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SYN547407 - Independent Laboratory Validation of Residue Method (GRM072.12A) for the Determination of SYN547407 and its Metabolites SYN549107, SYN549431, and SYN550738 in Soil by LC-MS/MS

Final Report

TEST GUIDELINE(S): EPA 850.6100
EC SANCO/3029/99 Rev 4
EC SANCO/825/00 Rev 8.1

2.0 INTRODUCTION

Described in this report is the independent laboratory validation (ILV) of Syngenta Analytical Method GRM072.12A entitled "SYN547407 - Analytical Method GRM072.12A for the Determination of SYN547407 and its Metabolites SYN549107, SYN549431 and SYN550738 in Soil" (Ref. 1) as performed by RTI International.

This study was designed to satisfy harmonized guideline requirements described in EPA 850.6100 (Ref. 2), EC SANCO/3029/99 Rev.4 (Ref. 3), and EC SANCO/825/00 Rev.8.1 (Ref. 4). This study was conducted in compliance with EPA FIFRA Good Laboratory Practice Standards, 40 CFR Part 160.

The residue analytical method is suitable for the determination of SYN547407 and its metabolites SYN549107, SYN549431 and SYN550738 in soil.

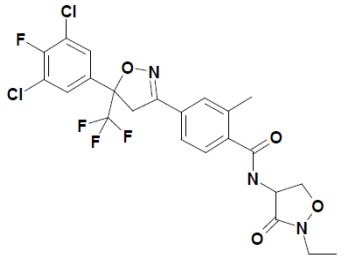
Soil samples (10 g) are extracted by shaking with acetonitrile/water (80/20 v/v) followed by centrifugation and decanting of the supernatant. The sample is extracted a second time with acetonitrile/water/acetic acid (80/20/0.1 v/v/v) with centrifugation and decanting of the supernatant. The supernatants are combined and an aliquot of the combined extracts is centrifuged again. An aliquot is diluted 1:1 with water and final determination is by ultra-performance liquid chromatography tandem mass spectrometry (UPLC-MS/MS). An optional solid phase extraction (SPE) procedure to concentrate and further extract the samples was also verified.

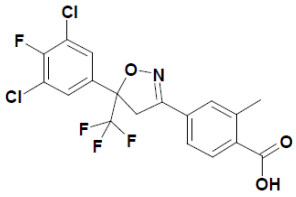
The validated limit of quantitation of the method is 0.001 mg/kg (0.001 ppm, 1 ppb).

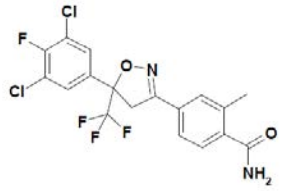
3.0 MATERIALS AND METHODS

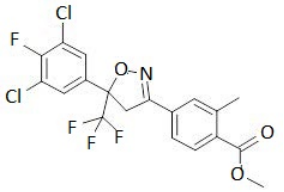
3.1 Test/Reference Substance

The test/reference substances were obtained from Syngenta Crop Protection, LLC. The following test/reference substances were used:

Compound Structure	
Syngenta Code:	SYN547407
Lot Number:	AMS 1521/3
IUPAC Name:	4-(5-(3,5-dichloro-4-fluorophenyl)-5-(trifluoromethyl)-4,5-dihydro-1,2-oxazol-3-yl)-N-(2-ethyl-3-oxo-1,2-oxazolidin-4-yl)-2-methylbenzamide
Molecular Formula:	$C_{23}H_{19}O_4N_3Cl_2F_4$
Molecular Weight:	548.3
Storage Condition:	Room temperature
Purity:	98.6%
Expiration Date:	February 28, 2021

Compound Structure	
Syngenta Code:	SYN549107
Lot Number:	MES 421/2
IUPAC Name:	4-[5-(3,5-dichloro-4-fluoro-phenyl)-5-(trifluoromethyl)-4H-isoxazol-3-yl]-2-methyl-benzoic acid
Molecular Formula:	$C_{18}H_{11}Cl_2F_4NO_3$
Molecular Weight:	436.2
Storage Condition:	Refrigerator
Purity:	94.3%
Expiration Date:	August 31, 2022

Compound Structure	
Syngenta Code:	SYN549431
Lot Number:	MES 431/3
IUPAC Name:	4-[5-(3,5-dichloro-4-fluorophenyl)-5-(trifluoromethyl)-4,5-dihydro-1,2-oxazol-3-yl]-2-methylbenzamide
Molecular Formula:	$C_{18}H_{12}Cl_2F_4N_2O_2$
Molecular Weight:	435.2
Storage Condition:	Refrigerator
Purity:	98%
Expiration Date:	June 30, 2021

Compound Structure	
Syngenta Code:	SYN550738
Lot Number:	MES 524/2
IUPAC Name:	Methyl 4-[5-(3,5-dichloro-4-fluorophenyl)-5-(trifluoromethyl)-4,5-dihydro-1,2-oxazol-3-yl]-2-methylbenzoate
Molecular Formula:	C19H13Cl2F4NO3
Molecular Weight:	450.2
Storage Condition:	Refrigerator
Purity:	96%
Expiration Date:	November 30, 2022

Characterization data for the test/reference standards are maintained by the Sponsor, Syngenta Crop Protection, LLC. The Certificates of Analysis are included in Appendix 2.

The test/reference substances (analytical standards) used in this study were procured from the Sponsor and stored as directed on their Certificates of Analysis. All solutions made from the reference substances (analytical standards) were stored according to the method.

3.2 Test System

The test system evaluated in this study was soil (Table 1). The control samples used in this study were provided by the Sponsor. Characterization data are provided in Appendix 3. Each test system was stored under freezer (-20 ºC) conditions when not in use. Unfortified control samples were checked during the ILV for contamination by employing the same extraction and detection method as described in Syngenta Method GRM072.12A.

3.3 Equipment and Reagents

The equipment and reagents used for the method validation were as outlined in the method. Identical or equivalent equipment and materials were used, as permitted by the method.

