

## Analytical method for tiafenacil (DCC-3825) and its metabolites M-01, M-12, M-13, M-36, and M-53 in water

**Reports:** ECM: EPA MRID No.: 50493807. Tasaki, S. 2017. Validation of an analytical method for the determination of DCC-3825 and its metabolites (M-01, M-12, M-13, M-36, and M-53) in Surface water and Drinking water. Document No.: MFT03717E. Unpublished study performed by Safety Science Research Laboratories, Ishihara Sangyo Kaisha, Ltd., Shiga-ken, Japan; sponsored by Ishihara Sangyo Kaisha, Ltd., Osaka, Japan, and FarmHannong Co., Ltd., Seoul, Korea; and submitted by ISK Biosciences Corporation, Concord, Ohio; 103 pages. Final report issued February 8, 2017.

ILV: EPA MRID No. 50493808. Perez, R. 2018. Independent Laboratory Validation of Method MFT03717E: "Validation of an analytical method for the determination of DCC-3825 and its metabolites (M-01, M-12, M-13, M-36, and M-53) in surface water and drinking water". ADPEN Study No. and Document No.: 17E1104. Report prepared by ADPEN Laboratories, Inc., Jacksonville, Florida; sponsored by Ishihara Sangyo Kaisha, Ltd., Osaka, Japan, and FarmHannong Co., Ltd., Seoul, Korea; and submitted by ISK Biosciences Corporation, Concord, Ohio; 292 pages. Final report issued January 25, 2018.

**Document No.:** MRIDs 50493807 & 50493808

**Guideline:** 850.6100

**Statements:** ECM: The study did not contain a statement which stated that it was conducted in accordance with Good Laboratory Practice (GLP) standards (p. 1C of MRID 50493807). Signed and dated No Data Confidentiality, GLP, and Authenticity statements were provided (pp. 1B-1C, 2). A Quality Assurance statement was not included.

ILV: The study was conducted in accordance with USEPA FIFRA GLP standards (40 CFR Part 160; pp. 1C, 3 of MRID 50493808). Signed and dated No Data Confidentiality, GLP, Quality Assurance, and Authenticity statements were provided (pp. 1B-1C, 2-4).

**Classification:** This analytical method is classified as **supplemental**. MRID 50493807 and 50493808 are reliable environmental chemistry methods (ECM) with an independent laboratory validation (ILV) to measure tiafenacil and the majority of degradates in water. ILV linearity for M-12 and M-36 were slightly below the recommended linearity value of 0.9950. The specificity of the method for M-53 was not supported by ILV representative chromatograms. The ECM did not report the method LOD.

**PC Code:** 012311

**EFED Final Reviewer:** Joshua Antoline, Ph.D.,  
Chemist

Signature:

**Antoline,  
Joshua** Digitally signed by  
Antoline, Joshua  
Date: 2020.07.17  
10:33:23 -04'00'


**CDM/CSS-Dynamac JV** Lisa Muto,  
Environmental Scientist

Signature:

*Lisa Muto*

**Reviewers:**

Joan Gaidos, Ph.D.,  
Environmental Scientist

Date: 12/18/2018  
Signature:   
Date: 12/21/2018

**EPA Reviewer:** Katrina White, Ph.D., Senior Scientist KATRINA WHITE Digitally signed by KATRINA WHITE  
Date: 2020.07.21 11:15:34 -04'00'

*This Data Evaluation Record may have been altered by the Environmental Fate and Effects Division subsequent to signing by CDM/CSS-Dynamac JV personnel. The CDM/CSS-Dynamac Joint Venture role does not include establishing Agency policies.*

**Executive Summary**

This analytical method, ISK Biosciences Analytical Method MFT03717E, is designed for the quantitative determination of tiafenacil (DCC-3825) and its metabolites M-01, M-12, M-13, M-36, and M-53 in water at the LOQ of 0.1 ng/mL using LC/MS/MS. The Limit of Quantitation (LOQ) for this method was 0.1 µg/L, which is less than the lowest aquatic toxicity endpoint in water (No observable adverse effect concentration of 0.769 µg/L for duckweed (*Lemna gibba*) (MRID 50486882)) for all analytes. The ECM and ILV used two characterized water matrices, surface and drinking waters.

For all analytes, the method was validated after the first trial with insignificant modifications of the analytical parameters and the use of matrix-matched standards. All submitted ILV and ECM data pertaining to precision, repeatability, and reproducibility was acceptable. ILV linearity was satisfactory for all analytes, but M-36; all ECM linearity >0.995. The specificity of the method for M-53 was not supported by ILV representative chromatograms because the analyte peak was not resolved from contaminants for accurate integration at LOQ. The specificity of the method for all other analytes was supported by ECM and ILV representative chromatograms; however, persistent nearby contaminants were observed in all M-12 and M-53 chromatograms. The LOD was not reported in the ECM.

