1.0 EXECUTIVE SUMMARY

1.1 Study Design

The study objective was to validate analytical method GRM042.05A (Syngenta) for the determination of residues of SYN545192 and its metabolite SYN546206 in two different soil types. The validation meets the requirements of guideline SANCO/825/00 rev. 8.1 (16/11/2010) and also complies with Regulations (EU) No 544/2011 and 545/2011 (10/06/2011), OECD ENV/JM/MONO(2007)17 and EPA guideline OPPTS 860.1340 (1996).

1.2 Results and Conclusions

The analytical method GRM042.05A (Syngenta) was validated by Eurofins Agroscience Services Chem GmbH, Germany for the determination of residues of SYN545192 and its metabolite SYN546206 in two different soil types (a silt loam and a clay loam).

Control specimens were analysed in duplicate, and fortified specimens were analysed in quintuplicate for each fortification level at the limit of quantification (LOQ) and at ten times that level for each soil type. Also one reagent blank (without matrix) was analysed.

For SYN545192 and its metabolite SYN546206, the limit of quantification (LOQ) was established at 0.001 mg/kg, with a limit of detection (LOD) of 0.0003 mg/kg.

No residues of SYN545192 or its metabolite SYN546206 were detected in any control specimen indicating that no interferences were present at the retention time of the analytes in the test systems.

For both fortification levels (0.001 mg/kg and 0.01 mg/kg), mean recoveries in both soils were in the range of 70 - 120 % with a relative standard deviation (RSD) of ≤ 20 % for both mass transitions monitored. For the silt loam soil, one specimen fortified at 0.01 mg/kg was defined as an outlier via Grubbs testing and therefore not included in the statistical evaluation.

The linearity of the detector response was confirmed by injecting matrix-matched standards covering the working range. The lower margin of the linearity test was 30 % of the LOQ and the higher margin above 20xLOQ concentrations for both primary and confirmatory mass transitions. These margins cover the range as demanded in SANCO/825/00 rev. 8.1. The coefficients of determination (R^2) were ≥ 0.9955 for SYN545192, and ≥ 0.9989 for the metabolite SYN546206.

Since two characteristic mass transitions are used to monitor both SYN545192 and its metabolite SYN546206, the method achieves a high level of specificity and no further confirmation on a different detector was necessary.

The stability of sample extracts was checked for a storage in a refrigerator at 3-8 °C. After at least eight days, stored samples that had been fortified at the LOQ were analysed against freshly prepared calibration standards. The overall mean recoveries in the stored fortified samples were within the acceptable range of 70-120 % with an RSD of ≤ 20 % for both soil types, indicating storage stability of the sample extracts under the storage conditions used.

The stability of standard solutions of SYN545192 and its metabolite SYN546206 in matrix was checked for a storage in a refrigerator at 3-8 °C for at least 7 days. The matrix matched standard solutions for both soil types were within ± 10% of the freshly prepared solutions, indicating storage stability under the storage conditions used.

2.0 INTRODUCTION

Analytical method GRM042.05A (Syngenta) has been validated by Eurofins Agroscience Services Chem GmbH, Germany, for the determination of residues of SYN545192 and its metabolite SYN546206 in two different soil types.

Test Systems and Analytes Validated in this Study

Test System	Validated for Analyte
Silt loam soil Clay loam soil	SYN545192 and its metabolite SYN546206

This study was conducted to validate analytical method GRM042.05A (Syngenta) and specifically:

a) To establish that the method will produce recovery values which are within an acceptable range (i.e. mean recoveries between 70 % and 120 %, with a relative standard deviation within a run lower than or equal to 20 % of the LOQ level, and lower than or equal to 20 % of the 10xLOQ level, respectively), for each fortification level and overall for both primary and confirmatory mass ion transitions.

b) To establish that the limit of quantification (LOQ) of the analytical method is 0.001 mg/kg for SYN545192 and its metabolite SYN546206.

c) To establish that residues in control samples and method blank are not present at levels above 30 % of the LOQ.

d) To investigate the relationship between instrument response and concentration over concentration ranges typical of those for which the method will be used.