3.3.1 Equipment

UPLC-MS/MS Instrument	Applied Biosystems API 5000 Mass Spectrometer with Waters Acquity (Classic) UPLC
HPLC Column	Phenomenex Kinetex C18 (2.1 x 50 mm, 2.6 µm) with SecurityGuard ULTRA fully porous C18
Laboratory Balance	Mettler-Toledo
Mechanical Pipettes with plastic disposable tips	Eppendorf Research plus: 200, 1000, 5000 µL
Vortex Mixer	Scientific Industries G-560
Orbital Shaker	VWR OS-500
Centrifuge	Eppendorf 5910R
SPE Cartridges	Waters Oasis HLB, 60 mg, 3cc
Nitrogen Evaporator	Zymark TurboVap LV
Vacuum Manifold	Spelco Visiprep DL

3.3.2 Reagents

Ultra-pure, HPLC-grade water was obtained from AlfaAesar. HPLC-grade acetonitrile and methanol were obtained from EMD. LC-MS grade acetic acid was obtained from Fisher Scientific.

3.3.3 Preparation of Reagents

- Acetonitrile/water (80/20 v/v) for extraction was prepared by mixing 1600 mL acetonitrile with 400 mL water in a glass bottle with a magnetic stir bar.
- Acetonitrile/water/acetic acid (80/20/0.1 v/v/v) for extraction was prepared by mixing 1600 mL acetonitrile and 400 mL water in a glass bottle with a magnetic stir bar, then adding 2 mL acetic acid while stirring.
- Acetonitrile/water (30/70 v/v) for SPE rinse was prepared by combining 60 mL acetonitrile with 140 mL water in a glass bottle and mixing by manual shaking.

- Acetonitrile/water (50/50 v/v) for sample dilution was typically prepared by mixing 50 mL acetonitrile with 50 mL water in a glass bottle with a magnetic stir bar.
- 0.1% acetic acid in water (Mobile Phase A) was typically prepared by adding 1 mL acetic acid to 1 L water in a glass bottle and mixing with a magnetic stir bar.
- 0.1% acetic acid in acetonitrile (Mobile Phase B) was typically prepared by adding 1 mL acetic acid to 1 L acetonitrile in a glass bottle and mixing with a magnetic stir bar.

3.4 Preparation of Standard Solutions

Standard solutions were prepared and stored as recommended in the method (Ref 1).

3.4.1 Stock Standards

Individual 1000 µg/mL stock solutions of SYN547407, SYN549107, SYN549431 and SYN550738 were prepared by weighing ~10 mg of each standard into separate 10-mL volumetric flasks. The stock solutions were dissolved with acetonitrile and brought to volume to yield approximate concentrations of 1000 µg/mL after adjusting for purities. The stock solutions were mixed well, transferred to amber glass vials, and stored refrigerated (1 - 10 °C) when not in use.

Compound	Weight (mg)	Purity (%)	Total Volume (mL)	Theoretical Conc. (µg/mL)
SYN547407	10.143	98.6	10	1000
SYN549107	10.571	94.3	10	996.8
SYN549431	10.231	98	10	1003
SYN550738	10.438	96	10	1002

3.4.2 Intermediate and Fortification Standards

Fortification solutions containing all four analytes were prepared by dilution of the stock solutions in acetonitrile and stored refrigerated (1 - 10 °C) when not in use.

Fortification Standard	Solution(s) Used	Vol. Transferred (mL)	Total Vol. (mL)	Theoretical Conc. (ng/mL)
High spike	1000 µg/mL stocks	0.1 (of each)	10	10,000
10X LOQ spike	High spike	1.0	10	1000
LOQ spike	High spike	0.1	10	100
Solvent Std B	LOQ spike	5.0	10	50
Solvent Std C	LOQ spike	0.5	10	5.0
Solvent Std D	LOQ spike	0.1	10	1.0
Solvent Std E	Solvent Std C	1.0	10	0.5
Solvent Std F	Solvent Std C	0.3	10	0.15

Fortification Standard	Solution(s) Used	Vol. Transferred (mL)	Total Vol. (mL)	Theoretical Conc. (ng/mL)
Spiking Std a	High spike	2.0	10	2000
Spiking Std b	High spike	1.0	10	1000
Spiking Std c	Spiking Std b	1.0	10	100
Spiking Std d	Spiking Std b	0.2	10	20
Spiking Std e	Spiking Std b	0.1	10	10
Spiking Std f	Spiking Std b	0.03	10	3.0

3.4.3 Calibration Standards

A diluted matrix extract was prepared by combining 10 mL control blank extract (see Section 3.3 of Analytical Method GRM072.12A) and 10 mL water in a glass vial and mixing by vortex. Matrix-matched calibration standards (6-point) were prepared by adding 0.1 mL of the appropriate fortification standard to 0.9 mL of diluted matrix extract and mixing by vortex. The calibration standards were stored refrigerated (1 - 10 °C) when not in use.

Calibration Standard	Fortification Solution Used	Vol. Transferred (mL)	Total Vol. (mL)	Theoretical Conc. (ng/mL)
Matrix Std A	LOQ spike	0.1	1	10
Matrix Std B	Solvent Std B	0.1	1	5.0
Matrix Std C	Solvent Std C	0.1	1	0.50
Matrix Std D	Solvent Std D	0.1	1	0.10
Matrix Std E	Solvent Std E	0.1	1	0.050
Matrix Std F	Solvent Std F	0.1	1	0.015

Another set of matrix-matched calibration standards were prepared specifically to test the SPE method. These were prepared at twice the concentration to account for the difference in extract dilution. The matrix-matched calibration standards (6-point) for SPE were prepared by adding 0.05 mL of the appropriate fortification standard to 4.95 mL of undiluted control blank extract and mixing by vortex. Each standard was then diluted by adding 10 mL water and mixing by inversion.

