MRID:51778718

202100486

TITLE

Independent Laboratory Validation of Valent Analytical Method RM-52S-4A: "Determination of V-10456, S-3100-CA, S-3100-DA, S-3100-CA-RD, S-3100-CA-UR, S-3100-DP, 4″-OH-S-3100-CA, and S-3100-DA-RD in Soil"

TEST GUIDELINE

850.6100

AUTHOR(S)

Vandaveer W,

Shepherd J

STUDY COMPLETION DATE

12/28/2021

PERFORMING LABORATORY

SynTech Research

17745 South Metcalf Ave.

Stilwell, KS 66085

USA

LABORATORY PROJECT ID

V-21-202602

TOTAL PAGES

282

INTRODUCTION

Summary

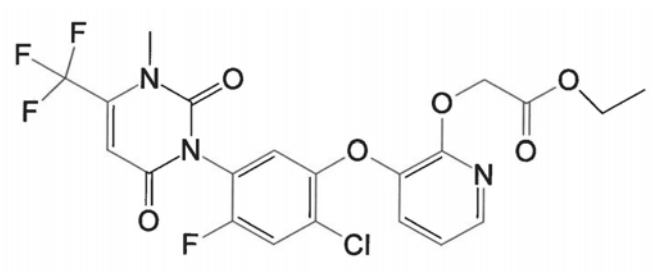
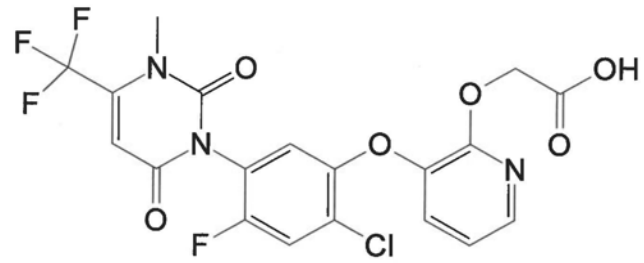
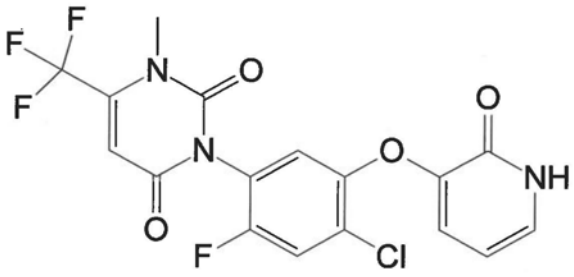
The purpose of this study was to independently validate the analytical method RM-52S-4A for V-10456, S-3100-CA, S-3100-DA, S-3100-CA-RD, S-3100-CA-UR, S-3100-DP, 4″-OH-S-3100-CA, and S-3100-DA-RD in soil. This Independent Laboratory Validation (ILV) was conducted according to the guidelines issued by the US EPA found in OCSPP 850.6100 and PR Notice 96-1 (1,2). Additionally, the ILV encompassed the guidelines found in European Commission SANTE/2020/12830, Rev.1 and SANTE/12682/2019 Guidance Document of Pesticide Residue Analytical Methods (3). This study was conducted in compliance with the EPA FIFRA Good Laboratory Practice Standards, 40 CFR Part 160 (4). The protocol and amendments are included in Appendix A. SynTech Research successfully independently validated Valent U.S.A. LLC analytical method RM-52S-4A entitled "Determination of Residues of V-10456, S-3100-CA, S-3100-DA, S-3100-CA-RD, S-3100-CA-UR, S-3100-DP, 4″-OH-S-3100-CA and S-3100-DA-RD in Soil" (5).

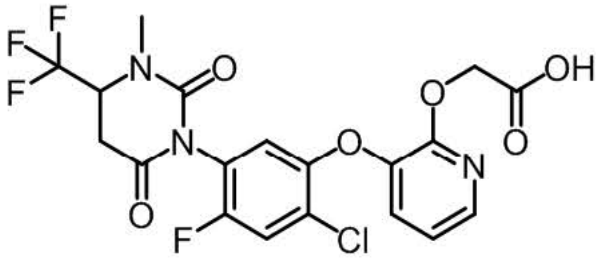
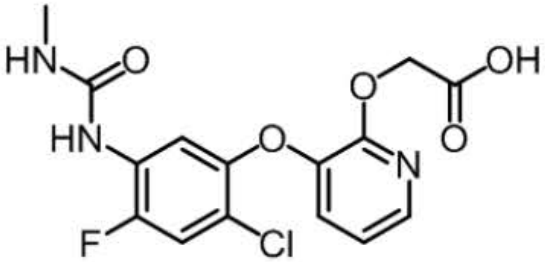
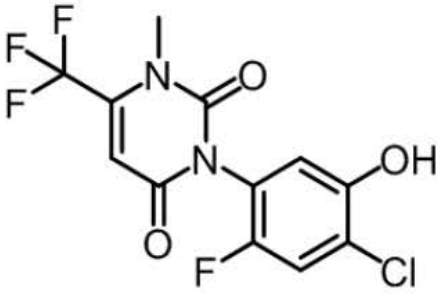
Method Principle

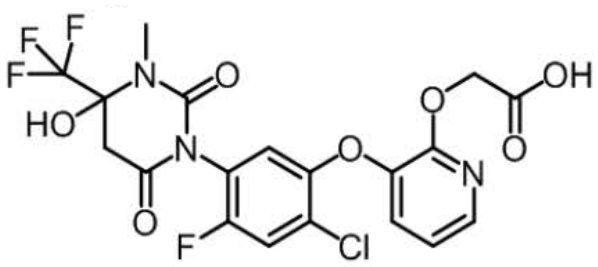
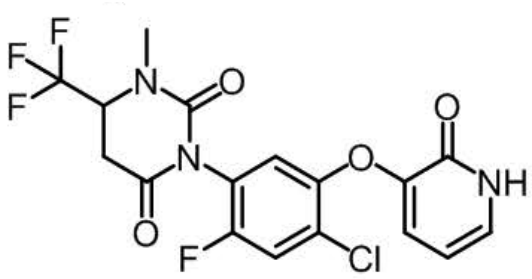
Residues of V-10456, S-3100-CA, S-3100-DA, S-3100-CA-RD, S-3100-CA-UR, S-3100-DP, 4″-OH-S-3100-CA, and S-3100-DA-RD were extracted from 20.0 g (±0.1g) of soil in a 50 mL polypropylene centrifuge tube using 25 mL of acetonitrile (ACN)/0.1 M HCl (5/1, v/v) and shaking on a reciprocating shaker for approximately 20 min. The sample was centrifuged at approximately 4000 rpm for 5 min and the supernatant was decanted into a 100 mL graduated cylinder. The sample was extracted a second and a third time with a separate 25 mL aliquot of ACN/0.1 M HCl (5/1, v/v), and each time the sample was centrifuged and the supernatant decanted and combined in the original 100 mL graduated cylinder. The sample extract was brought to a final volume of 100 mL with the extraction solvent. A 25 mL aliquot of the sample extract was transferred to a separatory funnel, 25 mL of HPLC water was added, and around 50 mL of dichloromethane was added and the sample was shaken for one minute. The layers were allowed to separate and the lower dichloromethane layer was drained into a 200 mL turbo vap tube. The extraction was repeated twice with fresh aliquots of dichloromethane (50 mL) and the extracts were combined. The aqueous layer was then discarded. The sample extract was concentrated to dryness using a turbo vap at ≤40 °C. The sample was reconstituted in 5.0 mL of ACN acidified with 0.1% formic acid and sonicated briefly. A 5.0 mL aliquot of HPLC-grade water acidified with 0.1% formic acid was added to the sample and sonicated to mix. Approximately 1.5 mL of the sample extract was filtered using 0.22 µm PTFE filter into a HPLC autosampler vial. The sample was analyzed by liquid chromatography tandem mass spectrometry (LC-MS/MS). The detailed method is included in a protocol amendment found in Appendix A.

The Test Reference Substances

The test reference substances were provided by Valent U.S.A. LLC. The certificates of analysis for the test reference substances are provided in Appendix B and details are provided below.

Common Name	Structural Formula and Chemical Name
V-10456 (S-3100) CAS Number: 353292-31-6 Molecular Formula: $C_{21}H_{16}ClF_4N_3O_6$ Molecular Weight: 517.82 g/mol Lot No.: AS 2429b Purity: 99.4% Expiration Date: 4/9/2023 Date of Analysis: 4/9/2021 Storage Conditions: Freezer Source: Valent U.S.A. LLC	 Ethyl [(3-{2-chloro-4-fluoro-5-[3-methyl-2,6-dioxo-4-(trifluoromethyl)-3,6-dihydropyrimidin-1(2H)-yl]phenoxy}pyridin-2-yl)oxy]acetate
S-3100-CA CAS Number: 353292-39-4 Molecular Formula: $C_{19}H_{12}ClF_4N_3O_6$ Molecular Weight: 489.77 g/mol Lot No.: AS 2432a Purity: 99.7% Expiration Date: 10/21/2021 Date of Analysis: 10/21/2019 Storage Conditions: Freezer Source: Valent U.S.A. LLC	 (3-{2-chloro-4-fluoro-5-[1,2,3,6-tetrahydro-3-methyl-2,6-dioxo-4-(trifluoromethyl)pyrimidin-1-yl]phenoxy}pyridine-2-yloxy) acetic acid
S-3100-DA CAS Number: 497866-09-8 Molecular Formula: $C_{17}H_{10}ClF_4N_3O_4$ Molecular Weight: 431.73 g/mol Lot No.: AS 2435a Purity: 95.7% Expiration Date: 11/11/2021 Date of Analysis: 11/11/2019 Storage Conditions: Freezer Source: Valent U.S.A. LLC	 3-[4-chloro-5-(1,2-dihydro-2-oxpyridin-3-yloxy)-2-fluorophenyl]-1,2,3,4-tetrahydro-1-methyl-6-(trifluoromethyl)pyrimidine-2,4-dione

Common Name	Structural Formula and Chemical Name
S-3100-CA-RD CAS Number: Not Given Molecular Formula: $C_{19}H_{14}ClF_4N_3O_6$ Molecular Weight: 491.78 g/mol Lot No.: AS 2433a Purity: 95.4% Expiration Date: 10/24/2021 Date of Analysis: 10/24/2019 Storage Conditions: Freezer Source: Valent U.S.A. LLC	 (3-{2-chloro-4-fluoro-5-[1,2,3,4,5,6-hexahydro-3-methyl-2,6-dioxo-4-(trifluoromethyl)pyrimidin-1-yl]phenoxy}pyridine-2-yloxy)acetic acid
S-3100-CA-UR CAS Number: Not Given Molecular Formula: $C_{15}H_{13}ClFN_3O_5$ Molecular Weight: 369.74 g/mol Lot No.: AS 2434a Purity: 97.1% Expiration Date:11/05/2021 Date of Analysis: 11/05/2019 Storage Conditions: Freezer Source: Valent U.S.A. LLC	 {3-[2-chloro-4-fluoro-5-(3-methylureido)phenoxy]pyridine-2-yloxyl}acetic acid
S-3100-DP CAS Number: Not Given Molecular Formula: $C_{12}H_7ClF_4N_2O_3$ Molecular Weight: 338.65 g/mol Lot No.: AS 2439a Purity: 99.2% Expiration Date:02/11/2022 Date of Analysis: 02/11/2020 Storage Conditions: Freezer Source: Valent U.S.A. LLC	 3-(4-chloro-2-fluoro-5-hydroxyphenyl)-1,2,3,4-tetrahydro-1-methyl-6-(trifluoromethyl)pyrimidine-2,4-dione

Common Name	Structural Formula and Chemical Name
4″-OH-S-3100-CA CAS Number: Not Given Molecular Formula: $C_{19}H_{14}ClF_4N_3O_7$ Molecular Weight: 507.78 g/mol Lot No.: AS 2438b Purity: 92.4% Expiration Date: 12/12/2021 Date of Analysis: 12/12/2019 Storage Conditions: Freezer Source: Valent U.S.A. LLC	 (3-{2-chloro-4-fluoro-5-[1,2,3,4,5,6-hexahydro-4-hydroxy-3-methyl-2,6-dioxo-4-(trifluoromethyl)pyrimidin-1-yl]phenoxy}pyridine-2-yloxy)acetic acid
S-3100-DA-RD CAS Number: Not Given Molecular Formula: $C_{17}H_{12}ClF_4N_3O_4$ Molecular Weight: 433.74 g/mol Lot No.: AS 2443a Purity: 99.7% Expiration Date: 04/27/2022 Date of Analysis: 04/27/2020 Storage Conditions: Freezer Source: Valent U.S.A. LLC	 3-[4-chloro-5-(1,2-dihydro-2-oxo-pyridin-3-yloxy)-2-fluorophenyl]-1,2,3,4,5,6-hexahydro-1-methyl-6-(trifluoromethyl)pyrimidine-2,4-dione

EXPERIMENTAL

Sample Origin, Numbering, and Storage

Untreated control samples of soil were provided by the sponsor and used for the study. The soil selected was the most difficult (highest organic matter) from the terrestrial field dissipation study. The samples were stored frozen at ≤ -18 °C except when removed for subsampling for extraction.

Samples and standard solutions were identified with unique sample names and numbers. Labels included the name of the compound, concentration and date mixed.

Accuracy and Precision

Method accuracy (recovery) and precision (data spread) were evaluated by fortifying control samples with V-10456, S-3100-CA, S-3100-DA, S-3100-CA-RD, S-3100-CA-UR, S-3100-DP, 4″-OH-S-3100-CA, and S-3100-DA-RD and analyzing the samples as described in the analytical method RM-52S-4A (Appendix A). The quantitative and qualitative MRM ion

transitions were monitored for the quantitation of V-10456, S-3100-CA, S-3100-DA, S-3100-CA-RD, S-3100-CA-UR, S-3100-DP, 4″-OH-S-3100-CA, and S-3100-DA-RD in the validation sample sets. The following transitions were monitored:

V-10456	m/z Q1/Q3 518 / 293 (quantitative)
	m/z Q1/Q3 518 / 250 (qualitative)
S-3100-CA	m/z Q1/Q3 490 / 250 (quantitative)
	m/z Q1/Q3 490 / 293 (qualitative)
S-3100-DA	m/z Q1/Q3 432 / 281 (quantitative)
	m/z Q1/Q3 432 / 82 (qualitative)
S-3100-CA-RD	m/z Q1/Q3 490 / 376 (quantitative)
	m/z Q1/Q3 490 / 319 (qualitative)
S-3100-CA-UR	m/z Q1/Q3 368 / 293 (quantitative)
	m/z Q1/Q3 368 / 337 (qualitative)
S-3100-DP	m/z Q1/Q3 337 / 186 (quantitative)
	m/z Q1/Q3 337 / 150 (qualitative)
4″-OH-S-3100-CA	m/z Q1/Q3 508.1 / 385.0 (quantitative)
	m/z Q1/Q3 508.1 / 489.9 (qualitative)
S-3100-DA-RD	m/z Q1/Q3 434.1 / 281.1 (quantitative)
	m/z Q1/Q3 434.1 / 323.0 (qualitative)

Calculation of Standard Calibration Curve

Calculation of a standard curve is executed by injection of a series of calibration standards as described in Appendix A and acquisition of peak areas for V-10456, S-3100-CA, S-3100-DA, S-3100-CA-RD, S-3100-CA-UR, S-3100-DP, 4″-OH-S-3100-CA, and S-3100-DA-RD. The linearity of detector response for V-10456, S-3100-CA, S-3100-DA, S-3100-CA-RD, S-3100-CA-UR, S-3100-DP, 4″-OH-S-3100-CA, and S-3100-DA-RD was evaluated using standard solutions prepared in solvent. Calibration curves were calculated using a second-order polynomial equation on the basis of peak area. Refer to Appendix C for calibration plots of the validation sets and to Appendix D for example calculations.

