Dongbu Farm Hannong

Dongbu Farm Hannong Co., Ltd., Dongbu Advanced Research Institute,
39-23, Dongan-ro 1113, Yeonmu-eup, Nonsan-si, Chungcheongnam-do, Republic of Korea 33010
Tel : +82-41-730-9169, Fax : +82-41- 742-8510

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

Dongbu Farm Hannong Co., Ltd., Dongbu Advanced Research Institute,
39-23, Dongan-ro 1113, Yeonmu-eup, Nonsan-si, Chungcheongnam-do, Republic of Korea 33010
Tel : +82-41-730-9169, Fax : +82-41- 742-8510

Chemical structure :

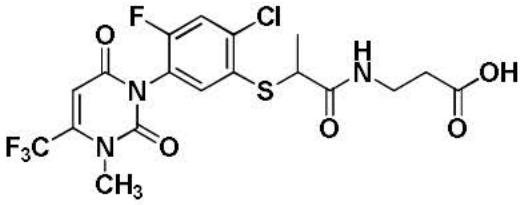
Molecular Weight : 511.88

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 :



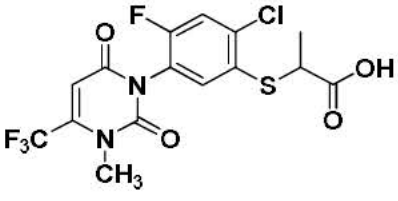
Molecular Weight : 497.85

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 %

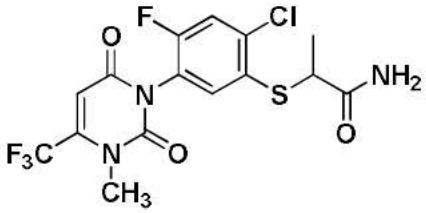
Dongbu Farm Hannong

Dongbu Farm Hannong Co., Ltd., Dongbu Advanced Research Institute,
39-23, Dongan-ro 1113, Yeonmu-eup, Nonsan-si, Chungcheongnam-do, Republic of Korea 33010
Tel : +82-41-730-9169, Fax : +82-41- 742-8510

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 :



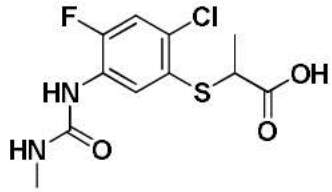
Molecular Weight : 425.79

Purity : 99.4 %

Identity : **DCC3825-M-20**

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

Chemical structure :



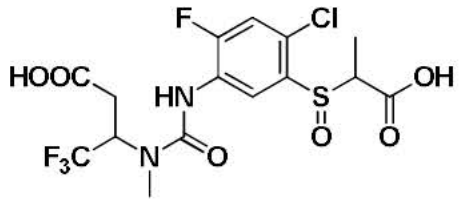
Molecular Weight : 306.74

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 %

Dongbu Farm Hannong

Dongbu Farm Hannong Co., Ltd., Dongbu Advanced Research Institute,
39-23, Dongan-ro 1113, Yeonmu-eup, Nonsan-si, Chungcheongnam-do, Republic of Korea 33010
Tel : +82-41-730-9169, Fax : +82-41- 742-8510

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 :

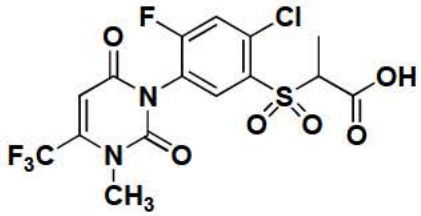
Molecular Weight : 478.80

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 :



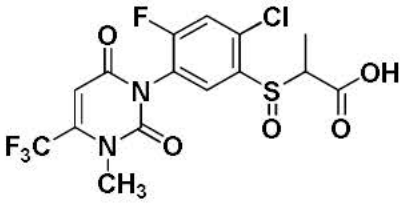
Molecular Weight : 458.77

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 %

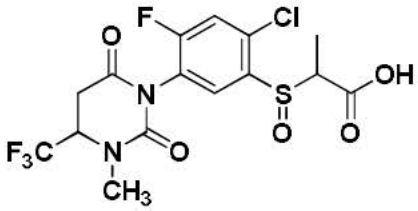
Dongbu Farm Hannong

Dongbu Farm Hannong Co., Ltd., Dongbu Advanced Research Institute,
39-23, Dongan-ro 1113, Yeonmu-eup, Nonsan-si, Chungcheongnam-do, Republic of Korea 33010
Tel : +82-41-730-9169, Fax : +82-41- 742-8510

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 :



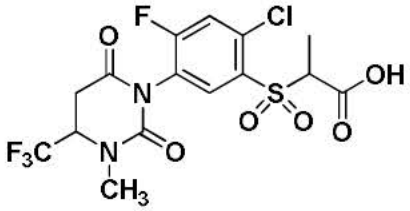
Molecular Weight : 444.79

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 %

Dongbu Farm Hannong

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

39-23, Dongan-ro 1113, Yeonmu-eup, Nonsan-si, Chungcheongnam-do, Republic of Korea 33010

Tel : +82-41-730-9169, Fax : +82-41- 742-8510

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 :

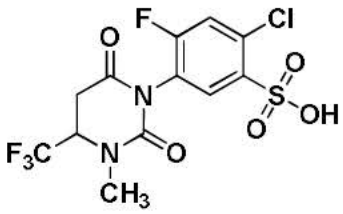
Molecular Weight : 402.71

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 %

Dongbu Farm Hannong Co., Ltd., Dongbu Advanced Research Institute,
39-23, Dongan-ro 1113, Yeonmu-eup, Nonsan-si, Chungcheongnam-do, Republic of Korea 33010
Tel : +82-41-730-9169, Fax : +82-41- 742-8510

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

Dongbu Farm Hannong Co., Ltd., Dongbu Advanced Research Institute,
39-23, Dongan-ro 1113, Yeonmu-eup, Nonsan-si, Chungcheongnam-do, Republic of Korea 33010
Tel : +82-41-730-9169, Fax : +82-41- 742-8510

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

Dongbu Farm Hannong Co., Ltd., Dongbu Advanced Research Institute,
39-23, Dongan-ro 1113, Yeonmu-eup, Nonsan-si, Chungcheongnam-do, Republic of Korea 33010
Tel : +82-41-730-9169, Fax : +82-41- 742-8510

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

Dongbu Farm Hannong Co., Ltd., Dongbu Advanced Research Institute,
39-23, Dongan-ro 1113, Yeonmu-eup, Nonsan-si, Chungcheongnam-do, Republic of Korea 33010
Tel : +82-41-730-9169, Fax : +82-41- 742-8510

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.

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