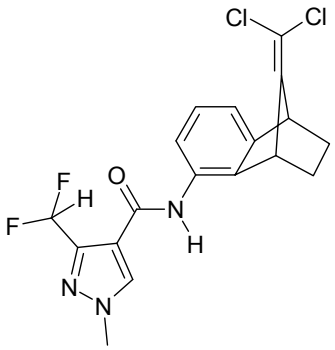
e) To assess the suppression or enhancement of instrument response in the presence of matrix.

f) To assess the stability in final extracts when stored at 3-8 °C.

g) To meet the requirements of guidelines SANCO/825/00 rev. 8.1 (16/11/2010)

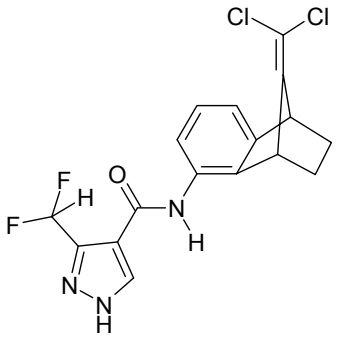
3.0 MATERIALS AND METHODS

3.1 Test Items

Compound Code Number:	**SYN545192**
Alternative Compound Code :	CSCD064398
Chemical Name (IUPAC):	3-Difluoromethyl-1-methyl-1H-pyrazole-4-carboxylic acid (9-di-chloromethen-1,2,3,4-tetrahydro-1,4-methano-naphthalen-5-yl)-amide
CAS-Registry-No.:	1072957-71-1
Molecular Formula:	$C_{18}H_{15}Cl_2F_2N_3O$
Molecular Mass:	398.2 g/mol
Structural Formula:	

Compound Code Number:	**SYN546206**
Alternative Compound Code :	CSCD711742
Chemical Name (IUPAC):	3-Difluoromethyl-1H-pyrazole-4-carboxylic acid (9-di-chloromethen-1,2,3,4-tetrahydro-1,4-methano-naphthalen-5-yl)-amide
CAS-Registry-No.:	-
Molecular Formula:	$C_{17}H_{13}Cl_2F_2N_3O$
Molecular Mass:	384.2 g/mol

Structural Formula:



3.2 Reference Items

The certified reference items of SYN545192 and SYN546206 were supplied by Syngenta Crop Protection Münchwilen AG, CH-4333 Münchwilen, Switzerland.

Name:	**SYN545192**
Lot:	AMS 1295/2
Purity:	99.4 %
Date of Certification:	18-Oct-2010
Storage Conditions (EAS Chem):	≤ -18 °C (in dark conditions)
Date of Expiry:	31-Oct-2014

Name:	**SYN546206**
Lot:	MES 156/2
Purity:	98 %
Date of Certification:	13-Oct-2011
Storage Conditions (EAS Chem):	≤ -18 °C (in dark conditions)
Date of Expiry:	31-Oct-2013

3.3 Test system

Specimen origin

The test system consisted of two different soil types derived from soil dissipation studies: A silt loam soil (from study S10-02292) and a clay loam soil (from study S10-02311). Details of the soil characteristics are given in Appendix 3. (Soil characterisation was performed outside the scope of this study).

Specimen preparation

Soils were stored at ≤ -18 °C until start of analysis. Prior to analysis, the soil material was defrosted completely and mixed thoroughly to ensure sample homogeneity.

3.3.1 Sample analysis

The specimens were analysed for residues of SYN545192 and its metabolite SYN546206 using analytical method GRM042.05A (Syngenta) and detected by means of liquid chromatography with mass spectrometric detection (LC-MS/MS). The limit of quantification (LOQ) was 0.001 mg/kg for each analyte. For SYN545192, the limit of detection (LOD) was estimated to be 0.0003 mg/kg (= 30 % of the LOQ). The LOD for SYN546206 was estimated to be below 0.0001 mg/kg (< 10 % of the LOQ).

Full details of the methodology and the chromatography conditions used in this study are in Section 3.4.

3.3.2 Preparation and stability of analytical standard solutions

Analytical grade reference items of SYN545192 and its metabolite SYN546206 were used for preparing the fortification and external standard solutions.

The solvent standard solutions were prepared in acetonitrile/water (50/50, v/v).

The matrix matched standard solutions were prepared using control matrix extracts.

All standards were prepared over an appropriate range (0.030 ng/mL to 5.0 ng/mL).

The analytical standard solutions were stored at the test facility between +3 and +8 °C.

Full details of the standard solutions are in Section 3.4.5.

