). Reduce sample volume to approximately 1 mL ± 0.1 mL by evaporation in a heating block set to 50°C under a steady stream of air. This takes approximately 1 to 1.5 hours.

d) Adjust the sample volume to 10 mL with ultra pure water, cap and ultrasonicate for a few seconds to ensure sample is mixed thoroughly.

3.5 Solid Phase Extraction

a) Take one Waters Oasis HLB SPE cartridge (60 mg, 3 mL size) for each sample to be analysed and place on a suitable vacuum manifold (e.g. IST Vacmaster). Add acetonitrile (2 mL) and allow to percolate through under gravity or draw through under vacuum to the level of the top frit at a rate of approximately 1 mL/min, discarding the cartridge eluate. Do not allow the cartridges to become dry. Add ultra pure water (2 mL) to the top of each cartridge and allow to percolate through

under gravity or draw through under vacuum to the level of the top frit at the same rate, again discarding the cartridge eluate. Do not allow the cartridges to become dry.

b) Using a suitable connector, attach a cartridge reservoir (e.g. 30 mL capacity) fitted with a 20 µm polyethylene frit to prevent blockage of the SPE cartridge with any particulate material in the extract.

c) Load the soil extracts from Section 3.4 (d) onto the SPE cartridges via the column reservoir and allow to percolate through under gravity or under low vacuum, at a rate of approximately 1-2 mL/min, to the level of the top frit. Do not allow cartridges to become dry.

d) On completion of loading, remove the cartridge reservoir and connector. Add ultra pure water (2 mL) to centrifuge tube, cap and shake well before adding the rinse to the top of the SPE cartridge. Allow to percolate through under gravity or draw through under vacuum to the level of the top frit at the same rate, again discarding the cartridge eluate. Remove the excess water and dry the cartridge by application of high vacuum for 15 minutes.

e) Place suitable disposable, plastic, graduated centrifuge tubes (15 mL size) under each port, as required, in the manifold rack. Add acetonitrile (2 mL) to the top of each cartridge and allow to percolate through under gravity. Collect the column eluate containing CSCC210616. Remove the excess solvent from the cartridges by application of positive pressure or vacuum, collecting the column eluate.

f) Evaporate the collected eluates to dryness under a stream of air in a sample concentrator with the heating block set at 40 ^{o}C.

g) Dissolve the residues in ultra pure water (1 mL) and mix sample thoroughly by ultrasonicating the contents of the centrifuge tube briefly.

h) Transfer the samples into suitable autosampler vials for final determination by LC-MS/MS. The final sample concentration is 2 g/mL.

3.6 Time Required for Analysis

The methodology can be performed with a batch of up 20 samples. One person can complete the analysis of 20 samples in 1 day (8 hour working period).

3.7 Method Stopping Points

The analytical procedure can be stopped at various points for overnight and weekend breaks unless otherwise specified in the analytical procedure. Acceptable method recoveries will validate any work flow interruptions. Samples should be stored refrigerated in sealed containers where the analysis cannot be completed in a single day.

3.8 Preparation of Calibration Standards for LC-MS/MS

No significant suppression or enhancement of the instrument response for CSCC210616 was observed in the soil types tested using the above procedure. Full details of matrix effects during method validation are presented in Table 4, Appendix 3. Non-matrix calibration standards should be prepared as described below. Samples should be quantified against non-matrix calibration standards where possible. Any significant matrix effects observed may be compensated for using matrix matched standards, at the discretion of the study director.

To prepare e.g., an LOQ equivalent calibration standard of CSCC210616 (0.0002 µg/mL), transfer ultra pure water (approximately 9 mL) into a 10 mL volumetric flask and add 20 µL of a 0.1 µg/mL CSCC210616 standard in acetonitrile. Adjust to the 10 mL mark with ultra pure water. Stopper the flask securely and shake to mix thoroughly. Transfer an aliquot of the standard into a suitable autosampler vial for analysis by LC-MS/MS.

A calibration curve may also be generated to quantify CSCC210616 residues. Standards over an appropriate concentration range should be prepared as described above, using the requisite volumes of CSCC210616 standards in acetonitrile.

4. FINAL DETERMINATION

The following instrumentation and conditions have been found to be suitable for this analysis. Other instrumentation can also be used, though optimisation may be required to achieve the desired separation and sensitivity. The operating manuals for the instruments should always be consulted to ensure safe and optimum use. The method has been developed for use on the Applied Biosystems API 4000 LC-MS/MS.

