SUMMARY

A method, based on the analytical method in soil and sediment (Reference1 and 2), was validated for the determination of DCC-3825 and its Metabolites (M-01, M-12, M-13, M-36, M-53) in two sediment types.

Samples were extracted with an acetonitrile/water/formic acid mixture, and clean-up with an OASIS HLB solid phase extraction (SPE) cartridge. Quantitation was performed using liquid chromatography with tandem mass spectrometric detection (LC/MS/MS).

Method validation

Since the correlation coefficient for the calibration curve was over 0.999 for DCC-3825 and its metabolites, linearity was demonstrated. Untreated samples of each sediment were analyzed using the analytical method and there was no apparent response (i.e. <30% of the LOQ) in the region in the chromatograms corresponding to the retention time of DCC-3825 and its metabolites. Therefore, specificity of the method was demonstrated. The recovery test was performed with fortification levels at 0.01 and 0.1 mg/kg for DCC-3825 and its metabolites. A LC/MS/MS scan with a different transition was used for confirmation. As a result, acceptable accuracy and precision were obtained (mean recovery in the range of 70-110 % and RSD<20%) for quantitation and confirmation monitored. The accuracy and precision data are summarized in the following tables.

1 OBJECTIVE

The objective of this study is to validate an analytical method for the determination of DCC-3825 and its metabolites (M-01, M-12, M-13, M-36, M-53) in sediment.

Guideline and Guidance

EPA (Environmental Protection Agency) US: Residue Analytical Method, OPPTS 860.1340, August 1996.

Sanco/3029/99 rev.4 (11/07/00): Guidance for generating and reporting methods of analysis in support of pre-registration data requirements for Annex II (part A, Section 4) and Annex III (part A, Section 5) of Directive 91/414.

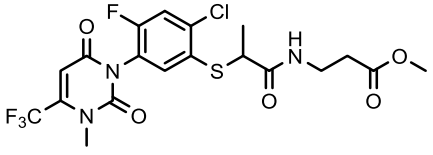
2 CONDUCT OF STUDY

The study was conducted at Ishihara Sangyo Kaisha, Ltd., Central Research Institute, Safety Science Research Laboratory, Environmental Sciences Group, 3-1, 2-Chome, Nishi-shibukawa Kusatsu-shi, Shiga-ken, 525-0025 Japan.

3 MATERIALS AND METHODS

3.1 Analytical standards

3.1.1 DCC-3825

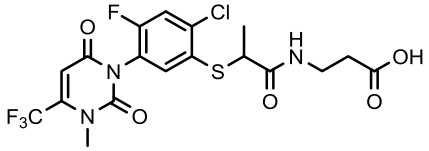
Identity:	DCC-3825
Common name:	Tiafenacil
Chemical name:	methyl 3-[(2*RS*)-2-{2-chloro-4-fluoro-5-[1,2,3,6-tetrahydro-3-methyl-2,6-dioxo-4-(trifluoromethyl)pyrimidin-1(6*H*)-yl] phenylthio}propionamido]propionate
Structure:	
Molecular weight:	511.87
Lot No.:	KILOLAB-140109
Purity:	98.7%

3.1.2 M-01

Identity: M-01

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

Structure:



Molecular weight: 497.85

Lot No.: K20066-01

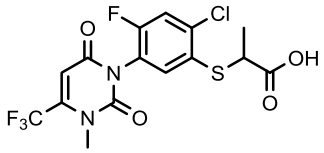
Purity: 96.1 %

3.1.3 M-12

Identity: M-12

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

Structure:



Molecular weight: 426.77

Lot No.: KM02478-01

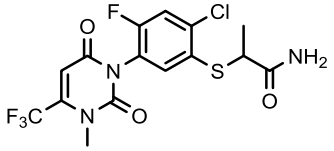
Purity: 97.4 %

3.1.4 M-13

Identity: M-13

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

Structure:

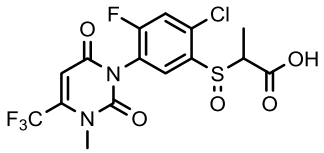


Molecular weight: 425.79

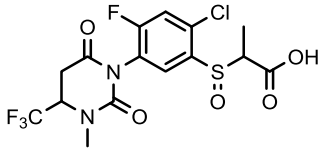
Lot No.: K20067-01

Purity: 98.6 %

3.1.5 M-36

Identity:	M-36
Chemical name:	2-((2-chloro-4-fluoro-5-(3-methyl-2,6-dioxo-4-(trifluoromethyl)-2,3-dihydropyrimidin-1(6*H*)-yl) phenyl)sulfinyl)propanoic acid
Structure:	
Molecular weight:	442.77
Lot No.:	K20268-01
Purity:	94.1 %

3.1.6 M-53

Identity:	M-53
Chemical name:	2-((2-chloro-4-fluoro-5-(3-methyl-2,6-dioxo-4-(trifluoromethyl)tetrahydropyrimidin-1(2*H*)-yl) phenyl)sulfinyl)propanoic acid
Structure:	
Molecular weight:	444.79
Lot No.:	K20389-01
Purity:	94.7 %

3.2 Sediment

Two type sediments were used in the study. These sediments were supplied from Envigo CRS Ltd in June 2016. The sediment characterization data are shown in Table 1.

3.3 Reagents

All reagents were of analytical, HPLC or LC/MS/MS grade.

3.4 Standard solutions

3.4.1 Stock solutions

Individual stock solutions (100 µg/mL) of DCC-3825 and its metabolites were prepared by dissolving an accurately weighed amount of each material in a suitable volume of methanol.

3.4.2 Fortification solutions

The stock solutions were further diluted with methanol to obtain fortification solutions with a concentration of 1.0 and 10.0 µg/mL. These fortification solutions were prepared by mixing DCC-3825 and its metabolites.

3.4.3 Calibration solutions

Calibration solutions, over the concentration range 0.1 to 10.0 ng/mL, were prepared by serial dilution of the mixed fortification solutions in methanol:water (70:30, v/v).

3.5 Fortification

To demonstrate the validity of the method used, untreated sediments were fortified with the following levels for DCC-3825 and its metabolites.

0.01 mg/kg	0.2 mL of the fortification solution (1.0 µg/mL) was added to 20 g (dry mass) sediment.
0.1 mg/kg	0.2 mL of the fortification solution (10.0 µg/mL) was added to 20 g (dry mass) sediment.

3.6 Analytical method

The analytical method in sediment was based on the methods used for soil and sediment in the following studies - The Transformation of [14C]-DCC-3825 in Four Soils Under Aerobic Conditions and The Transformation of [14C]-DCC-3825 in Two Aquatic Sediment Systems under Aerobic Conditions (Reference1 and 2).

3.6.1 Extraction

20 g of the untreated sediment sample was weighed into a 250 mL HDPE screw-top bottle. 90 mL of acetonitrile:water (80:20, v/v) and 0.9 mL of formic acid were added to the sediment sample. The sample was shaken for 30 minutes using a reciprocal shaker. The mixture was centrifuged at 3000 rpm for 5 minutes and supernatant was decanted. The sediment residue was re-extracted with 90 mL of acetonitrile:water (80:20, v/v) and 0.9 mL of formic acid were added for 30 minutes. The mixture was centrifuged and decanted likewise. The extracts were combined in glass flask and diluted to volume (200 mL) with acetonitrile:water (80:20, v/v).

3.6.2 Sample clean up on SPE

A SPE cartridge (OASIS HLB VAC RC, 60 mg) was placed onto a SPE vacuum manifold and conditioned using methanol (5 mL) followed by water:acetic acid (100:1, v/v) (5 mL). 5.0 mL of the extract and 30 mL of water:acetic acid (100:1, v/v) were mixed and transferred into the SPE cartridge. The aqueous sample solution was sucked through the column followed by 5 mL of water. All eluates were discarded. DCC-3825 and its metabolites were eluted with 9.5 mL of methanol:water (70:30, v/v). The eluate was collected and then filled up to 10 mL with methanol:water (70:30, v/v).

