

1.0 INTRODUCTION

1.1 Scope of the Method

Analytical method 1781.6992 (Syngenta Method GRM023.13A) is suitable for the determination of degradates SYN548031 and SYN548032 (Figures 1 and Figure 2) in ground and surface water. The limit of quantification (LOQ) of the method has been established at 0.10 µg/L (or 0.10 ppb).

This method satisfies EPA Guidance Document OCSPP 850.6100 (2012), OECD Guidance Document ENV/JM/MONO(2007)17 and EC Guidance Documents SANCO/3029/99 rev 4 and SANCO/825/00 rev 8.1.

1.2 Method Summary

Fortifications of SYN548031 and SYN548032 in water (ground or surface) are diluted with 20:80 acetonitrile:water matrix (where the water matrix is either ground or surface water) for concentrations <.50 µg/L. Fortifications ≥ 50 µg/L are diluted with either 20:80 acetonitrile:purified reagent water or 10:90 water matrix:(20:80 acetonitrile:purified reagent water). All samples are analyzed by liquid chromatography/mass spectrometry (LC-MS/MS).

The limit of quantification of the method is 0.10 µg/L for both SYN548031 and SYN548032 in ground and surface water.

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

Weigh out accurately, using a five figure balance, sufficient SYN548031 and SYN548032 analytical standard (0.0294 and 0.0268 g, respectively). Carefully transfer into a “Class A” volumetric flask (25.0 mL). Dilute to the mark with acetonitrile to give 1000 µg/mL stock solutions of SYN548031 and SYN548032.

Alternatively, the appropriate volume of acetonitrile to add to a known amount of standard material may be determined using the equation below. The standard concentration is corrected for its chemical purity.

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

- P = Standard purity in decimal form (P%)/100
V = Volume of acetonitrile required
W = Weight, in mg, of the solid analytical standard
C = Desired concentration of the final solution, (µg/mL)
1000 = Unit conversion factor

In this case, the standard material is weighed directly into an appropriate storage vessel.

2.3.2 Fortification Solutions

Sample fortification solutions containing SYN548031 and SYN548032 should be prepared by serial dilution in acetonitrile. It is recommended that the following solutions are prepared for each analyte: 100 µg/mL, 10.0 µg/mL and 1.0 µg/mL. It is recommended that the following mixed sub-stock solutions are prepared: 100 µg/mL, 10.0 µg/mL, 1.0 µg/mL, 0.10 µg/mL and 0.010 µg/mL.

2.3.3 Preparation of Calibration Standards for LC-MS/MS

Significant matrix effects of the instrument response for SYN548031 and SYN548032 were observed in the water types tested using the procedures described in Section 3 during method validation. Therefore matrix matched calibration standards should normally be used for quantification.

Calibration standards for SYN548031 and SYN548032 in ground water and surface water for fortifications at 50 µg/L and 5000 µg/L are prepared in 100 mL of 10:90 water

matrix:(20:80 acetonitrile:purified reagent water) by fortifying with a 1.0 or 10.0 µg/mL mixed sub-stock solution as shown in the table below:

Mixed Sub-Stock Concentration (µg/mL)	Fortification Volume (mL)	Final Volume (mL)	Calibration Standard Concentration (µg/L)
1.00	0.0750	100	0.750
	0.100	100	1.00
10.0	0.0250	100	2.50
	0.0500	100	5.00
	0.100	100	10.0
	0.250	100	25.0

Calibration standards for SYN548031 and SYN548032 in ground water and surface water for fortifications at 0.1 µg/L and 1.0 µg/L are prepared in 100 mL of 20:80 acetonitrile:water matrix by fortifying with a 0.010 or 0.10 µg/mL mixed sub-solution as shown in the table below:

Mixed Sub-Stock Concentration (µg/mL)	Fortification Volume (mL)	Final Volume (mL)	Calibration Standard Concentration (µg/L)
0.00995/0.0100 ^a	0.0500	10.0	0.0498/0.0500 ^a
	0.0750	10.0	0.0746/0.0750
	0.100	10.0	0.0995/0.100
0.0995/0.100	0.0250	10.0	0.249/0.250
	0.0500	10.0	0.498/0.500
	0.100	10.0	0.995/1.00
	0.250	10.0	2.49/2.50

^a Concentrations are presented as SYN548031/SYN548032, respectively.

