

**ANALYSIS REPORT**

Sponsor: [REDACTED]

Study No.: [REDACTED]

Study title: Ready Biodegradability Test of C4-FN

Testing facility: Environmental Risk Assessment Center, LSI Medience Corporation  
1000 Kamoshida-cho, Aoba-ku, Yokohama, Kanagawa, Japan

Purpose: This study was conducted to evaluate the ready biodegradability of the test substance.

Method: OECD Guideline for Testing of Chemicals 301D "Ready Biodegradability: CLOSED BOTTLE TEST" (1992).

Test substance: Chemical name: 2,3,3,3-Tetrafluoro-2-(trifluoromethyl)propanenitrile  
Alternate name: C4-FN  
Lot number: [REDACTED]  
Structural formula:  $(CF_3)_2CFCN$   
Molecular weight: 195.04  
Purity: 99.7%  
Name and content of impurities: 2,2,3,3,4,4,4-Heptafluorobutyronitrile: 0.3%  
Acetone: <100 ppm

Authentic standard: Alternate name: C4-FA  
Lot number: [REDACTED]  
Structural formula:  $(CF_3)_2CFCONH_2$   
Molecular weight: 213.05

Date of analysis: July 29, 2020 to August 28, 2020  
Measurement of pH; DO; residual amount of test substance: 30<sup>th</sup> day  
Measurement of amount of transformation product: 34<sup>th</sup> day

Test bottles: YDEG: Test suspension Mineral medium + inoculum + test substance  
YWS: Abiotic control Ultra-pure water + test substance  
test substance: 4.87 mg/L ; inoculum: 50 µL/L

Inoculum: Surface water  
Date of receipt: July 29, 2020

Apparatus: Incubator, M-210FN (No. 1), TAITEC (dark condition)

Measurements: • Biochemical oxygen demand (BOD) based on dissolved oxygen concentration  
• Residual amount of the test substance [measured with gas chromatograph / mass spectrometer (GC/MS)]  
• Amount of the transformation product (C4-FA) [measured with a liquid chromatograph / mass spectrometer (LC/MS)]

Measuring method: Refer to Appendix-1

Analytical results:

## 1) pH and BOD

Measurement		Test suspension	Abiotic control
pH		7.2	8.3
DO (start of exposure)	mgO <sub>2</sub> /L	8.87	8.76
DO (end of exposure)	mgO <sub>2</sub> /L	8.66	8.51

## 2) Residual amount of the test substance

Measurement		Test suspension		Abiotic control		Theoretical value
		1	2	1	2	
Test substance	mg	0.02	0.04	0.25	0.26	1.46
	%	1	3	17	18	-
Disappearance rate*1	%	99	97	-	-	-

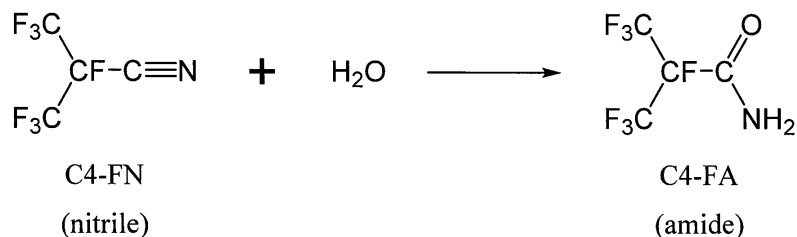
\*1 Since the residual amount of the test substance in the abiotic control was less than 90% of the theoretical value, disappearance rate was shown instead of degradability.

## 3) Amount of transformation product

Measurement		Test suspension	Abiotic control	Theoretical value
Transformation product	mg	1.63	1.43	1.59
(C4-FA)	%	103	90	-

Discussion:

Since the DO value did not decrease at the end of exposure, it can be concluded that the test substance is not readily biodegradable. In the test suspension, the residual rate of the test substance was av. 2%, and the detection rate of C4-FA was 103%. It was suggested that this result was due to the hydrolysis of nitrile (C4-FN) to the corresponding amide (C4-FA).



Study director:



Satoshi ICHIKAWA, Ph. D.