**Table 1. Analytical Method Summary**

MRID		EPA Review	Matrix	Method Date (dd/mm/yyyy)	Registrant	Analysis	Limit of Quantitation (LOQ)
Environmental Chemistry Method	Independent Laboratory Validation						
50493807 <sup>1</sup>	50493808 <sup>2</sup>	Supplemental	Water	08/02/2017	ISK Biosciences Corporation	LC/MS/MS	0.1 ng/mL

1 In the ECM, the surface water (pH not reported, 1.8 mg/L total organic carbon, 83.8 mg/L total calcium, 246 mg/L hardness as CaCO<sub>3</sub>) obtained from Calwich Abbey Lake (supplied by Envigo CRS Ltd.) and drinking water (pH 7.8, total organic carbon 0.8 mg/L, 39 mg/L harness as Ca and Mg) obtained from Kusatsu City in November were used in the study (p. 14; Table 1, p. 22 of MRID 50493807).

2 In the ILV, the surface water (Lab Code # 170110002-019; pH 7.4, total organic carbon not reported, 1312 ppm total dissolved solids, 21 ppm calcium, 62 mg/L hardness as CaCO<sub>3</sub>) obtained from Pond Creek Lake and drinking water (Lab Code # 170110002-020; pH 8.2, total organic carbon not reported, 406 ppm total dissolved solids, 86 ppm calcium, 362 mg/L hardness as CaCO<sub>3</sub>) obtained as finished tap water were used in the study (p.

14; Appendix B, pp. 150-154 of MRID 50493808). Water characterization was performed by Agvise Laboratories, Northwood, North Dakota. The source of the drinking water was not further specified.

## I. Principle of the Method

Water samples (50 mL) were fortified (0.5 mL of either 10 ng/mL or 100 ng/mL solutions) and acidified with 500  $\mu$ L of acetic acid (pp. 15-16 of MRD 50493807). An Oasis HLB VAC RC (60 mg) solid phase extraction (SPE) column was pre-conditioned with 5 mL each of methanol then water:acetic acid (100:1, v:v). The acidified sample was applied to and passed through the column. After the column was washed with 5 mL of water, the analytes were eluted with 9.5 mL of methanol:water (70:30, v:v). The volume of the eluate was adjusted to 10 mL with methanol:water (70:30, v:v).

Analytes were identified and quantified by LC/MS using a Waters Acquity UPLC system coupled to an AB Sciex API 5000 mass spectrometer (pp. 17-18 of MRID 50493807). The following conditions were employed for all analytes, but M-01: Kinetex Biphenyl column (2.1  $\times$  150 mm, 2.6  $\mu$ m; column temperature 40°C) eluted with an isocratic mobile phase of 0.1% formic acid in water:0.1% formic acid in methanol (25:75, v:v); injection volume of 4  $\mu$ L; and positive ESI ionization MRM scan mode at 600°C heater gas temperature. The following conditions were employed for M-01: Kinetex Biphenyl column (2.1  $\times$  150 mm, 2.6  $\mu$ m; column temperature 40°C) eluted with an isocratic mobile phase of 0.1% formic acid in water:0.1% formic acid in methanol (35:65, v:v); injection volume of 4  $\mu$ L; and positive ESI ionization MRM scan mode at 600°C heater gas temperature. Analytes were identified using two ion transitions (quantitation and confirmation, respectively):  $m/z$  512.2 $\rightarrow$ 381.0 and  $m/z$  512.2 $\rightarrow$ 152.2 for tiafenacil (DCC-3825),  $m/z$  498.1 $\rightarrow$ 381.0 and  $m/z$  498.1 $\rightarrow$ 359.1 for M-01,  $m/z$  427.2 $\rightarrow$ 380.7 and  $m/z$  427.2 $\rightarrow$ 152.0 for M-12,  $m/z$  426.1 $\rightarrow$ 380.9 and  $m/z$  426.1 $\rightarrow$ 152.0 for M-13,  $m/z$  443.1 $\rightarrow$ 218.1 and  $m/z$  443.1 $\rightarrow$ 353.0 for M-36, and  $m/z$  445.1 $\rightarrow$ 371.0 and  $m/z$  445.1 $\rightarrow$ 355.0 for M-53. Expected retention times were 1.74, 2.21, 1.63, 1.33, 1.43, and 1.11 minutes for tiafenacil (DCC-3825), M-01, M-12, M-13, M-36, and M-53, respectively.

In the ILV, the ECM was performed as written, except for minor LC/MS parameter modifications and the fact that the same parameters were employed for all analytes (pp. 13, 16, 26; Table 26, p. 55 of MRID 50493808). Additionally, matrix-matched standards were necessary to obtain acceptable recoveries. The following conditions were employed for all analytes: Kinetex Biphenyl column (2.1  $\times$  150 mm, 2.6  $\mu$ m; column temperature 40°C) eluted with an isocratic mobile phase of 0.1% formic acid in water:0.1% formic acid in methanol (25:75, v:v); injection volume of 40  $\mu$ L; and positive ESI ionization MRM scan mode at 500°C heater gas temperature. Analytes were identified using two ion transitions (quantitation and confirmation, respectively):  $m/z$  512 $\rightarrow$ 381 and  $m/z$  512 $\rightarrow$ 152 for tiafenacil (DCC-3825),  $m/z$  498 $\rightarrow$ 381 and  $m/z$  498 $\rightarrow$ 59 for M-01,  $m/z$  427 $\rightarrow$ 381 and  $m/z$  427 $\rightarrow$ 152 for M-12,  $m/z$  426 $\rightarrow$ 381 and  $m/z$  426 $\rightarrow$ 152 for M-13,  $m/z$  443 $\rightarrow$ 218 and  $m/z$  443 $\rightarrow$ 353 for M-36, and  $m/z$  445 $\rightarrow$ 371 and  $m/z$  445 $\rightarrow$ 355 for M-53. These MS transitions were similar to those of the ECM, except for the M-01 confirmation transition. Expected retention times were *ca.* 2.15, 1.63, 2.02, 1.61, 1.72, and 1.32 minutes for tiafenacil (DCC-3825), M-01, M-12, M-13, M-36, and M-53, respectively.