Calibration Standard for SPE	Fortification Solution Used	Vol. Transferred (mL)	Total Vol. (mL)	Theoretical Conc. (ng/mL)
SPE Std a	Spiking Std a	0.05	5	20
SPE Std b	Spiking Std b	0.05	5	10
SPE Std c	Spiking Std c	0.05	5	1.0
SPE Std d	Spiking Std d	0.05	5	0.20
SPE Std e	Spiking Std e	0.05	5	0.10
SPE Std f	Spiking Std f	0.05	5	0.0030

3.5 Analytical Procedures and Modifications

Analytical Method GRM072.12A (Ref. 1) was successfully validated as written using the procedures and instrumentation recommended by the method. Soil contents are extracted with acetonitrile/water (80/20 v/v) followed by centrifugation and decanting of the supernatant. The sample is extracted a second time with acetonitrile/water/acetic acid (80/20/0.1 v/v/v). The supernatants are combined, and an aliquot is centrifuged again. An aliquot is diluted 1:1 with water and analyzed by UPLC-MS/MS. An optional SPE procedure was also verified, whereby the diluted extract is further diluted with water, then loaded onto an SPE cartridge, washed with water/acetonitrile (70/30), and eluted with acetonitrile. SPE extracts are evaporated, diluted with water, and analyzed by UPLC-MS/MS. The limit of quantitation of the method is 0.001 mg/kg in soil.

3.5.1 Modifications

Syngenta Method GRM072.12A (Ref. 1) was followed as written with the following exceptions:

1. Section 3.3.a: HDPE bottles were used for extraction, rather than polypropylene, per correspondence with method author.
2. Section 3.4.f: The sample was concentrated to 1.5 mL instead of 0.5 mL; it was then reconstituted with 1.5 mL instead of 0.5 mL to keep the proportion the same.
3. Section 4.2: The guard column used was SecurityGuard ULTRA fully porous C18 (AJ0-9502), rather than SecurityGuard C18 (AJ0-4287).
4. Section 5.1.e: For the residue calculation, *average* nominal concentration of bracketing matrix standards was used for c1 to correspond to c2 (*average* calculated concentrations).

3.5.2 Fortifications

Untreated control soil samples were fortified using 0.1 mL of the appropriate fortification standard to LOQ and 10X LOQ concentrations as per method. Fortifications used in this ILV are as follows:

Matrix	Fortification Volume (µL)	Fortification Conc. (ng/mL)	Sample Weight (g)	Final Conc. (mg/kg)	Replicates
Soil	100	100	10	0.001 (LOQ)	5
Soil	100	1000	10	0.01 (10X LOQ)	5

3.5.3 Method Summary

As per Analytical Method GRM072.12A soil contents were extracted with acetonitrile/water (80/20 v/v), then again with acetonitrile/water/acetic acid (80/20/0.1 v/v/v). An aliquot is diluted with water and analyzed by UPLC-MS/MS. The optional SPE procedure was also verified in this ILV. The extraction method details are included in Appendix 4.

3.6 Instrument Conditions

UPLC System	:	Waters Acquity (Classic)
MS Detector	:	Applied Biosystems API-5000
Switching Valve	:	Valco Cheminert 09N-0301H
Data Software	:	AB Sciex Analyst 1.6.2

Flow rate: 0.6 mL/min
Column: Phenomenex Kinetex C18 (2.10 x 50 mm, 2.6 µm) w/ SecurityGuard ULTRA C18
Column temp.: 30 °C
Injection vol.: 30 µL
Autosampler temp.: 7 °C
Run time: 8 minutes
Retention times: SYN549431 = 2.8 min; SYN547407 = 3.2 min; SYN549107 = 3.6 min; SYN550738 = 4.6 min
Mobile phase A: Water with 0.1% acetic acid
Mobile phase B: Acetonitrile with 0.1% acetic acid

Gradient program:

Time (min)	%A	%B	Valve Position
0	60	40	B (To waste)
1.0	60	40	-
2.0	-	-	A (To MS)
5.5	-	-	B (To waste)
6.0	5	95	-
7.0	5	95	-
7.1	60	40	-
8.0	60	40	B (To waste)

Mass Spectrometer Conditions

	Period 1 (0 – 3.4 min)	Period 2 (3.4 – 8.0 min)
Ionization mode:	ESI, positive mode	ESI, negative mode
Curtain gas (CUR):	N_2, 30 psi	N_2, 30 psi
Collision gas (CAD):	8 psi	5 psi
Ionspray voltage (IS):	4500 V	-4500 V
Temperature (TEM):	500 °C	400 °C
Gas 1 (GS1):	20 psi	30 psi
Gas 2 (GS2):	50 psi	30 psi
Interface heater:	On	On
Dwell time:	55 msec	95 msec

MRM Transitions (primary transition on top; confirmatory transition on bottom)

Analyte (mode)	Precursor ion	Product ion	Declustering Potential (DP)	Entrance Potential (EP)	Collision Energy (CE)	Collision Cell Exit Potential (CXP)
SYN547407	548.1	418.0	105	10	31	16
(Positive)	548.1	160.1	105	10	62	11
SYN549431	435.1	392.1	141	10	33	14
(Positive)	435.1	160.1	141	10	53	26
SYN549107	434.1	238.0	-130	-10	-36	-15
(Negative)	434.1	354.0	-130	-10	-30	-15
SYN550738	448.1	378.0	-150	-13	-20	-14
(Negative)	448.1	69.0	-150	-13	-57	-11

3.7 Data Acquisition

Peak integration was performed using Analyst Software version 1.6.2. Microsoft Office Excel was used to derive a best-fit, linear regression equation which was used in conjunction with the analyte response in each sample to calculate the concentration of analyte. The square of correlation coefficients (R^2) for the calibration curves for each analytical set was greater than 0.99. Recovery results were computed for each sample.

A statistical treatment of the data includes the calculation of averages, standard deviations, and relative standard deviations. Mean percent recoveries, standard deviations, and relative standard deviations were calculated using Excel as described in the method (Ref. 1), except *average* nominal concentration of bracketing matrix standards was used for c1 in the residue calculation to correspond to c2 (*average* calculated concentrations).

