

## 1.0 INTRODUCTION

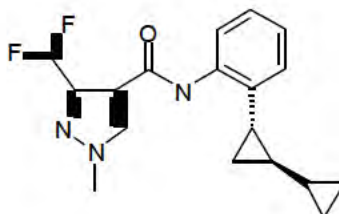
### 1.1 Scope and chemical structures

Analytical method GRM023.02A is suitable for the determination of SYN524464 as SYN508210 and SYN508211 (Figures 1 and 2 respectively) in soil. The limit of quantification (LOQ) of the method has been established at 0.0001 mg/kg.

This method satisfies EU guidelines SANCO/3029/99 rev. 4, SANCO/825/00 rev. 7 and US EPA guidelines OPPTS 850.7100 and OPPTS 860.1340

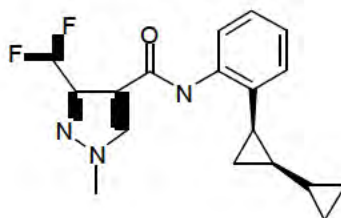
**Figure 1**

<b>Compound Code Number</b>	: SYN508210
<b>CAS Number</b>	: 599197-38-3
<b>IUPAC Name</b>	: 3-Difluoromethyl-1-methyl-1 <i>H</i> -pyrazole-4-carboxylic acid (( <i>IRS,2SR</i> )-2-bicyclopropyl-2-yl-phenyl)amide
<b>Molecular Formula</b>	: C <sub>18</sub> H <sub>19</sub> F <sub>2</sub> N <sub>3</sub> O
<b>Molecular Weight</b>	: 331.4



**Figure 2**

<b>Compound Code Number</b>	: SYN508211
<b>CAS Number</b>	: 599194-51-1
<b>IUPAC Name</b>	: 3-Difluoromethyl-1-methyl-1 <i>H</i> -pyrazole-4-carboxylic acid (( <i>IRS,2RS</i> )-2-bicyclopropyl-2-yl-phenyl)amide
<b>Molecular Formula</b>	: C <sub>18</sub> H <sub>19</sub> F <sub>2</sub> N <sub>3</sub> O
<b>Molecular Weight</b>	: 331.4



## **1.2 Method summary**

10 g sub samples of soil are extracted in 80:20 v/v acetonitrile:ultra pure water by shaking at room temperature. After centrifugation, aliquots of the soil extracts (2 g, equivalent to 10 mL) are diluted with water and taken through a solid phase extraction (SPE) procedure. SYN508210 and SYN508211 are eluted from the cartridge with acetonitrile and the eluate is evaporated. Samples are diluted with ultra pure water and aliquots transferred into autosampler vials. 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.

## **2.0 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 a 200 µg/mL stock solution for SYN508210 and SYN508211 by one of the following methods.

Weigh out accurately, using a five figure balance, sufficient SYN508210 and SYN508211 analytical standard into separate amber "Class A" volumetric flask (50 mL). Dilute to the mark with acetonitrile to give a 200 µg/mL stock solution of SYN508210 and SYN508211.

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 containing SYN508210 and SYN508211 should be prepared by serial dilution in acetonitrile. Mixed SYN508210 and SYN508211 standards may be prepared if required. It is recommended that the following solutions are prepared: 10.0 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 for SYN508210 and SYN508211 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

	Methanol	Acetonitrile	Formic acid
Harmful Vapour	9	9	9
Highly Flammable	9	9	8
Harmful by Skin Absorption	9	9	9
Irritant to respiratory system and eyes	9	9	9
Causes severe burns	8	8	9
Syngenta Hazard Category (SHC)	SHC-C, S	SHC-C, S	SHC-C, S
OES Short Term (mg/m <sup>3</sup> )	310	105	N/A
OES Long Term (mg/m <sup>3</sup> )	260	70	9

N/A not known

At present there are insufficient data available to assign a Syngenta Hazard Classification for SYN508210 and SYN508211. They should be treated as category SHC-C compounds 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 hazard category of S indicates the compound is a severe skin and eye irritant.

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

### 3.0 ANALYTICAL PROCEDURE

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

#### 3.1 Modifications and potential problems

- Bottled HPLC grade ultra pure water is used to prepare the LC mobile phase, which produces a lower background noise in the MS/MS chromatograms than water taken from a laboratory water purification system.