The Limit of Quantification (LOQ) was 0.1 ng/mL in the ECM and ILV (pp. 9, 20 of MRID 50493807; pp. 6, 13 of MRID 50493808). The Limit of Detection (LOD) for all analytes in water

was reported as 0.03 ng/mL (30% of the LOQ) in the ILV; the LOD was not specifically reported in the ECM, but <30% of the LOQ was reported as “no apparent response”.

## II. Recovery Findings

ECM (MRID 50493807): Mean recoveries and relative standard deviations (RSDs) were within guideline requirements (mean 70-120%; RSD  $\leq$ 20%) for analysis of tiafenacil (DCC-3825), M-01, M-12, M-13, M-36, and M-53 at fortification levels of 0.1 ng/mL (LOQ) and 1.0 ng/mL (10 $\times$ LOQ) in two water matrices (Tables 2-25, pp. 23-34; DER Attachment 2). All analytes were identified using two ion transitions; performance data (recovery results) for the quantitation and confirmation ion analyses were comparable. The surface water (pH not reported, 1.8 mg/L total organic carbon, 83.8 mg/L total calcium, 246 mg/L hardness as CaCO<sub>3</sub>) obtained from Calwich Abbey Lake (supplied by Envigo CRS Ltd.) and drinking water (pH 7.8, total organic carbon 0.8 mg/L, 39 mg/L harness as Ca and Mg) obtained from Kusatsu City in November were used in the study (p. 14; Table 1, p. 22).

ILV (MRID 50493808): Mean recoveries and RSDs were within guideline requirements for analysis of tiafenacil (DCC-3825), M-01, M-12, M-13, M-36, and M-53 at fortification levels of 0.1 ng/mL (LOQ) and 1.0 ng/mL (10 $\times$ LOQ) in two water matrices (pp. 7-10). All analytes were identified using two ion transitions; performance data (recovery results) for the quantitation and confirmation ion analyses were comparable. The surface water (Lab Code # 170110002-019; pH 7.4, total organic carbon not reported, 1312 ppm total dissolved solids, 21 ppm calcium, 62 mg/L hardness as CaCO<sub>3</sub>) obtained from Pond Creek Lake and drinking water (Lab Code # 170110002-020; pH 8.2, total organic carbon not reported, 406 ppm total dissolved solids, 86 ppm calcium, 362 mg/L hardness as CaCO<sub>3</sub>) obtained as finished tap water were used in the study (p. 14; Appendix B, pp. 150-154). Water characterization was performed by Agvise Laboratories, Northwood, North Dakota. The source of the drinking water was not further specified. For all analytes, the method was validated after the first trial with insignificant modifications of the analytical parameters and the use of matrix-matched standards (pp. 7, 26).

**Table 2. Initial Validation Method Recoveries for Tiafenacil (DCC-3825) and its Metabolites M-01, M-12, M-13, M-36, and M-53 in Water<sup>1,2</sup>**