Statistical Treatment of Data

Statistical treatment of data included the calculation of regression equations, coefficients of determination (r^2) for describing the linearity of calibration curves, and means, standard deviations, and relative standard deviations of the results for the fortified recovery samples.

RESULTS AND DISCUSSION

Assay Time

A typical analytical run would consist of a minimum of six calibration standards (at different concentration levels beginning at 50% of the LOQ or below on the low end), reagent blank, two untreated control samples, five fortified control samples at the LOQ and five fortified control samples at 10xLOQ. This typical sample set can be taken through the analytical

procedure in approximately 1 working day.

Selectivity

The method is selective for the determination of V-10456, S-3100-CA, S-3100-DA, S-3100-CA-RD, S-3100-CA-UR, S-3100-DP, 4″-OH-S-3100-CA, and S-3100-DA-RD by virtue of the chromatographic separation and MRM detection. Significant peak response (>30% of the LOQ peak area ratio) was not observed in extracts of untreated blank control samples at the expected retention times of the analytes. Unambiguous identification is ensured by the observation of a precursor ion plus a structurally significant product ion observed at the same retention time.

Confirmation of the presence of V-10456, S-3100-CA, S-3100-DA, S-3100-CA-RD, S-3100-CA-UR, S-3100-DP, 4″-OH-S-3100-CA, and S-3100-DA-RD was done by comparison of retention times (liquid chromatography) of recovery samples with the retention times of the calibration standards as well as by monitoring two structurally characteristic MRM transitions for the analyte by tandem mass spectrometry. Validation data obtained using the qualitative MRM transition(s) met the same acceptance criteria as the validation data generated using the quantitative MRM transition(s), therefore demonstrating that the analyte signal of the quantitative MRM transition is selective and not affected by interferences.

Linearity

The linearity of detector response to the analytes was evaluated using solvent standard solutions. Calibration curves were calculated using a second-order polynomial equation on the basis of peak area. Calibration curves resulting from the injection of six standards over the concentration range of 0.05 ng/mL to 25.0 ng/mL demonstrated linearity with coefficients of determination (r^2) of at least 0.995.

Typical calibration curves for the determination of V-10456, S-3100-CA, S-3100-DA, S-3100-CA-RD, S-3100-CA-UR, S-3100-DP, 4″-OH-S-3100-CA, and S-3100-DA-RD in soil are illustrated in detailed analytical spreadsheets in Appendix C.

Recovery Levels and Precision

The method validation study was conducted to determine the recovery levels and the precision of the method for the determination of V-10456, S-3100-CA, S-3100-DA, S-3100-CA-RD, S-3100-CA-UR, S-3100-DP, 4″-OH-S-3100-CA, and S-3100-DA-RD in soil. Samples were fortified at the limit of quantitation (LOQ) of 0.00015 mg/kg and 0.0015 mg/kg (10xLOQ) for V-10456, S-3100-CA, S-3100-DA, and S-3100-DP. Samples were fortified at the LOQ of 0.001 mg/kg and 0.01 mg/kg (10xLOQ) for S-3100-CA-RD, 4″-OH-S-3100-CA, and S-3100-DA-RD. Samples were fortified at the LOQ of 0.005 mg/kg and 0.05 mg/kg (10xLOQ) for S-3100-CA-UR. Two unfortified control samples were also included in each set.

REFERENCES

1. Ecological Effects Test Guidelines, OCSPP 850.6100, Environmental Chemistry Methods and Associated Independent Laboratory Validation, U.S. Environmental Protection Agency. U.S. Government Printing Office: Washington, DC, 2012; EPA-712-C-001.

2. Pesticide Regulation Notice 96-1, U.S. Environmental Protection Agency, Office of Pesticide Programs: Washington, DC 1996.

3. European Commission Guidelines, SANTE/2020/12830, Rev 1, SANTE/12682/2019 Guidance Document on Residue Analytical Methods

4. U.S. Environmental Protection Agency, Office of Compliance Monitoring. 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.

5. Method-No. RM-52S-4A, Determination of Residues of V-10456, S-3100-CA, S-3100-DA, S-3100-CA-RD, S-3100-CA-UR, S-3100-DP, 4″-OH-S-3100-CA and S-3100-DA-RD in Soil, Chen, Tiffany, 10-14-2021.

APPENDIX A PROTOCOL AND AMENDMENTS

SynTech Research 693SRUS21R0167

STUDY PROTOCOL

STUDY TITLE

Independent Laboratory Validation of Valent Analytical Method RM-52S-4: "Determination of V-10456, S-3100-CA, S-3100-DA, S-3100-CA-RD, S-3100-CA-UR, S-3100-DP, 4"-OH-S-3100-CA and S-3100-DA-RD in Soil"

Valent Study Number
V-21-202602

SynTech Research Study Number
693SRUS21R0167

Data Requirement

Ecological Effects Test Guideline
OCSPP 850.6100
Environmental Chemistry Methods and Associated Independent Laboratory Validation

European Commission Guidelines:
SANTE/2020/12830, Rev 1
SANTE/12682/2019
Guidance Document on
Residue Analytical Methods

Residue Chemistry Guidelines:
PR Notice 96-1, Tolerance Enforcement Methods, ILV

OECD ENV/JM/MONO(2007) 17-Guidance Document of Pesticide Residue Analytical Methods

SPONSOR

Valent USA, LLC
4600 Norris Canyon Road
San Ramon, CA 94583
USA

STUDY DIRECTOR

Michael FinleySynTech Research

TESTING FACILITY

SynTech Research
17745 South Metcalf Ave.
Stilwell, KS 66085

2. PURPOSE

The purpose of this study is to perform an Independent Laboratory Validation (ILV) of analytical method RM-52S-4, Determination of Residues of V-10456, S-3100-CA, S-3100-DA, S-3100-CA-RD, S-3100-CA-UR, S-3100-DP, 4"-OH-S-3100-CA and S-3100-DA-RD in Soil (1) (Appendix 1). Validation samples will be fortified and evaluated to demonstrate the feasibility of the method at a laboratory having no prior experience with the method.

SynTech Research will conduct the ILV according to the guidelines issued by the US EPA found in Ecological Effects Test GuidelineOCSPP 850.6100: Environmental Chemistry Methods and Associated Independent Laboratory Validation and PR Notice 96-1. Additionally, the ILV will encompass the guidelines found in European Commission SANTE/2020/12830, Rev.1 and SANTE/12682/2019 -Guidance Document of Pesticide Residue Analytical Methods. This study will be conducted in compliance with the EPA FIFRA Good Laboratory Practice Standards, 40 CFR Part 160.

The ILV of this method will be performed by SynTech Research, Stilwell, Kansas on soil from ND.

3. GOOD LABORATORY PRACTICE STATEMENT

This study will be performed in compliance with EPA's Good Laboratory Practice Standards as specified in 40 CFR 160. The Study Director is responsible for the overall conduct of the study.

4. QUALITY ASSURANCE

The quality assurance unit of SynTech Research will inspect and review the protocol and at least one phase of the study while the study is in progress, and the final report. Audit findings will be reviewed by the Study Director and appropriate management of the Testing Facility.

5. PROTOCOL AMENDMENTS AND DEVIATIONS

This protocol can be amended by mutual consent of the Study Director, Study Director Management and the Study Monitor. Detailed descriptions of all amendments will be signed by the Study Director, Study Director Management and Sponsor, and be effective at the time of the Study Director's signature.

Unplanned changes (deviations) to the protocol will be documented and established in the raw data, signed and dated by the Study Director. The written deviation must include a description of the deviation, the reason for the deviation, and the impact, if any, on the study. The written deviation will be placed in the study file.

6. SCHEDULE

Estimated experimental start Date:	Septamber 2021
Estimated experimental completion Date:	September 2021
Estimated Date of Final Report:	October 2021

7. MATERIALS AND METHODS

7.1 TEST/REFERENCE STANDARDS

Characterization data for each of the test/reference substances will be maintained by Valent USA, LLC., 4600 Norris Canyon Road, San Ramon, CA 94583, USA. Test/reference substances will be stored as per the recommended storage conditions specified on the label or GLP Certificate of Analysis (COA). Safety Data Sheets (SDS) or other information necessary for proper and safe handling of the test/reference materials will be provided by Valent USA, LLC., and maintained at SynTech Research. All records of test/reference substance storage conditions and records of weights and dilutions will be maintained in the study raw data file.

7.1.1 TARGET COMPOUNDS

Target Compound: V-10456 (S-3100)

Chemical Name:	Ethyl [(3-{2-chloro-4-fluoro-5-[3-methyl-2,6-dioxo-4-(trifluoromethyl)-3,6-dihydropyrimidin-1(2H)-yl]phenoxy}pyridin-2-yl)oxy]acetate
CAS Number:	353292-31-6
Molecular Formula:	$C_{21}H_{16}ClF_4N_3O_6$
Molecular Weight:	517.82
Lot No.:	AS 2429B
Purity:	99.4%
Expiration Date:	4/9/2023
Date of Analysis:	4/9/2021
Storage Conditions:	Freezer
Source:	Valent USA, LLC

Target Compound: S-3100-CA

Chemical Name:	2-[[3-[2-chloro-4-fluoro-5-[3-methyl-2,6-dioxo-4-(trifluoromethyl)pyrimidin-1-yl]phenoxy]-2-pyridyl]oxy]acetic acid
CAS Number:	353292-39-4
Molecular Formula:	$C_{19}H_{12}ClF_4N_3O_6$
Molecular Weight:	489.77
Lot No.:	AS 2432a
Purity:	99.7%
Expiration Date:	10/21/2021
Date of Analysis:	10/21/2019
Storage Conditions:	Freezer
Source:	Valent USA, LLC

Target Compound: S-3100-DA

Chemical Name:	3-[4-chloro-2-fluoro-5-[(2-oxo-1H-pyridin-3-yl)oxy]phenyl]-1-methyl-6-(trifluoromethyl)pyrimidine-2,4-dione
CAS Number:	497866-09-8
Molecular Formula:	$C_{17}H_{10}ClF_4N_3O_4$
Molecular Weight:	431.73
Lot No.:	AS 2435a
Purity:	95.7%
Expiration Date:	11/11/2021
Date of Analysis:	11/11/2019
Storage Conditions:	Freezer
Source:	Valent USA, LLC

Target Compound: S-3100-CA-UR

Chemical Name:	{3-[2-chloro-4-fluoro-5-(3-methylureido)phenoxy]pyridine-2-yloxyl}acetic acid
CAS Number:	Not Given
Molecular Formula:	$C_{15}H_{13}ClFN_3O_5$
Molecular Weight:	369.74
Lot No.:	AS 2434a
Purity:	97.1%
Expiration Date:	11/05/2021
Date of Analysis:	11/05/2019
Storage Conditions:	Freezer
Source:	Valent USA, LLC

Target Compound: S-3100-DP

Chemical Name:	3-(4-chloro-2-fluoro-5-hydroxyphenyl)-1,2,3,4-tetrahydro-1-methyl-6-(trifluoromethyl)pyrimidine-2,4-dione
Molecular Formula:	$C_{12}H_7ClF_4N_2O_3$
CAS Number:	Not Given
Molecular Weight:	338.65
Lot No.:	AS 2439a
Purity:	99.2%
Expiration Date:	02/11/2022
Date of Analysis:	02/11/2020
Storage Conditions:	Freezer
Source:	Valent USA, LLC

Target Compound: S-3100-CA-RD

Chemical Name:	(3-{2-chloro-4-fluoro-5-[1,2,3,4,5,6-hexahydro-3-methyl-2,6-dioxo-4-(trifluoromethyl)pyrimidin-1-yl]phenoxy}pyridine-2-yloxy)acetic acid
Molecular Formula:	$C_{19}H_{14}ClF_4N_3O_6$
CAS Number:	Not Given
Molecular Weight:	491.78
Lot No.:	AS 2433a
Purity:	95.4%
Expiration Date:	10/24/2021
Date of Analysis:	10/24/2019
Storage Conditions:	Freezer
Source:	Valent USA, LLC

Target Compound: 4"-OH-S-3100-CA

Chemical Name:	(3-{2-chloro-4-fluoro-5-[1,2,3,4,5,6-hexahydro-4-hydroxy-3-methyl-2,6-dioxo-4-(trifluoromethyl)pyrimidin-1-yl]phenoxy}pyridine-2-yloxy)acetic acid
Molecular Formula:	$C_{19}H_{14}ClF_4N_3O_7$
CAS Number:	Not Given
Molecular Weight:	507.78
Lot No.:	AS 2438b
Purity:	92.4%
Expiration Date:	12/12/2021
Date of Analysis:	12/12/2019
Storage Conditions:	Freezer
Source:	Valent USA, LLC

Target Compound: S-3100-DA-RD

Chemical Name:	3-[4-chloro-5-(1,2-dihydro-2-oxo-pyridin-3-yloxy)-2-fluorophenyl]-1,2,3,4,5,6-hexahydro-1-methyl-6-(trifluoromethyl)pyrimidine-2,4-dione
Molecular Formula:	$C_{17}H_{12}ClF_4N_3O_4$
CAS Number:	Not Given
Molecular Weight:	433.74
Lot No.:	AS 2443a
Purity:	99.7%
Expiration Date:	04/27/2022
Date of Analysis:	04/27/2020
Storage Conditions:	Freezer
Source:	Valent USA, LLC

7.2 TEST SYSTEMS

Control soil will be supplied by Valent USA, LLC. The source, along with any pertinent records will be documented and maintained in the raw data. Samples to be analyzed will be processed (e.g., homogenized in the presence of dry ice) as necessary using the appropriate SOPs.

Control soil samples will be stored frozen at all times in a temperature monitored freezer at less than or equal to an average temperature of -18 °C except when removed for weighing for analysis. Sample storage conditions during validation will be documented in the raw data.

Control soil will be fortified with the targeted analytes and extracted and analyzed using internal standard calibration according to procedures described in method RM-52S-4. Unfortified soils will also be analyzed to verify the absence of interfering compounds.

7.3 METHOD OF ANALYSIS

Prior to performing the ILV, it will be necessary for the SynTech personnel to establish the method (e.g., determine analyte retention times, perform instrument tuning, and determine instrument detection limits and linearity of the response to a range of analyte concentrations). In general, the ILV lab needs to verify control of the LC-MS/MS aspects of the method before initiating the ILV. Clarification of the method will be provided by the method developer if requested by the Study Director or his designee. All contacts made during the establishment of the method will be documented in writing and presented in the final report.

SynTech Research will analyze a validation set by following the procedure described in method RM-52S-4 as written, and according to the instructions found in this protocol. Comparable apparatus, solvents, and glassware may be substituted for those described in the method, except where specifically noted otherwise. Any substitutions must be pre-approved by the Study Monitor. In the event a substituted piece of equipment or technique is used, this use will be documented in the raw data and discussed in the analytical report. All contact with the Sponsor concerning questions on the methodology will be documented in the raw data and reported. Three ILV attempts are allowed for the soil.

7.4 METHOD PERFORMANCE

In general, calculations will be performed as described in the analytical method RM-52S-4.

Linearity will be assessed from the standard calibration curve. The linear response will extend over the same concentration as described in the original method. A representative standard curve is included in the original method (Appendix 1).

Accuracy, precision and repeatability will be assessed with the analysis of the fortified matrix: soil. At each fortification level, the mean recovery should be in the range of 70-120% with a %RSD <20. Interferences should be <30% compared to the LOQ. The measured values for the calibration standards at or above the LOQ should be within ± 25% of the nominal solution concentration. The above criteria apply to both the quantitation and confirmatory analysis. In this method, the confirmatory analysis will be an alternate SRM (Single Reaction Monitoring) transition on the LC-MS/MS.

