MRID: 51778713

202100291

TITLE

V-10456: Validation of Valent Method RM-52S-4, "Determination of Residues of V-10456 and its Degradates in Soil"

TEST GUIDELINE

850.6100

AUTHOR(S)

Chen T

STUDY COMPLETION DATE

1st Amended Date: 2021-10-14

Original 2021-08-25

PERFORMING LABORATORY

Valent U.S.A. LLC

4600 Norris Canyon Road

San Ramon, CA 94583

LABORATORY PROJECT ID

V-21-202220

TOTAL PAGES

162

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1 EXECUTIVE SUMMARY

The validation of residue analytical methods RM-52S-4, entitled "Determination of Residues of V-10456 and its Degradates in Soil", was completed successfully. RM-52S-4 has a limit of detection (LOD) of 0.0001 mg/kg and a limit of quantification (LOQ) of 0.00015 mg/kg for V-10456, S-3100-CA, S-3100-DA, and S-3100-DP. RM-52S-4 has a limit of detection (LOD) of 0.0002 mg/kg and a limit of quantification (LOQ) of 0.001 mg/kg for S-3100-CA-RD, S-3100-DA-RD, and 4″-OH-S-3100-CA. RM-52S-4 has a limit of detection (LOD) of 0.0010 mg/kg and a limit of quantification (LOQ) of 0.005 mg/kg for S-3100-CA-UR. The method was validated using at least five untreated North Dakota soil samples fortified at nominal concentrations, LOQ, and 10XLOQ each.

2 INTRODUCTION

The objective of this study was to validate Valent analytical method RM-52S-4, "Determination of Residues of V-10456 and Its Degradates in Soil". The protocol for the validation of the method is included in APPENDIX 1 and the analytical method is presented in APPENDIX 2. Individual recoveries for all analytes for RM-52S-4 can be found in Table 1 through Table 16. This study was designed to fulfill the requirements of the US EPA Ecological Effects Test Guidelines OPPTS 850.6100: Environmental Chemistry Methods and Associated Independent Residue Analytical Method (REFERENCE 1).

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.

3 MATERIALS AND METHOD

3.1 TEST SUBSTANCE/REFERENCE STANDARDS

The reference standard used for the validation described in APPENDIX 2. The certificates of analysis of the analytes are included in APPENDIX 3.

3.2 TEST SYSTEMS

The test system used for the validation of RM-52S-4 was a composite of North Dakota untreated control soil from study V-18-200320 "V-10456: Terrestrial Field Soil Dissipation on Bare Ground in California, Georgia, Ontario, and North Dakota". The North Dakota soil was classified according to the USDA classification system for soil as clay with 3.5% organic matter as per Walkley Black procedure. See APPENDIX 4 for soil characterization report.

3.3 EQUIPMENT AND REAGENTS

For the specific equipment and reagents used in the validation of RM-52S-4, refer to APPENDIX 2.

4 EXPERIMENTAL PROCEDURES

4.1 STANDARD SOLUTIONS AND SAMPLE PREPARATION

Refer to APPENDIX 2 for how standard solutions and samples were prepared.

4.2 SAMPLE ANALYSIS

All samples were analyzed as per the analytical method. For specifics, reference APPENDIX 2.

5 STATISTICAL METHODS

Means, standard deviations, coefficients of variation (%CV) and coefficients of determination (r^2) were used to evaluate the analytical method.

The protocol for the validation of this method is included in APPENDIX 1. Method RM-52S-4 is

presented in APPENDIX 2. The data are presented in APPENDIX 5. The certificates of analysis for reference standards used during the validation are included in APPENDIX 3. Example chromatograms are presented in Figure 1 through Figure 40.

7 CALCULATIONS

Reference APPENDIX 2 for how calculations were completed for RM-52S-4.

8 ANALYSIS TIME

A trained analyst, familiar with this method, can complete the analysis of a set of 17 samples in approximately 8 hours. The results are available within 36 hours of initiating the analysis.