A calibration curve may be generated to quantify SYN548031 and SYN548032 residues. Standards over an appropriate concentration range should be prepared as described above, using the requisite volume of SYN548031 and SYN548032 standard in the appropriate solvent.

2.3.4 Standard Solution Storage and Expiration

All stock solutions were stored in a refrigerator when not in use in amber glass bottles with Teflon-lined caps to prevent decomposition and/or concentration of the standard. Standard solutions should be allowed to equilibrate to room temperature prior to use. All sub-stocks should be discarded following use.

An expiration date of one month for SYN548031 and SYN548032 in acetonitrile is recommended unless additional data are generated to support a longer expiration date.

2.4 Safety Precautions and Hazards

The following information is included as an indication to the analyst of the nature and hazards of the reagents used in this procedure. If in any doubt, consult the appropriate

MSDS or a monograph such as ‘Hazards in the Chemical Laboratory’, edited by S G Luxon, The Chemical Society, London (Reference 1).

Solvent and Reagent Hazards

	Acetonitrile	Formic acid
Harmful Vapor	✓	✓
Highly Flammable	✓	✗
Harmful by Skin Absorption	✓	✓
Irritant to respiratory system and eyes	✓	✓
Causes severe burns	✗	✓
Smithers Visient Hazard Rating (HR)	1	2
OES Short Term (mg/m ³)	105	NA
OES Long Term (mg/m ³)	70	9

NA = Not Applicable.

HR “1” = Gloves, approved eye protection and lab coat required.

HR “2” = Gloves, labcoat, sleeves and facemask (or hood with approved eye protection) for handling raw material and primary stock solutions. Gloves, approved eye protection and labcoat for other operations (secondary stocks).

Test materials SYN548031 and SYN548032 have been assigned a Smithers Visient Hazard Rating 2.

3.0 ANALYTICAL PROCEDURE

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

3.1 Sample Preparation

- a) Transfer 10.0 mL of the water sample to be analyzed into a disposable glass vial with PTFE lined caps. Sample fortification, if required, is to be carried out at this time.

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-measured control water sample, add the appropriate amount of mixed sub-stock solution containing SYN548031 and SYN548032 in acetonitrile. At least two to three untreated control samples should be analyzed alongside each batch of samples to demonstrate acceptable performance of the method and allow recovery corrections to be made if desired. The fortification procedure is outlined in the table below:

(50 µg/L and 5000 µg/L):

Sample ID	Mixed Sub-Stock Concentration (µg/mL)	Fortification Volume (mL)	Matrix Volume (mL)	Fortified Concentration (µg/L)
Control C & D	NA ^a	NA	10.0	0.00
50 µg/L F, G, H, I & J	10.0	0.0500	10.0	50.0
5000 µg/L F, G, H, I & J	100	0.500	10.0	5000

^a NA = Not Applicable.

(0.1 µg/L and 1.0 µg/L):

Sample ID	Mixed Sub-Stock Concentration (µg/mL)	Fortification Volume (mL)	Matrix Volume (mL)	Fortified Concentration (µg/L)
Control E, F & G	NA ^a	NA	10.0	0.00
0.1 µg/L A, B & C	0.00995/0.0100 ^b	0.100	10.0	0.0995/0.100
1.0 µg/L A, B & C	0.0995/0.100	0.100	10.0	0.995/1.00

^a NA = Not Applicable.

^b Concentrations are presented as SYN548031/SYN548032.

b) The 50 µg/L and 5000 µg/L recovery samples were then diluted into the calibration standard range with the appropriate solvent as shown in the table below.

c) **(50 µg/L and 5000 µg/L):**

Sampled ID	Fortified Concentration (µg/L)	Sample Volume (mL)	Final Volume ^a (mL)	Sample Volume (mL)	Final Volume ^b (mL)	Dilution Factor
Control C & G	0.00	1.00	10.0	NA	NA	10.0
50 µg/L F, G, H, I & J	50.0	1.00	10.0	NA	NA	10.0
5000 µg/L F, G, H, I & J	5000	NA	NA	0.0500	50.0	1000

^a Diluted to volume with 20:80 acetonitrile:purified reagent water.

^b Diluted to volume with 10:90 water matrix water (20:80 acetonitrile:purified reagent water).

^c NA = Not Applicable.

d) The 0.1 µg/L and 1.0 µg/L were diluted with acetonitrile. The very low-level recovery samples were then further diluted into the calibration standard range with 20:80 acetonitrile:water matrix as shown in the table below.