Date:

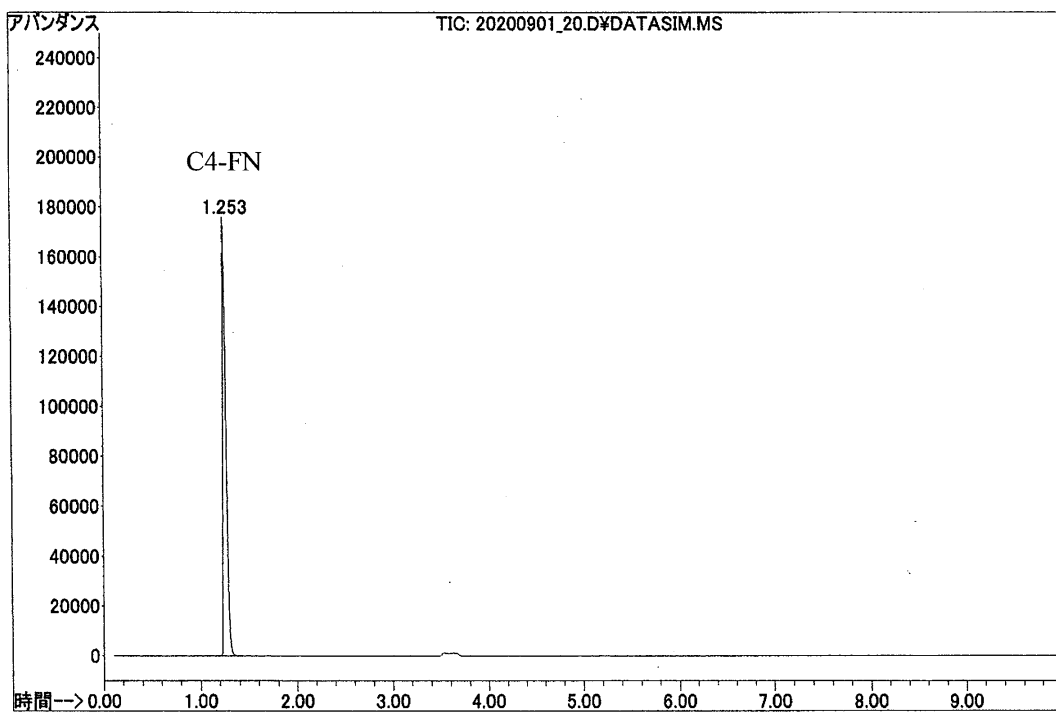
October 29, 2020

Figure 1 Chromatograms of the test substance and test solution

---Measurement of residual test substance amount

146 mg/L Standard solution

Study No. : ██████████ Vial No. : 2  
Test Substance: C4-FN Injection Vol.: 1  $\mu$ L  
Analyte: C4-FN Injection Date: 1 Sep 2020 14:08  
Sample Name: STD 146 mg/L Operator: オカ  
Method: D:\GCMSD\METHODS\████████\_BACK.M  
Data File: D:\GCMSD\DATA\████████\20200901\_20.D