3.3.3 Fortification

Recovery of SYN545192 and its metabolite SYN546206 through the analytical procedure was assessed by fortifying five aliquots of the untreated matrices with the fortification solution as detailed in the table below.

Specimen	Specimen weight [g]	Fortification Solution	Fortification Solution Volume [mL]	Fortification Level [mg/kg]
Soil	10	Z4 (0.010 µg/mL)	1.0	0.001
		Z3 (0.10 µg/mL)	1.0	0.01

3.3.4 Calibration information

Concentrations in the final extracts were calculated using the average response factor based on at least six matrix matched standards injected in a sample set, as well as the peak areas of the samples.

3.3.5 Example calculations

Full details of example calculations for fortification and quantification are in Section 3.4.6.

3.3.6 Limit of detection (LOD) and limit of quantification (LOQ)

The LOQ of the method is defined as the lowest analyte concentration in a sample at which the methodology has been successfully validated. A LOQ of 0.001 mg/kg was confirmed for SYN545192 and its metabolite SYN546206 in both tested matrices.

For SYN545192, the limit of detection (LOD) was estimated to be 0.0003 mg/kg (= 30 % of the LOQ). The LOD for SYN546206 was estimated to be below 0.0001 mg/kg (< 10 % of the LOQ).

3.3.7 Detector linearity

The linearity in all matrices was confirmed by injecting of matrix-matched standards covering the working range from 0.030 ng/mL to 2.5 ng/mL or 5.0 ng/mL (equivalent to 0.0003 mg/kg and 0.025 mg/kg or 0.050 mg/kg soil). The lower margin of the linearity was below 30 % of the LOQ, and the upper margin was above the 20x LOQ concentration in the final extracts.

3.3.8 Stability of sample extracts

The stability of the analytes was assessed by storing the final extracts refrigerated at 3-8 °C. The samples were then re-analysed by a second measurement, after at least eight days storage, against freshly prepared calibration standards.

Full details of the results are in Tables 5 and 6.

3.3.9 Matrix effects

Each sample set included appropriate matrix-matched standard solutions, prepared in control matrix extracts. External solvent standards were compared with standards prepared in sample matrix at the same concentration. The peak area ratios were determined and expressed as suppression or enhancement effects.

Full details of results generated in this study are in Tables 11 to 14.

3.4 Detailed Analytical Procedures

3.4.1 Apparatus and Equipment Used

- Ultrasonic bath (SONOREX SUPER 510, BANDELIN electronic GmbH & Co. KG, Berlin, Germany)
- Centrifuge Hettich Rotina 420R, Andreas Hettich GmbH & Co.KG, D-78532 Tuttlingen, Art.No. 4706
- Dilutor (Microlab 500, Hamilton Deutschland GmbH, Martinsried, Germany)
- Volumetric pipettes, e.g. 2.0 mL
- Volumetric pipettes, adjustable (Reference®, Eppendorf, Hamburg, Germany)
- Round-bottom flasks, 150 mL
- Volumetric flasks, e.g. 20 mL
- Test tubes, with ground stoppers and graduation marks at 2.5 mL, 5.0 mL and 10 mL
- Oasis HLB 3cc (60 mg), Waters, D-65760 Eschborn, Art.No. WAT 094226
- Reflux condenser (e.g. VWR)
- Common laboratory glassware

All glassware was rinsed with water (to remove detergents) and dried before use.

3.4.2 Reagents

- Acetic acid, Merck, Art.No. 1.00063.1011
- Acetonitrile gradient grade, e.g. ChromaSolv® Sigma-Aldrich 34851
- Distilled water (e.g. Braun Melsungen, Aqua ad iniectabilia, No. 536108)
- Methanol, Sigma Aldrich, Art.No. 34860
- Water Optigrade, LGC Promochem, Art.No. SO-9368-BO10

3.4.3 Sample Extraction

10 g of soil were weighed into round-bottom flasks (150 mL). Fortification solutions were added to the samples at this step, and allowed to soak into the matrix for 5 min. 50 mL of acetonitrile/ultra pure water (80/20, v/v) were added to the sample, and the weight of the flask and contents were recorded. The samples were heated at reflux for 1h, and subsequently allowed to cool down to room temperature. The weight of the flask was checked, and any losses were corrected for by addition of acetonitrile. The flask was swirled to mix the contents thoroughly. Soil and extract were then decanted into a centrifuge tube (50 mL), and centrifuged for 5 min at 4000 rpm.