Analyte	Fortification Level (ng/mL)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%) <sup>3</sup>	Relative Standard Deviation (%)
<b>Surface Water</b>						
Quantitation Ion Transition						
Tiafenacil (DCC-3825)	0.1	5	97.1-101.1	99	1.4	1.5
	1.0	5	97.9-100.6	99	1.1	1.1
M-01	0.1	5	94.5-100.2	98	2.2	2.2
	1.0	5	95.5-101.1	98	2.0	2.1
M-12	0.1	5	88.2-98.8	94	4.0	4.2
	1.0	5	96.4-99.4	97	1.2	1.3
M-13	0.1	5	98.2-100.0	99	0.7	0.7
	1.0	5	94.3-99.4	97	1.8	1.9
M-36	0.1	5	93.6-108.0	100	5.2	5.2
	1.0	5	97.0-99.4	99	1.1	1.1
M-53	0.1	5	93.5-100.9	99	3.2	3.2
	1.0	5	97.0-100.1	98	1.2	1.2
Confirmation Ion Transition						
Tiafenacil (DCC-3825)	0.1	5	95.2-99.2	97	1.8	1.8
	1.0	5	93.1-98.2	96	2.2	2.2
M-01	0.1	5	87.5-98.2	92	4.2	4.6
	1.0	5	91.8-96.9	95	2.1	2.2
M-12	0.1	5	89.6-96.0	93	2.4	2.6
	1.0	5	93.1-96.4	95	1.3	1.4
M-13	0.1	5	95.7-101.5	99	2.5	2.5
	1.0	5	96.7-100.3	99	1.5	1.5
M-36	0.1	5	91.4-101.2	97	4.4	4.5
	1.0	5	96.8-98.6	98	0.8	0.8
M-53	0.1	5	94.1-97.6	95	1.4	1.5
	1.0	5	98.6-102.5	100	1.5	1.4
<b>Drinking Water</b>						
Quantitation Ion Transition						
Tiafenacil (DCC-3825)	0.1	5	86.6-95.7	92	3.7	4.0
	1.0	5	92.6-98.4	96	2.8	2.9
M-01	0.1	5	94.7-99.3	97	1.8	1.8
	1.0	5	93.5-98.7	97	2.1	2.2
M-12	0.1	5	88.5-91.8	90	1.7	1.9
	1.0	5	91.8-96.0	94	1.9	2.0
M-13	0.1	5	94.8-98.0	97	1.4	1.4
	1.0	5	95.9-97.8	97	0.7	0.7
M-36	0.1	5	99.8-107.0	102	3.2	3.1
	1.0	5	96.2-98.9	97	1.1	1.2
M-53	0.1	5	91.2-99.2	95	3.5	3.8
	1.0	5	96.3-98.8	98	1.0	1.0

Analyte	Fortification Level (ng/mL)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%) <sup>3</sup>	Relative Standard Deviation (%)
Confirmation Ion Transition						
Tiafenacil (DCC-3825)	0.1	5	84.4-91.9	89	3.4	3.8
	1.0	5	89.6-95.7	93	2.8	3.0
M-01	0.1	5	86.1-94.6	89	3.1	3.9
	1.0	5	91.2-97.2	95	2.5	2.7
M-12	0.1	5	78.8-95.9	89	6.4	7.1
	1.0	5	92.0-94.9	93	1.2	1.2
M-13	0.1	5	93.6-98.2	96	2.1	2.1
	1.0	5	97.7-98.7	98	0.4	0.4
M-36	0.1	5	92.7-106.1	99	5.0	5.1
	1.0	5	96.1-99.6	97	1.3	1.4
M-53	0.1	5	90.4-102.6	97	5.0	5.1
	1.0	5	99.0-100.9	100	0.8	0.8

Data (uncorrected recovery results, p. 19) were obtained from Tables 2-25, pp. 23-34 of MRID 50493807 and DER Attachment 2.

1 The surface water (pH not reported, 1.8 mg/L total organic carbon, 83.8 mg/L total calcium, 246 mg/L hardness as CaCO<sub>3</sub>) obtained from Calwich Abbey Lake (supplied by Envigo CRS Ltd.) and drinking water (pH 7.8, total organic carbon 0.8 mg/L, 39 mg/L hardness as Ca and Mg) obtained from Kusatsu City in November were used in the study (p. 14; Table 1, p. 22).

2 Analytes were identified using two ion transitions (quantitation and confirmation, respectively): *m/z* 512.2→381.0 and *m/z* 512.2→152.2 for tiafenacil (DCC-3825), *m/z* 498.1→381.0 and *m/z* 498.1→359.1 for M-01, *m/z* 427.2→380.7 and *m/z* 427.2→152.0 for M-12, *m/z* 426.1→380.9 and *m/z* 426.1→152.0 for M-13, *m/z* 443.1→218.1 and *m/z* 443.1→353.0 for M-36, and *m/z* 445.1→371.0 and *m/z* 445.1→355.0 for M-53.

3 Standard deviations were reviewer-calculated since these values were not reported in the study report. Rules of significant figures were followed.

**Table 3. Independent Validation Method Recoveries for Tiafenacil (DCC-3825) and its Metabolites M-01, M-12, M-13, M-36, and M-53 in Water<sup>1,2</sup>**

Analyte	Fortification Level (ng/mL)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Surface Water						
Quantitation Ion Transition						
Tiafenacil (DCC-3825)	0.1	5	92-99	95	2.9	3.1
	1.0	5	88-93	91	1.9	2.1
M-01	0.1	5	117-121	118	1.7	1.4
	1.0	5	108-111	110	1.2	1.1
M-12	0.1	5	75-84	79	4.8	6.0
	1.0	5	74-81	77	3.1	4.1
M-13	0.1	5	82-87	83	2.2	2.7
	1.0	5	78-80	78	1.0	1.2
M-36	0.1	5	71-77	73	2.5	3.3
	1.0	5	76-80	77	2.3	3.0
M-53	0.1	5	93-119	100	10.7	10.7
	1.0	5	94-113	99	7.7	7.8
Confirmation Ion Transition						
Tiafenacil (DCC-3825)	0.1	5	96-102	98	2.6	2.7

