I. SUMMARY

ISK Biosciences Corporation and FarmHannong Co., Ltd. contracted Golden Pacific Laboratories, LLC (GPL) in Fresno, California, to conduct an Independent Laboratory Validation (ILV). The two objectives of this study were to validate the analytical method produced by Dongbu Farm Hannong Co., Ltd. (now FarmHannong Co., Ltd.) entitled "Residue Analytical Method of Tiafenacil and Its Metabolites in Soil," and to validate a modified method (i.e., method bridging) for use with the tiafenacil soil dissipation studies. The methods use solvent extraction and determination by Liquid Chromatography (LC) with tandem mass spectrometer (MS/MS) detection.

The method was successfully validated at 0.1 ppm and 1 ppm and met the acceptance criteria for all analytes in the first method trial attempt for each analyte (except for DCC-3825-M-69). The DCC-3825-M-69 mean recoveries at 0.1 ppm and 1 ppm did not meet the protocol acceptance criteria range of 70-120%

These mean recoveries were accepted by the Sponsors. The individual analytes were validated in two different analyte groups (Tier 1 vs. Tier 2) resulting in two full method trial attempts (one for each group). The Tier 1 analytes were defined as tiafenacil, and its metabolites DCC-3825-M-01, DCC-3825-M-12, DCC-3825-M-13, DCC-3825-M-36, and DCC-3825-M-53. The Tier 2 analytes were defined as DCC-3825-M-20, DCC-3825-M-29, DCC-3825-M-30, DCC-3825-M-35, DCC-3825-M-63, DCC-3825-M-69, DCC-3825-M-72, and DCC-3825-M-73.

As described in this report, two additional method bridging trials for both tiers of analytes were conducted using a modified method in order to support the modified method's use for the analysis of soil dissipation study samples. The modified method was successfully validated at 0.01 ppm, 0.1 ppm, and 1 ppm for all analytes using two transition ion pairs for each analyte

This study was designed to validate the analytical method to demonstrate method ruggedness and to meet US EPA Ecological Effects Test Guidelines, OCSPP 850.6100 Test Guidelines requirements for environmental chemistry methods and associated ILV. The study was conducted under EPA's Good Laboratory Practice Standards (GLPs) 40 CFR Part 160. The study protocol, protocol amendments, and deviation can be found in Appendix A for further information about the design of the study.

Independent Laboratory Validation (ILV) Trials

One control sample was used in this study. The soil sample was received from a site in North Dakota with an ongoing tiafenacil soil dissipation study. There was response of less than the Limit of Detection (LOD, 0.01 ppm for the Tier 1 analytes) for tiafeancil and DCC-3825-M-36 in one of the reagent blank samples for the ILV trial. Furthermore, there was responses of less than the LOD (0.002 ppm for the Tier 2 analytes) in one of the reagent blank samples for DCC-3825-M-20 and DCC-3825-M-73. For DCC-3825-M-29, DCC-3825-M-30, DCC-3825-M-35, DCC-3825-M-63, and DCC-3825-M-72, there was a

response in both the reagent blank and at least one of the control matrix sample. It is unknown whether these responses were analyte carry-over from a procedural step or interference from the matrix. The observed responses were always well under the LOD, and no further action was deemed necessary.

A control (untreated) soil sample was analyzed using the provided analytical method. Soil samples were extracted twice with acetonitrile/0.1% formic acid in water (80:20, v/v) and gravity filtered into a round-bottom flask. The combined extracts were evaporated to dryness, reconstituted, and taken through an Oasis HLB solid-phase extraction (SPE) clean-up. The resulting eluent was evaporated to dryness, reconstituted, and submitted for analysis by liquid chromatography with tandem mass spectrometric detection (LC-MS/MS). A single transition ion pair was quantitated for each analyte. For the Tier 1 analytes, all analytes were acquired during the same analytical run. For the Tier 2 analytes, analytes were acquired using three separate acquisition methods.

Method Bridging Trials

The same control sample was used for the ILV trials and for the modified method validation. There were no interferences in the control matrix samples in the chromatograms corresponding to the retention of the targeted analytes. No analyte residues were detected in the reagent blank or control samples. For the modified method, two separate procedures were employed. One was used to extract the Tier 1 and the second was used to extract the Tier 2 analytes.

For the Tier 1 analytes, soil samples were extracted twice with acetonitrile/water/formic acid (80:20:0.02, v/v/v) and centrifuged. The resulting supernatant was combined and brought up to volume. An aliquot of the combined extracts was evaporated to approximately 1 mL, brought up to volume, filtered, and submitted for analysis by LC-MS/MS. Two transition ion pairs were quantitated for each analyte. For the Tier 1 analytes, all analytes were acquired a single analytical run.

For the Tier 2 analytes, soil samples were extracted twice with acetonitrile/water/formic acid (79:20:1, v/v/v) and centrifuged. The resulting supernatant was combined and brought up to volume. An aliquot of the combined extracts was filtered, diluted 5-fold, and submitted for analysis by LC-MS/MS. Two transition ion pairs were quantitated for each analyte. For the Tier 2 analytes, analytes were acquired over two separate analytical runs.

II. MATERIALS

A. Equipment

The equipment that was used is listed below:

- Analytical balance: Mettler Toledo XS204
- Top Loading balances: Mettler Toledo MS3002S/03 and SB16000
- Volumetric flasks, glass: various sizes
- Bottles, amber glass with Teflon lined cap: various sizes
- Volumetric glass pipettes: various sizes
- Graduated cylinders: various volumes
- Micropipette, Drummond Wiretrol® disposable: various volumes
- Disposable pasteur pipettes, glass
- Repeating pipette: Eppendorf Stream
- HPLC vials and caps: 1.8 mL
- Platform shaker: Eberbach variable speed (Heavy Duty)
- Glass vials with Teflon lined screw-top cap: 8 mL
- Processor: Robot Coupe R 10 Cutter Mixer Model R 10 Series E
- Sonicator: Branson 5510 Ultrasonic Cleaner
- Column heater
- LC-MS/MS: AB Sciex API4000 LC-MS/MS with Shimadzu LC-20AD HPLC pumps, SCL-10A VP controller, and SIL-20AC HT autosampler
- LC-MS/MS: AB Sciex API5000 LC-MS/MS with Shimadzu LC-20AD XR HPLC Pumps, Shimadzu CBM-20A controller, and SIL-20AC XR autosampler

1. Equipment Unique to the ILV Trials

   - SPE manifold: Burdick & Jackson (24 position)
   - Polypropylene bottle: 250 mL
   - Filter paper: 802 fluted paper
   - Glass funnel
   - Round-bottom flask: 500 mL and 50 mL
   - SPE cartridges: Oasis HLB cartridges (500 mg, 6cc)
   - Rotary Evaporators: Labconco with a Welch W Series Model 8912A Direct Drive vacuum pump

2. Equipment Unique to the Method Bridging Trials

   - Evaporator System: TurboVap, Caliper Life Sciences TurboVap® LV
   - Centrifuge: Eppendorf Multipurpose Centrifuge 5810
   - Polypropylene tubes: BD Falcon 15 mL and VWR 50 mL
   - Syringe filters: 0.45 µm PTFE

B. Reagents and Standards

The following chemicals were used:

Chemical	Distributer	Part No:
Acetonitrile	Fisher	A996-4
Formic Acid (> 98%)	Fisher	A117-50
Formic Acid (88%)	Fisher	A118P-500
Methanol	VWR	MK304110
Water	Fisher	W5-4

1. Preparation of Reagent Solutions for the ILV Trials

The reagent solution preparation steps for the solutions used for the ILV trials are described below. Some solutions were prepared more than once. If different volumes were prepared, the quantities were scaled appropriately.

Mobile Phase A: 0.1% formic acid in acetonitrile (v/v): Prepared by adding 1 mL of concentrated formic acid (> 98%) to approximately 800 mL of acetonitrile in a 1000-mL volumetric flask. The solution was brought up to volume (1000 mL) with acetonitrile and mixed well.

Mobile Phase B: 0.1% formic acid in water (v/v): Prepared by adding 1 mL of concentrated formic acid (> 98%) to approximately 800 mL of HPLC-grade water in a 1000-mL volumetric flask. The solution was brought up to volume (1000 mL) with HPLC-grade water and mixed well.

acetonitrile/water (50:50, v/v): Prepared by combining 100 mL of acetonitrile and 100 mL of HPLC-grade water and mixing well.

Extraction Solvent: acetonitrile/0.1% formic acid in water (80:20, v/v): Prepared by combining 2400 mL of acetonitrile with 600 mL of 0.1% formic acid in water (v/v) and mixing well.

0.1% formic acid in acetonitrile/0.1 % formic acid in water (60:40, v/v): Prepared by combining 600 mL of 0.1% formic acid in acetonitrile (v/v) with 400 mL of 0.1% formic acid in water (v/v) and mixing well.

0.1% formic acid in acetonitrile/0.1 % formic acid in water (20:80, v/v): Prepared by combining 20 mL of 0.1% formic acid in acetonitrile (v/v) with 80 mL of 0.1% formic acid in water (v/v) and mixing well.

0.1% formic acid in acetonitrile/0.1 % formic acid in water (10:90, v/v): Prepared by combining 10 mL of 0.1% formic acid in acetonitrile (v/v) with 90 mL of 0.1% formic acid in water (v/v) and mixing well.

Needle-Wash, acetonitrile/water (50:50, v/v): Prepared by combining 500 mL of acetonitrile with 500 mL of HPLC-grade water and mixing well.

2. Preparation of Reagent Solutions for the Method Bridging Trials

The reagent solution preparation steps for the solutions used for the method bridging trials are described below. Some solutions were prepared more than once. If different volumes were prepared, the quantities were scaled appropriately.

Mobile Phase A: 0.2% formic acid in acetonitrile (v/v): Prepared by adding 2 mL of concentrated formic acid (88%) to approximately 800 mL of acetonitrile in a 1000-mL volumetric flask. The solution was brought up to volume (1000 mL) with acetonitrile and mixed well.

Mobile Phase B: 0.2% formic acid in water (v/v): Prepared by adding 2 mL of concentrated formic acid (88%) to approximately 800 mL of HPLC-grade water in a 1000-mL volumetric flask. The solution was brought up to volume (1000 mL) with HPLC-grade water and mixed well.

acetonitrile/water (50:50, v/v): Prepared by combining 100 mL of acetonitrile and 100 mL of HPLC-grade water and mixing well.

Tier 1 Analyte Extraction Solvent: acetonitrile/water/formic acid (80:20:0.02, v/v/v): Prepared by combining 1200 mL of acetonitrile with 300 mL of HPLC-grade water and 300 µL of concentrated formic acid (88%) and mixing well.

Tier 2 Analyte Extraction Solvent: acetonitrile/water/formic acid (79:20:1, v/v/v): Prepared by combining 1200 mL of acetonitrile with 300 mL of HPLC-grade water and 15 mL of concentrated formic acid (88%) and mixing well.

acetonitrile/water/formic acid (5:95:0.1, v/v/v): Prepared by combining 10 mL of acetonitrile with 190 mL of HPLC-grade water and 0.2 mL of concentrated formic acid (88%) and mixing well.

acetonitrile/water/formic acid (20:80:0.1, v/v/v):
Prepared by combining 400 mL of acetonitrile with 1600 mL of HPLC-grade water and 2 mL of concentrated formic acid (88%) and mixing well.

Needle-Wash, acetonitrile/water (50:50, v/v): Prepared by combining 500 mL of acetonitrile with 500 mL of HPLC-grade water and mixing well.

3. Reference Substances

The analytical reference substances Tiafenacil (DCC-3825), DCC-3825-M-(01, 12, 13, 36, and 53) were received on July 3, 2015. DCC-3825-M-(20, 29, 35, 69, and 73) were received on December 9, 2015. DCC-3825-M-(30, 63, and 72) were received on December 22, 2015. All reference substances were received in good condition along with their Safety Data Sheets (SDS). For some reference substances, the GLP Certificate of Analysis was received alongside the reference substance. For others, it was received by email at a later date. A copy of the certificate of analysis (COA) for each reference substance is located in Appendix B of this report. The following table contains detailed information on each analytical reference substance used in this study.

Tier 1 Analytes					
Analytical Standard	**Description**	**Batch/ Lot #**	**CAS Number**	**Purity (%)**	**Expiration Date**
Tiafenacil[1]	Light powder w/ solid pieces	RS-3825-02	1220411-29-9	99.87	Aug. 27, 2016
M-01[2]	Light powder w/ solid pieces	K20066-01	NA	96.1	Mar. 26, 2017
M-12[2]	Light powder w/ solid pieces	KM02478-01	NA	97.4	Apr. 13, 2017
M-13[2]	Light powder w/ solid pieces	K20067-01	NA	98.6	Apr. 13, 2017
M-36[2]	Light powder w/ solid pieces	K20268-01	NA	94.1	Mar. 26, 2017
M-53[2]	Light powder w/ solid pieces	K20389-01	NA	94.7	Apr. 13, 2017

[1]Tiafenacil is also referred to as DCC-3825.
[2]Analyte prefix is "DCC-3825-"

Tier 2 Analytes					
Analytical Standard	**Description**	**Batch/ Lot #**	**CAS Number**	**Purity (%)**	**Expiration Date**
M-20[1]	White powder w/ small solid pieces	DB15-0616-01	NA	99.2	Oct. 25, 2018
M-29[1]	White powder w/ small solid pieces	DB15-0702-01	NA	91.3	Oct. 28, 2018
M-30[1]	White powder w/ small solid pieces	DB15-0709-01	NA	95.7	Oct. 28, 2018
M-35[1]	White powder w/ small solid pieces	K20267-01	NA	98.6	Oct. 28, 2018
M-63[1]	White powder w/ small solid pieces	DB15-1014-02	NA	93.1	Dec. 16, 2018
M-69[1]	White powder w/ small solid pieces	DB15-0727-01	NA	96.5	Oct. 27, 2018
M-72[1]	White powder w/ small solid pieces	K20623-02	NA	95.0	Oct. 27, 2018
M-73[1]	White powder w/ small solid pieces	K20687-02	NA	86.7	Oct. 28, 2018

[1]Analyte prefix is "DCC-3825-"

Upon receipt, the Tiafenacil, DCC-3825-M-(01, 12, 13, 36, and 53) reference substances were stored in a freezer set to maintain ≤ -10 °C (frozen). The reference substances DCC-3825-M-(20, 29, 30, 35, 63, 69, 72, and 73) were stored in a refrigerator set to maintain a range of 4 ± 5 °C (refrigerated). The DCC-3825-M-(29, 69, 72, and 73) reference substances were stored under nitrogen as per the COAs. DCC-3825-M-63 was inadvertently stored refrigerated from December 22, 2015 until February 17, 2016. (The COA was received after the material was received). On February 17, 2016, the material was relocated to frozen storage. After discussion with the Sponsors, it was agreed upon that this period of storage at refrigerated conditions had no impact on the integrity or results of the study.

Characterizations and stability of the reference substances is the responsibility of the Sponsors. The Sponsors are responsible for the archival of the reference substance retention sample.