4.0 RESULTS AND DISCUSSION

4.1 Method Establishment/Pre-Validation Evaluation

Using the instrument parameters as described in the method, the retention times of the analytes, instrument detection limits and response linearity were established by injecting a series of calibration reference standards, for extraction with and without SPE. In addition, the control matrix was found to be free of interferences.

4.2 Independent Laboratory Results

The method was successfully validated on the first attempt for SYN547407, SYN549107, SYN549431, and SYN550738 on soil at the method LOQ and 10X LOQ concentration levels, using the method as written, both with and without SPE. The stated LOQ of the method for all analytes is 0.001 mg/kg (1 ppb). The successful validation results for both soil matrices, with and without SPE, are summarized below.

was not detected in the reagent blanks. SYN547407 was detected in the Soil 2 control blanks for only the quantitation ion, both with and without SPE, but the peak areas were < 2% of the LOQ peak areas. SYN547407 was also detected in the reagent blanks for the quantitation ion.

4.5 Time Required for Analysis

Two analysts were needed to complete a set of 13 samples for extraction both with and without SPE in one extended working day (~10 hours) with UPLC-MS/MS analysis performed overnight.

4.6 Deviations

One protocol deviation occurred during this ILV. The provided CoA for reference standard SYN549107 did not match the lot that was received, so incorrect information was listed in the protocol. The correct CoA for the lot of material received was eventually provided by the Study Sponsor and is included in Appendix 2. Two method deviations also occurred. For the residue calculation, *average* nominal concentration of bracketing matrix standards was used for c1 to correspond to c2 (*average* calculated concentrations). In addition, increased volume was used for the sample concentration and reconstitution step after solid phase extraction, to allow more control over having consistent volumes. The sample was concentrated to 1.5 mL instead of 0.5 mL; it was then reconstituted with 1.5 mL instead of 0.5 mL to keep the proportion the same.

4.7 Circumstances Affecting Data

No circumstances occurred during this ILV that affected quality or integrity of the data.

5.0 CONCLUSIONS

Analytical Method GRM072.12A "SYN547407 - Analytical Method GRM072.12A for the Determination of SYN547407 and its Metabolites SYN549107, SYN549431 and SYN550738 in Soil" was successfully validated by RTI International.

The method was demonstrated to be suitable for the determination of SYN547407, SYN549107, SYN549431 and SYN550738 in two types of soil, for extraction both with and without SPE. An LOQ of 0.001 mg/kg was demonstrated for all analytes.

STUDY OBJECTIVE

Syngenta Analytical Method GRM072.12A (Reference 1) is suitable for the determination of SYN547407 and its metabolites SYN549107, SYN549431, and SYN550738 in soil. The limit of quantitation (LOQ) of the method has been established at 0.001 mg/kg (1 ppb). This method satisfies US EPA Guideline OCSPP 850.6100 and EC Guidance Documents SANCO/3029/99 Rev.4 and SANCO/825/00 Rev.8.1. The objective of this study is to perform an independent laboratory validation (ILV) of the analytical method using representative soil samples.

REFERENCE STANDARDS

Reference standards SYN547407, SYN549107, SYN549431, and SYN550738 will be supplied by Syngenta. A Safety Data Sheet (SDS) and certificate of analysis (COA) should accompany the reference standard, if available. The following information will be provided with the standard:

STANDARD	LOT NUMBER	PURITY (%)	EXPIRATION DATE	STORAGE
SYN547407	AMS 1521/3	98.6	February 28, 2021	Room Temp.
SYN549107	MES 421/3	93	November 30, 2022	Refrigerator
SYN549431	MES 431/3	98	June 30, 2021	Refrigerator
SYN550738	MES 524/2	96	November 30, 2022	Refrigerator

Source: Syngenta Crop Protection, LLC

MATERIALS/EQUIPMENT

LC-MS/MS instrumentation will be used with two MS/MS transitions monitored as specified in the method. The sources and grades of the solvents and chemical reagents will be selected per the requirements from the method. If replacements have to be made, the sources, grades, and part numbers will be documented in the study records.

TEST SYSTEM

The test system for this study will consist of soil types associated with product use. Untreated control samples (UTC) will be provided by Syngenta for independent laboratory validation of the analytical method (Reference 1), along with the characterization information. Control samples should be stored frozen prior to analysis.

SAMPLE	MATRIX	SAMPLE DESCRIPTION
3DA2A.IA.0-3.A.420	Soil 0-3”	Control Plot-K1, Frontage Laboratories, TK0421897
PA.CA.K.BS.0-3”	Soil 0-3”	Untreated Control Bare Soil, Golden Pacific Labs, TK0421893

EXPERIMENTAL DESIGN

Prior to conducting the ILV, RTI will establish method control not limited to but including analyte retention time, linearity, instrument response, instrument detection limits, procedures and verification that the control matrix is free of interferences. RTI will demonstrate method control by performing assessment tests before proceeding to method validation trials. More than one assessment test may be made depending on the number and type of substitutions. Data and results of any assessment test shall be included in the study records, but not in the final report.

Clarification or interpretation of the method will be provided by the sponsor or method developer if requested by the Study Director. All contacts made during the establishment of the method will be documented in writing by the performing laboratory and presented in the final report.

Control samples will be fortified with known amounts of SYN547407, SYN549107, SYN549431, and SYN550738 and analyzed using the procedures outlined in the analytical method (Reference 1).

Validation Set (per soil type):

1x Reagent Blank (matrix free sample submitted to procedures outlined in method)

2x Control Samples (untreated control soil)

5x LOQ Recovery Samples (5 replicates at the target LOQ)

5x 10X LOQ Recovery Samples (5 replicates at 10X the target LOQ)

RTI should verify that the matrix control materials are free of interferences at the appropriate retention time or detector setting by examining the control samples under the instrumental conditions specified in the method. A response greater than 30% of each proposed LOQ constitutes a significant interference. If this is observed, the Study Sponsor will be contacted for direction on how to proceed.