#### 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 SYN508210 and SYN508211 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 representative amounts of soil (10 g) into a plastic centrifuge bottles (250 mL size).
- b) Add 80:20 v/v acetonitrile:ultra pure water (50 mL) and attach the bottle lids securely. Shake at room temperature for 1 hour using a mechanical shaker, at a speed which visibly agitates the samples.
- c) Centrifuge the samples at a speed which separates the supernatant from the soil e.g. 4000 rpm for 5 minutes. The sample concentration is 0.2 g/mL.
- d) Transfer 10 mL aliquots (equivalent to 2 g soil) into clean, plastic graduated centrifuge tubes (50 mL size). Dilute to 50 mL with ultra pure water. Cap the tubes securely and shake to mix thoroughly.

### **3.5 Solid phase extraction procedure.**

- 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 methanol (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 column 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 column eluate. Do not allow the cartridges to become dry.
- b) Using a suitable column connector, attach a column reservoir (70 mL capacity) fitted with a 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 column reservoir and connector. Add 90:10 v/v ultra pure water:acetonitrile (2 mL) to the top of the SPE 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 column eluate. Remove the excess water under vacuum by application of high vacuum for a few seconds.
- e) Place suitable disposable, plastic, graduated centrifuge tubes (15 mL size) under each port, as required, in the manifold rack. Add acetonitrile (1 mL) to the top of

each cartridge and allow to percolate through under gravity. Collect the column eluate containing SYN508210 and SYN508211. Remove the excess solvent from the cartridges by application of positive pressure or vacuum, collecting the column eluate.

- f) Evaporate the collected eluates to  $0.5 \text{ mL} \pm 0.1 \text{ mL}$  under a stream of air in a sample concentrator with the heating block set at  $30 \text{ }^\circ\text{C}$ .
- g) Adjust the final volume to 1 mL with ultra pure water and mix sample thoroughly by ultrasonication of the contents of centrifuge tube briefly.
- h) Transfer the samples into suitable autosampler vials ready for final determination by LC-MS/MS. The final sample concentration is 2 g/mL.

### **3.6 Time required for analysis**

The methodology is normally performed with a batch of 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 SYN508210 and SYN508211 has been observed in the soil types tested using the above procedure during method development and non-matrix standards should normally be used for calibration. For example, to prepare a  $0.2 \text{ ng/mL}$  calibration standard, transfer  $20 \text{ } \mu\text{L}$  of a  $0.1 \text{ } \mu\text{g/mL}$  mixed SYN508210 and SYN508211 standard in acetonitrile to 50:50 v/v acetonitrile:ultra pure water in a 10 mL volumetric flask. Adjust to the 10 mL mark with 50:50 v/v acetonitrile:ultra pure water. Stopper the flask securely and shake gently to mix thoroughly. Transfer an aliquot to a suitable autosampler vial for analysis by LC-MS/MS.

A calibration curve may also be generated to quantify SYN508210 and SYN508211 residues. Standards over an appropriate concentration range should be prepared as described above, using the requisite volumes of SYN508210 and SYN508211 in acetonitrile.

During method validation, there was insufficient sensitivity to accurately quantify SYN508210 and SYN508211 residues at the LOQ using a  $10 \text{ } \mu\text{L}$  injection volume. The injection volume was increased to  $50 \text{ } \mu\text{L}$  to achieve the desired sensitivity, which resulted in an increase in matrix effects. Matrix matched standards were used to compensate for these effects. To prepare e.g. a  $0.2 \text{ ng/mL}$  matrix matched calibration standard take an

additional control aliquot through the procedure from Section 3.4 (d) to 3.5 (g). At this point add 20 µL of a 0.01 µg/mL mixed SYN508210 and SYN508211 standard in acetonitrile and adjust the final volume to 1 mL with ultra pure water.

#### **4.0 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 an Applied Biosystems API4000.

#### **4.1 Instrument description**

Pump	: Agilent 1100 series quaternary pump model number G1311A
Degasser	: Agilent 1100 series model number G1322A
Column Oven	: Agilent 1100 series model number G1316A fitted with column switching valve
Detector	: Applied Biosystems API 4000 triple quadrupole mass spectrometer with Analyst™ software version 1.4.1
Autosampler	: CTC HTS PAL
Gas Supply	: Peak Scientific NM20ZA gas station

#### **4.2 Chromatography conditions for SYN508210 and SYN508211 used for method validation**

Column	: Phenomenex Luna C18 (2) 3 µm 50 x 3 mm
Column Oven Temperature	: 50°C
Injection volume	: 10-50 µL
Stop Time	: 9.1 minutes
Injection protocol	: Analyse calibration standard after 3 to 4 sample injections
Mobile phase	: Solvent 1 = 65:35:0.05 v/v/v methanol:ultra pure water:formic acid Solvent 2 = acetonitrile

### Mobile phase composition used for method validation

Time (mins)	% solvent 1	% solvent 2	Flow rate (mL/min)
0.0	100	0	0.3
6.0	100	0	0.3
6.2	0	100	0.3
7.1	0	100	0.3
9.1	100	0	0.3

Notes : The column eluate is diverted to waste for the first 0.75 min to prevent ionic material from the sample contaminating the mass spectrometer front plate. A secondary pump providing flow of mobile phase to the mass spectrometer when the column eluate is switched to waste has been found to be unnecessary. Under these conditions the retention time of SYN508210 is 4.2 minutes and SYN508211 is 5.0 minutes.