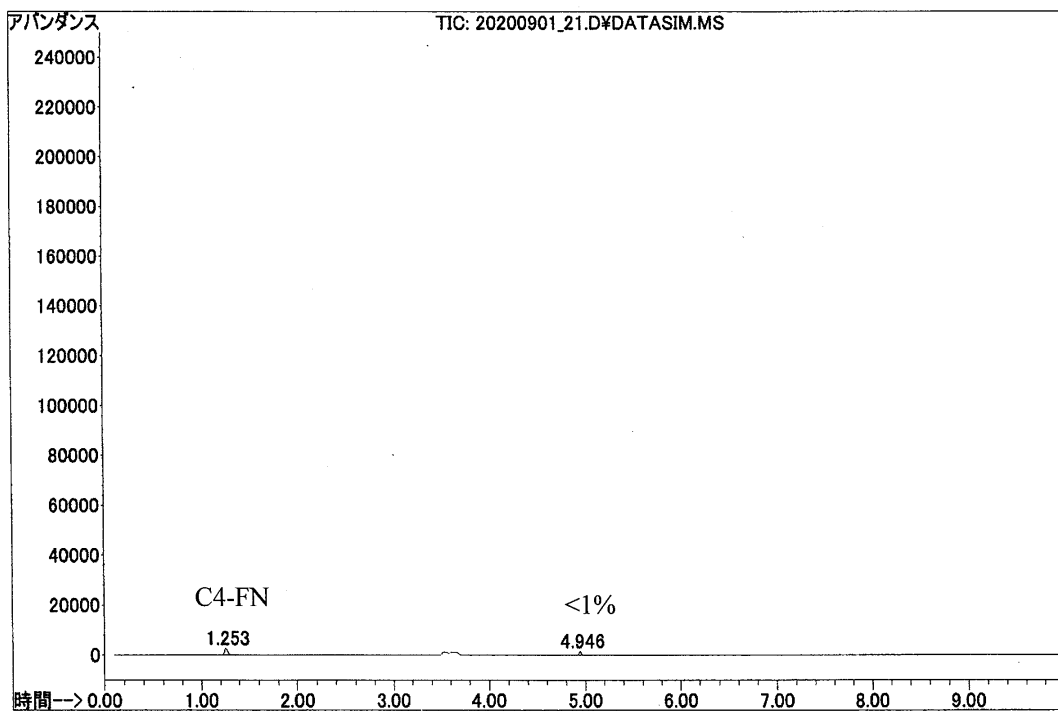
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STD 146 mg/L

ピーク#	R. T.	タイプ	半値幅	面積	開始時間	終了時間
1	1.253	M	0.036	3986329	1.211	1.426

Figure 1 Continued

Test suspension-1

Study No.: XXXXXXXXXX Vial No.: 3  
 Test Substance: C4-FN Injection Vol.: 1  $\mu$ L  
 Analyte: C4-FN Injection Date: 1 Sep 2020 14:21  
 Sample Name: YDEG-2 Operator: *カ*  
 Method: D:\GCMSD\METHODS\ XXXXXXXXXX\_BACK.M  
 Data File: D:\GCMSD\DATA\ XXXXXXXXXX\20200901\_21.D



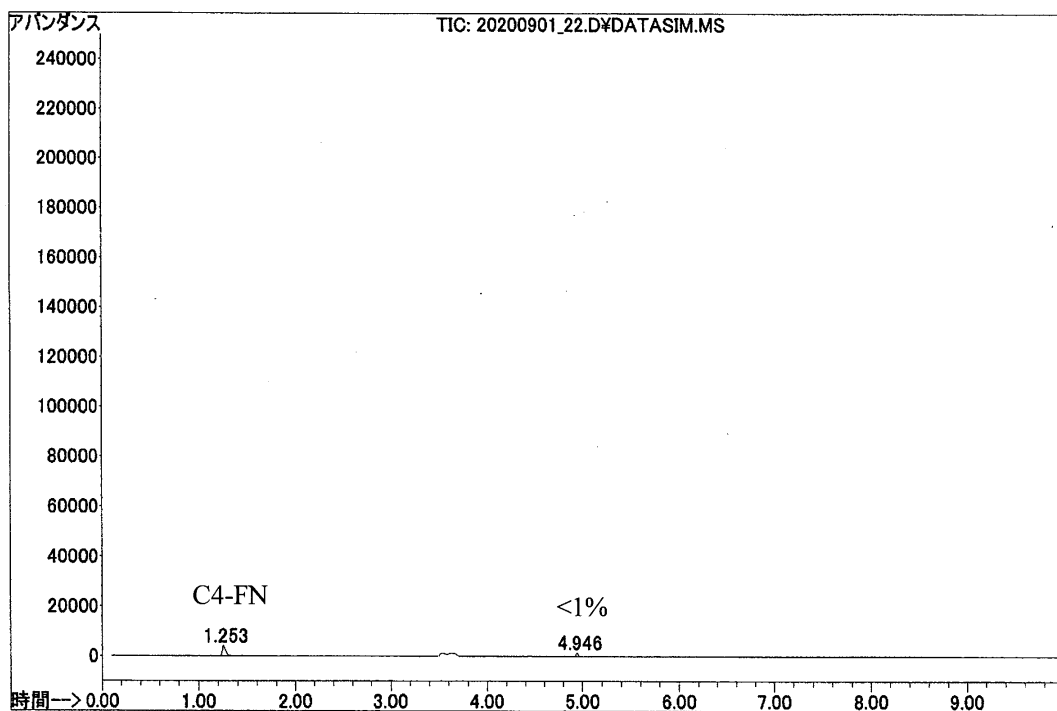
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 YDEG-2