For the soil, once the Study Director and Study Monitor have judged that first sample set of the three permitted has met the criteria; the study is complete. If the results are determined to be unsuccessful in the soil, the Study Director will consult with the Sponsor to clarify directions given in the method. This communication will be documented by the Testing Facility and presented in the final report. If the second attempt is also unsuccessful, the Study Monitor will again be contacted before attempting a third set. If all three sample sets fail, the method validation will be terminated and a final report written. In the report an explanation of what failed in each attempt (i.e. low recoveries, high recoveries) should be included; however, no speculation should be included in this discussion.

If validations are acceptable in the soil, a final report presenting the results will be written by the Test Facility.

7.5 LIMIT OF QUANTITATION

The limit of quantitation (LOQ) of the method for the ILV in soil is 0.00015 mg/kg (ppm) for V-10456, S-3100-CA, S-3100-DA, and S-3100-DP. The limit of quantitation (LOQ) of the method for the ILV in soil is 0.001 mg/kg (ppm) for S-3100-CA-RD, S-3100-DA-RD and 4"-OH-S-3100-CA. The limit of quantitation (LOQ) of the method for the ILV in soil is 0.005 mg/kg (ppm) for S-3100-CA-UR.

7.6 EXPERIMENTAL PROCEDURES

7.6.1 STANDARD PREPARATION

All calibration and fortification standard solutions must be made from the test/reference standards using solvents specified in the method. Preparation of all stock and serially diluted standards solutions will be documented. The documentation will be included in the raw data study file.

7.6.2 FORTIFICATION PROCEDURE

Aliquots of untreated control sample will be fortified with volume amounts of appropriate test/reference standard solutions. During the fortification, the standard solution will be evenly distributed over the exposed sample as much as possible.

Control sample of each soil will be fortified according to the following scheme:

Matrix	Sample Type	Fortifying Compound	Fortification Level (ppm) [mg/kg]	# of Samples
Reagent Blank	Blank	None	--	1
Soil(5)	Control	None	--	2
Soil(5)	Fortified Control	(1)	0.00015 (LOQ)	(4)
Soil(5)	Fortified Control	(1)	0.0015 (10XLOQ)	(4)
Soil(5)	Fortified Control	(2)	0.001 (LOQ)	(4)
Soil(5)	Fortified Control	(2)	0.010 (10XLOQ)	(4)
Soil(5)	Fortified Control	(3)	0.005 (LOQ)	(4)
Soil(5)	Fortified Control	(3)	0.050 (10XLOQ)	(4)

(1) Mixtue of V-10456, S-3100-CA, S-3100-DA, S-3100-DP
(2) Mixture of S-3100-CA-RD, S-3100-DA-RD and 4"-OH-S-3100-CA
(3) Mixture of S-3100-CA-UR
(4) The LOQ is at 3 different levels but will be spiked with separate standards into only 5 total samples. For example, The 5 LOQ samples will be at 0.00015, 0.001 and 0.005 mg/kg.
(5) North Dakota soil

7.6.3 CALIBRATION PROCEDURE

To determine system linearity and calculate sample residues, calibration curves will be generated. Each analytical run will begin and end with a calibration curve. A maximum of five (5) set injections, including quality control and samples, will be made between calibration curves.

8. STANDARD OPERATING PROCEDURES (SOPs)

The appropriate SynTech Research SOPs will be used. A list of applicable SOPs (name and number) will be included in the raw data study file.

9. EVALUATION OF RESULTS

The quantity of the residues in each sample shall be determined using standard calibration curves with appropriate weighting, as described in the method.

All residue results will be calculated in accordance with the provisions of the analytical method being used. In order to accommodate correction for control contribution, the actual percent recovery for the analytes will be determined using the following equation:

$$\% \text{ Recovery} = \frac{(\text{mg/kg found in fortified control}) - (\text{ave. mg/kg found in untreated control})}{(\text{mg/kg } added)} x100\%$$

Average % Recovery and % RSD for each analyte will be calculated with both primary (quantitation) transitions and secondary (confirmatory) transitions.

10. COMMUNICATIONS

All communications between the Independent Laboratory and the Study Monitor and/or method developer will be directed through the Study Director (or designee) and fully documented. Under no circumstances will personnel familiar with the method be allowed to visit the Independent Laboratory to observe or offer assistance.

11. STATISTICAL METHODS

Only descriptive statistics, including means, standard deviations, and relative standard deviations will be used to evaluate the data.

12. CONTROL OF BIAS

Bias will be controlled by taking representative samples from a homogenous mixture for analysis and replicate analysis of all control and fortified samples.

13. ARCHIVING

All original raw data generated during the conduct of the 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 SynTech Research Quality Assurance Unit in conjunction with the final report prior to submission to the Sponsor.

The final report, analytical raw data, computer generated listings of raw data and supporting documentation will be sent to the Sponsor. A copy of the afore-mentioned documents and facility records will be retained in the Archives of SynTech Research, 17745 South Metcalf Ave. Stilwell, KS 66085 (or at a location specified by the Testing Facility).

14. REPORTING

A final report will be prepared for the independent laboratory validation study by the Study Director or their designee. The report will be written in compliance with all applicable reporting requirements.

15. REFERENCES

1. Method-No. RM-52S-4, Determination of Residues of V-10456, S-3100-CA, S-3100-DA, S-3100-CA-RD, S-3100-CA-UR, S-3100-DP, 4"-OH-S-3100-CA and S-3100-DA-RD in Soil, Chen, Tiffany, 8-25-2021.

ANALYTICAL CHEMISTRY STUDY NOTIFICATION

Independent Laboratory Validation of Valent Analytical Method RM-52S-4: "Determination of Residues of V-10456, S-3100-CA, S-3100-DA, S-3100-CA-RD, S-3100-Ca-UR, S-3100-DP, 4"-OH-S-3100-CA and S-3100-DA-RD in Soil	
Test Matrix (system): Soil	
Type of Study: Independent Laboratory Validation	
Testing Facility Manager: Walter Vandaveer	
Study Director: Michael Finley	
Valent Study Number: V-21-202602	
Syntech Study Number: 693SRUS21R0167	
Proposed Schedule of Activities:	
Estimated Date	Activity
September 2021 September, 2021 October, 2021 October, 2021	Protocol Signed Begin Lab Validation Draft Report Final Report

[redacted]

APPENDIX 1 ANALYTICAL METHOD RM-52S-4

VALENT U.S.A. LLC

DETERMINATION OF RESIDUES OF V-10456, S-3100-CA, S-3100-DA, S-3100-CA-RD, S-3100-CA-UR, S-3100-DP, 4″-OH-S-3100-CA, and S-3100-DA-RD IN SOIL

Method: **RM-52S-4** Date: August 25, 2021

1. INTRODUCTION

This method determines residues of V-10456 and its metabolites S-3100-CA, S-3100-DA, S-3100-CA-RD, S-3100-CA-UR, S-3100-DP, 4″-OH-S-3100-CA, and S-3100-DA-RD in soil.

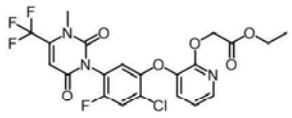
Briefly, the residues are extracted from soil using three extractions of acetonitrile/0.1 M HCl (5/1, v/v). The residues are then partitioned into dichloromethane, concentrated, and the residues are re-dissolved and samples are analyzed by LC-MS/MS.

The method limit of quantitation is 0.00015 mg/kg (0.15 ng/g) for V-10456, S-3100-CA, S-3100-DA, and S-3100-DP and was based on the lowest endpoint for a terrestrial plant (EC_{25} = 0.34 g a.i./ha from the vegetative vigor study). The method limit of quantitation is 0.001 mg/kg (1.0 ng/g) for S-3100-CA-RD, S-3100-DA-RD and 4″-OH-S-3100-CA and the method limit of quantitation is 0.005 mg/kg (5.0 ng/g) for S-3100-CA-UR; these limits of quantitation levels were set based on the lowest achievable level by state-of-the-art instrumentation.

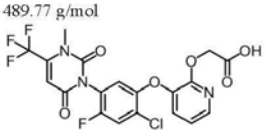
2. MATERIALS

2.1. ANALYTICAL REFERENCE STANDARDS

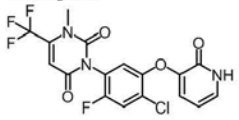
Common name:	V-10456, S-3100
Chemical name:	ethyl [(3-{2-chloro-4-fluoro-5-[3-methyl-2,6-dioxo-4-(trifluoromethyl)-3,6-dihydropyrimidin-1(2*H*)-yl]phenoxy}-2-pyridyl)oxy]acetate
Molecular formula:	$C_{21}H_{16}ClF_4N_3O_6$
Molecular weight:	517.82 g/mol



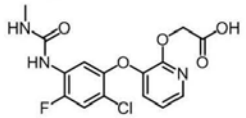
Common name: S-3100-CA
Chemical name: (3-{2-chloro-4-fluoro-5-[1,2,3,6-tetrahydro-3-methyl-2,6-dioxo-4-(trifluoromethyl)pyrimidin-1-yl]phenoxy}pyridine-2-yloxy)acetic acid
Molecular formula: $C_{19}H_{12}ClF_4N_3O_6$
Molecular weight: 489.77 g/mol



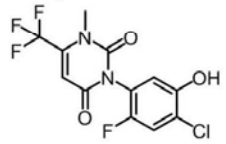
Common name: S-3100-DA
Chemical name: 3-[4-chloro-5-(1,2-dihydro-2-oxpyridin-3-yloxy)-2-fluorophenyl]-1,2,3,4-tetrahydro-1-methyl-6-(trifluoromethyl)pyrimidine-2,4-dione
Molecular formula: $C_{17}H_{10}ClF_4N_3O_4$
Molecular weight: 431.73 g/mol



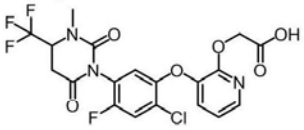
Common name: S-3100-CA-UR
Chemical name: {3-[2-chloro-4-fluoro-5-(3-methylureido)phenoxy]pyridine-2-yloxy}acetic acid
Molecular formula: $C_{15}H_{13}ClFN_3O_5$
Molecular weight: 369.74 g/mol



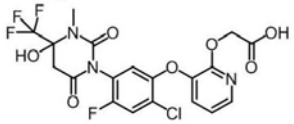
Common name: S-3100-DP
Chemical name: 3-(4-chloro-2-fluoro-5-hydroxyphenyl)-1,2,3,4-tetrahydro-1-methyl-6-(trifluoromethyl)pyrimidine-2,4-dione
Molecular formula: $C_{12}H_7ClF_4N_2O_3$
Molecular weight: 338.65 g/mol



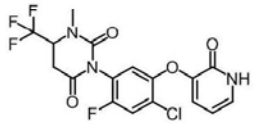
Common name: S-3100-CA-RD
Chemical name: (3-{2-chloro-4-fluoro-5-[1,2,3,4,5,6-hexahydro-3-methyl-2,6-dioxo-4-(trifluoromethyl)pyrimidin-1-yl]phenoxy}pyridine-2-yloxy)acetic acid
Molecular formula: $C_{19}H_{14}ClF_4N_3O_6$
Molecular weight: 491.78 g/mol



Common name: 4″-OH-S-3100-CA
Chemical name: (3-{2-chloro-4-fluoro-5-[1,2,3,4,5,6-hexahydro-4-hydroxy-3-methyl-2,6-dioxo-4-(trifluoromethyl)pyrimidin-1-yl]phenoxy}pyridine-2-yloxy)acetic acid
Molecular formula: $C_{19}H_{14}ClF_4N_3O_7$
Molecular weight: 507.78 g/mol



Common name: S-3100-DA-RD
Chemical name: 3-[4-chloro-5-(1,2-dihydro-2-oxo-pyridin-3-yloxy)-2-fluorophenyl]-1,2,3,4,5,6-hexahydro-1-methyl-6-(trifluoromethyl)pyrimidine-2,4-dione
Molecular formula: $C_{17}H_{12}ClF_4N_3O_4$
Molecular weight: 433.74 g/mol



2.2. ANALYTICAL REFERENCE STANDARDs

Other concentrations may be prepared and other containers and measuring devices (*e.g.*, vials and pipets) may be used as long as proportions are maintained and documented.

Stock Solutions

V-10456, S-3100-CA, S-3100-DA, 4″-OH-S-3100-CA and S-3100-DA-RD (200 µg/mL):
For each analyte, accurately weigh *ca.* 10 mg (weighed to an accuracy of 0.01 mg and corrected the amount for chemical purity) and quantitatively transfer to a 50 mL volumetric flask. Dilute with acetonitrile to the final volume and mix the solution thoroughly. Store the stock solutions in a refrigerator or freezer when not in use.

S-3100-CA-UR, S-3100-CA-RD, and S-3100-DP (200 µg/mL):
For each analyte, accurately weigh *ca.* 10 mg (weighed to an accuracy of 0.01 mg and corrected the amount for chemical purity) and quantitatively transfer to a 50 mL volumetric flask. Dilute with acetonitrile/HPLC-grade water (1/1, v/v) to the final volume and mix the solution thoroughly. Store the stock solutions in a refrigerator or freezer when not in use.

Fortification Solutions

V-10456, S-3100-CA, S-3100-DA, and S-3100-DP (1.00 µg/mL)
Transfer a 0.5 mL aliquot of the 200 µg/mL individual stock solutions of V-10456, S-3100-CA, S-3100-DA, and S-3100-DP to a 100 mL volumetric flask and dilute to the final volume with acetonitrile. Mix the solution thoroughly. Store this solution in a refrigerator or freezer when not in use.

V-10456, S-3100-CA, S-3100-DA, and S-3100-DP (0.10 µg/mL)

Transfer a 5.0 mL aliquot of the 1.00 µg/mL fortification solution of V-10456, S-3100-CA, S-3100-DA, and S-3100-DP to a 50 mL volumetric flask and dilute to the final volume with acetonitrile. Mix the solution thoroughly. Store this solution in a refrigerator or freezer when not in use.

V-10456, S-3100-CA, S-3100-DA, and S-3100-DP (0.01 µg/mL)

Transfer a 5.0 mL aliquot of the 0.10 µg/mL fortification solution of V-10456, S-3100-CA, S-3100-DA, and S-3100-DP to a 50 mL volumetric flask and dilute to the final volume with acetonitrile. Mix the solution thoroughly. Store this solution in a refrigerator or freezer when not in use.

4″-OH-S-3100-CA, S-3100-DA-RD, and S-3100-CA-RD (1.00 µg/mL)

Transfer a 0.5 mL aliquot of the 200 µg/mL stock solution of 4″-OH-S-3100-CA, S-3100-DA-RD, an S-3100-CA-RD to a 100 mL volumetric flask and dilute to the final volume with acetonitrile. Mix the solution thoroughly. Store this solution in a refrigerator or freezer when not in use.

4″-OH-S-3100-CA, S-3100-DA-RD, and S-3100-CA-RD (0.10 µg/mL)

Transfer a 5.0 mL aliquot of the 1.00 µg/mL fortification solution of 4″-OH-S-3100-CA, S-3100-DA-RD, an S-3100-CA-RD to a 50 mL volumetric flask and dilute to the final volume with acetonitrile. Mix the solution thoroughly. Store this solution in a refrigerator or freezer when not in use.

S-3100-CA-UR (10.0 µg/mL)

Transfer a 5.0 mL aliquot of the 200 µg/mL stock solution of S-3100-CA-UR to a 100 mL volumetric flask and dilute to the final volume with acetonitrile. Mix the solution thoroughly. Store this solution in a refrigerator or freezer when not in use.

S-3100-CA-UR (1.00 µg/mL)

Transfer a 5.0 mL aliquot of the 10.0 µg/mL fortification solution of S-3100-CA-UR to a 50 mL volumetric flask and dilute to the final volume with acetonitrile. Mix the solution thoroughly. Store this solution in a refrigerator or freezer when not in use.

Calibration Standard Solutions

Calibration standard solutions may be prepared in the following manner. Additional dilutions, alternative volumes and/or concentrations may be prepared to generate appropriate standards. The volumes shown below are examples for preparing the standards.