### 4.3 Alternative chromatography conditions for SYN508210 and SYN508211

Column : ACE C18 5  $\mu$ m 50 x 3.0 mm i.d. or Phenomenex  
Column Oven Temperature : 40°C  
Injection volume : 10  $\mu$ L  
Stop Time : 7.5 minutes  
Injection protocol : Analyse calibration standard after 3 to 4 sample injections  
Mobile phase : Solvent 1 = methanol  
Solvent 2 = 0.2% v/v formic acid in ultra pure water

### Alternative mobile phase composition

Time (mins)	% solvent 1	% solvent 2	Flow rate (mL/min)
0.0	50	50	0.5
5.0	90	10	0.5
6.0	90	10	0.5
6.1	50	50	0.5
7.5	50	50	0.5

Notes : The column eluate is diverted to waste for the first 0.75 min to prevent ionic material from the sample contaminating the mass spectrometer front plate. A secondary pump providing flow of mobile phase to the mass spectrometer when the column eluate is switched to waste has been found to be unnecessary. Under these conditions the retention time of SYN508210 is 4.7 minutes and SYN508211 is 5.1 minutes.



#### **4.4 Mass spectrometer conditions for SYN508210 and SYN508211 used for method validation**

Interface : TurboIonSpray  
Polarity : Negative  
Curtain gas (CUR) : Nitrogen set at 40 (arbitrary units)  
Temperature (TEM) : 450°C  
Ionspray voltage : -4500V  
Collision gas setting (CAD) : Nitrogen set at 6 (arbitrary units)  
Gas 1 (GS1) : Air set at 70 (arbitrary units)  
Gas 2 (GS2) : Air set at 60 (arbitrary units)  
Interface heater (ihe) : On  
Scan type : MRM

#### **4.5 Alternative mass spectrometer conditions for SYN508210 and SYN508211**

Interface : TurboIonSpray  
Polarity : Negative  
Curtain gas (CUR) : Nitrogen set at 15 (arbitrary units)  
Temperature (TEM) : 450°C  
Ionspray voltage : -4500V  
Collision gas setting (CAD) : Nitrogen set at 8 (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		<b>SYN508210 and SYN508211 primary transition</b>	<b>SYN508210 and SYN508211 confirmatory transition</b>
Q1 <i>m/z</i>	:	330	330
Q3 <i>m/z</i>	:	131	91
Dwell time	:	150 ms	150 ms
Resolution Q1	:	Unit	Unit
Resolution Q3	:	Unit	Unit
Declustering potential (DP)	:	-80 V	-80 V
Entrance potential (EP)	:	-10 V	-10 V
Collision energy (CE)	:	-26 V	-48 V
Collision cell exit potential (CXP)	:	-12 V	-7 V

Typical chromatograms are shown in Appendix 4.

## **5.0 CALCULATION OF RESULTS**

### **5.1 Single point calibration procedure**

SYN508210 and SYN508211 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 SYN508210 and SYN508211 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 areas obtained for SYN508210 and SYN508211.
- b) Make an injection of each sample solution and measure the areas of the peaks corresponding to SYN508210 and SYN508211.
- c) Re-inject the standard solution after a maximum of four injections of sample solutions.
- d) Calculate the SYN508210 and SYN508211 residues 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 ( $\mu\text{g/mL}$ )

Sample Conc. = Sample concentration ( $\text{g/mL}$ )

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

SYN508210 and SYN508211 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 SYN508210 and SYN508211. 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 SYN508210 and SYN508211 residues in the sample, expressed as mg/kg, as follows

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

Where analyte found ( $\mu\text{g/mL}$ ) 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.

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

## 6.0 CONTROL AND RECOVERY SAMPLES

Control samples should be analysed with each set of samples to verify that the sample used to prepare recovery samples is free from contamination. A minimum of one control should be analysed with each batch of samples.

At least two recovery samples (control samples accurately fortified with known amounts of SYN508210 and SYN508211 in acetonitrile) should also be analysed alongside each set of samples. Provided the recovery values are acceptable they may be used to correct any residues found. The fortification 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  $\leq 20\%$ .