3.6.3 Quantitation

Quantitation of the concentration of the DCC-3825 and its metabolites was performed by LC/MS/MS using the external standard method. The calibration standards at six concentrations (0.1, 0.5, 1.0, 2.0, 5.0 and 10.0 ng/mL) were used for construction of a calibration curve. The calibration curve was constructed by plotting the peak areas against the injected amount of standard. From the calibration curve, the concentration of DCC-3825 and its metabolites in sediment sample was determined.

3.7 LC/MS/MS conditions

Part A; HPLC

Instrument:	ACQUITY UPLC System (Waters)			
Column:	Kinetex Biphenyl 2.1×150 mm, 2.6 μm			
Column temp.:	40°C			
Mobile phase:	0.1% formic acid in water:0.1% formic acid in methanol (25:75, v/v)			
Flow rate:	0.4 mL/min			
Injection volume:	4 μL			
Retention time:	DCC-3825;	1.74 min	M-13;	1.33 min
	M-01;	1.34 min	M-36;	1.43 min
	M-12;	1.63 min	M-53;	1.11 min

Part B; MS/MS

Instrument:	API5000 (AB SCIEX)
Ionization mode:	ESI
Scan mode:	MRM
Mass resolution	Q1; unit, Q3; unit
Heater gas temp.:	600°C
Ion voltage:	5000 V
Gas flow settings:	Gas1; 60, Gas2; 80, CUR; 10, CAD; 11

Quantitation transition monitored

Analyte	Ion Polarity	Precursor Ion (m/z)	Product Ion (m/z)	CE	DP	EP	CXP
DCC-3825	Pos. $[M+H]^+$	512.2	381.0	37	141	10	28
M-01	Pos. $[M+H]^+$	498.1	381.0	35	101	10	10
M-12	Pos. $[M+H]^+$	427.2	380.7	23	81	10	26
M-13	Pos. $[M+H]^+$	426.1	380.9	27	131	10	12
M-36	Pos. $[M+H]^+$	443.1	218.1	51	121	10	12
M-53	Pos. $[M+H]^+$	445.1	371.0	33	81	10	26

Confirmation transition monitored

Analyte	Ion Polarity	Precursor Ion (m/z)	Product Ion (m/z)	CE	DP	EP	CXP
DCC-3825	Pos. $[M+H]^+$	512.2	152.2	57	141	10	8
M-01	Pos. $[M+H]^+$	498.1	359.1	79	101	10	10
M-12	Pos. $[M+H]^+$	427.2	152.0	45	81	10	20
M-13	Pos. $[M+H]^+$	426.1	152.0	47	131	10	18
M-36	Pos. $[M+H]^+$	443.1	353.0	25	121	10	26
M-53	Pos. $[M+H]^+$	445.1	355.0	23	81	10	34

3.8 Calculation

The concentration of DCC-3825 and its metabolites in sediment was calculated according to equation 1.

$$C = \frac{X \times V_F \times D \times CF}{V_I \times V_S} \quad (1)$$

Where

C = Concentration of DCC-3825 and its metabolites in sediment sample [mg/kg]

X = Injected amount of DCC-3825 and its metabolites [pg]

V_I = Injection volume [4 μL]

V_F = Final volume [10 mL]

D = Dilution factor [if applicable]

V_S = Aliquot of sediment sample [0.5 g]

CF = Conversion factor ($\times 10^3$: from mL to μL, $\times 10^{-6}$: from pg to μg)

The recovery of DCC-3825 and its metabolites was calculated according to equation 2.

$$R = \frac{C \times 100}{F} \quad (2)$$

Where

R = Recovery of DCC-3825 and its metabolites [%]

C = Concentration of DCC-3825 and its metabolites in sediment sample [mg/kg]

F = Fortification level [mg/kg]