CERI

Receipt number	682-13-N-6047
Study number	46047

FINAL REPORT

Bioconcentration study of 13F-SFA in common carp

This is a correct copy of the original.
Chemicals Evaluation and Research Institute, Japan, Kurume (CERI Kurume)

Date July 2, 2014

Study Director

June, 2014

Chemicals Evaluation and Research Institute, Japan, Kurume

GLP STATEMENT

Chemicals Evaluation and
Research Institute, Japan, Kurume

Sponsor DAIKIN INDUSTRIES, LTD.

Title Bioconcentration study of 13F-SFA in common carp

Study number 46047

The study described in this report was conducted in compliance with the following GLP principles:
OECD Principles of Good Laboratory Practice, November 26, 1997, ENV/MC/CHEM (98)17

This final report reflects the raw data accurately and it has been confirmed that the test data are valid.

Date June 27, 2014

Study Director

QUALITY ASSURANCE STATEMENT

Chemicals Evaluation and
Research Institute, Japan, Kurume

Sponsor DAIKIN INDUSTRIES, LTD.

Title Bioconcentration study of 13F-SFA in common carp

Study number 46047

I assure that the final report accurately describes the test methods and procedures, and that the reported results accurately reflect the raw data of the study.

The inspections of this study were carried out and the results were reported to the Study Director and the Test Facility Management by Quality Assurance Unit as follows.

Item of inspection	Date of inspection	Date of report to Study Director and Test Facility Management
Study plan (draft)	April 18, 2014	April 18, 2014
Study plan	April 21, 2014	April 21, 2014
Acute toxicity test	April 22, 2014	April 22, 2014
Recovery test for analysis of test water	April 23, 2014	April 24, 2014
Recovery test for analysis of test fish	April 24, 2014	April 24, 2014
Preparation of stock solutions	April 30, 2014	April 30, 2014
Analysis of test water	May 1, 2014	May 1, 2014
Measurement of lipid content in test fish	May 1, 2014 May 2, 2014	May 2, 2014
Start of exposure	May 2, 2014	May 2, 2014
Analysis of test fish	May 15, 2014	May 15, 2014
Study plan amendment No.1	May 16, 2014	May 16, 2014
Analysis of test water	May 16, 2014	May 16, 2014
Analysis of test fish	May 16, 2014	May 16, 2014
Raw data and final report (draft)	June 27, 2014	June 27, 2014
Final report	June 27, 2014	June 27, 2014

Date June 27, 2014

Head of Quality Assurance Unit

CONTENTS

Tables

Figures

1. Title

   Bioconcentration study of 13F-SFA in common carp

2. Sponsor

   Name DAIKIN INDUSTRIES, LTD.

   Address 1-1, Nishi-Hitotsuya, Settsu-shi, Osaka 566-8585, Japan

3. Test facility

   Name Chemicals Evaluation and Research Institute, Japan, Kurume (CERI Kurume)

   Address 3-2-7 Miyanojin, Kurume-shi, Fukuoka 839-0801, Japan

4. Objective

   This study was aimed at evaluating the bioconcentration potential of 13F-SFA in common carp.

5. Test method

   "305-I : Aqueous Exposure Bioconcentration Fish Test" stipulated in the OECD Guidelines for Testing of Chemicals, No.305, October 2, 2012, "Bioaccumulation in Fish : Aqueous and Dietary Exposure"

6. GLP principles

   OECD Principles of Good Laboratory Practice, November 26, 1997, ENV/MC/CHEM (98)17

7. Dates

Study initiation date	April 21, 2014
Experimental starting date	May 2, 2014
Experimental completion date	May 30, 2014
Study completion date	June 27, 2014

8. Storage of test item, raw data, etc.

The study plan (original), the final report (original), raw data, documents about the study presented by the sponsor and necessary materials will be stored in a storage room at this laboratory for 10 years from study completion date. The stability of the test item is not confirmed during the storage period. Treatment of raw data, etc. after the storage period will be discussed with the sponsor. The test item will be returned to the sponsor.

9. Personnel

Study Director (Section 3)
Study personnel (Operation of bioconcentration test)
Study personnel (Operation of acute toxicity test)
Person in charge of fish care

10. Approval of final report

Date June 27, 2014

Study Director

11. Summary

Test conditions

a) Acute toxicity test

Test fish	Common carp (*Cyprinus carpio*)
Duration of exposure	96 hours
Exposure method	Semi static system (Renewal of test water, at every 8 - 16 hours)

b) Bioconcentration test

Test fish	Common carp (*Cyprinus carpio*)	
Nominal concentrations of test item		
	High exposure level (Level 1)	10 μg/L
	Low exposure level (Level 2)	1 μg/L
Duration of exposure	28 days	
Exposure method	Flow-through system	
Analytical method	Gas chromatography-mass spectrometry	

Results

a) Acute toxicity test

96-hour LC_{50} value >20.0 mg/L

b) Bioconcentration test

	Bioconcentration factor
Level 1	<8.0-10
Level 2	<80

12. Test material

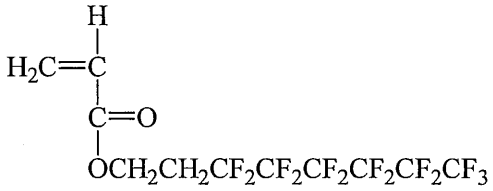
12.1 Test item

a) Chemical name etc.

Chemical name	3,3,4,4,5,5,6,6,7,7,8,8,8-Tridecafluorooctyl acrylate
Alias	13F-SFA
CAS number	17527-29-6

b) Chemical structure etc.

Structural formula



Molecular formula	$C_{11}H_7F_{13}O_2$
Molecular weight	418.15

c) Test sample

Purity of test item	99.8%
Impurity	Unknown 0.2%
Supplier	DAIKIN INDUSTRIES, LTD.
Lot number	6SFAD31003

The test item was treated as 100% in purity.

d) Physicochemical properties

Boiling point	78℃ (measurement pressure 8 mmHg)
Appearance	Clear and colorless liquid

Solubility in solvent

Solvent	Solubility	Stability in solvent
Dimethylsulfoxide	Soluble	-
Acetone	Soluble	-

Density 1.554 g/cm^3 (25℃)

Hydrolyzability CH_2=CHCOOCH$_2$CH$_2$C$_6$F$_{13}$ → C$_6$F$_{13}$CH$_2$CH$_2$OH + CH_2=CHCOOH

e) Storage conditions

The test sample was stored in a dark storage place at room temperature.

f) Identification and stability of test item

The infrared (IR) spectrum of the test item measured at this laboratory was confirmed to be identical to that provided by the sponsor (see Fig. 13, Reference 2). The stability of the test item was confirmed by comparing the IR spectrum of the test item before the experimental start and after the experimental completion (see Fig. 13).

g) Stability under testing conditions

A stability of the test item under the testing conditions was confirmed by a preliminary test.

h) Caution when handling

In order to avoid inhalation and contact with the skin and eyes, chemically resistant gloves, mask, safety glasses, and white coats are worn when handling all chemicals.

12.2 Dispersant

The following dispersants were used to prepare stock solutions for acute toxicity test and bioconcentration test.

N,N-dimethylformamide

a) Chemical name, etc.

Products name	*N,N*-dimethylformamide
CAS number	68-12-2

b) Supplier and grade

Supplier	NACALAI TESQUE
Grade	EXTRA PURE REAGENT

12.3 Test fish

a) Acute toxicity test

Species	Common carp (*Cyprinus carpio*) Reason for selection: The same fish species for the bioconcentration study
Supplier	CERI Kurume
Lot No.	TFC-140401
Weight	0.51-0.86 g
Length	3.9-4.8 cm

b) Bioconcentration test

Species	Common carp (*Cyprinus carpio*) Reason for selection: The previous data conducted with this species can be compared and the size of this species is adequate for handling.
Supplier	CERI Kurume
Conditions for acclimatization	The external disinfection was carried out in an aqueous solution containing OTC for fisheries (oxytetracycline hydrochloride, Kyoritsu Seiyaku) and sodium chloride (The Salt Industry Center of Japan). Thereafter fish were acclimatized in the following conditions. Period: 50 days Temperature: 25 less than ±2°C Mortality during acclimatization was less than 5%.
Lot No.	TFC-140218
Length	7.7-10.1 cm (at the beginning of exposure 7.7-8.1 cm)
Age	One-year-old fish
Feeding	Feed: Feed for fry of carp Composition: Proteins content ≥43.0% Lipid content ≥3.0% Manufacturer: Nippon Formula Feed Mfg. Feeding amount and interval: Amount corresponding to about 3% of total body weight was fed twice a day in halves (once a day in all at holiday). The fish were starved for 24 hours before sampling.

13. Performance of acute toxicity test

13.1 Test method

The test was performed in accordance with Japanese Industrial Standard (JIS K 0102-2010-71.).

13.2 Certification

The 48-hour LC_{50} value of the reference substance (PCP-Na: pentachlorophenol sodium salt, CAS number 131-52-2, Tokyo Kasei Kogyo, Lot No. GE01) for the fish of the same lot was 0.250 mg/L.

13.3 Dilution water for test

a) Origin

Groundwater from the premises of CERI Kurume

b) Water quality assessment

The results for the water quality assessment of the dilution water, taken out on January 8, 2014 and March 10, 2014, are shown in Reference 1. It was confirmed that the dilution water met the requirements of at least one of the following standards, except for the alkalinity and electric conductivity.

①Ministerial ordinance of the Ministry of Health, Labour and Welfare No.101 (Revised May 30, 2003)

② OECD Guidelines for Testing of Chemicals, No.210, July 17, 1992, "Fish, Early-life Stage Toxicity Test"

③ Water quality criteria for fisheries (Japan Fisheries Resource Conservation Association, March 1983)

④ Environmental Quality Standards for Water Pollutants No.14 (Revised February 22, 1999, Environment Agency)

⑤ OECD Guidelines for Testing of Chemicals, No.305, June 14, 1996, "Bioconcentration : Flow-through Fish Test"

13.4 Preparation of stock solution

Test sample (4.00 g) was dissolved in *N,N*-dimethylformamide to prepare 20.0 g/L stock solution (200 mL).

13.5 Test conditions

Test concentrations	20.0 mg/L and control	
Test tank	Glass bottle (hermetically-sealed condition)	
Volume of test water	3.50 L×5 / level	
Number of fish	10 fish / level (2 fish / test tank ×5)	
Temperature of test water	At the initial of exposure	24.2℃
	Before the renewal of test water (first)	24.4-24.5℃
Concentration of dissolves oxygen in test water		
	At the initial of exposure	7.8 mg/L
	Before the renewal of test water (first)	5.3-5.4 mg/L
pH of test water	At the initial of exposure	7.8-7.9
	Before the renewal of test water (first)	7.7
Duration of exposure	96 hours	
Exposure method	Semi static system (Renewal of test water, at every 8 - 16 hours)	
Aeration	No aeration	
Time of irradiation with light	16 hours light / 8 hours dark (artificial light of white fluorescent lamp)	

13.6 Performance of test

Place Aquatron room B

Date April 21, 2014 - April 25, 2014

13.7 Estimation of 96-hour LC_{50} value

The value of LC_{50} was indicated "> test concentration" because a mortality rate of more than 50% was not observed.

13.8 Test result

96-hour LC_{50} value of the test item >20.0 mg/L (see Fig. 3)

No diseases were observed in control.

The concentration of the dispersant (*N,N*-dimethylformamide) used at this time was about 1000 mg/L. Taking into account of the toxicity of the dispersant, the test was not performed at a higher concentration, because the 96-hour LC_{50} value of the dispersant was 11200 mg/L.

14. Performance of bioconcentration test

14.1 Dilution water for test

The same as described in Section 13.3

14.2 Conditions of test and circumstances

Supply of test water: Flow-through system assembled at this laboratory was used.

Test tank: Level 1 and 2 70-L glass tank for volatile item

Control 70-L glass tank

Flow rate of test water: 0.04 mL mL/min for stock solution and 2000 mL/min for dilution water, 2880 L/day of test water, were supplied.

Stock solution bottle: 1-L glass brown bottle

(Frequency of renewal 1-2 times / 2 weeks)

Temperature of test water: Before exposure of test item

Level 1 24.6-24.8℃

Level 2 24.6-24.8℃

Control 24.6-24.8℃

Duration of exposure

Level 1 24.2-25.0℃

Level 2 24.4-25.0℃

Control 24.3-25.0℃

Concentrations of dissolved oxygen in test water

Before exposure of test item

Level 1 7.8 mg/L

Level 2 7.8 mg/L

Control 8.0 mg/L

Duration of exposure

Level 1 6.5-7.7 mg/L

Level 2 6.2-7.1 mg/L

Control 7.2-7.5 mg/L

pH of test water	Before exposure of test item	
	Level 1	7.9
	Level 2	7.9
	Control	7.9
	Duration of exposure	
	Level 1	7.8, 7.5
	Level 2	7.8, 7.5
	Control	7.9, 7.5
Concentrations of total organic carbon in test water		
	48h and 24h before the exposure of test item	
	Level 1	10.4, 11.2 mgC/L
	Level 2	7.20, 11.8 mgC/L
	Control	17.5, 17.4 mgC/L
	Duration of exposure	
	Level 1	9.14-12.1 mgC/L
	Level 2	9.39-12.0 mgC/L
	Control	9.29-11.1 mgC/L
Total hardness of test water	Duration of exposure	
	Level 1	20.1 mg $CaCO_3$/L
	Control	22.1 mg $CaCO_3$/L
Aeration	No aeration	
Time of irradiation with light		
	14 hours light /10 hours dark (artificial light of white fluorescent lamp)	
Number of fish (at the beginning of exposure)		
	Level 1 and 2	26
	Control	19
Duration of exposure	28 days	
	Reason : A steady-state was reached after 28 days.	
Place	Aquatron room A	

14.3 Preparation of stock solutions

a) Level 1

Test sample (500 mg) was dissolved in *N,N*-dimethylformamide to prepare 500 mg/L stock solution (1 L).

b) Level 2

The 50.0 mg/L stock solution (1 L) was prepared from 500 mg/L stock solution (100 mL) described in Level 1 by dilution with *N,N*-dimethylformamide.

c) Control

N,N-dimethylformamide was used at the same concentration as used in Levels 1 and 2 (0.02 mL/L).

14.4 Test concentrations

Test nominal concentrations of the test item were set as follows. The control was set as a blank test.

Level 1	10 μg/L
Level 2	1 μg/L

14.5 Observation, measurement and cleaning

Observation, measurement and cleaning were conducted as following table. Excreta of common carp, dirt on test tank were removed for about once a day in test period.

Items	Frequency
Observation of test fish	Twice a day (once a day in holiday) in test period
Water temperature	Daily in test period
Flow rate of test water	Once a day in test period
Dissolved oxygen	Once before addition of test fish, and once or twice a week in test period
pH	Once before addition of test fish, and twice in test period
Total organic carbon	Before addition of the test fish (24h and 48h before the exposure) and, once a week in test period
Total hardness	Once in the test period for Control and Level 1

14.6 Analysis of test water and test fish

Analyses of the test item in test water and test fish were performed with Gas chromatography-mass spectrometry (GC-MS).

14.6.1 Frequency of analysis

a) Test water analysis

The test water of Level 1, Level 2 and Control were analyzed once before the experiment start and at the same time as analysis of test fish thereafter. Control water analyses were performed before the experiment start and after the experiment completion. One sample was analyzed at each sampling time.

b) Test fish analysis

Analysis of test fish was performed five times during the exposure period. Four fish per treatment level were taken out at each sampling time and divided into two groups (two fish per group) because one fish was not enough for the analytical sensitivity of the test item.

Test fish collection at final four successive analyses was carried out at intervals of more than 48 hours, and the final exposure period was 28 days.

Analysis of control fish was performed before the experiment start and after the experiment completion. Four fish were taken out at the sampling time and divided into two groups, and analyzed individually. Analysis of control fish was performed before the experiment start was taken out from tank of acclimatization.

14.6.2 Pretreatment for analysis

a) Test water

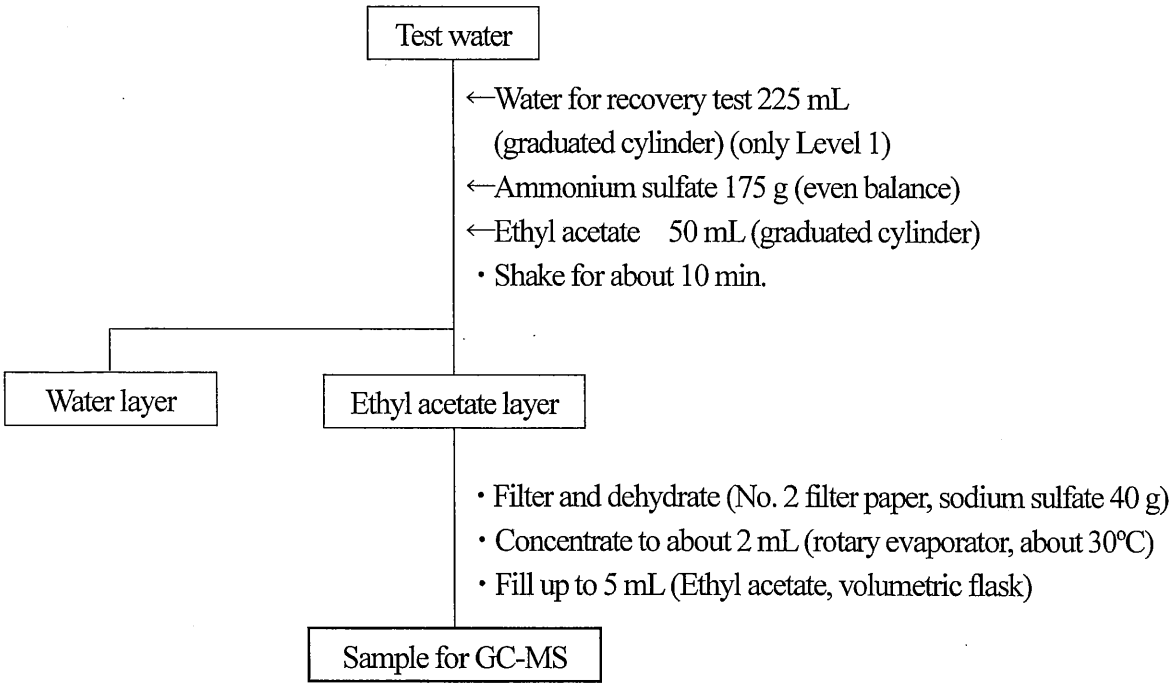
An aliquot of the test water,

Level 1 25 mL (graduated cylinder)

Level 2 250 mL (graduated cylinder)

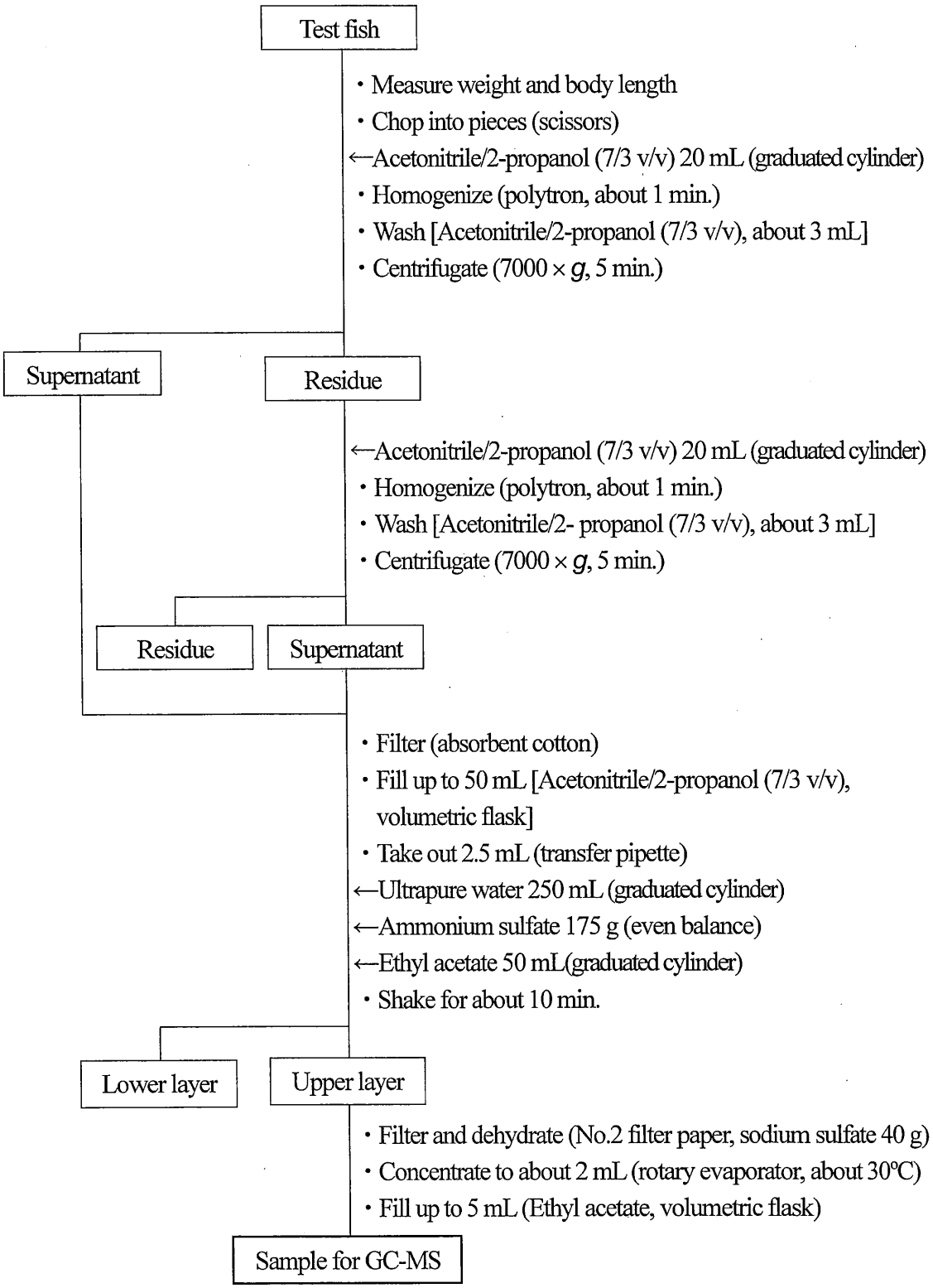
Control 250 mL (graduated cylinder)

was taken from each test tank and pretreated for GC-MS analysis as follows :



b) Test fish

Test fish were taken from each test tank and pretreated for GC-MS analysis as follows:



14.6.3 Quantitative analysis for test item

a) Quantitative method

The test item was determined with absolute calibration curve method using one concentration of standard solution.

In order to confirm the validity of this determination method, the calibration curve was made using four concentrations of standard solution, 5.00, 25.0, 50.0 and 100 μg/L (see Figs. 4, 7). The obtained correlation coefficient of the linear regression line was approximately 1 and the y-intercept does not differ from zero. These results represent very good linearity of the method, therefore the method can be considered to be validated on the linearity parameter.

b) Analytical conditions

Instrument	Gas chromatograph-mass spectrometer GC-MS QP2010 (Shimadzu)

Conditions of gas chromatograph

Column	HP-INNOWAX (30 m × 0.25 mm I.D., pore size 0.25 μm, Agilent technologies)
Column temperature	40°C (5 min) → 140°C (0 min) (Temperature rate 10°C /min*1)
Carrier gas	Helium
Control mode	Pressure 54.8 kPa
Injection temperature	200°C
Injection volume	1 μL
Injection method	Splitless
Sampling time	2 min

Conditions of mass spectrometer

Ionization mode	Electron ionization (EI)
Detection mode	Selected ion monitoring (SIM)
Mass range	*m/z* 55, 99
Ion source temperature	230°C
Desolvation temperature	250°C
Cone voltage	70 V

*1 The following analysis was performed by temperature rate 20°C/min. Since there was no change in pretreatment for analysis about the recovery test, the result performed by this condition was employed in this study (see Section 14.6.4 2)).

