Cyclobutrifluram

Independent Laboratory Validation - SYN549522 - Analytical Method GRM076.04A for the Determination of SYN549522 in Water

Final Report

TEST GUIDELINE(S): EPA 850.6100

AUTHOR(S): Johnson I. Jutson, Ph.D.

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PERFORMING LABORATORY: Smithers
790 Main Street
Wareham, MA 02571-1037 USA

LABORATORY PROJECT ID: Report Number: 1781-7389
Smithers Study Number: 1781-7389
Task Number: TK0592066

SPONSOR(S): Syngenta Crop Protection, LLC
410 Swing Road
Post Office Box 18300
Greensboro, NC 27419-8300 USA

1.0 EXECUTIVE SUMMARY

The purpose of this study was to conduct an independent laboratory validation (ILV) for Syngenta analytical method, GRM076.04A, entitled "SYN549522 – Analytical Method GRM076.04A for the Determination of SYN549522 in Water". This study was designed to satisfy guideline requirements described in US EPA OCSPP 850.6100.

The method was successfully validated on the first attempt for each matrix evaluated (surface water and ground water) for SYN549522 at the method stated Limit of Quantitation, LOQ [0.0250 ppb (µg/L)] and 10X LOQ [0.250 ppb (µg/L)] concentration levels using external solvent calibration.

Interferences were not observed in relation to LOQ for the test matrices. The overall mean recoveries were in the range of 70-120% with a relative standard deviation (RSD) of ≤20% in both matrices. The analytical method was successfully validated and has demonstrated to be suitable for the determination of SYN549522 in representative water matrices.

No significant matrix suppression or enhancement was observed.

A single analyst can complete two sets of 13 samples in 1 working day (8 hours).

2.0 INTRODUCTION

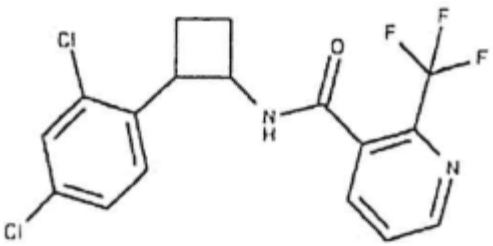
The purpose of this study was to conduct an independent laboratory validation (ILV) for Syngenta analytical method, GRM076.04A, entitled "SYN549522 – Analytical Method GRM076.04A for the Determination of SYN549522 in Water" [**1**]. This study was designed to satisfy guideline requirements described in US EPA OCSPP 850.6100 [**2**]. This study was conducted by Smithers (Study No. 1781-7389) for Syngenta (TK0592066) under the protocol entitled, "Independent Laboratory Validation: SYN549522 - Analytical Method GRM076.04A for the Determination of SYN549522 in Water" (**Appendix 1**). Surface and ground water were selected for evaluation in this validation study.

This study was conducted in compliance with US EPA FIFRA Good Laboratory Practice Standards, 40 CFR Part 160 [**3**].

3.0 MATERIALS AND METHODS

3.1 Test Substance/Reference Substance

The test substance (also the reference substance), SYN549522, was received on 25 February 2021 from Syngenta Crop Protection, LLC, Greensboro, North Carolina USA. The following information was provided:

Compound Structure	
Syngenta Code:	SYN549522
CAS Number:	1460292-16-3
Batch Number:	AMS 1573/2
Molecular Weight:	389.2
Structural Formula	$C_{17}H_{13}C_{l2}F_3N_2O$
Storage Conditions:	Room temperature
Purity:	99.9% w/w
Recertification Date:	31 January 2023

Upon receipt, the test substance (SYN549522) was stored at room temperature in the original container. Concentration of SYN549522 primary stock (100 µg/mL) was adjusted for the purity of the test substance.

All solutions made from the test substance were stored according to the method.

3.2 Test Systems

The test systems evaluated in this study were surface water and ground water. These matrices were chosen because they are representative of the intended use for the method.

Surface water used for this study was collected on 17 February 2021 from the Weweantic River, West Wareham, Massachusetts, USA. The water was collected from an area of the river with approximately 30 to 60 cm of overlying water. Characterization results for surface water are presented in **Tables 1** and **3**.

Ground water collected on 3 March 2021 consists of unadulterated water from a 100-meter bedrock well collected on site at Smithers, Wareham, MA, USA, prepared by filtering to remove any potential organic contaminants. Characterization results for ground water are presented in **Tables 1** and **2**.

Test water matrices were stored refrigerated at approximately 4.0 ± 1 °C except for the periods during which the samples were removed for use in analysis. Refrigerator temperature was monitored on a daily basis.

3.3 Equipment and Reagents/Supplies

The equipment and reagents/supplies used for the ILV were as outlined in the method. Identical or equivalent equipment and materials were used, as permitted by the method. The equivalent equipment and reagents used were as follows:

3.3.1 Equipment

1.	Instrument:	MDS Sciex™ API 6500+ QTRAP mass spectrometer equipped with an ESI Turbo V Sciex Shimadzu™ SIL-20ACXR autoinjector Shimadzu DGU-20A5R vacuum degasser Shimadzu LC-20ADXR solvent delivery pumps Shimadzu CTO-20AC column compartment Shimadzu CBM-20A communications bus Analyst™ 1.6.3 software for data acquisition
2.	Balance:	Mettler Toledo™ XSE205DU
3.	Centrifuge:	Beckman™ Centrifuge Avanti J-20XP
4.	Laboratory equipment:	Volumetric flasks, graduated cylinders, disposable glass pipets, disposable glass vials, positive displacement pipets, stir bars, stir plates, sonicator, vortexer, 15-mL and 50-mL centrifuge tubes, amber HPLC vials with caps, and amber glass bottles with Teflon-lined caps

3.3.2 Reagents

1.	Acetonitrile:	EMD™, reagent grade
2.	Methanol:	EMD, reagent grade
3.	Acetic acid:	EMD, reagent grade
4.	Ultra-pure reagent water:	Fisher, reagent grade
5.	Purified reagent water:	Prepared from a Millipore™ MilliQ Direct 8 water purification system (meets ASTM Type II requirements)

3.4 Preparation of Standard Solutions

The preparation of SYN549522 standard solutions used for this study is described below. The stock solution was stored refrigerated (2 to 8 °C) in amber glass bottles fitted with Teflon-lined caps. The intermediate and fortification standard solutions were prepared fresh daily and stored refrigerated for possible future use.

3.4.1 Stock Standard Solution

Ten (10) milligrams (corrected for purity) of SYN549522 test substance were accurately weighed and quantitatively transferred to a 100-mL volumetric flask. The contents were brought to volume with acetonitrile to make a stock standard solution of SYN549522 having a concentration of 100 µg/mL.