Prepare the following analytical calibration standards daily with each set of samples for analysis:

50.0 ng/mL: Transfer a 0.50 mL aliquot of the S-3100-CA-UR 1.00 µg/mL fortification solution to a 10 mL volumetric flask and dilute to the final volume with acetonitrile/HPLC-grade water (1/1, v/v) acidified with 0.1% formic acid. Mix the solution thoroughly.

25.0 ng/mL: Transfer 5 mL aliquot of the 50.0 ng/mL analytical solution, 0.25 mL aliquot of V-10456, S-3100-CA, S-3100-DA, and S-3100-DP 1.00 µg/mL fortification solution, and 0.25 mL aliquot of 4″-OH-S-3100-CA, S-3100-CA-RD, and S-3100-DA-RD 1.00 µg/mL fortification solution to a 10 mL volumetric flaks and dilute to the final volume with acetonitrile/HPLC-grade water (1/1, v/v) acidified with 0.1% formic acid. Mix the solution thoroughly.

5.00 ng/mL: Transfer a 2 mL aliquot of the 25.0 ng/mL analytical standard solution to a 10 mL volumetric flask and dilute to the final volume with acetonitrile/HPLC-grade water (1/1, v/v) acidified with 0.1% formic acid. Mix the solution thoroughly.

1.00 ng/mL: Transfer a 2 mL aliquot of the 5.00 ng/mL analytical standard solution to a 10 mL volumetric flask and dilute to the final volume with acetonitrile/HPLC-grade water (1/1, v/v) acidified with 0.1% formic acid. Mix the solution thoroughly.

0.50 ng/mL: Transfer a 5 mL aliquot of the 1.00 ng/mL analytical standard solution to a 10 mL volumetric flask and dilute to the final volume with acetonitrile/HPLC-grade water (1/1, v/v) acidified with 0.1% formic acid. Mix the solution thoroughly.

0.25 ng/mL: Transfer a 5 mL aliquot of the 0.50 ng/mL analytical standard solution to a 10 mL volumetric flask and dilute to the final volume with acetonitrile/HPLC-grade water (1/1, v/v) acidified with 0.1% formic acid. Mix the solution thoroughly.

0.10 ng/mL: Transfer a 4 mL aliquot of the 0.25 ng/mL analytical standard solution to a 10 mL volumetric flask and dilute to the final volume with acetonitrile/HPLC-grade water (1/1, v/v) acidified with 0.1% formic acid. Mix the solution thoroughly.

0.05 ng/mL: Transfer a 5 mL aliquot of the 0.10 ng/mL analytical standard solution to a 10 mL volumetric flask and dilute to the final volume with acetonitrile/HPLC-grade water (1/1, v/v) acidified with 0.1% formic acid. Mix the solution thoroughly.

2.3. REAGENTS

Acetonitrile, LC-MS grade or equivalent

Dichloromethane, HPLC-grade

Formic acid, LC-MS grade

Hydrochloric acid, 12 N

Methanol, LC-MS grade or equivalent

Water, LC-MS grade

2.4. REAGENT SOLUTION PREPARATION

Reagent solutions may be prepared in the following manner. Other volumes may be used, provided that the correct proportions are maintained. All prepared solutions should be well mixed and stored at room temperature.

Acetonitrile/0.1 M HCl (5/1, v/v):
Add 5 parts of acetonitrile and 1 part of 0.1 M HCl. For example, add 750 mL of acetonitrile and 150 mL of 0.1 M HCl sequentially into a reagent bottle. Mix the solution thoroughly. Store this solution at ambient temperature when not in use.

0.1 M HCl:
Add 8.3 mL of 12 N HCl into approximately 800 mL of HPLC-grade water. Fill with HPLC-grade water to a final volume of 1 L. Mix the solution thoroughly. Store this solution at ambient temperature when not in use.

Acetonitrile/ HPLC-grade water (1/1, v/v) with 0.1% Formic Acid:
Add 500 mL of acetonitrile, 500 mL of HPLC-grade water and 1000 µL of formic acid. Mix the solution thoroughly. Store this solution at ambient temperature when not in use.

Methanol, 0.05% formic acid:
Add 0.5 mL of formic acid into 1 L of methanol. Mix the solution thoroughly. Store this solution at ambient temperature when not in use.

Water, 0.05% formic acid:
Add 0.5 mL of formic acid into 1 L of HPLC-grade water. Mix the solution thoroughly. Store this solution at ambient temperature when not in use.

2.5. EQUIPMENT AND MATERIALS

Analytical Column, Kinetex® Biphenyl, 5µm, 100 Å, 150 mm x 4.6 mm, Phenomenex (part# 00F-4627-E0)

Balances, analytical and top loading

Sample homogenizers, Robot Coupe® Food Chopper R25T or equivalent

Centrifuge tubes, 50 mL polypropylene

Centrifuge, Sorvall centrifuge or equivalent

Disposable syringes, 3 mL

Graduated cylinders, various sizes

Glass vials with caps, assorted volumes

LC-MS/MS: Agilent 1290 Infinity II UHPLC series system with a binary pump, a multisampler, thermostat and a SCIEX Triple Quad™ 6500+ mass spectrometer with an electrospray ionization interface (or an equivalent system)

Pasteur pipets, various sizes

Pipettor, automatic, capable of accurately dispensing 0.100 mL to 10 mL volumes, Rainin or equivalent

Reciprocating shaker, Eberbach or equivalent

Round Bottom Flasks (500 mL or equivalent)

Separatory Funnel (250 mL or equivalent)

Syringe Filter Disc (PTFE Filter, 0.22 µm E&K Scientific#6057-06 or equivalent)

Vials, autosampler (with caps)

Volumetric flasks, assorted volumes for preparing analytical standards

Volumetric pipets, assorted volumes

Vortex

Note: Equivalent equipment may be substituted for the above items.

3. ANALYTICAL PROCEDURE

A schematic diagram describing method is presented in ATTACHMENT 1.

A. Sample Preparation

A bulk soil sample from the field must be homogenized before use for analysis. For that, mix by hand or homogenize the bulk sample in the presence of dry ice to obtain a homogeneous sample. If homogenized, allow the dry ice to sublime from the sample before taking a subsample for analysis.

Weigh 20.0 g (± 0.1 g) of the homogenized sample into a 50 mL polypropylene centrifuge tube. At this point, if required by the testing facility, control samples for method recovery should be fortified with V-10456, S-3100-CA, S-3100-DA, S-3100-CA-UR, S-3100-CA-RD, S-3100-DP, 4″-OH-S-3100-CA, and S-3100-DA-RD (See Note 1). For example, at a LOQ fortification level, an untreated sample (20.0 g) is spiked with 300 µL of the 0.010 µg/mL fortification standard containing V-10456, S-3100-CA, S-3100-DA, and S-3100-DP; 200 µL of the 0.100 µg/mL fortification standard containing S-3100-CA-RD, S-3100-DA-RD, and 4″-OH-S-3100-CA; and 100 µL of the 10.0 µg/mL fortification standard containing S-3100-CA-UR. At a 10XLOQ fortification level, an untreated sample (20.0 g) is spiked with 300 µL of the 0.100 µg/mL fortification standard containing V-10456, S-3100-CA, S-3100-DA, and S-3100-DP; 200 µL of the 1.00 µg/mL fortification standard containing S-3100-CA-RD, S-3100-DA-RD, and 4″-OH-S-3100-CA; and 100 µL of the 100 µg/mL fortification standard containing S-3100-CA-UR.

If moisture correction is required, weigh *ca.* 20 g of the homogenized sample into a tared drying vessel and place samples in an oven at (nominally) 135°C for at least 2 hours. Reweigh samples with drying vessel.

B. Sample Extraction

Add 25 mL of acetonitrile/0.1 M HCl mixture (5/1, v/v) to the centrifuge tube containing the sample and shake the sample on a reciprocating shaker for *ca.* 20 minutes. Centrifuge the sample for about 5 minutes at *ca.* 4000 rpm or as needed to separate the solids from the extraction solvent. Decant the sample extract into a 100 mL graduated cylinder with a stopper.

Break the soil pellet (with spatula or shaking pellet loose) and repeat the extraction twice using a fresh portion of solvent (25 mL of acetonitrile/0.1 M HCl mixture (5/1, v/v)). Combine all three extracts in the 100 mL graduated cylinder and then dilute them up to 100 mL with acetonitrile/0.1 M HCl mixture (5/1, v/v) and mix.

C. Dichloromethane Partition

Note: No more than four samples can be extracted at the same time during this partition step.

Pipet 25.0 mL of the extract and 25.0 mL of HPLC-grade water into a 250 mL separatory funnel. Add 50.0 mL of dichloromethane to the separatory funnel. Immediately shake the separatory funnel for about one minute and allow layers to separate. Drain the lower dichloromethane layer into a round bottom flask. Repeat extraction twice by adding a fresh portion of 50 mL dichloromethane to the separatory funnel containing the aqueous phase. Combine all dichloromethane extracts into the round bottom flask. Discard the aqueous layer after the third extraction.

D. Final Volume

Evaporate dichloromethane phase of the sample to dryness using a rotary-evaporator and water bath set to ≤ 40°C.

Re-dissolve the extract in 5.0 mL of acetonitrile acidified with 0.1% formic acid and sonicate briefly. Then add 5.0 mL of HPLC-grade water acidified with 0.1% formic acid to the sample extract. Sonicate briefly again.

Filter approximately 1.5 mL of the extract into an autosampler vial using PTFE 0.22 µm filter. Cap and vortex.

If samples cannot be analyzed immediately, store solutions in refrigerator (or freezer). After freezer storage, sample should be brought to room temperature and sonicated prior to analysis.

Prepare a set of calibration standards, as described previously, with each set of samples and store the standards under the same conditions as the samples if not analyzed immediately.

E. Sample Analyses

Analytical Sequence: Condition the LC-MS/MS instrument with at least six injections of a sample extract. (This number of conditions may be reduced if the set is analyzed consecutively behind another set). Prepare an analytical sequence that contains at least five calibration standard concentrations to establish the response of the instruments. A typical sequence would include 0.05, 0.10, 0.25, 0.50, 1.00, 5.00, and 25.0 ng/mL calibration standards and a continuing calibration standard, typically one of the mid-level concentration calibration standards (*i.e.,* 0.50 ng/mL calibration standard). An analytical sequence must begin (after the conditioning injections) and end with a continuing calibration standard. The continuing calibration standard must also be injected at least once within the sequence to verify the instrument reproducibility (See Note 2). Samples and calibration standards are interspersed within the sequence, so that a calibration standard is injected after every one to five sample injections (unless <5 samples then there may be no sample in between two standards). A typical sequence would be as follows: 6 conditioning injections, continuing calibration standard, one to five samples, calibration standard, one to five sample injections, calibration standard, … and ending with the continuing calibration standard. Any sample having detector response greater than the largest calibration standard response must be appropriately diluted with acetonitrile/HPLC-grade water (1/1, v/v) so that the analyte response will be within the calibration standard range. The diluted sample is then analyzed along with the untreated control sample and the high fortification sample from the set.

HPLC Conditions:

For analysis of V-10456, S-3100-CA, S-3100-DA, and S-3100-DA-RD:

Column Oven Temperature: 30 ± 0.8 °C
Mobile Phase: Water, 0.05% formic acid
Acetonitrile

Gradient Program:

Time, min	% Water, 0.05% formic acid	% Acetonitrile
0.0	77	23
1.0	77	23
7.5	10	90
18.0	10	90
19.0	77	23
25.0	77	23

Flow Rate Program: 600 µL/min
Injection Drawing Speed: 100 µL/minute
Injection Volume: 5 µL
Ejecting Speed: 400 µL/minute

Divert Program:

Total Time (min)	Position
0.1	Waste
5.5	MS
10.0	Waste

Typical MS-MS Parameters:

Period 1

Runtime: 7.5 min
Scan Type: MRM
Polarity: Positive
Ion Source: TurboSpray® Ion Drive Electro Spray interface
Probe Type: Electrospray
Collision gas: 9 psi (N_2)
Curtain gas: 20 psi (N_2)
Ion source gas 1: 55 psi (Zero Grade Air)
Ion source gas 2: 50 psi (Zero Grade Air)
Ion spray voltage: 5500 V
Temperature: 700 °C

Retention time (approximate):
S-3100-DA 7.15 minutes
S-3100-DA-RD 6.83 minutes

Analyte	Precursor ion Q1 (amu)	Product ion Q3 (amu)	Scan time (ms)	DP (V)	EP (V)	CE (V)	CXP (V)
S-3100-DA	432.0	281.0	100	80	10	40	10
S-3100-DA Qual	432.0	82.0	100	50	5	60	10
S-3100-DA-RD	434.1	281.1	100	80	5	40	15
S-3100-DA-RD Qual	434.1	323.0	100	70	15	50	15

Period 2

Runtime:	1.0 min
Scan Type:	MRM
Polarity:	Positive
Ion Source:	TurboSpray® Ion Drive Electro Spray interface
Probe Type:	Electrospray
Collision gas:	9 psi (N_2)
Curtain gas:	20 psi (N_2)
Ion source gas 1:	60 psi (Zero Grade Air)
Ion source gas 2:	30 psi (Zero Grade Air)
Ion spray voltage:	5500 V
Temperature:	700 °C

Retention time (approximate): 8.17 minutes

Analyte	Precursor ion Q1 (amu)	Product ion Q3 (amu)	Scan time (ms)	DP (V)	EP (V)	CE (V)	CXP (V)
S-3100-CA	490.0	250.0	100	40	10	40	15
S-3100-CA Qual	490.0	293.0	100	30	15	40	15

Period 3

Runtime:	16.5 min
Scan Type:	MRM
Polarity:	Positive
Ion Source:	TurboSpray® Ion Drive Electro Spray interface
Probe Type:	Electrospray
Collision gas:	9 psi (N_2)
Curtain gas:	20 psi (N_2)
Ion source gas 1:	60 psi (Zero Grade Air)
Ion source gas 2:	40 psi (Zero Grade Air)
Ion spray voltage:	4500 V
Temperature:	700 °C

Retention time (approximate): 9.30 minutes

Analyte	Precursor ion Q1 (amu)	Product ion Q3 (amu)	Scan time (ms)	DP (V)	EP (V)	CE (V)	CXP (V)
V-10456	518.0	293.0	100	50	15	50	15
V-10456 Qual	518.0	250.0	100	30	10	40	15

For analysis of S-3100-CA-RD, S-3100-CA-UR, and S-3100-DP:

Column Oven Temperature: 30 ± 0.8 °C
Mobile Phase: Water, 0.05% formic acid
Acetonitrile

Gradient Program:

Time, min	% Water, 0.05% formic acid	% Acetonitrile
0.0	85	15
1.0	85	15
9.0	5	95
13.0	5	95
13.5	85	15
20.0	85	15

Flow Rate Program: 600 µL/min
Injection Drawing Speed: 100 µL/minute
Injection Volume: 15 µL
Ejecting Speed: 400 µL/minute

Divert Program:

Total Time (min)	Position
0.1	Waste
6.0	MS
10.0	Waste

Typical MS-MS Parameters:

Period 1

Runtime: 8.0 minutes
Scan Type: MRM
Polarity: Negative
Ion Source: TurboSpray® Ion Drive Electro Spray interface
Probe Type: Electrospray
Collision gas: 9 psi (N_2)
Curtain gas: 20 psi (N_2)
Ion source gas 1: 50 psi (Zero Grade Air)
Ion source gas 2: 30 psi (Zero Grade Air)
Ion spray voltage: -4500 V
Temperature: 600 °C
Entrance potential: -10 V

Retention time (approximate): 7.68 minutes

Analyte	Precursor ion Q1 (amu)	Product ion Q3 (amu)	Scan time (ms)	DP (V)	EP (V)	CE (V)	CXP (V)
S-3100-CA-UR	368.0	293.0	500	-60	-10	-20	-5
S-3100-CA-UR Qual	368.0	337.0	200	-50	-15	-20	-15