METHOD PERFORMANCE

A successful ILV will require performance data on at least one complete set of samples meeting the necessary criteria described in guidelines EPA 850.6100 of that matrix type. Generally, this requires mean recovery at each spiking level to be within 70-110% for that matrix type. The relative standard deviation (RSD) of replicate measurements of recoveries should be $\leq$ 20% at or above the method LOQ. Upon successful completion, the Study Director or delegate will proceed to write the final report. If the mean results fall outside the 70% to 110% range, the Study Director must consult the Study Sponsor to determine if further trials are required. A maximum of three (3) validation trials for that matrix type may be analyzed to show the subject method is valid.

Communication between the Study Director and the Study Sponsor should occur after each validation set is analyzed, and must be fully documented in the study records.

PROPOSED STATISTICAL METHODS

The statistical method for a regression analysis of a standard curve and quantification of residues are described in the method. The accuracy will be determined in terms of a mean and relative standard deviation for the recovery results from this study. Precision will be demonstrated by calculating the mean, range, standard deviation, and relative standard deviation of the sample recoveries.

MODIFICATIONS

During the 1st method trial, the method procedure should be followed as written, with the exceptions noted below. If the performance data on the first attempt is unsuccessful, the independent laboratory may contact the study sponsor to clarify the directions given in the method. Any correspondence must be documented in writing in the final report. If the second attempt is unsuccessful, the performing laboratory may contact the study sponsor for further clarification. Failure of the third attempt will terminate the study and a report will be issued to the study sponsor, Syngenta, explaining why the method failed.

Planned Method Modifications:

1. Section 3.3.a : HDPE bottles will be used for extraction, rather than polypropylene, per correspondence with method author.
2. Section 4.2: The guard column will be SecurityGuard ULTRA fully porous C18 (AJ0-9502), rather than SecurityGuard C18 (AJ0-4287).

ROUTE OF ADMINISTRATION

The test and reference material will be prepared as outlined in the analytical method.

JUSTIFICATION OF THE TEST SYSTEM

The control samples will be analyzed with the method for evaluation of substrate-related interferences, and the fortified samples will be analyzed using the method for evaluation of method performance via procedural recoveries.

CONTROL OF BIAS

Bias will be controlled by experimental design and statistical methods performed on procedural recoveries from a homogeneous mixture by fortification.

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syngenta

SYN547407

SYN547407 - Analytical Method GRM072.12A for the Determination of SYN547407 and its Metabolites SYN549107, SYN549431 and SYN550738 in Soil

Analytical Method

TEST GUIDELINE(S): EPA OCSPP 850.6100 (2012)
EC SANCO/3029/99 rev 4 (2000)
EC SANCO/825/00 rev 8.1 (2010)

Abbreviations and Symbols

Abbreviation	Definition
ACN	acetonitrile
°C	degrees Celsius or Centigrade
CAS	Chemical Abstract Services
cm	centimetre
EPA	Environmental Protection Agency (U.S.)
EC	European Commission
EU	European Union
g	gram
GRM	Global Residue Method
HPLC	high performance liquid chromatography
i.d.	internal diameter
IUPAC	International Union of Pure and Applied Chemistry
kg	kilogram
L	litre
LC-MS/MS	tandem liquid chromatography/mass spectrometry/mass spectrometry
LOD	limit of detection
LOQ	limit of quantification
m	metre
MeCN	acetonitrile
µg	microgram
µL	microlitre
µm	micrometre
mg	milligram
mL	millilitre
mm	millimetre
mmol	millimole
min	minute
mol	mole
ms	millisecond
MS/MS	tandem mass spectrometry/mass spectrometry
mV	millivolt
MW	molecular weight
m/z	mass to charge ratio

Abbreviation	Definition
N/A (or n/a)	not applicable
ND (or nd)	not detectable (below limit of detection)
ng	nanogram
No.	number
OCSPP	Office of Chemical Safety and Pesticide Pollution
OES	Occupational Exposure Standards
ppb	parts per billion or micrograms per kilogram or micrograms per litre
ppm	parts per million or milligrams per kilogram or milligrams per litre
pg	picogram
R (or r)	correlation coefficient
R^2 (or r^2)	coefficient of determination or square of correlation coefficient
RSD	relative standard deviation
Rt (or RT)	retention time
s (or sec)	second
SD	standard deviation
SPE	Solid Phase Extraction
UPW	Ultra-pure water
V	volt
Vol (or vol)	volume

1.0 INTRODUCTION

1.1 Scope of the Method

Analytical method GRM072.12A is suitable for the determination of SYN547407 (Figure 1) and its metabolites SYN549107 (Figure 2), SYN549431 (Figure 3), and SYN550738 (Figure 4) in soil. The limit of quantification (LOQ) of the method has been established at 0.001 mg/kg (or 0.001 ppm, 1 ppb).

This method satisfies US EPA guideline OCSPP 850.6100 and EC Guidance Documents SANCO/3029/99 rev 4 and SANCO/825/00 rev 8.1.

1.2 Stereospecificity

SYN547407 is comprised of a diastereomeric pair; this method does not resolve the diastereomers or the R and S enantiomers of each diastereomeric pair and will detect and quantify all enantiomers of SYN547407 as a single chromatographic peak. SYN549107, SYN549431 and SYN550738 have one chiral centre comprised of R and S enantiomers; this method does not resolve the enantiomers of each metabolite and will detect and quantify each individual compound as a single chromatographic peak.

1.3 Method Summary

Soil samples (10g) are extracted by shaking with acetonitrile/ultra-pure water (80/20 v/v) followed by centrifugation and decanting of the supernatant. The sample is extracted a second time with acetonitrile/ ultra-pure water /acetic acid (80:20:0.1 v/v/v) followed by centrifugation and decanting of the supernatant. The supernatants are combined and then an aliquot of the combined extracts is centrifuged once more. An aliquot is then diluted 1:1 with ultra-pure water and final determination is by ultra-performance liquid chromatography with triple quadrupole mass spectrometric detection (LC-MS/MS). Note: The recommended instrumentation for analysis is AB Sciex Q Trap® 6500 or instrumentation with equivalent sensitivity.