Final determination by LC-MS/MS with 2 transitions is considered to be highly specific; hence no further confirmatory conditions are included.

4.1 Instrument Description

HPLC system	:	Shimadzu Prominence
Pump	:	Shimadzu LC-20 AD
Degasser	:	Shimadzu DGU-20A5
Column Oven	:	Shimadzu CTO-20A
Detector	:	Applied Biosystems API 4000 triple quadrupole mass spectrometer with Analyst™ software version 1.4.1
Autosampler	:	Shimadzu SIL-HTC

4.2 Chromatography Conditions

Column	:	Phenomenex Synergi 4µm Hydro-RP 80A, 150 mm x 3 mm i.d.,
Column Oven Temperature	:	30°C
Injection volume	:	50 µL
Stop Time	:	9.0 minutes
Injection protocol	:	Analyse calibration standard after 3-4 sample injections
Mobile phase	:	Solvent 1 = 0.1% v/v acetic acid in methanol Solvent 2 = 0.1% v/v acetic acid in ultra pure water

Mobile phase composition

Time (min)	% Solvent 1	% Solvent 2	Flow, mL/min
0.0	20	80	0.3
3.0	90	10	0.3
7.0	90	10	0.3
7.1	20	80	0.3
9.0	20	80	0.3

Under these conditions the retention time of CSCC210616 is approximately 5.1 minutes.

Note : It is not necessary to reduce the flow rate into the mass spectrometer when using the API 4000.

4.3 Mass Spectrometer Conditions

Interface	:	TurboIonSpray
Polarity	:	Positive
Curtain gas (CUR)	:	Nitrogen set at 50 (arbitrary units)
Temperature (TEM)	:	550 °C
Ionspray voltage	:	5500 V
Collision gas setting (CAD)	:	Nitrogen set at 6 (arbitrary units)
Gas 1 (GS1)	:	Air set at 60 (arbitrary units)
Gas 2 (GS2)	:	Air set at 60 (arbitrary units)
Interface heater (ihe)	:	On
Scan type	:	MRM

MRM Conditions		**CSCC210616 Primary Transition**	**CSCC210616 Confirmatory Transition**
Q1 *m/z*	:	176	176
Q3 *m/z*	:	156	136
Dwell time	:	350 ms	350 ms
Resolution Q1	:	Unit	Unit
Resolution Q3	:	Unit	Unit
Declustering potential (DP)	:	45 V	45 V
Entrance potential (EP)	:	10 V	10 V
Collision energy (CE)	:	14 V	24 V
Collision cell exit potential (CXP)	:	12 V	12 V

Typical chromatograms are shown in Appendix 4.

5. CALCULATION OF RESULTS

5.1 Single Point Calibration Procedure

CSCC210616 residues may be calculated in mg/kg for each sample using a mean standard response from each of the injections bracketing the sample as follows.

a) Make repeated injections of a standard containing CSCC210616 at an appropriate concentration into the LC-MS/MS operated under conditions as described in Section 4. When a consistent response is obtained, measure the peak area obtained for CSCC210616.

b) Make an injection of each sample solution and measure the areas of the peaks corresponding to CSCC210616.

c) Re-inject the standard solution after a maximum of four injections of sample solutions.

d) Calculate the CSCC210616 residue in the sample, expressed as mg/kg using a mean standard response from each of the injections bracketing the sample as follows.

$$\text{Residue (mg/kg)} = \frac{\text{PK area (SA)}}{\text{PK area (STD)}} \times \frac{\text{Standard Conc.}}{\text{Sample Conc.}}$$

PK area (SA) = Peak response for sample

PK area (STD) = Average peak response for bracketing standards

Standard Conc. = Concentration of standard (μg/mL)

Sample Conc. = Sample concentration (g/mL)

If residues need to be corrected for average percentage recovery e.g. for storage stability studies, then the equation below should be used.

$$\text{Corrected Residue} = \frac{\text{Residue} \times 100}{\text{Average percentage Recovery}} \text{ (mg/kg)}$$

Although single point calibration may be used to quantify residues it is recommended that a calibration curve is generated with each analytical run to demonstrate the linearity of instrument response (Reference 2).