3.4.2 Intermediate and Fortification Standard Solutions

10.0-µg/mL:	1.00 mL of a 100-µg/mL SYN549522 stock standard solution was transferred to a disposable glass vials. The volume was brought to 10.0 mL with acetonitrile and mixed well.
1.00-µg/mL:	1.00 mL of a 10.0-µg/mL fortification solution was transferred to a disposable glass vials. The volume was brought to 10.0 mL with acetonitrile and mixed well.
0.0250-µg/mL:	0.250 mL of a 1.00-µg/mL fortification solution was transferred to a disposable glass vials. The volume was brought to 10.0 mL with purified reagent water and mixed well.
0.0100-µg/mL:	4.00 mL of a 0.0250-µg/mL fortification solution was transferred to a disposable glass vials. The volume was brought to 10.0 mL with purified reagent water and mixed well.
0.00100-µg/mL:	1.00 mL of a 0.0100-µg/mL fortification solution was transferred to a disposable glass vials. The volume was brought to 10.0 mL with purified reagent water and mixed well.

3.4.3 Calibration Standard Solutions

Calibration standards were prepared fresh daily from the fortification solutions and were stored refrigerated for possible future use.

1.00 ng/mL:	0.400 mL of a 0.0250 µg/mL fortification solution diluted to 10 mL with purified reagent water
0.500 ng/mL:	0.200 mL of a 0.0250 µg/mL fortification solution diluted to 10 mL with purified reagent water
0.250 ng/mL:	0.250 mL of a 0.0100 µg/mL fortification solution diluted to 10 mL with purified reagent water
0.100 ng/mL:	0.100 mL of a 0.0100 µg/mL fortification solution diluted to 10 mL with purified reagent water
0.0250 ng/mL:	0.250 mL of a 0.00100 µg/mL fortification solution diluted to 10 mL with purified reagent water
0.0120 ng/mL:	0.120 mL of a 0.00100 µg/mL fortification solution diluted to 10 mL with purified reagent water
0.00800 ng/mL:	0.0800 mL of a 0.00100 µg/mL fortification solution diluted to 10 mL with purified reagent water

3.5 Analytical Method

See **Appendix 2** for the complete text of the method. The following is a summary of that method:

> "The surface water and ground water samples were centrifuged at 10,000 rpm for 2 minutes prior to the fortification with the appropriate fortification standard solutions. Two aliquots of surface water and two aliquots of ground water were left unfortified to serve as control." An aliquot of this sample was transferred to an autosampler vial. The LC vial was capped then submitted for LC-MS/MS analysis. The limit of quantitation (LOQ) is 0.0250 ppb for SYN549522.

Exceptions to the method GRM076.04A [**1**] as written and performed by Smithers are as follows:

- Syngenta method GRM076.04A indicates for LC-MS/MS an AB Sciex API 6500 system was used. For this ILV, an AB MDS Sciex API 6500+ QTRAP instrument was utilized, which had comparable sensitivity and was deemed appropriate for this study.

- Syngenta method GRM076.04A indicates an injection volume of 50.0 µL was used. For this ILV, a lower injection volume of 15.0 µL was used to achieve desired chromatography and sensitivity.
- Syngenta method GRM076.04A indicates for HPLC Shimadzu LC-30AD (pressure tolerance up to ~1300 bar) was used. For this ILV, HPLC Shimadzu LC-20ADXR (pressure tolerance up to ~660 bar) used in the ILV study.
- Syngenta method GRM076.04A indicates a Water Acquity UPLC C8, 2.1 × 50 mm, 1.7 µm particle size column was used. For this ILV, a Water Xbridge BEH C8, 2.1 × 50 mm, 2.5 µm particle size column was used. The stationary phase of the column was kept the same as Syngenta method, however 2.5 µm particle size column was used in order to make it compatible with the HPLC system available at ILV lab. This modification was approved by the method developer prior to use, and comparable chromatography was achieved and was deemed appropriate for this study.

Residue calculations were performed as specified in the analytical method and was conducted using Analyst™ 1.6.3 software for data acquisition to prepare the calibration curve with 1/x weighting. The calculation worksheet can be found in **Appendix 4**.

3.5.1 Fortifications

Untreated control surface and ground water samples were fortified using microliter amounts of the appropriate SYN549522 fortification standard for LOQ and 10X LOQ concentrations as per method. Fortifications used are as follows:

Matrix	Fortification Volume (µL)	Fortification Conc. (µg/mL)	Final Volume (mL)	Final Conc. (µg/L)	Replicates
Surface	250	0.00100	10.0	0.0250 LOQ	5
	250	0.0100	10.0	0.250 10X LOQ	5
Ground	250	0.00100	10.0	0.0250 LOQ	5
	250	0.0100	10.0	0.250 10X LOQ	5

After fortification, the sample was mixed thoroughly before vialing.

The analytical sets consisted of the following samples:

Matrix	Sample Type	Fortifying Compound	Fortification Level (ppb)	# of Samples
Surface Water	Reagent blank	None	0.0	1
	Control	None	0.0	2
	Fortified control	SYN549522	0.0250 LOQ	5
	Fortified control	SYN549522	0.250 10X LOQ	5
Ground Water	Reagent blank	None	0.0	1
	Control	None	0.0	2
	Fortified control	SYN549522	0.0250 LOQ	5
	Fortified control	SYN549522	0.250 10X LOQ	5

3.6 Instrumentation Conditions

All samples were analyzed using an AB MDS Sciex API 6500+ QTRAP LC-MS/MS. Typical conditions were as follows:

Instrument Description

Pump:	Shimadzu LC-20ADXR solvent delivery pumps
Degasser:	Shimadzu DGU-20A5R vacuum degassers
Column compartment:	Shimadzu CTO-20AC column compartment
Autoinjector:	Shimadzu SIL-20ACXR autoinjector
Communication bus:	Shimadzu CBM-20A communications bus
Detector:	AB MDS Sciex API 6500+ QTRAP mass spectrometer equipped with an ESI Turbo V ion source
Software:	Analyst version 1.6.3 software for data acquisition

Chromatography Conditions

Column:	Waters™ Xbridge BEH C8 2.1 × 50 mm, 2.5 µm
Column Oven Temperature:	30 °C
Autosampler Temperature:	10 °C
Injection Volume:	15.0 µL
Stop Time:	5.0 minutes
Injection Protocol:	Two sets of calibration standards were analyzed with each sample set. Calibration standards were interspersed among analysis of the recovery samples, approximately every three to eight injections. Injection of recovery samples and calibration standards onto the chromatographic system was performed by programmed automated injection.
Autosampler Rinse Solution:	30/30/40 acetonitrile/methanol/purified reagent water (v/v/v)
Mobile Phases:	(Solvent A) 0.1% acetic acid in ultra-pure reagent water (Solvent B) 0.1% acetic acid in acetonitrile

Mobile Phase Composition

Time (minutes)	Solvent A (%)	Solvent B (%)	Flow Rate (mL/min)
0.01	90	10	0.600
1.00	90	10	0.600
2.50	10	90	0.600
4.10	10	90	0.600
5.00	90	10	0.600

Mass Spectrometer Conditions

Ion Source Parameters:

Interface:	TurboIonSpray (ESI)
Polarity:	Negative
Curtain Gas (CUR):	Set at 20.0 (arbitrary units)
Temperature (TEM):	500 °C
Ionspray Voltage (IS):	-4500 V
Collision Gas Setting (CAD):	Medium
Gas 1 (GS1):	Set at 50.0 (arbitrary units)
Gas 2 (GS2):	Set at 40.0 (arbitrary units)
Entrance Potential (EP):	-10.0 V
Declustering Potential (DP):	-25.0 V
Resolution Q1:	Unit
Resolution Q3:	Unit
Scan Type:	MRM
Dwell Time:	50.0 ms

Note: The mass spectrometer tuning parameters shown here are for reference only. The analyst should always consult with instrument operation manual to obtain optimum conditions for all the analytes prior to residue analysis.