9 CONCLUSION

The validation of residue analytical method, RM-52S-4, entitled "Determination of Residues of V-10456 and Its Degradates in Soil", was completed successfully.

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.

10 REFERENCE

1. US EPA Office of Prevention, Pesticides and Toxic Substances, Ecological Effects Tests Guidelines OPPTS 850.6100: Environmental Chemistry Methods and Associated Independent Laboratory Validation, June 2012.

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STUDY PROTOCOL:

V-10456: Validation of Valent Method RM-52S-4, "Determination of Residues of V-10456 and its Degradates in Soil"

DATA REQUIREMENT:

EPA Ecological Effects Test Guidelines,
OCSPP 850.6100, Environmental Chemistry Methods and Associated Independent Laboratory Validation

SPONSOR:

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San Ramon, CA 94583
U.S.A.

TESTING FACILITY/ ANALYTICAL LABORATORY:

Valent U.S.A. LLC
4600 Norris Canyon Road
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U.S.A.

STUDY IDENTIFICATION NUMBERS:

Valent Study Number: V-21-202220

TOTAL PAGES:

7

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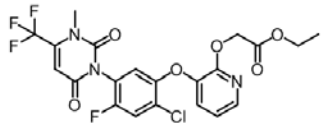
2. GENERAL INFORMATION

PURPOSE: To validate Valent method RM-52S-4, "Determination of Residues of V-10456 and its Degradates in Soil" following OCSPP 850.6100 guidelines.

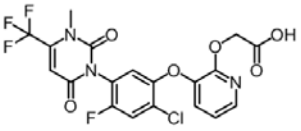
A. VALENT PROTOCOL NUMBER: V-21-202220

B. TEST / REFERENCE SUBSTANCES:

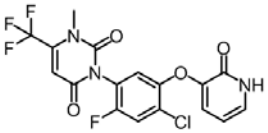
Common name: V-10456, S-3100
Chemical name: Ethyl [(3-{2-chloro-4-fluoro-5-[3-methyl-2,6-dioxo-4-(trifluoromethyl)-3,6-dihydropyrimidin-1(2*H*)-yl]phenoxy}-2-pyridyl)oxy]acetate
Molecular formula: $C_{21}H_{16}ClF_4N_3O_6$
Molecular weight: 517.82 g/mol



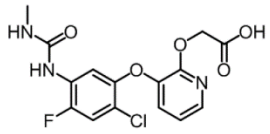
Common name: S-3100-CA
Chemical name: (3-{2-chloro-4-fluoro-5-[1,2,3,6-tetrahydro-3-methyl-2,6-dioxo-4-(trifluoromethyl)pyrimidin-1-yl]phenoxy}pyridine-2-yloxy)acetic acid
Molecular formula: $C_{19}H_{12}ClF_4N_3O_6$
Molecular weight: 489.77 g/mol



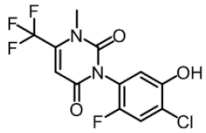
Common name: S-3100-DA
Chemical name: 3-[4-chloro-5-(1,2-dihydro-2-oxpyridin-3-yloxy)-2-fluorophenyl]-1,2,3,4-tetrahydro-1-methyl-6-(trifluoromethyl)pyrimidine-2,4-dione
Molecular formula: $C_{17}H_{10}ClF_4N_3O_4$
Molecular weight: 431.73 g/mol



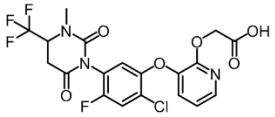
Common name: S-3100-CA-UR
Chemical name: {3-[2-chloro-4-fluoro-5-(3-methylureido)phenoxy]pyridine-2-yloxy}acetic acid
Molecular formula: $C_{15}H_{13}ClFN_3O_5$
Molecular weight: 369.74 g/mol