4. Preparation of Standard Solutions

The various reference substances were used in the preparation of the fortification and calibration solutions. Preparation and dilution data forms

pertaining to the stock solutions, spiking solutions, and calibration standards are located in the raw data. All solutions were stored frozen when not in use. Solutions were assigned an expiration period of three months (except stock solutions).

The storage units that stored the reference substances, stock solutions, spiking solutions, and calibration standards were temperature monitored. Temperature records showing weekly temperature ranges are located in the raw data package.

a. Stock Solutions

When necessary, stock solutions were sonicated to completely dissolve the analytical reference substance into solution.

(1) Tier 1 Analytes

Solutions of Tiafenacil, DCC-3825-M-(01, 12, 13, 36, 53, 20, 29, 30, 35, 63, 69, 72, and 73) were prepared individually in acetonitrile for use as stock solutions.

On August 5, 2015, approximately 10 to 15 mg of each Tier 1 analyte reference substance was individually weighed directly into a 100-mL volumetric flask and diluted to the mark with acetonitrile. The resulting stock solutions contained approximately 100 µg/mL of each individual analyte when corrected for purity (using the purity that accounted for water content when applicable). The table below describes the details for each weighing.

Tier 1 Stock Solution Preparation Details				
Analytical Standard	**Net Weight (g)**	**Weight Corrected for Purity (mg)**	**Final Volume (mL)**	**Final Conc. (µg/mL)**
Tiafenacil[1]	0.0104	10.38648	100	104
M-01[2]	0.0108	10.3788	100	104
M-12[2]	0.0113	11.0062	100	110
M-13[2]	0.0127	12.5222	100	125
M-36[2]	0.0136	12.7976	100	128
M-53[2]	0.0108	10.2276	100	102

[1]Tiafenacil is also referred to as DCC-3825.
[2]Analyte prefix is "DCC-3825-"

On December 17, 2015, additional Tier 1 analyte stock solutions were prepared using the same procedure as described above. These solutions were used to conduct the re-analysis of the 1 ppm method bridging samples.

(2) Tier 2 Analytes

On February 17, 2016, approximately 10 to 15 mg of each Tier 2 analyte reference substance was individually weighed directly into a 100-mL volumetric flask and diluted to the mark with acetonitrile. The resulting stock solutions contained approximately 100 µg/mL of each individual analyte when corrected for purity (using the purity that accounted for water content when applicable). The table below describes the details for each weighing.

Tier 2 Stock Solution Preparation Details				
Analytical Standard	**Net Weight (g)**	**Weight Corrected for Purity (mg)**	**Final Volume (mL)**	**Final Conc. (µg/mL)**
M-20[1]	0.0115	11.408	100	114
M-29[1]	0.0117	10.6821	100	107
M-30[1]	0.0135	12.9195	100	129
M-35[1]	0.0103	10.1558	100	102
M-63[1]	0.0120	11.172	100	112
M-69[1]	0.0108	10.422	100	104
M-72[1]	0.0117	11.115	100	111
M-73[1]	0.0121	10.4907	100	105

[1]Analyte prefix is "DCC-3825-"

b. Fortification Solutions

(1) Tier 1 Analytes

An aliquot of each the six individual stock solutions was combined in a 50-mL volumetric flask. A 0.5-mL aliquot of concentrated formic acid was added and the solution was brought up to 50 mL with acetonitrile/water (50:50, v/v) resulting in a mixed solution with a concentration of approximately 10 µg/mL for each analyte. This solution was used to fortify the Tier 1, 1 ppm samples for the ILV trial and the Tier 1, 0.1 ppm and 1 ppm samples for the method bridging trial.

This solution was further diluted 10-fold with acetonitrile/water (50:50, v/v) acidified with 1 mL of formic acid (conc. ≥ 98%) resulting in a mixed solution with a concentration of approximately 1 µg/mL for each analyte. This second solution was used to fortify the Tier 1, 0.1 ppm samples for the ILV trial, and the Tier 1, 0.01 ppm samples for the method bridging trial.

The details of the preparation of these solutions are listed in the table below.

Initial Soln. Description	Initial Soln. Conc. (µg/mL)	Aliquot Volume (mL)	Final Volume (mL)	Final Conc. (µg/mL) (Tiafenacil/ DCC-3825-M-01/12/13/36/53)
Tiafenacil Stock	104	4.8	50	9.98/9.98/ 10.1/10.0/9.98/ 10.0
DCC-3825-M-01 Stock	104	4.8		
DCC-3825-M-12 Stock	110	4.6		
DCC-3825-M-13 Stock	125	4.0		
DCC-3825-M-36 Stock	128	3.9		
DCC-3825-M-53 Stock	102	4.9		
10 µg/mL mixed fortification solution	9.98/9.98/ 10.1/10.0/ 9.98/10.0	10	100	0.998/0.998/ 1.01/1.00/ 0.998/1.00

(2) Tier 2 Analytes

The same processes and solvents were used for the preparation of the Tier 2 fortification solutions as the Tier 1.

The details of the preparation of this solution are listed in the table below.

Initial Soln. Description	Initial Soln. Conc. (µg/mL)	Aliquot Volume (mL)	Final Volume (mL)	Final Conc. (µg/mL) (DCC-3825-M-20/29/30/35/63/ 69/72/73)
DCC-3825-M-20 Stock	114	4.4	50	10.0/10.1/10.1/ 10.0/10.1/10.2/ 10.2/10.1
DCC-3825-M-29 Stock	107	4.7		
DCC-3825-M-30 Stock	129	3.9		
DCC-3825-M-35 Stock	102	4.9		
DCC-3825-M-63 Stock	112	4.5		
DCC-3825-M-69 Stock	104	4.9		
DCC-3825-M-72 Stock	111	4.6		
DCC-3825-M-73 Stock	105	4.8		
10 µg/mL mixed fortification solution	10.0/10.1/ 10.1/10.0/ 10.1/10.2/ 10.2/10.1	10	100	1.00/1.01/1.01/ 1.00/1.01/1.02/ 1.02/1.01

c. Intermediate and Calibration Solutions

(1) Tier 1 Analytes, ILV Trial

Subsequent dilutions of the spiking solutions were prepared in 0.1% formic acid in acetonitrile/0.1% formic acid in water

(60:40, v/v) to create the calibration curve standard solutions. The details are described in the table below.

Initial Soln. Description	Initial Soln. Conc. (µg/mL) (Tiafenacil/ DCC-3825-M-01/12/13/36/53)	Aliquot Volume (mL)	Final Volume (mL)	Final Conc. (µg/mL) (Tiafenacil/ DCC-3825-M-01/12/13/36/53)
10 µg/mL mixed fortification solution	9.98/9.98/ 10.1/10.0/ 9.98/10.0	5	50	0.998/0.998/ 1.01/1.00/ 0.998/1.00
10 µg/mL mixed fortification solution	9.98/9.98/ 10.1/10.0/ 9.98/10.0	5	100	0.499/0.499/0.505/ 0.500/0.499/0.500
0.5 µg/mL mixed calibration standard solution	0.499/0.499/0.505/ 0.500/0.499/0.500	10	20	0.250/0.250/ 0.253/0.250/ 0.250/0.250
10 µg/mL mixed fortification solution	9.98/9.98/ 10.1/10.0/ 9.98/10.0	1	100	0.0998/0.0998/ 0.101/0.100/ 0.0998/0.100
10 µg/mL mixed fortification solution	9.98/9.98/ 10.1/10.0/ 9.98/10.0	0.5	100	0.0499/0.0499/ 0.0505/0.0500/ 0.0499/0.0500

(2) Tier 2 Analytes, ILV Trial

Subsequent dilutions of the spiking solutions were prepared in 0.1% formic acid in acetonitrile/0.1% formic acid in water (60:40, v/v) to create the calibration curve standard solutions. The details are described in the table below.

Initial Soln. Description	Initial Soln. Conc. (µg/mL) (DCC-3825-M-20/29/30/35/63/69 /72/73)	Aliquot Volume (mL)	Final Volume (mL)	Final Conc. (ng/mL) (DCC-3825-M-20/29/30/35/63/ 69/72/73)
10 µg/mL mixed fortification solution	10.0/10.1/10.1/ 10.0/10.1/10.2/ 10.2/10.1	1	100	100/101/101/ 100/101/102/ 102/101
10 µg/mL mixed fortification solution	10.0/10.1/10.1/ 10.0/10.1/10.2/ 10.2/10.1	0.5	100	50.0/50.5/50.5/ 50.0/50.5/51.0/ 51.0/50.5
10 µg/mL mixed fortification solution	10.0/10.1/10.1/ 10.0/10.1/10.2/ 10.2/10.1	0.1	100	10.0/10.1/10.1/ 10.0/10.1/10.2/ 10.2/10.1
50 ng/mL mixed calibration standard solution	50.0/50.5/50.5/ 50.0/50.5/51.0/ 51.0/50.5	1	10	5.00/5.05/5.05/ 5.00/5.05/5.10/ 5.10/5.05
10 ng/mL mixed calibration standard solution	10.0/10.1/10.1/ 10.0/10.1/10.2/ 10.2/10.1	1	10	1.00/1.01/1.01/ 1.00/1.01/1.02/ 1.02/1.01

(3) Tier 1 Analytes, Method Bridging Trial

Subsequent dilutions of the 0.1 µg/mL ILV calibration standard were prepared in acetonitrile/water/formic acid (20:80:0.1, v/v/v) to create the calibration curve standard solutions. The details are described in the table below.

Initial Soln. Description	Initial Soln. Conc. (ng/mL) (Tiafenacil/ DCC-3825-M-01/12/13/36/53)	Aliquot Volume (mL)	Final Volume (mL)	Final Conc. (ng/mL) (Tiafenacil/ DCC-3825-M-01/12/13/36/53)
0.1 µg/mL mixed ILV calibration standard solution	99.8/99.8/101/100/ 99.8/100	10	100	9.98/9.98/10.1/ 10.0/9.98/10.0
0.1 µg/mL mixed ILV calibration standard solution	99.8/99.8/101/100/ 99.8/100	5	100	4.99/4.99/5.05/ 5.00/4.99/5.00
0.1 µg/mL mixed ILV calibration standard solution	99.8/99.8/101/100/ 99.8/100	1	50	2.00/2.00/2.02/ 2.00/2.00/2.00
0.1 µg/mL mixed ILV calibration standard solution	99.8/99.8/101/100/ 99.8/100	0.5	50	0.998/0.998/ 1.01/1.00/0.998/ 1.00
0.1 µg/mL mixed ILV calibration standard solution	99.8/99.8/101/100/ 99.8/100	0.5	100	0.499/0.499/ 0.505/0.500/ 0.499/0.500
0.1 µg/mL mixed ILV calibration standard solution	99.8/99.8/101/100/ 99.8/100	0.5	200	0.250/0.250/ 0.253/0.250/ 0.250/0.250

Additional calibration standards were prepared to conduct the re-analysis of the 1 ppm Tier 1 method bridging samples. Those standards were prepared similarly. The preparation details for those standards are located in the raw data.

(4) Tier 2 Analytes, Method Bridging Trial

Subsequent dilutions of the spiking solutions were prepared in 0.1% formic acid in acetonitrile/0.1% formic acid in water (60:40, v/v) to create an intermediate solution. The calibration curve standard solutions were prepared from the intermediate. The details are described in the table below.

Initial Soln. Description	Initial Soln. Conc. (µg/mL) (DCC-3825-M-20/29/30/35/63/69/72/73)	Aliquot Volume (mL)	Final Volume (mL)	Final Conc. (ng/mL) (DCC-3825-M-20/29/30/35/63/69/72/73)
1 µg/mL mixed fortification solution	1.00/1.01/1.01/1.00/1.01/1.02/1.02/1.01	5	50	100/101/101/100/101/102/102/101
100 ng/mL mixed intermediate solution	100/101/101/100/101/102/102/101	5	100	5.00/5.05/5.05/5.00/5.05/5.10/5.10/5.05
100 ng/mL mixed intermediate solution	100/101/101/100/101/102/102/101	2	100	2.00/2.02/2.02/2.00/2.02/2.04/2.04/2.02
100 ng/mL mixed intermediate solution	100/101/101/100/101/102/102/101	1	100	1.00/1.01/1.01/1.00/1.01/1.02/1.02/1.01
100 ng/mL mixed intermediate solution	100/101/101/100/101/102/102/101	0.5	100	0.500/0.505/0.505/0.500/0.505/0.510/0.510/0.505
100 ng/mL mixed intermediate solution	100/101/101/100/101/102/102/101	0.2	100	0.200/0.202/0.202/0.200/0.202/0.204/0.204/0.202
100 ng/mL mixed intermediate solution	100/101/101/100/101/102/102/101	0.1	100	0.100/0.101/0.101/0.100/0.101/0.102/0.102/0.101

C. Safety and Health

Material Safety Data Sheets (MSDS) and/or Safety Data Sheets (SDS) were available. Proper personal protective equipment was worn during the execution of this method. Staff avoided breathing chemical vapor and avoided chemical contact with eyes and skin. Caution was used when handling concentrated formic acid. There were no other procedural steps that required special precautions to avoid safety or health hazards.

III. METHODS

A. Principle of Analytical Method

The objective of this study was to validate the analytical method titled "Residue Analytical Method of Tiafenacil and Its Metabolites in Soil provided by Dongbu Farm Hannong Co., Ltd. (now FarmHannong Co., Ltd.) which is located in Appendix C.

The limit of quantitation (LOQ) for all analytes for the ILV was 0.1 ppm (µg/g). The limit of detection (LOD) was 0.01 ppm for the Tier 1 analytes and 0.002 ppm for the Tier 2 analytes. The individual analytes were validated in two different

analyte groups (Tier 1 vs. Tier 2) resulting in two full method trial attempts (one for each group). The Tier 1 analytes were defined as tiafenacil, and its metabolites DCC-3825-M-01, DCC-3825-M-12, DCC-3825-M-13, DCC-3825-M-36, and DCC-3825-M-53. The Tier 2 analytes were defined as DCC-3825-M-20, DCC-3825-M-29, DCC-3825-M-30, DCC-3825-M-35, DCC-3825-M-63, DCC-3825-M-69, DCC-3825-M-72, and DCC-3825-M-73.

All of the ILV samples were extracted in four analytical sets (2 for each analyte Tier). Each set consisted of one reagent blank sample, two control samples, and five LOQ laboratory fortification samples, or five 10x LOQ laboratory fortification samples. Prior to extraction, a unique laboratory code designation was assigned by GPL to each sample. The laboratory code consisted of the last three digits of the GPL study number; the sample set designation and a sample number (e.g., 608ILV01-5).

Two additional method bridging trials for both tiers of analytes were conducted using a modified method in order to support its use for the analysis of soil dissipation study samples. The LOQ and LOD for all analytes for the method bridging trials were 0.01 ppm and 0.005 pm, respectively. For the modified method, two separate procedures were employed. One was used for the extraction of the Tier 1 analytes and the second was used for the extraction of the Tier 2 analytes.

All of the method bridging samples were extracted in two analytical sets (1 for each analyte Tier). Each set consisted of one reagent blank sample, two control samples, five LOQ laboratory fortification samples, five 10x LOQ laboratory fortification samples, and 100x LOQ laboratory fortification samples. Prior to extraction, a unique laboratory code designation was assigned by GPL to each sample. The laboratory code consisted of the last three digits of the GPL study number; the sample set designation and a sample number (e.g., 608MV01-5).