MRM Operating Parameters:

SYN549522	MS/MS Transition	CE (Volts)	CXP (Volts)
Quantification	387.0 → 146.0	-38.0	-10.0
Confirmation	387.0 → 215.0	-16.0	-13.0

3.7 Modifications, Interpretations, and Critical Steps

Exceptions to the method GRM076.04A [**1**] as written and performed by Smithers are as follows:

- Syngenta method GRM076.04A indicates for LC-MS/MS an AB Sciex API 6500 system was used. For this ILV, an AB MDS Sciex API 6500+ QTRAP instrument was utilized, which had comparable sensitivity and was deemed appropriate for this study.
- Syngenta method GRM076.04A indicates an injection volume of 50.0 µL was used. For this ILV, a lower injection volume of 15.0 µL was used to achieve desired chromatography and sensitivity.
- Syngenta method GRM076.04A indicates for HPLC Shimadzu LC-30AD (pressure tolerance up to ~1300 bar) was used. For this ILV, HPLC Shimadzu LC-20ADXR (pressure tolerance up to ~660 bar) used in the ILV study.
- Syngenta method GRM076.04A indicates a Water Acquity UPLC C8, 2.1 × 50 mm, 1.7 µm particle size column was used. For this ILV, a Water Xbridge BEH C8, 2.1 × 50 mm, 2.5 µm particle size column was used. The stationary phase of the column was kept the same as Syngenta method, however 2.5 µm particle size column was used in order to make it compatible with the HPLC system available at ILV lab. This modification was approved by the method developer prior to use, and comparable chromatography was achieved and was deemed appropriate for this study.

3.8 Statistics

Statistical methods used were limited to calculations of the mean, range, standard deviation, 1/x weighting of linear regression and relative standard deviation. Software programs, Microsoft Excel® and Analyst version 1.6.3, were employed to develop all regression analysis and statistical data.

4.0 RESULTS AND DISCUSSION

4.1 Pre-Validation Evaluations

Prior to analysis of actual validation samples, the control samples initially selected for use in the study were analyzed per the method to determine if any interferences were present in the area of SYN549522. The result of this evaluation indicated that the control samples were below the limit of detection (LOD), and were free of any interference that would affect the analyte responses.

Control Suitability Evaluation	
Matrix	**Residue (ppb)[a]**
Surface Water	<LOD
Ground Water	<LOD

[a] LOD = Limit of Detection

The overall mean recoveries were in the range of 70-120% with a relative standard deviation (RSD) of ≤20% in both matrices. Interferences were <30% of the LOQ. See **Tables 4** and **5** for individual recovery data for SYN549522.

Representative chromatography and linearity of standards for SYN549522 are provided in the **Figures 1** through **20**.

4.3 Detector Linearity

The linearity of the detector response was assessed using a calibration curve generated with the analysis sequence for SYN549522. The LC-MS/MS detector response for SYN549522 in surface water has a correlation coefficient of 0.9998 and 0.9997 for the primary transition in surface and ground water, respectively; and correlation coefficient of 0.9997 for the confirmatory transition in both surface and ground water. The linear concentration ranged from 0.00800 ng/mL (0.00800 ppb) to 1.00 ng/mL (1.00 ppb) when a 15.0 µL injection volume was used. This is equivalent to [32%] LOQ to [40X] LOQ.

The calibration curves for the analyte primary and confirmatory transition are presented in the **Figures 21** through **24**.

4.4 Matrix Effects

No significant matrix effects (<20% difference) were observed during method validation and non-matrix standards should generally be used for quantification. A summary of the matrix effects is included in **Table 6**.

4.5 Communications

Prior to the first attempt per US EPA OCSPP 850.6100 guidance [**2**], allowable communications occurred with the Sponsor Study Monitor and Sponsor Method Contact to discuss items including: approval of the protocol, modification of the method, matrices, and timing updates. Additionally, after the first attempt, the results of the first attempt of the ILV were communicated. A complete summary list of communications is provided in **Appendix 6**.

4.6 Potential Interferences

Interferences were not observed in relation to the LOQ (limit of quantification) in the matrices tested in this validation.

No significant matrix suppression or enhancement in the water matrices tested was observed.

4.7 Time Requirements

A single analyst can complete two sets of 13 samples in 1 working day (8 hours).

4.8 Method Exceptions and Protocol/SOP Deviations

Exceptions to the method GRM076.04A [**1**] as written and performed by Smithers are as follows:

- Syngenta method GRM076.04A indicates for LC-MS/MS an AB Sciex API 6500 system was used. For this ILV, an AB MDS Sciex API 6500+ QTRAP instrument was utilized, which had comparable sensitivity and was deemed appropriate for this study.
- Syngenta method GRM076.04A indicates an injection volume of 50.0 µL was used. For this ILV, a lower injection volume of 15.0 µL was used to achieve desired chromatography and sensitivity.
- Syngenta method GRM076.04A indicates for HPLC, a Shimadzu LC-30AD (pressure tolerance up to ~1300 bar) was used. For this ILV, a HPLC Shimadzu LC-20ADXR (pressure tolerance up to ~660 bar) was used.
- Syngenta method GRM076.04A indicates a Water Acquity UPLC C8, 2.1 × 50 mm, 1.7 µm particle size column was used. For this ILV, a Water Xbridge BEH C8, 2.1 × 50 mm, 2.5 µm particle size column was used. The stationary phase of the column was kept the same as Syngenta method; however, a 2.5 µm particle size column was used in order to make it compatible with the HPLC system available at ILV lab. This modification was approved by the Sponsor Method Contact prior to use, and comparable chromatography was achieved and was deemed appropriate for this study.

No protocol or SOP deviations occurred for this study.

5.0 CIRCUMSTANCES AFFECTING DATA

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

6.0 CONCLUSION

Syngenta Analytical Method GRM076.04A entitled “SYN549522 – Analytical Method GRM076.04A for the Determination of SYN549522 in Water," was successfully validated on the first attempt. No significant matrix suppression or enhancement was observed in the matrices tested. The method was demonstrated to be suitable for the determination of SYN549522 in surface and ground water at an LOQ of 0.0250 ppb (µg/L) and 10X LOQ 0.250 ppb (µg/L).

