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Cyclobutrifluram

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

Analytical Method

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

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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/30°C
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 $\leq$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.

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).

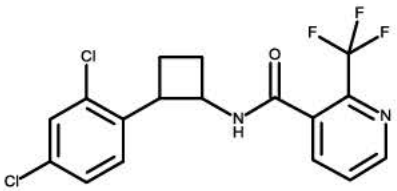
This method satisfies US EPA guideline OCSPP 850.6100 and EC Guidance Documents SANCO/3029/99 rev 4 and SANCO/825/00 rev 8.1.

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 2 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

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Centrifuge water sample > 10K RPM for 2 minutes

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

↓

Submit autosampler vial for LC-MS/MS analysis