Solid Phase Extraction

An aliquot (2.5 mL) of the centrifuged extract was transferred into another centrifuge tube (50 mL). The extract was diluted to 25 mL ± 2 mL with ultra pure water and mixed thoroughly.

An SPE cartridge was placed on a vacuum manifold and conditioned successively with 2 mL methanol and 2 mL ultra pure water, allowing the volumes to percolate through under gravity or under vacuum at a rate of approximately 1 mL/min to the level of the top frit. The column eluates were discarded, and the cartridge was not allowed to become dry.

For the sample application, a reservoir (30 mL) was attached to the SPE cartridge and the extract solution was loaded onto the cartridge via the reservoir. The extract solution was allowed to percolate through to the level of the top frit under gravity or a vacuum at a rate of 1-2 mL/min. The column eluate was discarded, and the cartridge was not allowed to become dry.

The reservoir was removed, and 2 mL acetonitrile/ultra pure water (90/10, v/v) were added to the column and allowed to percolate through to the level of the top frit under gravity or a vacuum at a rate of 1-2 mL/min. The column eluate was discarded, and the cartridge was not allowed to become dry. Then the remaining water was removed from the column by applying an higher vacuum for about 3 seconds.

The analytes were eluted by adding 2 mL acetonitrile to the column and allowing to perculate through under gravity. The eluate was collected in a 10 mL test tube. Remaining acetonitrile was removed from the column by applying an higher vacuum for about 3 seconds, and collecting the eluate.

The eluate was made up to 5.0 mL with acetonitrile/ultra pure water (20/80, v/v) (resulting in an acetonitrile/ultra pure water-ratio of approx. 50/50, v/v), and mixed thoroughly using an ultrasonic bath. The final solution was transferred into an autosampler vial for determination by LC-MS/MS.

3.4.4 Detection

Chromatography and Analysis – SYN545192 and its metabolite SYN546206

The final extracts were analysed for SYN545192 and its metabolite SYN546206 using an HPLC (Agilent Technologies) coupled to a AB-Sciex API 4000 tandem mass spectrometer with electrospray nebuliser. Typical HPLC and mass spectral operating conditions are summarized in the following tables.

HPLC Conditions

System:	Agilent Series 1200 HPLC (Agilent Technologies)			
Column:	ACE 5 C18, 100 mm × 3 mm, 5 µm			
Column Temperature:	40 °C			
Injection Volume:	50 µL			
Mobile Phase Conditions:	A: Acetonitrile B: Water + 0.2 % acetic acid			
	Time (min)	% A	% B	Flow (mL/min)
Injection:	0.0	20	80	1.0
	0.0 → 3.0	20 → 90	80 → 10	1.0
	3.0 → 4.0	90	10	1.0
	4.0 → 4.1	90 → 20	10 → 80	1.0
	4.1 → 6.0	20	80	1.0
Retention Time (approx.):	3.1 min for SYN546206, and 3.3 min for SYN545192			

Mass Spectrometer Conditions

MS System:		AB-Sciex API 4000 Tandem mass spectrometer			
Ionisation type:		Electrospray (ESI, TurboIon Spray)			
Polarity:		Negative ion mode			
Scan type:		MS/MS, Multiple Reaction Monitoring (MRM)			
Capillary voltage (IS):		-3000 V			
Collision gas (CAD):		5 (arbitrary units)			
Ionspray Turbo Heater (TEM):		500 °C			
Curtain gas (CUR):		25 (arbitrary units)			
Gas Flow 1 (GS1):		65 (arbitrary units)			
Gas Flow 2 (GS2):		75 (arbitrary units)			
Analyte Monitored	Transitions Monitored	Declustering Potential (DP) [V]	Collision Energy (CE) [eV]	Cell Exit Potential (CXP) [V]	Dwell Time [s]
SYN545192	396/368	-80	-30	-2	0.250
	396/91	-80	-66	-1	0.250
SYN546206	382/342	-20	-22	-5	0.250
	382/362	-20	-14	-1	0.250

The mass spectrometer was operated in negative ionisation mode. Integrated peak areas were used for quantification.

The mass transitions 396 → 368 and 396 → 91 are both suitable for SYN545192. For the metabolite SYN546206, mass transitions 382 → 342 and 382 → 362 were used. No preference could be seen in the results for both analytes.