## **7.0 SPECIFICITY**

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

### **7.1 Matrix**

LC-MS/MS is a highly specific detection technique. Interference arising from the 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**

This method uses mainly disposable labware. All reusable glassware should be detergent washed and then 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.

Polypropylene centrifuge bottles, 250 mL capacity, available from Fisher Scientific UK, Bishop Meadow Road, Loughborough, Leicestershire LE11 5RG.

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.

Polypropylene centrifuge tubes, 50 mL capacity, available from Fisher Scientific UK, Bishop Meadow Road, Loughborough, Leicestershire LE11 5RG.

Isolute<sup>®</sup> Vacmaster-20<sup>®</sup> sample processing station, available from Argonaut Technologies, Tir-y-Berth Industrial Estate, New Road, Hengoed, Mid Glamorgan, CF8 8AU.

Column connectors, available from Argonaut Technologies, Tir-y-Berth Industrial Estate, New Road, Hengoed, Mid Glamorgan, CF8 8AU.

Column reservoirs, 70 mL size, available from Varian Ltd, 28 Manor Road, Walton-on-Thames, Surrey, KT12 2QF.

Oasis HLB solid phase extraction cartridges 60 mg, 3 mL size, available from Waters 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 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.

Agilent 1100 HPLC system equipped with quaternary pump, vacuum degasser and column compartment with column switching valve, available from Agilent Technologies

UK Limited, Chemical Analysis Group, Lakeside Heath, Cheadle Royal Business Park, Stockport, Cheshire SK8 3GR.

CTC HTS PAL autosampler, available from Presearch Ltd, System House, 59-61 Knowlpiece, Hitchin, Herts SG4 0TY.

HPLC column, either ACE 5 C18 5 µm 50 mm × 3.0 mm i.d., available from Hichrom Ltd., 1 The Markham Centre, Station Road, Theale, Reading, Berkshire RG47 4PE ([www.hichrom.co.uk](http://www.hichrom.co.uk)) or Phenomenex Luna C18(2) 3 µm 50 x 3 mm, available from Phenomenex, Queens Avenue, Hurdsfield Ind. Est., Macclesfield, Cheshire SK10 2BN

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.

Polypropylene centrifuge bottles, 250 mL capacity 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, Liberty Lane, Hampton, NH 03842.

Polypropylene centrifuge tubes, 50 mL capacity available from Fisher Scientific, Liberty Lane, Hampton NH 03842.

Isolute<sup>®</sup> Vacmaster-20<sup>®</sup> sample processing station, available from Argonaut Technologies - Order Processing, 1101 Chess Drive, Foster City, CA 94404.

Oasis<sup>™</sup> HLB solid phase extraction columns, 3 mL 60 mg size, available from Waters Corporation, 34 Maple Street, Milford, Massachusetts, 01757-3696, USA

Column connectors, available from Argonaut Technologies, Order Processing, 1101 Chess Drive, Foster City, CA 94404.

Column reservoirs, 70 mL size, available from Varian Inc., 2700 Mitchell Drive, Walnut Creek, CA 94598.

Plastic disposable pipettes, 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.

Agilent 1100 HPLC system equipped with quaternary pump, vacuum degasser and column compartment with column switching valve, available from Agilent Technologies, 395 Page Mill Road, Palo Alto, CA 94304.

CTC HTS PAL autosampler, available from LEAP Technologies Inc., P.O. Box 969, Carrboro, NC 27510.

HPLC column, either ACE 5 C18 5  $\mu\text{m}$  50 mm  $\times$  3.0 mm i.d, available from [www.Hichrom.co.uk](http://www.Hichrom.co.uk) or Phenomenex Luna C18(2) 3  $\mu\text{m}$  50 x 3 mm available from 411 Madrid Ave., Torrance, CA 90501-1430.

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



## APPENDIX 2 REAGENTS

### UK suppliers

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

Analytical grade concentrated formic acid available from Sigma-Aldrich, The Old Brickyard, New Road, Gillingham, Dorset SP8 4XT or [www.sigmaaldrich.com](http://www.sigmaaldrich.com)

SYN508210 and SYN508211 analytical standard, available from GLP Testing Facility, Syngenta, CH-4333, Munchweilen, Switzerland.

### US suppliers

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

Ultra pure water (HPLC grade) from e.g. Fluka via Sigma-Aldrich [www.sigmaaldrich.com](http://www.sigmaaldrich.com)

Analytical grade concentrated formic acid available from [www.sigmaaldrich.com](http://www.sigmaaldrich.com)

SYN508210 and SYN508211 analytical standard, available from Syngenta Crop Protection, Inc., P.O. Box 18300, Greensboro, NC 27419-8300.