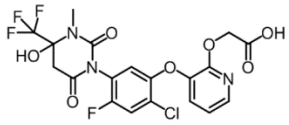
Common name: S-3100-DP
Chemical name: 3-(4-chloro-2-fluoro-5-hydroxyphenyl)-1,2,3,4-tetrahydro-1-methyl-6-(trifluoromethyl)pyrimidine-2,4-dione
Molecular formula: $C_{12}H_7ClF_4N_2O_3$
Molecular weight: 338.65 g/mol



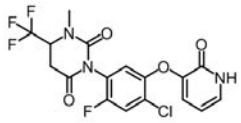
Common name: S-3100-CA-RD
Chemical name: (3-{2-chloro-4-fluoro-5-[1,2,3,4,5,6-hexahydro-3-methyl-2,6-dioxo-4-(trifluoromethyl)pyrimidin-1-yl]phenoxy}pyridine-2-yloxy)acetic acid
Molecular formula: $C_{19}H_{14}ClF_4N_3O_6$
Molecular weight: 491.78 g/mol



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



C. TEST SYSTEM: The test system will be North Dakota untreated control soil (V-18-200320-D) from study V-18-200320 "V-10456: Terrestrial Field Soil Dissipation on Bare Ground in California, Georgia, Ontario, and North Dakota".

D. TEST SYSTEM JUSTIFICATION: To determine the performance of a residue method to analyze V-10456 and metabolites in soil.

E. STUDY DIRECTOR:
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F. ANALYTICAL LABORATORY:
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G. VALENT SPONSOR REPRESENTATIVE / TESTING FACILITY MANAGEMENT:
Eric Tamichi
Valent U.S.A. LLC
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Phone: (925) 256-2743
E-mail: eric.tamichi@valent.com

H. PROPOSED EXPERIMENTAL START DATE: July 19, 2021

I. PROPOSED EXPERIMENTAL TERMINATION DATE: July 31, 2021
(Last date of data collection)

J. GOOD LABORATORY PRACTICES: This study shall be conducted in accordance with FIFRA Good Laboratory Practices Standards (40 CFR Part 160).

K. QUALITY ASSURANCE:

Vincella Erickson
Valent U.S.A. LLC
4600 Norris Canyon Road
San Ramon, CA 94583
Phone: (925) 948-2938
E-mail: Vincella.Erickson@valent.com

L. METHODS:

1. All procedures involved in the implementation of this protocol will be covered by general SOPs, except where otherwise stated in this document.

M. STATISTICAL ANALYSIS:

1. Means and standard deviations will be calculated, where appropriate. Analysis of the data and statistical methods used in this study will be described in the final report.

3. ANALYTICAL LABORATORY REQUIREMENTS

A. MATERIALS

1. **Test System:** The test system used for the validation will be a North Dakota untreated control composite soil (V-18-200320-D) from study V-18-200320 "V-10456: Terrestrial Field Soil Dissipation on Bare Ground in California, Georgia, Ontario, and North Dakota".

2. **Test Substance/ Reference Standard:** Test Substance/Reference standards will be obtained from Valent U.S.A. LLC. The test substance will be used to prepare fortifying solutions. The lot number and percent purity of the reference standard will be recorded in the raw data and the final report. A copy of the Certificate of Analysis for the reference standards will be included in the final report.

B. ANALYSES

1. The procedure for Valent method RM-52S-4, "Determination of Residues of V-10456 and its Degradates in Soil" will be validated. The preparation sheet should provide sufficient detail for the reconstruction of the method.
2. The validation set will contain a reagent blank, two control samples, and at least five control samples fortified at the proposed limit of quantitation (LOQ) and at least five control samples fortified at 10 times the LOQ. The target LOQ for V-10456, S-3100-CA, S-3100-DA, and S-3100-DP is 0.00015 mg/kg (0.15 ng/g). The target LOQ for S-3100-CA-RD, S-3100-DA-RD, and 4″-OH-S-3100-CA is 0.001 mg/kg (1.00 ng/g). The target LOQ for S-3100-CA-UR is 0.005 mg/kg (5.00 ng/g). The target LOQ values were derived from the lowest ecotoxicity endpoints.