Analyte	Fortification Level (ng/mL)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
	1.0	5	94-95	95	0.4	0.4
M-01	0.1	5	113-117	114	1.7	1.5
	1.0	5	111-114	112	1.2	1.1
M-12	0.1	5	73-99	83	10.4	12.6
	1.0	5	74-89	81	7.1	8.7
M-13	0.1	5	78-85	80	2.7	3.4
	1.0	5	77-80	78	1.2	1.6
M-36	0.1	5	69-74	71	2.2	3.1
	1.0	5	75-81	77	2.3	3.0
M-53	0.1	5	95-116	103	9.1	8.8
	1.0	5	92-112	98	7.9	8.1
<b>Drinking Water</b>						
Quantitation Ion Transition						
Tiafenacil (DCC-3825)	0.1	5	94-110	106	7.0	6.6
	1.0	5	97-104	100	2.6	2.6
M-01	0.1	5	104-113	110	3.9	3.6
	1.0	5	104-106	105	0.9	0.9
M-12	0.1	5	86-107	97	8.5	8.8
	1.0	5	83-94	90	4.8	5.3
M-13	0.1	5	102-117	112	6.3	5.6
	1.0	5	101-109	105	3.1	2.9
M-36	0.1	5	88-98	94	3.9	4.2
	1.0	5	96-99	97	1.1	1.1
M-53	0.1	5	104-113	108	3.5	3.2
	1.0	5	103-111	106	3.2	3.0
Confirmation Ion Transition						
Tiafenacil (DCC-3825)	0.1	5	95-111	107	6.6	6.2
	1.0	5	99-103	101	1.5	1.4
M-01	0.1	5	99-111	107	4.7	4.4
	1.0	5	104-106	105	0.9	0.8
M-12	0.1	5	86-98	94	5.0	5.4
	1.0	5	89-102	95	5.8	6.1
M-13	0.1	5	103-118	112	6.1	5.4
	1.0	5	102-110	107	3.2	3.0
M-36	0.1	5	85-99	94	6.0	6.4
	1.0	5	95-98	97	1.2	1.2
M-53	0.1	5	106-129	117	8.4	7.2
	1.0	5	104-115	107	4.3	4.0

Data (uncorrected recovery results, Appendix D, pp. 257-280) were obtained from pp. 7-10 of MRID 50493808.

1 The surface water (Lab Code # 170110002-019; pH 7.4, total organic carbon not reported, 1312 ppm total dissolved solids, 21 ppm calcium, 62 mg/L hardness as CaCO<sub>3</sub>) obtained from Pond Creek Lake and drinking water (Lab Code # 170110002-020; pH 8.2, total organic carbon not reported, 406 ppm total dissolved solids, 86 ppm calcium, 362 mg/L hardness as CaCO<sub>3</sub>) obtained as finished tap water were used in the study (p. 14;

Appendix B, pp. 150-154). Water characterization was performed by Agvise Laboratories, Northwood, North Dakota. The source of the drinking water was not further specified.

2 Analytes were identified using two ion transitions (quantitation and confirmation, respectively):  $m/z$  512→381 and  $m/z$  512→152 for tiafenacil (DCC-3825),  $m/z$  498→381 and  $m/z$  498→59 for M-01,  $m/z$  427→381 and  $m/z$  427→152 for M-12,  $m/z$  426→381 and  $m/z$  426→152 for M-13,  $m/z$  443→218 and  $m/z$  443→353 for M-36, and  $m/z$  445→371 and  $m/z$  445→355 for M-53. These MS transitions were similar to those of the ECM, except for the M-01 confirmation transition.

### III. Method Characteristics

The LOQ for all analytes in water was 0.1 ng/mL in the ECM and ILV (pp. 9, 20 of MRID 50493807; pp. 6, 13, 26 of MRID 50493808). In the ECM, the LOQ was defined as the lowest fortification level tested which yielded acceptable results (mean recovery 70-110%, RSD <20%) and blanks not exceeding 30%. The LOQ was reported in the ILV from the ECM without justification. No calculations were reported to support the LOQ. The LOD for all analytes in water was reported as 0.03 ng/mL (30% of the LOQ) in the ILV; the LOD was not specifically reported in the ECM, but <30% of the LOQ was reported as “no apparent response”. In the ILV, the LOD was defined as the absolute amount of analyte injected into the LC/MS/MS using the lowest calibration standard (0.1 ng/mL). The ILV also reported that acceptable signal-to-noise ratios (S/N >3:1) were demonstrated for all analytes at the LOD; no calculations for the LOD were provided in the ILV.