7.0 REFERENCES

1. Hewa, K., 2021. SYN549522 - Analytical Method GRM076.04A for the Determination of SYN549522 in Water, Report Number: GRM076.04A/Task N0 TK0540379/PASC Report N0: PASC-TMS-1564, V03, Primera Analytical Solution Corp. 259 Prospect Plains Road, Building E Cranbury, NJ 08512, Sponsor: Syngenta Crop Protection, LLC, 410 Swing Road Greensboro, NC 27409 USA.

2. US EPA OCSPP 850.6100, 2012. Environmental Chemistry Methods and Associated Independent Laboratory Validation, United State Environmental Protection Agency Office of Chemical Safety and Pollution Prevention (7101), EPA 712 C-001, January 2012

3. U.S. EPA, 1989. 40 CFR, Part 160. Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA). Good Laboratory Practices Standards; Final Rule. Office of the Federal Register, National Archives, and Records Administration. U.S. Government Printing Office, Washington, D.C.

4. Taylor John K., 1987. Quality assurance of chemical measurements. Lewis Publishers, Inc., Table C.3, page 267

5. U.S. EPA, 2011. Pesticide Registration (PR) Notice 2011-3 Standard Format for Data Submitted Under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) and Certain Provisions of the Federal Food, Drug, and Cosmetic Act (FFDCA). US Environmental Protection Agency Office of Pesticide Programs. November 30, 2011.

APPENDIX 1 Study Protocol

SYN549522

Independent Laboratory Validation:

SYN549522 - Analytical Method GRM076.04A for the Determination of SYN549522 in Water

Study Protocol

DATA REQUIREMENTS: EPA OCSPP 850.6100

PERFORMING LABORATORY: Smithers
790 Main Street
Wareham, Massachusetts 02571 USA

LABORATORY PROJECT ID: Smithers Study Number: 1781-7389
Syngenta Study Number: TK0592066

STUDY SPONSOR: Syngenta Crop Protection, LLC
410 Swing Road
Post Office Box 18300
Greensboro, NC 27419-8300 USA

1.0 STUDY OBJECTIVE

The objective of this study is to perform an independent laboratory validation (ILV) of Syngenta ECM analytical method: *SYN549522 - Analytical Method GRM076.04A for the Determination of SYN549522 in Water* [1]. This study will be conducted in accordance to guideline and reporting requirements described in EPA OCSPP 850.6100 - Environmental Chemistry Methods and Associated Independent Laboratory Validation [2]. This study will be conducted in compliance with EPA FIFRA Good Laboratory Practice Standards, 40 CFR Part 160 [3]. Successful ILV will be achieved by obtaining satisfactory procedural recoveries of SYN549522, in fortified control surface water and ground water samples at levels of 0.025 ppb, the method limit of quantitation (LOQ), and 0.25 ppb (10X the LOQ) within the OCSPP 850.6100 guideline requirements. For the ILV to be considered successful, the mean recovery for each matrix, at each fortification level, will fall between 70-120% with a relative standard deviation (RSD) of ≤20%. In addition, acceptable results must occur within the guideline stipulated three attempts at validation.

2.0 TEST AND REFERENCE SUBSTANCES

Code Name:	SYN549522
Common Name:	Cyclobutrifluram
IUPAC Name:	N-[(1S,2S)-2-(2,4-dichlorophenyl)cyclobutyl]-2-(trifluoromethyl)pyridine-3-carboxamide
CAS Number:	1460292-16-3
Source:	Syngenta Crop Protection, LLC

Characterization data for the test and reference substances will be maintained by Syngenta Crop Protection, LLC, Greensboro, NC USA.

The test and reference substances used in this study will be stored under the conditions stated in the COA received with the test and reference substances. All solutions made from the test and reference substances will be stored according to the method.

3.0 EXPERIMENTAL DESIGN

3.1 Test System

Surface water and ground water will be used in this study as representative water types the method is designed for. Complete sample identification information will be documented in the study raw data and final report. Furthermore, GLP characterization of each water type will be performed and the results included in the final report.

From EPA OCSPP 850.6100 (2012):
"*(3) **Test matrix.***
(i) To assess the ECM, the ILV test includes normal test conditions which include

the presence of other compounds expected to be present. Matrix or matrices (e.g., soil, water, plant or animal tissue) to be sampled as part of the laboratory and/or field study to generate residue data should be identified and/or supplied to the independent lab by the registrant. Chemists at the independent lab should use these samples to prepare matrix spikes for the validation study."

"(ii) An ILV is conducted for each ECM for a given sample matrix for each parent compound, significant metabolites, and/or degradates. A method used for more than one study should meet the same performance standards as the original method. For a given sample matrix, the registrant should select the most difficult analytical sample condition from the study (e.g., high organic content versus low organic content in a soil matrix) to analyze from the study to demonstrate how well the method performs."

3.2 Test System Justification and Bias Control

Control surface water and ground water samples will be fortified with SYN549522 and analyzed according to Syngenta method number GRM076.04A. This method was developed for the determination SYN549522 in water according to the data requirements under EPA guidelines for registration of new pesticide active ingredients.

Method bias will be evaluated by including, in each analytical set, control matrix blank and reagent blank samples. Control matrix blank samples, without fortification, will be analyzed to demonstrate that potentially interfering components are not present in interfering/confounding amounts. Reagent blank analyses, using purified reagent water (Milli-Q™ water), will serve to demonstrate that no interfering artifacts are generated in the analytical procedure or from the method reagents.

3.3 Sample Identification

Each sample used for validation will be assigned a unique code number and documented in the raw data.

3.4 Establishment of the Method

Prior to performing the ILV, it will be necessary to establish the method (e.g., determine analyte retention times, instrument detection limits, linearity of instrument responses to a range of analyte concentrations, and verify that the sample matrices are free of interferences at the appropriate retention times). In general, an analytical laboratory is expected to demonstrate that the method is under control before initiating the ILV.

Clarification of the method will be provided by the "Sponsor Method Contact" if requested by the Study Director. All method clarification contacts made during the establishment of the method will be documented in writing and presented in the final report in the form of a log that clearly documents any verbal communication by time and date or by email. All communication will be provided in an appendix to the final report.