Detailed method flow charts for the ILV trials and the method bridging trials can be found in Appendix D.

1. ILV Trials

   Soil samples were extracted twice with acetonitrile/0.1% formic acid in water (80:20, v/v) and gravity filtered into a round-bottom flask. The combined extracts were evaporated to dryness, reconstituted, and taken through an Oasis HLB solid-phase extraction (SPE) cleanup. The resulting eluent was evaporated to dryness, reconstituted, and submitted for analysis by LC-MS/MS. A single transition ion pair was quantitated for each analyte. For the Tier 1 analytes, all analytes were acquired during the same analytical run. For the Tier 2 analytes, analytes were acquired using three separate acquisition methods.

2. Tier 1 Analytes, Method Bridging Trial

Soil samples were extracted twice with acetonitrile/water/formic acid (80:20:0.02, v/v/v) and centrifuged. The resulting supernatant was combined and brought up to volume. An aliquot of the combined extracts was evaporated to approximately 1 mL, brought up to volume, filtered, and submitted for analysis by LC-MS/MS. Two transition ion pairs were quantitated for each analyte. For the Tier 1 analytes, all analytes were acquired a single analytical run.

3. Tier 2 Analytes, Method Bridging Trial

Soil samples were extracted twice with acetonitrile/water/formic acid (79:20:1, v/v/v) and centrifuged. The resulting supernatant was combined and brought up to volume. An aliquot of the combined extracts was filtered, diluted 5-fold, and submitted for analysis by LC-MS/MS. Two transition ion pairs were quantitated for each analyte. For the Tier 2 analytes, analytes were acquired over two separate analytical runs.

B. Analytical Procedure

1. Control Matrix

The soil control matrix sample was obtained from the Northwood, ND field site of the ND tiafenacil soil dissipation study conducted at GPL. The sample was provided for use on this study. GLP soil characterization of the soil was provided in an email from the Study Director and is listed below:

AGVISE Sample ID	Depth	Texture	Sand (%)	Silt (%)	Clay (%)	OC (%)	pH	CEC	WHC 1/3 Bar (%)	Bulk Density (g/cc)
Northwood, ND (Trial ID PSM-15-06-03, GPL Study # 150614)										
15-1291	0-6"	Sandy Loam	63	18	19	1.7	6.4	15.6	22.4	1.10
15-1292	6-12"	Sandy Loam	63	20	17	1.3	6.9	14.9	23.1	1.09
15-1293	12-18"	Sandy Loam	65	18	17	0.42	7.6	13.7	21.5	1.08
15-1294	18-24"	Sandy Clay Loam	63	16	21	0.42	8.1	14.2	21.7	0.99
15-1295	24-30"	Sandy Loam	65	16	19	0.22	8.4	14.5	19.6	1.07
15-1296	30-36"	Sandy Loam	63	22	15	0.22	8.4	14.0	20.0	1.11

The sample was stored frozen when not in use.

2. Preparation of Samples

a. ILV Trials

Sub-samples (10.0 ± 0.02 g) of the control soil matrix were measured into 250-mL polypropylene bottles.

b. Method Bridging Trials

Sub-samples (5.00 ± 0.03 g) of the control soil matrix were measured into 50-mL plastic centrifuge tubes.

3. Fortifications

a. Tier 1, ILV Trial

Samples were fortified at the LOQ (0.1 ppm) or 10x the LOQ (1 ppm). Fortifications were performed using serological pipettes to directly fortify the 10-g samples as follows:

Fortification Level	Amount and Concentration of Spiking Solution Used (µg/mL) (Tiafenacil/DCC-3825-M-01/12/13/36/53)
LOQ (0.1 ppm)	1 mL of a 0.998/0.998/1.01/1.00/0.998/1.00
10x LOQ (1 ppm)	1 mL of a 9.98/9.98/10.1/10.0/9.98/10.0

b. Tier 2, ILV Trial

Samples were fortified at the LOQ (0.1 ppm) or 10x the LOQ (1 ppm). Fortifications were performed using serological pipettes to directly fortify the 10-g samples as follows:

Fortification Level	Amount and Concentration of Spiking Solution Used (µg/mL) (DCC-3825-M-20/29/30/35/63/69/72/73)
LOQ (0.1 ppm)	1 mL of a 1.00/1.01/1.01/1.00/1.01/1.02/1.02/1.01
10x LOQ (1 ppm)	1 mL of a 10.0/10.1/10.1/10.0/10.1/10.2/10.2/10.1

c. Tier 1, Method Bridging Trial

Samples were fortified at the LOQ (0.01 ppm), 10x the LOQ (0.1 ppm), or 100x the LOQ (1 ppm). Fortifications were performed using Wiretrol® micropipettes to directly fortify the 5-g samples as follows:

Fortification Level	Amount and Concentration of Spiking Solution Used (µg/mL) (Tiafenacil/DCC-3825-M-01/12/13/36/53)
LOQ (0.01 ppm)	50 µL of a 0.998/0.998/1.01/1.00/0.998/1.00
10x LOQ (0.1 ppm)	50 µL of a 9.98/9.98/10.1/10.0/9.98/10.0
100x LOQ (1 ppm)	500 µL of a 9.98/9.98/10.1/10.0/9.98/10.0

d. Tier 2, Method Bridging Trial

Samples were fortified at the LOQ (0.01 ppm), 10x the LOQ (0.1 ppm), or 100x the LOQ (1 ppm). Fortifications were performed using Wiretrol® micropipettes to directly fortify the 5-g samples as follows:

Fortification Level	Amount and Concentration of Spiking Solution Used (µg/mL) (DCC-3825-M-20/29/30/35/63/69/72/73)
LOQ (0.01 ppm)	50 µL of a 1.00/1.01/1.01/1.00/1.01/1.02/1.02/1.01
10x LOQ (0.1 ppm)	50 µL of a 10.0/10.1/10.1/10.0/10.1/10.2/10.2/10.1
100x LOQ (1 ppm)	500 µL of a 10.0/10.1/10.1/10.0/10.1/10.2/10.2/10.1

4. Extraction

a. ILV Trials

After fortification, 50 mL of extraction solvent (acetonitrile/0.1% formic acid in water (80:20, v/v) was added to each sample. Each sample bottle was capped and shaken on a platform shaker for 30 minutes at approximately 200 rpm. After shaking, the supernatant was decanted through a reeve angel 802 fluted filter paper (in a glass funnel) into a 500-mL round-bottom flask. The filter paper was rinsed with 25 mL of extraction solvent.

An additional 50 mL of extraction solvent was added to each sample. Each sample bottle was capped and shaken on a platform shaker for 30 minutes at approximately 200 rpm. The supernatant was decanted through the same filter apparatus combining the extracts into the same 500-mL round-bottom flask. The filter paper was rinsed with 25 mL of extraction solvent.

The combined extract was evaporated to dryness using a rotary evaporator set at 40 °C. The residues were reconstituted in 5 mL of

0.1% formic acid in acetonitrile/0.1% formic acid in water (10:90, v/v).

b. Tier 1 Analytes, Method Bridging Trial

Samples were extracted with 25 mL of extraction solvent (acetonitrile/water/formic acid (80:20:0.02, v/v/v)) and shaken on a platform shaker for 30 minutes at approximately 200 rpm**.** After shaking, the samples were spun in a centrifuge set for three minutes and 3000 rpm. The supernatants were transferred into clean 50-mL centrifuge tubes. The remaining solids in the samples were re-extracted using 25 mL of extraction solvent following the same steps as the first extraction. The supernatants were transferred into the same 50-mL centrifuge tubes combining the extracts. The extracts were brought to a final volume of 50 mL with extraction solvent.

Aliquots (5 mL) of the sample extracts were transferred into 15-mL plastic centrifuge tubes, and the extracts were concentrated down to approximately 1 mL under nitrogen using a TurboVap with the water bath set to 40 °C. The samples were reconstituted to 10 mL using acetonitrile/water/formic acid (20:80:0.1, v/v/v) and syringe filtered through a 0.45 µm polytetrafluoroethylene (PTFE) filter prior to analysis by LC-MS/MS. Sample extracts requiring dilution were diluted using acetonitrile/water/formic acid (20:80:0.1, v/v/v).

c. Tier 2 Analytes, Method Bridging Trial

Samples were extracted with 25 mL of extaction solvent (acetonitrile/water/formic acid (79:20:1, v/v/v)) and shaken on a platform shaker for 30 minutes at approximately 200 rpm**.** After shaking, the samples were spun in a centrifuge set for three minutes and 3000 rpm. The supernatants were transferred into clean 50-mL centrifuge tubes. The remaining solids in the samples were re-extracted using 25 mL of extraction solvent following the same steps as the first extraction. The supernatants were transferred into the same 50-mL centrifuge tubes combining the extracts. The extracts were brought to a final volume of 50 mL with extraction solvent.

Samples were syringe filtered through a 0.45-µm PTFE filter, and aliquots of the filtered extracts were diluted fivefold using acetonitrile/water/formic (5:95:0.1, v/v/v). Samples were then submitted for analysis by LC-MS/MS. Sample extracts requiring dilution were diluted using acetonitrile/water/formic acid (5:95:0.1, v/v/v).

5. Clean-Up (ILV Trials)

SPE (Oasis HLB, 500 mg, 6cc) cartridges were conditioned with 5 mL of 0.1% formic acid in acetonitrile followed by equilibration using 5 mL of 0.1% formic acid in water. The eluate was discarded. The reconstituted extract (from 4a) was loaded onto the SPE cartridge. The eluate was discarded. Once the sample was loaded onto the cartridge, the cartridge was washed with 5 mL 0.1% formic acid in acetonitrile/0.1% formic acid in water (20:80, v/v). The residues were eluted into a 15-mL graduated plastic tube using a 10-mL aliquot of 0.1% formic acid in acetonitrile/0.1% formic acid in water (60:40, v/v).

The cleaned-up extract was transferred to a 50-mL round-bottom flask and was evaporated to dryness using a rotary evaporator set at 40 °C. The residues were reconstituted in 2 mL of 0.1% formic acid in acetonitrile/0.1% formic acid in water (60:40, v/v). Sample extracts requiring dilution were diluted using 0.1% formic acid in acetonitrile/0.1% formic acid in water (60:40, v/v). The extracts were vialed for analysis by LC-MS/MS.

C. Instrumentation

For the Tier 1 and 2 analyte LC-MS/MS analysis (for both the ILV trial and method bridging trial), a Sciex API4000 MS/MS and Sciex API5000 was used, respectively. Both MS/MS systems were coupled with Shimadzu HPLC pumps, controller, and autosampler. The LC-MS/MS conditions were further optimized after the ILV trials before the method bridging trials. As a result, different LC-MS/MS conditions were used for each trial, for each set of analytes. Due to the complexity of the LC-MS/MS conditions, they are summarized on the raw data forms in Appendix E.

D. Potential Interferences

1. Matrix Interference

No matrix interferences were observed for the 14 analytes in soil. However, a matrix effect is described in the Modification or Potential Problems section of this report.

2. Reagent and Solvent Interference

High purity solvents and reagents were used for this assay. No interferences were observed.

3. Labware Interference

This method uses disposable labware and washable glassware. No interferences from the labware or glassware use were observed. However, there may be the potential for the carry-over of residues if the rotary evaporator stations are not rinsed between each use. This may have been the source of the very low residues found in reagent blank and control samples.

E. Confirmatory Techniques

The independent laboratory validation sets were run by LC-MS/MS with quantitation of a single transition pair for each of the 14 analytes. Only a single product ion was listed for each analyte. No confirmatory ion pair was quantitated for the ILV trials.

The method bridging validation sets were run by LC-MS/MS with monitoring of two ion transition pairs for all 14 analytes. As this method is highly selective, no additional confirmatory technique was used.

F. Time Required for Analysis

1. ILV Trials

Approximately 6 to 8 hours were required for one person to prepare an analysis set (8 samples) from the time samples were prepared to LC-MS/MS analysis. Automated LC-MS/MS analysis was performed overnight. Since the LC-MS/MS parameters were compatible for different analyte acquisition methods, for the Tier 2 analytes, the 3 analytical runs were set up to run one after another. An additional 0.5 hours per analyte was spent on data calculation and tabulation the following day. An analytical set of 8 samples can be prepared, analyzed and tabulated over two calendar days (provided the set contains only 6-8 analytes, if all 14 analytes are being tabulated, it may take more than two calendar days).

2. Tier 1 Analytes, Method Bridging Trial

Approximately 6 to 8 hours were required for one person to prepare an analysis set from the time samples were prepared to LC-MS/MS analysis. Automated LC-MS/MS analysis was performed overnight. An additional 0.5 hours per analyte (per transition ion pair) was spent on data calculation and tabulation the following day. An analytical set of 13 samples can be prepared, analyzed and tabulated over two calendar days.

3. Tier 2 Analytes, Method Bridging Trial

Approximately 4 to 6 hours were required for one person to prepare an analysis set from the time samples were prepared to LC-MS/MS analysis. Automated LC-MS/MS analysis was performed overnight. An additional 0.5 hours per analyte (per transition ion pair) was spent on data calculation and tabulation the following day. An analytical set of 13 samples can be prepared, analyzed and tabulated over two calendar days.

G. Modification or Potential Problems

Over the course of this study, four different reference method versions were received from the Sponsors. All four of these method versions are included in the raw data alongside documentation of when they were received. The following discussions regarding the differences between the method and the procedures used to generate the data for this study are referring to differences between the procedures used and the final reference method issued March 31, 2016, which is the version appended to this report.

1. Fortification Procedure

According to the reference method: "All fortifications were made directly to the 100 g of soil and operated 3 replications." This was interpreted as stating that 100 g of control soil was fortified and that the fortified sample was further sub-sampled to produce each of the replicate samples. For the ILV and method bridging trials, each soil sample replicate was fortified individually (i.e., first the sub-sample was weighed out from the control bulk sample and then the sub-sample was fortified). This change did not affect the results of this study.

2. Different Rinsing Solvent Used to Rinse Filter Paper

After each extraction step, the method specifies that the filter paper be rinsed with 25 mL of acetonitrile. For the ILV trials, the chemist inadvertently used 25 mL of acetonitrile/0.1% formic acid in water (80:20, v/v) to rinse the filter paper for all sets for all analytes. This change did not affect the results of this study. The acceptance criteria were met for all analytes except DCC-3825-M-69. The cause for the recovery problems for DCC-3825-M-69 was unrelated.

3. Method Documentation Details Omitted

a. Precursor Ions Not Listed

For the mass spectrometry parameters, although the product ion was listed for each analyte, the precursor ion was not listed. As a result,

for the Tier 1 ILV trial, instead of using the protonated exact mass for the monitored ion (M + 1), the M + 2 mass was used inadvertently. This was because the molecular weight was used to calculate M + 1 instead of the exact mass. Although this error can be avoided by calculating the exact mass to determine the appropriate precursor ion, it is industry standard to include both the precursor and product ion masses in an analytical method. It is suggested that this information be added to the method. Furthermore, due to the way the instrumentation and masses are listed in the method, it is confusing as to whether the method was completed using MSD or MS/MS. As a result, this should also be clarified.

b. Representative Chromatograms Not Present

The reference method did not contain representative chromatograms. It is industry standard to include representative chromatograms to determine during method transfer whether the qualitative performance of the method is comparable to the original laboratory's validation data. It is suggested that chromatograms be added to the method.