External matrix matched standards were prepared for both matrices by diluting a working solution with control matrix extract using volumetric pipettes to reach final concentrations of 0.030 ng/mL, 0.10 ng/mL, 0.25 ng/mL, 0.50 ng/mL, 1.0 ng/mL, 2.5 ng/mL and 5.0 ng/mL.

Due to significant matrix effects found for both soils, all extracts were analysed against matrix matched standards.

3.4.5 Standard preparation

Stock solution no. 7191: 200 µg SYN545192/mL

10.06 mg of SYN545192, analytical reference standard AMS 1295/2 (purity = 99.4 %, see section 3.2) = 10.0 mg of SYN545192 (100%) were dissolved in acetonitrile and diluted to 50 mL in a volumetric flask.

Stock solution no. 7158: 200 µg SYN546206/mL

10.20 mg SYN546206, analytical reference standard MES 156/2 (purity = 98%, see section 3.2) = 10.0 mg of SYN546206 (100%) were dissolved in acetonitrile and diluted to 50 mL in a volumetric flask.

Standard solutions

Solvent working and standard solutions were prepared in acetonitrile/water (50/50, v/v) using a dilutor as follows:

Solution used	Volume used [mL]	Dilution volume [mL]	Concentration obtained [ng/mL]	Working / Standard Solution
Stock solutions no. 7191 & 7158	0.050 each	5.0	2000 each	DL3
DL3	0.12	2.4	100 each	DL4
DL4	0.12	2.4	5.0 each	L8
DL4	0.060	2.4	2.5 each	L9
DL4	0.050	5.0	1.0 each	L10
L8	0.20	2.0	0.50 each	L11
L8	0.12	2.4	0.25 each	L12
L8	0.20	10	0.10 each	L13
L10	0.15	5.0	0.030 each	L14
Stock solutions no. 7191 & 7158	0.050 each	5.0	2000 each	DL5
Stock solutions no. 7191 & 7158	0.050 each	5.0	2000 each	DL8

Matrix matched working solutions

Matrix matched working solutions were prepared by diluting a solvent working solution with control matrix extracts from soil specimens using volumetric pipettes as presented below:

Solvent Working Solution used	Volume used [µL]	Dilution volume [µL]	Concentration obtained [ng/mL]	Matrix matched Working Solution
DL3	50	2000	50	DM3 (silt loam soil)
DL3	50	2000	50	DM4 (clay loam soil)
DL5	50	2000	50	DM6 (clay loam soil)
DL8	50	2000	50	DM8 (silt loam soil)

Preparation of matrix matched standard solutions

For the determination of SYN454192 and SYN546206 in soil extracts, standards were prepared in matrix extracts of untreated (control) soil specimens as follows:

Working / Calibration Solution used	Volume used [µL]	Dilution Volume [µL]	Concentration obtained [ng/mL]	Matrix matched Calibration Solution
DM3	100	1000	5.0	M15 (silt loam soil)
DM3	50	1000	2.5	M16 (silt loam soil)
M15	100	500	1.0	M17 (silt loam soil)
M15	100	1000	0.50	M18 (silt loam soil)
M15	50	1000	0.25	M19 (silt loam soil)
M17	50	500	0.10	M20 (silt loam soil)
M18	60	1000	0.030	M21 (silt loam soil)
DM4	100	1000	5.0	M22 (clay loam soil)
DM4	50	1000	2.5	M23 (clay loam soil)
M22	100	500	1.0	M24 (clay loam soil)
M22	100	1000	0.50	M25 (clay loam soil)
M22	50	1000	0.25	M26 (clay loam soil)
M24	50	500	0.10	M27 (clay loam soil)
M25	60	1000	0.030	M28 (clay loam soil)

Preparation of matrix matched standard solutions (continued)

Working / Calibration Solution used	Volume used [µL]	Dilution Volume [µL]	Concentration obtained [ng/mL]	Matrix matched Calibration Solution
DM6	100	1000	5.0	M38 (clay loam soil)
DM6	50	1000	2.5	M39 (clay loam soil)
M38	100	500	1.0	M40 (clay loam soil)
M38	50	500	0.50	M41 (clay loam soil)
M38	50	1000	0.25	M42 (clay loam soil)
M40	50	500	0.10	M43 (clay loam soil)
M41	60	1000	0.030	M44 (clay loam soil)
DM8	100	1000	5.0	M52 (silt loam soil)
DM8	50	1000	2.5	M53 (silt loam soil)
M52	100	500	1.0	M54 (silt loam soil)
M52	50	500	0.50	M55 (silt loam soil)
M52	50	1000	0.25	M56 (silt loam soil)
M54	50	500	0.10	M57 (silt loam soil)
M55	60	1000	0.030	M58 (silt loam soil)

Fortification solutions

Fortification solutions were prepared in acetonitrile using a dilutor, a volumetric pipette and volumetric flasks.