5.2 Multi Point Calibration Procedure

CSCC210616 residues may be calculated in mg/kg for each sample as follows.

a) Prepare standard solutions over a concentration range appropriate to the expected residues in the samples (for example, 50% LOQ to 10 x LOQ). An appropriate number of different concentrations within this range should be prepared (at least four).

b) Make an injection of each sample solution and measure the areas of the peaks corresponding to CSCC210616. Calibration standard solutions should be interspersed throughout the analysis, after a maximum of four injections of sample solutions.

c) Generate calibration curve parameters using an appropriate regression package.

d) The following equation can be rearranged and used to calculate residues as follows:

$$y = mx + c$$

Where y is the instrument response value, x is the standard concentration, m is the gradient of the line of best fit ("X-variable 1" in MS Excel) and c is the intercept value. An example of this equation generated using the experimental values of m and c should be included in the raw data, as should the "R-Squared" value for the regression.

Re-arrangement for *x* gives

$$x = \frac{y - c}{m}$$

e) Alternatively (depending on the regression analysis software available) a quadratic equation may be used to fit the data. In this case the following general equation should be re-arranged and used to calculate residues:

$$y = a + bx + cx^2$$

Where *y* is the instrument response value, x is the standard concentration and *a*, *b*, *c* are constants.

f) Calculate the CSCC210616 residue in the sample, expressed as mg/kg as follows

$$\text{Residue (mg/kg)} = \frac{\text{Analyte found (µg/mL)}}{\text{Sample conc. (g/mL)}}$$

Where analyte found (µg/mL) is calculated from the standard calibration curve and sample conc. is the final sample concentration in g/mL.

If residues need to be corrected for average percentage recovery e.g. for storage stability studies, then the equation below should be used

$$\text{Corrected Residue} = \frac{\text{Residue} \times 100}{\text{Average percentage Recovery}} \text{ (mg/kg)}$$

6. CONTROL AND RECOVERY SAMPLES

Control samples should be completed as detailed in Section 3 for each set of samples analysed to verify that samples are free from CSCC210616 contamination. A minimum of one control should be analysed with each batch of samples.

At least two recovery samples (control samples accurately fortified with a known amount of CSCC210616 prior to extraction) should also be completed alongside each batch of samples. Provided the recovery values are acceptable they may be used to correct any CSCC210616 residues found. The recovery levels should be appropriate to the residue levels expected.

Recovery efficiency is generally considered acceptable when the mean values are between 70% and 110% and with a relative standard deviation of $\leq$20%.

7. SPECIFICITY

It is recommended that reagent blank samples be included in a sample set if contamination is suspected.

7.1 Matrix Interference

LC-MS/MS is a highly specific detection technique. Interference arising from the soil matrices tested has not been observed.

7.2 Reagent and Solvent Interference

Using high purity solvents and reagents no interference has been found.

7.3 Labware Interference

All reusable glassware should be detergent washed and rinsed with HPLC grade methanol, acetone or acetonitrile prior to use.

APPENDIX 1 APPARATUS

UK suppliers

General glassware, available from Fisher Scientific UK, Bishop Meadow Road, Loughborough, Leicestershire LE11 5RG

Plastic centrifuge bottles, 250 mL size, available from Fisher Scientific UK, Bishop Meadow Road, Loughborough, Leicestershire LE11 5RG, UK

Mechanical shaker, available from Fisher Scientific UK, Bishop Meadow Road, Loughborough, Leicestershire LE11 5RG

Laboratory centrifuge, available from Fisher Scientific UK, Bishop Meadow Road, Loughborough Leicestershire LE11 5RG.

Isolute Vacmaster-20™ sample processing station, available from Biotage, Tir-y-Berth Industrial Estate, New Road, Hengoed, Mid Glamorgan, CF8 8AU

Column reservoirs fitted with 20µm polyethylene frits, 30 mL size, available from Agilent Technologies UK Limited, Chemical Analysis Group, Lakeside Heath, Cheadle Royal Business Park, Stockport, Cheshire. SK8 3GR

Oasis HLB solid phase extraction cartridges 60 mg, 3 mL size, available from Waters U.K. Ltd., 730-740 Centennial Court, Elstree, Hertfordshire, WD6 3SZ

Plastic disposable pipettes, available from Fisher Scientific UK, Bishop Meadow Road, Loughborough, Leicestershire, LE11 5RG.