ピーク#	R. T.	タイプ	半値幅	面積	開始時間	終了時間
1	1.253	M	0.037	64883	1.209	1.382
2	4.946	M	0.022	21259	4.910	5.062

Figure 1 Continued

Test suspension-2

Study No. : ██████████ Vial No. : 4  
 Test Substance: C4-FN Injection Vol. : 1 μL  
 Analyte: C4-FN Injection Date: 1 Sep 2020 14:35  
 Sample Name: YDEG-3 Operator: オカ  
 Method: D:¥GCMSD¥METHODS¥████████\_BACK.M  
 Data File: D:¥GCMSD¥DATA¥████████¥20200901\_22.D



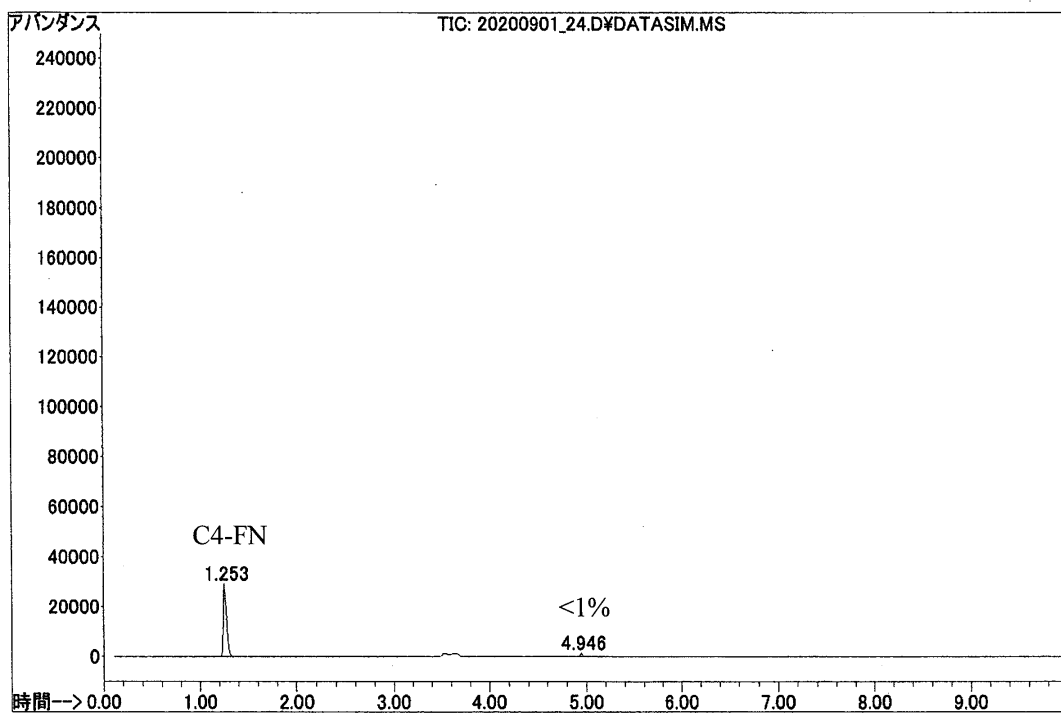
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 YDEG-3

ピーク#	R. T.	タイプ	半値幅	面積	開始時間	終了時間
1	1.253	M	0.037	96970	1.212	1.387
2	4.946	M	0.023	20742	4.909	5.033