Solution used	Volume used [mL]	Dilution volume [mL]	Concentration obtained [µg/mL]	Working / Fortification Solution
Stock solutions no. 7191 & 7158	0.25 each	5.0	10.0 each	DZ2
DZ2	0.20	20	0.10	Z3
Z3	2.0	20	0.010	Z4

All solutions were stored at +3 to +8 °C in the dark.

3.4.6 Example Calculations

The evaluation of the results was based on the average response factor which was calculated from the calibration standards. Two ion mass transitions were evaluated for each analyte.

Residues (R) in mg/kg were calculated according to the following equation:

$$R = \frac{A_A \times AvF \times V_{Ex} \times V_{End} \times D_F}{G \times V_{R1} \times C_F}$$

where:

R: Residue of the analyte in mg/kg

A_A: Peak area of the analyte in final solution in counts

AvF: Average response factor: average of standard conc. (ng/mL) / peak area calculated from the calibration standards in each sequence.

The average response factor was calculated as follows:

$$AvF = \frac{(C_{St1} / A_{St1} + C_{St2} / A_{St2} + ... C_{StN} / A_{StN})}{N}$$

C_{St}: Concentration of analyte in external standard solution, in ng/mL

A_{St}: Peak area of analyte in external standard solution, in counts

N: Number of external standard solutions

V_{Ex}: Volume of extract (= 50 mL)

V_{R1}: Volume of aliquot (= 2.5 mL)

V_{End}: Final volume (= 5.0 mL)

G: Sample weight in g (= 10 g)

D_F: Dilution factor (= 1)

C_F: Conversion factor for ng into µg (= 1000)

Percent recovery from fortified specimen was calculated using the following expressions:

$$\text{Recovery (\%)} = \frac{R_{fortified}}{F} \times 100\%$$

$R_{fortified}$: Residues of fortified specimen, in mg/kg

F: Fortification, in mg/kg

3.4.7 Calculation example

For a 0.001 mg/kg fortified silt loam soil specimen (sample no. 7-W, *m/z* 396/368), the concentration of SYN545192 found was calculated as follows:

$$R = \frac{1080 \times 8.5301E\text{-}5 \times 50 \times 5.0 \times 1}{10 \times 2.5 \times 1000} = \underline{0.00092 \text{ mg/kg}} \text{ (rounded)}$$

The percent recovery found was calculated as follows:

$$\text{Recovery (\%)} = \frac{0.00092 \text{ mg/kg}}{0.00100 \text{ mg/kg}} \times 100$$

Recovery = <u>92 %</u>

Limit of quantification (LOQ): 0.001 mg/kg

Limit of detection (LOD): 0.0003 mg/kg

TABLE 11 Determination of Matrix Effects for SYN545192 in Silt Loam Soil

Matrix	Mass Transition	Sequence	Standard Name Solvent / Matrix	Standard Concentration (ng/mL) Solvent Standard	 Matrix Standard	Solutions in Matrix Peak Area	Solutions in Solvent Peak Area	Matrix Effect %
Silt Loam Soil	396/368	SYN1168B	L8 / M15	5.0	5.0	52300	79300	- 34.0
			L9 / M16	2.5	2.5	28500	38400	- 25.8
			L10 / M17	1.0	1.0	12400	16400	- 24.4
			L11 / M18	0.50	0.50	5870	8340	- 29.6
			L12 / M19	0.25	0.25	3070	3800	- 19.2
			L13 / M20	0.10	0.10	1230	1400	- 12.1
							Mean:	**- 24.2**
Silt Loam Soil	396/91	SYN1168B	L8 / M15	5.0	5.0	47300	72200	- 34.5
			L9 / M16	2.5	2.5	26000	32800	- 20.7
			L10 / M17	1.0	1.0	10600	14700	- 27.9
			L11 / M18	0.50	0.50	5350	7370	- 27.4
			L12 / M19	0.25	0.25	2900	3420	- 15.2
			L13 / M20	0.10	0.10	1120	1400	-20.0
							Mean:	**- 24.3**