3. All samples used in this study will be labeled in a manner that precludes error in the recording and storage of data.
4. Prior to experimental start date, standard solutions for fortifications and analyses will be prepared and documented according to Valent SOP.

C. CRITERIA FOR VALIDATION

1. The mean recovery at each fortification level, at or above the LOQ, should lie between 70 and 120% of the known quantity of the analyte fortified into the test system blanks during the method validation.
2. The relative standard deviation (RSD) of replicate measurements of recoveries should not exceed 20%.
3. Interferences with peak areas that are less than 50 percent (%) of the LOD are considered not significant.

D. DATA AND FINAL REPORT

1. All original data, (or certified copies thereof), the original protocol, amendments and deviations, and the original report will be archived in the Valent U.S.A. LLC archive at or before the conclusion of this study.
2. A final report will be written and signed by the Study Director. Minimally, it will include all of the applicable items in 40 CFR Part 160.185 and OCSPP 850.6100.

4. REPORTING REQUIREMENTS

A. PROTOCOL AMENDMENTS

Planned changes to this protocol will require the approval of both the Testing Facility Management/ Sponsor Representative and the Study Director. The Study Director will authorize a written amendment reflecting the changes. It will be signed by the Testing Facility Management/ Sponsor Representative and the Study Director. It will be reviewed by Valent QAU.

B. PROTOCOL AND SOP DEVIATIONS

Changes from procedures as outlined in this protocol, if not amended as described in Protocol Amendments and similar changes to SOPs are considered deviations. The deviation notice will be prepared immediately after discovery, include a description of the deviation, the reason for the deviation, and an assessment of the impact on the study. The deviation will be signed by the Study director. Copies of the protocol deviation notices will be placed in the Study File.

C. QUALITY ASSURANCE ACTIVITY

The Valent Quality Assurance Unit (QAU) may audit at least one critical phase of the study, the raw data, and the final report. All findings from the QAU audits and reviews will be reported to the Study Director and Testing Facility Management.

APPENDIX 2
Analytical Method RM-52S-4 and Analytical Method RM-52S-4A

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.

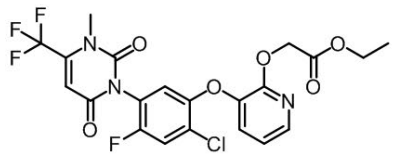
Briefly, the residues are extracted from soil using three extractions of acetonitrile/0.1 M HCl (5/1, v/v). The residues are then partitioned into dichloromethane, concentrated, and the residues are re-dissolved and samples are analyzed by LC-MS/MS.

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.

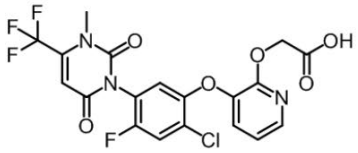
2. MATERIALS

2.1. ANALYTICAL REFERENCE STANDARDS

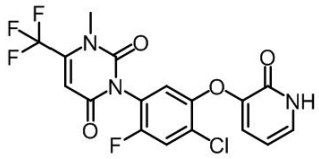
Common name:	V-10456, S-3100
Chemical name:	ethyl [(3-{2-chloro-4-fluoro-5-[3-methyl-2,6-dioxo-4-(trifluoromethyl)-3,6-dihydropyrimidin-1(2*H*)-yl]phenoxy}-2-pyridyl)oxy]acetate
Molecular formula:	$C_{21}H_{16}ClF_4N_3O_6$
Molecular weight:	517.82 g/mol