(0.1 µg/L and 1.0 µg/L):

Sampled ID	Fortified Concentration (µg/L)	Sample Volume (mL)	Final Volume ^a (mL)	Sample Volume (mL)	Final Volume ^b (mL)	Dilution Factor
Control C & G	0.00	4.00	5.00	NA ^c	NA	1.25
0.1 µg/L F, G, H, I & J	0.0995/0.100 ^d	4.00	5.00	NA	NA	1.25
1.0 µg/L F, G, H, I & J	0.995/1.00	4.00	5.00	1.00	10.0	12.5

^a Diluted to volume with acetonitrile.

^b Diluted to volume with 20:80 acetonitrile: water matrix.

^c NA = Not Applicable.

^d Concentrations are presented as SYN548031/SYN548032.

- e) Transfer an aliquot to a suitable autosampler vial ready for final determination by LC-MS/MS.

3.2 Experimental Precautions

- a) To prevent contamination of the instrument and to minimize 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 0.005 µg/mL. It may also be useful to include multiple blank injections of the final diluent after high level samples to clear any observed carry-over greater than 10% of the LOQ.
- b) Handle controls with clean gloves only; change gloves when returning to a low concentration from a high concentration, etc.

3.3 Time Required for Analysis

The methodology is normally performed with a batch of approximately 9 to 12 samples. One person can complete the analysis of 12 samples in one day (eight hour working period).

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. Acceptable method recoveries will validate any work flow interruptions. Samples should be stored refrigerated in sealed containers where the analysis cannot be completed in a single day.

4.0 FINAL DETERMINATION

The method has been developed for use on an Applied Biosystems Sciex API5000 mass spectrometer. The following instrumentation and conditions have been found to be suitable for this analysis. Other instrumentation can also be used, though optimization 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

Pump	:	Acquity Binary Solvent Manager
Column Oven	:	Acquity Column Compartment
Turbo Ion Spray	:	Applied Biosystems Sciex Turbo V Ion Source ESI
Autosampler	:	Acquity Sample Manager - FTN
Software	:	Analyst 1.6

4.2 Chromatography Conditions

Column	:	XBridge C18 2.5 μ m, 2.1 \times 50 mm
Column Oven Temperature	:	40.0 $^{\circ}$ C
Injection volume	:	50 μ L
Stop Time	:	5.00 min
Injection protocol	:	Bracket samples with calibration standards
Mobile phase	:	0.1% Formic acid in purified reagent water 0.1% Formic acid in acetonitrile

Mobile Phase Composition

Time (min)	% Solvent 1	% Solvent 2	Flow, mL/min
0.00	95.0	5.0	0.300
0.50	95.0	5.0	0.300
3.50	0.0	100.0	0.300
4.00	0.0	100.0	0.300
4.10	95.0	5.0	0.300
5.00	95.0	5.0	0.300

Notes: The column eluate is diverted to waste for the first 1.4 minute to prevent ionic material from the sample contaminating the mass spectrometer front plate. A secondary pump providing flow of mobile phase to the mass spectrometer when the column eluate is switched to waste has been found to be unnecessary.

Under these conditions the retention SYN548031 and SYN548032 is approximately 2.4 minutes and 2.1 minutes, respectively.

4.3 Mass Spectrometer Conditions for SYN548031 and SYN548032

Interface : Turbo V Ion Source - ESI
Polarity : Negative

Curtain gas (CUR) : Nitrogen set at 18.00 (arbitrary units)
Temperature (TEM) : 500 °C
Ionspray voltage : -4500.00 V

Collision gas setting (CAD) : Nitrogen set at 6.00 (arbitrary units)
Gas 1 (GS1) : Air set at 65.00 (arbitrary units)
Gas 2 (GS2) : Air set at 65.00 (arbitrary units)
Interface heater (ihe) : On
Scan type : MRM