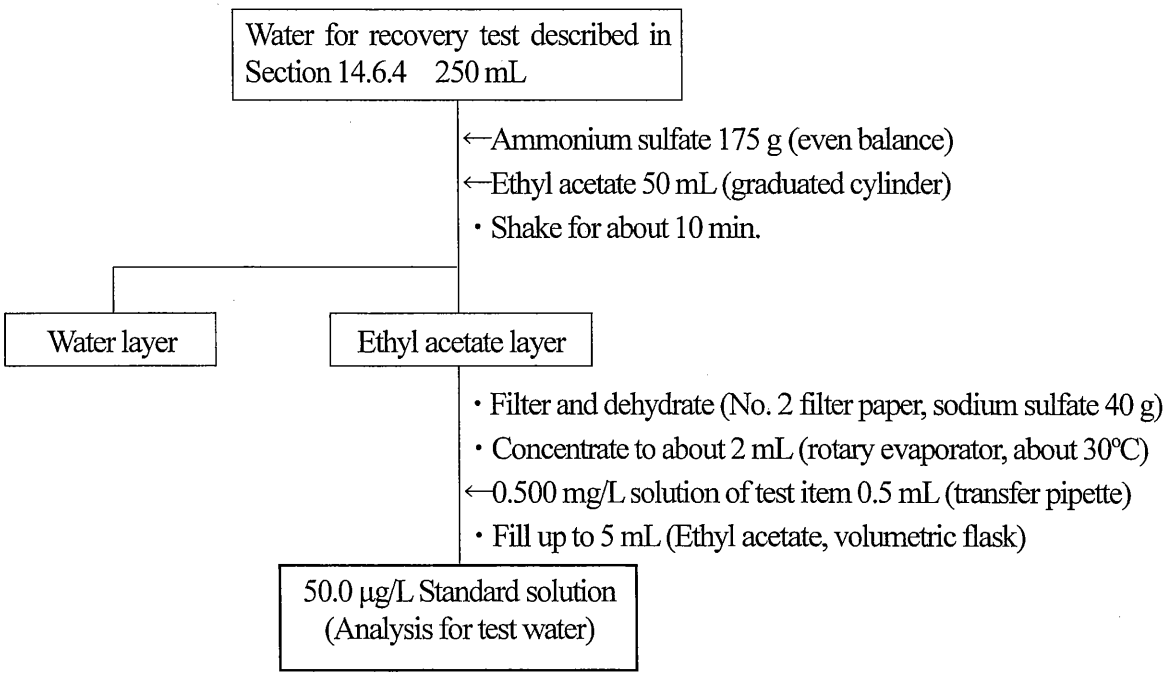
1) The test water of Level 1, Level 2 and Control before the experiment start
2) The test fish of Control before the experiment start
3) Recovery and blank test

c) Preparation of standard solution and calculation of the test item concentration in sample

1) Test water

Test sample (100 mg) was accurately weighed with an electronic analytical balance and dissolved in ethyl acetate to obtain 1000 mg/L solution of the test item (100 mL). This was diluted with ethyl acetate to prepare 0.500 mg/L solution. This solution was pretreated as follows to prepare 50.0 μg/L standard solution.

The concentration of the test item in the sample for GC-MS analysis was calculated proportionally by comparing the peak area on the chromatogram of the sample for GC-MS analysis with that of 50.0 μg/L standard solution (see Tables-4, 5, Fig. 6).

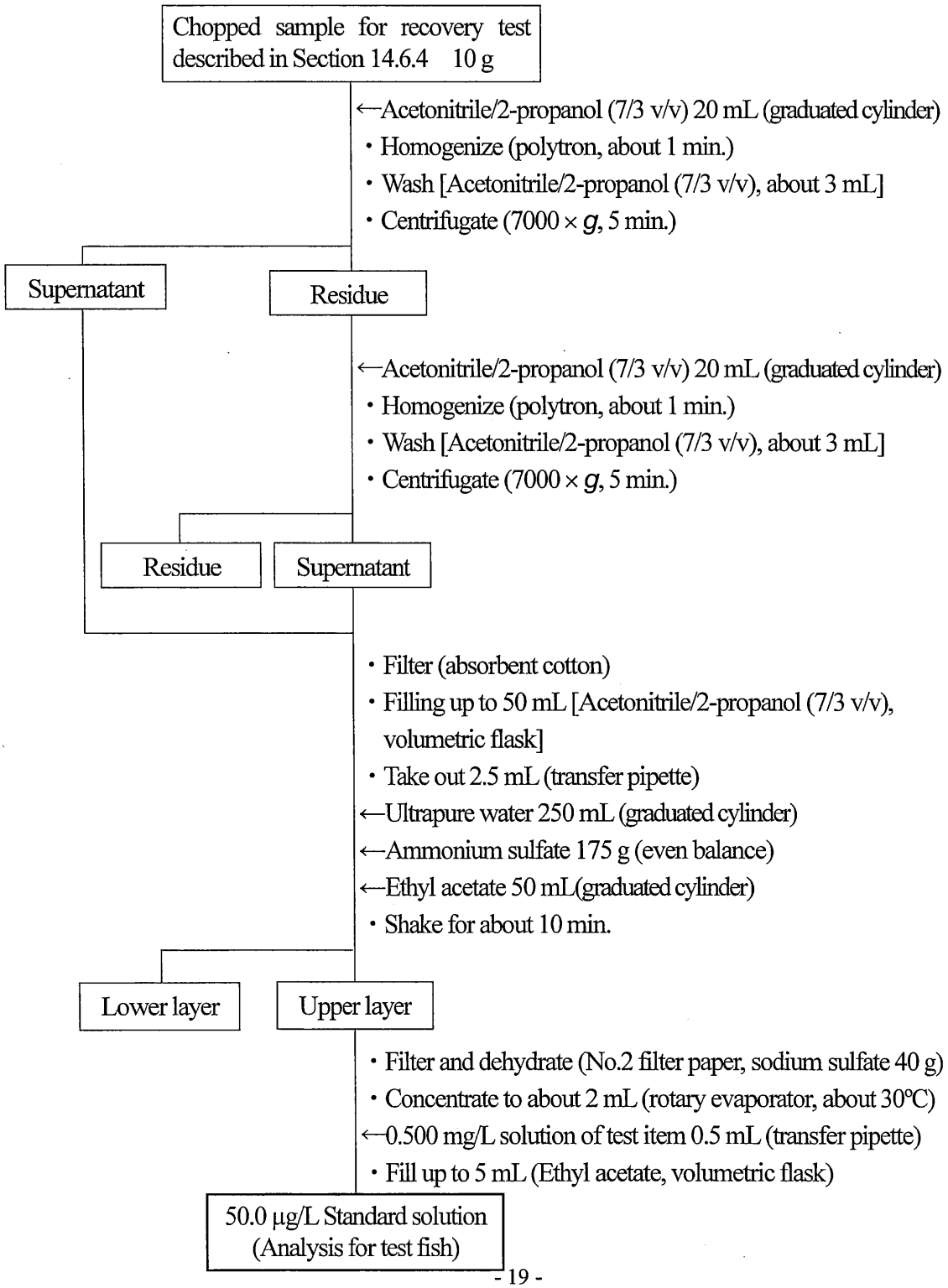


2) Test fish

Test sample (100 mg) was accurately weighed with an electronic analytical balance and dissolved in Ethyl acetate to obtain 1000 mg/L solution of the test item (100 mL). This was diluted with ethyl acetate to prepare 0.500 mg/L solution. This solution was pretreated as follows to prepare 50.0 μg/L standard solution.

The concentration of the test item in the sample for GC-MS analysis was calculated proportionally by comparing the peak area on the chromatogram of the sample for GC-MS analysis with that of 50.0 μg/L standard solution (see Tables-7, 8, 9, Figs. 9, 10, 11).

The limit of quantitation of the test item in the sample for GC-MS analysis was regarded as 5.00 μg/L, which corresponded to the lowest concentration of calibration curve.



14.6.4 Recovery and blank test

a) Method

Water and chopped sample of fish (10 g) were spiked with a specified amount of the test item and pretreated by the same way as described in Section 14.6.2 for the recovery tests. The blank tests were also performed in the same manner but without adding the test item. All the recovery and blank tests were performed in duplicate.

b) Results of recovery test

In the blank tests, the chromatogram of GC-MS had no peaks interfering with determination of the test item concentration. The duplicate recovery rates and the average of them in the pretreatment are shown below (see Tables-3, 6 and Figs. 5, 8). The average recovery rate was used as correction factors for the determination of the test item concentrations in the analytical samples.

Recovery rate for each treatment

For analysis of test water

Amount of test item spiked (250 ng)

= Nominal concentration of Level 2 (1 μg/L) × Amount of sampling water (250 mL)

Addition method (100 μL of 2.50 mg/L stock solution of test item)

80.5%, 84.8% average 82.7%

For analysis of test fish

Amount of test item spiked (5000 ng)

= Nominal concentration of Level 2 (1 μg/L)

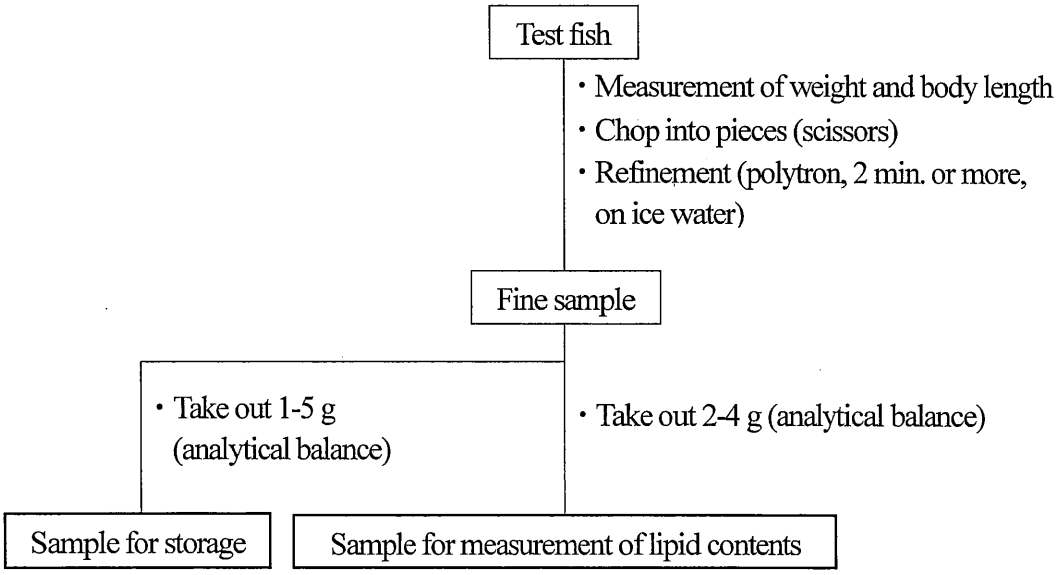
× Assumed bioconcentration factor (500 times) × Fish weight (10 g)

Addition method (100 μL of 50.0 mg/L stock solution of test item)

65.6%, 68.2% average 66.9%

14.6.5 Lipid content in test fish

To confirm whether the fish lipid after the experiment completion is within ±25% of the fish lipid before the experiment start, lipid contents of fish were determined using control fish before and after the experiment. Three fish were taken out at each sampling time. Then each sample was pretreated as follows, and lipid contents were determined individually with gravimetric analysis after chloroform-methanol extraction.



14.6.6 Calculation of the test item concentration in sample and limit of quantitation (LOQ)

a) Calculation of the test item concentration in test water

The equations in Tables-3 and 4 were used to obtain the concentrations, and they were rounded to 3 figures.

b) The LOQ of the test item in test water

The LOQ *2 of the test item in test water was calculated on the basis of that obtained from the calibration curve in Sections 14.6.3 c) as follows.

Level 1 1.2 μg/L

Level 2 0.12 μg/L

c) Calculation of the test item concentration in test fish

The equations in Tables-6, 7 and 8 were used to obtain the concentrations, and they were rounded to 3 figures.

d) Determination limit of the test item in test fish

Assuming the fish to be 10 g, the LOQ *2 of the test item in test fish was calculated to be 75 ng/g on the basis of that obtained from the calibration curve in Sections 14.6.3 c).

*2 LOQ of the test item (ng/g) $= \dfrac{A}{\dfrac{B}{100} \times \dfrac{C \times E}{D}}$

A : LOQ of the test item on the calibration curve (μg/L)

B : Recovery rate (%)

C : Sampling volume of test water (mL) or fish (g)

D : Final volume of sample solution (mL)

E : Ratio of the portion, used for analysis to whole volume

Results were rounded to 2 figures.

14.7 Calculation of results

14.7.1 Calculation of average concentration of the test item in test water

$\overline{Cwt} = \{Cw(1) + \ldots\ldots + Cw(n)\} / n$

$\overline{Cwt}$: The average concentration of the test item in test water (μg/L)

n : Number of analysis for test water (measurement times)

Cw(1) : Concentration of the test item in 1st analysis of test water (μg/L)

Cw(n) : Concentration of the test item in *n*-th analysis of test water (μg/L)

14.7.2 Calculation of bioconcentration factor (BCF)

Bioconcentration factor (BCF) was calculated as follows.

a) Calculation of bioconcentration factor

$BCF = Cf / \overline{Cwt}$

BCF : Bioconcentration factor

Cf : Concentration of the test item in test fish (subtract FB) (ng/g)

$\overline{Cwt}$: The average concentration of the test item in test water (μg/L)

FB : The average concentration of blank in analysis of control or seemingly (blank) concentration of test item (ng/g)

b) The average bioconcentration factor in *m*-th analysis

$BCFm = (BCFa + BCFb) / n$

BCFm : The average bioconcentration factor in *m*-th analysis (number of group 2 (a,b))

BCFa,b : Each bioconcentration factor in *m*-th analysis of test fish

n : Number of group in *m*-th analysis of test fish

BCFm was not calculated when one or more concentrations of the test item in test fish at *m*-th analysis were not higher than minimum determination limit.

14.7.3 Definition of steady-state

The steady-state of BCF is defined to reach when the variation of BCFs in four successive analyses at intervals of more than 48 hours is within ±20%. When BCFs are less than 100, it is evaluated that a steady-state has been reached after 28 days even if the variation of BCFs are over ±20%.

14.7.4 Calculable BCF

On the basis of the LOQ for the test item in Section 14.6.6 d), BCF can be obtained when BCF exceeds the following. The average concentration of the test item in test water obtained from all the analyzed sample was used to calculate the following calculable BCF.

Level 1 8.0

Level 2 80

14.7.5 Calculation of growth rate constant (k_g)

All individual weight data were converted to natural logarithms and ln(weight) was plotted vs. time (day), then a linear least squares correlation was calculated for this plot. Growth rate constant for the study (k_g) was calculated as the slope of the linear correlation.

14.7.6 Calculation of lipid content

Lipid contents were calculated according to following equation.

$\text{Lipid content (\%)} = (T - T_0) / S \times 100$

T_0 : Weight of vessel (g)

T : Weight of sample for gravimetric analysis containing vessel (g)

S : Weight of fine sample taken out for analysis of lipid content (g)

14.8 Treatment of numerical values

Values were rounded off in accordance with JIS Z 8401:1999 rule B. Furthermore, numerical values were used for calculation without being rounded.

The concentration values of the test item in test water and fish were rounded off to 3 figures. BCF values were rounded off to 2 figures.

15. Factors that affected reliability of test

No adverse effects on the reliability of this test were noted.

16. Results and discussion

16.1 Concentration of the test item in test water

The measured concentrations of the test item in test water are shown in Table-1. Concentrations of the test item were maintained ≥88% of nominal concentrations and the variations were within ±20% of the average measured concentrations. Concentrations of the test item in test water before the experimental starting are as follows.

Level 1 9.54 μg/L

Level 2 0.959 μg/L

Table-1 Measured concentrations of test item in test water (Unit : μg/L)

Level	After 13 days	After 19 days	After 21 days	After 26 days	After 28 days	Average (Standard deviation)	Table	Fig.
1	8.98	9.20	8.79	9.98	9.68	9.33 (0.495)	4	6
2	0.888	0.895	0.885	0.983	1.04	0.939 (0.0710)	5	

16.2 Bioconcentration factors

BCFs are shown in Table-2. These BCFs plotted against the duration of exposure are shown in Figs. 1 and 2. BCFs of the test item were as follows.

Level 1 <8.0-10

Level 2 <80

Table-2 BCFs

Level	After 13 days	After 19 days	After 21 days	After 26 days	After 28 days	Table	Fig.
1	10 <8.0	<8.0 <8.0	<8.0 <8.0	<8.0 <8.0	<8.0 <8.0	7	9
2	<80 <80	<80 <80	<80 <80	<80 <80	<80 <80	8	10

16.3 BCFs at a steady-state (BCFss)

Because the test item in all test fish at last four successive analyses were not more than the LOQ, BCFss was not calculated. However, because all BCFs were less than 100, it was evaluated that a steady-state was reached after 28 days.

16.4 Growth rate constant

There was no statistically significant difference in the weight data analysis for duration of exposure Levels 1, 2 and Control, all the fish weight data (Levels 1, 2 and Control) was calculated as the overall slope of the linear correlation. The k_g calculated with the data for duration of exposure are shown as follows. Since Control was only two points (before the experiment start and after the experiment completion), it showed only inclination.

Level 1 0.0156±0.0037

Level 2 0.0144±0.0059

Control 0.0199

16.5 Lipid content in test fish

The measured lipid contents in the test fish are shown below. The change of lipid contents of after the experimental completion versus those before the experimental starting was -3% which was within ±25%.

Before initiation of experiment 3.90%
After termination of experiment 3.77%

16.6 Results of test fish observation

No abnormality in behavior or appearance was noted.

16.7 Discussion

The validity criteria of test method

In this test, the validity criteria of test method were applied as following conditions. Therefore we concluded that it was reasonable test condition to estimate bioconcentration potential of test item.

a) The temperature variation was less than ±2°C of set water temperature 25°C.
b) The concentration of dissolved oxygen does not fall below 60% of saturated concentration 8.1 mg/L at 25°C.
c) The concentration of the test item in the test tank was maintained within ±20% of the mean of the measured values during the test period.
d) The mortality or other adverse effects/disease in both control and test group was not observed.

17. Remarks

Instruments, apparatuses and reagents, etc. for the test

a) Instruments for fish care

Micro quantitative pump for supplying stock solution :
NIHON SEIMITSU KAGAKU CO., LTD type SP-D-2500(01)
NIHON SEIMITSU KAGAKU CO., LTD type SP-Y-2500(01)

Instrument for measuring concentration of dissolved oxygen :
Iijima Electronics Co., Ltd. type ID-100

pH meter : DKK-TOA CORPORATION type HM-21P

b) Instruments, apparatuses and reagents

Instruments and apparatuses

Gas chromatography-mass spectrometer :
see page 17

Total organic carbon analyzer : Shimadzu type TOC-LCPH
Electronic analytical balance : Sartorius AG type BP301S
A&D type FX-2000i
Infrared spectrophotometer : Shimadzu type IR Affinity-1S
Homogenizer (polytron) : Kinematica AG type PT3100
Centrifuge : Hitachi Koki type CR21G
Rotary evaporator : Tokyo Rika Kikai type N-1000K2
Mechanical shaker : Taitec type SR-2DW

Reagents

Ultrapure water : City water was treated with ultrapure water system.

Purified water (Japanese Pharmacopeia) : Takasugi Pharmaceutical

Acetonitrile (guaranteed reagent) : Wako Pure Chemical Industries

Ethyl acetate (extra pure) : Kanto Chemical Co., Inc.

2-propanol (EXTRA PURE REAGENT) : NACALAI TESQUE

N,N-dimethylformamide (EXTRA PURE REAGENT) : NACALAI TESQUE

Ammonium sulfate (extra pure) : Kanto Chemical Co., Inc.

Sodium sulfate (extra pure) : Kanto Chemical Co., Inc.

Buffer Solution pH10 (NH_4OH-NH_4Cl) [for Chelatometric titration] : Tokyo Chemical Inductry Co., Ltd.

Eriochrome Black T : Tokyo Chemical Inductry Co., Ltd.

Ethylenediaminetetraacetic acid disodium salt solution (volumetric, 0.01 M EDTA-Na, in H_2O) : Sigma-Aldrich Japan K.K.

2 mol/L Hydrochloric acid (extra pure) : Kanto Chemical Co., Inc.

c) Instruments, apparatuses and reagents for gravimetric analysis of lipid content in test fish

Instruments and apparatus

Homogenizer (polytron) : Kinematica AG type PT3100

Electronic analytical balance : Mettler-Toledo International type AB204-S

Vacuum pump : ULVAC KIKO type DA-20D

Vacuum desiccator : Iuchiseieido type VL

Homogenizer (autocellmaster) : AS ONE type CM-200

Rotary evaporator : Tokyo Rika Kikai type N-1000K2

Reagents

Methanol (Wako 1st grade) : Wako Pure Chemical Industries

Chloroform (guaranteed reagent) : Wako Pure Chemical Industries, Ltd.

Sodium sulfate (extra pure) : Kanto Chemical Co., Inc.

Table-3 Calculation table for recovery and blank test (analysis of test water)

Study No. 46047

Sample description	A	B	C	D	E	F
Standard 50.0μg/L	133979					
Recovery a	107862	1	5	-	201	80.5
Recovery b	113635	1	5	-	212	84.8
						Average
Standard 50.0μg/L	132914					82.7
Blank a	n.d.	1	5	-	-	
Blank b	n.d.	1	5	-	-	
				Average		
				-		

(a, b : individual sample)

A : Peak area

A(std) : Standard solution A(t) : Sample

B : Ratio of portion used for analysis

C : Final volume (mL)

D : Amount of blank in test water (ng)

E : Amount of test item recovered (ng)

$E = P \times (A(t) / A(std)) / B \times C - D$

F : Recovery rate (%)

$F = E / Q \times 100$

P : Concentration of test item in standard solution 50.0μg/L

Q : Amount of test item added (250ng)

See Fig. 5

June 19, 2014 Name _____

Table-4 Calculation table for analysis of test water (Level 1)

Study No. 46047

Sample description	A	I
Standard 50.0μg/L	68677	
Before exposure	54174	9.54
Standard 50.0μg/L	58979	
Test water after 13 days	43758	8.98
Standard 50.0μg/L	47766	
Test water after 19 days	36332	9.20
Standard 50.0μg/L	51656	
Test water after 21 days	37534	8.79
Standard 50.0μg/L	71729	
Test water after 26 days	59195	9.98
Standard 50.0μg/L	64202	
Test water after 28 days	51357	9.68

Average concentration of test item in test water after exposure 9.33 (S.D. 0.495)

A: Peak area

A(std) : Standard solution A(t) : Sample

B: Ratio of portion used for analysis 1

C: Final volume 5mL

F: Recovery rate 82.7%

H: Volume of test water taken out 25mL

I: Concentration of test item in test water (μg/L)

$I = P \times (A(t) / A(std)) / B \times C / F \times 100 / H$

J: Average concentration of test item in test water after exposure (μg/L)

$J = (I(1) + ... + I(n)) / n$

n : Number of test water analyses (n = 5)

I (1) : First analysis of test water I (n) : Last analysis of test water

$$S.D. = \sqrt{\frac{n \times \sum_{i=1}^{n} I(i)^2 - \left(\sum_{i=1}^{n} I(i) \right)^2}{n \times (n-1)}}$$

P: Concentration of test item in standard solution 50.0μg/L

See Fig. 6

June 19, 2014 Name _____

Table-5 Calculation table for analysis of test water (Level 2)

Study No. 46047

Sample description	A	I
Standard 50.0μg/L	68677	
Before exposure	54444	0.959
Standard 50.0μg/L	58979	
Test water after 13 days	43283	0.888
Standard 50.0μg/L	47766	
Test water after 19 days	35330	0.895
Standard 50.0μg/L	51656	
Test water after 21 days	37783	0.885
Standard 50.0μg/L	71729	
Test water after 26 days	58307	0.983
Standard 50.0μg/L	64202	
Test water after 28 days	55326	1.04

Average concentration of test item in test water after exposure 0.939 (S.D. 0.0710)

A: Peak area

A(std) : Standard solution A(t) : Sample

B: Ratio of portion used for analysis 1

C: Final volume 5mL

F: Recovery rate 82.7%

H: Volume of test water taken out 250mL

I: Concentration of test item in test water (μg/L)

$I = P \times (A(t) / A(std)) / B \times C / F \times 100 / H$

J: Average concentration of test item in test water after exposure (μg/L)

$J = (I(1) + ... + I(n)) / n$

n : Number of test water analyses (n = 5)

I (1) : First analysis of test water I (n) : Last analysis of test water

$$S.D. = \sqrt{\frac{n \times \sum_{i=1}^{n} I(i)^2 - \left(\sum_{i=1}^{n} I(i) \right)^2}{n \times (n-1)}}$$

P: Concentration of test item in standard solution 50.0μg/L

See Fig. 6

June 19, 2014 Name _____

Table-6 Calculation table for recovery and blank test (analysis of test fish)

Study No. 46047

Sample description	A	C	D	E	F	G
Standard 50.0μg/L	154677					
Recovery a	101393	2.5/50	5	-	3280	65.6
Recovery b	105521	2.5/50	5	-	3410	68.2
						Average
						66.9
Standard 50.0μg/L	153764					
Blank a	n.d.	2.5/50	5	-	-	
Blank b	n.d.	2.5/50	5	-	-	
				Average		
				-		

(a, b : individual sample)