Period 2

Runtime:	12.0 minutes
Scan Type:	MRM
Polarity:	Negative
Ion Source:	TurboSpray® Ion Drive Electro Spray interface
Probe Type:	Electrospray
Collision gas:	8 psi (N_2)
Curtain gas:	40 psi (N_2)
Ion source gas 1:	40 psi (Zero Grade Air)
Ion source gas 2:	40 psi (Zero Grade Air)
Ion spray voltage:	-4500 V
Temperature:	700 °C
Entrance potential:	-10 V

Retention times (approximate):

S-3100-CA-RD	8.77 minutes
S-3100-DP	8.46 minutes

Analyte	Precursor ion Q1 (amu)	Product ion Q3 (amu)	Scan time (ms)	DP (V)	EP (V)	CE (V)	CXP (V)
S-3100-CA-RD	490.0	376.0	100	-30	-10	-30	-5
S-3100-CA-RD Qual	490.0	319.0	100	-30	-10	-45	-5
S-3100-DP	337.0	186.0	100	-28	-10	-33	-14
S-3100-DP Qual	337.0	150.0	100	-28	-10	-50	-14

For analysis of 4″-OH-S-3100-CA:

Column Oven Temperature:	40 ± 0.8 °C
Mobile Phase:	Water, 0.05% formic acid Methanol, 0.05% formic acid

Gradient Program:

Time, min	Flow Rate, µL/min	% Water, 0.05% formic acid	% Methanol, 0.05% formic acid
0.0	600	60	40
1.0	600	60	40
1.5	400	10	90
12.0	400	10	90
12.5	600	60	40
18.0	600	60	40

Injection Drawing Speed: 100 µL/minute
Injection Volume: 15 µL
Ejecting Speed: 400 µL/minute

Divert Program:

Total Time (min)	Position
0.1	Waste
6.0	MS
8.0	Waste

Typical MS-MS Parameters:

Scan Type: MRM
Polarity: Positive
Ion Source: TurboSpray® Ion Drive Electro Spray interface
Probe Type: Electrospray
Collision gas: 9 psi (N_2)
Curtain gas: 20 psi (N_2)
Ion source gas 1: 40 psi (Zero Grade Air)
Ion source gas 2: 40 psi (Zero Grade Air)
Ion spray voltage: 4500 V
Temperature: 600 °C

Retention time (approximate): 6.78 minutes

Analyte	Precursor ion Q1 (amu)	Product ion Q3 (amu)	Scan time (ms)	DP (V)	EP (V)	CE (V)	CXP (V)
4″-OH-S-3100-CA	508.1	385.0	100	60	5	40	50
4″-OH-S-3100-CA Qual	508.1	489.9	100	70	5	30	20

The instrument parameters shown above are given only as a guide. They may be modified as needed to optimize the chromatography, to resolve matrix interferences (if observed), or to utilize other types of LC-MS/MS instruments.

F. Residue Calculations

The concentrations of the analytes (V-10456, S-3100-CA, S-3100-DA, S-3100-CA-RD, S-3100-CA-UR, S-3100-DP, 4″-OH-S-3100-CA, and S-3100-DA-RD) in each sample extract are calculated using a weighted (1/standard conc.) second-order polynomial equation. The data are presented graphically as concentration of the calibration standards verses their corresponding peak areas. Typically, an Excel® spreadsheet running a Sciex Analyst® curve fitting algorithm is used to determine the curve coefficients a, b, c, r, and so the sample residue concentrations. However, other quantification software that generate curve fit data may also be used. The area responses are entered in the following equation to calculate concentrations in the sample solutions:

$$C_{analyte}\left(^{ng}/_{mL}\right) = \boldsymbol{A} \times [\text{Detector Response}]^2 + \boldsymbol{B} \times [\text{Detector Response}] + \boldsymbol{C}$$

For example, a set of calibration standards covering 25.0 ng/mL to 0.10 ng/mL range gives peak areas as follows:

ng/mL	Area
25.0	4,710,000
5.00	966,000
1.00	193,000
0.50	98,500
0.25	49,900
0.10	20,900

The resulting equation from the Excel spreadsheet is as follows:

$$C_{analyte}\left({}^{ng}/_{mL}\right) = \boldsymbol{A} \times [\text{Detector Response}]^2 + \boldsymbol{B} \times [\text{Detector Response}] + \boldsymbol{C}$$

$A = 3.0945 \times 10^{-14}$
$B = 5.1634 \times 10^{-6}$
$C = -7.4962 \times 10^{-3}$

To ensure that the equation is appropriate, the areas of the calibration standards are entered into this equation and the standard concentrations are calculated. For each standard, the calculated concentration must each be within 15% of the actual concentration. In addition, the coefficient of determination (r^2) must be greater than 0.99.

For example (from the above data), the 1 ng/mL standard has an area of 193,000 and the calculated concentration (using the equation) is 0.99 ng/mL. This is acceptable as this is 99% of the known concentration. The criteria listed above for recalculated concentrations and the coefficient of determination must be met for acceptance of each analytical set, unless approved by the chemist responsible for the analysis. Sample extract concentrations are also calculated using the equation from the calibration standards. For example, a sample extract for S-3100-DA-RD with a peak area of 1,010,000 would have a concentration as follows:

$$C_{\text{analyte}}\left({}^{ng}/_{mL}\right) = \mathbf{3.0945 \times 10^{-14}} \times [1{,}010{,}000]^2 + \mathbf{5.1634 \times 10^{-6}} \times [1{,}010{,}000] - \mathbf{7.4962 \times 10^{-3}} = 5.239$$

Concentrations of each analyte (V-10456, S-3100-CA, S-3100-DA, S-3100-CA-RD, S-3100-CA-UR, S-3100-DP, 4″-OH-S-3100-CA, and S-3100-DA-RD) in the sample is calculated using the following formula:

$$C_{soil}\left({}^{mg}/_{kg}\right) = \frac{C_{\text{analyte}} \times \text{EV} \times \text{FV} \times \text{DF}}{\text{AV} \times \text{W}}$$

where:
$C_{analyte}$ = Concentration of analyte in solution (in ng/mL, from equation)
C_{soil} = Concentration of analyte in the soil sample (in mg/kg, from equation)
EV = Total extraction volume (100 mL)

FV = Final volume of extract (10 mL)
DF = Dilution factor (1)
W = Sample weight analyzed (20 g)
AV = Aliquot volume (25.0 mL)

For example, the concentration in the above example sample (with a calculated extract concentration of 5.239 ng/mL) would be calculated as follows:

$$C_{soil}\left(^{mg}/_{kg}\right) = \frac{5.239\ ^{ng}/_{mL} \times 100\ \text{mL} \times 10\ \text{mL} \times 1}{25\ \text{mL} \times 20\ \text{g}} \left(\frac{1000\ \text{g}}{1\ \text{kg}}\right)\left(\frac{1\ \text{mg}}{1 \times 10^6\ \text{ng}}\right) = 0.0104$$

The concentration of each analyte (V-10456, S-3100-CA, S-3100-DA, S-3100-CA-RD, S-3100-CA-UR, S-3100-DP, 4″-OH-S-3100-CA, and S-3100-DA-RD) in the fortified soil sample is calculated by the above calculation. The percent recovery is calculated by taking the concentration found in fortified sample subtracting the concentration found in the untreated control (any negative values are set to 0) and dividing by the amount the sample was fortified with and multiplying by 100. If the peak area of the untreated sample is ≤ 10% of the peak area of the lowest standard, no correction was applied when calculating the percent recovery. The percent recovery for fortified samples are calculated using the formula:

$$\%\ \text{Recovery} = \frac{C_{\text{fortified sample}} - C_{\text{control sample}}}{\text{Fortification level}} \times 100\%$$

For a compound fortified at 0.01 mg/kg, with a peak area of 1,010,000, the following values were utilized to calculate the recovery of each analyte in the sample:

mg/kg found in fortified sample = 0.0104 mg/kg
mg/kg found in untreated control sample = 0 mg/kg

$$\%\ \text{Recovery} = \frac{0.0104\ ^{mg}/_{kg} - 0\ ^{mg}/_{kg}}{0.01\ ^{mg}/_{kg}} \text{ x } 100\% = 104\%$$

Fortification samples are not corrected for moisture. Samples that require moisture content on a dry weight basis.

$$\%\ \text{water content} = \frac{\text{sample wet weight (g)} - \text{sample dry weight (g)}}{\text{sample dry weight (g)}} \text{ x } 100\%$$

$$\text{Soil dry weight (g)} = \frac{\text{sample wet weight (g)}}{(100\% + \%\ \text{water content})} \times 100\%$$

For example, a soil with a wet weight of 23.516 g and a dry weight of 17.422 g then the following values would be obtained:

$$\%\ \text{water content} = \frac{23.516\ \text{(g)} - 17.422\ \text{(g)}}{17.422\ \text{(g)}} \times 100\% = 34.98\%$$

$$\text{Soil dry weight (g)} = \frac{23.516(\text{g})}{(100\%+34.98\%)} \times 100\% = 17.422\text{g}$$

The concentration (mg/kg) found in the soil is then corrected to concentration (mg/kg) found in dry soil.

$$C_{dry\ soil}\left({}^{mg}/_{kg}\right) = \frac{C_{soil} \times (100\% + \%\ \text{water content})}{100\%}$$

If a wet weight concentration of 0.5 mg/kg was found in a sample and a water content of 34.98% then the following dry weight concentration would be obtained:

$$C_{dry\ soil}\left({}^{mg}/_{kg}\right) = \frac{0.5\ \text{mg/kg} \times (100\% + 34.98\%)}{100\%} = 0.675\left({}^{mg}/_{kg}\right)$$

4. ANALYTICAL LIMITS

A. Limit of Detection

The limit of detection (LOD) of this method is 0.0001 mg/kg for V-10456, S-3100-CA, S-3100-DA, and S-3100-DP. The LOD of this method is 0.0002 mg/kg for S-3100-CA-RD, S-3100-DA-RD, and 4″-OH-S-3100-CA. The LOD of this method is 0.0010 mg/kg for S-3100-CA-UR. This LOD is calculated by dividing the lowest calibration standard concentration by the effective matrix concentration in the sample extracts. This is based on a 20 g sample, a 25 mL aliquot (of the 100 mL total volume after extraction), a 10 mL final volume, a 1X dilution, and a 0.05 ng/mL or 0.10 ng/mL standard in the calibration.

$$\text{LOD}\left({}^{mg}/_{kg}\right) = \frac{0.10\ {}^{ng}/_{mL} \times 100\ \text{mL} \times 10\ \text{mL} \times 1}{25\ \text{mL} \times 20\ \text{g}}\left(\frac{1000\ \text{g}}{1\ \text{kg}}\right)\left(\frac{1\ \text{mg}}{1 \times 10^6\ \text{ng}}\right) = 0.0002$$

B. Limit of Quantitation

The method limit of quantitation (LOQ) is 0.00015 mg/kg for V-10456, S-3100-CA, S-3100-DA, and S-3100-DP. The method limit of quantitation is 0.001 mg/kg for S-3100-CA-RD, S-3100-DA-RD and 4″-OH-S-3100-CA. The method limit of quantitation is 0.005 mg/kg for S-3100-CA-UR.

5. NOTES

A. Note 1

A typical quality control set consists of one untreated control sample (UTC) and two fortified samples at the LOQ and 10×LOQ levels. The levels of fortification are generally at the method LOQ (0.00015 mg/kg for V-10456, S-3100-CA, S-3100-DA, and S-3100-DP, 0.001 mg/kg for S-3100-CA-RD, S-3100-DA-RD, and 4″-OH-S-3100-CA, and 0.005 mg/kg for S-3100-CA-UR) and/or at 10 times the LOQ (0.0015 mg/kg for V-10456, S-3100-CA, S-3100-DA, and S-3100-DP, 0.01 mg/kg for S-3100-CA-RD, S-3100-DA-RD, and 4″-OH-S-3100-CA, and 0.05 mg/kg for S-3100-

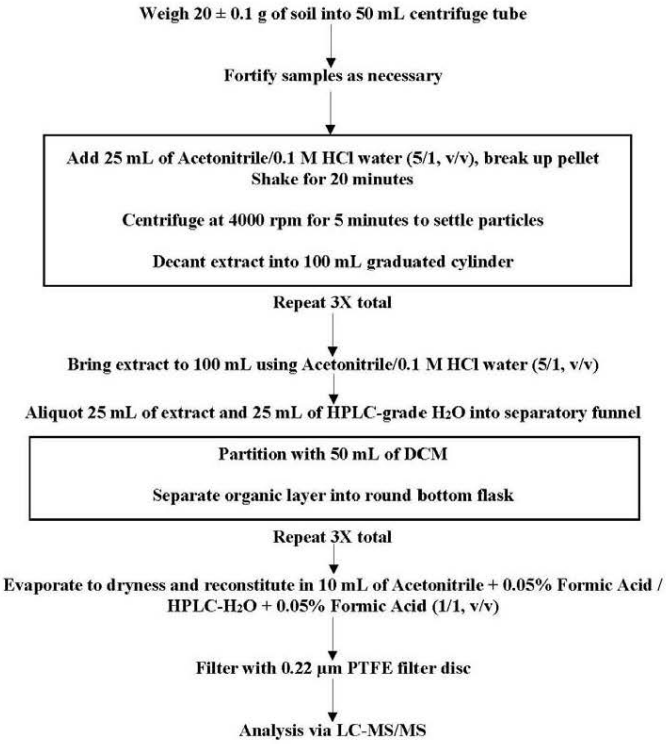
CA-UR). If residues higher than 10 times LOQ are anticipated or observed, then fortifications should be made at a higher concentration (typically slightly above the highest residues). Method recoveries must be 70% to 120% with the coefficient of variation (CV) of replicate measurements of recoveries of plus or minus 20% to be acceptable unless approved by the chemist responsible for the analysis. If the testing facility does not require concurrent analysis of fortified control samples, or if a UTC sample is not available, this method requirement may be waived.

B. **Note 2**

At Valent U.S.A. LLC, reproducibility of an analytical run is determined by calculating the coefficient of variation (CV) from the calculated concentration obtained from the continuing calibration standards analyzed in the analytical sequence (set). For the analytical set to be acceptable, these CV's must be 15% or less unless approved by the chemist responsible for the analysis.

C. **Note 3**

For the instrument calibration set to be acceptable, the coefficient of determination (r^2) of the weighted polynomial calibration curve must be greater than or equal to 0.99, and the back-calculated concentration for each standard must be within 15% (20% for the lowest standard) of the labeled concentration for the instrument to be considered within a polynomial fit unless approved by the chemist responsible for the analysis.


Weigh 20 ± 0.1 g of soil into 50 mL centrifuge tube
Fortify samples as necessary
Add 25 mL of Acetonitrile/0.1 M HCl water (5/1, v/v), break up pellet
Shake for 20 minutes
Centrifuge at 4000 rpm for 5 minutes to settle particles
Decant extract into 100 mL graduated cylinder
Repeat 3X total
Bring extract to 100 mL using Acetonitrile/0.1 M HCl water (5/1, v/v)
Aliquot 25 mL of extract and 25 mL of HPLC-grade H2O into separatory funnel
Partition with 50 mL of DCM
Separate organic layer into round bottom flask
Repeat 3X total
Evaporate to dryness and reconstitute in 10 mL of Acetonitrile + 0.05% Formic Acid /
HPLC-H2O + 0.05% Formic Acid (1/1, v/v)
Filter with 0.22 µm PTFE filter disc
Analysis via LC-MS/MS

Study/Protocol Number V-21-202602
SR Project Number 693SRUS21R0167
Protocol Amendment No. 1

VALENT U.S.A. LLC

DETERMINATION OF RESIDUES OF V-10456, S-3100-CA, S-3100-DA, S-3100-CA-RD, S-3100-CA-UR, S-3100-DP, 4″-OH-S-3100-CA, and S-3100-DA-RD IN SOIL

Method: **RM-52S-4A** Date: October 14, 2021

1. INTRODUCTION

This method determines residues of V-10456 and its metabolites S-3100-CA, S-3100-DA, S-3100-CA-RD, S-3100-CA-UR, S-3100-DP, 4″-OH-S-3100-CA, and S-3100-DA-RD in soil.