Table 4. Method Characteristics

Analyte		Tiafenacil (DCC-3825)	M-01	M-12	M-13	M-36	M-53	
Limit of Quantitation (LOQ)		0.1 ng/mL						
Limit of Detection (LOD)	ECM	<b>Not reported</b> <sup>1</sup>						
	ILV	0.03 ng/mL						
Linearity (calibration curve $r^2$ and concentration range) <sup>2</sup>	ECM	$r^2 = 0.9990$ (Q) $r^2 = 0.9998$ (C)	$r^2 = 1.0000$ (Q) $r^2 = 0.9990$ (C)	$r^2 = 0.9996$ (Q & C)	$r^2 = 0.9996$ (Q) $r^2 = 0.9992$ (C)	$r^2 = 0.9998$ (Q) $r^2 = 0.9996$ (C)	$r^2 = 0.9998$ (Q & C)	
	ILV	Surface	$r^2 = 0.9990$ (Q) $r^2 = 0.9988$ (C)	$r^2 = 0.9996$ (Q) $r^2 = 0.9999$ (C)	$r^2 = 0.9991$ (Q) $r^2 = \mathbf{0.9936}$ (C)	$r^2 = 0.9998$ (Q & C)	$r^2 = \mathbf{0.9908}$ (Q) $r^2 = \mathbf{0.9924}$ (C)	$r^2 = 0.9999$ (Q) $r^2 = 0.9998$ (C)
		Drinking	$r^2 = 0.9992$ (Q & C)	$r^2 = 1.0000$ (Q) $r^2 = 0.9998$ (C)	$r^2 = 0.9986$ (Q) $r^2 = 0.9992$ (C)	$r^2 = 0.9998$ (Q) $r^2 = 1.0000$ (C)	$r^2 = \mathbf{0.9918}$ (Q) $r^2 = \mathbf{0.9922}$ (C)	$r^2 = 0.9998$ (Q & C)
	Concentration range	0.10-10 ng/mL						
Repeatable	ECM <sup>3</sup>	Yes at LOQ and 10×LOQ (characterized surface and drinking waters).						
	ILV <sup>4,5</sup>							
Reproducible		Yes at LOQ and 10×LOQ.						
Specificity	ECM	Yes, matrix interferences were <2% of the LOQ (based on peak area).	Yes, matrix interferences were <2% of the LOQ (based on peak area).	Yes, no matrix interferences were observed. Persistent contaminants at RT - 0.3 min of analyte were observed in all chromatograms.	Yes, matrix interferences were <3% of the LOQ (based on peak area).	Yes, matrix interferences were <3% of the LOQ (based on peak area). Peak fronting was noted.	Yes, matrix interferences were <4% of the LOQ (based on peak area). Persistent contaminants at RT ± 0.3 min of analyte were observed in all chromatograms.	
	ILV	Yes, no matrix interferences were observed.	Yes, no matrix interferences were observed.	Yes, no matrix interferences were observed. Elevated, sloping baseline was noted. Elevated baseline noise was noted around the analyte peak at LOQ. Persistent contaminants at RT	Yes, matrix interferences were <1% of the LOQ (based on peak area). Elevated baseline noise was noted around the analyte peak at LOQ.	Yes, matrix interferences were <2% of the LOQ (based on peak area). Peak fronting was noted.	<b>No</b> , matrix interferences were not observed in Q ion and <i>ca.</i> 12% of the LOQ in C ion (based on peak area); however, analyte peak was not resolved for accurate integration	

				- 0.4 min of analyte were observed in all chromatograms.			at LOQ <sup>7</sup> . Persistent contaminants at RT ± 0.3 min of analyte were observed in all chromatograms.
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Data were obtained from pp. 9, 20 (LOQ/LOD); Tables 2-25, pp. 23-34 (recovery data); Figures 1-12, pp. 35-40 (calibration curve); Figures 13-120, pp. 41-94 (chromatograms) of MRID 50493807; pp. 6, 13, 26 (LOQ/LOD); pp. 7-10 (recovery data); Figures 1-6, pp. 57-62 (calibration curves); Figures 7-54, pp. 63-140 (chromatograms); Appendix D, pp. 257-280 (calibration data) of MRID 50493808; DER Attachment 2. Q = Quantitation ion transition; C = Confirmatory ion transition.

- 1 The LOD was not specifically reported in the ECM, but <30% of the LOQ was reported as “no apparent response” (p. 20 of MRID 50493807).
- 2 Reported correlation coefficients were reviewer-calculated from r values reported in the study report (Figures 1-12, pp. 35-40 of MRID 50493807; Figures 1-6, pp. 57-62 and Appendix D, pp. 257-280 of MRID 50493808; DER Attachment 2). Solvent standards were used in the ECM (p. 15 of MRID 50493807). Matrix-matched standards were used in the ILV (p. 9 of MRID 50493808). Significant figures of  $r^2$  were limited to four.
- 3 In the ECM, the surface water (pH not reported, 1.8 mg/L total organic carbon, 83.8 mg/L total calcium, 246 mg/L hardness as  $\text{CaCO}_3$ ) obtained from Calwich Abbey Lake (supplied by Envigo CRS Ltd.) and drinking water (pH 7.8, total organic carbon 0.8 mg/L, 39 mg/L harness as Ca and Mg) obtained from Kusatsu City in November were used in the study (p. 14; Table 1, p. 22 of MRID 50493807).
- 4 In the ILV, the surface water (Lab Code # 170110002-019; pH 7.4, total organic carbon not reported, 1312 ppm total dissolved solids, 21 ppm calcium, 62 mg/L hardness as  $\text{CaCO}_3$ ) obtained from Pond Creek Lake and drinking water (Lab Code # 170110002-020; pH 8.2, total organic carbon not reported, 406 ppm total dissolved solids, 86 ppm calcium, 362 mg/L hardness as  $\text{CaCO}_3$ ) obtained as finished tap water were used in the study (p. 14; Appendix B, pp. 150-154 of MRID 50493808). Water characterization was performed by Agvise Laboratories, Northwood, North Dakota. The source of the drinking water was not further specified.
- 5 For all analytes, the method was validated after the first trial with insignificant modifications of the analytical parameters and the use of matrix-matched standards (pp. 7, 26 of MRID 50493808).
- 6 See Figures 49-66, pp. 59-67; Figures 103-120, pp. 86-94 of MRID 50493807; and Figures 23-24, pp. 109-110; Figures 35-36, pp. 121-122; Figures 44-45, pp. 130-131; Figures 52-53, pp. 138-139 of MRID 50493808.
- 7 Based on Figure 35, p. 121 and Figure 53, p. 139 of MRID 50493808. Linearity is satisfactory when  $r^2 \geq 0.995$ .