MRM Conditions	SYN548031 primary transition	SYN548031 confirmatory transition #1	SYN548031 confirmatory transition #2	SYN548032 primary transition	SYN548032 confirmatory transition #1	SYN548032 confirmatory transition #1
Q1 <i>m/z</i>	: 364.171	364.171	364.171	338.119	338.119	338.119
Q3 <i>m/z</i>	: 175.100	131.200	91.200	278.200	91.200	131.100
Dwell time	: 150 ms	150 ms	150 ms	150 ms	150 ms	150 ms
Resolution Q1	: Unit	Unit	Unit	Unit	Unit	Unit
Resolution Q3	: Unit	Unit	Unit	Unit	Unit	Unit
Declustering potential (DP)	: -270.00 V	-270.00 V	-270.00 V	-270.00 V	-270.00 V	-270.00 V
Entrance potential (EP)	: -10.00 V	-10.00V	-10.00 V	-10.00 V	-10.00 V	-10.00 V
Collision energy (CE)	: -26.00 V	-34.00 V	-58.00 V	-20.00 V	-56.00 V	-40.00 V
Collision cell exit potential (CXP)	: -21.00 V	-1.00 V	-17.00 V	-27.00 V	-9.00 V	-15.00 V

Typical chromatograms for ground and surface water are shown in the Figures Section.

4.4 Confirmatory Procedures for SYN548031 and SYN548032

Final determination by LC-MS/MS with three 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

SYN548031 and SYN548032 residues may be calculated in $\mu\text{g/L}$ for each sample as follows.

- a) Prepare standard solutions over a concentration range appropriate to the expected residues in the samples (for example, 50% LOQ to 10 x LOQ). An appropriate number of different concentrations within this range should be prepared (at least five).
- b) Make an injection of each sample solution and measure the areas of the peaks corresponding to SYN548031 and SYN548032. Calibration standard solutions should be run prior to and following the analysis of the recovery solutions.
- c) Generate calibration curve parameters using an appropriate regression package.
- d) A calibration curve was constructed by plotting the analyte concentration ($\mu\text{g/L}$) in the calibration standards against the peak area of the calibration standards. The equation of the line (equation 1) was algebraically manipulated to give equation 2. The concentration of the test substance within each recovery sample was determined using the regression coefficients from the quadratic equation, the peak area of the recovery sample, and the dilution factor. Equations 2 and 3 were then used to calculate measured concentrations and analytical results.

$$(1) \quad y = ax^2 + bx + c$$

$$(2) \quad \text{DC}(x) = \frac{-b + \sqrt{b^2 - 4aC}}{2a}$$

$$(3) \quad A = \text{DC} \times \text{DF}$$

where:

- y = detector response (peak area) for analyte
- a, b and c = regression constants
- DC(x) = detected concentration ($\mu\text{g/L}$) in the sample
- C = constant c minus the peak area; $C = (c - y)$
- DF = dilution factor (the final sample volume divided by the original sample volume)
- A = concentration of the analyte in the original sample

6.0 CONTROL AND RECOVERY SAMPLES

Control samples should be analyzed 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 analyzed with each batch of samples.

At least two recovery samples (control samples accurately fortified with known amounts of SYN548031 and SYN548032 in acetonitrile) should also be analyzed 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 80% and 120% 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 and then rinsed with HPLC grade methanol, acetone or acetonitrile prior to use.

CHEMICAL STRUCTURES

FIGURE 1 **SYN548031**

Compound Code Number : SYN548031
Alternative compound code number : CSCD668094
CAS Number : Not Applicable
IUPAC Name : Not Applicable
Molecular Formula : C₁₈H₂₀F₂N₃O₃
Molecular Weight : 364.147 (Exact Mass)

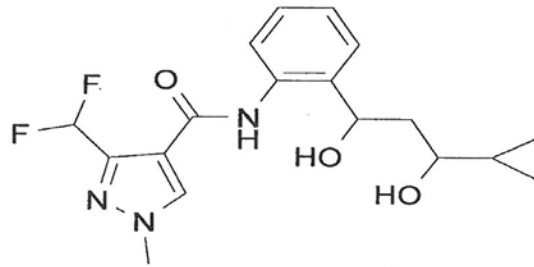
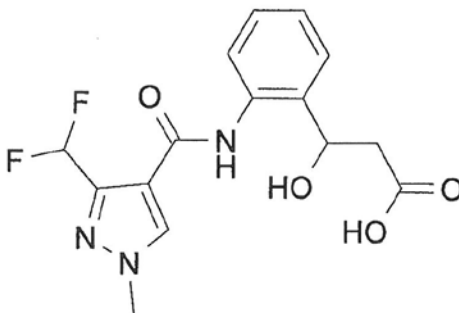


FIGURE 2 SYN548032

Compound Code Number : SYN548032
Alternative compound code number : CSCD668095
CAS Number : Not Applicable
IUPAC Name : Not Applicable
Molecular Formula : C₁₅H₁₅F₂N₃O₄
Molecular Weight : 339.103 (Exact Mass)