Briefly, the residues are extracted from soil using three extractions of acetonitrile/0.1 M HCl (5/1, v/v). The residues are then partitioned into dichloromethane, concentrated, and the residues are re-dissolved and samples are analyzed by LC-MS/MS.

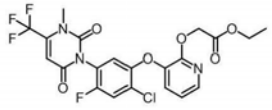
The method limit of quantitation is 0.00015 mg/kg (0.15 ng/g) for V-10456, S-3100-CA, S-3100-DA, and S-3100-DP and was based on the lowest endpoint for a terrestrial plant (EC_{25} = 0.34 g a.i./ha from the vegetative vigor study). The method limit of quantitation is 0.001 mg/kg (1.0 ng/g) for S-3100-CA-RD, S-3100-DA-RD and 4″-OH-S-3100-CA and the method limit of quantitation is 0.005 mg/kg (5.0 ng/g) for S-3100-CA-UR; these limits of quantitation levels were set based on the lowest achievable level by state-of-the-art instrumentation.

RM-52S-4A replaces RM-52S-4 to correct typographical errors in section 3.

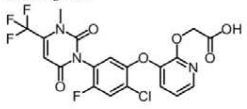
2. MATERIALS

2.1. ANALYTICAL REFERENCE STANDARDS

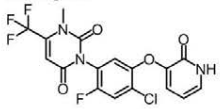
Common name:	V-10456, S-3100
Chemical name:	ethyl [(3-{2-chloro-4-fluoro-5-[3-methyl-2,6-dioxo-4-(trifluoromethyl)-3,6-dihydropyrimidin-1(2*H*)-yl]phenoxy}-2-pyridyl)oxy]acetate
Molecular formula:	$C_{21}H_{16}ClF_4N_3O_6$
Molecular weight:	517.82 g/mol



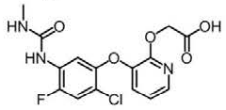
Common name:	S-3100-CA
Chemical name:	(3-{2-chloro-4-fluoro-5-[1,2,3,6-tetrahydro-3-methyl-2,6-dioxo-4-(trifluoromethyl)pyrimidin-1-yl]phenoxy}pyridine-2-yloxy)acetic acid
Molecular formula:	$C_{19}H_{12}ClF_4N_3O_6$
Molecular weight:	489.77 g/mol



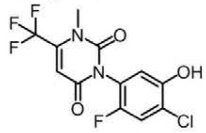
Common name:	S-3100-DA
Chemical name:	3-[4-chloro-5-(1,2-dihydro-2-oxpyridin-3-yloxy)-2-fluorophenyl]-1,2,3,4-tetrahydro-1-methyl-6-(trifluoromethyl)pyrimidine-2,4-dione
Molecular formula:	$C_{17}H_{10}ClF_4N_3O_4$
Molecular weight:	431.73 g/mol



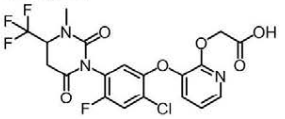
Common name:	S-3100-CA-UR
Chemical name:	{3-[2-chloro-4-fluoro-5-(3-methylureido)phenoxy]pyridine-2-yloxy}acetic acid
Molecular formula:	$C_{15}H_{13}ClFN_3O_5$
Molecular weight:	369.74 g/mol



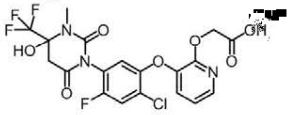
Common name:	S-3100-DP
Chemical name:	3-(4-chloro-2-fluoro-5-hydroxyphenyl)-1,2,3,4-tetrahydro-1-methyl-6-(trifluoromethyl)pyrimidine-2,4-dione
Molecular formula:	$C_{12}H_7ClF_4N_2O_3$
Molecular weight:	338.65 g/mol



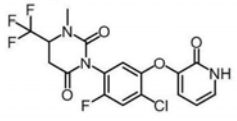
Common name:	S-3100-CA-RD
Chemical name:	(3-{2-chloro-4-fluoro-5-[1,2,3,4,5,6-hexahydro-3-methyl-2,6-dioxo-4-(trifluoromethyl)pyrimidin-1-yl]phenoxy}pyridine-2-yloxy)acetic acid
Molecular formula:	$C_{19}H_{14}ClF_4N_3O_6$
Molecular weight:	491.78 g/mol



Common name:	4″-OH-S-3100-CA
Chemical name:	(3-{2-chloro-4-fluoro-5-[1,2,3,4,5,6-hexahydro-4-hydroxy-3-methyl-2,6-dioxo-4-(trifluoromethyl)pyrimidin-1-yl]phenoxy}pyridine-2-yloxy)acetic acid
Molecular formula:	$C_{19}H_{14}ClF_4N_3O_7$
Molecular weight:	507.78 g/mol



Common name:	S-3100-DA-RD
Chemical name:	3-[4-chloro-5-(1,2-dihydro-2-oxo-pyridin-3-yloxy)-2-fluorophenyl]-1,2,3,4,5,6-hexahydro-1-methyl-6-(trifluoromethyl)pyrimidine-2,4-dione
Molecular formula:	$C_{17}H_{12}ClF_4N_3O_4$
Molecular weight:	433.74 g/mol



2.2. ANALYTICAL REFERENCE STANDARDs

Other concentrations may be prepared and other containers and measuring devices (*e.g.*, vials and pipets) may be used as long as proportions are maintained and documented.

Stock Solutions

V-10456, S-3100-CA, S-3100-DA, 4″-OH-S-3100-CA and S-3100-DA-RD (200 µg/mL):
For each analyte, accurately weigh *ca.* 10 mg (weighed to an accuracy of 0.01 mg and corrected the amount for chemical purity) and quantitatively transfer to a 50 mL volumetric flask. Dilute with acetonitrile to the final volume and mix the solution thoroughly. Store the stock solutions in a refrigerator or freezer when not in use.

S-3100-CA-UR, S-3100-CA-RD, and S-3100-DP (200 µg/mL):
For each analyte, accurately weigh *ca.* 10 mg (weighed to an accuracy of 0.01 mg and corrected the amount for chemical purity) and quantitatively transfer to a 50 mL volumetric flask. Dilute with acetonitrile/HPLC-grade water (1/1, v/v) to the final volume and mix the solution thoroughly. Store the stock solutions in a refrigerator or freezer when not in use.

Fortification Solutions

V-10456, S-3100-CA, S-3100-DA, and S-3100-DP (1.00 µg/mL)
Transfer a 0.5 mL aliquot of the 200 µg/mL individual stock solutions of V-10456, S-3100-CA, S-3100-DA, and S-3100-DP to a 100 mL volumetric flask and dilute to the final volume with acetonitrile. Mix the solution thoroughly. Store this solution in a refrigerator or freezer when not in use.

V-10456, S-3100-CA, S-3100-DA, and S-3100-DP (0.10 µg/mL)

Transfer a 5.0 mL aliquot of the 1.00 µg/mL fortification solution of V-10456, S-3100-CA, S-3100-DA, and S-3100-DP to a 50 mL volumetric flask and dilute to the final volume with acetonitrile. Mix the solution thoroughly. Store this solution in a refrigerator or freezer when not in use.

V-10456, S-3100-CA, S-3100-DA, and S-3100-DP (0.01 µg/mL)

Transfer a 5.0 mL aliquot of the 0.10 µg/mL fortification solution of V-10456, S-3100-CA, S-3100-DA, and S-3100-DP to a 50 mL volumetric flask and dilute to the final volume with acetonitrile. Mix the solution thoroughly. Store this solution in a refrigerator or freezer when not in use.

4″-OH-S-3100-CA, S-3100-DA-RD, and S-3100-CA-RD (1.00 µg/mL)

Transfer a 0.5 mL aliquot of the 200 µg/mL stock solution of 4″-OH-S-3100-CA, S-3100-DA-RD, an S-3100-CA-RD to a 100 mL volumetric flask and dilute to the final volume with acetonitrile. Mix the solution thoroughly. Store this solution in a refrigerator or freezer when not in use.

4″-OH-S-3100-CA, S-3100-DA-RD, and S-3100-CA-RD (0.10 µg/mL)

Transfer a 5.0 mL aliquot of the 1.00 µg/mL fortification solution of 4″-OH-S-3100-CA, S-3100-DA-RD, an S-3100-CA-RD to a 50 mL volumetric flask and dilute to the final volume with acetonitrile. Mix the solution thoroughly. Store this solution in a refrigerator or freezer when not in use.

S-3100-CA-UR (10.0 µg/mL)

Transfer a 5.0 mL aliquot of the 200 µg/mL stock solution of S-3100-CA-UR to a 100 mL volumetric flask and dilute to the final volume with acetonitrile. Mix the solution thoroughly. Store this solution in a refrigerator or freezer when not in use.

S-3100-CA-UR (1.00 µg/mL)

Transfer a 5.0 mL aliquot of the 10.0 µg/mL fortification solution of S-3100-CA-UR to a 50 mL volumetric flask and dilute to the final volume with acetonitrile. Mix the solution thoroughly. Store this solution in a refrigerator or freezer when not in use.

Calibration Standard Solutions

Calibration standard solutions may be prepared in the following manner. Additional dilutions, alternative volumes and/or concentrations may be prepared to generate appropriate standards. The volumes shown below are examples for preparing the standards.

Prepare the following analytical calibration standards daily with each set of samples for analysis:

50.0 ng/mL: Transfer a 0.50 mL aliquot of the S-3100-CA-UR 1.00 µg/mL fortification solution to a 10 mL volumetric flask and dilute to the final volume with acetonitrile/HPLC-grade water (1/1, v/v) acidified with 0.1% formic acid. Mix the solution thoroughly.

25.0 ng/mL: Transfer 5 mL aliquot of the 50.0 ng/mL analytical solution, 0.25 mL aliquot of V-10456, S-3100-CA, S-3100-DA, and S-3100-DP 1.00 µg/mL fortification solution, and 0.25 mL aliquot of 4″-OH-S-3100-CA, S-3100-CA-RD, and S-3100-DA-RD 1.00 µg/mL fortification solution to a 10 mL volumetric flaks and dilute to the final volume with acetonitrile/HPLC-grade water (1/1, v/v) acidified with 0.1% formic acid. Mix the solution thoroughly.

5.00 ng/mL: Transfer a 2 mL aliquot of the 25.0 ng/mL analytical standard solution to a 10 mL volumetric flask and dilute to the final volume with acetonitrile/HPLC-grade water (1/1, v/v) acidified with 0.1% formic acid. Mix the solution thoroughly.

1.00 ng/mL: Transfer a 2 mL aliquot of the 5.00 ng/mL analytical standard solution to a 10 mL volumetric flask and dilute to the final volume with acetonitrile/HPLC-grade water (1/1, v/v) acidified with 0.1% formic acid. Mix the solution thoroughly.

0.50 ng/mL: Transfer a 5 mL aliquot of the 1.00 ng/mL analytical standard solution to a 10 mL volumetric flask and dilute to the final volume with acetonitrile/HPLC-grade water (1/1, v/v) acidified with 0.1% formic acid. Mix the solution thoroughly.

0.25 ng/mL: Transfer a 5 mL aliquot of the 0.50 ng/mL analytical standard solution to a 10 mL volumetric flask and dilute to the final volume with acetonitrile/HPLC-grade water (1/1, v/v) acidified with 0.1% formic acid. Mix the solution thoroughly.

0.10 ng/mL: Transfer a 4 mL aliquot of the 0.25 ng/mL analytical standard solution to a 10 mL volumetric flask and dilute to the final volume with acetonitrile/HPLC-grade water (1/1, v/v) acidified with 0.1% formic acid. Mix the solution thoroughly.

0.05 ng/mL: Transfer a 5 mL aliquot of the 0.10 ng/mL analytical standard solution to a 10 mL volumetric flask and dilute to the final volume with acetonitrile/HPLC-grade water (1/1, v/v) acidified with 0.1% formic acid. Mix the solution thoroughly.

2.3. REAGENTS

Acetonitrile, LC-MS grade or equivalent

Dichloromethane, HPLC-grade

Formic acid, LC-MS grade

Hydrochloric acid, 12 N

Methanol, LC-MS grade or equivalent

Water, LC-MS grade

2.4. REAGENT SOLUTION PREPARATION

Reagent solutions may be prepared in the following manner. Other volumes may be used, provided that the correct proportions are maintained. All prepared solutions should be well mixed and stored at room temperature.

Acetonitrile/0.1 M HCl (5/1, v/v):
Add 5 parts of acetonitrile and 1 part of 0.1 M HCl. For example, add 750 mL of acetonitrile and 150 mL of 0.1 M HCl sequentially into a reagent bottle. Mix the solution thoroughly. Store this solution at ambient temperature when not in use.

0.1 M HCl:
Add 8.3 mL of 12 N HCl into approximately 800 mL of HPLC-grade water. Fill with HPLC-grade water to a final volume of 1 L. Mix the solution thoroughly. Store this solution at ambient temperature when not in use.

Acetonitrile/ HPLC-grade water (1/1, v/v) with 0.1% Formic Acid:
Add 500 mL of acetonitrile, 500 mL of HPLC-grade water and 1000 µL of formic acid. Mix the solution thoroughly. Store this solution at ambient temperature when not in use.

Methanol, 0.05% formic acid:
Add 0.5 mL of formic acid into 1 L of methanol. Mix the solution thoroughly. Store this solution at ambient temperature when not in use.

Water, 0.05% formic acid:
Add 0.5 mL of formic acid into 1 L of HPLC-grade water. Mix the solution thoroughly. Store this solution at ambient temperature when not in use.

2.5. EQUIPMENT AND MATERIALS

Analytical Column, Kinetex® Biphenyl, 5µm, 100 Å, 150 mm x 4.6 mm, Phenomenex (part# 00F-4627-E0)

Balances, analytical and top loading

Sample homogenizers, Robot Coupe® Food Chopper R25T or equivalent

Centrifuge tubes, 50 mL polypropylene

Centrifuge, Sorvall centrifuge or equivalent

Disposable syringes, 3 mL

Graduated cylinders, various sizes

Glass vials with caps, assorted volumes

LC-MS/MS: Agilent 1290 Infinity II UHPLC series system with a binary pump, a multisampler, thermostat and a SCIEX Triple Quad™ 6500⁺ mass spectrometer with an electrospray ionization interface (or an equivalent system)

Pasteur pipets, various sizes

Pipettor, automatic, capable of accurately dispensing 0.100 mL to 10 mL volumes, Rainin or equivalent

Reciprocating shaker, Eberbach or equivalent

Round Bottom Flasks (500 mL or equivalent)

Separatory Funnel (250 mL or equivalent)

Syringe Filter Disc (PTFE Filter, 0.22 µm E&K Scientific#6057-06 or equivalent)

Vials, autosampler (with caps)

Volumetric flasks, assorted volumes for preparing analytical standards

Volumetric pipets, assorted volumes

Vortex

Note: Equivalent equipment may be substituted for the above items.

3. ANALYTICAL PROCEDURE

A schematic diagram describing method is presented in ATTACHMENT 1.

A. Sample Preparation

A bulk soil sample from the field must be homogenized before use for analysis. For that, mix by hand or homogenize the bulk sample in the presence of dry ice to obtain a homogeneous sample. If homogenized, allow the dry ice to sublime from the sample before taking a subsample for analysis.