4. Different Instrumentation Used

The Ultra Performance Liquid Chromatography-Mass spectrometer (UPLC/MSD) system used in the method is different that the instrumentation used for this study. The method states that the UPLC was an HPLC H-Class and that the mass spectrometer was a Xevo TQ with MassLynx as the data system. Both pieces of equipment and data system are manufactured by Waters, USA. For this study, Shimazdu HPLC systems were used along with Sciex 4000 (for Tier 1 analytes) and Sciex 5000 (for Tier 2 analytes) mass spectrometers and Analyst data systems. Since different instrumentation was used, the instrument settings were optimized for each compound. Injection volumes were altered where necessary.

For DCC-3825-M-20, due to the instrumentation difference, there was insufficient sensitivity to use the DCC-3825-M-20 product ion listed in the method (197); instead, a different product ion was used (274), which resulted in acceptable sensitivity. This is the only way the change in instrumentation affected the results of this study. This did not affect the results of this study as the method was successfully validated.

5. Matrix Matching

The method specifies the use of matrix-matched standards. At the request of the Sponsor Representative, matrix-matched standards were not employed. This did not affect the results of this study as the method was successfully validated.

6. Cross-Talk (Mass Overlap)

Due to the number of structurally similar compounds included in this analytical method, multiple occurrences of cross-talk, (which was specifically a result of mass overlap), were observed in the mass chromatograms. Mass overlap can occur when compounds have similar molecular weights and are chlorinated. Due to the wide natural abundance of Cl^{35} (~76%) and Cl^{37} (~24%) isotopes, chlorinated compounds have the potential to have multiple Q1 and Q3 transition ion pairs. These multiple pairs can overlap the pairs of other unique analytes. This is even more of a problem and can affect the specificity of a method when compounds that generate mass overlaps are not chromatographically resolved from others. Since not all 14 compounds were validated simultaneously, it is possible that cross-talk that was not observed during this study can occur between Tier 1 and Tier 2 analytes. Furthermore, it is significant to note that the parent compound (tiafenacil) is chlorinated. As a result, compounds with structural similarities resulting in a difference of 2 amu in the molecular weight will show mass spectrometry mass overlap, which can cause signal identification issues for both the Q1 and Q3 masses when the compounds are not chromatographically resolved.

For example, the chemical structures for DCC-3825-M-36 and DCC-3825-M-53 only differ by a single double bond making the molecular weights different by only 2 amu. As a result, their signals cross-talk and they do not fully chromatographically resolve. For the method bridging trials, the Cl^{37} mass isotope was used for DCC-3825-M-53 for Q1 mass to reduce the cross-talk. In addition, the DCC-3825-M-53 peak was integrated on an angle to mitigate the DCC-3825-M-36 contribution.

DCC-3825-M-72 and DCC-3825-M-73 was observed to show some cross-talk. In addition, due to cross-talk into the method recommended DCC-3825-M-63 product ion suggested in the method (351) a different product ion (112) was used for quantitation.

As a result, of this potential problem, it is imperative to evaluate any possible cross-talk during method transfer and validation.

7. Matrix Effects on Recovery

After the Tier 2 analyte ILV trial was completed, non-GLP experiments were conducted to determine whether the modified Tier 1 analyte extraction procedure would also produce acceptable results for the Tier 2 analytes. In addition, alongside the experiment to see if the modified method would perform acceptably, experiments were undertaken to improve the DCC-3825-M-69 recoveries.

Six fortified soil aliquots were extracted. The steps from the Tier 1 modified method were used up until the production of the 50 mL combined extract (refer to the Tier 1 method bridging flow chart). However, different solvents were used to extract the different sample aliquots. Two sample aliquots were extracted with the original solvents used in the Tier 1 modified method for both extractions (i.e., acetonitrile/water/formic acid (80:20:0.02, v/v/v). Two sample aliquots were extracted first with acetonitrile/water/formic acid (80:20:0.02, v/v/v) and the second extraction was performed with acetonitrile/water/formic acid (79:20:1, v/v/v). Two additional sample aliquots were extracted with acetonitrile/water/formic acid (79:20:1, v/v/v) for both extractions. After the extractions, all aliquots were brought up to 50 mL with acetonitrile/water/formic acid (80:20:0.02, v/v/v).

At that point, all six extracts were either filtered and diluted 10-fold or taken through the rest of the Tier 1 analyte modified method (i.e., TurboVap, bring up to volume, filter).

Extracts that were filtered and diluted recovered better than those that were taken through the Tier 1 analyte modified method.

Recoveries increased with the amount of acid used in the extraction solvent. The DCC-3825-M-69 recoveries improved approximately 20%. Increasing the acidity of the extraction solvent likely increased the extraction efficiency by decreasing the pH of the extraction solvent.

From these results, it was decided that for the Tier 2 analyte method bridging trial, the soil would be extracted with acetonitrile/water/formic acid (79:20:1, v/v/v) for both extractions and that the extracts would be filtered and diluted rather than concentrated (as per the Tier 1 modified method).

These experiments indicate that the soil type may have an effect on analyte recoveries if the soil type affects the pH of the extract.

H. Methods of Calculation

Analyst Chromatography Data System version 1.5 and 1.6, a product of AB Sciex, was used to acquire, integrate and calculate the concentrations of each analyte as µg/mL (for the Tier 1 analyte ILV trial) or ng/mL using the linear regression function with 1/x weighting. The calibration curve was not forced through the origin. For the regression calculations, concentration was designated as the independent variable and plotted on the x-axis. Peak area response was designated as the dependent variable and plotted on the y-axis. From this regression curve, a slope, a correlation coefficient and other parameters of the standard curve were calculated. Calibration standards were injected every three to five sample injections as well as at the beginning and end of the injection sequence. Five different standard concentrations were injected within the Tier 1 and Tier 2 ILV analytical sets and the Tier 1 method bridging analytical set. For the Tier 2 method bridging analytical sets, six different standard concentrations were injected.

Recovery of each of the analytes from fortified samples was calculated as follows:

$$\% \text{ Recovery} = \frac{\text{Measured Concentration (ppm)}}{\text{Theoretical Concentration (ppm)}} \text{ X } 100$$

1. Tier 1 Analytes, ILV Trial

The concentrations (µg/mL) of the Tier 1 analytes detected in ILV sample extracts were interpolated from the standard calibration curve. The concentration as ppm of residue found in samples was then calculated with Microsoft® Excel using the following equation:

$$ppm = \frac{(\mu g/mL \text{ from curve}) \times (\text{Final Volume (mL)})}{\text{Sample Amount (g)}}$$

An example calculation for soil, for a tiafenacil ILV laboratory fortification sample in set 608ILV01, sample 608ILV01-3 LOQ sample fortified at 0.0998 ppm, is as follows:

standard curve equation: $y = 3.01 \times 10^6 (x) + 4.51 \times 10^4$

where x = tiafenacil concentration in µg/mL and

y = peak response = 1292565.0

tiafenacil concentration from the curve = 0.414 µg/mL

$$ppm = \frac{(0.414 \ \mu g/mL) \times (2 \ mL)}{(10.00 \ g)} = 0.0828 \ ppm$$

$$\% \text{ Recovery} = \frac{0.0828 \ ppm}{0.0998 \ ppm} \text{ X } 100 = 83.0\%$$

Residues <LOD for several analytes were measured in a few of the reagent blank and control samples. Laboratory fortification samples were not corrected for control responses.

2. Tier 2 Analytes, ILV Trial

The concentrations (ng/mL) of the Tier 2 analytes detected in ILV sample extracts were interpolated from the standard calibration curve. The concentration as ppm of residue found in samples was then calculated with Microsoft® Excel using the following equation:

$$ppm = \frac{(ng/mL \text{ from curve}) \times (\text{Final Volume (mL)}) \times 1\ \mu g}{\text{Sample Amount (g)} \times 1000\ ng}$$

An example calculation for soil, for a DCC-3825-M-20 ILV laboratory fortification sample in set 608ILV03, sample 608ILV03-4 LOQ sample fortified at 0.100 ppm, is as follows:

standard curve equation: $y = 2.14 \times 10^4 (x) + 1.89 \times 10^3$

where x = DCC-3825-M-20 concentration in ng/mL and

y = peak response = 847502.8

DCC-3825-M-20 concentration from the curve = 39.5 ng/mL

$$ppm = \frac{(39.5\ ng/mL) \times (20\ mL) \times 1\ \mu g}{(10.00\ g) \times 1000\ ng} = 0.0790\ ppm$$

$$\%\ Recovery = \frac{0.0790\ ppm}{0.100\ ppm} \times 100 = 79.0\%$$

Residues <LOD for several analytes were measured in a few of the reagent blank and control samples. Laboratory fortification samples were not corrected for control responses.

3. Tier 1 Analytes, Method Bridging Trial

The concentrations (ng/mL) of the Tier 1 analytes detected in method bridging sample extracts were interpolated from the standard calibration curve. The concentration as ppm of residue found in samples was then calculated with Microsoft® Excel using the following equations:

$$ppm = \frac{(ng/mL \text{ from curve}) \times \text{Aliquot Factor} \times (\text{Final Volume (mL)}) \times 1\ \mu g}{\text{Sample Amount (g)} \times 1000\ ng}$$

Aliquot Factor = Extraction Volume (mL) ÷ Aliquot Volume (mL)

Aliquot Factor = 50 mL ÷ 5 mL = 10

An example calculation for soil, for a DCC-3825-M-01 (498.2/381.0) method bridging laboratory fortification sample in set 608MV01, sample 608MV01-5 LOQ sample fortified at 0.00994 ppm, is as follows:

standard curve equation: $y = 1.99 \times 10^5 (x) + (-5.46 \times 10^3)$

where x = DCC-3825-M-01 concentration in ng/mL and

y = peak response = 79231.4

DCC-3825-M-01 concentration from the curve = 0.426 ng/mL

$$ppm = \frac{(0.426\ ng/mL) \times (10) \times (10\ mL) \times 1\ \mu g}{(5.02\ g) \times 1000\ ng} = 0.00849\ ppm$$

$$\%\ Recovery = \frac{0.00849\ ppm}{0.00994\ ppm} X\ 100 = 85.4\%$$

No residues were detected in any reagent blank or control samples. Laboratory fortification samples were not corrected for control responses.

4. Tier 2 Analytes, Method Bridging Trial

The concentrations (ng/mL) of the Tier 2 analytes detected in method bridging sample extracts were interpolated from the standard calibration curve. The concentration as ppm of residue found in samples was then calculated with Microsoft® Excel using the following equation:

$$ppm = \frac{(ng/mL\ from\ curve) \times (Final\ Volume\ (mL)) \times 1\ \mu g}{Sample\ Amount\ (g) \times 1000\ ng}$$

An example calculation for soil, for a DCC-3825-M-29 method bridging laboratory fortification sample in set 608MV02, sample 608MV02-17 100x LOQ sample fortified at 1.01 ppm, is as follows:

standard curve equation: $y = 2.09 \times 10^5 (x) + (-546)$

where x = DCC-3825-M-29 concentration in ng/mL and

y = peak response = 211866.1

DCC-3825-M-29 concentration from the curve = 1.02 ng/mL

$$ppm = \frac{(1.02\ ng/mL) \times (5000\ mL) \times 1\ \mu g}{(5.00\ g) \times 1000\ ng} = 1.02\ ppm$$

$$\%\ Recovery = \frac{1.02\ ppm}{1.01\ ppm} X\ 100 = 101\%$$

No residues were detected in any reagent blank or control samples. Laboratory fortification samples were not corrected for control responses.

VII. AMENDMENTS/DEVIATIONS

Amendments and deviations for this study are described below. There was no negative effect on the results or the integrity of this study as a result of any of the amendments and deviations.

Protocol Amendment 1:
Amendment 1 changed Section III.C. of the protocol to delete the requirement for GLP soil characterization for the soil sample used in this study. At the request of the Sponsor Representative, the requirement was deleted because a sub-sample of the bulk control soil was supplied from a Northwood, ND field site conducting a GLP tiafenacil soil dissipation study. Soil from the field site was characterized according to GLPs, which is reported herein.

Protocol Amendment 2:
Amendment 2 changed Section III.D.1. of the protocol redefining the definition of a validation set in order to allow a validation trial to be split into two separate smaller extraction sets. The resulting definition of a validation set is consistent with the OCSPP 850.6100 guidelines, which allows the complete validation set to be split into two subsets for efficient handling.

Protocol Amendment 3:
Amendment 3 revised the protocol in its entirety to incorporate several changes. These were to: 1) add Dongbu Farm Hannong Co., Ltd. as a Sponsor in the protocol, 2) clarify that the method was supplied by ISK Biosciences Corporation but was prepared by Dongbu Farm Hannong Co., Ltd. (which included changing the title of the study), 3) update the proposed experimental start and termination date, 4) incorporate text changes from protocol amendments 1 and 2, 5) add six more metabolite analytes to the protocol, as well as add an additional ILV trial and method bridging set for the analysis of these analytes, 6) clarify that the report and raw data archival location will be documented in the final report, 7) delete the page number and total page number off of the title page of the protocol, and 8) correct the typographical errors in the description of the five original metabolite listings.

Protocol Amendment 4:
Amendment 4 added DCC-3825-M-30 and DCC-3825-M-72 to the protocol as test substances/analytes. In places where the number of analytes was listed, the text was amended to include the two additional analytes.

Protocol Amendment 5:
Amendment 5 changed the fortification levels for the method bridging trials, changed the co-Sponsor's name (Dongbu Farm Hannong Co., Ltd. to FarmHannong Co., Ltd.) and address, corrected the chemical name for DCC-3825-M-72, corrected the molecular weight for DCC-3825-M-53, and updated the study title on protocol amendments #3 and #4 as the study title change was previously overlooked.

Protocol Amendment 6:
Amendment 6 changed the Sponsor Representative from Jason A. McDonald, Ph.D. to Mark D. Gelin as Jason McDonald is no longer employed by ISK Biosciences Corporation.

Dongbu Farm Hannong Co., Ltd., Dongbu Advanced Research Institute,
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1. PURPOSE

The purpose of this study is the determination of residue analytical method of Tiafenacil (DCC-3825) and its metabolites (DCC3825-M-01, M-12, M-13, M-20, M-29, M-30, M-35, M-36, M-53, M-63, M-69, M-72 and M-73) in soil.

2. EXPERIMENTAL PROCEDURE

2.1. Equipments

Ultra Performance Liquid Chromatography-Mass spectrometer (UPLC/MSD) :

UPLC :	Model HPLC H-Class (WATERS, USA)
Mass spectrometer :	Model Xevo TQ (WATERS, USA)
	Data handling MassLynx

2.2. Reagents

Acetonitrile :	HPLC grade (MERCK, GERMANY)
Water :	HPLC grade (Burdick & Jackson, USA)
Formic acid (FA) :	98.0 + % (ACROS ORGANICS, USA)
OASIS HLB cartridge :	500 mg, 6 cc (WATERS, USA)

0.1% FA in water solution (Mobile phase for UPLC/MSD) :
Prepare by dissolving FA (1 mL) in water (1 L). The solution is filtered through a membrane filter
and then sonicated.