Polypropylene centrifuge tubes, 15 mL capacity, available from Fisher Scientific, Bishop Meadow Road, Loughborough, Leicestershire LE11 5RG

Ultrasonic bath e.g. Ultrawave U300/D, available from Fisher Scientific UK, Bishop Meadow Road, Loughborough, Leicestershire, LE11 5RG,.

Crimp cap autosampler vials and caps available from Agilent Technologies UK Limited, Chemical Analysis Group, Lakeside Heath, Cheadle Royal Business Park, Stockport, Cheshire. SK8 3GR

API 4000 LC-MS/MS system equipped with a TurboIonSpray source, available from Applied Biosystems, 120 Birchwood Boulevard, Warrington, Cheshire WA3 7PB.

Shimadzu Prominence HPLC system equipped with autosampler, dual piston pump, vacuum degasser and column compartment with column switching valve, available from Shimadzu UK Limited, Mill Court, Featherstone Road, Wolverton Mill South, Milton Keynes MK12 5RD.

HPLC column, Phenomenex Synergi 4µ Hydro-RP 80A, 150 mm x 3 mm i.d., (Part No. 00F-4375-Y0) available from Phenomenex, Queens Avenue, Hurdsfield Industrial Estate, Macclesfield, Cheshire, SK10 2BN, UK

Peak Scientific NM20ZA gas station, available from Peak Scientific Instruments Ltd., Fountain Crescent, Inchinnan Business Park, Inchinnan, Renfrew PA9 4RE.

US suppliers

General glassware, available from Fisher Scientific, Liberty Lane, Hampton NH 03842

Plastic centrifuge bottles, 250 mL size, available from Fisher Scientific, Liberty Lane, Hampton NH 03842

Mechanical shaker, available from Fisher Scientific, Liberty Lane, Hampton NH 03842

Laboratory centrifuge, available from Fisher Scientific Fisher Scientific, Liberty Lane, Hampton NH 03842

Isolute Vacmaster-20^{TM} sample processing station available from Jones Chromatography USA Ltd., PO Box 280 329, Lakewood, Colorado, 8022-0329

Column reservoirs, 30 mL size fitted with 20µm polyethylene frits, available from available from Agilent Technologies, 395 Page Mill Road, Palo Alto, CA 94304.

Oasis HLB solid phase extraction cartridges 60 mg, 3 mL size, available from Waters Corporation,34 Maple Street, Milford, MA 01757.

Plastic disposable pipettes, available from Fisher Scientific, Liberty Lane, Hampton NH 03842

Polypropylene centrifuge tubes, 15 mL capacity, available from Fisher Scientific, Liberty Lane, Hampton NH 03842

Ultrasonic bath e.g. Ultrawave U300/D, available from Fisher Scientific, Liberty Lane, Hampton NH 03842

Crimp cap auto sampler vials and caps, available from Agilent Technologies, 395 Page Mill Road, Palo Alto, CA 94304

API 4000 LC-MS/MS system equipped with a TurboIonSpray source, available from Applied Biosystems, 850 Lincoln Center, Foster City, CA 94404-1128

Shimadzu Prominence HPLC system equipped with autosampler, dual piston pump, vacuum degasser and column compartment with column switching valve, available from Shimadzu Scientific Instruments, 7102 Riverwood Drive, Columbia, MD 21046

HPLC column, Phenomenex Synergi 4µ Hydro-RP 80A, 150 mm x 3 mm i.d., (Part No. 00F-4375-Y0), available from Phenomenex, 411 Madrid Ave, Torrence, CA90501-1430

Peak Scientific NM20ZA gas station, available from Peak Scientific Instruments, 1300 West Belmont Ave., Chicago IL 60657

APPENDIX 2 REAGENTS

UK suppliers

Solvents: Acetonitrile, methanol and ultra pure water (HPLC grade), available from Rathburn Chemicals Ltd., Walkerburn, Scotland EH43 6AU

Analytical grade concentrated glacial acetic acid available from Sigma-Aldrich, The Old Brickyard, New Road, Gillingham, Dorset. SP8 4XT or www.sigmaaldrich.com

CSCC210616 analytical standard, available from GLP Testing Facility, Syngenta, CH-4333, Munchweilen, Switzerland.