The limit of quantification of the method is 0.001 mg/kg (0.001 ppm, 1 ppb).

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 individual 1000 µg/mL stock solutions for SYN547407, SYN549107, SYN549431 and SYN550738 by one of the following methods.

Note: the amount weighed out must be corrected for the purity of the analytical standard as indicated on the certificate of analysis and also any salt content, where the analytical standard is received as a salt e.g. Na^+, Cl^- etc..

Weigh out accurately, using a four figure balance, sufficient SYN547407, SYN549107, SYN549431 and SYN550738 analytical standard into separate volumetric flasks (10 mL size). Dilute to the mark with acetonitrile to give an 1000 µg/mL stock solutions of SYN547407, SYN549107, SYN549431 and SYN550738.

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 and any salt content where the analytical standard is received as a salt e.g. Na^+, Cl^- etc.

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

P	=	Standard purity (including correction for salt content where the analytical standard is received as a salt e.g. Na^+, Cl^- etc.) 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

The standard material is weighed into a volumetric flask (10 mL size) and the required volume of acetonitrile is added. Note that the amount of solvent added must still allow for the volumetric flask to be sealed and mixed.

2.3.2 Fortification Solutions

Mixed sample fortification solutions containing SYN547407, SYN549107, SYN549431 and SYN550738 should be prepared by serial dilution in acetonitrile. It is recommended that the following solutions are prepared: 10.0 µg/mL, 1.0 µg/mL and 0.1 µg/mL. Individual standards of SYN547407, SYN549107, SYN549431 and SYN550738 may be prepared if desired.

2.3.3 Preparation of Calibration Standards for LC-MS/MS

No significant suppression or enhancement of the instrument response for SYN547407, SYN549107, SYN549431 and SYN550738 have been observed in the soil types tested using the procedures described in Section 3 during method validation. However, to compensate for possible matrix effects matrix matched standards should normally be used for calibration.

Solvent based calibration standards should be prepared by serial dilution of stock or fortification solutions to a final solution of acetonitrile/ultra-pure water (50/50 v/v).

For example, to prepare a mixed standard solution containing SYN547407, SYN549107, SYN549431 and SYN550738 at 10 µg/mL, transfer an aliquot (0.1 mL) of each 1000 µg/mL stock standard solution into a 10 mL volumetric flask and dilute to 10 mL mark using acetonitrile/ultra-pure water (50/50 v/v).

Matrix matched calibration standards should be prepared by serial dilution of mixed standard solutions in acetonitrile/water (50:50, v/v) into a final solution of representative matrix blank extract which was generated by completing the procedure described in section 3.3 with untreated matrix.

Recommended calibration range for analysis using instrumentation described in Section 4 over an appropriate range: 0.015 ng/mL to 10 ng/mL (0.45 pg to 300 pg on column based on a 30µL injection volume).

A calibration curve should be generated to quantify SYN547407, SYN549107, SYN549431 and SYN550738 residues. Standards over an appropriate concentration range should be prepared as described above, using the requisite volumes of SYN547407, SYN549107, SYN549431 and SYN550738 in matrix.

2.3.4 Standard Solution Storage and Expiration

All stock solutions should be stored refrigerated in the dark 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.

A stability of 27 days was demonstrated for fortification solutions during method validation (Reference 1).

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 3).

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	✗	✗	✓
OES Short Term (mg/m^3)	105	310	37
OES Long Term (mg/m^3)	70	260	25

Suitable personal protective equipment should be worn when handling chemicals and reagents. The appropriate SDS should be consulted for each reagent and a local risk assessment should be carried out. In all cases avoid breathing vapour. Avoid contact with eyes and skin.

3.0 ANALYTICAL PROCEDURE

3.1 Sample Preparation

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

3.2 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 SYN547407, SYN549107, SYN549431 and SYN550738 in acetonitrile. Let each sample stand for at least five minutes after fortification to allow the spiking solution to soak into the soil before proceeding with the extraction. At least one untreated control and two fortified control samples should be analysed with each sample set.

3.3 Extraction (Mechanical Shake)

A summary of the method is included in flow-chart form in Appendix 4.

a) For extraction weigh 10 g of soil into a 250 mL polypropylene bottle; fortification solution is added at this step if required and the sample left for 5 min

b) Add 50 mL of acetonitrile/water (80:20 v/v).

c) Samples were shaken for 1 hour using a flatbed shaker at a rate of 250 rpm.

d) Centrifuge samples at 3500 rpm for 3 minutes.

e) Decant the supernatant into a 100 mL volumetric flask.

f) Extract the remaining soil with 50 mL of acetonitrile/ultra-pure water/acetic acid (80:20:0.1 v/v/v) by shaking the sample as above for 1 hour.

g) Centrifuge samples at 3500 rpm for 3 minutes.

h) Decant the supernatant again into the same 100 mL volumetric flask as above. Adjusted the volume to 100 mL with acetonitrile/water (80:20, v/v) and the solution was homogenised by shaking by hand.

i) Transfer an aliquot of the combined solution into a 50 mL centrifuge tube and centrifuged for 5 min at 10000 rpm.

j) Dilute 500 µL of the supernatant with 500 µL of ultra-pure water in a HPLC vial and analyze by LC-MS/MS. If further concentration is required and/or clean-up due to matrix, proceed to Section 3.4.