#### IV. Method Deficiencies and Reviewer's Comments

1. In the ILV, the linearity was not satisfactory for M-36 [surface water,  $r^2 = 0.9908$  (Q) and  $0.9924$  (C); drinking water,  $r^2 = 0.9918$  (Q) and  $0.9922$  (C)], as well as the confirmation ion analysis of M-12 ( $r^2 = 0.9936$ ; Figures 1-6, pp. 57-62 and Appendix D, pp. 257-280 of MRID 50493808; DER Attachment 2). Linearity is satisfactory when  $r^2 \geq 0.995$ . The contracted reviewer noted that linearity deviations in the confirmation ion analyses do not affect the validity of the method since a confirmation method is not usually required when LC/MS or GC/MS is used as the primary method to generate study data.
2. The specificity of the method for M-53 was not supported by ILV representative chromatograms because the analyte peak was not resolved from contaminants for accurate integration at LOQ (Figure 35, p. 121 and Figure 53, p. 139 of MRID 50493808). The LC parameters should be modified to enhance the separation of the analyte from the contaminants.
3. The LOD was not specifically reported in the ECM, but <30% of the LOQ was reported as “no apparent response” (pp. 9, 20 of MRID 50493807).
4. Persistent contaminants at RT – 0.3 to 0.4 min. of M-12 and  $\pm 0.3$  min of M-53 were observed in all M-12 and M-53 chromatograms of the ECM and ILV (Figures 49-66, pp. 59-67; Figures 103-120, pp. 86-94 of MRID 50493807; and Figures 23-24, pp. 109-110; Figures 35-36, pp. 121-122; Figures 44-45, pp. 130-131; Figures 52-53, pp. 138-139 of MRID 50493808). The study author did not address these contaminants. The contracted reviewer noted that the chemical purities of M-12 and M-53 were 97.4-98.6% and 93.9-94.7%, respectively, in the ECM and ILV (pp. 13-14 of MRID 50493807; pp. 15-16 of MRID 50493808).
5. The estimation of LOQ and LOD in ECM and ILV was not based on scientifically acceptable procedures as defined in 40 CFR Part 136 (pp. 9, 20 of MRID 50493807; pp. 6, 13, 26 of MRID 50493808). In the ECM, the LOQ was defined as the lowest fortification level tested which yielded acceptable results (mean recovery 70-110%, RSD <20%) and blanks not exceeding 30%. The LOQ was reported in the ILV from the ECM without justification. No calculations were reported to support the LOQ. In the ILV, the LOD was defined as the absolute amount of analyte injected into the LC/MS/MS using the lowest calibration standard (0.1 ng/mL). The ILV also reported that acceptable signal-to-noise ratios (S/N >3:1) were demonstrated for all analytes at the LOD; no calculations for the LOD were provided in the ILV. Detection limits should not be based on arbitrary values.
6. In the ECM, the pH of the surface water matrix was not reported (p. 14; Table 1, p. 22 of MRID 50493807).
7. The ILV reported that no communication between the ILV Study Director and Study Monitor occurred, and the sponsors did not visit the testing facility during the study (p. 26 of MRID 50493808).

8. In the ILV, the time required to complete the extraction of one set of 13 samples required *ca.* 18 hours of work, including calculation of results (p. 25 of MRID 50493808).

## V. References

U.S. Environmental Protection Agency. 2012. Ecological Effects Test Guidelines, OCSPP 850.6100, Environmental Chemistry Methods and Associated Independent Laboratory Validation. Office of Chemical Safety and Pollution Prevention, Washington, DC. EPA 712-C-001.

40 CFR Part 136. Appendix B. Definition and Procedure for the Determination of the Method Detection Limit-Revision 1.11, pp. 317-319.