A: Peak area

A(std) : Standard solution A(t) : Sample

B: Ratio of portion used for analysis 1

C: Ratio of portion used for analysis (extracted solution)

D: Final volume (mL)

E: Amount of blank in test fish (ng)

F: Amount of test item recovered (ng)

$F = P \times (A(t) / A(std)) / B / C \times D - E$

G: Recovery rate (%)

$G = F / Q \times 100$

P: Concentration of test item in standard solution 50.0μg/L

Q: Amount of test item added (5000ng)

See Fig. 8

June 19, 2014 Name _____

Table-7 Calculation table for analysis of test fish (Level 1)

Study No. 46047

Sample description	A	D	G	K	J	M
Standard 50.0µg/L	67589					
Test fish after 13 days a	12078	1	14.0	95.4	10	-
Test fish after 13 days b	10023	1	17.2	64.5	-	
Standard 50.0µg/L	48146					
Test fish after 19 days a	1202	1	19.0	9.82	-	-
Test fish after 19 days b	1042	1	17.3	9.35	-	
Standard 50.0µg/L	51065					
Test fish after 21 days a	778	1	18.1	6.29	-	-
Test fish after 21 days b	1235	1	17.3	10.5	-	
Standard 50.0µg/L	70243					
Test fish after 26 days a	1411	1	17.6	8.53	-	-
Test fish after 26 days b	1951	1	19.0	10.9	-	
Standard 50.0µg/L	59765					
Test fish after 28 days a	1099	1	17.1	8.04	-	-
Test fish after 28 days b	1004	1	18.2	6.90	-	

(a, b : individual sample)

A: Peak area
A(std) : Standard solution A(t) : Sample
B: Ratio of portion used for analysis 2.5/50
C: Final volume 5mL
D: Dilution factor
E: Average concentration of blank in analysis of control 0ng/g
F: Recovery rate 66.9%
G: Weight of test fish (g)
K: Concentration of test item in test fish (ng/g)
$K = \{ P \times (A(t) / A(std)) / B \times D \times C / G - E \} / F \times 100$
H: Average concentration of test item in test water (µg/L) 9.33
I: Concentration of test item in test water (µg/L)
J: BCF
$J = K / H$
M: Average value of BCF(a) and BCF(b)
$M = \{ BCF(a) + BCF(b) \} / 2$
P: Concentration of test item in standard solution 50.0µg/L

See Fig. 9

June 19, 2014 Name _____

Table-8 Calculation table for analysis of test fish (Level 2)

Study No. 46047

Sample description	A	D	G	K	J	M
Standard 50.0μg/L	68664					
Test fish after 13 days a	9227	1	18.2	55.2	-	-
Test fish after 13 days b	9024	1	17.7	55.5	-	
Standard 50.0μg/L	48117					
Test fish after 19 days a	n.d.	1	18.6	-	-	-
Test fish after 19 days b	n.d.	1	19.8	-	-	
Standard 50.0μg/L	51597					
Test fish after 21 days a	n.d.	1	20.0	-	-	-
Test fish after 21 days b	n.d.	1	19.1	-	-	
Standard 50.0μg/L	71377					
Test fish after 26 days a	n.d.	1	16.8	-	-	-
Test fish after 26 days b	n.d.	1	16.8	-	-	
Standard 50.0μg/L	60729					
Test fish after 28 days a	n.d.	1	17.5	-	-	-
Test fish after 28 days b	n.d.	1	16.7	-	-	

(a, b : individual sample)

A: Peak area
A(std) : Standard solution A(t) : Sample
B: Ratio of portion used for analysis 2.5/50
C: Final volume 5mL
D: Dilution factor
E: Average concentration of blank in analysis of control 0ng/g
F: Recovery rate 66.9%
G: Weight of test fish (g)
K: Concentration of test item in test fish (ng/g)
$K = \{ P \times (A(t) / A(std)) / B \times D \times C / G - E \} / F \times 100$
H: Average concentration of test item in test water (μg/L) 0.939
I: Concentration of test item in test water (μg/L)
J: BCF
$J = K / H$
M: Average value of BCF(a) and BCF(b)
$M = \{ BCF(a) + BCF(b) \} / 2$
P: Concentration of test item in standard solution 50.0μg/L

See Fig. 10

June 19, 2014 Name _____

Table-9 Calculation table for analysis of test fish (Control)

Study No. 46047

Sample description	A	E	G	I
Standard 50.0μg/L	67313			
Before the experimental start a	n.d.	-	11.5	-
Before the experimental start b	n.d.	-	11.8	-
Standard 50.0μg/L	82664			
After the experimental completion a	n.d.	-	19.5	-
After the experimental completion b	n.d.	-	19.4	-
				Average
				-
(a, b : individual sample)				

A: Peak area

A(std) : Standard solution A(t) : Sample

B: Ratio of portion used for analysis 2.5/50

C: Final volume 5mL

E: Amount of blank in analysis of control (ng)

$E = P \times (A(t) / A(std)) / B \times C$

G: Weight of test fish (g)

I: Concentration of blank in test fish (ng/g)

$I = E / G$

P: Concentration of test item in standard solution 50.0μg/L

See Fig. 11

June 19, 2014 Name _____ _____

Reference 1 Analytical results of dilution water (Underground water)

Sampling date January 8, 2014 and March 10, 2014[*1]

Item	Unit	Measured value	Standard value	Determination limit
Total hardness (Ca, Mg)	mg/L	12	< 300 [*2]	1
Suspended solid	mg/L	< 1	< 20 [*3]	1
pH	—	7.9	6.5 ~ 8.5 [*4]	—
Total organic carbon	mg/L	0.7	< 2 [*3]	0.5
Chemical oxygen demand	mg/L	1.8	< 5 [*4]	1
Residual chlorine	mg/L	< 0.02	< 0.02 [*4]	0.02
Ammonium ion	mg/L	< 0.1	< 1 [*4]	0.1
Total cyanide	mg/L	< 0.05	n.d.[*4]	0.05
Alkalinity	mg/L	110	—	1
Electric conductivity	μS/cm	280	—	1
Organic phosphorus	mg/L	< 0.01	n.d.[*4]	0.01
Alkylmercury	mg/L	< 0.0005	n.d.[*4]	0.0005
Total mercury	mg/L	< 0.0005	< 0.0005 [*4]	0.0005
Cadmium	mg/L	< 0.001	< 0.01 [*4]	0.001
Chromium (VI)	mg/L	< 0.01	< 0.05 [*4]	0.01
Lead	mg/L	< 0.001	< 0.1 [*4]	0.001
Arsenic	mg/L	< 0.005	< 0.05 [*4]	0.005
Iron	mg/L	< 0.1	< 1.0 [*4]	0.1
Copper	mg/L	< 0.001	< 0.005 [*4]	0.001
Cobalt	mg/L	< 0.001	< 0.001 [*6]	0.001
Manganese	mg/L	0.007	< 0.05 [*2]	0.005
Zinc	mg/L	< 0.1	< 1.0 [*2]	0.1
Aluminium	mg/L	0.03	< 0.2 [*2]	0.02
Nickel	mg/L	< 0.001	< 0.001 [*6]	0.001
Silver	mg/L	< 0.0001	< 0.0001 [*6]	0.0001
Organochlorine pesticides				
1,2-Dichloropropane	mg/L	< 0.002	< 0.06 [*5]	0.002
Chlorothalonil	mg/L	< 0.004	< 0.04 [*5]	0.004
Propyzamide	mg/L	< 0.0008	< 0.008 [*5]	0.0008
Chlornitrofen	mg/L	< 0.0001	< 0.0001 [*2]	0.0001
Simazine	mg/L	< 0.0003	< 0.003 [*5]	0.0003
Thiobencarb	mg/L	< 0.002	< 0.02 [*5]	0.002
Organophosphorous pesticides				
Diazinon	mg/L	< 0.0005	< 0.005 [*5]	0.0005
Isoxathion	mg/L	< 0.0008	< 0.008 [*5]	0.0008
Fenitrothion	mg/L	< 0.0003	< 0.003 [*5]	0.0003
EPN	mg/L	< 0.0006	< 0.006 [*5]	0.0006
Dichlorvos	mg/L	< 0.001	< 0.01 [*5]	0.001
Iprobenfos	mg/L	< 0.0008	< 0.008 [*5]	0.0008
PCB	mg/L	< 0.0005	n.d.[*5]	0.0005
Coliform bacteria count	—	n.d.	n.d.[*2]	—
Fluorine compound	mg/L	1.1	< 1.5 [*4]	0.1
Anionic surfactant	mg/L	< 0.02	< 0.2 [*2]	0.02

*1 Sample of dilution water for analysis of nickel and that for analysis of other items were taken on March 10, 2014 and January 8, 2014, respectively.

*2 Ministerial ordinance of the Ministry of Health, Labour and Welfare No.101 (Revised May 30, 2003)

*3 OECD Guidelines for Testing of Chemicals, Fish, Early-life Stage Toxicity Test (Guideline 210, July 17, 1992)

*4 Water quality criteria for fisheries (Japan Fisheries Resource Conservation Association, March 1983)

*5 Environmental Quality Standards for Water Pollutants No.14 (Revised February 22, 1999, Environment Agency)

*6 OECD Guidelines for Testing of Chemicals, Bioconcentration : Flow-through Fish Test (Guideline 305, June 14, 1996)

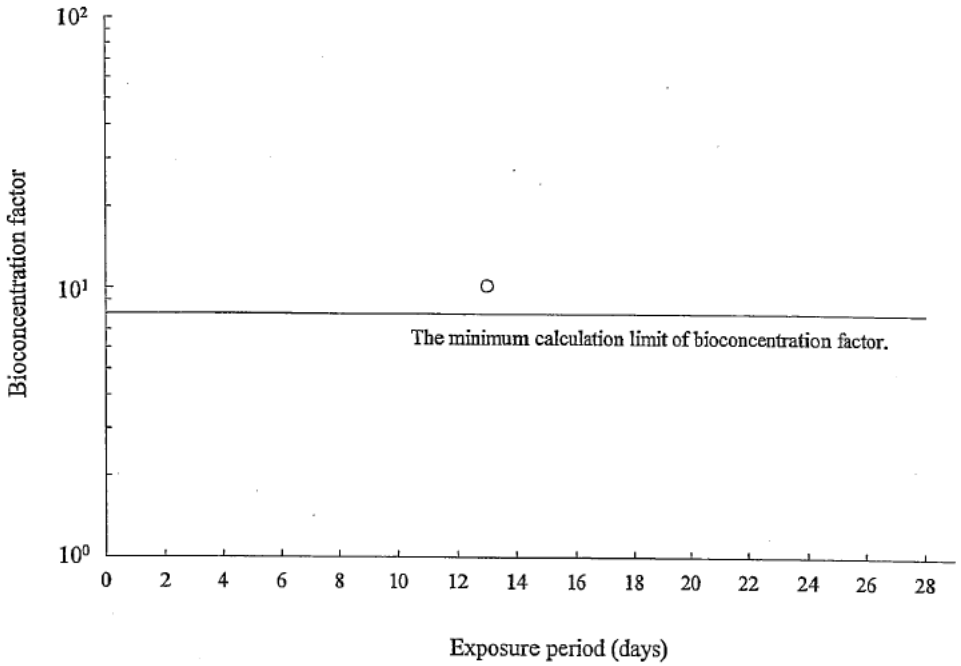


Fig.1 Correlation between exposure period and bioconcentration factor (Level 1).

Nine data after 13, 19, 21, 26 and 28 days were lower than detection limit.

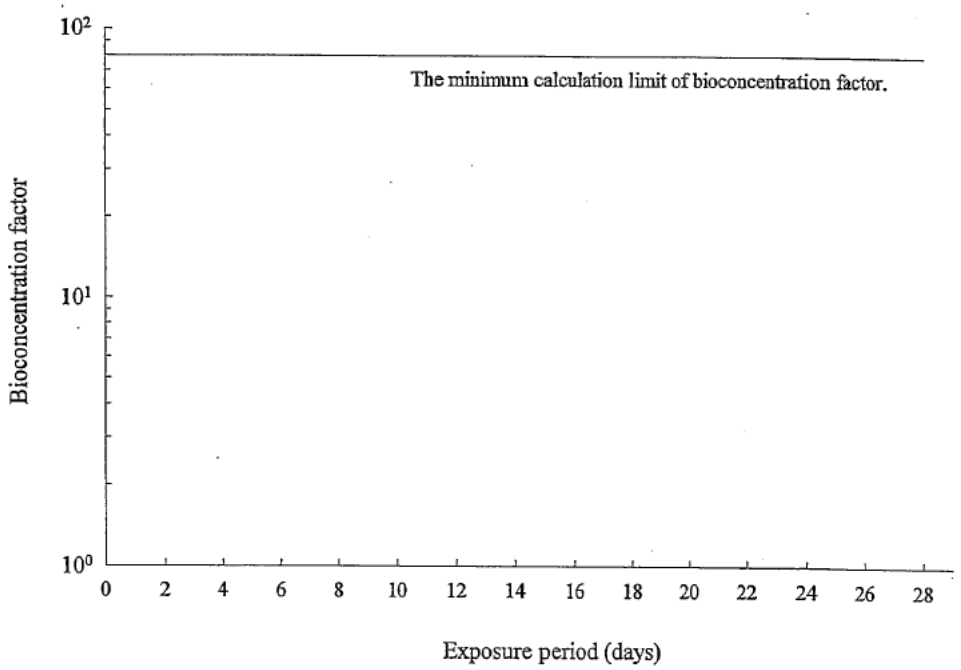
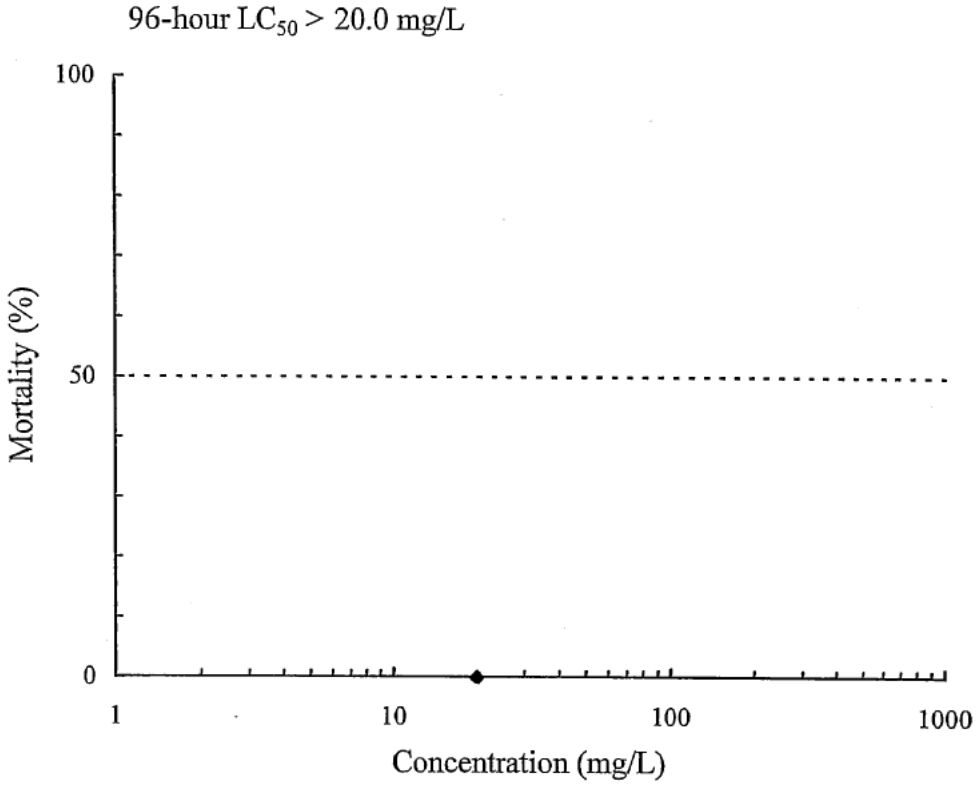


Fig.2 Correlation between exposure period and bioconcentration factor (Level 2).

Ten data after 13, 19, 21, 26 and 28 days were lower than detection limit.

June 20, 2014 Name _____



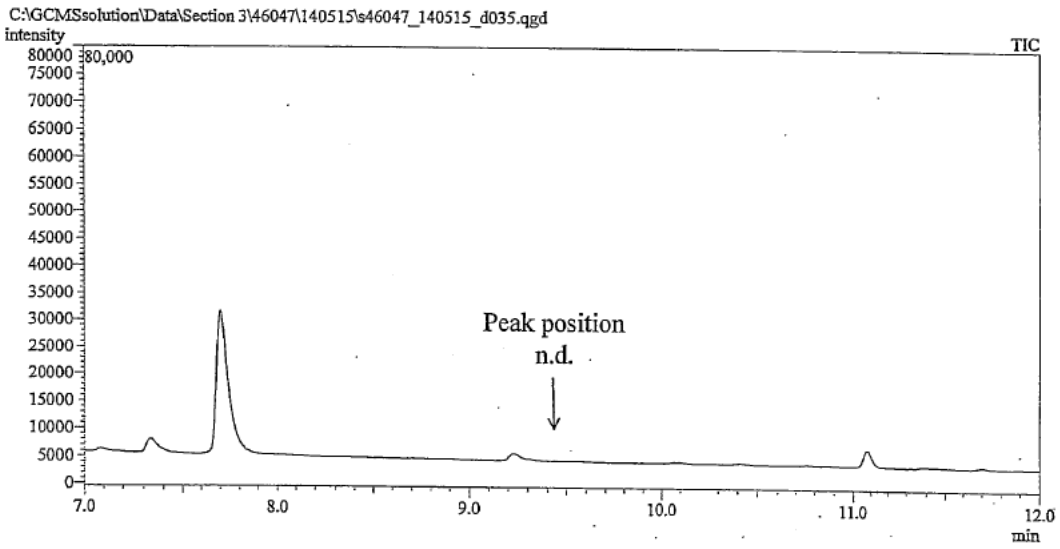
Concentration (mg/L)	Cumulative Mortality (%)			
	24 hours	48 hours	72 hours	96 hours
Control	0	0	0	0
20.0	0	0	0	0

Fig. 3 Concentration - mortality curve.

Date : April 29, 2014 Name

Solvent blank (w)

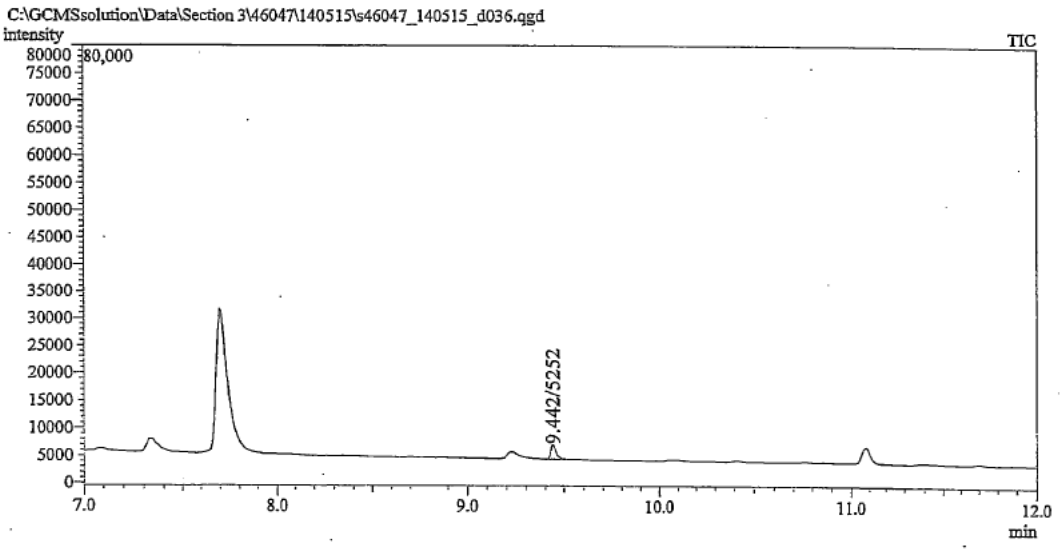
Operating date : May. 16, 2014
File name : C:\GCMSsolution\Data\Section 3\46047\140515\s46047_140515_d035.qgd



Date : May. 19, 2014 Name :

Standard solution 5.00 ug/L (w)

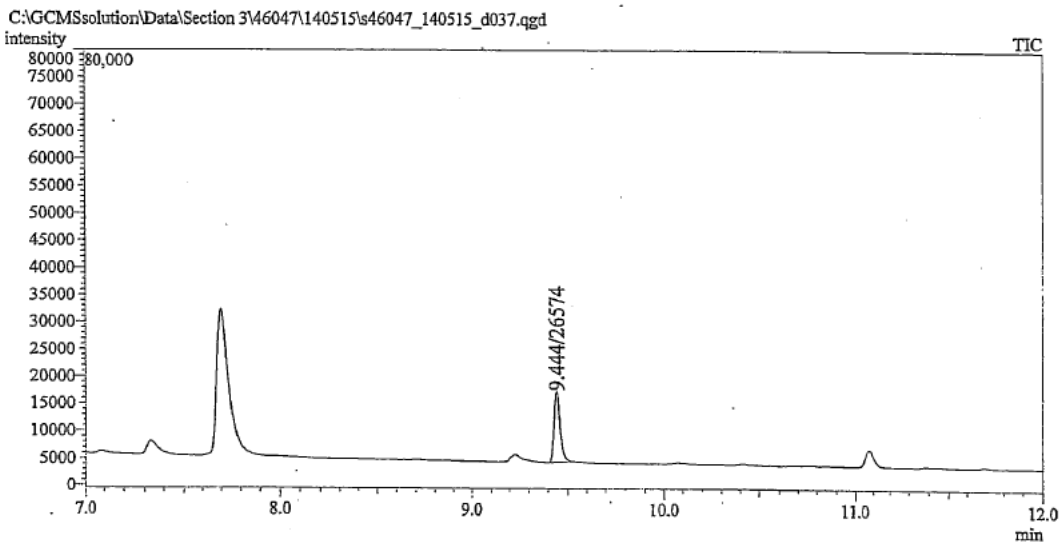
Operating date : May. 16, 2014
File name : C:\GCMSsolution\Data\Section 3\46047\140515\s46047_140515_d036.qgd



Date : May. 19, 2014 Name :

Fig. 4-1 Chromatograms of GC-MS analysis for calibration curve (analysis of test water).