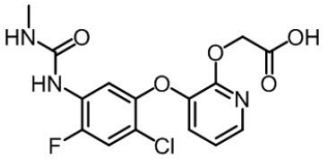
Common name: S-3100-CA
Chemical name: (3-{2-chloro-4-fluoro-5-[1,2,3,6-tetrahydro-3-methyl-2,6-dioxo-4-(trifluoromethyl)pyrimidin-1-yl]phenoxy}pyridine-2-yloxy)acetic acid
Molecular formula: $C_{19}H_{12}ClF_4N_3O_6$
Molecular weight: 489.77 g/mol



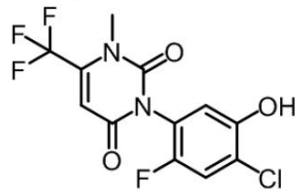
Common name: S-3100-DA
Chemical name: 3-[4-chloro-5-(1,2-dihydro-2-oxpyridin-3-yloxy)-2-fluorophenyl]-1,2,3,4-tetrahydro-1-methyl-6-(trifluoromethyl)pyrimidine-2,4-dione
Molecular formula: $C_{17}H_{10}ClF_4N_3O_4$
Molecular weight: 431.73 g/mol



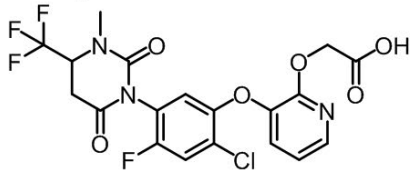
Common name: S-3100-CA-UR
Chemical name: {3-[2-chloro-4-fluoro-5-(3-methylureido)phenoxy]pyridine-2-yloxy}acetic acid
Molecular formula: $C_{15}H_{13}ClFN_3O_5$
Molecular weight: 369.74 g/mol



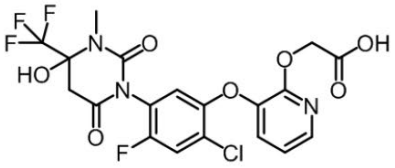
Common name: S-3100-DP
Chemical name: 3-(4-chloro-2-fluoro-5-hydroxyphenyl)-1,2,3,4-tetrahydro-1-methyl-6-(trifluoromethyl)pyrimidine-2,4-dione
Molecular formula: $C_{12}H_7ClF_4N_2O_3$
Molecular weight: 338.65 g/mol



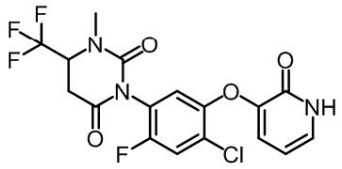
Common name: S-3100-CA-RD
Chemical name: (3-{2-chloro-4-fluoro-5-[1,2,3,4,5,6-hexahydro-3-methyl-2,6-dioxo-4-(trifluoromethyl)pyrimidin-1-yl]phenoxy}pyridine-2-yloxy)acetic acid
Molecular formula: $C_{19}H_{14}ClF_4N_3O_6$
Molecular weight: 491.78 g/mol



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:

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

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:

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

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-

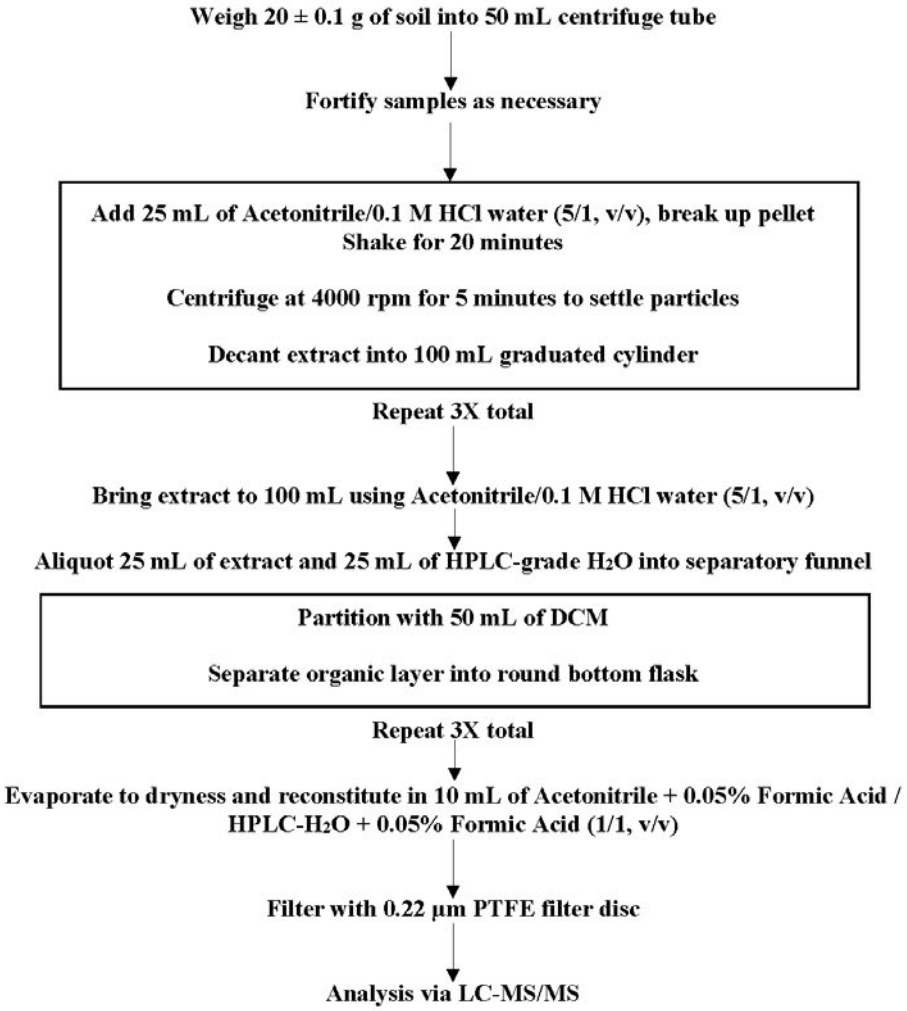
CA-UR). If residues higher than 10 times LOQ are anticipated or observed, then fortifications should be made at a higher concentration (typically slightly above the highest residues). Method recoveries must be 70% to 120% with the coefficient of variation (CV) of replicate measurements of recoveries 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.


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

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.

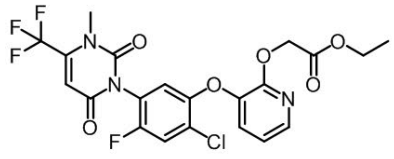
The method limit of quantitation is 0.00015 mg/kg (0.15 ng/g) for V-10456, S-3100-CA, S-3100-DA, and S-3100-DP and was based on the lowest endpoint for a terrestrial plant (EC_{25} = 0.34 g a.i./ha from the vegetative vigor study). The method limit of quantitation is 0.001 mg/kg (1.0 ng/g) for S-3100-CA-RD, 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.

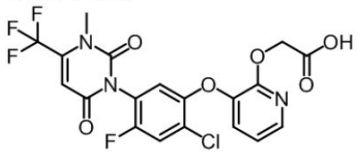
2. MATERIALS

2.1. ANALYTICAL REFERENCE STANDARDS

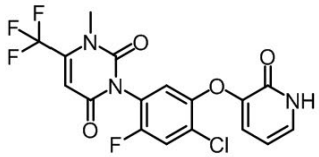
Common name:	V-10456, S-3100
Chemical name:	ethyl [(3-{2-chloro-4-fluoro-5-[3-methyl-2,6-dioxo-4-(trifluoromethyl)-3,6-dihydropyrimidin-1(2*H*)-yl]phenoxy}-2-pyridyl)oxy]acetate
Molecular formula:	$C_{21}H_{16}ClF_4N_3O_6$
Molecular weight:	517.82 g/mol