Figure 1 Continued

Abiotic control-1

Study No. : ██████████ Vial No. : 5  
 Test Substance: C4-FN Injection Vol. : 1  $\mu$ L  
 Analyte: C4-FN Injection Date: 1 Sep 2020 15:02  
 Sample Name: YWS-2 Operator: オカ  
 Method: D:\GCMSD\METHODS\████████\_BACK.M  
 Data File: D:\GCMSD\DATA\████████\20200901\_24.D



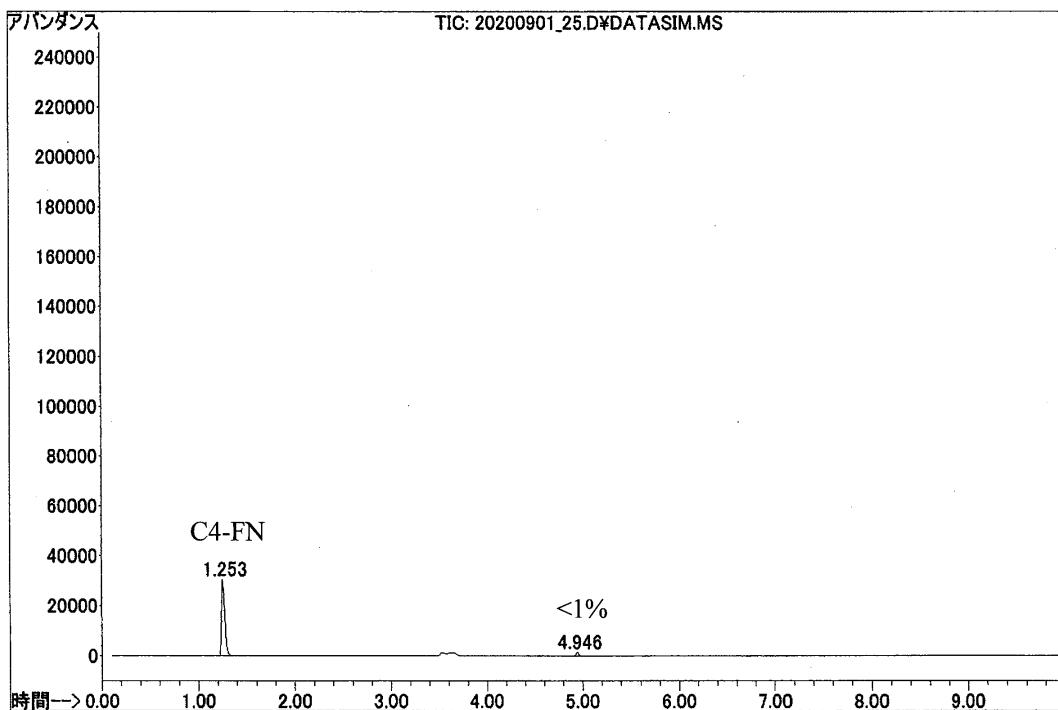
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 YWS-2

ピーク#	R. T.	タイプ	半値幅	面積	開始時間	終了時間
1	1.253	M	0.038	688508	1.207	1.424
2	4.946	M	0.022	19167	4.903	5.009