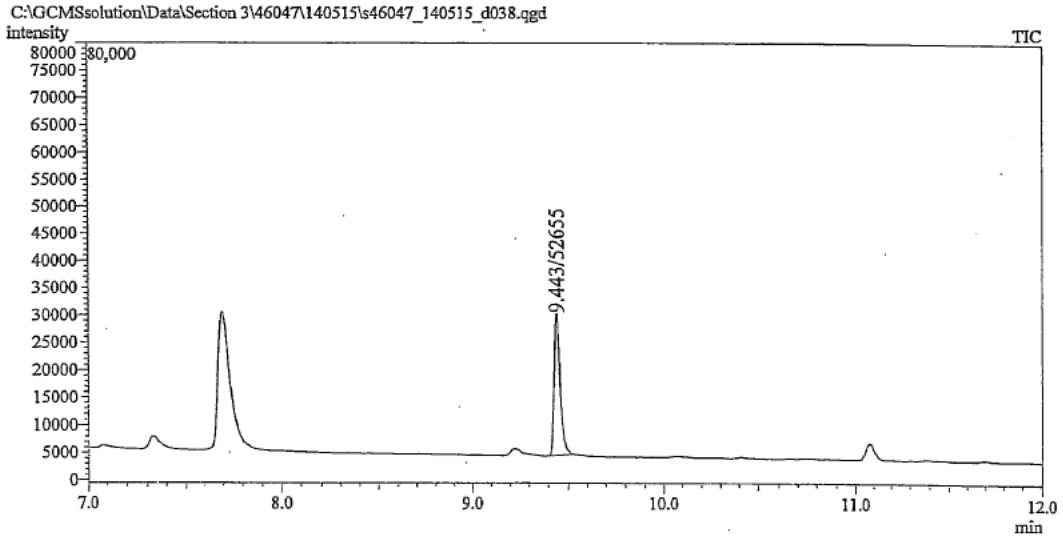
Standard solution 25.0 ug/L (w)
Operating date : May. 16, 2014
File name : C:\GCMSsolution\Data\Section 3\46047\140515\s46047_140515_d037.qgd



Date : May. 19, 2014 Name :

Study No. 46047

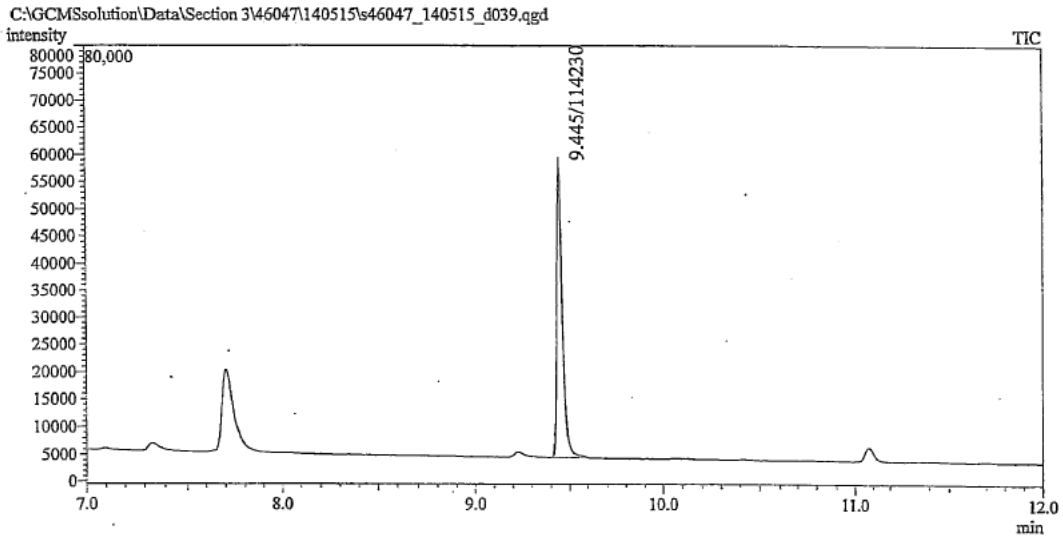
Standard solution 50.0 ug/L (w)
Operating date : May. 16, 2014
File name : C:\GCMSsolution\Data\Section 3\46047\140515\s46047_140515_d038.qgd



Date : May. 19, 2014 Name :

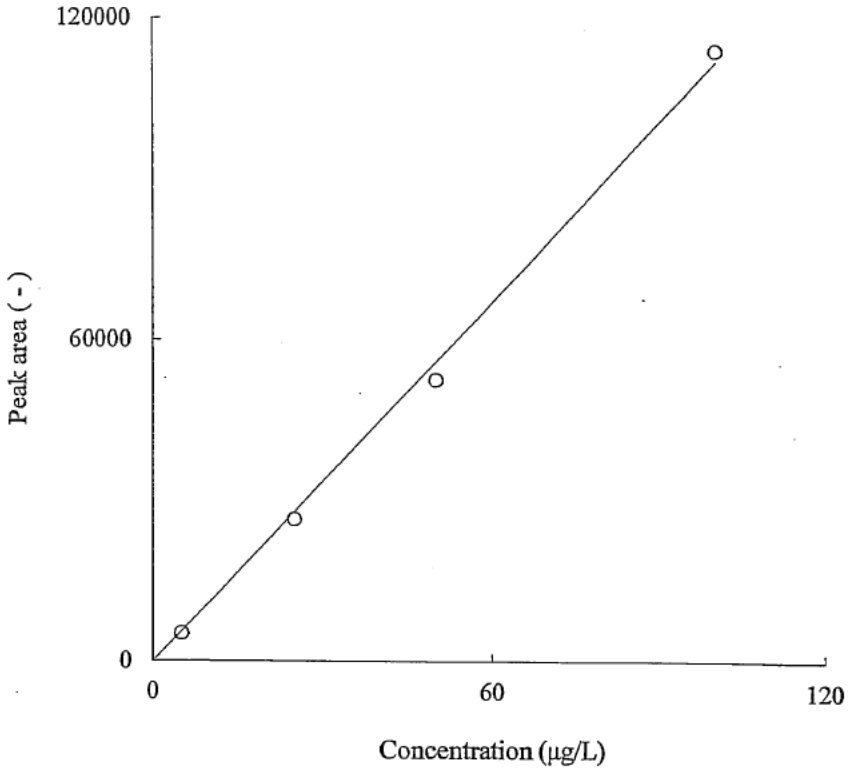
Fig. 4-2 Chromatograms of GC-MS analysis for calibration curve (analysis of test water).

Standard solution 100 ug/L (w)
Operating date : May. 16, 2014
File name : C:\GCMSsolution\Data\Section 3\46047\140515\s46047_140515_d039.qgd



Date : May. 19, 2014 Name :

Fig. 4-3 Chromatograms of GC-MS analysis for calibration curve (analysis of test water).



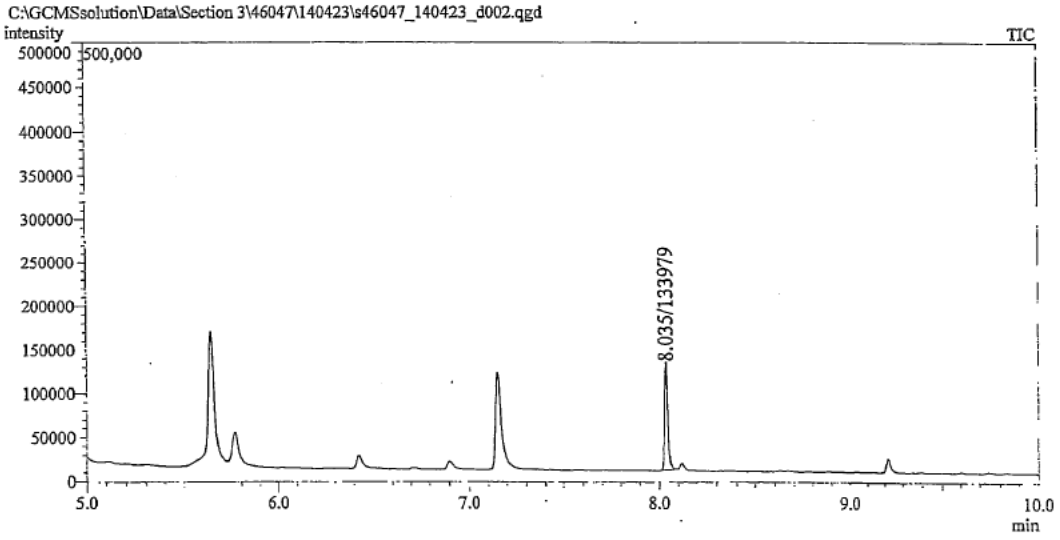
y = 1121x
r = 0.999

Concentration (μg/L)	Peak area (-)
5.00	5252
25.0	26574
50.0	52655
100	114230

Fig. 4- 4 Calibration curve of test item (analysis of test water).

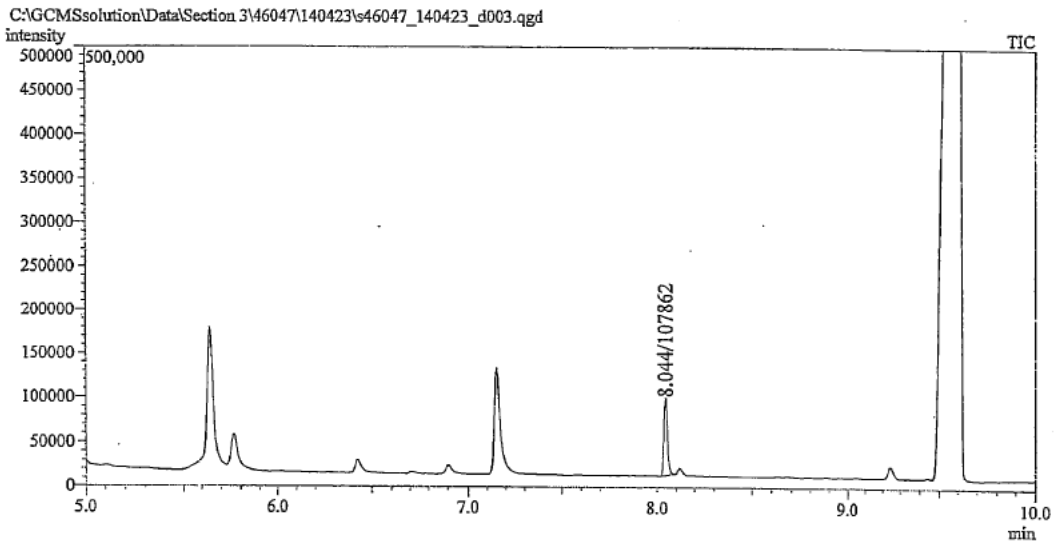
June 19, 2014 Name _____

Standard solution 50.0 ug/L (w)
Operating date : Apr. 23, 2014
File name : C:\GCMSsolution\Data\Section 3\46047\140423\s46047_140423_d002.qgd



Date : Apr. 24, 2014 Name :

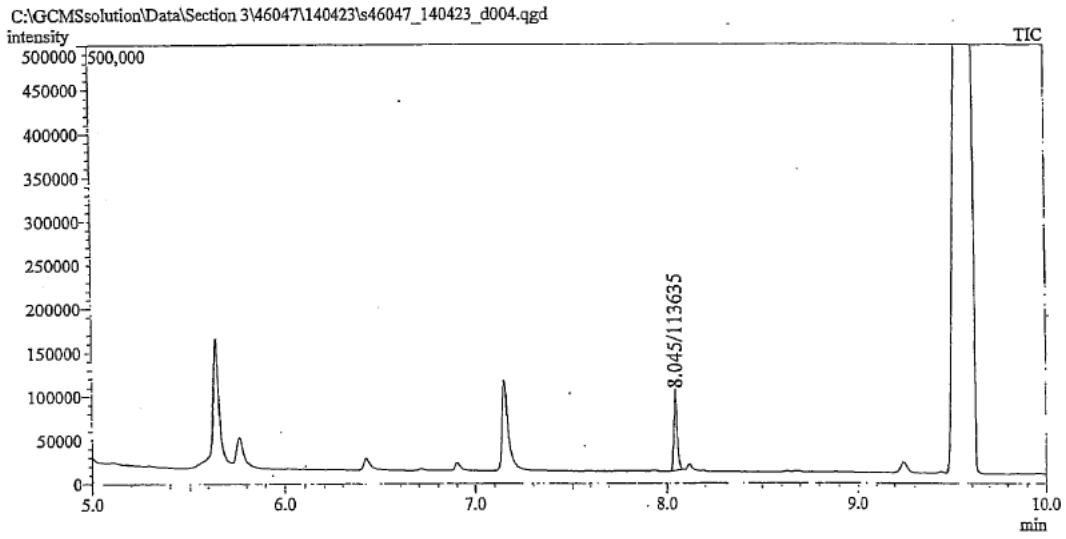
Recovery test from test water a
Operating date : Apr. 23, 2014
File name : C:\GCMSsolution\Data\Section 3\46047\140423\s46047_140423_d003.qgd



Date : Apr. 24, 2014 Name :

Fig. 5-1 Chromatograms of GC-MS analysis for recovery and blank test (analysis of test water).

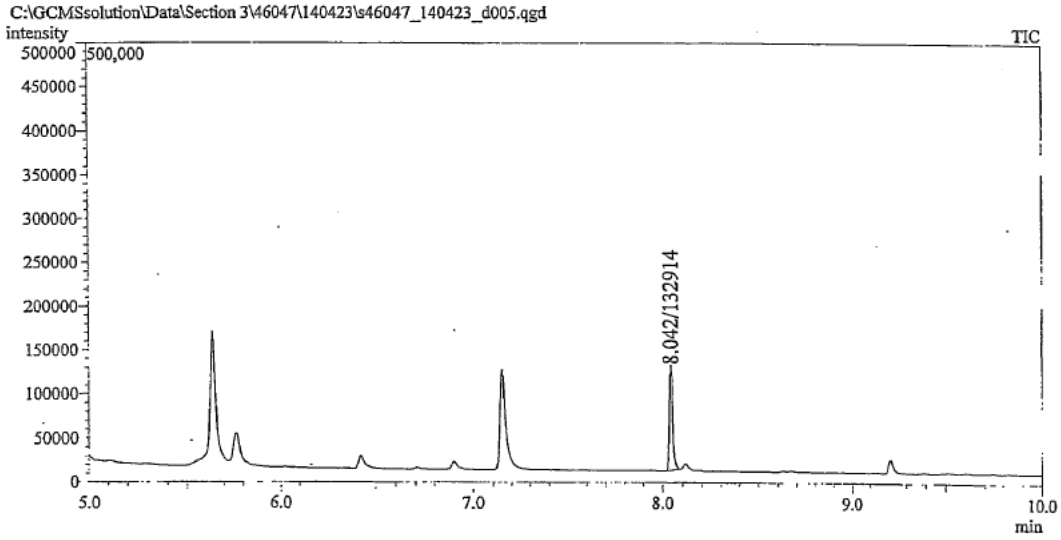
Recovery test from test water b
Operating date : Apr. 23, 2014
File name : C:\GCMSsolution\Data\Section 3\46047\140423\s46047_140423_d004.qgd



Date : Apr. 24, 2014 Name :

Fig. 5-2 Chromatograms of GC-MS analysis for recovery and blank test (analysis of test water).

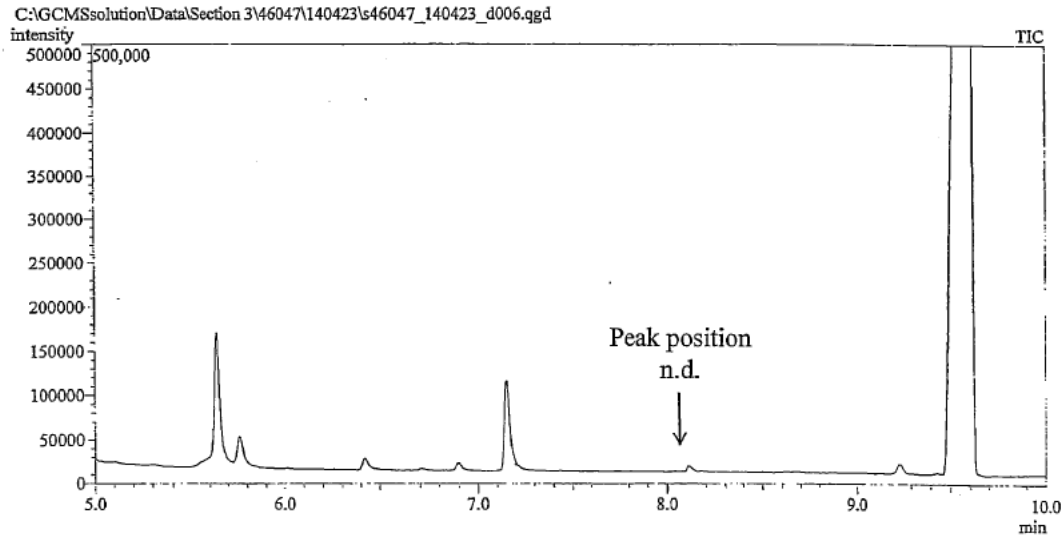
Standard solution 50.0 ug/L (w)
Operating date : Apr. 23, 2014
File name : C:\GCMSsolution\Data\Section 3\46047\140423\s46047_140423_d005.qgd



Date : Apr. 24, 2014 Name :

Study No. 46047

Blank test of test water a
Operating date : Apr. 23, 2014
File name : C:\GCMSsolution\Data\Section 3\46047\140423\s46047_140423_d006.qgd

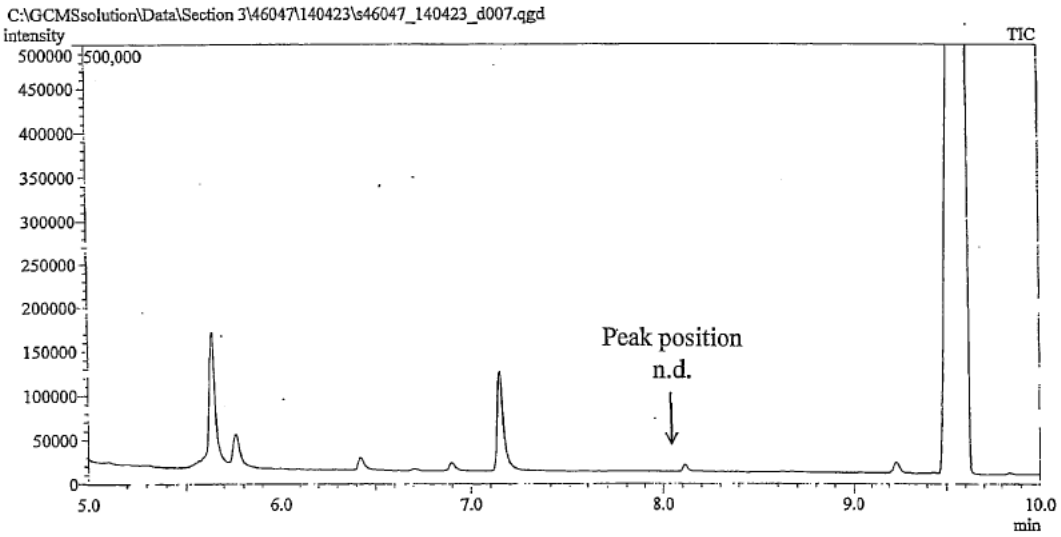


Date : Apr. 24, 2014 Name :

Fig. 5-3 Chromatograms of GC-MS analysis for recovery and blank test (analysis of test water).

Blank test of test water b

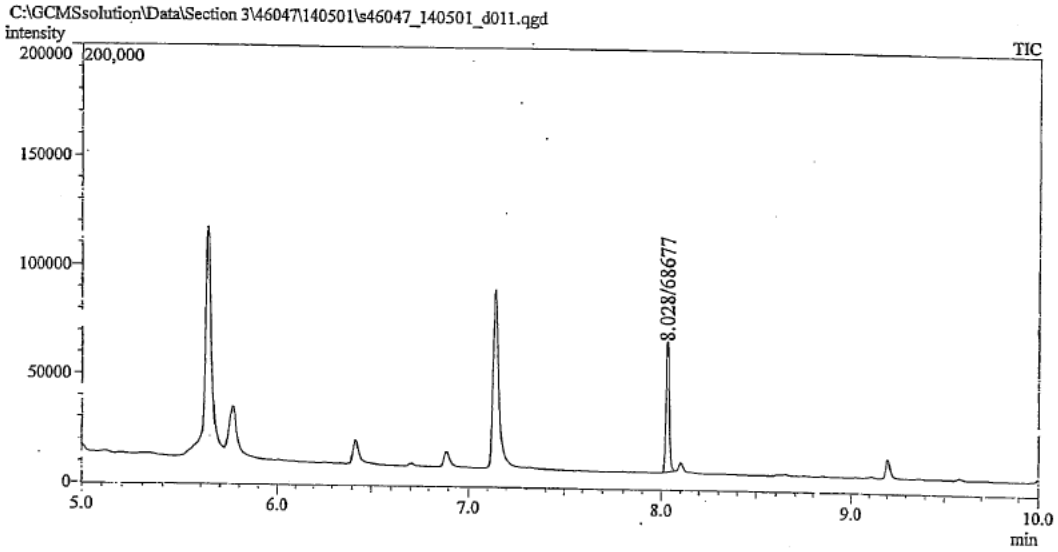
Operating date : Apr. 23, 2014
File name : C:\GCMSsolution\Data\Section 3\46047\140423\s46047_140423_d007.qgd



Date : Apr. 24, 2014 Name :

Fig. 5-4 Chromatograms of GC-MS analysis for recovery and blank test (analysis of test water).

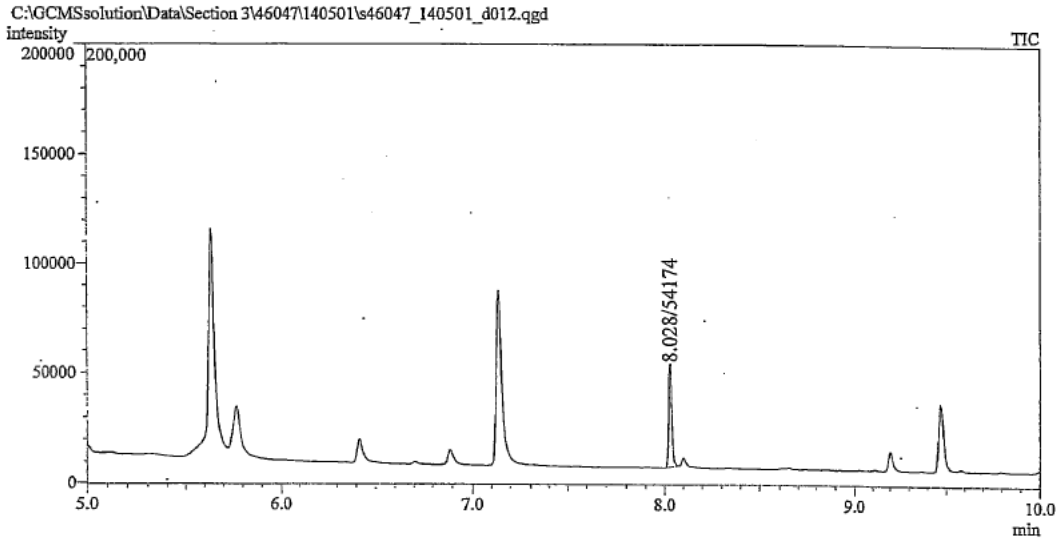
Standard solution 50.0 ug/L (w)
Operating date : May. 01, 2014
File name : C:\GCMSsolution\Data\Section 3\46047\140501\s46047_140501_d011.qgd



Date : May. 02, 2014 Name :

Study No. 46047

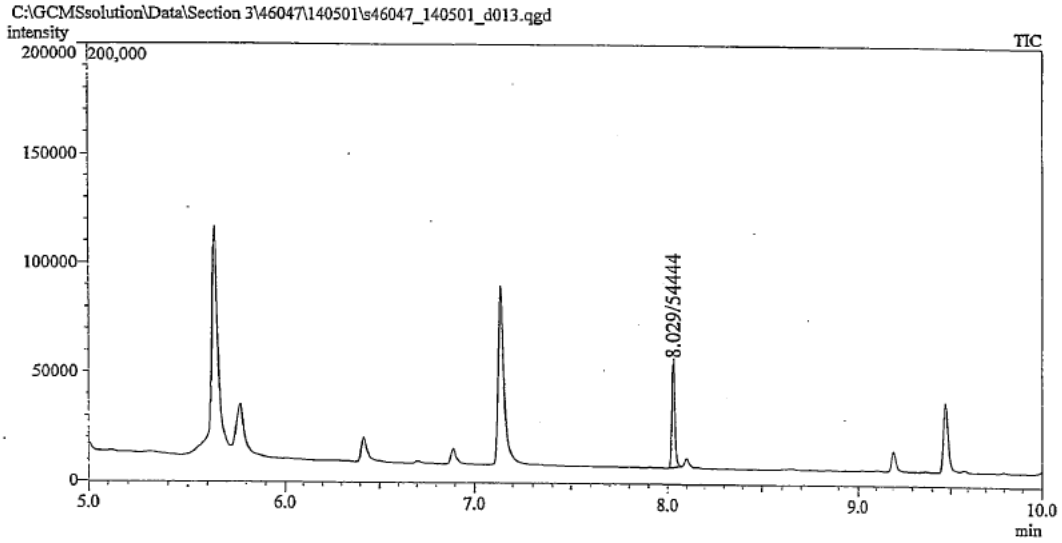
Test water before exposure (Level 1)
Operating date : May. 01, 2014
File name : C:\GCMSsolution\Data\Section 3\46047\140501\s46047_140501_d012.qgd



Date : May. 02, 2014 Name :

Fig. 6-1 Chromatograms of GC-MS analysis for test water.