US suppliers

Solvents: Acetonitrile and methanol available from B & J Brand Solvents, from Scientific Products Division of Baxter Healthcare Corporation, USA.

Ultra pure HPLC grade water from e.g. Fluka via Sigma-Aldrich www.sigmaaldrich.com

Analytical grade concentrated glacial acetic acid, available from www.sigmaaldrich.com

CSCC210616 analytical standards, available from Syngenta Crop Protection, Inc., P.O. Box 18300, Greensboro, NC 27419-8300.

Preparation of reagents

1. 80/20 v/v acetonitrile/ultra pure water: Add 800 mL of acetonitrile to 200 mL ultra pure water in a 1 L volumetric flask. Stopper the flask securely and shake to mix thoroughly.

2. 0.1% v/v acetic acid in ultra pure water: Add 1 mL glacial acetic acid to ultra pure water in a 1 L volumetric flask. Adjust to the 1 L mark with ultra pure water. Stopper the flask securely and shake to mix thoroughly.

3. 0.1% acetic acid in methanol: Add 1 mL glacial acetic acid to methanol in a 1 L volumetric flask. Adjust to the 1 L mark with methanol. Stopper the flask securely and shake to mix thoroughly

Determination of LC-MS/MS matrix effects

The effect of soil matrices on the LC-MS/MS signal was assessed by preparing standards in the presence of soil matrix and comparing the peak area of CSCC210616 against non-matrix standards at an equivalent concentration. Non-matrix calibration standards are prepared as described in Section 3.8.

To prepare for example, a 0.002 µg/mL matrix-matched standard, add 20 µL of a 0.1 µg/mL CSCC210616 standard in acetonitrile to a 980 µL aliquot of a control extract from Section 3.5 (g) in a suitable autosampler vial. Cap the vial securely and shake gently to mix. Analyse by LC-MS/MS.

A summary of the data is presented below.

Table 4 :

Soil Type	CSCC210616 nominal concentration (ng/mL)	% Matrix Effect for CSCC210616	
		m/z 176 → 156	*m/z* 176 → 136
Clay (soil type 6S)	2	+ 3	+ 3
	0.2	- 4	- 2
Loamy sand (soil type 2.2)	2	+ 3	+ 3
	0.2	- 2	+ 2

Matrix effects were not significant and non-matrix standards were used for quantification during method validation.

APPENDIX 6 API4000 MS/MS TUNING PROCEDURE

Calibration of instrument

The instrument must be mass calibrated on a regular basis using polypropylene glycol (PPG) solutions according to the manufacturer's instructions. Calibrate both mass resolving quadrupoles (Q1 and Q3).

Tuning instrument for CSCC210616

Infuse separate standard solutions of CSCC210616 (0.1 to 1.0 µg/mL in mobile phase, see section 4) directly into the mass spectrometer interface at a rate at of about 10 µL/min. Roughly adjust interface parameters (sprayer position, spray, heater/auxiliary gas flows, as well as voltages of spray, orifice, and focusing ring) for a sufficiently high parent ion signal at *m/z* 176 in positive ionisation mode for Using the Analyst 1.4 software quantitative optimisation routine, tune the instrument for CSCC210616, ensuring that the correct ions are selected (initial Q1 *m/z* 176 and product ions *m/z* 156 and 136). Alternatively, the instrument ion optics and collision energy may be tuned manually for CSCC210616 to ensure maximum sensitivity.

Note: If problems are encountered in tuning the instrument for these ions, the ions should be entered in the method as detailed in Section 4.3 and tuning performed manually.

Finally, connect the LC-pump via the autosampler directly to the MS/MS instrument. Perform repetitive flow injections of CSCC210616 standards in mobile phase and at the flow rate to be used. Tune the interface parameters (sprayer position, spray and heater gas flows, spray, orifice, and focusing ring voltages) and the collision gas flow for maximum sensitivity.

In positive ionisation mode, the protonated molecular ions generated in the ion source (*m/z* 176) are selected and subjected to further fragmentation by collisional activation. The two most sensitive daughter ions (*m/z* 156 and *m/z* 136) are then selected and used for quantitative analysis. The daughter ions correspond to loss of HF and 2 x HF respectively.

Final determination by LC-MS/MS with 2 transitions is considered to be highly specific; hence no further confirmatory conditions are included.

APPENDIX 8 METHOD FLOWCHART