3.4 Optional (Solid Phase Extraction Procedure)

Note: SPE should only be performed if concentration of sample results in significant matrix effect that impacts residue determination using matrix-match calibration.

a) Take one Oasis HLB cartridge (3 mL, 60 mg) for each sample to be analyzed 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) Transfer 5 mL of soil extract to a 15 mL conical polypropylene tube (Falcon Tube) and add 10 mL of ultra-pure water.

c) Load diluted sample (15 mL) into SPE cartridge using a pipette or by attaching a column reservoir. Allow sample to percolate through under gravity or draw through under slight 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.

d) Rinse the empty sample container with 3 mL of ultra-pure water/acetonitrile (70/30 v/v), transfer to SPE and allow to percolate through under gravity or draw through under slight vacuum to the level of the top frit at the same rate, again discarding the column eluate. Apply high vacuum for 3 minutes to remove any remaining water.

e) Elute SYN547407, SYN549107, SYN549431 and SYN550738 from SPE cartridge into a 15 mL tube using 5 mL acetonitrile under gravity or slight vacuum. Elute any remaining volume using high vacuum for 10 seconds.

f) Evaporate samples to 0.5 mL under a gentle stream of nitrogen or air in a sample concentrator with a heating block set to 40°C. Reconstitute to 1 mL using 0.5 mL of ultra-pure water, ultrasonicate/vortex to mix thoroughly. If sample is allowed to evaporate to dryness or near dryness, low recoveries may be observed.

k) Transfer the samples into suitable autosampler vials ready for final determination by LC-MS/MS. The final sample concentration is 0.3 g/mL.

3.5 Experimental Precautions

a) 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 0.008 µg/mL.

3.6 Time Required for Analysis

The methodology is normally performed with a batch of 12 samples. One person can complete the analysis of 1 batch (12 samples) in 1 day (8 hour working period).

3.7 Method Stopping Points

Stopping point were not tested during method validation, sample preparation should therefore be finalised within on working day.

4.0 FINAL DETERMINATION

The method has been developed for use on an Applied Biosystems QTRAP® 6500. 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.

4.1 Instrument Description

LC System	:	Sciex QTRAP 6500+
Detector	:	Agilent Infinity II
Gas Supply	:	House Supply

4.2 Chromatography Conditions for SYN547407, SYN549107, SYN549431 and SYN550738

Column	:	Kinetex C18 (50 mm x 2.1 mm, 2.6 µm, 00B-4462-AN, Phenomenex) with pre-column (KJ0-4282, Phenomenex) with 4 mm C18 cartridge (AJ0-4287, Phenomenex)
Alternate Column	:	ACE Excel C18-Amide (50 mm x 2.1 mm, 2 µm, EXL10120502U, MAC-MOD)
Column Oven Temperature	:	30 °C
Injection volume	:	30 µL
Stop Time	:	8 min
Injection protocol	:	Analyse calibration standard after 3 - 5 sample injections
Mobile phase	:	(A) Ultra-Pure Water + 0.1% Acetic Acid (B) Acetonitrile + 0.1 % Acetic Acid

Mobile Phase Composition

Time (mins)	% solvent A	% solvent B	Flow rate (mL/min)
0	60	40	0.6
1.0	60	40	0.6
6.0	5	95	0.6
7.0	5	95	0.6
7.1	60	40	0.6
8.0	60	40	0.6

Valve Switching program

Time (mins)	
0	To waste
2.7	To mass spectrometer
5.5	To waste

Under these conditions, SYN549431, SYN547407, SYN549107 and SYN550738 eluted after 3.5, 3.9, 4.3 and 5.2 minutes respectively.

4.3 Mass Spectrometer Conditions for SYN547407 and SYN549431

Interface	:	TurboIonSpray
Polarity	:	Positive (+)
Curtain gas (CUR)	:	30 (arbitrary units)
Temperature (TEM)	:	550 °C
Ionspray voltage	:	4500 V in positive mode; -4500 V in negative mode
Collision gas setting (CAD)	:	8 (arbitrary units)
Gas 1 (GS1)	:	30 (arbitrary units)
Gas 2 (GS2)	:	60 (arbitrary units)
Interface heater (ihe)	:	MRM
Scan type	:	TurboIonSpray

MRM Conditions		SYN547407 primary transition	SYN547407 confirmatory transition	SYN549431 primary transition	SYN549431 confirmatory transition
Q1 *m/z*	:	548	548	435	435
Q3 *m/z*	:	418	160	392	160
Dwell time	:	25 ms	25 ms	25 ms	25 ms
Resolution Q1	:	Unit	Unit	Unit	Unit
Resolution Q3	:	Unit	Unit	Unit	Unit
Declustering potential (DP)	:	60 V	60V	60 V	60V
Entrance potential (EP)	:	14 V	14V	14 V	14V
Collision energy (CE)	:	30 V	60 V	30 V	45 V
Collision cell exit potential (CXP)	:	15 V	15 V	15 V	15 V

Typical chromatograms are shown in the Figures Section.

4.4 Mass Spectrometer Conditions for SYN549107 and SYN550738

Interface	:	TurboIonSpray
Polarity	:	Period 1: 0.0 - 4.5 min: Positive/negative switching mode (+/-) Period 2: 4.5 - 8.0 min: Negative mode (-)
Curtain gas (CUR)	:	30 (arbitrary units)
Temperature (TEM)	:	550 °C
Ionspray voltage	:	4500 V in positive mode; -4500 V in negative mode
Collision gas setting (CAD)	:	8 (arbitrary units)
Gas 1 (GS1)	:	30 (arbitrary units)
Gas 2 (GS2)	:	60 (arbitrary units)
Interface heater (ihe)	:	MRM
Scan type	:	TurboIonSpray

MRM Conditions		SYN549107 primary transition	SYN549107 confirmatory transition	SYN550738 primary transition	SYN550738 confirmatory transition
Q1 *m/z*	:	434	434	448	448
Q3 *m/z*	:	238	354	378	69
Dwell time	:	100 ms	100 ms	100 ms	100 ms
Resolution Q1	:	Unit	Unit	Unit	Unit
Resolution Q3	:	Unit	Unit	Unit	Unit
Declustering potential (DP)	:	-60 V	-60 V	-60 V	-60 V
Entrance potential (EP)	:	-14 V	-14 V	-14 V	-14 V
Collision energy (CE)	:	-35 V	-30 V	-18 V	-60 V
Collision cell exit potential (CXP)	:	-15 V	-15 V	-15 V	-15 V

Typical chromatograms are shown in the Figures Section.