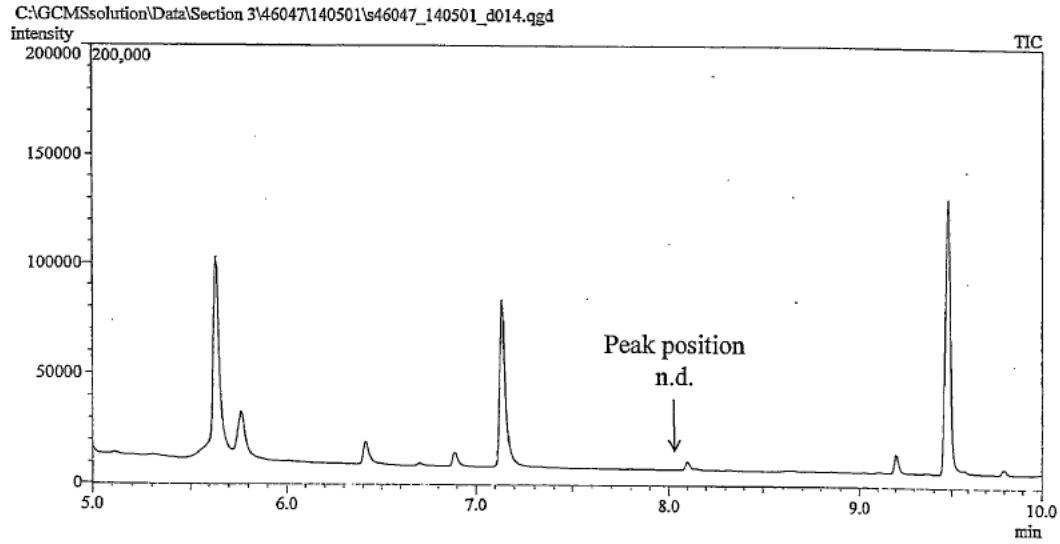
Test water before exposure (Level 2)
Operating date : May. 01, 2014
File name : C:\GCMSsolution\Data\Section 3\46047\140501\s46047_140501_d013.qgd



Date : May. 02, 2014 Name :

Study No. 46047

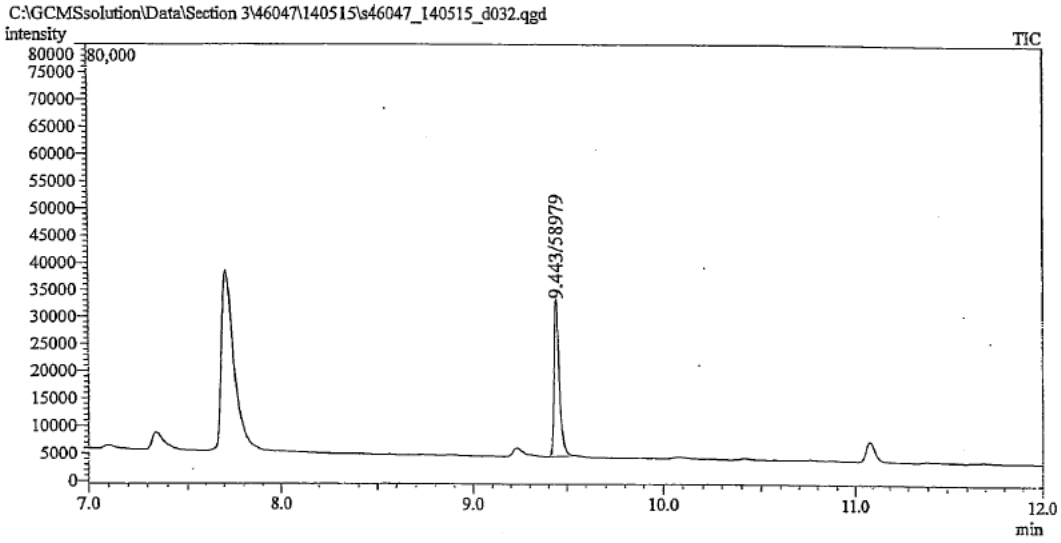
Test water before exposure (Control)
Operating date : May. 01, 2014
File name : C:\GCMSsolution\Data\Section 3\46047\140501\s46047_140501_d014.qgd



Date : May. 02, 2014 Name :

Fig. 6-2 Chromatograms of GC-MS analysis for test water.

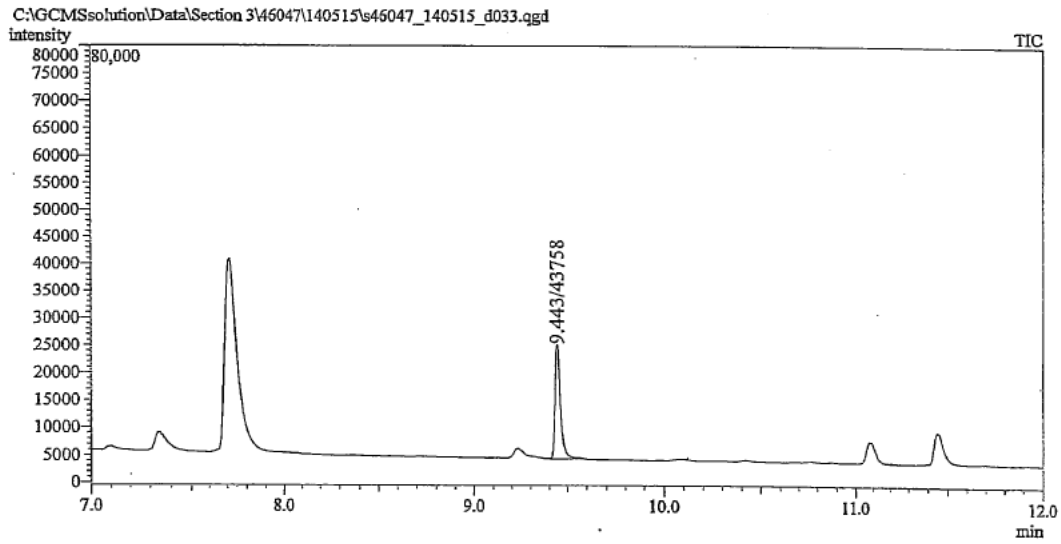
Standard solution 50.0 ug/L (w)
Operating date : May. 16, 2014
File name : C:\GCMSsolution\Data\Section 3\46047\140515\s46047_140515_d032.qgd



Date : May. 19, 2014 Name :

Study No. 46047

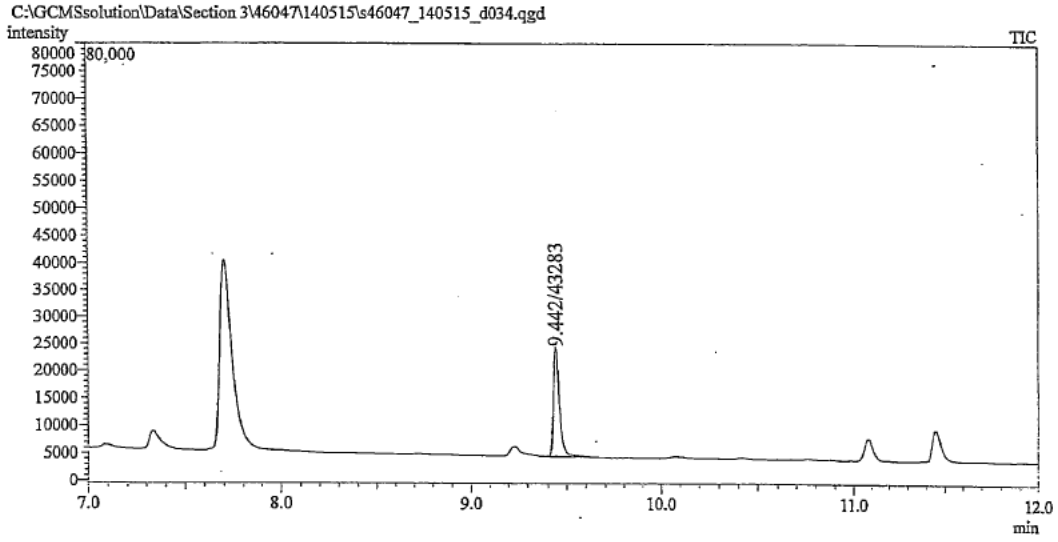
Test water after 13 days (Level 1)
Operating date : May. 16, 2014
File name : C:\GCMSsolution\Data\Section 3\46047\140515\s46047_140515_d033.qgd



Date : May. 19, 2014 Name :

Fig. 6-3 Chromatograms of GC-MS analysis for test water.

Test water after 13 days (Level 2)
Operating date : May. 16, 2014
File name : C:\GCMSsolution\Data\Section 3\46047\140515\s46047_140515_d034.qgd

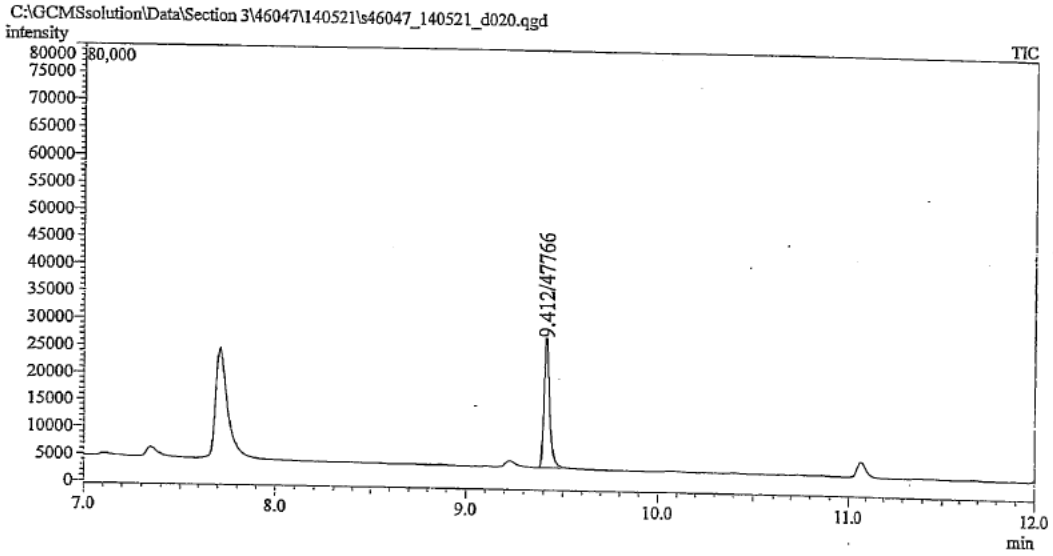


Date : May. 19, 2014 Name :

Fig. 6-4 Chromatograms of GC-MS analysis for test water.

Standard solution 50.0 ug/L (w)

Operating date : May. 22, 2014
File name : C:\GCMSsolution\Data\Section 3\46047\140521\s46047_140521_d020.qgd

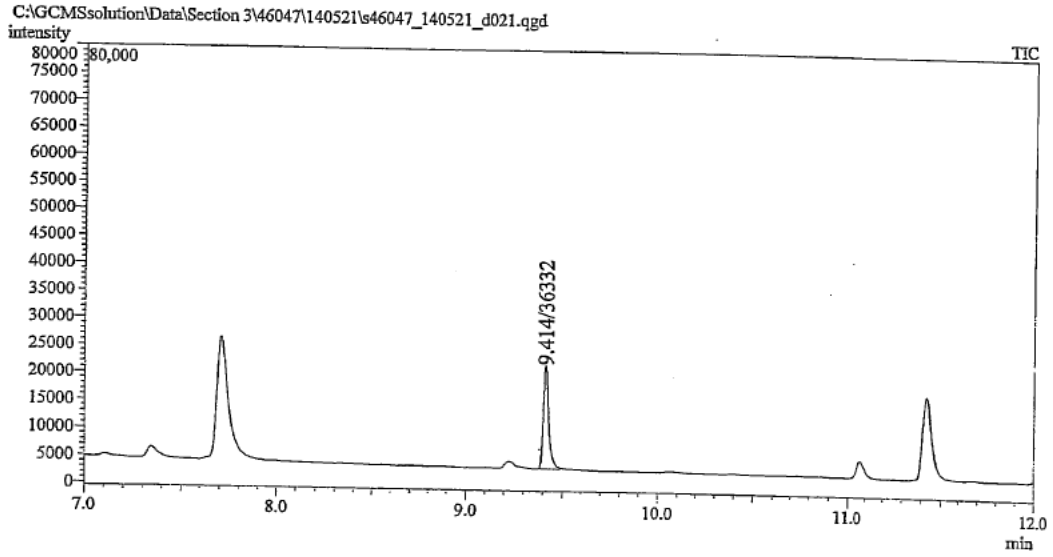


Date : May. 22, 2014 Name :

Study No. 46047

Test water after 19 days (Level 1)

Operating date : May. 22, 2014
File name : C:\GCMSsolution\Data\Section 3\46047\140521\s46047_140521_d021.qgd



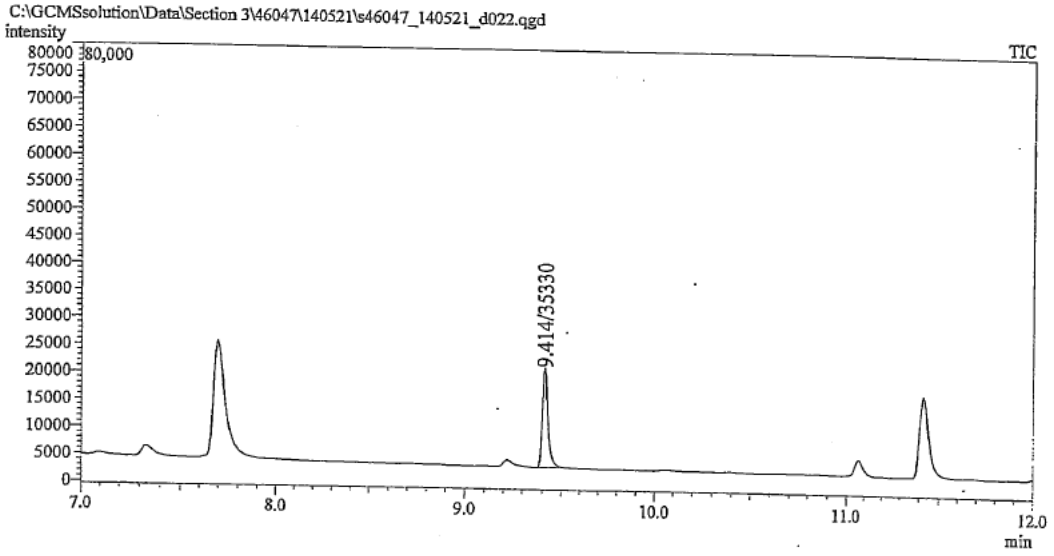
Date : May. 22, 2014 Name :

Fig. 6-5 Chromatograms of GC-MS analysis for test water.

Test water after 19 days (Level 2)

Operating date : May. 22, 2014

File name : C:\GCMSsolution\Data\Section 3\46047\140521\s46047_140521_d022.qgd

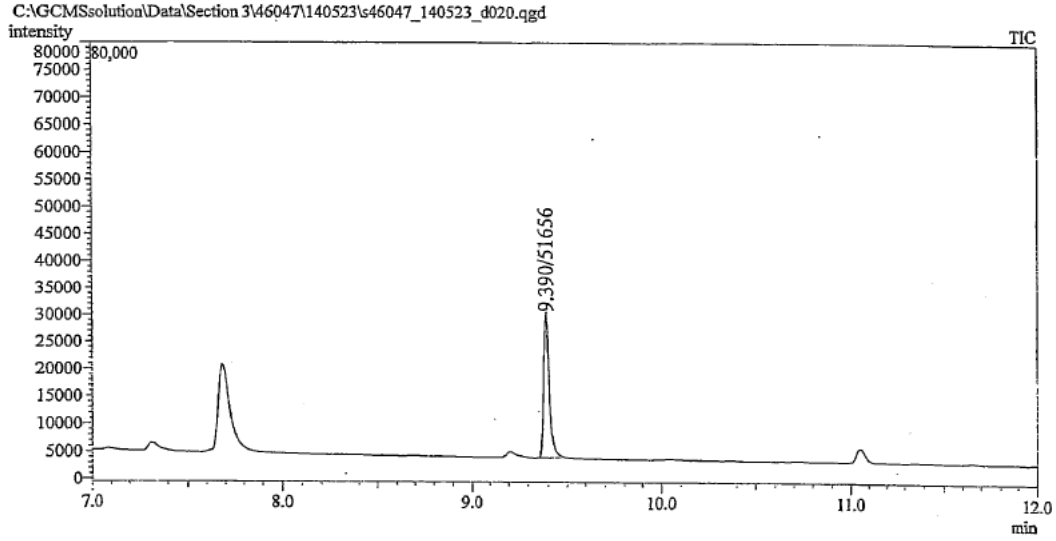


Date : May. 22, 2014 Name :

Fig. 6-6 Chromatograms of GC-MS analysis for test water.

Standard solution 50.0 ug/L (w)

Operating date : May. 24, 2014
File name : C:\GCMSsolution\Data\Section 3\46047\140523\s46047_140523_d020.qgd

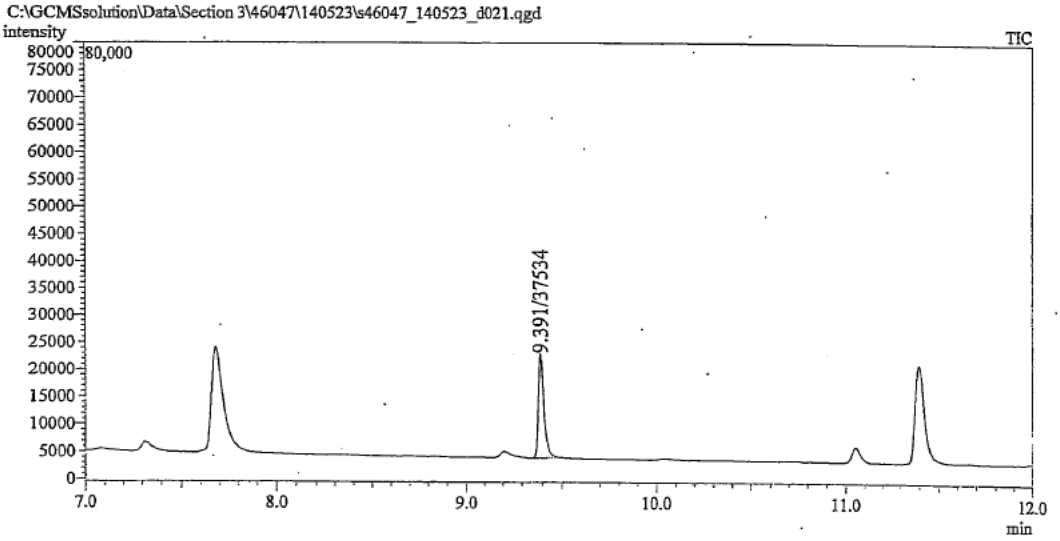


Date : May. 26, 2014 Name :

Study No. 46047

Test water after 21 days (Level 1)

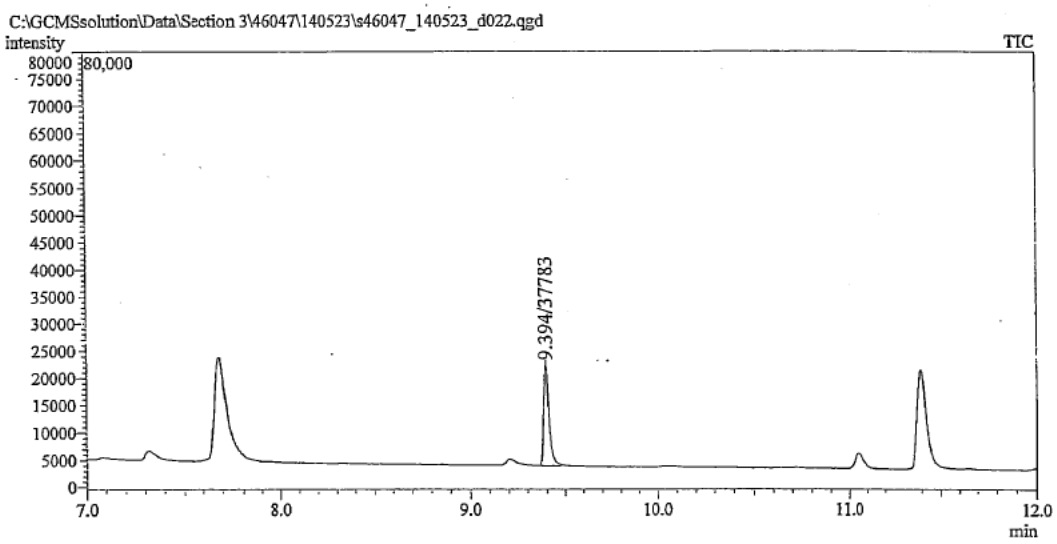
Operating date : May. 24, 2014
File name : C:\GCMSsolution\Data\Section 3\46047\140523\s46047_140523_d021.qgd



Date : May. 26, 2014 Name :

Fig. 6-7 Chromatograms of GC-MS analysis for test water.

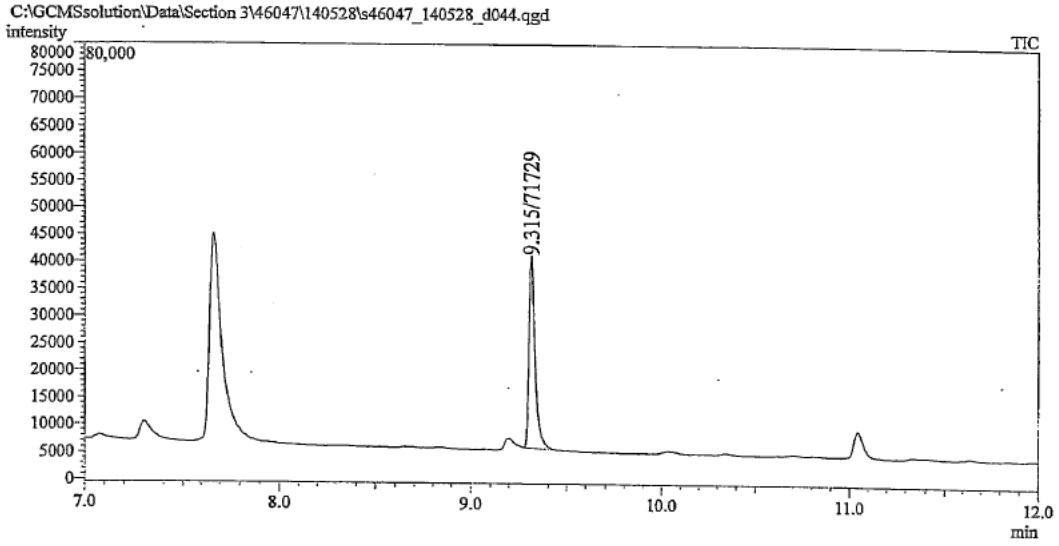
Test water after 21 days (Level 2)
Operating date : May. 24, 2014
File name : C:\GCMSsolution\Data\Section 3\46047\140523\s46047_140523_d022.qgd



Date : May. 26, 2014 Name :

Fig. 6-8 Chromatograms of GC-MS analysis for test water.

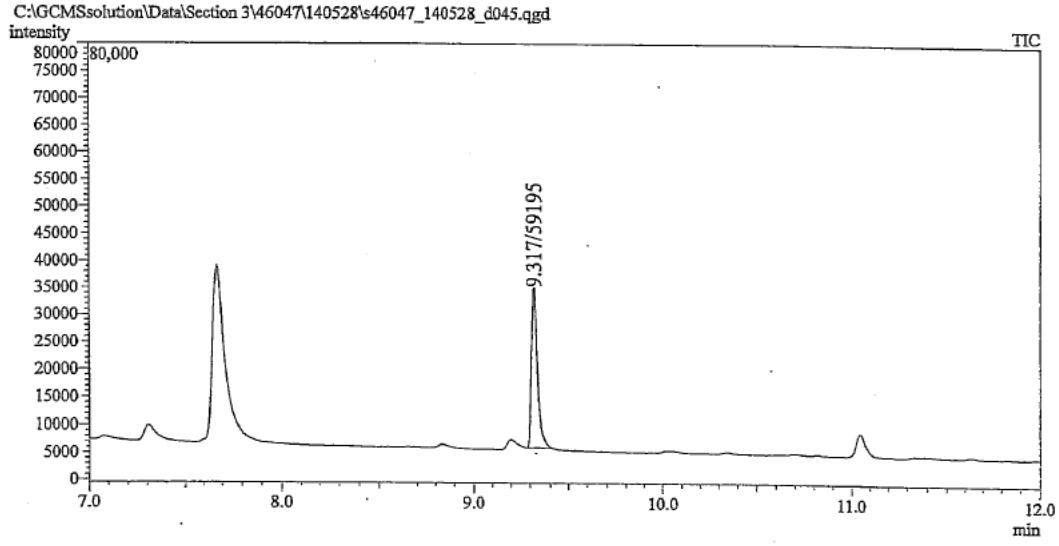
Standard solution 50.0 ug/L (w)
Operating date : May. 30, 2014
File name : C:\GCMSsolution\Data\Section 3\46047\140528\s46047_140528_d044.qgd



Date : May. 30, 2014 Name :

Study No. 46047

Test water after 26 days (Level 1)
Operating date : May. 30, 2014
File name : C:\GCMSsolution\Data\Section 3\46047\140528\s46047_140528_d045.qgd

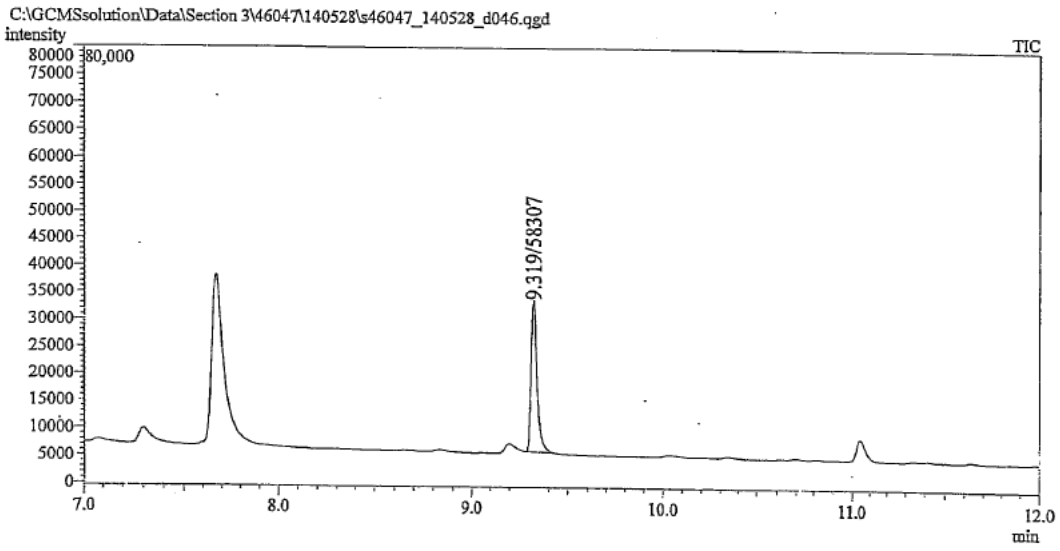


Date : May. 30, 2014 Name :

Fig. 6-9 Chromatograms of GC-MS analysis for test water.