0.1 % FA in acetonitrile solution (Mobile phase for UPLC/MSD) :
Prepare by dissolving FA (1 mL) in acetonitrile (1 L). The solution is filtered through a membrane filter and then sonicated.

2.3. TEST Items

Identity :	**Tiafenacil**
Chemical name :	3-{2-[2-Chloro-4-fluoro-5-(3-methyl-2,6-dioxo-4-trifluoromethyl-3,6-dihydro-2*H*-pyrimidin-1-yl)-phenylsulfanyl]-propionylamino}-propionic acid methyl ester

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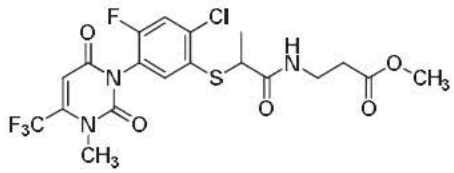
Chemical structure :



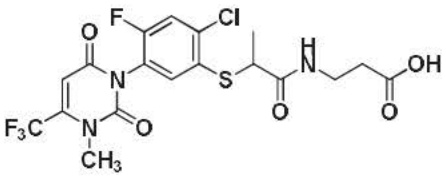
Molecular Weight : 511.88

Assay : 99.8 %

Identity : **DCC3825-M-01**

Chemical name : 3-(2-(2-chloro-4-fluoro-5-(3-methyl-2,6-dioxo-4-(trifluoromethyl)-2,3-dihydropyrimidin-1(6H)-yl) phenylthio)propanamido)propanoic acid

Chemical structure :



Molecular Weight : 497.85

Purity : 98.4 %

Identity : **DCC3825-M-12**

Chemical name : 2-(2-chloro-4-fluoro-5-(3-methyl-2,6-dioxo-4-(trifluoromethyl)-2,3-dihydropyrimidin-1(6H)-yl) phenylthio)propanoic acid

Chemical structure :

Molecular Weight : 426.77

Purity : 98.8 %

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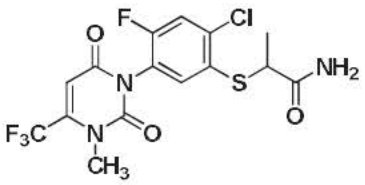
Identity : **DCC3825-M-13**

Chemical name : 2-(2-chloro-4-fluoro-5-(3-methyl-2,6-dioxo-4-(trifluoromethyl)-2,3-dihydropyrimidin-1(6H)-yl) phenylthio)propanamide

Chemical structure :



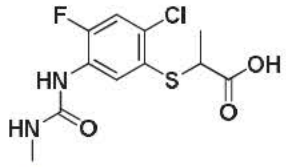
Molecular Weight : 425.79

Purity : 99.4 %

Identity : **DCC3825-M-20**

Chemical name : 2-(2-chloro-4-fluoro-5-(3-methylureido)phenylthio) propanoic acid

Chemical structure :



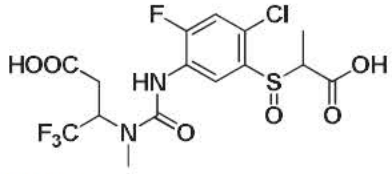
Molecular Weight : 306.74

Purity : 99.2%

Identity : **DCC3825-M-29**

Chemical name : 3-(3-(5-(1-carboxyethylsulfinyl)-4-chloro-2-fluorophenyl)-1-methylureido)-4,4,4-trifluoro-butanoic acid

Chemical structure :



Molecular Weight : 462.80

Purity : 86.5 %

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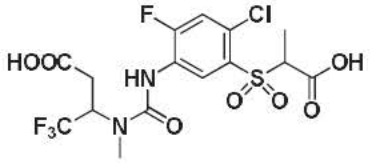
Identity : **DCC3825-M-30**

Chemical name : 3-(3-(5-(1-carboxyethylsulfonyl)-4-chloro-2-fluorophenyl)-1-methylureido)-4,4,4-trifluoro-butanoic acid

Chemical structure :



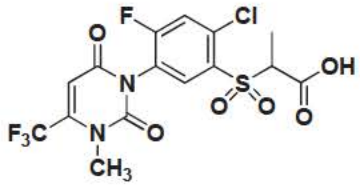
Molecular Weight : 478.80

Purity : 99.7 %

Identity : **DCC3825-M-35**

Chemical name : 2-(2-chloro-4-fluoro-5-(3-methyl-2,6-dioxo-4-(trifluoromethyl)-2,3-dihydropyrimidin-1(6H)-yl)phenyl sulfonyl)propanoic acid

Chemical structure :



Molecular Weight : 458.77

Purity : 90.3 %

Identity : **DCC3825-M-36**

Chemical name : 2-(2-chloro-4-fluoro-5-(3-methyl-2,6-dioxo-4-(trifluoromethyl)-2,3-dihydropyrimidin-1(6H)-yl)phenylsulfinyl)propanoic acid

Chemical structure :

Molecular Weight : 442.77

Purity : 98.2 %

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Identity : **DCC3825-M-53**

Chemical name : 2-(2-chloro-4-fluoro-5-(3-methyl-2,6-dioxo-4-(trifluoromethyl)tetrahydropyrimidin-1(2H)-yl)phenyl sulfinyl)propanoic acid

Chemical structure :

Molecular Weight : 444.79

Purity : 98.3 %

Identity : **DCC3825-M-63**

Chemical name : 2-(2-chloro-4-fluoro-5-(3-methyl-2,6-dioxo-4-(trifluoromethyl)tetrahydropyrimidin-1(2H)-yl) phenylsulfonyl)propanoic acid

Chemical structure :

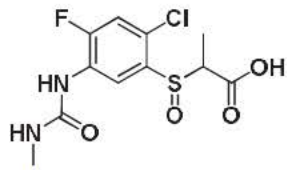
Molecular Weight : 460.79

Purity : 99.9 %

Identity : **DCC3825-M-69**

Chemical name : 2-(2-chloro-4-fluoro-5-(3-methylureido) phenylsulfinyl)propanoic acid

Chemical structure :



Molecular Weight : 322.74

Purity : 99.4 %

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Identity :	**DCC3825-M-72**
Chemical name :	2-chloro-4-fluoro-5-(3-methyl-2,6-dioxo-4-(trifluoromethyl)-2,3-dihydropyrimidin-1(6H)-yl) benzenesulfonic acid
Chemical structure :	
Molecular Weight :	402.71
Purity :	99.9 %

Identity :	**DCC3825-M-73**
Chemical name :	2-chloro-4-fluoro-5-(3-methyl-2,6-dioxo-4-(trifluoromethyl)tetra hydropyrimidin-1(2H)-yl) benzenesulfonic acid
Chemical structure :	
Molecular Weight :	404.72
Purity :	99.0 %

2.4. Analytical Procedure

2.4.1. Instrument Conditions

1) Tiafenacil, DCC3825-M-01, M-12, M-13, M-30, M-36, M-53, M-72

Instrument : UPLC/MSD

Column : Acquity UPLC® BEH C18
50 mm (L) * 2.1 mm (I.D.), 1.7 ㎛

Mobile phase :

Time (min)	0.1 % FA in acetonitrile	0.1 % FA in water
0	45 %	55 %
6	45 %	55 %

Flow rate : 0.2 mL/min

Injection volume : 5 ㎕

Column temperature : 40 ℃

Retention time :

Tiafenacil	~ 4.49 min
DCC3825-M-01	~ 2.73 min
DCC3825-M-12	~ 4.66 min
DCC3825-M-13	~ 2.96 min
DCC3825-M-30	~ 0.89 min
DCC3825-M-36	~ 2.49 min
DCC3825-M-53	~ 2.13 min
DCC3825-M-72	~ 0.77 min

Mass conditions

Ion source : ESI

Capillary voltage : 4 kV

Desolvation temperature : 500 ℃

Desolvation gas flow : 1,000 L/hr, Argon

Product ion, cone voltage and collision voltage

Compound	Polarity	Product ion (m/z)	Cone voltage (V)	Collision voltage (V)
Tiafenacil	Positive	381.295	19	29
DCC3825-M-01	Positive	381.257	21	27
DCC3825-M-12	Positive	152.055	13	31
DCC3825-M-13	Positive	110.069	21	47
DCC3825-M-30	Positive	112.053	20	42
DCC3825-M-36	Positive	218.043	19	35

Compound	Polarity	Product ion (m/z)	Cone voltage (V)	Collision voltage (V)
DCC3825-M-53	Positive	229.097	21	37
DCC3825-M-72	Negative	249.874	42	30

2) DCC3825-M-20, M-35 and M-63

Instrument : UPLC/MSD

Column : Acquity UPLC® BEH C18

50 mm (L) * 2.1 mm (I.D.), 1.7 ㎛

Mobile phase :

Time (min)	0.1 % FA in acetonitrile	0.1 % FA in water
0	25 %	75 %
4	65 %	35 %
6	100 %	0 %
6.1	25 %	75 %
8.1	25 %	75 %

Flow rate : 0.2 mL/min

Injection volume : 5 ㎕

Column temperature : 40 ℃

Retention time : DCC3825-M-20 ~ 3.06 min
DCC3825-M-35 ~ 3.85 min
DCC3825-M-63 ~ 3.58 min

Mass condition

Ion source : ESI

Capillary voltage : 3.7 kV

Desolvation temperature : 500 ℃

Desolvation gas flow : 1000 L/hr, Argon

Product ion, cone voltage and collision voltage

Compound	Polarity	Product ion (m/z)	Cone voltage (V)	Collision voltage (V)
DCC3825-M-20	Negative	197.095	15	23
DCC3825-M-35	Positive	198.090	18	36
DCC3825-M-63	Positive	351.158	18	22

3) DCC3825- M-29, M-69 and M-73

Instrument : UPLC/MSD

Column : Acquity UPLC® BEH C18
50 mm (L) * 2.1 mm (I.D.), 1.7 ㎛

Mobile phase :

Time (min)	0.1 %-FA in acetonitrile	0.1 %-FA in water
0	50 %	50 %
6	50 %	50 %

Flow rate : 0.2 mL/min

Injection volume : 5 ㎕

Column temperature : 40 ℃

Retention time : DCC3825-M-29 ~ 0.84 min
DCC3825-M-69 ~ 0.74 min
DCC3825-M-73 ~ 0.73 min

Mass condition

Ion source : ESI

Capillary voltage : 3.5 kV

Desolvation temperature : 500 ℃

Desolvation gas flow : 1000 L/hr, Argon

Product ion, cone voltage and collision voltage

Compound	Polarity	Product ion (m/z)	Cone voltage (V)	Collision voltage (V)
DCC3825-M-29	Positive	111.603	18	52
DCC3825-M-69	Positive	144.940	14	44
DCC3825-M-73	Negative	186.034	50	36

2.4.2. Preparation of Sample Solution

1) Source of soil

Soil was obtained in Daejeon (Korea). The soil characteristics are shown in the following table;

pH [1] (1:5)	EC [2] (dS/m)	OM [3] (%)	Exchangeable cation ($cmol^+/Kg$)				CEC [4] ($cmol^+/Kg$)
			Na	Mg	K	Ca	
6.96	1.334	1.8	0.29	0.28	2.2	6.8	11.98

[1] Soil pH : soil/water ratio 1/5 (w/w)
[2] EC : Electric conductivity
[3] OM : Soil organic matter
[4] CEC : Cation Exchange Capacity

2) Preparation of test item solution

Prepare 100 μg/mL standard stock solutions of each test item into individual 100 mL volumetric flask using an analytical balance. Dissolve the test item in approximately 50 mL of HPLC grade acetonitrile. After dissolving, bring the solution to a volume of 100 mL using HPLC grade acetonitrile and invert the volumetric flask to mix the solution to homogeneity. 2 mL of each 100 μg/mL stock standard are pipetted into a 100 mL volumetric flask. Dilute the solution to approximately 50 mL with acetonitrile/water (50/50, v/v) and add 1.0 mL of concentrated formic acid. Bring to the volume of 100 mL using HPLC grade acetonitrile/water (50/50, v/v) and mix to homogeneity. This solution was called "Mix standard solution". The final concentration of the each test item in the "Mix standard solution" is 2 μg/mL.

3) Preparation of fortification

All fortifications were made directly to the 100 g of soil and operated 3 replications. Fortified soil samples were prepared using "Mix standard solution".

Fortification Level (mg/kg)	Volume of "Mix standard solution" (mL)
0.1	5.0

4) Extraction

10 g (±0.1g) of fortified soil accurately weighed into a 250 mL HDPE bottle. Then 50 mL of acetonitrile/0.1%FA in water (80/20, v/v) was added into the HDPE bottle. The bottle was shaken vigorously for 30 minutes. After shaking, the sample was filtered through filter paper and rinsed with 25 mL of acetonitrile. The extraction was replicated twice. The extracted solutions were collected to the same flask, and evaporated at 40 ℃ under reduced pressure. After evaporation, residues were dissolved in 5 mL of 0.1% FA in acetonitrile/0.1% FA in water (10/90, v/v). This solution was called solution A.

5) Purification

OASIS HLB Cartridge (500 mg, 6 cc) was conditioned with 5 mL 0.1% FA in acetonitrile and then 5 mL 0.1% FA in water. Then the "solution A" was applied to HLB cartridge and rinsed with 5 mL of 0.1% FA in acetonitrile/0.1% FA in water (20/80, v/v). And the rinsate was discarded. Subsequently, the HLB cartridge was eluted with 10 mL of 0.1% FA in acetonitrile / 0.1% FA in water (60/40, v/v). The eluate was concentrated under reduced pressure at 40 ℃ and dissolved in 2 mL of 0.1% FA in acetonitrile/0.1% FA in water (60/40, v/v).

6) Determination

Residues of Tiafenacil and its metabolites (DCC3825-M-01, M-12, M-13, M-20, M-29, M-30, M-35, M-36, M-53, M-63, M-69, M-72 and M-73) in soil were determined using UPLC/MSD as the conditions of 2.4.1. The peak area was measured. And the amounts of Tiafenacil and its

metabolites in the sample were quantified by comparison to those of reference standards.

7) Preparation of reference standard solutions

The reference standard solutions were prepared in the table below. These reference standard solutions were prepared by mixing the "Mix standard solution" and control solution. These reference solutions were used to obtain the calibration curve of each test item for quantification.

Concentration of reference standard solutions (μg/mL)	Mix standard solution prepared (μg/mL)	Volume of Mix standard solution (μL)	Volume of Control Solution* (μL)
0.01	0.2	50	950
0.05	1.0	50	950
0.1	2.0	50	950
0.5	10.0	50	950
1.0	20.0	50	950

*The control solution was prepared from the blank soil sample that operated by the procedure 2.4.2. 4)~5).