### Preparation of reagents

- a) 80:20 v/v acetonitrile:ultra pure water:  
Measure 800 mL acetonitrile into a 1 L volumetric flask. Add 200 mL ultra pure water. Stopper flask securely and shake to mix thoroughly.
- b) 90:10 /v ultra pure water:acetonitrile:  
Add 10 mL acetonitrile to 90 mL ultra pure water in a 100 mL volumetric flask. Stopper the flask securely and shake to mix thoroughly.
- c) 50:50 /v ultra pure water:acetonitrile:  
Add 50 mL acetonitrile to 50 mL ultra pure water in a 100 mL volumetric flask. Stopper the flask securely and shake to mix thoroughly
- d) 65:35:0.05 v/v/v methanol:ultra pure water:formic acid in ultra pure water.  
Add concentrated formic acid (0.5 mL) to 650 mL methanol and 350 mL ultra pure water in a 1L volumetric flask. Stopper flask securely and shake to mix thoroughly.

- e) 0.2% v/v formic acid in ultra pure water.  
Add concentrated formic acid (2 mL) to 1 L ultra pure water in a 1L volumetric flask. Stopper 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 areas of SYN508210 and SYN508211 against non-matrix standards at an equivalent concentration.

For example, to prepare a 0.0002 µg/mL calibration standard, transfer 20 µL of a 0.01 µg/mL mixed SYN508210 and SYN508211 standard in acetonitrile to a control sample at point 3.5 (f) in the method, after evaporation. Adjust final volume to 1 mL with ultra pure water and ultrasonicate briefly to mix thoroughly. Transfer to a suitable autosampler vial. Standard is ready for analysis by LC-MS/MS.

**Table 6 : LC-MS/MS Matrix Effects**

Analyte	Matrix Effect			
	Silt loam		Silty clay loam	
	60 ng/mL*	0.2 ng/mL**	60 ng/mL*	0.2 ng/mL**
SYN508210 <i>m/z</i> = 330 Æ 131	+4%	+65%	+5%	+35%
SYN508210 <i>m/z</i> = 330 Æ 91	+5%	+58%	+4%	+26%
SYN508211 <i>m/z</i> = 330 Æ 131	+1%	+2%	-3%	-15%
SYN508211 <i>m/z</i> = 330 Æ 91	+1%	+2%	-3%	-15%

\*10 µL injection volume

\*\*50 µL injection volume

The magnitude of the matrix effects were considered not to be significant when a 10 µL injection volume is used and non-matrix standards should normally be used for calibration. Due to poor instrument sensitivity during method validation, it was not possible to accurately quantify residues of SYN508210 and SYN508211 at the LOQ using a 10 µL injection volume and the injection volume was increased to 50 µL. When a 50 µL injection volume was used however, significant matrix effects for SYN508210 were observed for SYN508210 in both soil types and matrix matched standards were used to compensate for these effects. Matrix matched standards may be used to compensate for any significant effects, at the discretion of the study director.

## APPENDIX 6 LC-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 SYN508210 and SYN508211

Infuse a standard solutions of SYN508210 and SYN508211 (0.01 to 1.0 µg/mL) in mobile phase (see section 4) directly into the mass spectrometer interface at a rate at of approximately 10-20 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  $m/z = 330$  for SYN508210 and SYN508211 in negative ionisation mode.

Using the Analyst software quantitative optimisation routine, tune the instrument for SYN508210 and SYN508211, ensuring that the correct ion is selected. If desired, manual tuning of the ion optics and collision energy can be carried out to ensure maximum sensitivity.

Finally, connect the LC-pump via the autosampler directly to the MS/MS instrument. Perform repetitive flow injection of a SYN508210 and SYN508211 standard using mobile phase 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 negative ionisation mode, the deprotonated molecular ion generated in the ion source ( $m/z = 330$ ) is selected and subjected to further fragmentation by collisional activation. The two most sensitive daughter ions ( $m/z = 131$  and  $m/z = 91$ ) are then selected and used for quantitative analysis.

The fragment  $m/z = 131$  corresponds to the 3-difluoromethyl-1-methyl-pyrazole fragment following cleavage at the amide linkage and  $m/z = 91$  corresponds to loss of two moles of HF from this fragment ion.

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

## APPENDIX 8 METHOD FLOW CHART

Shake 10 g soil sample with 80:20 v/v acetonitrile:ultra pure water  
at room temperature for 1 hour



Centrifuge



Dilute 10 mL of extract (= 2g soil) with ultra pure water



Oasis HLB SPE procedure



Evaporate eluate and dilute with ultra pure water



Analyse by LC-MS/MS