APPENDICES SECTION

APPENDIX 1 Apparatus

Recommended Suppliers

Equipment	Description	Supplier
General glassware	General glassware	www.vwrsp.com
Amber autosampler vials	General laboratory supplies	www.vwrsp.com
Amber glass bottles	General laboratory supplies	www.vwrsp.com
Teflon [®] -lined caps	General laboratory supplies	www.vwrsp.com
Balance	AG285	Mettler Toledo
Positive Displacement Pipets	Various sizes	Rainin
LC-MS/MS system	API 5000	AB Sciex
Binary Pump	Acquity Sample Manager	Waters
Autosampler	Acquity Binary Solvent Manager	Waters
Colum Oven	Acquity Column Compartment	Waters
Turbo Ion Spray	Turbo V ESI Ion Source	AB Sciex
Software	Analyst 1.6	AB Sciex

APPENDIX 2 Reagents

Recommended Suppliers

Reagent	Description	Supplier
Purified reagent water	Reagent grade	prepared from a Millipore Milli-Q [®] Direct 8 Water Purification system (meets ASTM Type II requirements)
Acetonitrile	reagent grade	EMD
Formic acid	reagent grade	EMD
SYN548031 and SYN548032 analytical standards	GLP certified	Syngenta Crop Protection, Inc., P.O. Box 18300, Greensboro, NC 27419-8300.

Preparation of Reagents

- a) A 20:80 acetonitrile:purified reagent water (v:v) liquid reagent solution was typically prepared by combining 800 mL of purified reagent water with 200 mL of acetonitrile. The solution was then mixed using a stir bar and stir plate.
- b) A 10:90 ground water:(20:80) acetonitrile:purified reagent water (v:v) liquid reagent solution was typically prepared by combining 100 mL of ground water with 900 mL of 20:80 acetonitrile:purified reagent water (v:v). The solution was then mixed using a stir bar and stir plate.
- c) A 10:90 surface water:(20:80) acetonitrile:purified reagent water (v:v) liquid reagent solution was typically prepared by combining 100 mL of surface water with 900 mL of 20:80 acetonitrile:purified reagent water (v:v). The solution was then mixed using a stir bar and stir plate.
- d) A 20:80 acetonitrile:ground water (v:v) liquid reagent solution was typically prepared by combining 80.0 mL of ground water with 20 mL of acetonitrile. The solution was then mixed using a stir bar and stir plate.
- e) A 20:80 acetonitrile:surface water (v:v) liquid reagent solution was typically prepared by combining 80.0 mL of surface water with 20.0 mL of acetonitrile. The solution was then mixed using a stir bar and stir plate.
- f) A 0.1% formic acid in purified reagent water mobile phase solution was typically prepared by adding 1.00 mL of concentrated formic acid to 1.0 L of purified reagent water and mixed well. The solution was degassed under vacuum with sonication.
- g) A 0.1% formic acid in acetonitrile mobile phase solution was typically prepared by adding 1.00 mL of concentrated formic acid to 1.0 L of acetonitrile and mixed well. The solution was degassed under vacuum with sonication.

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 quadruples (Q1 and Q3).

Tuning Instrument for SYN548031 and SYN548032

Infuse standard solutions of SYN548031 and SYN548032 (1.0 to 10.0 µg/mL) in mobile phase (see section 4) directly into the mass spectrometer interface at a rate of approximately 10 to 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 under negative ionization mode.

Using the Analyst software quantitative optimization routine, tune the instrument for SYN548031 and SYN548032, 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.

Analyte	Parent Ion	Daughter ion Primary Transition	Daughter ion Confirmatory Transition	Daughter ion Confirmatory Transition
SYN548031	364	175	131	91
SYN548032	338	278	91	131

Finally, connect the LC-pump via the autosampler directly to the MS/MS instrument. Perform repetitive flow injection of a SYN548031 and SYN548032 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.

The parent ion is subjected to further fragmentation by collisional activation. The three most sensitive daughter ions are then selected and used for quantitative analysis.

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

APPENDIX 4 Method Flow Chart

Analyze directly by LC-MS/MS after dilution with 20:80 acetonitrile:purified reagent water, 20:80 acetonitrile: water matrix, or 10:90 acetonitrile:(20:80 acetonitrile:purified reagent water).