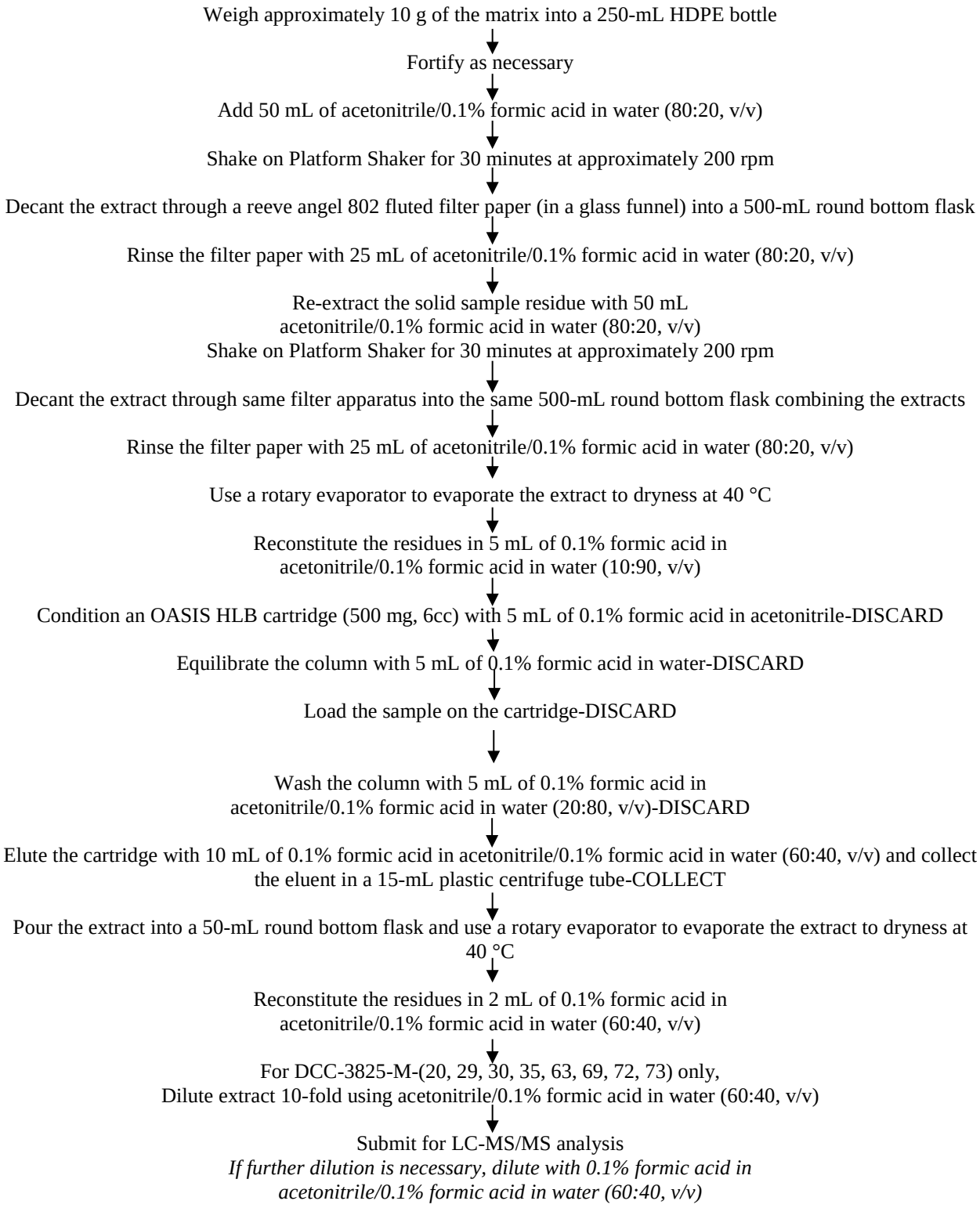
3. Result

3.1. Minimum detectable amount and Limit of Quantitation (LOQ)

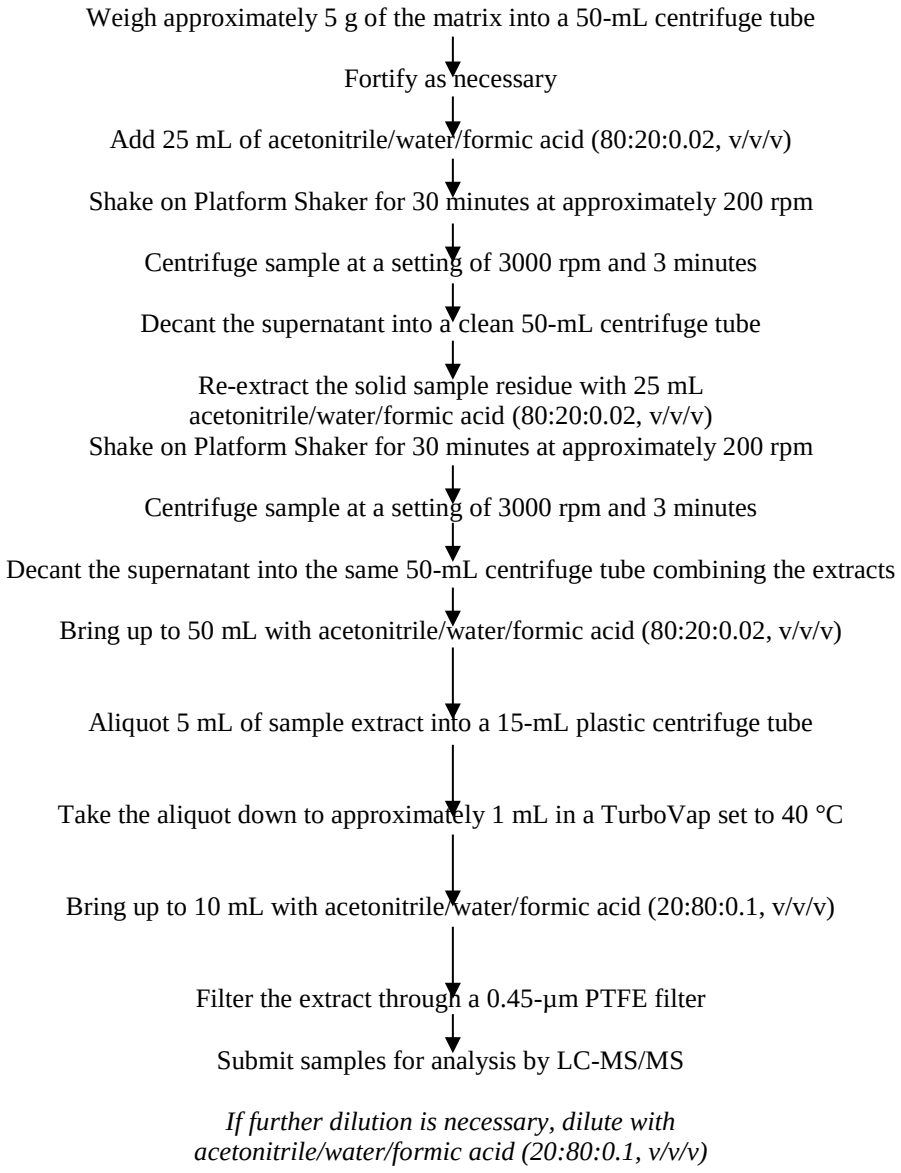
- Minimum detectable amount of Tiafenacil and its metabolites (DCC3825-M-01, M-12, M-13, M-20, M-29, M-30, M-35, M-36, M-53, M-63, M-69, M-72 and M-73) : 0.05 ng
- LOQ of Tiafenacil and its metabolites (DCC3825-M-01, M-12, M-13, M-20, M-29, M-30, M-35, M-36, M-53, M-63, M-69, M-72 and M-73) : 0.002 mg/kg as calculated by using the equation.

$$0.05\text{ng} \times \frac{2\text{ mL}}{5\ \mu\text{L}} \times \frac{1}{10\text{ g}} = 0.002\text{ mg/kg}$$

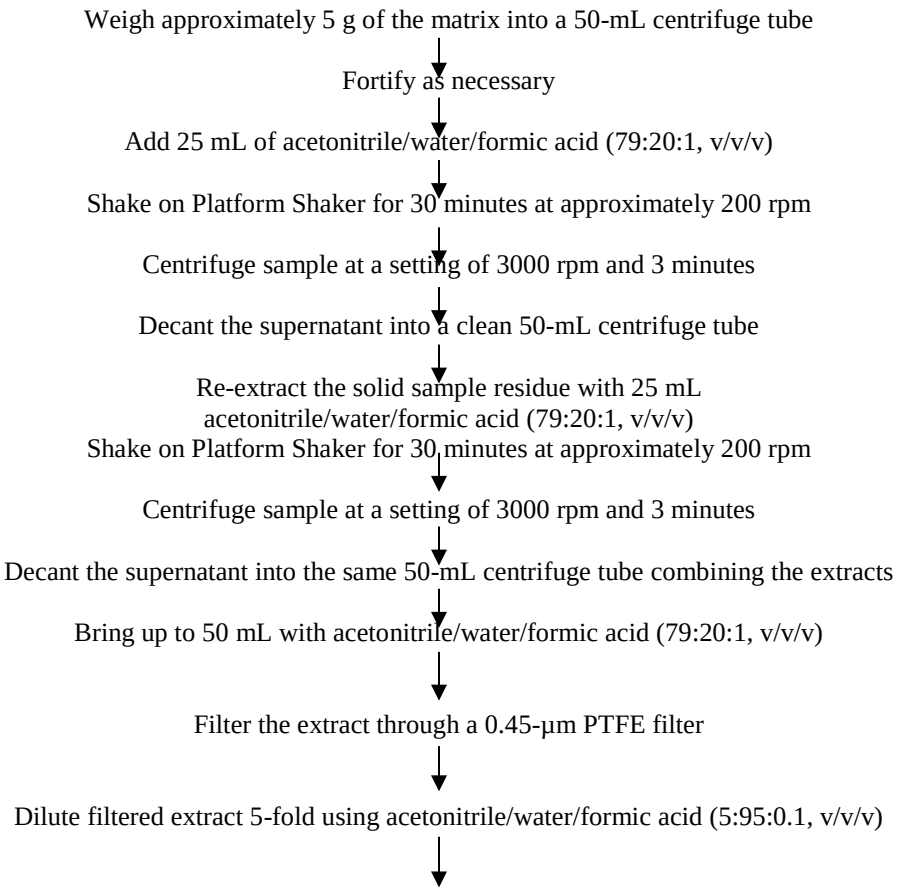
Analysis of Tiafenacil and 13 Metabolites in Soil by LC-MS/MS (Procedure for ILV Trials)



Analysis of Tiafenacil, DCC-3825-M-(01, 12, 13, 36, 53) in Soil by LC-MS/MS
(Procedure for Modified Method Validation)



Analysis of DCC-3825-M-(20, 29, 30, 35, 63, 69, 72, 73) in Soil by LC-MS/MS (Procedure for Modified Method Validation)



LC-MS/MS Conditions for Tier 1 ILV Analytes

Golden Pacific Laboratories, LLC **Form #GPL-402**

HPLC Parameters Form

GPL Study Number: 150608 Sponsor Study Number: NA

Sample Set I.D.: 608ILV01 Matrix: ~~Soil~~ ③ Soil

Instrument I.D.: LC/MS/MS #2, Sciex API4000 LC/MS/MS with Shimadzu LC-20AD HPLC Pumps, Shimadzu SCL-10A Controller, Shimadzu SIL-20AC HT Autosampler and Analyst Data System Ver. 1.5.2

Column: Acquity UPLC® BEH C18 (2.1 mm x 50 mm, 1.7 µm)
Serial#: 01763007415522 Part No. 186002350

Guard Column: Acquity UPLC® BEH C18 (2.1 mm x ~~50 mm~~ 5mm, 1.7 µm) VanGuard Pre-Column ⑤ EAS 01102118

Flow Rate: 0.2 mL/minute **Injection Volume:** 5 µL

Mobile Phase:

A = 0.1% Formic Acid in Acetonitrile ID# EAS-080615
B = 0.1% Formic Acid in Water ID# EAS-080615

Isocratic Program:

(0.0 minutes) 45% Mobile Phase A: 55% Mobile Phase B
(6.0 minutes) 45% Mobile Phase A: 55% Mobile Phase B

Run Time: 6.0 minutes with the column heater set to 40 °C.

MS/MS Parameters:

Analyte	Transition Ions	Dwell Time (msec)	DP	CE
Tiafenacil	513.1/381.1	50	75	38
DCC-3825-M-01	499.3/381.1	50	65	34
DCC-3825-M-12	428.0/152.1	50	65	45
DCC-3825-M-13	427.0/110.0	50	65	71
DCC-3825-M-36	444.2/218.0	50	70	53
DCC-3825-M-53	446.2/229.1	50	70	51

MRM scan using TurboIonSpray®: Positive polarity, Unit/Unit Resolution

CUR:	**25**	**IS:**	**5500**	**TEM:**	**500**
GS1:	**40**	**GS2:**	**40**	**ihe:**	**ON**
CAD:	**10**	**CXP:**	**10**	**EP:**	**10**

Divert Valve:

0.0	**minutes**	**Waste**
0.9	**minutes**	**LC/MS/MS**
4.0	**minutes**	**Waste**

Calibration Standards:	**Concentration①**	**Standard I.D.**	**Expiration Date**
	0.0499/0.0499/0.0505/0.0500/0.0499/0.0500 µg/mL	1518M-6	12/01/2015
	0.0998/0.0998/0.101/0.100/0.0998/0.100 µg/mL	1518M-5	12/01/2015
	0.250/0.250/0.253/0.250/0.250/0.250 µg/mL	1518M-9	12/01/2015
	0.499/0.499/0.505/0.500/0.499/0.500 µg/mL	1518M-4	12/01/2015
	0.998/0.998/1.01/1.00/0.998/1.00 µg/mL	1518M-3	12/01/2015

① The concentrations are listed in the following order: Tiafenacil, DCC-3825-M-(01, 12, 13, 36, 53)

Prepared By: Elizabeth D Quinonez Date: 09/01/15

Retention Time(s): Tiafenacil: ~ 3.09 **min., DCC-3825-M-01: ~** 1.95 **min., DCC-3825-M-12: ~** 3.20 **min., DCC-3825-M-13: ~** 2.10 **min., DCC-3825-M-36: ~** 1.81 **min., DCC-3825-M-53: ~** 1.59 **min.,**
Recorded by /Date: RR 09/03/15

Revised: 08/04/2011

②Ⓔ RR 09/03/15 ③Ⓔ AM 09/09/15

LC-MS/MS Conditions for Tier 2 ILV Analytes: DCC-3825-M-30 and DCC-3825-M-72

Although multiple sets of transition ion pairs were acquired for each analyte, the following pairs were quantitated and the resulting data are reported herein:

Analyte	Quantitated and Reported Transition Ion Pairs
DCC-3825-M-30	479.2/112.1
DCC-3825-M-72	400.9/249.8

Golden Pacific Laboratories, LLC **Form #GPL-402**

HPLC Parameters Form

GPL Study Number: 150608 Sponsor Study Number: NA

Sample Set I.D.: 6081V03 Matrix: Soil

Instrument I.D.: LC/MS/MS #3, Sciex API5000 LC/MS/MS with Shimadzu LC-20AD XR HPLC Pumps, Shimadzu CBM-20A Controller, Shimadzu SIL-20AC XR Autosampler and Analyst Data System Ver. 1.6.3

Column: Waters Acquity UPLC BEH C18 (50 x 2.1 mm 1.7 micron)
Serial#: 02713600815854 Part No. 186002350

Guard Column: Waters VanGuard UPLC BEH C18 1.7 µm 2.1 x 5 mm Part No. 186003975

Flow Rate: 0.2 mL/minute **Injection Volume:** 2 µL

Mobile Phase:

A = 0.1 % Formic Acid in Acetonitrile ID# EAS-022216
B = 0.1% Formic Acid in Water ID# EAS-022216

Isocratic Program:

(0.0 minutes) 45% Mobile Phase A: 55% Mobile Phase B
(6.0 minutes) 45% Mobile Phase A: 55% Mobile Phase B

Run Time: 6.0 minutes with a column heater set to 40 °C.