TABLE 12 Determination of Matrix Effects for SYN546206 in Silt Loam Soil

Matrix	Mass Transition	Sequence	Standard Name	Standard Concentration (ng/mL)		Solutions in Matrix	Solutions in Solvent	Matrix Effect
			Solvent / Matrix	Solvent Standard	Matrix Standard	Peak Area	Peak Area	%
Silt Loam Soil	382/342	SYN1168D	L9 / M16	2.5	2.5	85400	103000	- 17.1
			L10 / M17	1.0	1.0	34900	44900	- 22.3
			L11 / M18	0.50	0.50	18100	22900	- 21.0
			L12 / M19	0.25	0.25	9210	12600	- 26.9
			L13 / M20	0.10	0.10	3730	4490	- 16.9
							Mean:	**- 20.8**
Silt Loam Soil	382/362	SYN1168D	L9 / M16	2.5	2.5	76400	95800	- 20.3
			L10 / M17	1.0	1.0	32500	40400	- 19.6
			L11 / M18	0.50	0.50	16200	20800	- 22.1
			L12 / M19	0.25	0.25	8300	11200	- 25.9
			L13 / M20	0.10	0.10	3380	4470	- 24.4
							Mean:	**- 22.4**

TABLE 13 Determination of Matrix Effects for SYN545192 in Clay Loam Soil

Matrix	Mass Transition	Sequence	Standard Name	Standard Concentration (ng/mL)		Solutions in Matrix	Solutions in Solvent	Matrix Effect
			Solvent / Matrix	Solvent Standard	Matrix Standard	Peak Area	Peak Area	%
Clay Loam Soil	396/368	SYN1168C	L8 / M22	5.0	5.0	45200	81100	- 44.3
			L9 / M23	2.5	2.5	22800	41300	- 44.8
			L10 / M24	1.0	1.0	10500	16000	- 34.4
			L11 / M25	0.50	0.50	5300	8590	- 38.3
			L12 / M26	0.25	0.25	2640	4560	- 42.1
			L13 / M27	0.10	0.10	1060	2010	- 47.3
							Mean:	**- 41.9**
Clay Loam Soil	396/91	SYN1168C	L8 / M22	5.0	5.0	40800	71400	- 42.9
			L9 / M23	2.5	2.5	20000	35400	- 43.5
			L10 / M24	1.0	1.0	8850	14400	- 38.5
			L11 / M25	0.50	0.50	4520	7600	- 40.5
			L12 / M26	0.25	0.25	2270	3830	- 40.7
			L13 / M27	0.10	0.10	981	1450	- 32.3
							Mean:	**- 39.8**

TABLE 14 Determination of Matrix Effects for SYN546206 in Clay Loam Soil

Matrix	Mass Transition	Sequence	Standard Name	Standard Concentration (ng/mL)		Solutions in Matrix	Solutions in Solvent	Matrix Effect
			Solvent / Matrix	Solvent Standard	Matrix Standard	Peak Area	Peak Area	%
Clay Loam Soil	382/342	SYN1168C	L8 / M22	5.0	5.0	783000	971000	- 19.4
			L9 / M23	2.5	2.5	416000	545000	- 23.7
			L10 / M24	1.0	1.0	177000	213000	- 16.9
			L11 / M25	0.50	0.50	91800	113000	- 18.8
			L12 / M26	0.25	0.25	46900	59200	- 20.8
			L13 / M27	0.10	0.10	19700	24400	- 19.3
							Mean:	**- 19.8**
Clay Loam Soil	382/362	SYN1168C	L8 / M22	5.0	5.0	944000	1200000	- 21.3
			L9 / M23	2.5	2.5	498000	652000	- 23.6
			L10 / M24	1.0	1.0	215000	270000	- 20.4
			L11 / M25	0.50	0.50	114000	141000	- 19.1
			L12 / M26	0.25	0.25	56700	76800	- 26.2
			L13 / M27	0.10	0.10	22800	29300	- 22.2
							Mean:	**- 22.1**