Test water after 26 days (Level 2)

Operating date : May. 30, 2014
File name : C:\GCMSsolution\Data\Section 3\46047\140528\s46047_140528_d046.qgd

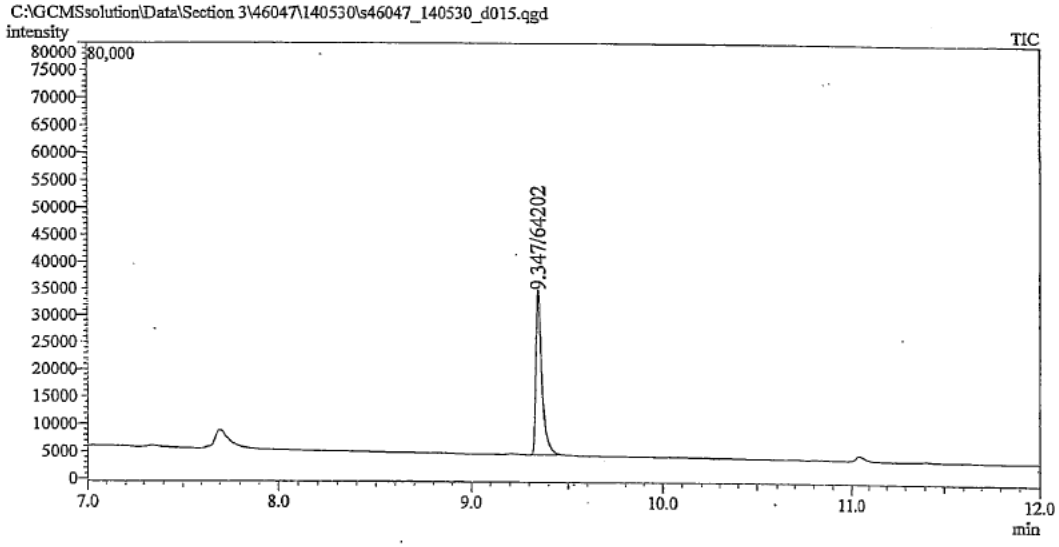


Date : May. 30, 2014 Name :

Fig. 6-10 Chromatograms of GC-MS analysis for test water.

Standard solution 50.0 ug/L (w)

Operating date : May. 31, 2014
File name : C:\GCMSsolution\Data\Section 3\46047\140530\s46047_140530_d015.qgd

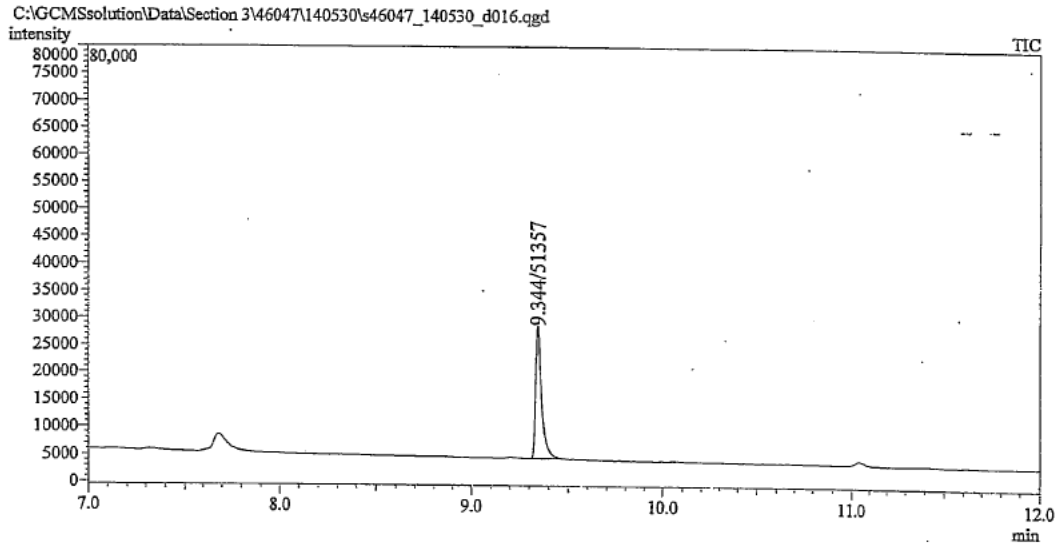


Date : Jun. 03, 2014 Name :

Study No. 46047

Test water after 28 days (Level 1)

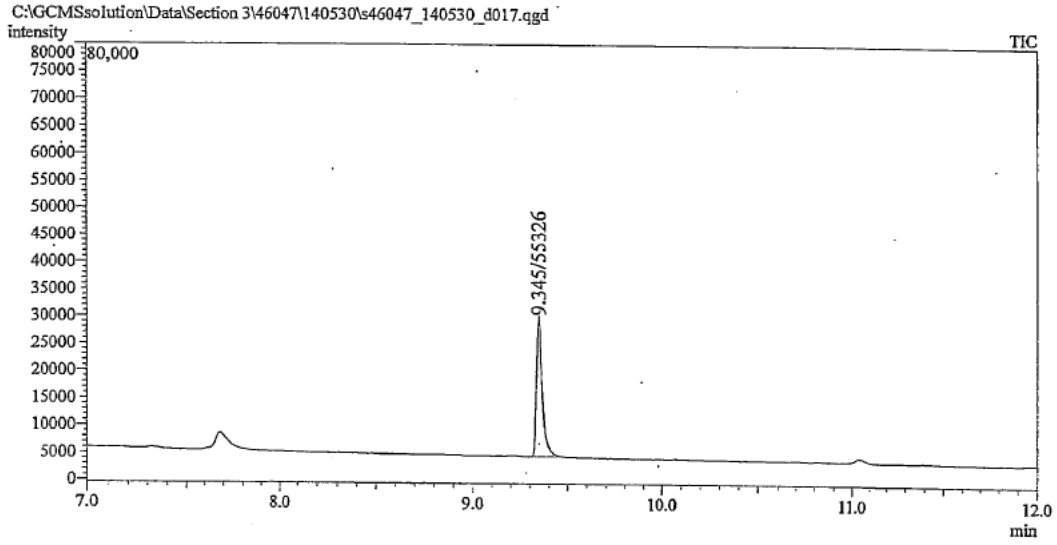
Operating date : May. 31, 2014
File name : C:\GCMSsolution\Data\Section 3\46047\140530\s46047_140530_d016.qgd



Date : Jun. 03, 2014 Name :

Fig. 6-11 Chromatograms of GC-MS analysis for test water.

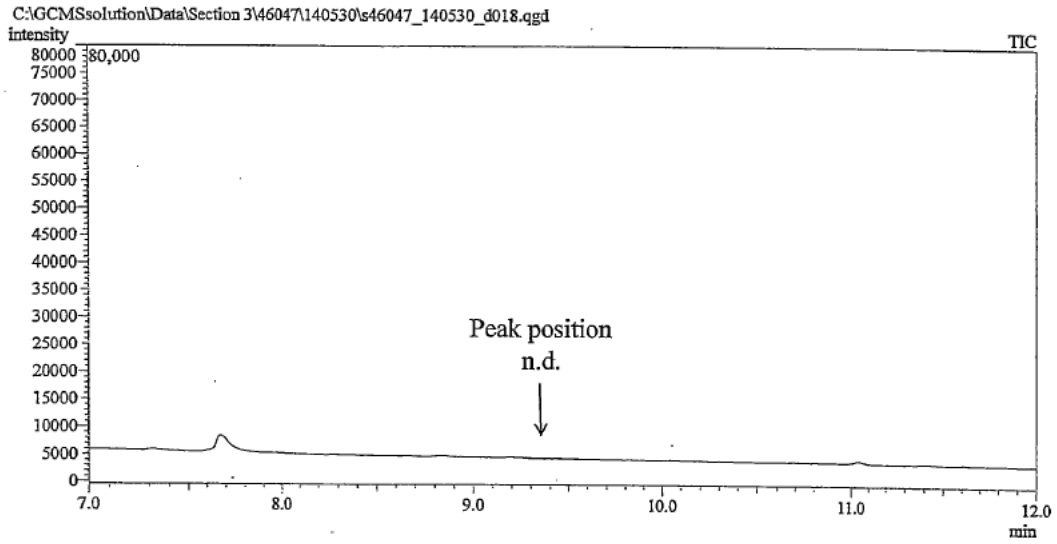
Test water after 28 days (Level 2)
Operating date : May. 31, 2014
File name : C:\GCMSsolution\Data\Section 3\46047\140530\s46047_140530_d017.qgd



Date : Jun. 03, 2014 Name :

Study No. 46047

Test water after 28 days (Control)
Operating date : May. 31, 2014
File name : C:\GCMSsolution\Data\Section 3\46047\140530\s46047_140530_d018.qgd

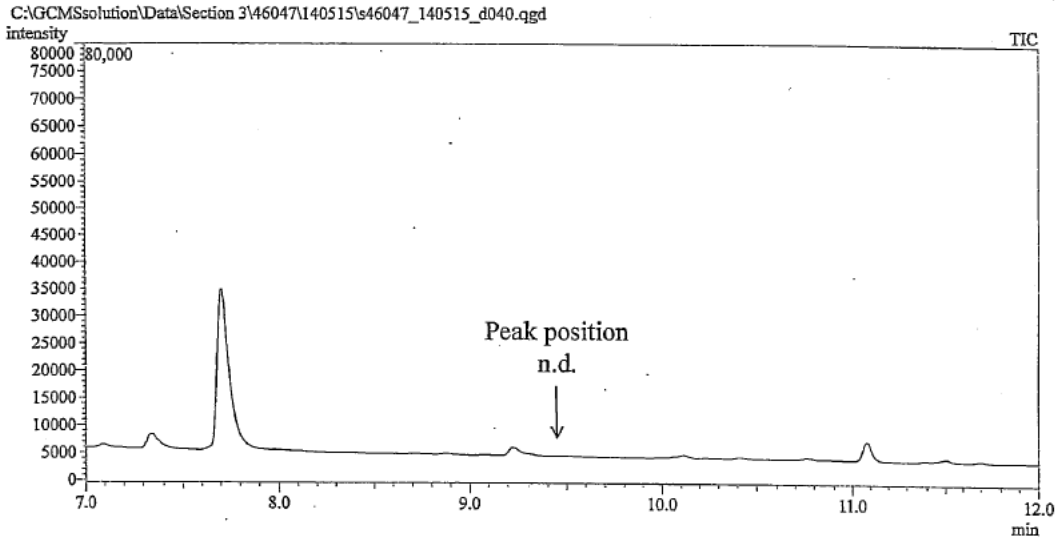


Date : Jun. 03, 2014 Name :

Fig. 6-12 Chromatograms of GC-MS analysis for test water.

Solvent blank (f)

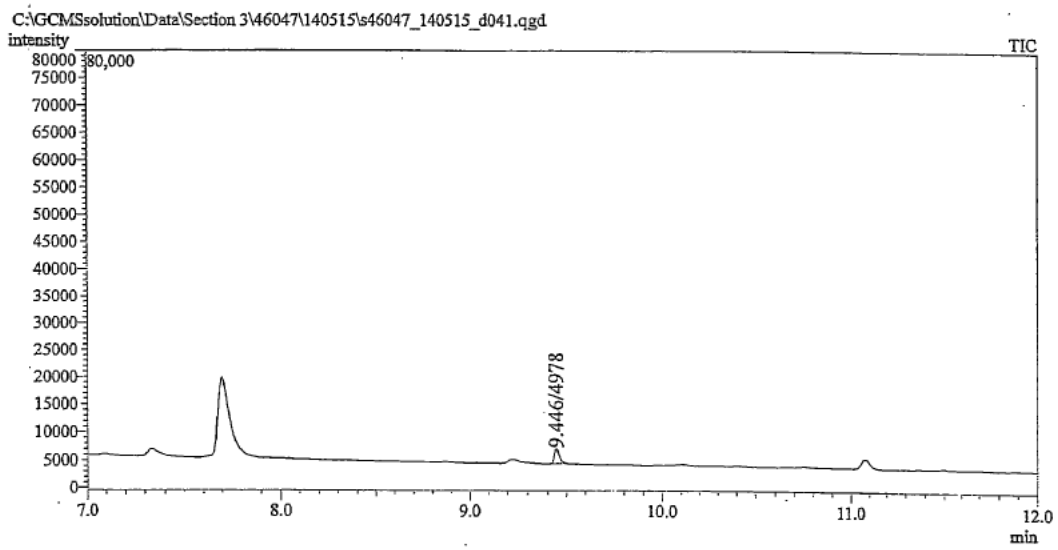
Operating date : May. 17, 2014
File name : C:\GCMSsolution\Data\Section 3\46047\140515\s46047_140515_d040.qgd



Date : May. 19, 2014 Name :

Standard solution 5.00 ug/L (f)

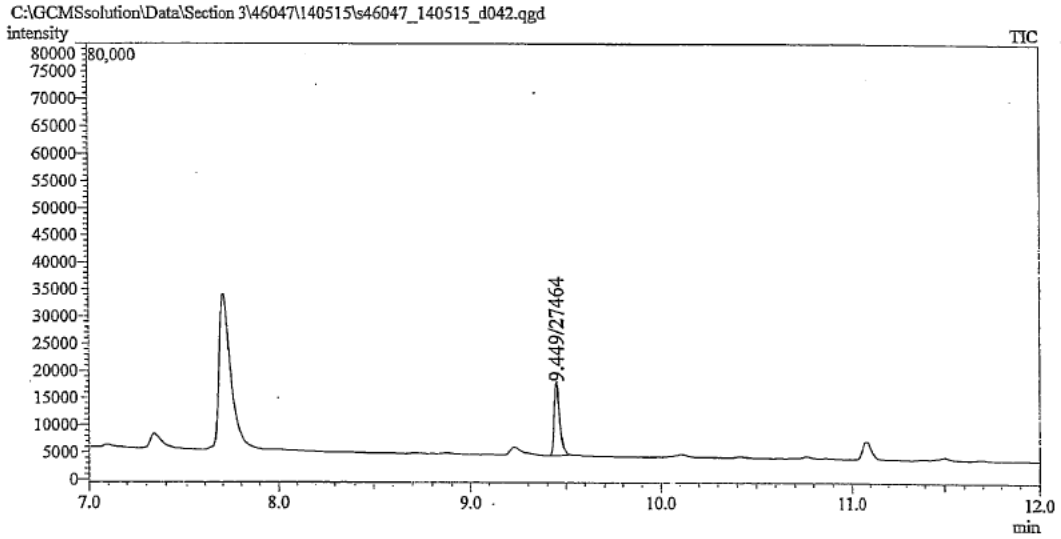
Operating date : May. 17, 2014
File name : C:\GCMSsolution\Data\Section 3\46047\140515\s46047_140515_d041.qgd



Date : May. 19, 2014 Name :

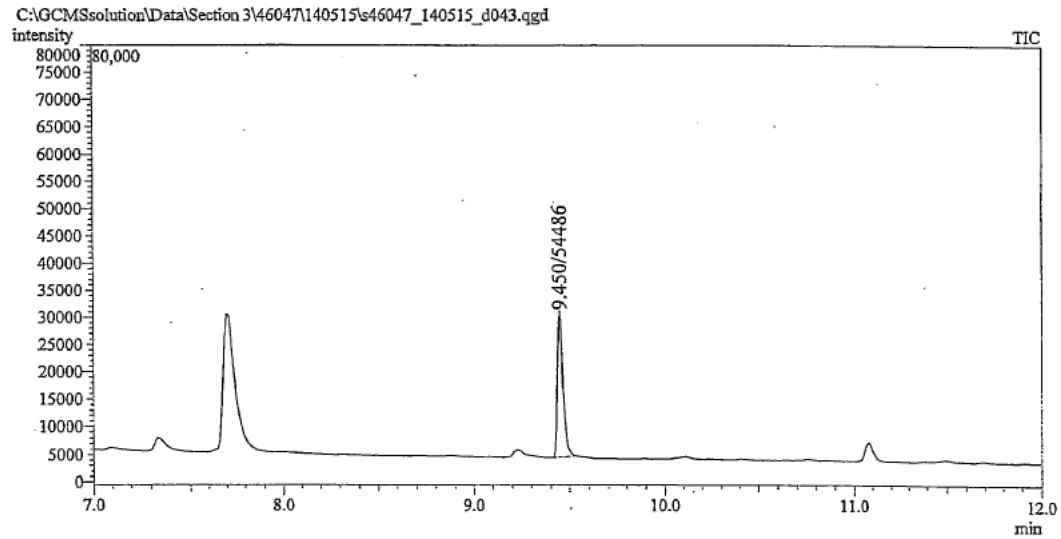
Fig. 7-1 Chromatograms of GC-MS analysis for calibration curve (analysis of test fish).

Standard solution 25.0 ug/L (f)
Operating date : May. 17, 2014
File name : C:\GCMSsolution\Data\Section 3\46047\140515\s46047_140515_d042.qgd



Date : May. 19, 2014 Name :

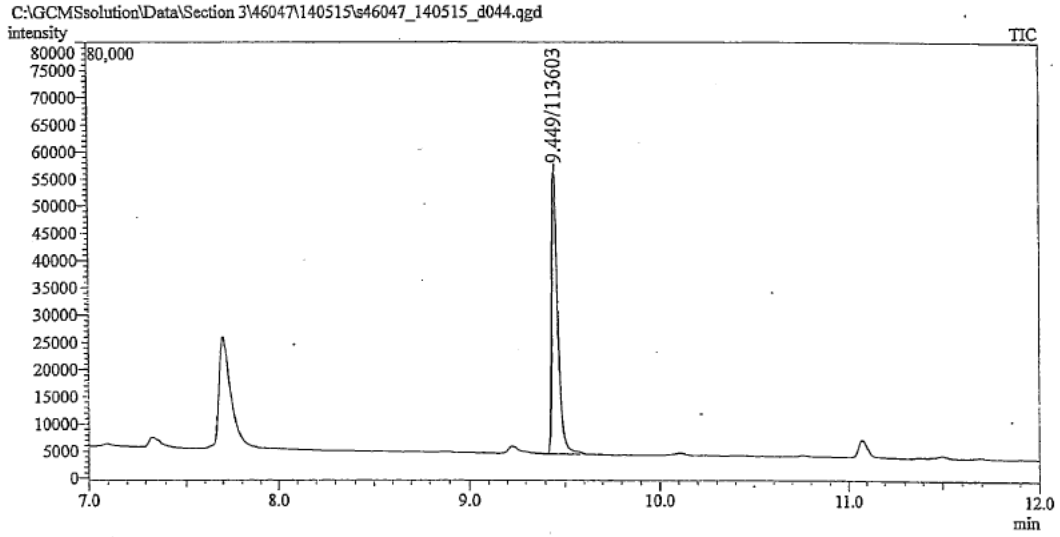
Standard solution 50.0 ug/L (f)
Operating date : May. 17, 2014
File name : C:\GCMSsolution\Data\Section 3\46047\140515\s46047_140515_d043.qgd



Date : May. 19, 2014 Name :

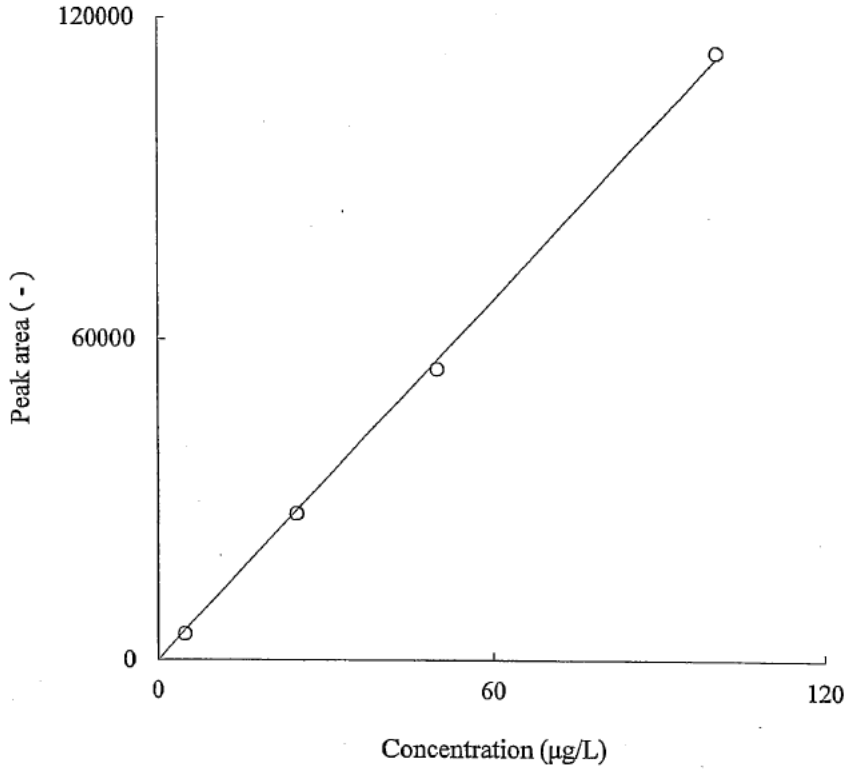
Fig. 7-2 Chromatograms of GC-MS analysis for calibration curve (analysis of test fish).

Standard solution 100 ug/L (f)
Operating date : May. 17, 2014
File name : C:\GCMSsolution\Data\Section 3\46047\140515\s46047_140515_d044.qgd



Date : May. 19, 2014 Name :

Fig. 7-3 Chromatograms of GC-MS analysis for calibration curve (analysis of test fish).



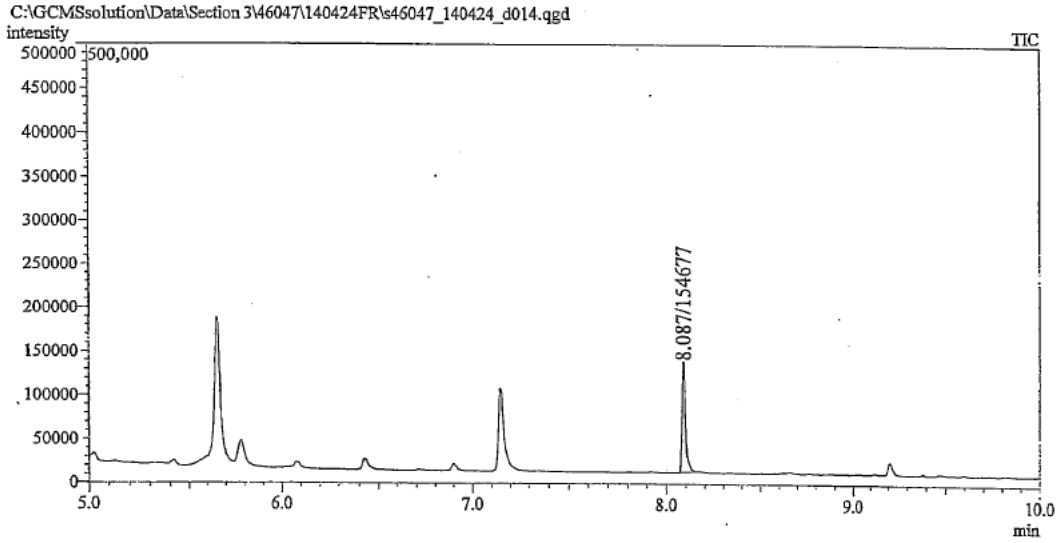
y = 1125x
r = 1.000

Concentration (µg/L)	Peak area (-)
5.00	4978
25.0	27464
50.0	54486
100	113603

Fig. 7-4 Calibration curve of test item (analysis of test fish).