MS/MS Parameters (Experiment 1):

Analyte	Transition Ions	MRM Detection Window (sec)	Target Scan Time (sec)	Retention Time	DP	CE
DCC-3825-M-72	400.9/249.8	90	2	1.30	-50	-42
DCC-3825-M-72	400.9/185.9	90	2	1.30	-50	-56

Scheduled MRM scan using TurboIonSpray®, Negative Polarity, Unit/Unit Resolution

CUR:	**35**	**IS:**	**-4500**	**TEM:**	**500**
GS1:	**40**	**GS2:**	**40**	**ihe:**	**ON**
CAD:	**12**	**EP:**	**-10**	**CXP:**	**-10**

MS/MS Parameters (Experiment 2):

Analyte	Transition Ions	MRM Detection Window (sec)	Target Scan Time (sec)	Retention Time	DP	CE
DCC-3825-M-30	479.2/112.1	90	2	1.50	140	59
DCC-3825-M-30	479.2/171.8	90	2	1.50	140	25

Scheduled MRM scan using TurboIonSpray®, Positive Polarity, Unit/Unit Resolution

CUR:	**35**	**IS:**	**5500**	**TEM:**	**500**
GS1:	**40**	**GS2:**	**40**	**ihe:**	**ON**
CAD:	**12**	**EP:**	**10**	**CXP:**	**10**

Prepared By: [signature] Date: 02/29/16

Revised: 08/04/2011

Golden Pacific Laboratories, LLC — **Form #GPL-402**

HPLC Parameters Form

GPL Study Number: 150608 Sponsor Study Number: NA

Sample Set I.D.: 608ILV03 Matrix: Soil

Instrument I.D.: LC/MS/MS #3, Sciex API5000 LC/MS/MS with Shimadzu LC-20AD XR HPLC Pumps, Shimadzu CBM-20A Controller, Shimadzu SIL-20AC XR Autosampler and Analyst Data System Ver. 1.6.3

Divert Valve:

0.0	**minutes**	**Waste**
0.3	**minutes**	**LC/MS/MS**
3.0	**minutes**	**Waste**

Calibration Standards:

Concentration①	Standard I.D.	Expiration Date
1.00/1.01/1.01/1.00/1.01/1.02/1.02/1.01 ng/mL	1598M-9	05/24/2016
5.00/5.05/5.05/5.00/5.05/5.10/5.10/5.05 ng/mL	1598M-8	05/24/2016
10.0/10.1/10.1/10.0/10.1/10.2/10.2/10.1 ng/mL	1598M-7	05/24/2016
50.0/50.5/50.5/50.0/50.5/51.0/51.0/50.5 ng/mL	1598M-6	05/24/2016
100/101/101/100/101/102/102/101 ng/mL	1598M-5	05/24/2016

① **The concentrations are listed in the following order: DCC-3825-M-(20, 29, 30, 35, 63, 69, 72, 73).**

Prepared By: Elizabeth D Lukosnan Date: 02/29/16

Retention Time(s):

DCC-3825-M-30 ~ 1.29 **minutes,** **DCC-3825-M-72 ~** 1.18 **minutes**

Recorded by /Date: EDL 02/29/16

Revised: 08/04/2011

LC-MS/MS Conditions for Tier 2 ILV Analytes: DCC-3825-M-29, DCC-3825-M-69, and DCC-3825-M-73

Although multiple sets of transition ion pairs were acquired for each analyte, the following pairs were quantitated and the resulting data are reported herein:

Analyte	Quantitated and Reported Transition Ion Pairs
DCC-3825-M-29	463.1/112.0
DCC-3825-M-69	322.9/144.9
DCC-3825-M-73	402.9/185.9

Golden Pacific Laboratories, LLC **Form #GPL-402**

HPLC Parameters Form

GPL Study Number: 150608 Sponsor Study Number: NA

Sample Set I.D.: 6081LV03A Matrix: Soil

Instrument I.D.: LC/MS/MS #3, Sciex API5000 LC/MS/MS with Shimadzu LC-20AD XR HPLC Pumps, Shimadzu CBM-20A Controller, Shimadzu SIL-20AC XR Autosampler and Analyst Data System Ver. 1.6.3

Column: Waters Acquity UPLC BEH C18 (50 x 2.1 mm 1.7 micron)
Serial#: 02713600815854 Part No. 186002350

Guard Column: Waters VanGuard UPLC BEH C18 1.7 µm 2.1 x 5 mm Part No. 186003975

Flow Rate: 0.2 mL/minute **Injection Volume:** 5 µL

Mobile Phase:

A = 0.1 % Formic Acid in Acetonitrile ID# EAS-022216
B = 0.1% Formic Acid in Water ID# EAS-022216

Isocratic Program:

(0.0 minutes) 50% Mobile Phase A: 50% Mobile Phase B
(6.0 minutes) 50% Mobile Phase A: 50% Mobile Phase B

Run Time: 6.0 minutes with a column heater set to 40 °C.

MS/MS Parameters (Experiment 1):

Analyte	Transition Ions	MRM Detection Window (sec)	Target Scan Time (sec)	Retention Time	DP	CE
DCC-3825-M-73	402.9/249.9	90	2	1.20	-60	-50
DCC-3825-M-73	402.9/185.9	90	2	1.20	-60	-56

Scheduled MRM scan using TurboIonSpray®, Negative Polarity, Unit/Unit Resolution

CUR:	**35**	**IS:**	**-4500**	**TEM:**	**500**
GS1:	**40**	**GS2:**	**40**	**ihe:**	**ON**
CAD:	**12**	**EP:**	**-10**	**CXP:**	**-10**

MS/MS Parameters (Experiment 2):

Analyte	Transition Ions	MRM Detection Window (sec)	Target Scan Time (sec)	Retention Time	DP	CE
DCC-3825-M-29	463.1/112.0	90	2	1.40	140	69
DCC-3825-M-29	463.1/373.1	90	2	1.40	140	23
DCC-3825-M-69	322.9/144.9	90	2	1.10	125	55

Scheduled MRM scan using TurboIonSpray®, Positive Polarity, Unit/Unit Resolution

CUR:	**35**	**IS:**	**5500**	**TEM:**	**500**
GS1:	**40**	**GS2:**	**40**	**ihe:**	**ON**
CAD:	**12**	**EP:**	**10**	**CXP:**	**10**

Prepared By: Elisabeth D Andreasen Date: 02/29/16

Revised: 08/04/2011

Golden Pacific Laboratories, LLC	Form #GPL-402

HPLC Parameters Form

GPL Study Number: 150608 Sponsor Study Number: NA

Sample Set I.D.: 6081LV03A Matrix: Soil

Instrument I.D.: LC/MS/MS #3, Sciex API5000 LC/MS/MS with Shimadzu LC-20AD XR HPLC Pumps, Shimadzu CBM-20A Controller, Shimadzu SIL-20AC XR Autosampler and Analyst Data System Ver. 1.6.3

Divert Valve:

0.0	**minutes**	**Waste**
0.1	**minutes**	**LC/MS/MS**
2.5	**minutes**	**Waste**

Calibration Standards:

Concentration①	Standard I.D.	Expiration Date
1.00/1.01/1.01/1.00/1.01/1.02/1.02/1.01 ng/mL	1598M-9	05/24/2016
5.00/5.05/5.05/5.00/5.05/5.10/5.10/5.05 ng/mL	1598M-8	05/24/2016
10.0/10.1/10.1/10.0/10.1/10.2/10.2/10.1 ng/mL	1598M-7	05/24/2016
50.0/50.5/50.5/50.0/50.5/51.0/51.0/50.5 ng/mL	1598M-6	05/24/2016
100/101/101/100/101/102/102/101 ng/mL	1598M-5	05/24/2016

① **The concentrations are listed in the following order: DCC-3825-M-(20, 29, 30, 35, 63, 69, 72, 73).**

Prepared By: Elisabeth D Lucero Date: 02/29/16

Retention Time(s): DCC-3825-M-29 ~ 1.07 **minutes, DCC-3825-M-69 ~** 0.90 **minutes**

DCC-3825-M-73 ~ 1.03 **minutes** Recorded by /Date: EDL 02/29/16

Revised: 08/04/2011

LC-MS/MS Conditions for Tier 2 ILV Analytes: DCC-3825-M-20, DCC-3825-M-35, and DCC-3825-M-63

Although multiple sets of transition ion pairs were acquired for each analyte, the following pairs were quantitated and the resulting data are reported herein:

Analyte	Quantitated and Reported Transition Ion Pairs
DCC-3825-M-20	305.0/273.9
DCC-3825-M-35	459.0/198.0
DCC-3825-M-63	461.0/111.8

Golden Pacific Laboratories, LLC **Form #GPL-402**

HPLC Parameters Form

GPL Study Number: 150608 Sponsor Study Number: NA

Sample Set I.D.: 608LV033 Matrix: Soil

Instrument I.D.: LC/MS/MS #3, Sciex API5000 LC/MS/MS with Shimadzu LC-20AD XR HPLC Pumps, Shimadzu CBM-20A Controller, Shimadzu SIL-20AC XR Autosampler and Analyst Data System Ver. 1.6.3

Column: Waters Acquity UPLC BEH C18 (50 x 2.1 mm 1.7 micron)
Serial#: 02713600815854 Part No. 186002350

Guard Column: Waters VanGuard UPLC BEH C18 1.7 µm 2.1 x 5 mm Part No. 186003975

Flow Rate: 0.2 mL/minute **Injection Volume:** 10 µL

Mobile Phase:

A = 0.1 % Formic Acid in Acetonitrile ID# EAS-022216
B = 0.1% Formic Acid in Water ID# EAS-022216

Gradient Program:

(0.0 minutes) 25% Mobile Phase A: 75% Mobile Phase B
(4.0 minutes) 65% Mobile Phase A: 35% Mobile Phase B
(6.0 minutes) 100% Mobile Phase A: 0% Mobile Phase B
(6.1 minutes) 25% Mobile Phase A: 75% Mobile Phase B
(8.1 minutes) 25% Mobile Phase A: 75% Mobile Phase B

Run Time: 8.1 minutes with a column heater set to 40 °C.

MS/MS Parameters (Experiment 1):

Analyte	Transition Ions	MRM Detection Window (sec)	Target Scan Time (sec)	Retention Time	DP	CE
DCC-3825-M-20	305.0/273.9	110	2	3.33	-70	-13
DCC-3825-M-20	305.0/203.9	110	2	3.33	-70	-24
DCC-3825-M-20	305.0/197.0	110	2	3.33	-45	-24

Scheduled MRM scan using TurboIonSpray®, Negative Polarity, Unit/Unit Resolution

CUR:	**35**	**IS:**	**-4500**	**TEM:**	**500**
GS1:	**40**	**GS2:**	**40**	**ihe:**	**ON**
CAD:	**12**	**EP:**	**-10**	**CXP:**	**-10**

MS/MS Parameters (Experiment 2):

Analyte	Transition Ions	MRM Detection Window (sec)	Target Scan Time (sec)	Retention Time	DP	CE
DCC-3825-M-35	459.0/349.0	110	2	4.11	130	31
DCC-3825-M-35	459.0/198.0	110	2	4.11	130	49
DCC-3825-M-63	461.0/351.0	110	2	3.75	140	33
DCC-3825-M-63	461.0/111.8	110	2	3.75	140	43

Scheduled MRM scan using TurboIonSpray®, Positive Polarity, Unit/Unit Resolution

Prepared By: Elizabeth D Donovan Date: 02/29/16

Revised: 08/04/2011

Golden Pacific Laboratories, LLC	Form #GPL-402

HPLC Parameters Form

GPL Study Number: 150608 Sponsor Study Number: NA

Sample Set I.D.: 6081LU03B Matrix: Soil

Instrument I.D.: LC/MS/MS #3, Sciex API5000 LC/MS/MS with Shimadzu LC-20AD XR HPLC Pumps, Shimadzu CBM-20A Controller, Shimadzu SIL-20AC XR Autosampler and Analyst Data System Ver. 1.6.3

MS/MS Parameters (Experiment 2)-*Continued*:

CUR:	**35**	**IS:**	**5500**	**TEM:**	**500**
GS1:	**40**	**GS2:**	**40**	**ihe:**	**ON**
CAD:	**12**	**EP:**	**10**	**CXP:**	**12**

Divert Valve:	0.0	**minutes**	**Waste**
	2.0	**minutes**	**LC/MS/MS**
	5.1	**minutes**	**Waste**

Calibration Standards:

Concentration①	Standard I.D.	Expiration Date
1.00/1.01/1.01/1.00/1.01/1.02/1.02/1.01 ng/mL	1598M-9	05/24/2016
5.00/5.05/5.05/5.00/5.05/5.10/5.10/5.05 ng/mL	1598M-8	05/24/2016
10.0/10.1/10.1/10.0/10.1/10.2/10.2/10.1 ng/mL	1598M-7	05/24/2016
50.0/50.5/50.5/50.0/50.5/51.0/51.0/50.5 ng/mL	1598M-6	05/24/2016
100/101/101/100/101/102/102/101 ng/mL	1598M-5	05/24/2016

① **The concentrations are listed in the following order: DCC-3825-M-(20, 29, 30, 35, 63, 69, 72, 73).**

Prepared By: Elisabeth D Thomas Date: 02/29/16

Retention Time(s): DCC-3825-M-20 ~ 3.39 **minutes, DCC-3825-M-35 ~** 4.07 **minutes**

DCC-3825-M-63 ~ 3.80 **minutes,** Recorded by /Date: ET 02/29/16

Revised: 08/04/2011

LC-MS/MS Conditions for Tier 1 Method Bridging Analytes

Although multiple sets of transition ion pairs were acquired for each analyte, the following pairs were quantitated and the resulting data are reported herein:

Analyte	Quantitated and Reported Transition Ion Pairs
Tiafenacil	512.0/479.8
	512.0/381.2
DCC-3825-M-01	498.2/381.0
	498.2/480.0
DCC-3825-M-12	426.9/381.2
	426.9/152.0
DCC-3825-M-13	425.9/381.0
	425.9/152.0
DCC-3825-M-36	443.1/369.0
	443.1/218.0
DCC-3825-M-53	447.1/373.1
	447.1/428.9

Golden Pacific Laboratories, LLC — **Form #GPL-402**

HPLC Parameters Form — **Page 1 of 2**

GPL Study Number: 150608 Sponsor Study Number: NA

Sample Set I.D.: 608mv01A Matrix: Soil

Instrument I.D.: LC/MS/MS #2, Sciex API4000 LC/MS/MS with Shimadzu LC-20AD HPLC Pumps, Shimadzu SCL-10A Controller, Shimadzu SIL-20AC HT Autosampler and Analyst Data System Ver. 1.6.3