## Attachment 1: Chemical Names and Structures

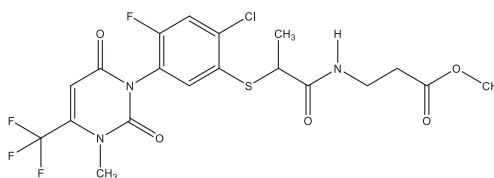
## Tiafenacil (DCC-3825)

**IUPAC Name:** Methyl 3-[(2RS)-2-{2-chloro-4-fluoro-5-[1,2,3,6-tetrahydro-3-methyl-2,6-dioxo-4-(trifluoromethyl)pyrimidin-1(6H)-yl]phenylthio}propionamido]propionate

**CAS Name:** Methyl N-[2-[[2-chloro-5-[3,6-dihydro-3-methyl-2,6-dioxo-4-(trifluoromethyl)-1(2H)-pyrimidinyl]-4-fluorophenyl]thio]-1-oxopropyl]-β-alaninate

**CAS Number:** 1220411-29-9

**SMILES String:** O=C(C=C(C(F)(F)F)N1C)N(C2=C(F)C=C(Cl)C(SC(C)C(N([H])CCC(O)C)=O)=O)=C2)C1=O



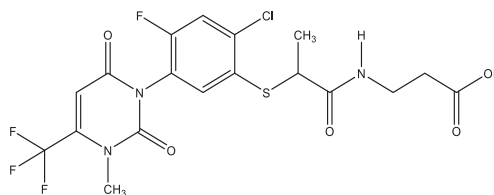
## M-01 (DCC-3825-M-01)

**IUPAC Name:** 3-(2-((2-Chloro-4-fluoro-5-(3-methyl-2,6-dioxo-4-(trifluoromethyl)-3,6-dihydropyrimidin-1(2H)-yl)phenyl)thio)propanamido)propanoic acid

**CAS Name:** Not reported

**CAS Number:** Not reported

**SMILES String:** O=C(C=C(C(F)(F)F)N1C)N(C2=C(F)C=C(Cl)C(SC(C)C(N([H])CCC(O)=O)=O)=O)=C2)C1=O



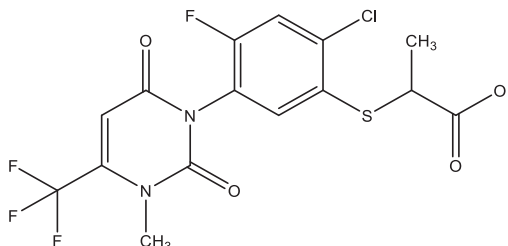
**M-12 (DCC-3825-M-12)**

**IUPAC Name:** 2-((2-Chloro-4-fluoro-5-(3-methyl-2,6-dioxo-4-(trifluoromethyl)-3,6-dihydropyrimidin-1(2H)-yl)phenyl)thio)propanoic acid

**CAS Name:** Not reported

**CAS Number:** Not reported

**SMILES String:** O=C(C=C(C(F)(F)F)N1C)N(C2=C(F)C=C(Cl)C(SC(C)C(O)=O)=C2)C1=O

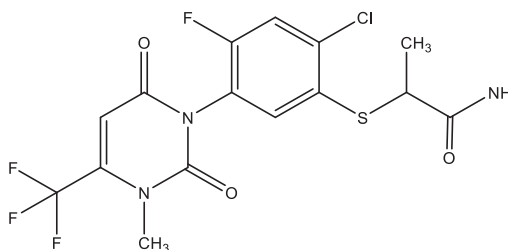
**M-13 (DCC-3825-M-13)**

**IUPAC Name:** 2-((2-Chloro-4-fluoro-5-(3-methyl-2,6-dioxo-4-(trifluoromethyl)-3,6-dihydropyrimidin-1(2H)-yl)phenyl)thio)propanamide

**CAS Name:** Not reported

**CAS Number:** Not reported

**SMILES String:** O=C(C=C(C(F)(F)F)N1C)N(C2=C(F)C=C(Cl)C(SC(C)C(N)=O)=C2)C1=O



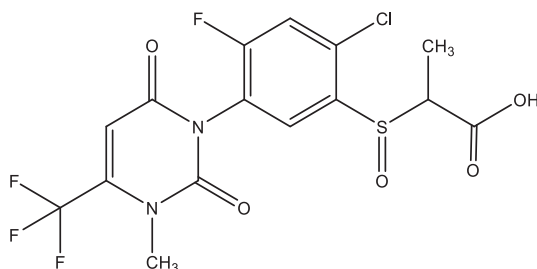
**M-36 (DCC-3825-M-36)**

**IUPAC Name:** 2-((2-Chloro-4-fluoro-5-(3-methyl-2,6-dioxo-4-(trifluoromethyl)-3,6-dihydropyrimidin-1(2H)-yl)phenyl)sulfinyl)propanoic acid

**CAS Name:** Not reported

**CAS Number:** Not reported

**SMILES String:** CN(C(N1C2=C(F)C=C(Cl)C(S(C(C)C(O)=O)=O)=C2)=O)C(C(F)(F)F)=CC1=O

**M-53 (DCC-3825-M-53)**

**IUPAC Name:** 2-((2-Chloro-4-fluoro-5-(3-methyl-2,6-dioxo-4-(trifluoromethyl)tetrahydropyrimidin-1(2H)-yl)phenyl)sulfinyl)propanoic acid

**CAS Name:** Not reported

**CAS Number:** Not reported

**SMILES String:** CN(C(N1C2=C(F)C=C(Cl)C(S(C(C)C(O)=O)=O)=C2)=O)C(C(F)(F)F)C1=O