Weigh 20.0 g (± 0.1 g) of the homogenized sample into a 50 mL polypropylene centrifuge tube. At this point, if required by the testing facility, control samples for method recovery should be fortified with V-10456, S-3100-CA, S-3100-DA, S-3100-CA-UR, S-3100-CA-RD, S-3100-DP, 4″-OH-S-3100-CA, and S-3100-DA-RD (See Note 1). For example, at a LOQ fortification level, an untreated sample (20.0 g) is spiked with 300 µL of the 0.010 µg/mL fortification standard containing V-10456, S-3100-CA, S-3100-DA, and S-3100-DP; 200 µL of the 0.100 µg/mL fortification standard containing S-3100-CA-RD, S-3100-DA-RD, and 4″-OH-S-3100-CA; and 100 µL of the 1.00 µg/mL fortification standard containing S-3100-CA-UR. At a 10XLOQ fortification level, an untreated sample (20.0 g) is spiked with 300 µL of the 0.100 µg/mL fortification standard containing V-10456, S-3100-CA, S-3100-DA, and S-3100-DP; 200 µL of the 1.00 µg/mL fortification standard containing S-3100-CA-RD, S-3100-DA-RD, and 4″-OH-S-3100-CA; and 100 µL of the 10.0 µg/mL fortification standard containing S-3100-CA-UR.

If moisture correction is required, weigh *ca.* 20 g of the homogenized sample into a tared drying vessel and place samples in an oven at (nominally) 135°C for at least 2 hours. Reweigh samples with drying vessel.

B. Sample Extraction

Add 25 mL of acetonitrile/0.1 M HCl mixture (5/1, v/v) to the centrifuge tube containing the sample and shake the sample on a reciprocating shaker for *ca.* 20 minutes. Centrifuge the sample for about 5 minutes at *ca.* 4000 rpm or as needed to separate the solids from the extraction solvent. Decant the sample extract into a 100 mL graduated cylinder with a stopper.

Break the soil pellet (with spatula or shaking pellet loose) and repeat the extraction twice using a fresh portion of solvent (25 mL of acetonitrile/0.1 M HCl mixture (5/1, v/v)). Combine all three extracts in the 100 mL graduated cylinder and then dilute them up to 100 mL with acetonitrile/0.1 M HCl mixture (5/1, v/v) and mix.

C. Dichloromethane Partition

Note: No more than four samples can be extracted at the same time during this partition step.

Pipet 25.0 mL of the extract and 25.0 mL of HPLC-grade water into a 250 mL separatory funnel. Add 50.0 mL of dichloromethane to the separatory funnel. Immediately shake the separatory funnel for about one minute and allow layers to separate. Drain the lower dichloromethane layer into a round bottom flask. Repeat extraction twice by adding a fresh portion of 50 mL dichloromethane to the separatory funnel containing the aqueous phase. Combine all dichloromethane extracts into the round bottom flask. Discard the aqueous layer after the third extraction.

D. Final Volume

Evaporate dichloromethane phase of the sample to dryness using a rotary-evaporator and water bath set to ≤ 40°C.

Re-dissolve the extract in 5.0 mL of acetonitrile acidified with 0.1% formic acid and sonicate briefly. Then add 5.0 mL of HPLC-grade water acidified with 0.1% formic acid to the sample extract. Sonicate briefly again.

Filter approximately 1.5 mL of the extract into an autosampler vial using PTFE 0.22 µm filter. Cap and vortex.

If samples cannot be analyzed immediately, store solutions in refrigerator (or freezer). After freezer storage, sample should be brought to room temperature and sonicated prior to analysis.

Prepare a set of calibration standards, as described previously, with each set of samples and store the standards under the same conditions as the samples if not analyzed immediately.

E. Sample Analyses

Analytical Sequence: Condition the LC-MS/MS instrument with at least six injections of a sample extract. (This number of conditions may be reduced if the set is analyzed consecutively behind another set). Prepare an analytical sequence that contains at least five calibration standard concentrations to establish the response of the instruments. A typical sequence would include 0.05, 0.10, 0.25, 0.50, 1.00, 5.00, and 25.0 ng/mL calibration standards and a continuing calibration standard, typically one of the mid-level concentration calibration standards (*i.e.*, 0.50 ng/mL calibration standard). An analytical sequence must begin (after the conditioning injections) and end with a continuing calibration standard. The continuing calibration standard must also be injected at least once within the sequence to verify the instrument reproducibility (See Note 2). Samples and calibration standards are interspersed within the sequence, so that a calibration standard is injected after every one to five sample injections (unless <5 samples then there may be no sample in between two standards). A typical sequence would be as follows: 6 conditioning injections, continuing calibration standard, one to five samples, calibration standard, one to five sample injections, calibration standard, … and ending with the continuing calibration standard. Any sample having detector response greater than the largest calibration standard response must be appropriately diluted with acetonitrile/HPLC-grade water (1/1, v/v) so that the analyte response will be within the calibration standard range. The diluted sample is then analyzed along with the untreated control sample and the high fortification sample from the set.

HPLC Conditions:

For analysis of V-10456, S-3100-CA, S-3100-DA, and S-3100-DA-RD:

Column Oven Temperature: 30 ± 0.8 °C
Mobile Phase: Water, 0.05% formic acid
Acetonitrile

Gradient Program:

Time, min	% Water, 0.05% formic acid	% Acetonitrile
0.0	77	23
1.0	77	23
7.5	10	90
18.0	10	90
19.0	77	23
25.0	77	23

Flow Rate Program: 600 µL/min
Injection Drawing Speed: 100 µL/minute
Injection Volume: 5 µL
Ejecting Speed: 400 µL/minute

Divert Program:

Total Time (min)	Position
0.1	Waste
5.5	MS
10.0	Waste

Typical MS-MS Parameters:

Period 1

Runtime: 7.5 min
Scan Type: MRM
Polarity: Positive
Ion Source: TurboSpray® Ion Drive Electro Spray interface
Probe Type: Electrospray
Collision gas: 9 psi (N_2)
Curtain gas: 20 psi (N_2)
Ion source gas 1: 55 psi (Zero Grade Air)
Ion source gas 2: 50 psi (Zero Grade Air)
Ion spray voltage: 5500 V
Temperature: 700 °C

Retention time (approximate):
S-3100-DA 7.15 minutes
S-3100-DA-RD 6.83 minutes

Analyte	Precursor ion Q1 (amu)	Product ion Q3 (amu)	Scan time (ms)	DP (V)	EP (V)	CE (V)	CXP (V)
S-3100-DA	432.0	281.0	100	80	10	40	10
S-3100-DA Qual	432.0	82.0	100	50	5	60	10
S-3100-DA-RD	434.1	281.1	100	80	5	40	15
S-3100-DA-RD Qual	434.1	323.0	100	70	15	50	15

Period 2

Runtime:	1.0 min
Scan Type:	MRM
Polarity:	Positive
Ion Source:	TurboSpray® Ion Drive Electro Spray interface
Probe Type:	Electrospray
Collision gas:	9 psi (N_2)
Curtain gas:	20 psi (N_2)
Ion source gas 1:	60 psi (Zero Grade Air)
Ion source gas 2:	30 psi (Zero Grade Air)
Ion spray voltage:	5500 V
Temperature:	700 °C

Retention time (approximate): 8.17 minutes

Analyte	Precursor ion Q1 (amu)	Product ion Q3 (amu)	Scan time (ms)	DP (V)	EP (V)	CE (V)	CXP (V)
S-3100-CA	490.0	250.0	100	40	10	40	15
S-3100-CA Qual	490.0	293.0	100	30	15	40	15

Period 3

Runtime:	16.5 min
Scan Type:	MRM
Polarity:	Positive
Ion Source:	TurboSpray® Ion Drive Electro Spray interface
Probe Type:	Electrospray
Collision gas:	9 psi (N_2)
Curtain gas:	20 psi (N_2)
Ion source gas 1:	60 psi (Zero Grade Air)
Ion source gas 2:	40 psi (Zero Grade Air)
Ion spray voltage:	4500 V
Temperature:	700 °C

Retention time (approximate): 9.30 minutes

Analyte	Precursor ion Q1 (amu)	Product ion Q3 (amu)	Scan time (ms)	DP (V)	EP (V)	CE (V)	CXP (V)
V-10456	518.0	293.0	100	50	15	50	15
V-10456 Qual	518.0	250.0	100	30	10	40	15

For analysis of S-3100-CA-RD, S-3100-CA-UR, and S-3100-DP:

Column Oven Temperature: 30 ± 0.8 °C
Mobile Phase: Water, 0.05% formic acid
Acetonitrile

Gradient Program:

Time, min	% Water, 0.05% formic acid	% Acetonitrile
0.0	85	15
1.0	85	15
9.0	5	95
13.0	5	95
13.5	85	15
20.0	85	15

Flow Rate Program: 600 µL/min
Injection Drawing Speed: 100 µL/minute
Injection Volume: 15 µL
Ejecting Speed: 400 µL/minute

Divert Program:

Total Time (min)	Position
0.1	Waste
6.0	MS
10.0	Waste

Typical MS-MS Parameters:

Period 1

Runtime: 8.0 minutes
Scan Type: MRM
Polarity: Negative
Ion Source: TurboSpray® Ion Drive Electro Spray interface
Probe Type: Electrospray
Collision gas: 9 psi (N_2)
Curtain gas: 20 psi (N_2)
Ion source gas 1: 50 psi (Zero Grade Air)
Ion source gas 2: 30 psi (Zero Grade Air)
Ion spray voltage: -4500 V
Temperature: 600 °C
Entrance potential: -10 V

Retention time (approximate): 7.68 minutes

Analyte	Precursor ion Q1 (amu)	Product ion Q3 (amu)	Scan time (ms)	DP (V)	EP (V)	CE (V)	CXP (V)
S-3100-CA-UR	368.0	293.0	500	-60	-10	-20	-5
S-3100-CA-UR Qual	368.0	337.0	200	-50	-15	-20	-15

Period 2

Runtime:	12.0 minutes
Scan Type:	MRM
Polarity:	Negative
Ion Source:	TurboSpray® Ion Drive Electro Spray interface
Probe Type:	Electrospray
Collision gas:	8 psi (N_2)
Curtain gas:	40 psi (N_2)
Ion source gas 1:	40 psi (Zero Grade Air)
Ion source gas 2:	40 psi (Zero Grade Air)
Ion spray voltage:	-4500 V
Temperature:	700 °C
Entrance potential:	-10 V

Retention times (approximate):

S-3100-CA-RD	8.77 minutes
S-3100-DP	8.46 minutes

Analyte	Precursor ion Q1 (amu)	Product ion Q3 (amu)	Scan time (ms)	DP (V)	EP (V)	CE (V)	CXP (V)
S-3100-CA-RD	490.0	376.0	100	-30	-10	-30	-5
S-3100-CA-RD Qual	490.0	319.0	100	-30	-10	-45	-5
S-3100-DP	337.0	186.0	100	-28	-10	-33	-14
S-3100-DP Qual	337.0	150.0	100	-28	-10	-50	-14

For analysis of 4″-OH-S-3100-CA:

Column Oven Temperature:	40 ± 0.8 °C
Mobile Phase:	Water, 0.05% formic acid
	Methanol, 0.05% formic acid

Gradient Program:

Time, min	Flow Rate, µL/min	% Water, 0.05% formic acid	% Methanol, 0.05% formic acid
0.0	600	60	40
1.0	600	60	40
1.5	400	10	90
12.0	400	10	90
12.5	600	60	40
18.0	600	60	40

Injection Drawing Speed: 100 µL/minute
Injection Volume: 15 µL
Ejecting Speed: 400 µL/minute

Divert Program:

Total Time (min)	Position
0.1	Waste
6.0	MS
8.0	Waste

Typical MS-MS Parameters:

Scan Type: MRM
Polarity: Positive
Ion Source: TurboSpray® Ion Drive Electro Spray interface
Probe Type: Electrospray
Collision gas: 9 psi (N_2)
Curtain gas: 20 psi (N_2)
Ion source gas 1: 40 psi (Zero Grade Air)
Ion source gas 2: 40 psi (Zero Grade Air)
Ion spray voltage: 4500 V
Temperature: 600 °C

Retention time (approximate): 6.78 minutes

Analyte	Precursor ion Q1 (amu)	Product ion Q3 (amu)	Scan time (ms)	DP (V)	EP (V)	CE (V)	CXP (V)
4″-OH-S-3100-CA	508.1	385.0	100	60	5	40	50
4″-OH-S-3100-CA Qual	508.1	489.9	100	70	5	30	20

The instrument parameters shown above are given only as a guide. They may be modified as needed to optimize the chromatography, to resolve matrix interferences (if observed), or to utilize other types of LC-MS/MS instruments.

F. Residue Calculations

The concentrations of the analytes (V-10456, S-3100-CA, S-3100-DA, S-3100-CA-RD, S-3100-CA-UR, S-3100-DP, 4″-OH-S-3100-CA, and S-3100-DA-RD) in each sample extract are calculated using a weighted (1/standard conc.) second-order polynomial equation. The data are presented graphically as concentration of the calibration standards verses their corresponding peak areas. Typically, an Excel® spreadsheet running a Sciex Analyst® curve fitting algorithm is used to determine the curve coefficients a, b, c, r, and so the sample residue concentrations. However, other quantification software that generate curve fit data may also be used. The area responses are entered in the following equation to calculate concentrations in the sample solutions:

$$C_{analyte}\ (^{ng}/_{mL}) = \boldsymbol{A} \times [\text{Detector Response}]^2 + \boldsymbol{B} \times [\text{Detector Response}] + \boldsymbol{C}$$

For example, a set of calibration standards covering 25.0 ng/mL to 0.10 ng/mL range gives peak areas as follows:

ng/mL	Area
25.0	4,710,000
5.00	966,000
1.00	193,000
0.50	98,500
0.25	49,900
0.10	20,900

The resulting equation from the Excel spreadsheet is as follows:

$$C_{analyte}\ (^{ng}/_{mL}) = \boldsymbol{A} \times [\text{Detector Response}]^2 + \boldsymbol{B} \times [\text{Detector Response}] + \boldsymbol{C}$$

$A = 3.0945 \times 10^{-14}$
$B = 5.1634 \times 10^{-6}$
$C = -7.4962 \times 10^{-3}$

To ensure that the equation is appropriate, the areas of the calibration standards are entered into this equation and the standard concentrations are calculated. For each standard, the calculated concentration must each be within 15% of the actual concentration. In addition, the coefficient of determination (r^2) must be greater than 0.99.