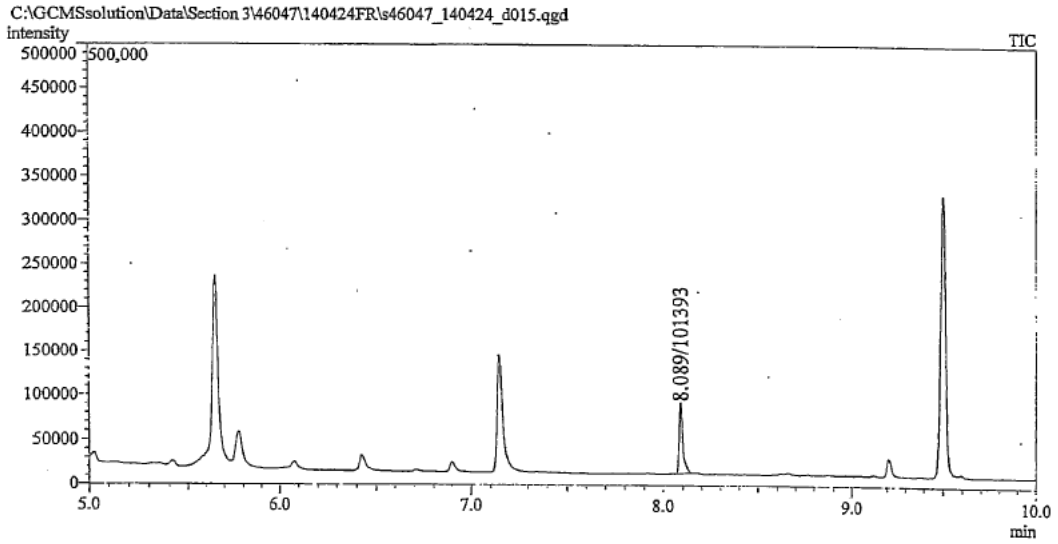
June 19, 2014 Name _____

Standard solution 50.0 ug/L (f)
Operating date : Apr. 24, 2014
File name : C:\GCMSsolution\Data\Section 3\46047\140424FR\s46047_140424_d014.qgd



Date : Apr. 25, 2014 Name :

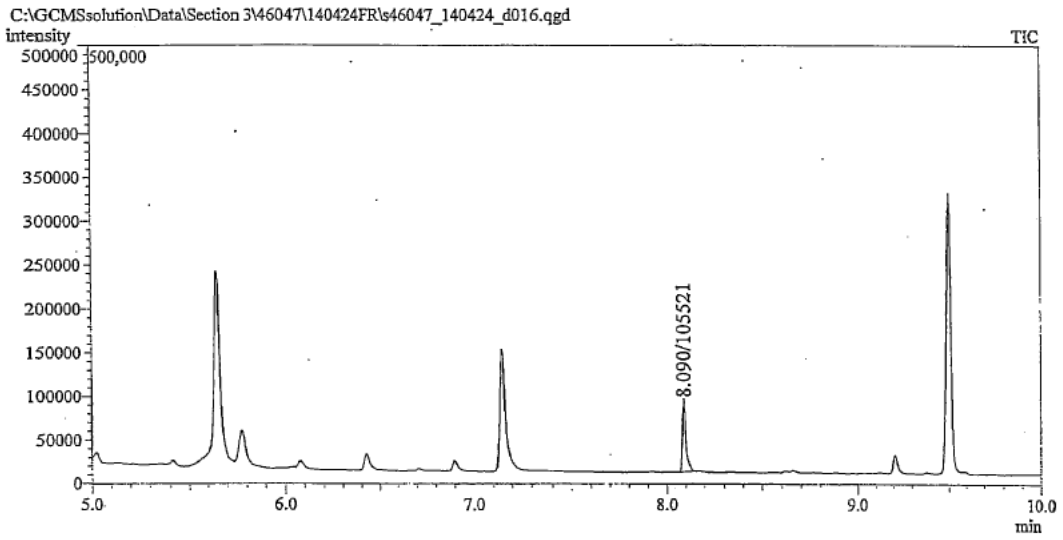
Recovery test from test fish a
Operating date : Apr. 24, 2014
File name : C:\GCMSsolution\Data\Section 3\46047\140424FR\s46047_140424_d015.qgd



Date : Apr. 25, 2014 Name :

Fig. 8-1 Chromatograms of GC-MS analysis for recovery and blank test (analysis of test fish).

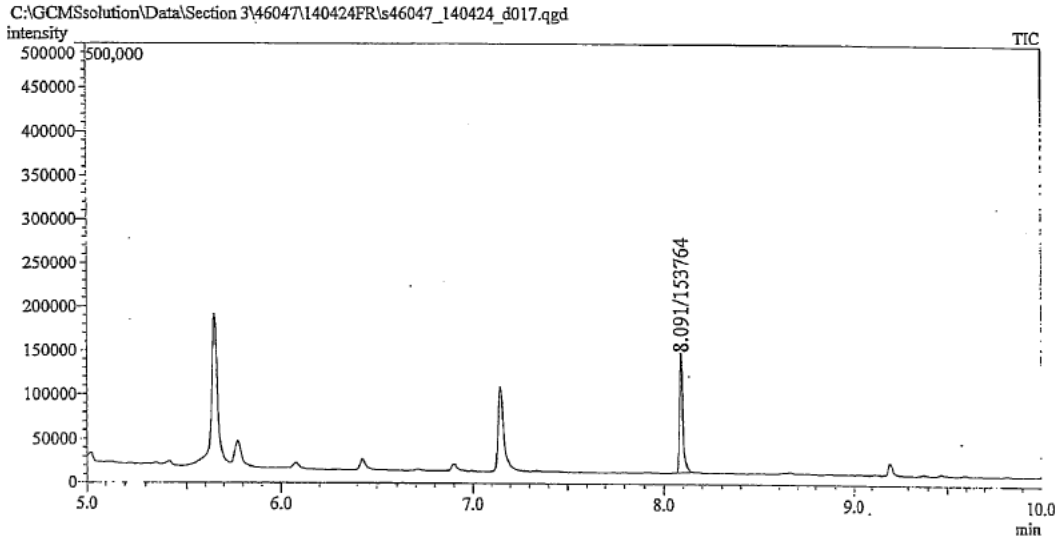
Recovery test from test fish b
Operating date : Apr. 25, 2014
File name : C:\GCMSsolution\Data\Section 3\46047\140424FR\s46047_140424_d016.qgd



Date : Apr. 25, 2014 Name :

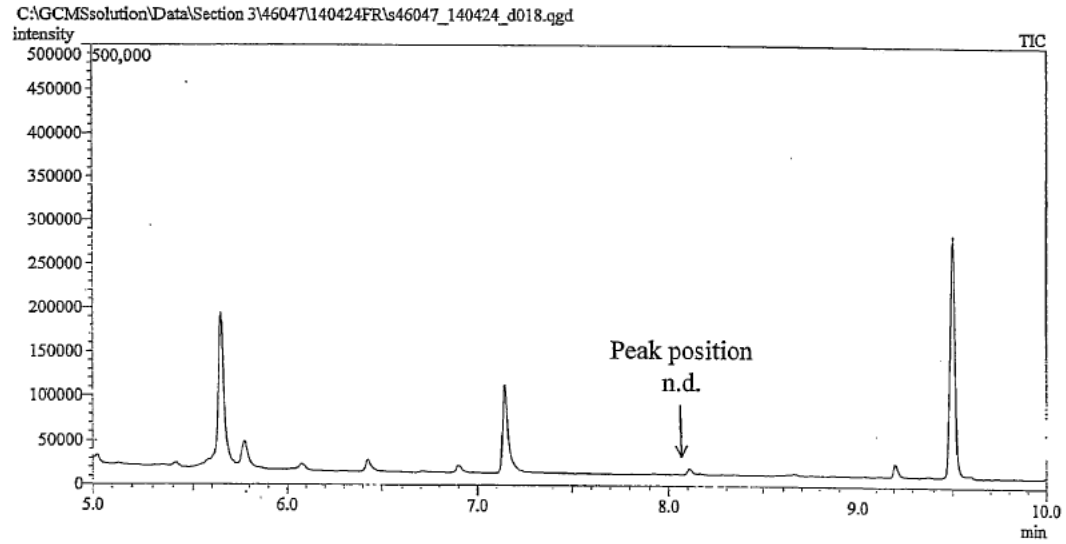
Fig. 8-2 Chromatograms of GC-MS analysis for recovery and blank test (analysis of test fish).

Standard solution 50.0 ug/L (f)
Operating date : Apr. 25, 2014
File name : C:\GCMSsolution\Data\Section 3\46047\140424FR\s46047_140424_d017.qgd



Date : Apr. 25, 2014 Name :

Blank test of test fish a
Operating date : Apr. 25, 2014
File name : C:\GCMSsolution\Data\Section 3\46047\140424FR\s46047_140424_d018.qgd

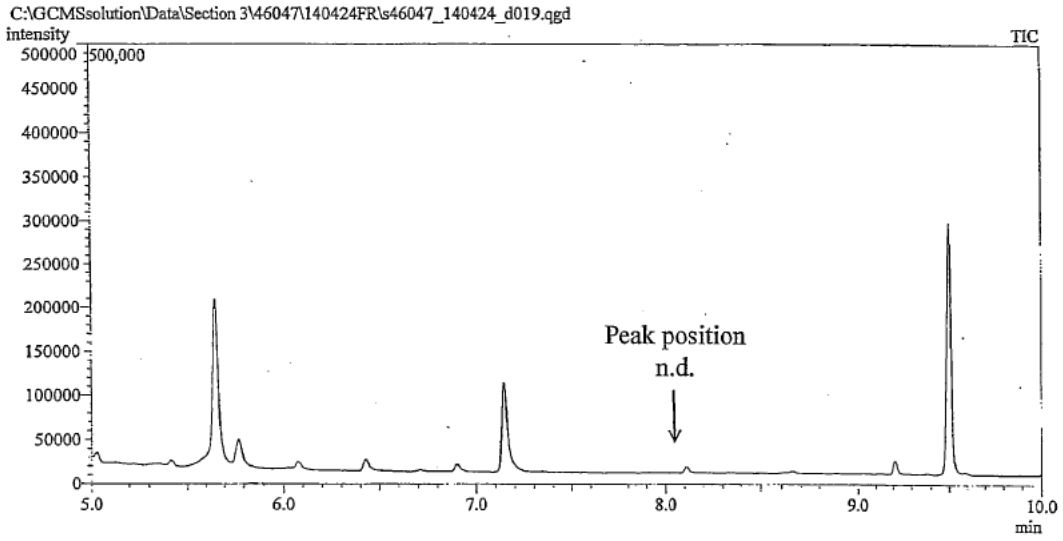


Date : Apr. 25, 2014 Name :

Fig. 8-3 Chromatograms of GC-MS analysis for recovery and blank test (analysis of test fish).

Blank test of test fish b

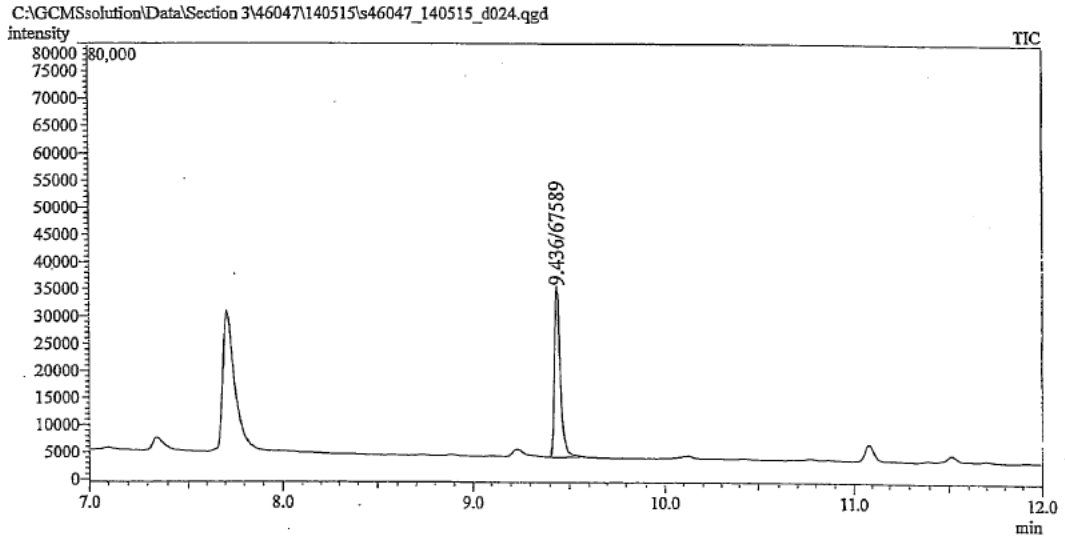
Operating date : Apr. 25, 2014
File name : C:\GCMSsolution\Data\Section 3\46047\140424FR\s46047_140424_d019.qgd



Date : Apr. 25, 2014 Name :

Fig. 8-4 Chromatograms of GC-MS analysis for recovery and blank test (analysis of test fish).

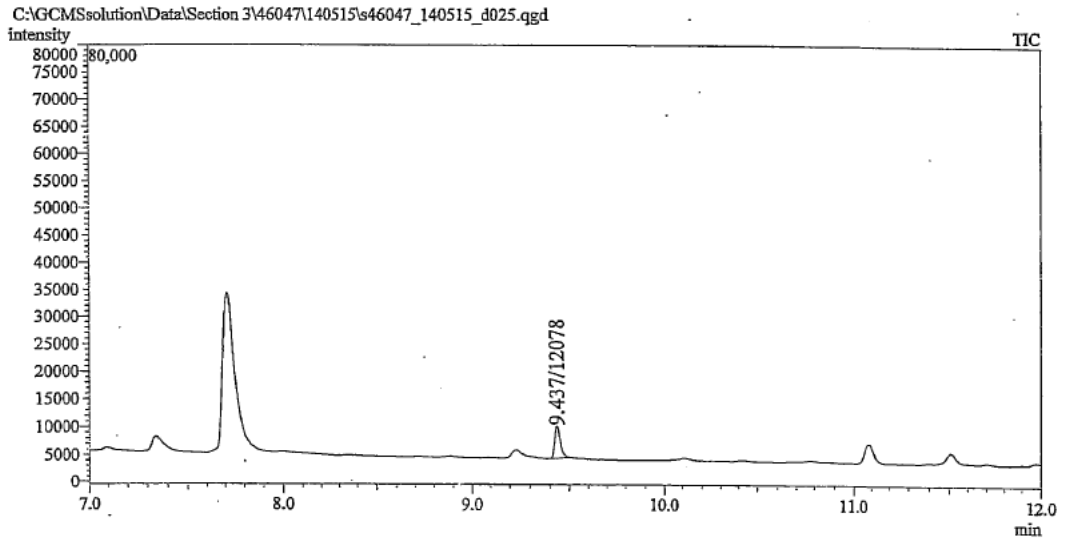
Standard solution 50.0 ug/L (f)
Operating date : May. 16, 2014
File name : C:\GCMSsolution\Data\Section 3\46047\140515\s46047_140515_d024.qgd



Date : May. 19, 2014 Name :

Study No. 46047

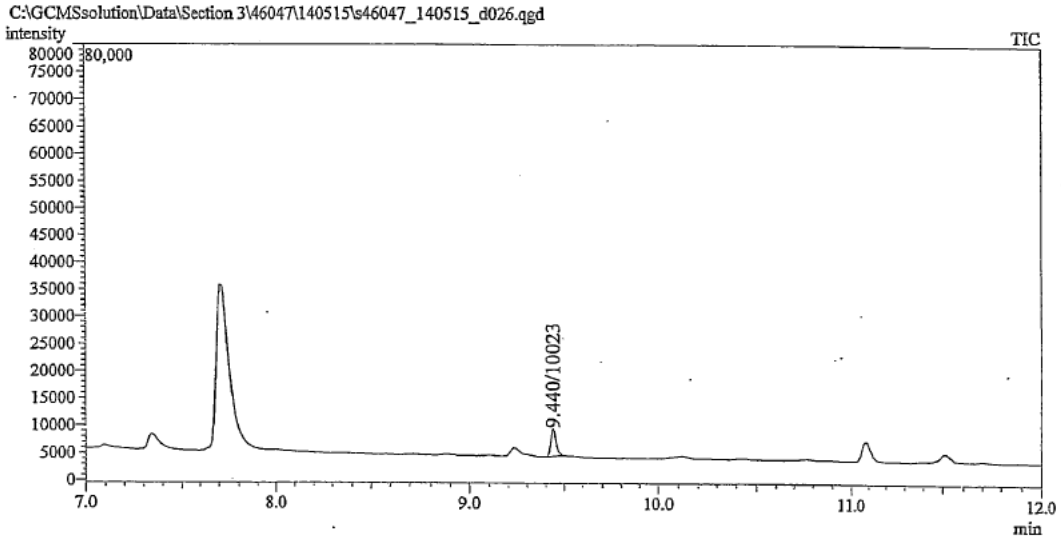
Test fish after 13 days (Level 1-a)
Operating date : May. 16, 2014
File name : C:\GCMSsolution\Data\Section 3\46047\140515\s46047_140515_d025.qgd



Date : May. 19, 2014 Name :

Fig. 9-1 Chromatograms of GC-MS analysis for test fish (Level 1).

Test fish after 13 days (Level 1-b)
Operating date : May. 16, 2014
File name : C:\GCMSsolution\Data\Section 3\46047\140515\s46047_140515_d026.qgd

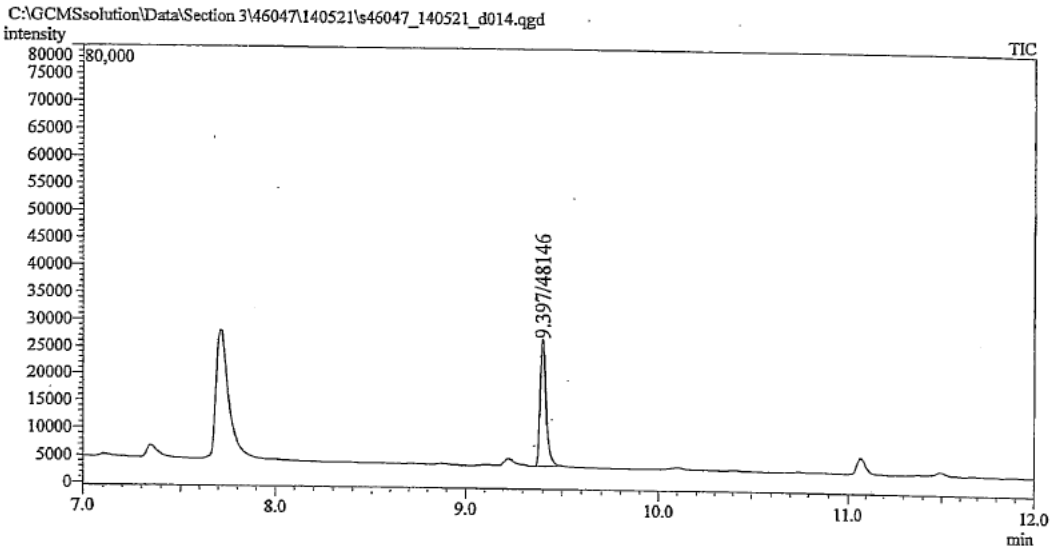


Date : May. 19, 2014 Name :

Fig. 9-2 Chromatograms of GC-MS analysis for test fish (Level 1).

Standard solution 50.0 ug/L (f)

Operating date : May. 22, 2014
File name : C:\GCMSsolution\Data\Section 3\46047\140521\s46047_140521_d014.qgd

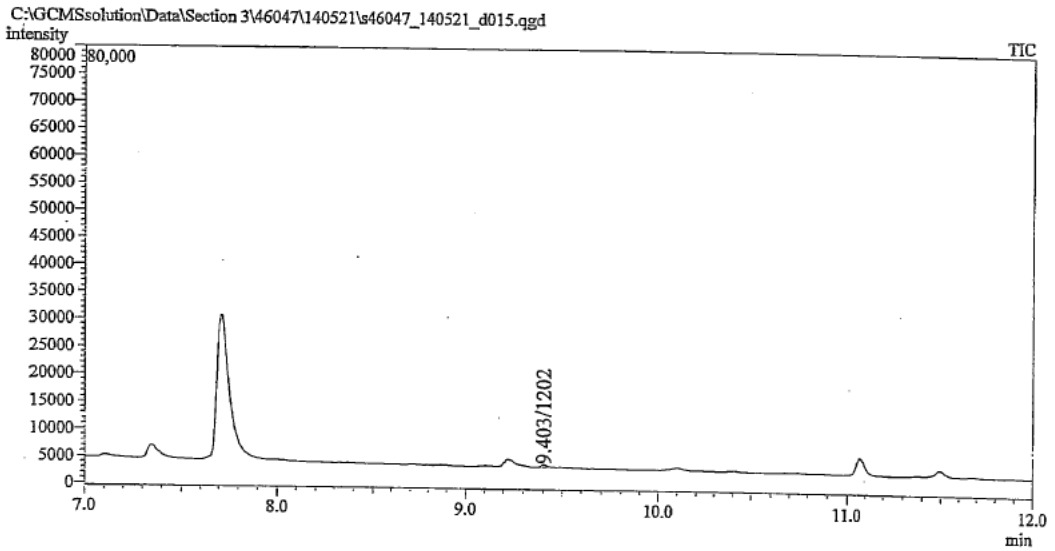


Date : May. 22, 2014 Name :

Study No. 46047

Test fish after 19 days (Level 1-a)

Operating date : May. 22, 2014
File name : C:\GCMSsolution\Data\Section 3\46047\140521\s46047_140521_d015.qgd



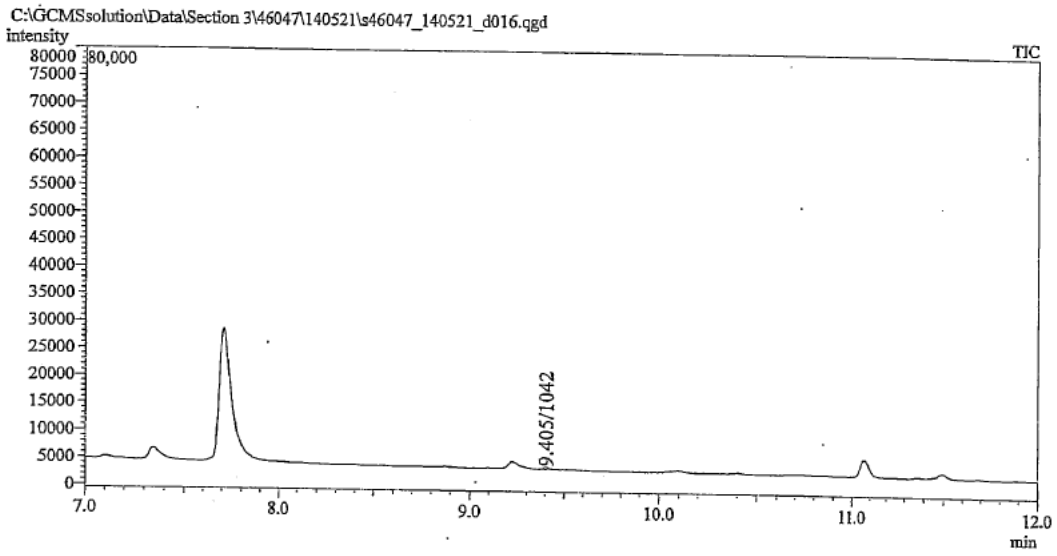
Date : May. 22, 2014 Name :

Fig. 9-3 Chromatograms of GC-MS analysis for test fish (Level 1).