From EPA OCSPP 850.6100 (2012):
"*(ii) The laboratory conducting the ILV trial may contact the developers or previous users of the method prior to running the first set of samples, but all communications should be logged and reported to the Agency documenting that such communication did not compromise the independent evaluation. The ILV itself is likely to be invalid if anyone from the petitioner, developer, or any previous users are allowed to visit the laboratory during the ILV trial to observe, offer help, or assist the chemists or technicians.*"

"*(5) **ILV.** The independent laboratory establishes the relationship between the instrument responses and concentrations of analytes and verifies that matrix control samples are free of interferences at the appropriate retention time, wavelength or detector setting. All quality control conditions should be satisfied in order to demonstrate that the method is under control and before the independent laboratory analyzes any performance samples to be reported to the Agency. Any contact with the registrant or developers during the establishment of the method should be documented in writing in the final report submitted by the independent laboratory*

3.5 Method Performance - Validation Sets

Per the EPA OCSPP 850.6100 guidance, each validation set (for ground water and surface water) will consist of two control samples, a single reagent blank, five fortified control samples at 0.025 ppb, the limit of quantitation (LOQ), and five fortified control samples fortified at 0.25 ppb, or 10X LOQ. Reagent blank samples will be prepared using purified reagent water (PRW, Milli-Q water).

From EPA OCSPP 850.6100 (2012):
"*A) **Sample sets.** A maximum of three sample sets are used by an independent laboratory to validate the method as written. A complete sample set consists at a minimum of a reagent blank, two unspiked matrix control samples, five matrix control samples spiked at the LOQ and another five matrix control samples spiked at ten times the LOQ (10xLOQ) for each distinct matrix. A complete set may include more than thirteen samples depending on the number of reagent, unspiked and spiked matrix control samples. It may be necessary, however, to divide a complete set into two subsets for efficient handling. Each subset should contain a reagent blank, two unspiked matrix control samples, and five matrix control samples spiked at the LOQ or 10xLOQ. The independent laboratory will use the predetermined values from the registrant for the LOQ and 10xLOQ to establish appropriate spiking levels.*"

3.6 Method Validation

Syngenta method number GRM076.04A will be followed as written. No changes or modifications to the method are permissible unless indicated in the method, this protocol, or brought about by clarification of the method with the "Sponsor Method Contact" during the initial method establishment step. Any modifications of the method after the first validation trial will be documented in the raw data and in the final report. The ILV experiments will be

considered successful if the mean recovery for each matrix at each fortification level falls between 70-120% with an RSD of ≤20%.

IMPORTANT - No contact is allowed between the Study Director and the "Sponsor Method Contact" or Sponsor Study Monitor during the actual conduct of a validation set.

Validation set 1. Results of the validation trial will be reviewed by the Study Director and the "Sponsor Method Contact". If the results are determined to be acceptable, a final report will be written. If the validation trial is unsuccessful, the Study Director will consult with the "Sponsor Method Contact" to clarify procedures given in the method. This contact will be documented in writing and presented in the final report in the form of a log that clearly documents any verbal communication by time and date or by email. All communication will be provided in an appendix to the final report. A second set of validation samples will then be analyzed.

Validation set 2. If a second validation trial is conducted, the results will be reviewed by the Study Director and the "Sponsor Method Contact". If the results are determined to be acceptable, a final report will be written. If the second validation trial is unsuccessful, the Study Director will again consult with the "Sponsor Method Contact" to further clarify directions given in the method. This contact will be documented in writing and presented in the final report in the form of a log that clearly documents any verbal communication by time and date or by email. All communication will be provided in an appendix to the final report. A third set of validation samples will then be analyzed.

Validation set 3. If a third validation trial is conducted, the results will be reviewed by the Study Director and the "Sponsor Method Contact". If the results are acceptable, a final report will be written. If the third validation trial is unsuccessful, the ILV will be terminated and a final report will be written. Any contact after the 3rd attempt, will be documented in writing and presented in the final report in the form of a log that clearly documents any verbal communication by time and date or by email. All communication will be provided in an appendix to the final report.

3.7 Data Reported and Evaluation

Evaluation of the data will include but not be limited to calculations of percent recovery, mean percent recovery, standard deviation, relative standard deviation and confidence intervals (95 or 99%) for the true average recoveries for each analyte at each fortification level. The report will include but not be limited to the reporting requirements outlined in EPA OCSPP 850.6100 - Environmental Chemistry Methods and Associated Independent Laboratory Validation [**2**].

From EPA OCSPP 850.6100 (2012):
"*(B) Results reported should include:*
(1) Results of each performance sample set — LOD or the MDL, the established LOQ, and accuracy and precision measured.
(2) The mean and individual values for recoveries and standard

deviations for the pesticide parent, toxicologically significant metabolites and/or degradates in fortified matrix control samples at each spiking level.
(3) The confidence intervals (95 or 99%) for the true average recoveries at each spiking level.
(4) Individual values for recoveries at each spiking level.
(C) Any matrix or solvent effects that result in signal enhancement, masking or suppression and the impact those effects have on the test results is described."

4.0 FINAL REPORT

A final report will be prepared for the ILV study for submission to the US EPA. The report will include but not be limited to the reporting requirements outlined in EPA OCSPP 850.6100 - Environmental Chemistry Methods and Associated Independent Laboratory Validation [**2**].

From EPA OCSPP 850.6100 (2012):
"(7) ***Results/Discussion.***
(i) Method validation results (include tables of test levels and results of analysis).
(ii) Accuracy (mean, range of recoveries, standard deviations and confidence limits for specific concentration levels, such as the LOQ or 10×LOQ).
(iii)) Precision. Provide the RSD at each specific concentration level.
(iv) Limit of detection. Provide a clearly written explanation of how this value is calculated and cite the reference.
(v) Limit of quantitation. Provide a clearly written explanation of how this value is calculated and cite the reference.
(vi) Ruggedness testing (if performed).
(vii) Discussion of selectivity and specificity of method.
(viii) Limitations.
(ix) Interference/calibration.

5.0 DATA RETENTION

All original raw data generated during the conduct of this study will be maintained in an appropriate format. Analysts will maintain laboratory records which detail all procedures, observations, and other analytical activities relevant to the experimental work. Chromatograms, computer printouts, etc. will be clearly labeled and maintained by the analysts until the conclusion of the study. Raw data will be reviewed and approved by the Study Director and audited by the Smithers Quality Assurance Unit prior to finalization.

Upon completion of the final study report, the original final report, raw data and study related documentation will initially be archived at the Smithers archives and subsequently transferred to the Syngenta Crop Protection Archives, Greensboro, North Carolina for final archiving.

Test materials, samples, and sample extracts will be retained by the analytical laboratory until the final report has been signed.

6.0 PROTOCOL CHANGES

Any required changes or revisions to this protocol will be documented as a protocol amendment or deviation. The amendment or deviation will include a description of the change, the reason for the change, the effect of the change on the study, and the effective date of the change. The protocol amendment or deviation will be signed and dated by the Study Director and the Study Director management.

7.0 QUALITY ASSURANCE

Smithers Quality Assurance Unit will inspect one or more critical phases of the study to assure that facilities, equipment, personnel, procedures, and records conform to Good Laboratory Practice Standards, 40 CFR Part 160. The results of these inspections will be reported to the Study Director and the Study Director's management. The final report for this study will be audited for protocol and GLP compliance, as well as to assure that the methods and standard operating procedures utilized were followed and that the content of the report reflects the raw data. A signed Quality Assurance statement will be included in the final report specifying when audits/inspections were performed and when they were reported to the Study Director and the Study Director's management.