Figure 1 Continued

Abiotic control-2

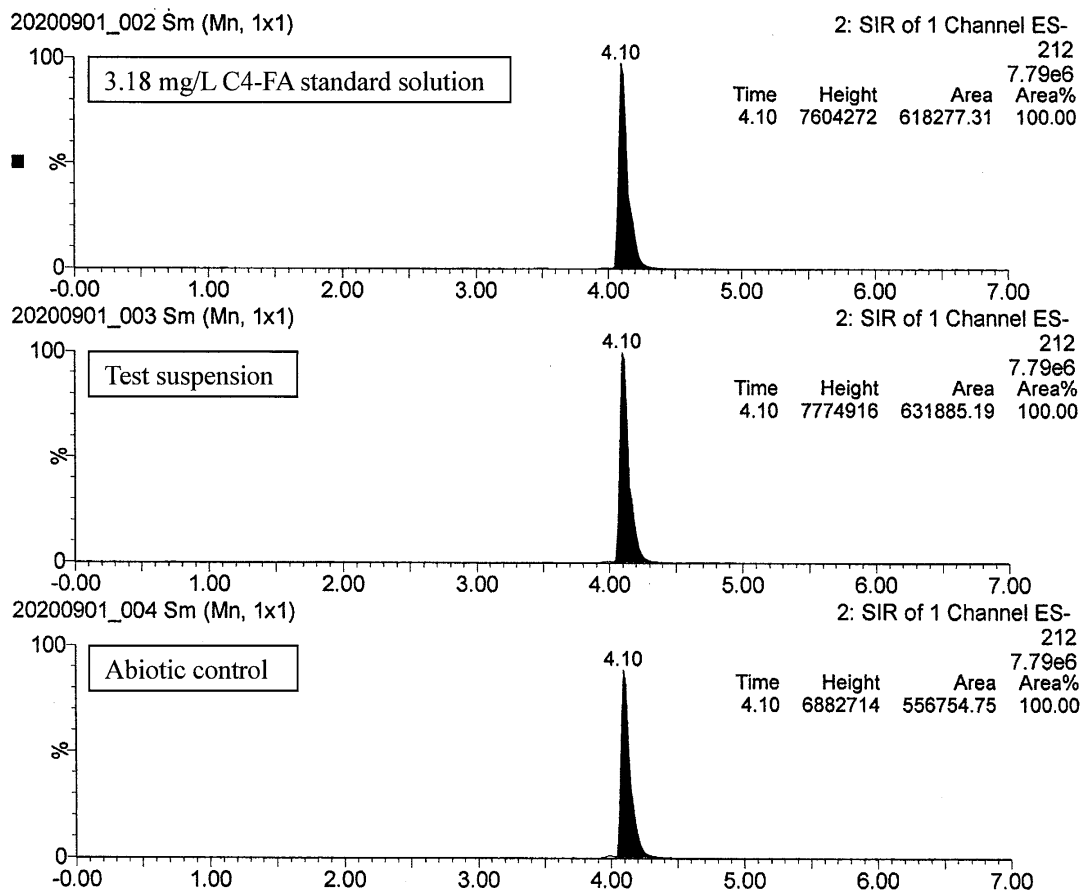
Study No. : ██████████ Vial No. : 6  
 Test Substance: C4-FN Injection Vol. : 1 μL  
 Analyte: C4-FN Injection Date: 1 Sep 2020 15:16  
 Sample Name: YWS-3 Operator: ㊦  
 Method: D:\GCMSD\METHODS\A200731\_BACK.M  
 Data File: D:\GCMSD\DATA\████████\20200901\_25.D



TIC: 20200901\_25.D\DATASIM.MS  
 YWS-3

ピーク#	R. T.	タイプ	半値幅	面積	開始時間	終了時間
1	1.253	M	0.037	713691	1.208	1.422
2	4.946	M	0.023	20797	4.905	5.021

Figure 2 Chromatograms of C4-FA and test solution ---Measurement of C4-FA amount

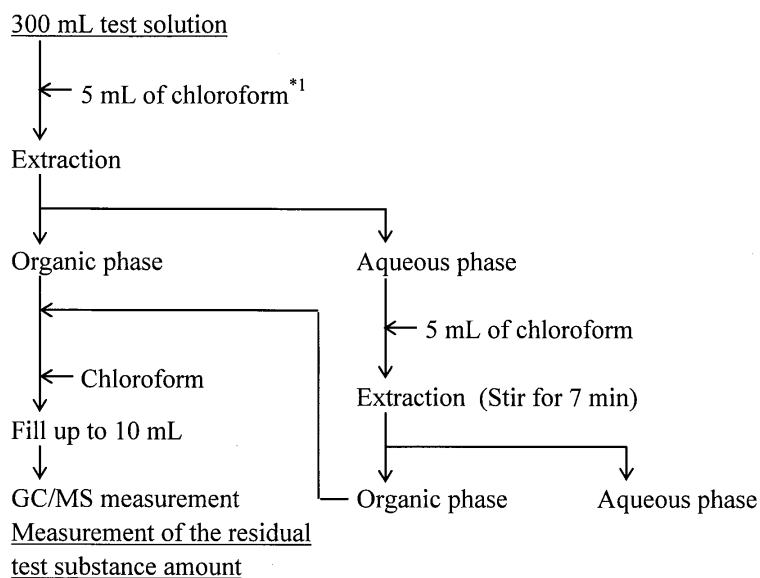




## Appendix-1 Measuring method

## 1. Measurement of the residual test substance amount

## 1) Pre-treatment of the test solutions



\*1 Remove the sealing water on the collar of the incubator bottle, pour 5 mL of chloroform onto the collar, lift up the stopper of the incubator bottle slightly, and pour chloroform into the incubator bottle in small quantities.