For example (from the above data), the 1 ng/mL standard has an area of 193,000 and the calculated concentration (using the equation) is 0.99 ng/mL. This is acceptable as this is 99% of the known concentration. The criteria listed above for recalculated concentrations and the coefficient of determination must be met for acceptance of each analytical set, unless approved by the chemist responsible for the analysis. Sample extract concentrations are also calculated using the equation from the calibration standards. For example, a sample extract for S-3100-DA-RD with a peak area of 1,010,000 would have a concentration as follows:

$$C_{analyte}\ (^{ng}/_{mL}) = \mathbf{3.0945 \times 10^{-14}} \times [1{,}010{,}000]^2 + \mathbf{5.1634 \times 10^{-6}} \times [1{,}010{,}000] - \mathbf{7.4962 \times 10^{-3}} = 5.239$$

Concentrations of each analyte (V-10456, S-3100-CA, S-3100-DA, S-3100-CA-RD, S-3100-CA-UR, S-3100-DP, 4″-OH-S-3100-CA, and S-3100-DA-RD) in the sample is calculated using the following formula:

$$C_{soil}\ (^{mg}/_{kg}) = \frac{C_{analyte} \times EV \times FV \times DF}{AV \times W}$$

where:

$C_{analyte}$ = Concentration of analyte in solution (in ng/mL, from equation)
C_{soil} = Concentration of analyte in the soil sample (in mg/kg, from equation)
EV = Total extraction volume (100 mL)

FV = Final volume of extract (10 mL)
DF = Dilution factor (1)
W = Sample weight analyzed (20 g)
AV = Aliquot volume (25.0 mL)

For example, the concentration in the above example sample (with a calculated extract concentration of 5.239 ng/mL) would be calculated as follows:

$$C_{soil}\left({}^{mg}/_{kg}\right) = \frac{5.239\ {}^{ng}/_{mL} \times 100\ \text{mL} \times 10\ \text{mL} \times 1}{25\ \text{mL} \times 20\ \text{g}}\left(\frac{1000\ \text{g}}{1\ \text{kg}}\right)\left(\frac{1\ \text{mg}}{1 \times 10^{6}\ \text{ng}}\right) = 0.0104$$

The concentration of each analyte (V-10456, S-3100-CA, S-3100-DA, S-3100-CA-RD, S-3100-CA-UR, S-3100-DP, 4″-OH-S-3100-CA, and S-3100-DA-RD) in the fortified soil sample is calculated by the above calculation. The percent recovery is calculated by taking the concentration found in fortified sample subtracting the concentration found in the untreated control (any negative values are set to 0) and dividing by the amount the sample was fortified with and multiplying by 100. If the peak area of the untreated sample is ≤ 10% of the peak area of the lowest standard, no correction was applied when calculating the percent recovery. The percent recovery for fortified samples are calculated using the formula:

$$\%\ \text{Recovery} = \frac{C_{\text{fortified sample}} - C_{\text{control sample}}}{\text{Fortification level}} \times 100\%$$

For a compound fortified at 0.01 mg/kg, with a peak area of 1,010,000, the following values were utilized to calculate the recovery of each analyte in the sample:

mg/kg found in fortified sample = 0.0104 mg/kg
mg/kg found in untreated control sample = 0 mg/kg

$$\%\ \text{Recovery} = \frac{0.0104\ {}^{mg}/_{kg} - 0\ {}^{mg}/_{kg}}{0.01\ {}^{mg}/_{kg}}\ \text{x}\ 100\% = 104\%$$

Fortification samples are not corrected for moisture. Samples that require moisture content on a dry weight basis.

$$\%\ \text{water content} = \frac{\text{sample wet weight (g)} - \text{sample dry weight (g)}}{\text{sample dry weight (g)}}\ \text{x}\ 100\%$$

$$\text{Soil dry weight (g)} = \frac{\text{sample wet weight (g)}}{(100\% + \%\ \text{water content})} \times 100\%$$

For example, a soil with a wet weight of 23.516 g and a dry weight of 17.422 g then the following values would be obtained:

$$\%\ \text{water content} = \frac{23.516\ \text{(g)} - 17.422\ \text{(g)}}{17.422\ \text{(g)}} \times 100\% = 34.98\%$$

$$\text{Soil dry weight (g)} = \frac{23.516(\text{g})}{(100\%+34.98\%)} \times 100\% = 17.422\text{g}$$

The concentration (mg/kg) found in the soil is then corrected to concentration (mg/kg) found in dry soil.

$$C_{dry\ soil}\left({}^{mg}/_{kg}\right) = \frac{C_{soil} \times (100\% + \%\ \text{water content})}{100\%}$$

If a wet weight concentration of 0.5 mg/kg was found in a sample and a water content of 34.98% then the following dry weight concentration would be obtained:

$$C_{dry\ soil}\left({}^{mg}/_{kg}\right) = \frac{0.5\ \text{mg/kg} \times (100\% + 34.98\%)}{100\%} = 0.675\left({}^{mg}/_{kg}\right)$$

4. ANALYTICAL LIMITS

A. Limit of Detection

The limit of detection (LOD) of this method is 0.0001 mg/kg for V-10456, S-3100-CA, S-3100-DA, and S-3100-DP. The LOD of this method is 0.0002 mg/kg for S-3100-CA-RD, S-3100-DA-RD, and 4″-OH-S-3100-CA. The LOD of this method is 0.0010 mg/kg for S-3100-CA-UR. This LOD is calculated by dividing the lowest calibration standard concentration by the effective matrix concentration in the sample extracts. This is based on a 20 g sample, a 25 mL aliquot (of the 100 mL total volume after extraction), a 10 mL final volume, a 1X dilution, and a 0.05 ng/mL or 0.10 ng/mL standard in the calibration.

$$\text{LOD}\left({}^{mg}/_{kg}\right) = \frac{0.10\ {}^{ng}/_{mL} \times 100\ \text{mL} \times 10\ \text{mL} \times 1}{25\ \text{mL} \times 20\ \text{g}}\left(\frac{1000\ \text{g}}{1\ \text{kg}}\right)\left(\frac{1\ \text{mg}}{1 \times 10^6\ \text{ng}}\right) = 0.0002$$

B. Limit of Quantitation

The method limit of quantitation (LOQ) is 0.00015 mg/kg for V-10456, S-3100-CA, S-3100-DA, and S-3100-DP. The method limit of quantitation is 0.001 mg/kg for S-3100-CA-RD, S-3100-DA-RD and 4″-OH-S-3100-CA. The method limit of quantitation is 0.005 mg/kg for S-3100-CA-UR.

5. NOTES

A. Note 1

A typical quality control set consists of one untreated control sample (UTC) and two fortified samples at the LOQ and 10×LOQ levels. The levels of fortification are generally at the method LOQ (0.00015 mg/kg for V-10456, S-3100-CA, S-3100-DA, and S-3100-DP, 0.001 mg/kg for S-3100-CA-RD, S-3100-DA-RD, and 4″-OH-S-3100-CA, and 0.005 mg/kg for S-3100-CA-UR) and/or at 10 times the LOQ (0.0015 mg/kg for V-10456, S-3100-CA, S-3100-DA, and S-3100-DP, 0.01 mg/kg for S-3100-CA-RD, S-3100-DA-RD, and 4″-OH-S-3100-CA, and 0.05 mg/kg for S-3100-

CA-UR). If residues higher than 10 times LOQ are anticipated or observed, then fortifications should be made at a higher concentration (typically slightly above the highest residues). Method recoveries must be 70% to 120% with the coefficient of variation (CV) of replicate measurements of recoveries of plus or minus 20% to be acceptable unless approved by the chemist responsible for the analysis. If the testing facility does not require concurrent analysis of fortified control samples, or if a UTC sample is not available, this method requirement may be waived.

B. **Note 2**

At Valent U.S.A. LLC, reproducibility of an analytical run is determined by calculating the coefficient of variation (CV) from the calculated concentration obtained from the continuing calibration standards analyzed in the analytical sequence (set). For the analytical set to be acceptable, these CV's must be 15% or less unless approved by the chemist responsible for the analysis.

C. **Note 3**

For the instrument calibration set to be acceptable, the coefficient of determination (r^2) of the weighted polynomial calibration curve must be greater than or equal to 0.99, and the back-calculated concentration for each standard must be within 15% (20% for the lowest standard) of the labeled concentration for the instrument to be considered within a polynomial fit unless approved by the chemist responsible for the analysis.

APPENDIX D EXAMPLE CALCULATION

All quantitation calculations performed by the Analyst software were based on the mg/kg (µg/g, ppm) sample equivalents of the calibration standard solutions, with results expressed as mg/kg sample concentrations. The mg/kg sample equivalent concentration for a given calibration standard solution for V-10456, S-3100-CA, S-3100-DA, S-3100-CA-RD, S-3100-CA-UR, S-3100-DP, 4″-OH-S-3100-CA, and S-3100-DA-RD was calculated from the method as follows:

$$mg/kg = \frac{(\mu g/ml\ cal\ std) \times (initial\ vol\ mL) \times (final\ vol.\ mL)}{(grams\ sample) \times (aliquot\ vol.\ mL)}$$

As an example, the 0.0001 µg/mL calibration standard (closest standard to LOQ of 0.00015 mg/kg) for V-10456, S-3100-CA, S-3100-DA, and S-3100-DP is equivalent to mg/kg sample equivalents as calculated below:

$$mg/kg = \frac{(0.0001\ \mu g/mL) \times (100\ mL) \times (10.0\ mL)}{(20\ g) \times (25.0\ mL)}$$

$$= 0.0002\ mg/kg$$

The concentrations of the analytes (V-10456, S-3100-CA, S-3100-DA, S-3100-CA-RD, S-3100-CA-UR, S-3100-DP, 4″-OH-S-3100-CA, and S-3100-DA-RD) in each sample extract were calculated using a second-order polynomial equation on the basis of peak area:

$$Y = A \times (\text{Detector Response})^2 + B \times (\text{Detector Response}) + C$$

EXAMPLE:
Sample Name: V-18-200320-NDSoil-LOQ-01, Set ID: SMM211009
Calibration Curve for V-10456: A = 8.8869E-16; B = 7.9537E-07; C = -2.7523E-03
Peak Area for V-10456: 104090

$$\text{V-10456 mg/kg} = 8.8869E\text{-}16 \times (104090)^2 + 7.9537E\text{-}07 \times (104090) + -2.7523E\text{-}03$$

$$\text{V-10456 mg/kg} = 0.00016$$

$$\text{Percent Recovery} = \frac{0.00016\ \text{mg/kg}}{0.00015\ \text{mg/kg}} \times 100$$

$$\text{Percent Recovery} = 107\ \%$$

APPENDIX F INSTRUMENT CONDITIONS

Analytical Condition log SCIEX Q-Trap 6500+

Study ID: 693SRUS21R0167

Analytes: V-10456, S-3100-CA, S-3100-DA, & S-3100-DA-RD

Instrument ID: SCIEX Q-Trap 6500+ Serial # CG23321704

ExionLC AC Autosampler Serial# ABCXR5670470

Pump ID: ExionLC AC Pump A Serial #ABDXR5671011
ExionLC AC Pump B Serial #ABDXR5671012

Quantitation Software Version: Analyst 1.7

Column make: Phenomenex Kinetex Biphenyl
Column Measurements: 150 x 4.6 mm, 5 µm
Column Temperature (°C): 30

Injection Volume (µL): 5
Aqueous Mobile Phase ID: Optima LC-MS Water containing 0.05 % LC-MS Formic Acid
Organic Mobile Phase ID: ACN LC-MS
Syringe Wash Solvent: ACN/Water (1:1)

HPLC Gradient Program

Time (min)	% Aqueous	% Organic	Flow Rate (mL/min)
0.00	77	23	0.600
1.00	77	23	0.600
7.50	10	90	0.600
18.0	10	90	0.600
19.0	77	23	0.600
25.0	77	23	0.600

Divert Program

0.1 min to 5.5 min to Waste
5.5 min to 10.0 min to Instrument
10.0 min to 25.0 min to Waste

Compound ID	S-3100-DA	S-3100-DA Qual	S-3100-DA-RD	S-3100-DA-RD Qual	S-3100-CA	S-3100-CA Qual	V-10456	V-10456 Qual
Ionization Mode	MRM (MRM)							
Polarity	Positive							
Q1 Mass (m/z)	432.0	432.0	434.1	434.1	490.0	490.0	518.0	518.0
Q3 Mass (m/z)	281.0	82.0	281.1	323.0	250.0	293.0	293.0	250.0
(DP) Declustering Potential (v)	80	50	80	70	40	30	50	30
(CE) Collision Energy (v)	40	60	40	50	40	40	50	40
(CXP) Collision Cell Energy Potential (v	10	10	15	15	15	15	15	15
(EP) Entrance Potential	10	5	5	15	10	15	15	10
Dwell Time (ms)	100							
(CUR) Curtain Gas	20							
(GS1) Ion Gas Source 1	55							
(GS2) Ion Gas Source 2	50							
(IS) Ion Spray Voltage	4500							
(TEM) Temperature (°C)	600							
(CAD) Collision Gas	Medium							

Analytical Condition log SCIEX Q-Trap 6500+

Study ID: 693SRUS21R0167
Analytes: S-3100-CA-UR, S-3100-CA-RD, & S-3100-DP

Instrument ID: SCIEX Q-Trap 6500+ Serial # CG23321704
ExionLC AC Autosampler Serial# ABCXR5670470
Pump ID: ExionLC AC Pump A Serial #ABDXR5671011
ExionLC AC Pump B Serial #ABDXR5671012

Quantitation Software Version: Analyst 1.7

Column make: Phenomenex Kinetex Biphenyl
Column Measurements: 150 x 4.6 mm, 5 µm
Column Temperature (°C): 30

Injection Volume (µL): 15
Aqueous Mobile Phase ID: Optima LC-MS Water containing 0.05 % LC-MS Formic Acid
Organic Mobile Phase ID: ACN LC-MS
Syringe Wash Solvent: ACN/Water (1:1)

HPLC Gradient Program

Time (min)	% Aqueous	% Organic	Flow Rate (mL/min)
0.00	85	15	0.600
1.00	85	15	0.600
9.00	5	95	0.600
13.0	5	95	0.600
13.5	85	15	0.600
20.0	85	15	0.600

Divert Program

0.1 min to 6.0 min to Waste
6.0 min to 10.0 min to Instrument
10.0 min to 20.0 min to Waste

Compound ID	S-3100-CA-UR	S-3100-CA-UR Qual	S-3100-CA-RD	S-3100-CA-RD Qual	S-3100-DP	S-3100-DP Qual
Ionization Mode	MRM (MRM)					
Polarity	Negative					
Q1 Mass (m/z)	368.0	368.0	490.0	490.0	337.0	337.0
Q3 Mass (m/z)	293.0	337.0	376.0	319.0	186.0	150.0
(DP) Declustering Potential (v)	-60	-50	-30	-30	-28	-28
(CE) Collision Energy (v)	-20	-20	-30	-45	-33	-50
(CXP) Collision Cell Energy Potential (v)	-5	-15	-5	-5	-14	-14
(EP) Entrance Potential	-10	-15	-10	-10	-10	-10
Dwell Time (ms)	500	200	100			
(CUR) Curtain Gas	20					
(GS1) Ion Gas Source 1	55					
(GS2) Ion Gas Source 2	50					
(IS) Ion Spray Voltage	-4500					
(TEM) Temperature (°C)	600					
(CAD) Collision Gas	Medium					

Analytical Condition log SCIEX Q-Trap 6500+

Study ID: 693SRUS21R0167
Analyte: 4"-OH-S-3100-CA

Instrument ID: SCIEX Q-Trap 6500+ Serial # CG23321704
ExionLC AC Autosampler Serial# ABCXR5670470
Pump ID: ExionLC AC Pump A Serial #ABDXR5671011
ExionLC AC Pump B Serial #ABDXR5671012

Quantitation Software Version: Analyst 1.7

Column make: Phenomenex Kinetex Biphenyl
Column Measurements: 150 x 4.6 mm, 5 µm
Column Temperature (°C): 40

Injection Volume (µL): 15
Aqueous Mobile Phase ID: Optima LC-MS Water containing 0.05 % LC-MS Formic Acid
Organic Mobile Phase ID: MeOH with 0.05% LC-MS Formic Acid
Syringe Wash Solvent: ACN/Water (1:1)

HPLC Gradient Program

Time (min)	% Aqueous	% Organic	Flow Rate (mL/min)
0.00	60	40	0.600
1.00	60	40	0.600
1.50	10	90	0.400
12.0	10	90	0.400
12.5	60	40	0.600
18.0	60	40	0.600

Divert Program
0.1 min to 6.0 min to Waste
6.0 min to 8.0 min to Instrument
8.0 min to 18.0 min to Waste

Compound ID	4"-OH-S-3100-CA	4"-OH-S-3100-CA Qual
Ionization Mode	MRM (MRM)	
Polarity	Positive	
Q1 Mass (m/z)	508.1	508.1
Q3 Mass (m/z)	385.0	489.9
(DP) Declustering Potential (v)	60	70
(CE) Collision Energy (v)	40	30
(CXP) Collision Cell Energy Potential (v)	50	20
(EP) Entrance Potential	5	5
Dwell Time (ms)	100	
(CUR) Curtain Gas	20	
(GS1) Ion Gas Source 1	40	
(GS2) Ion Gas Source 2	40	
(IS) Ion Spray Voltage	4500	
(TEM) Temperature (°C)	600	
(CAD) Collision Gas	Medium	