8.0 REFERENCES

1. Hewa, K. 2021. SYN549522 - Analytical Method GRM076.04A for the Determination of SYN549522 in Water, Report Number: GRM076.04A/Task No. TK0540379/PASC Report No: PASC-TMS-1564, V03, Primera Analytical Solutions Corp. 259 Prospect Plains Road, Building E Cranbury, NJ 08512, Sponsor: Syngenta Crop Protection, LLC 410 Swing Road, Greensboro, NC 27409 USA.

2. OCSPP 850.6100: Environmental Chemistry Methods and Associated Independent Laboratory Validation, United States Environmental Protection Agency Office of Chemical Safety and Pollution Prevention (7101), EPA 712-C-001, January 2012.

3. U.S. Environmental Protection Agency, Office of Compliance Monitoring. 1989. Federal Insecticide, Fungicide and Rodenticide Act (FIFRA); Good Laboratory Practice Standards; Final Rule, 40 CFR, Part 160. Federal Register, Vol. 54, No. 158: pp. 34052-34074.

APPENDIX 2 Analytical Method

syngenta

SYN549522

SYN549522 – Analytical Method GRM076.04A for the Determination of SYN549522 in Water

Analytical Method

TEST GUIDELINE(S):	EPA 850.6100 (2012) EC SANCO/3029/99 rev 4 (2000) EC SANCO/825/00 rev 8.1 (2010)
AUTHOR(S):	Kosala Thenna Hewa, Ph.D.
COMPLETION DATE:	January 28, 2021
PERFORMING LABORATORY:	Primera Analytical Solutions Corp. 259 Prospect Plains Road, Building E Cranbury, NJ 08512
LABORATORY PROJECT ID:	Report Number: GRM076.04A PASC Project ID: 141-6426 PASC Report No: PASC-TMS-1564, V03 Task Number: TK0540379
SPONSOR:	Syngenta Crop Protection, LLC 410 Swing Road Post Office Box 18300 Greensboro, NC 27419-8300 USA

1.0 INTRODUCTION

1.1 Scope of the Method

This analytical method is suitable for the determination of residues of SYN549522 in water. The limit of quantification (LOQ) of the method has been established at 0.025 ng/mL (0.025 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 Isomers

1.2.1 Stereoisomers

This method is non-enantiospecific and will detect and quantify SYN549522 as a single chromatographic peak.

1.3 Method Summary

Transfer water samples into HPLC vials and perform final determination by ultra-high-performance liquid chromatography using a C8 UPLC column separation with triple quadrupole mass spectrometric detection (LC-MS/MS). The limit of quantification (LOQ) of the method has been established at 0.025 ng/mL (0.025 ppb) for SYN549522 in water.

2.0 MATERIALS AND APPARATUS

2.1 Apparatus

The equipment and apparatus used in method development are listed in the appendix section. Equipment with equivalent performance specifications may be substituted.

2.2 Reagents

All solvents and other reagents used in method development were 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 for the method development along with details of preparation of solutions are included in appendix section.

2.3 Preparation of Analytical Standard Solutions

2.3.1 Stock solution

Prepare individual 100 µg/mL stock solution of SYN549522 in acetonitrile using one of the following methods.

Either weigh out accurately, using a five-figure balance, sufficient analytical standard into an amber "Class A" volumetric flasks (50 mL size) to give the appropriate concentration

and then dilute to the mark with solvent. Note: the amount of analytical standard required must allow for its chemical purity (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.).

Alternatively, dissolve a known amount of analytical standard material in an appropriate volume of solvent. The volume of solvent required to achieve the desired concentration can be calculated using the formula below noting that amount of analytical standard taken must allow for its chemical purity (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.).

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

Where:

V = Volume of solvent required in mL

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 (i.e. 0.989 = 98.9%)

M = Mass (in mg) of analytical standard taken

C = Desired concentration of the final standard (in µg/mL)

2.3.2 Fortification Solutions

Sample fortification solution containing SYN549522 should be prepared with serial dilution with deionized (Milli-Q) water. Solution concentrations containing 25 ng/mL and 2.5 ng/mL should be prepared for fortification purposes.

2.3.3 Preparation of Calibration Standards for LC-MS/MS

Significant suppression of the instrument response has not been observed in the matrices tested using the procedures described below during method development. The calibration solutions should be prepared in deionized (Milli-Q) water. A typical calibration curve covering the range 0.008 ng/mL to 1 ng/mL (equivalent to 32% of LOQ to 40X LOQ) can be prepared using the following dilution sequence. At least five different (non-zero) calibration solutions must be prepared.

Standard name	Source	Source Concentration (ng/mL)	Source Volume (µL)	Milli-Q Water Volume (µL)	Conc. (ng/mL)	Conc. (ppb)
STD 1	STD 4	0.1	80	920	0.008	0.008
STD 2	STD 4	0.1	120	880	0.012	0.012
STD 3	STD 4	0.1	250	750	0.025	0.025
STD 4	STD 7	1	100	900	0.1	0.1
STD 5	STD 7	1	250	750	0.25	0.25
STD 6	Fortification Solution II	2.5	200	800	0.5	0.5
STD 7	Fortification Solution II	2.5	400	600	1	1

Matrix matched standards may be required if significant matrix effect is observed and when no internal standard is available.

2.3.4 Stock and Standard Solution Storage and Expiration

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

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 Safety Data Sheet (SDS).

2.4.1 Solvent and Reagent Hazards

The following information is included as an indication of the hazards associated with the reagents used in this procedure. The procedure is intended to be used by trained and competent personnel, well versed in laboratory safety. Users of the method should ensure they have sufficient information to conduct an appropriate risk assessment that fulfils local requirements. If in any doubt, consult a suitably qualified expert for further advice. It is recommended that the following precautions should be taken when handling the analytical standards and reagents.

- Avoid breathing dust or vapours; ensure good ventilation at all times.
- Avoid skin contact; wear suitable gloves when handling.
- Prevent contamination of skin and clothing: wear a laboratory coat.
- Avoid contact with mouth; ensure good laboratory hygiene practice.
- Avoid spillages; wash any contaminated area immediately with tissue soaked with an appropriate solvent.

The following hazards have been identified for the solvents and reagents utilised in this method.

Compound	Hazard Phrases
Acetonitrile	H225: Highly flammable liquid and vapour H302 + H312 + H332: Harmful if swallowed, in contact with skin or if inhaled H319: Causes serious eye irritation
Acetic acid	H226: Flammable liquid and vapour H314: Causes severe skin burns and eye damage

2.4.2 Analytical Standard Material Hazards

Analyte	Hazard Phrases
SYN549522	Not Available

It is recommended that all analytical standard materials are treated as hazardous and appropriate control measures are used to reduce the risk of exposure. The following minimum precautions should be taken when handling the analytical materials directly.