## 2) Apparatus

Gas chromatograph / mass spectrometer (GC/MS) :

5975C, Agilent technologies (No.1)

Work station: MSD ChemStation

Gas chromatograph(GC): 6890A

Auto injector: 7683B, (Back Inlet)

Mass selective detector (MSD): 5975C inertXL

## 3) Conditions

[GC]

Column: J&W, DB-5.625, 30m×0.25 mm i.d.×1.0 μm (film thickness)

Oven temperature: 40°C (2 min) → 20°C/min → 160°C (2 min)

Injector: Split (Split ratio 10:1)

Injector temperature: 200°C

Carrier gas: He

Column flow: 1 mL/min (Mode: Constant flow)

Injection volume: 1 μL

[MSD]

Temperature: Ion source 230°C

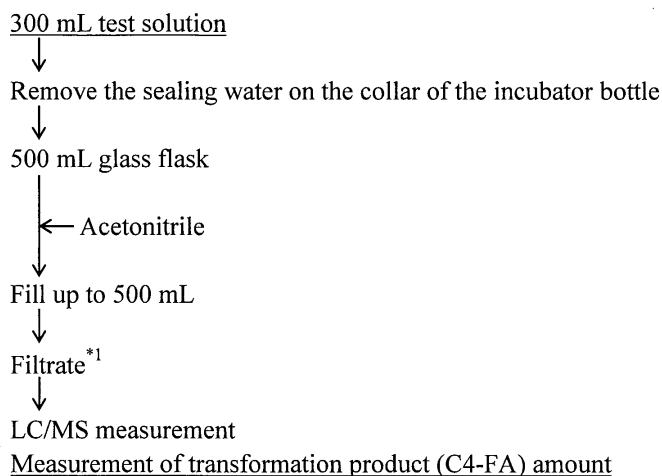
Quadrupole 150°C

Detection mode: Selected ion monitoring (SIM)  $m/z$  69

## Appendix-1 Measuring method

## 2. Measurement of transformation product (C4-FA) amount

## 1) Pre-treatment of the test solutions



\*1 Syringe filtr PTFE-HI, 25 mm, 0.2µm, Agilent technologies

## 2) Apparatus

High performance liquid chromatograph mass spectrometer (LC/MS) :

	UPLC SQD2 (No.1) , Waters Co.
Workstation:	Masslynx 4.1
Pump:	Quaternary Solvent Manager
Auto injector:	Sample Manager-FTN
Mass spectrometric detector (MS):	SQ Detector 2

## 3) Conditions

[LC]

Column: ACQUITY UPLC BEH C18, 1.7 µm, 2.1 mm i.d.× 100 mm, Waters Co.

Mobile phase: A 0.028% Ammonia solution<sup>\*2</sup>

B Acetonitrile

Gradient condition:	Time (min)	A (%)	B (%)
	0.00	95	5
	0.50	95	5
	5.00	5	95
	7.00	5	95
	7.10	95	5
	10.00	95	5

Flow rate: 0.4 mL/min

Column temperature: 40°C

Injection volume: 20 µL

\*2 In preparation for the solution, 0.5 mL of 28% ammonia solution was added to 500 mL of ultra-pure water.



Appendix-1 Measuring method

[MS]

Detection mode:	SIR	
Ion mode:	Electrospray Negative $m/z$ 212	
Voltage:	Capillary:	1.00 kV
	Cone:	10 V
Temperatures:	Source Temp:	150°C
	Desolvation Temp:	500°C
Gas Flow:	Desolvation:	1000 L/hr
	Cone:	50 L/hr