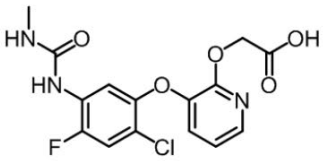
Common name: S-3100-CA
Chemical name: (3-{2-chloro-4-fluoro-5-[1,2,3,6-tetrahydro-3-methyl-2,6-dioxo-4-(trifluoromethyl)pyrimidin-1-yl]phenoxy}pyridine-2-yloxy)acetic acid
Molecular formula: $C_{19}H_{12}ClF_4N_3O_6$
Molecular weight: 489.77 g/mol



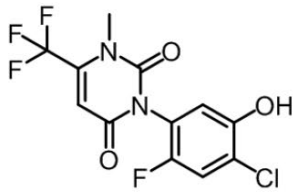
Common name: S-3100-DA
Chemical name: 3-[4-chloro-5-(1,2-dihydro-2-oxpyridin-3-yloxy)-2-fluorophenyl]-1,2,3,4-tetrahydro-1-methyl-6-(trifluoromethyl)pyrimidine-2,4-dione
Molecular formula: $C_{17}H_{10}ClF_4N_3O_4$
Molecular weight: 431.73 g/mol



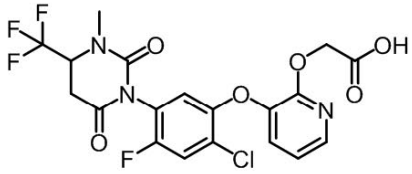
Common name: S-3100-CA-UR
Chemical name: {3-[2-chloro-4-fluoro-5-(3-methylureido)phenoxy]pyridine-2-yloxy}acetic acid
Molecular formula: $C_{15}H_{13}ClFN_3O_5$
Molecular weight: 369.74 g/mol



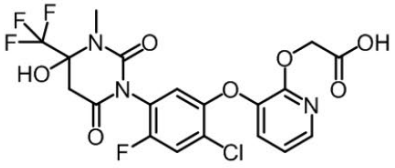
Common name: S-3100-DP
Chemical name: 3-(4-chloro-2-fluoro-5-hydroxyphenyl)-1,2,3,4-tetrahydro-1-methyl-6-(trifluoromethyl)pyrimidine-2,4-dione
Molecular formula: $C_{12}H_7ClF_4N_2O_3$
Molecular weight: 338.65 g/mol



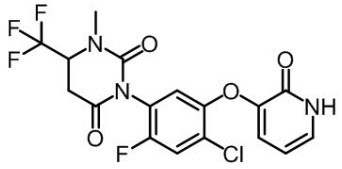
Common name: S-3100-CA-RD
Chemical name: (3-{2-chloro-4-fluoro-5-[1,2,3,4,5,6-hexahydro-3-methyl-2,6-dioxo-4-(trifluoromethyl)pyrimidin-1-yl]phenoxy}pyridine-2-yloxy)acetic acid
Molecular formula: $C_{19}H_{14}ClF_4N_3O_6$
Molecular weight: 491.78 g/mol



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:

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

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:

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

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:

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

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.

6. DISCUSSION

The method was developed for 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.

RM-52S-4 has a limit of detection (LOD) of 0.0001 mg/kg and a limit of quantification (LOQ) of 0.00015 mg/kg for V-10456, S-3100-CA, S-3100-DA, and S-3100-DP. RM-52S-4 has a limit of detection (LOD) of 0.0002 mg/kg and a limit of quantification (LOQ) of 0.001 mg/kg for S-3100-CA-RD, S-3100-DA-RD, and 4″-OH-S-3100-CA. RM-52S-4 has a limit of detection (LOD) of 0.0010 mg/kg and a limit of quantification (LOQ) of 0.005 mg/kg for S-3100-CA-UR.