- Avoid breathing dust or vapours; ensure good ventilation at all times.

- Avoid skin contact; wear suitable gloves when handling.
- Prevent contamination of skin and clothing: wear a laboratory coat.
- Avoid contact with mouth; ensure good laboratory hygiene practice.
- Avoid spillages; wash any contaminated area immediately with tissue soaked with an appropriate solvent.

3.0 ANALYTICAL PROCEDURE

A summary flow-chart of the analytical procedures for sample preparation is included in the appendix section.

3.1 Sample Preparation

If water samples are received deep frozen, they should be allowed to defrost completely at room temperature. Defrosted samples should be shaken thoroughly to ensure sample homogeneity.

1. Transfer 1.5 mL of water sample to a microcentrifuge tube
2. Centrifuge water sample at > 10k RPM for 2 minutes to remove any suspended solids. Fortify procedural recoveries if required
3. Taking care not to disturb or re-suspend any particles, transfer sample from upper portion of centrifuged sample and dispense into a glass autosampler vial
4. Submit autosampler vial for LC-MS/MS 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 an untreated control sample, add the appropriate volume of fortification standard solution to fortify at approximately the anticipated residue level (or at the LOQ and 10X LOQ if the residue level cannot be estimated). Fortification with between 100 µL and 500 µL of standard solution (see Section 2.3.2) in 10 mL of matrix can be made without the need to adjust the volume of the extraction solvent. 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. At least one untreated control and two recovery samples (fortified control samples) should be analysed with each sample batch.

Note: For procedural recovery purposes in all water types, fortification should be performed into water that has been previously centrifuged at >10K for 2 minutes to remove particulates due to compound binding.

3.3 Sample Determination

Transfer an aliquot (~1 mL) from the sample to a HPLC injection vial for instrument analysis (see Section 4.0).

3.4 Method Stopping Points

The analytical procedure can be stopped at various points for overnight and weekend breaks unless otherwise specified in the analytical procedure. Results from recovery samples can be used to validate any workflow interruptions. Samples should be stored refrigerated in sealed containers when the analysis cannot be completed in a single day.

4.0 FINAL DETERMINATION

The method has been developed using an Applied Biosystems API 6500 LC/MS/MS. The method is not suitable for instruments without similar performance and sensitivity. The following instrumentation and conditions have been found to be suitable for this analysis.

4.1 Instrumentation Description

Pump	:	SHIMADZU LC-30AD
Column Oven	:	SHIMADZU 20AC
Autosampler	:	SHIMADZU SIL-30ACMP
Detector	:	AB Sciex API 6500 Mass Spectrometer
Gas supply	:	Nitrogen, house supply

4.2 Chromatography Conditions for SYN549522

The following instrumental conditions have been demonstrated to be suitable for the determination of all the analytes within the scope of this method development procedure.

Column Phase	:	Waters Acquity UPLC C8
Column Dimension	:	2.1 X 50mm, 1.7 µm Particle size
Column Oven Temperature	:	Ambient/30ºC
Flow rate	:	0.600 mL/min
Injection volume	:	50.00 µL
Stop Time	:	5 mins
Injection protocol	:	Inject a calibration standard after every 6 sample injections
Elution	:	Gradient
Mobile phase A	:	0.1% Acetic acid in water
Mobile phase B	:	0.1% Acetic acid in acetonitrile

Mobile Phase Composition

Time (mins)	MPA (%)	MPB (%)
0.00	90	10
1.00	90	10
2.50	10	90
4.00	10	90
4.10	90	10
5.00	90	10

Divert Valve Switching Programme:

Time (min)	Position
0	To waste
1.2	To mass spectrometer
3.5	To waste

Expected Retention Times: 2.5 minute

Divert valve switching programme can be adjusted based on instrument performance.

4.3 Mass Spectrometer Conditions for SYN549522

Interface	:	TurboIonSpray
Curtain gas (CUR)	:	Nitrogen set at 20 (arbitrary units)
Temperature (TEM)	:	500 °C
Collision gas setting (CAD)	:	Nitrogen set at 8
Gas 1 (GS1)	:	Air set at 40 (arbitrary units)
Gas 2 (GS2)	:	Air set at 50 (arbitrary units)
Interface heater (ihe)	:	On
Duration (minutes)	:	5
Ionspray voltage	:	-4500 V
Scan type	:	MRM
Polarity	:	Negative

Analyte Name	MRM Conditions						
	Q1 Mass	Q3 Mass	Time (msec.)	DP	EP	CE	CXP
SYN549522	386.966	146.000	25	-110	-10	-40	-19
SYN549522 Confirmation	386.966	215.000	25	-110	-10	-16	-13

Note: The mass spectrometer parameters should be established by tuning of the instrument. Differences from the above parameters are not considered a method deviation.

4.4 Confirmatory Procedures for SYN549522

Final determination by LC-MS/MS with two transitions is considered to be highly specific; hence no further confirmatory conditions are included for SYN549522 in this method.

5.0 CALCULATION OF RESULTS

5.1 Multi-Point Calibration Procedure

Calculate residues for each sample as follows.

a) Repeatedly inject a low to mid-range calibration solution (or fortified sample) until a consistent instrument response is observed. Typically, three injections have been found to be sufficient.
b) Inject the calibration standards prepared in Section 2.4.3 above and measure the peak areas corresponding to the correct peaks.
c) Make an injection of each sample/ recovery solution and measure the peak areas corresponding to the correct peaks.
d) After a maximum of six injections of sample solutions, make a repeat injection of a suitable calibration solution.
e) Generate calibration curve parameters using an appropriate regression package. The response is expected to be linear and 1/x weighting should be used to provide a line of best fit.

   The following equation should be generated with can the experimental values of m and c and should be included in the raw data:

   $$y=mx+c$$

   Where y is the instrument response, x is standard concentration, m is the gradient of the line of best fit and c is the intercept value.

   Include in the raw data the coefficient of determination, R^2 (square of correlation coefficient). Alternatively, the correlation coefficient, R, can be recorded.
f) Calculate the concentration of the analytes in the sample/recovery solutions using a suitable method.
g) Residues in each sample is expressed in ng/mL (ppb).

5.2 Recovery Correction Procedure

Under certain circumstances (for example in storage stability studies), measured residues may need to be corrected for average percentage recovery. In such cases, the equation below should be used.

$$\text{Corrected Residue} = \frac{\text{Sample Residue (ng/mL)}}{\text{Mean \% Recovery}} \times 100$$

6.0 CONTROL AND RECOVERY SAMPLES

When possible, a minimum of one control (untreated) sample should be analysed with each set of samples. The control sample should verify that the sample used to prepare recovery samples is free from contamination.