4.5 Confirmatory Procedures for SYN547407, SYN549107, SYN549431 and SYN550738

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

5.0 CALCULATION OF RESULTS

5.1 Multi-Point Calibration Procedure

SYN547407, SYN549107, SYN549431 and SYN550738 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 (30% LOQ to at least 20% above the highest fortified level as a minimum). An appropriate number of different concentrations within this range should be prepared (at least five).

b) Make an injection of each sample solution and measure the areas of the peaks corresponding to SYN547407, SYN549107, SYN549431 and SYN550738. Calibration standard solutions should be interspersed throughout the analysis, after a maximum of five 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:

$$y \quad mx + c$$

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

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

e) Calculate the SYN547407, SYN549107, SYN549431 and SYN550738 residues in the sample, expressed as mg/kg, as follows

$$\text{Residue (mg/kg)} \quad \frac{c_1 \cdot c_{sample} \cdot V_{ex} \cdot V_{end}}{c_2 \cdot W \cdot V_{ali} \cdot 1000}$$

With:

c_1	Nominal concentration of bracketing matrix standards (ng/mL)
c_2	Average calculated concentration of matrix standards bracketed between samples, obtained from the matrix calibration function (ng/mL)

c_{sample}	Analysed concentration of the sample, as calculated from the matrix calibration function (ng/mL)
V_{ex}	Extraction volume (100 mL)
V_{end}	Final volume (1 mL)
W_{Sample}	weight (10 g)
V_{ali}	Aliquot (0.5 mL)
1000	Conversion from ng/g to mg/kg

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 SYN547407, SYN549107, SYN549431 and SYN550738 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%.

Where the method is used for monitoring purposes, control and recovery samples are not required where suitable control samples are not available.

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 prior to use.

8.0 METHOD VALIDATION

8.1 Extractability

SYN547407, SYN549107, SYN549431 and SYN550738 have been shown to be efficiently extracted in a validation study using the conditions described in Section 3 of the analytical procedure (Reference 2).

8.2 Hydrolysis

Hydrolysis is not required for the determination of residues of SYN547407, SYN549107, SYN549431 and SYN550738 in soil.

8.3 Derivatization

Derivatization is not required for the determination of residues of SYN547407, SYN549107, SYN549431 and SYN550738 in soil.

8.4 Recovery Data and Repeatability

Method validation has been carried out on the procedures described in Sections 3 and 4. The method validation data are summarized in Tables 2 - 9.

8.5 Limit of Quantification (LOQ)

The limit of quantification of the method is defined as the lowest analyte concentration in a sample at which the methodology has been validated and a mean recovery of 70-110% with a relative standard deviation of ≤ 20% has been obtained.

The limit of quantification has been set at 0.001 mg/kg (0.001 ppm, 1 ppb).

8.6 Limit of Detection (LOD)

The limit of detection of the method is defined as 30 % of the LOQ
(LOD = 0.0003 mg/kg).

Note that the LOD may vary between runs and from instrument to instrument.

The LOD was determined as 0.45 pg injected on column, equivalent to 0.015 ng/mL when using a 30 µL injection volume. LOD is based on the lowest concentration sample injected.

8.7 Matrix Effects

No significant matrix effects was observed for all analytes in the soils types tested during method validation. The method includes procedures to reduce matrix effects as far as practically possible, however matrix effects in some matrices may be still observed. Therefore matrix matched standards should be used to compensate for possible the matrix effects at the discretion of the study director. A summary of the matrix effects is included in Table 10.

8.8 Detector Linearity

For accurate quantification of residue concentrations, analyses should be carried out within the linear range of the detector. For multi-point calibration, detector range and linearity will be demonstrated within each sample set.

The linearity of the LC-MS/MS detector response for SYN547407, SYN549107, SYN549431 and SYN550738 was tested in the range from 0.045 pg to 300 pg injected on column (equivalent to 0.015 ng/mL to 10 ng/mL standards when using a 30 µL injection volume. This range corresponds to equivalent sample concentrations of 0.0003 mg/kg to 0.2 mg/kg) and was found to be linear. This is equivalent to 30% LOQ to >120% of the highest validated level.

If a residue beyond the tested concentration range is expected, dilute the sample appropriately to bring it within the tested linear range prior to quantification.

Inject standards in the range of 30% LOQ – 20% above highest concentration required and plot the peak area against the standard concentration, using Microsoft Excel for both primary and confirmatory transitions for SYN547407, SYN549107, SYN549431 and SYN550738.

Detector linearity graphs are presented in the Figures Section.

8.9 Extract Stability

Final soil extracts in acetonitrile/ultra-pure water (50/50 v/v) retained in vials and stored at 1 °C to 10 °C in the dark were suitable for SYN547407, SYN549107, SYN549431 and SYN550738 residue analysis, for storage periods of up to 8 days. A summary of the stability data obtained during method validation is presented in Table 13 - 16.

9.0 LIMITATIONS

The method has been tested on representative soil types. It can reasonably be assumed that the method can be applied to other soil types not tested in this method provided successful recovery tests at the relevant levels validate the suitability of the method.

10.0 CONCLUSIONS

This procedure has been demonstrated to be a reliable and accurate procedure for the determination of SYN547407, SYN549107, SYN549431 and SYN550738 residues in soil. Only commercially available laboratory equipment and reagents are required. The analysis of 12 soil samples for SYN547407, SYN549107, SYN549431 and SYN550738 can be completed by one person in 1 day (8 working hour period). Untreated and fortified samples should be analysed with each set of samples to demonstrate absence of any interference and adequate recovery, if possible. The limit of quantification of the method is 0.001 mg/kg (0.001 ppm, 1 ppb).

This method satisfies US EPA guideline EPA OCSPP 850.6100 and EC Guidance Documents SANCO/3029/99 rev 4 and SANCO/825/00 rev 8.1.

APPENDIX 4 Method Flow Chart