Column: Phenomenex Kinetex 2.6 µm C18 100A, 100 x 3.0 mm
Serial#: 740698-39 Part No. 00D-4462-Y0

Guard Column: SecurityGuard ULTRA Cartridge UHPLC C18 for 3.00 mm Part No. AJ0-8775

Flow Rate: 0.2 mL/minute **Injection Volume:** 50 µL

Mobile Phase:

A = 0.2% Formic Acid in Acetonitrile ID# EAS - 100215
B = 0.2% Formic Acid in Water ID# EAS - 101415

Gradient Program:

(0.0 minutes) 55% Mobile Phase A: 45% Mobile Phase B
(4.0 minutes) 55% Mobile Phase A: 45% Mobile Phase B
(5.5 minutes) 100% Mobile Phase A: 0% Mobile Phase B
(6.5 minutes) 100% Mobile Phase A: 0% Mobile Phase B
(6.6 minutes) 55% Mobile Phase A: 45% Mobile Phase B
(8.0 minutes) 55% Mobile Phase A: 45% Mobile Phase B

Run Time: 8.0 minutes, w/column heater set to 40°C. EAS 10/21/15

MS/MS Parameters:

Analyte	Transition Ions	Dwell Time (msec)	DP	CE
Tiafenacil	512.0/479.8	50	75	23
DCC-3825-M-01	498.2/381.0	50	65	35
DCC-3825-M-12	426.9/381.2	50	65	21
DCC-3825-M-13	425.9/381.0	50	65	26
DCC-3825-M-36	443.1/369.0	50	70	28
DCC-3825-M-53	447.1/373.1	50	70	27
Tiafenacil	512.0/381.2	50	75	36
DCC-3825-M-01	498.2/480.0	50	65	22
DCC-3825-M-12	426.9/152.0	50	65	45
DCC-3825-M-13	425.9/152.2	50	65	47
DCC-3825-M-36	443.1/218.0	50	70	53
DCC-3825-M-53	447.1/428.9	50	70	~~27~~ 19 (E) EAS 01/02/18
DCC-3825-M-53	445.1/371.1	100	70	29
DCC-3825-M-53	445.1/427.0	50	70	19

MRM scan using TurboIonSpray®: Positive polarity, Unit/Unit Resolution

CUR:	**25**	**IS:**	**5500**	**TEM:**	**500**
GS1:	**40**	**GS2:**	**40**	**ihe:**	**ON**
CAD:	**10**	**CXP:**	**10**	**EP:**	**10**

Prepared By: Elizabeth L Johnson Date: 10/21/15

Revised: 08/04/2011

Golden Pacific Laboratories, LLC	Form #GPL-402
HPLC Parameters Form	Page 2 of 2

GPL Study Number: 150608 Sponsor Study Number: NA
Sample Set I.D.: 608 mv01A Matrix: Soil

Instrument I.D.: LC/MS/MS #2, Sciex API4000 LC/MS/MS with Shimadzu LC-20AD HPLC Pumps, Shimadzu SCL-10A Controller, Shimadzu SIL-20AC HT Autosampler and Analyst Data System Ver. 1.6.3

Divert Valve:

0.0	minutes	Waste
2.6	minutes	LC/MS/MS
5.7	minutes	Waste

Calibration Standards:

Concentration①	Standard I.D.	Expiration Date
0.250/0.250/0.253/0.250/0.250/0.250 ng/mL	1518M-15	12/01/2015
0.499/0.499/0.505/0.500/0.499/0.500 ng/mL	1518M-14	12/01/2015
0.998/0.998/1.01/1.00/0.998/1.00 ng/mL	1518M-13	12/01/2015
2.00/2.00/2.02/2.00/2.00/2.00 ng/mL	1518M-12	12/01/2015
4.99/4.99/5.05/5.00/4.99/5.00 ng/mL	1518M-11	12/01/2015

① **The concentrations are listed in the following order: Tiafenacil, DCC-3825-M-(01, 12, 13, 36, 53)**

Prepared By: Elizabeth S Johnson Date: 10/21/15

Retention Time(s): Tiafenacil: ~ 5.06 **min., DCC-3825-M-01: ~** 3.75 **min., DCC-3825-M-12: ~** 5.06 **min., DCC-3825-M-13: ~** 4.03 **min., DCC-3825-M-36: ~** 3.70 **min., DCC-3825-M-53: ~** 3.40 **min.,**
Recorded by /Date: ESJ 12/13/15

Revised: 08/04/2011

LC-MS/MS Conditions for Tier 2 Method Bridging Analytes: DCC-3825-M-29, DCC-3825-M-30, DCC-3825-M-35, DCC-3825-M-63, and DCC-3825-M-69

Although multiple sets of transition ion pairs were acquired for each analyte, the following pairs were quantitated and the resulting data are reported herein:

Analyte	Quantitated and Reported Transition Ion Pairs
DCC-3825-M-29	463.1/373.1
	463.1/112.0
DCC-3825-M-30	479.2/112.1
	479.2/198.0
DCC-3825-M-35	459.0/349.0
	459.0/441.1
DCC-3825-M-63	461.0/111.8
	461.0/351.0
DCC-3825-M-69	322.9/200.1
	322.9/144.9

Golden Pacific Laboratories, LLC **Form #GPL-402**

HPLC Parameters Form

GPL Study Number: 150608 Sponsor Study Number: NA

Sample Set I.D.: 608MV02 - POS Matrix: SOIL

Instrument I.D.: LC/MS/MS #3, Sciex API5000 LC/MS/MS with Shimadzu LC-20AD XR HPLC Pumps, Shimadzu CBM-20A Controller, Shimadzu SIL-20AC XR Autosampler and Analyst Data System Ver. 1.6.3

Column: Phenomenex Kinetex 2.6 µm C18 100A, 100 x 3.0 mm
Serial#: H16-065173 Part No. 00D-4462-Y0

Guard Column: SecurityGuard ULTRA Cartridge UHPLC C18 for 3.00 mm Part No. AJ0-8775

Flow Rate: 0.2 mL/minute **Injection Volume:** 50 µL

Mobile Phase:

A = 0.2% Formic Acid in Acetonitrile ID# EAS-031116
B = 0.2% Formic Acid in Water ID# EAS-031116

Gradient Program:

(0.0 minutes) 50% Mobile Phase A: 50% Mobile Phase B
(3.7 minutes) 50% Mobile Phase A: 50% Mobile Phase B
(5.5 minutes) 100% Mobile Phase A: 0% Mobile Phase B
(6.5 minutes) 100% Mobile Phase A: 0% Mobile Phase B
(6.6 minutes) 50% Mobile Phase A: 50% Mobile Phase B
(10.0 minutes) 50% Mobile Phase A: 50% Mobile Phase B

Run Time: 10.0 minutes, with a column heater set to 40 °C.

MS/MS Parameters:

Analyte	Transition Ions	Dwell Time (msec)	DP	CE
DCC-3825-M-29	463.1/373.1	75	140	23
DCC-3825-M-29	463.1/112.0	25	140	69
DCC-3825-M-30	479.2/112.1	75	140	59
DCC-3825-M-30	479.2/198.0	25	140	25
DCC-3825-M-35	459.0/349.0	75	130	31
DCC-3825-M-35	459.0/441.1	25	130	20
DCC-3825-M-63	461.0/111.8	75	140	43
DCC-3825-M-63	461.0/351.0	25	140	33
DCC-3825-M-63	461.0/442.9	25	140	20
DCC-3825-M-69	322.9/200.1	75	125	31
DCC-3825-M-69	322.9/144.9	25	125	55

MRM scan using TurboIonSpray®, Positive polarity, Unit/Unit Resolution

CUR:	**35**	**IS:**	**5500**	**TEM:**	**500**
GS1:	**40**	**GS2:**	**40**	**ihe:**	**ON**
CAD:	**12**	**EP:**	**10**	**CXP:**	**10**

Prepared By: Elisabeth A Schoeman Date: 03/19/16

Revised: 08/04/2011

Golden Pacific Laboratories, LLC **Form #GPL-402**

HPLC Parameters Form

GPL Study Number: 150608 Sponsor Study Number: NA

Sample Set I.D.: 608MV02-POS Matrix: SOIL

Instrument I.D.: LC/MS/MS #3, Sciex API5000 LC/MS/MS with Shimadzu LC-20AD XR HPLC Pumps, Shimadzu CBM-20A Controller, Shimadzu SIL-20AC XR Autosampler and Analyst Data System Ver. 1.6.3

Divert Valve:

0.0	**minutes**	**Waste**
1.8	**minutes**	**LC/MS/MS**
4.8	**minutes**	**Waste**

Calibration Standards:

Concentration①	Standard I.D.	Expiration Date
0.100/0.101/0.101/0.100/0.101/0.102/0.102/0.101 ng/mL	1598M-16	05/24/2016
0.200/0.202/0.202/0.200/0.202/0.204/0.204/0.202 ng/mL	1598M-15	05/24/2016
0.500/0.505/0.505/0.500/0.505/0.510/0.510/0.505 ng/mL	1598M-14	05/24/2016
1.00/1.01/1.01/1.00/1.01/1.02/1.02/1.01 ng/mL	1598M-13	05/24/2016
2.00/2.02/2.02/2.00/2.02/2.04/2.04/2.02 ng/mL	1598M-12	05/24/2016
5.00/5.05/5.05/5.00/5.05/5.10/5.10/5.05 ng/mL	1598M-11	05/24/2016

① **The concentrations are listed in the following order: DCC-3825-M-(20, 29, 30, 35, 63, 69, 72, 73).**

Prepared By: Elizabeth A Lunaman Date: 03/19/16

Retention Time(s): **DCC-3825-M-29:** ~ 2.92 **minutes, DCC-3825-M-30:** ~ 3.18 **minutes,**
DCC-3825-M-35: ~ 4.31 **minutes, DCC-3825-M-63:** ~ 3.96 **minutes,**
DCC-3825-M-69: ~ 2.52 **minutes,** Recorded by /Date: EAL 03/21/16

Revised: 08/04/2011

LC-MS/MS Conditions for Tier 2 Method Bridging Analytes: DCC-3825-M-20, DCC-3825-M-72, and DCC-3825-M-73

Although multiple sets of transition ion pairs were acquired for each analyte, the following pairs were quantitated and the resulting data are reported herein:

Analyte	Quantitated and Reported Transition Ion Pairs
DCC-3825-M-20	305.0/273.9
	305.0/203.9
DCC-3825-M-72	400.9/249.8
	400.9/185.9
DCC-3825-M-73	402.9/249.9
	402.9/185.9

Golden Pacific Laboratories, LLC **Form #GPL-402**

HPLC Parameters Form

GPL Study Number: 150608 Sponsor Study Number: NA

Sample Set I.D.: 608MV02-NEG Matrix: SOIL

Instrument I.D.: LC/MS/MS #3, Sciex API5000 LC/MS/MS with Shimadzu LC-20AD XR HPLC Pumps, Shimadzu CBM-20A Controller, Shimadzu SIL-20AC XR Autosampler and Analyst Data System Ver. 1.6.3

Column: Phenomenex Kinetex 2.6 µm C18 100A, 100 x 3.0 mm
Serial#: H16-065173 Part No. 00D-4462-Y0

Guard Column: SecurityGuard ULTRA Cartridge UHPLC C18 for 3.00 mm Part No. AJ0-8775

Flow Rate: 0.2 mL/minute **Injection Volume:** 10 µL

Mobile Phase:

A = 0.2% Formic Acid in Acetonitrile ID# EAS-031116
B = 0.2% Formic Acid in Water ID# EAS-031116

Gradient Program:

(0.0 minutes) 50% Mobile Phase A: 50% Mobile Phase B
(3.8 minutes) 50% Mobile Phase A: 50% Mobile Phase B
(3.9 minutes) 100% Mobile Phase A: 0% Mobile Phase B
(4.9 minutes) 100% Mobile Phase A: 0% Mobile Phase B
(5.0 minutes) 50% Mobile Phase A: 50% Mobile Phase B
(8.5 minutes) 50% Mobile Phase A: 50% Mobile Phase B

Run Time: 8.5 minutes, with a column heater set to 40 °C.

MS/MS Parameters:

Analyte	Transition Ions	Dwell Time (msec)	DP	CE
DCC-3825-M-20	305.0/273.9	150	-70	-13
DCC-3825-M-20	305.0/203.9	50	-70	-24
DCC-3825-M-72	400.9/249.8	150	-50	-42
DCC-3825-M-72	400.9/185.9	50	-50	-56
DCC-3825-M-73	402.9/249.9	150	-60	-50
DCC-3825-M-73	402.9/185.9	50	-60	-56

MRM scan using TurboIonSpray®, Negative polarity, Unit/Unit Resolution

CUR:	**35**	**IS:**	**-4500**	**TEM:**	**500**
GS1:	**40**	**GS2:**	**40**	**ihe:**	**ON**
CAD:	**12**	**EP:**	**-10**	**CXP:**	**-10**

Divert Valve:

0.0	**minutes**	**Waste**
1.8	**minutes**	**LC/MS/MS**
3.6	**minutes**	**Waste**

Prepared By: Elisabeth D Ackerman Date: 03/19/16

Revised: 08/04/2011

Golden Pacific Laboratories, LLC	Form #GPL-402

HPLC Parameters Form

GPL Study Number: 150608 Sponsor Study Number: NA

Sample Set I.D.: 608MV02-NEG Matrix: SOIL

Instrument I.D.: LC/MS/MS #3, Sciex API5000 LC/MS/MS with Shimadzu LC-20AD XR HPLC Pumps, Shimadzu CBM-20A Controller, Shimadzu SIL-20AC XR Autosampler and Analyst Data System Ver. 1.6.3

Calibration Standards:

Concentration①	Standard I.D.	Expiration Date
0.100/0.101/0.101/0.100/0.101/0.102/0.102/0.101 ng/mL	1598M-16	05/24/2016
0.200/0.202/0.202/0.200/0.202/0.204/0.204/0.202 ng/mL	1598M-15	05/24/2016
0.500/0.505/0.505/0.500/0.505/0.510/0.510/0.505 ng/mL	1598M-14	05/24/2016
1.00/1.01/1.01/1.00/1.01/1.02/1.02/1.01 ng/mL	1598M-13	05/24/2016
2.00/2.02/2.02/2.00/2.02/2.04/2.04/2.02 ng/mL	1598M-12	05/24/2016
5.00/5.05/5.05/5.00/5.05/5.10/5.10/5.05 ng/mL	1598M-11	05/24/2016

① **The concentrations are listed in the following order: DCC-3825-M-(20, 29, 30, 35, 63, 69, 72, 73).**

Prepared By: Elizabeth D Peterson Date: 03/19/16

Retention Time(s): DCC-3825-M-20: ~ 3.07 **minutes, DCC-3825-M-72:** ~ 2.56 **minutes, DCC-3825-M-73:** ~ 2.38 **minutes,** Recorded by /Date: EDP 03/21/16

Revised: 08/04/2011