At least two recovery samples (control samples accurately fortified with known amounts of all relevant analytes) should be analysed within each set of samples. The fortification levels should be appropriate to the residue levels expected.

Recovery efficiency is generally considered acceptable when the mean recovery values are between 70% and 120% and with a relative standard deviation of ≤20%.
Provided the mean recovery values are acceptable they may be used to correct any residues found.

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

7.0 SPECIFICITY

7.1 Matrix

Control samples without fortification of analyte should be included in each sample set to confirm that no interferences are observed.

7.2 Reagent and Solvent Interference

A reagent blank sample should be included in each sample set to confirm that no interferences are observed.

Using high purity solvents and reagents, no interferences have been found during the method development.

8.0 METHOD VALIDATION

8.1 Extractability

Residues of SYN549522 in water are analysed by direct injection extractability is not relevant here. Acceptable precision and calibration data achieved during method validation (Reference 1) are presented in Tables 1 - 4.

8.2 Recovery Data and Repeatability

Method development has been carried out on the procedures described in sections 3.0 and 4.0. Summary of the method development data are available in the Tables section below.

8.3 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 developed. The estimated LOQ was taken as not less than ten times of the background noise (NLT 10).

The limit of quantification has been set at 0.025 ng/mL (0.025 ppb).

8.4 Limit of Detection (LOD)

The limit of detection of the method is defined as the lowest analyte concentration detectable above the mean amplitude of the background noise in an untreated sample at the corresponding retention time. An estimate of the LOD was taken as three times background noise (NLT3). Note that the LOD varies between each set of samples, for each analyte and from instrument to instrument.

The limit of detection has been set at 0.008 ng/mL (0.008 ppb).

8.5 Matrix Effects

No significant matrix effects were observed in the water 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. In these instances, matrix matched standards may be used to compensate for the matrix effects at the discretion of the study director.

8.6 Detector Linearity

The detector response for SYN549522 has been tested during method development and was found to be linear within the range equivalent to LOQ to 100X LOQ for SYN549522. Full details of the linearity data achieved during method development is presented in the Tables Section below. Calibration graphs are also presented in the Figures Section.

For accurate quantification of residue concentrations, analyses should be carried out within the linear range of the detector used. For multi-point calibration, detector range and linearity will be demonstrated within each sample set. If a residue beyond the measured concentration range is observed, dilute the sample appropriately to bring it within the linear range prior to quantification.

8.7 Extract Stability

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

Up to three (3) days of extract stability was established during the method validation. Achieved extract stability data are presented in Table 5.

9.0 LIMITATIONS

The method has been developed via analysis of representative sample matrix. It can reasonably be assumed that the method can be applied to other samples not previously tested provided that successful recovery samples at the relevant levels verify the performance of the method.

10.0 CONCLUSIONS

This procedure has been demonstrated to be a reliable and accurate procedure for the determination of residues of SYN549522 in water. Only commercially available laboratory equipment and reagents were used for the method development procedure. The limit of quantification of the method is 0.025 ng/mL (0.025 ppb).

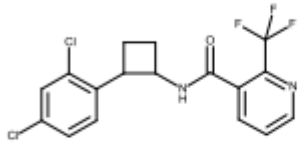
This method satisfies US EPA guideline OCSPP 850.6100 and EC Guidance Documents SANCO/3029/99 rev 4 and SANCO/825/00 rev 8.1.

11.0 REFERENCES

1. Draghi A, Hewa K (2020): SYN549522 - Validation of Residue Method GRM076.04A for the Determination of SYN549522 in Water. PASC-REP-3452, 141-6426. TK0286587.
2. Luxon S G (1992): Hazards in the Chemical Laboratory 5th Edition. The Royal Society of Chemistry. Thomas Graham House, The Science Park, Cambridge CB4 4WF, UK. ISBN 0-85186-229-2.
3. Cardone M J, Palermo P J and Sybrand L B: Potential error in single point ratio calculations based on linear calibration curves with a significant intercept. Anal Chem., 52 pp 1187-1191, 1980

CHEMICAL STRUCTURES

FIGURE 1: Cyclobutrifluram

Company code:	SYN549522
CAS Number:	1460292-16-3
IUPAC Name:	N-[(1S,2S)-2-(2,4-dichlorophenyl)cyclobutyl]-2-(trifluoromethyl)pyridine-3-carboxamide
Molecular formula	$C_{17}H_{13}Cl_2F_3N_2O$
Formula weight:	389.2
Structure:	

APPENDIX 1 APPARATUS

Recommended Suppliers

Equipment	Description	Supplier
General glassware	General glassware	Fisher Scientific
Plastic ware	15 mL PP Centrifuge Tube	Corning Falcon Tube
Centrifuge	Avanti J-25	Beckman Coulter
Vortex mixer	Vortex Genie 2	VWR
Ultrasonic bath	8150	Branson
LC-MS/MS system	AB Sciex API 6500 equipped with a Turbo Ion Spray source	Applied Biosystems
HPLC system	Shimadzu L20	Shimadzu
Autosampler	Shimadzu 30 series	Shimadzu
HPLC column	Waters Acquity UPLC C8, 2.1X50mm, 1.7 µm (P/N: 186002877)	Waters
Nitrogen	Liquid Nitrogen Tank	Air Gas

APPENDIX 1 REAGENTS

Recommended Suppliers

Reagent	Description	Supplier
Acetic acid	ACS grade	Sigma Aldrich
Acetonitrile	HPLC grade	J T Baker
Water	Deionized water	Milli-Q
	HPLC grade	PHARMCO
SYN549522 Analytical standard	GLP certified	GLP Testing Facility, Syngenta, CH-4333, Münchweilen, Switzerland or Syngenta Crop Protection, Inc., P.O. Box 18300 Greensboro, NC 27419-8300

Preparation of Reagents

a) 0.1% Acetic Acid in Acetonitrile – prepared by mixing 1 mL of acetic acid with 1000 mL of acetonitrile.

b) 0.1% Acetic Acid in HPLC grade Water– prepared by mixing 1 mL of acetic acid with 1000 mL of HPLC grade water.

APPENDIX 3 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

Infuse a standard solution of each compound (0.1 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:

m/z 387 for SYN549522 in negative ionization mode.

Using the Analyst software quantitative optimization routine, tune the instrument for SYN549522 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 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.

For SYN549522, in negative ionization mode, the deprotonated molecular ion generated in the ion source (*m/z* 387) is selected and subjected to further fragmentation by collisional activation. The two most sensitive daughter ions (*m/z* 146 and *m/z* 215) are then selected and used for quantitative analysis.

APPENDIX 4 METHOD FLOW CHART

Transfer 1.5 mL of water sample to microcentrifuge tube

↓

Centrifuge water sample > 10K RPM for 2 minutes

↓

Transfer sample from upper portion of centrifuged water to an autosampler vial

↓

Submit autosampler vial for LC-MS/MS analysis