Test fish after 19 days (Level 1-b)

Operating date : May. 22, 2014

File name : C:\GCMSsolution\Data\Section 3\46047\140521\s46047_140521_d016.qgd

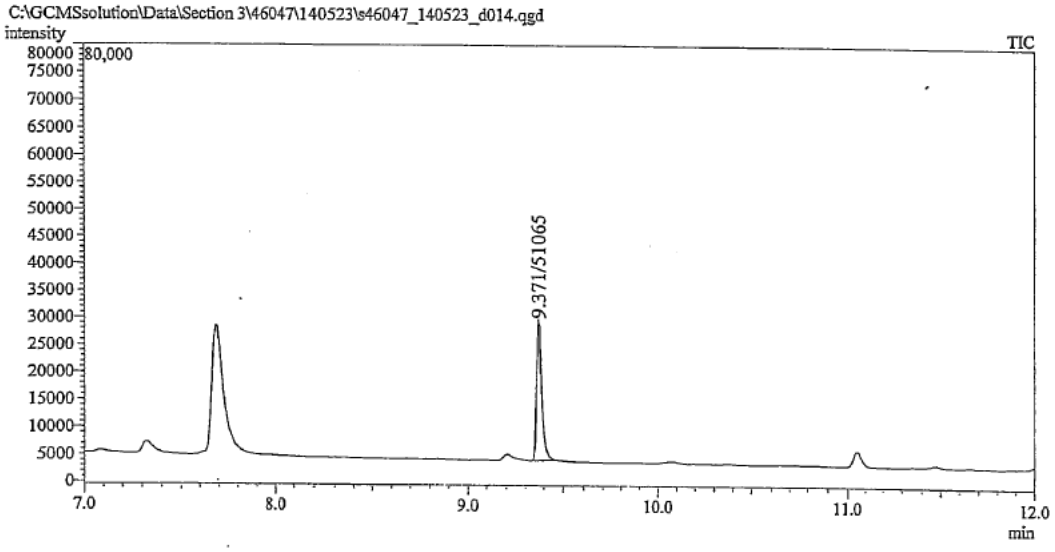


Date : May. 22, 2014 Name :

Fig. 9-4 Chromatograms of GC-MS analysis for test fish (Level 1).

Standard solution 50.0 ug/L (f)

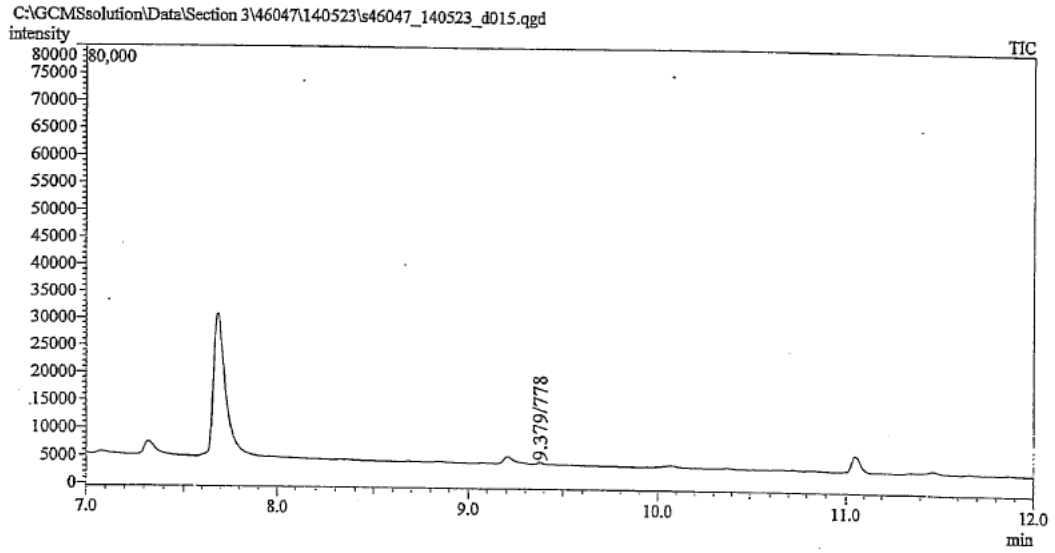
Operating date : May. 23, 2014
File name : C:\GCMSsolution\Data\Section 3\46047\140523\s46047_140523_d014.qgd



Date : May. 26, 2014 Name :

Test fish after 21 days (Level 1-a)

Operating date : May. 24, 2014
File name : C:\GCMSsolution\Data\Section 3\46047\140523\s46047_140523_d015.qgd



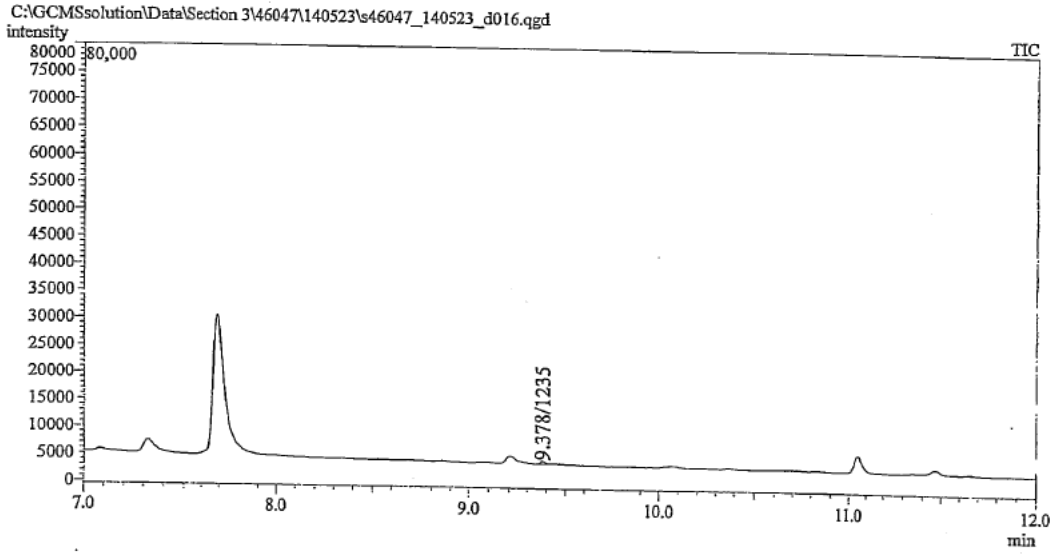
Date : May. 26, 2014 Name :

Fig. 9-5 Chromatograms of GC-MS analysis for test fish (Level 1).

Test fish after 21 days (Level 1-b)

Operating date : May. 24, 2014

File name : C:\GCMSsolution\Data\Section 3\46047\140523\s46047_140523_d016.qgd

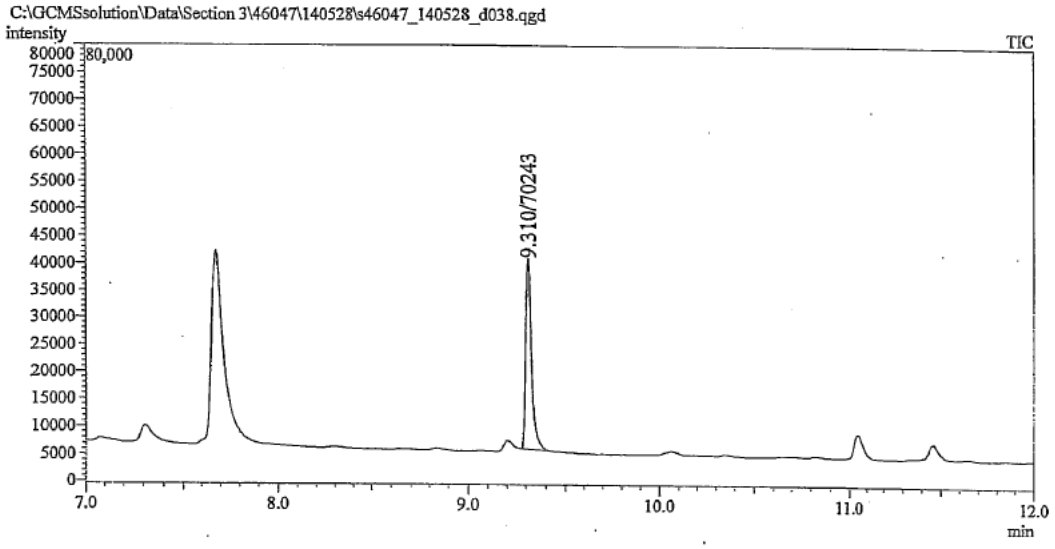


Date : May. 26, 2014 Name :

Fig. 9-6 Chromatograms of GC-MS analysis for test fish (Level 1).

Standard solution 50.0 ug/L (f)

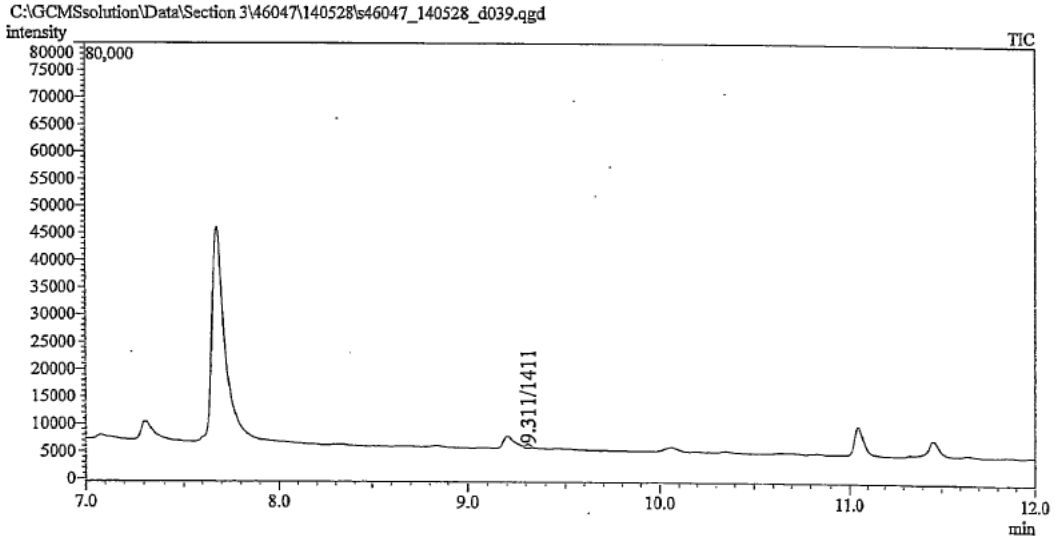
Operating date : May. 30, 2014
File name : C:\GCMSsolution\Data\Section 3\46047\140528\s46047_140528_d038.qgd



Date : May. 30, 2014 Name :

Test fish after 26 days (Level 1-a)

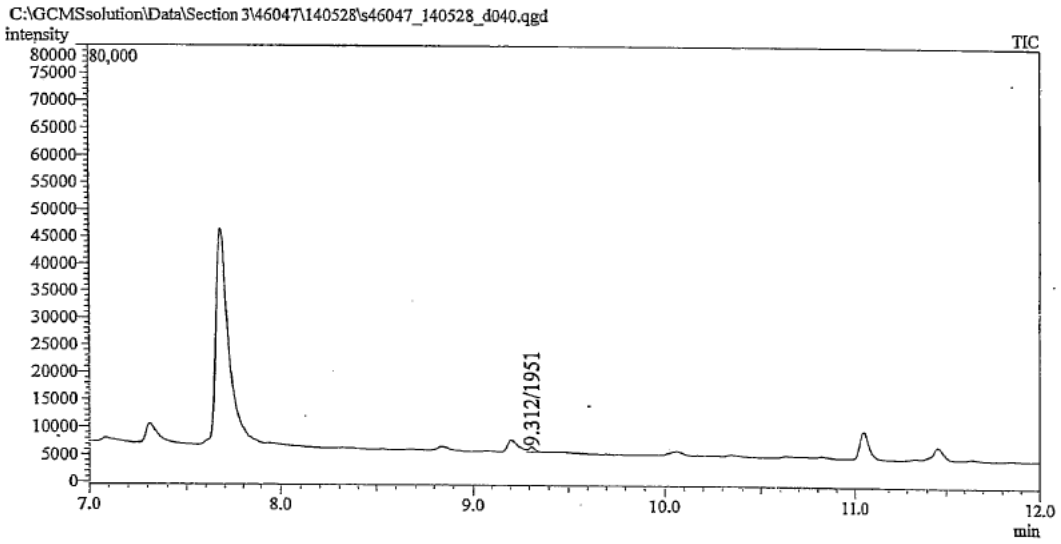
Operating date : May. 30, 2014
File name : C:\GCMSsolution\Data\Section 3\46047\140528\s46047_140528_d039.qgd



Date : May. 30, 2014 Name :

Fig. 9-7 Chromatograms of GC-MS analysis for test fish (Level 1).

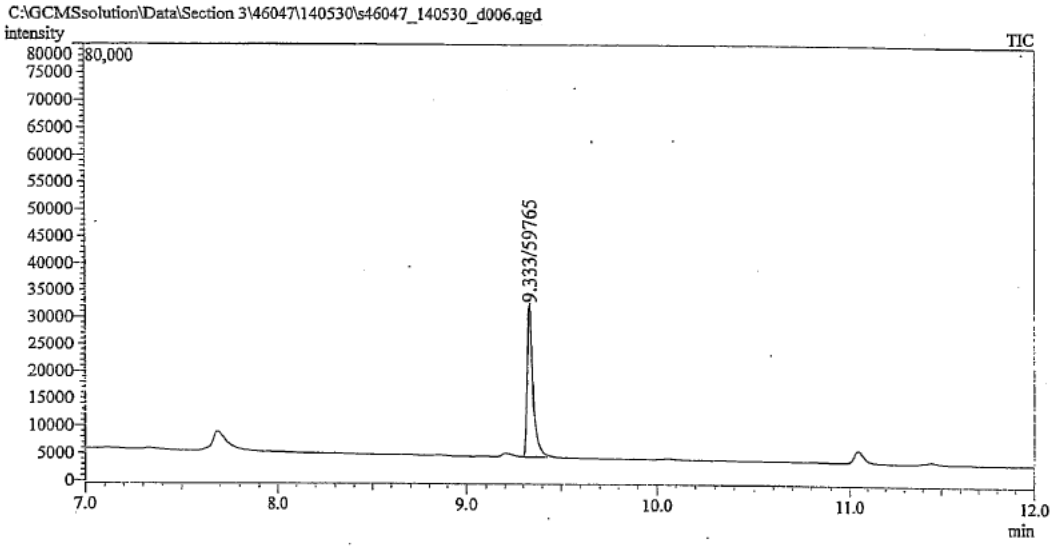
Test fish after 26 days (Level 1-b)
Operating date : May. 30, 2014
File name : C:\GCMSsolution\Data\Section 3\46047\140528\s46047_140528_d040.qgd



Date : May. 30, 2014 Name :

Fig. 9-8 Chromatograms of GC-MS analysis for test fish (Level 1).

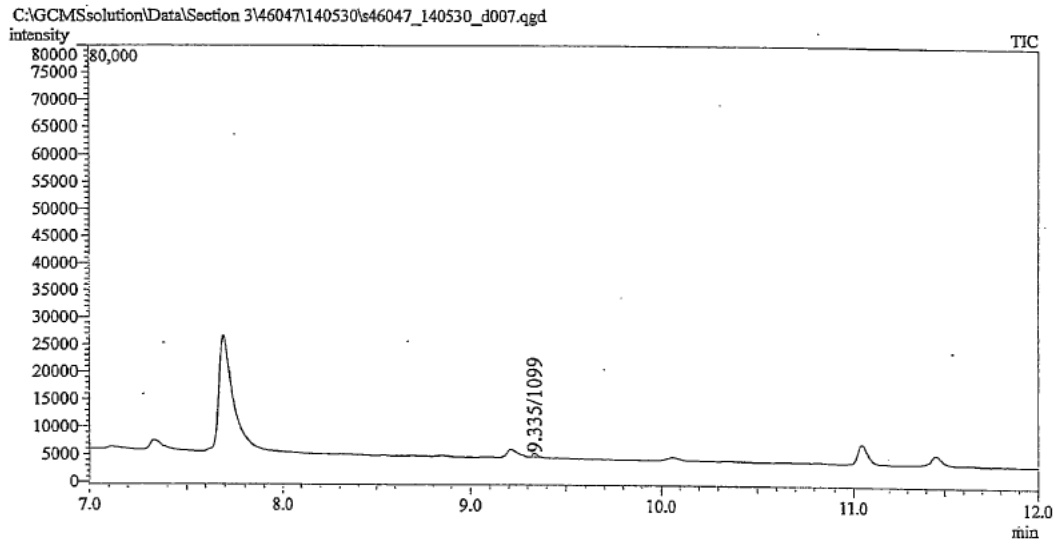
Standard solution 50.0 ug/L (f)
Operating date : May. 30, 2014
File name : C:\GCMSsolution\Data\Section 3\46047\140530\s46047_140530_d006.qgd



Date : Jun. 03, 2014 Name :

Study No. 46047

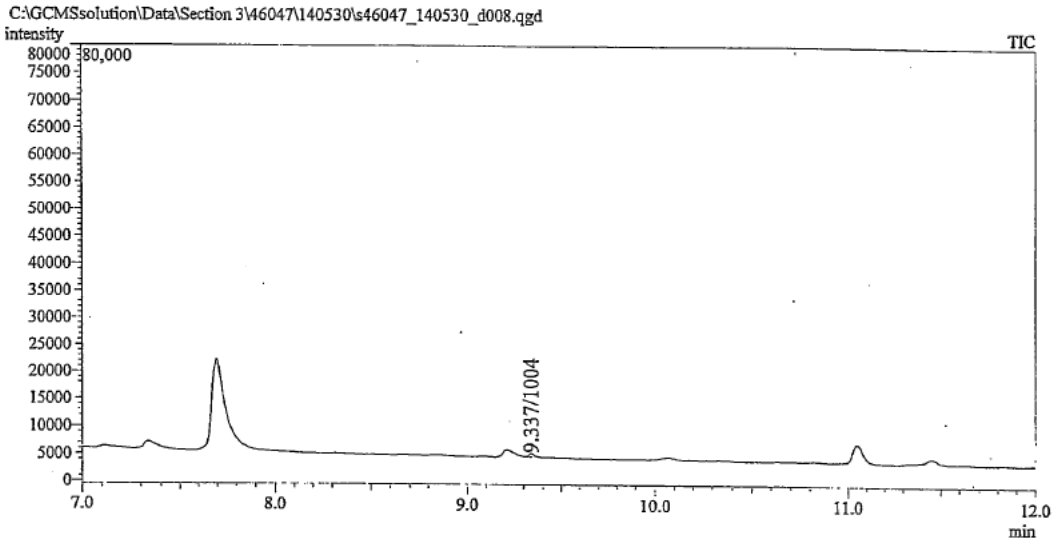
Test fish after 28 days (Level 1-a)
Operating date : May. 31, 2014
File name : C:\GCMSsolution\Data\Section 3\46047\140530\s46047_140530_d007.qgd



Date : Jun. 03, 2014 Name :

Fig. 9-9 Chromatograms of GC-MS analysis for test fish (Level 1).

Test fish after 28 days (Level 1-b)
Operating date : May. 31, 2014
File name : C:\GCMSsolution\Data\Section 3\46047\140530\s46047_140530_d008.qgd

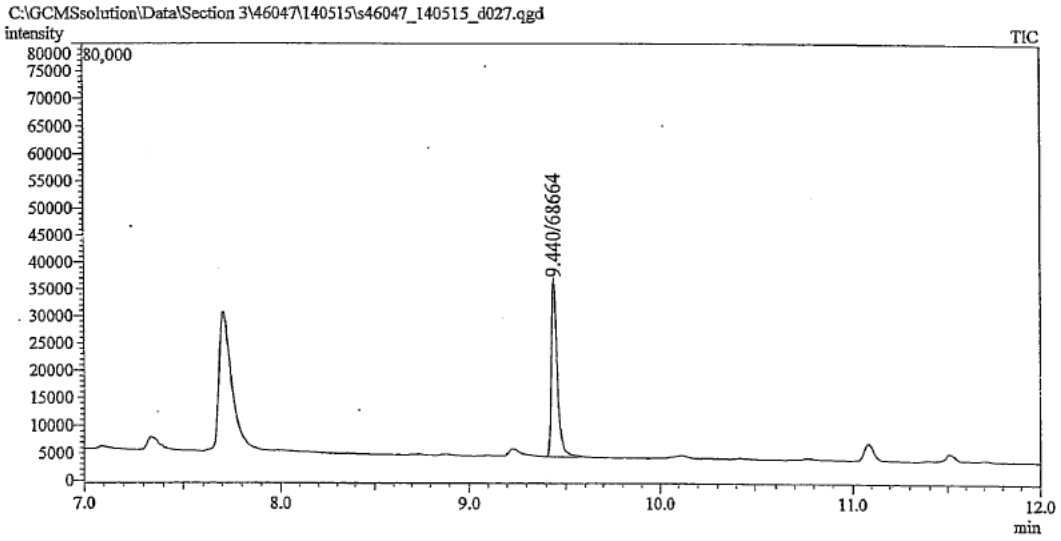


Date : Jun. 03, 2014 Name :

Fig. 9-10 Chromatograms of GC-MS analysis for test fish (Level 1).

Standard solution 50.0 ug/L (f)

Operating date : May. 16, 2014
File name : C:\GCMSsolution\Data\Section 3\46047\140515\s46047_140515_d027.qgd

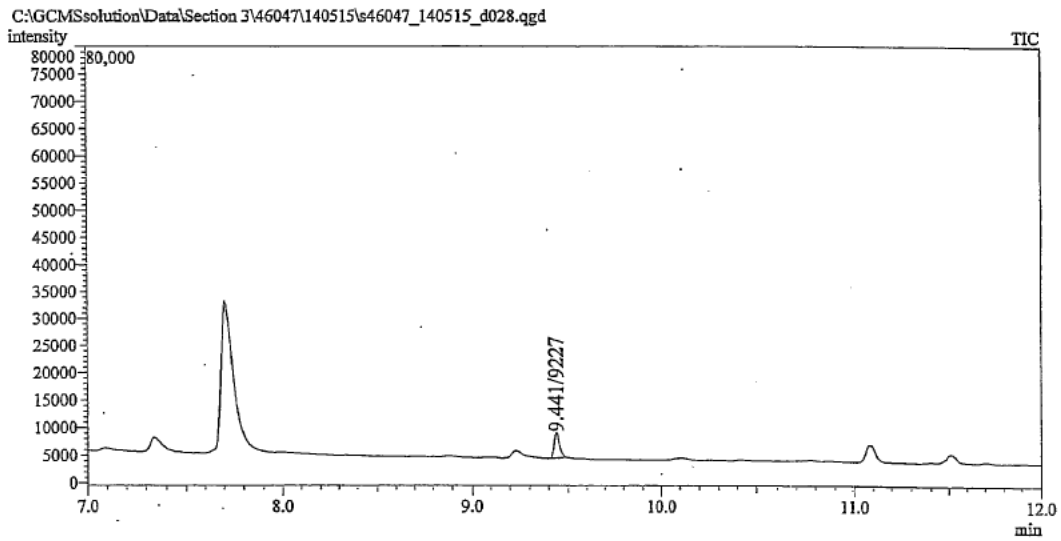


Date : May. 19, 2014 Name :

Study No. 46047

Test fish after 13 days (Level 2-a)

Operating date : May. 16, 2014
File name : C:\GCMSsolution\Data\Section 3\46047\140515\s46047_140515_d028.qgd



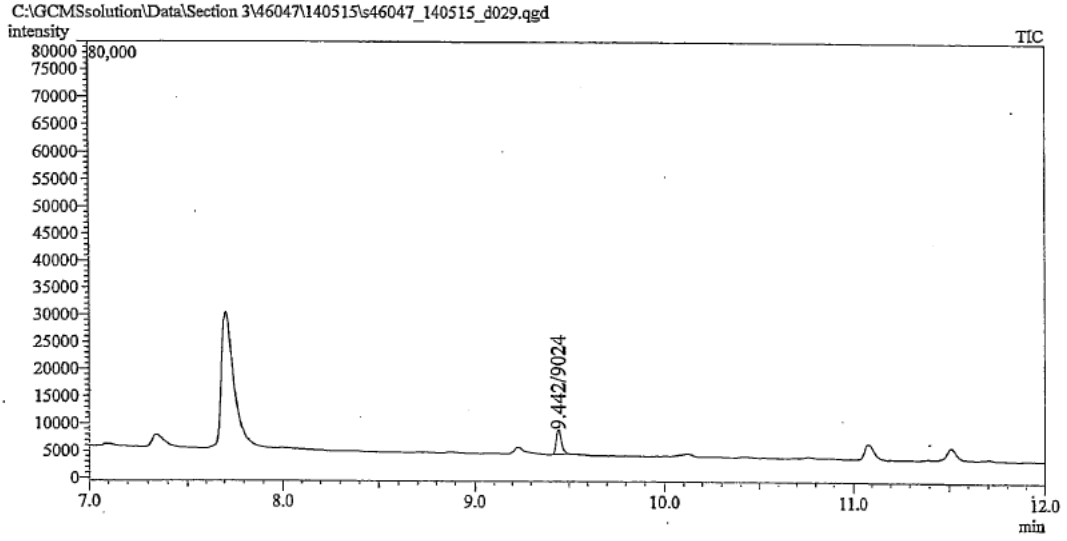
Date : May. 19, 2014 Name :

Fig. 10-1 Chromatograms of GC-MS analysis for test fish (Level 2).

Test fish after 13 days (Level 2-b)

Operating date : May. 16, 2014

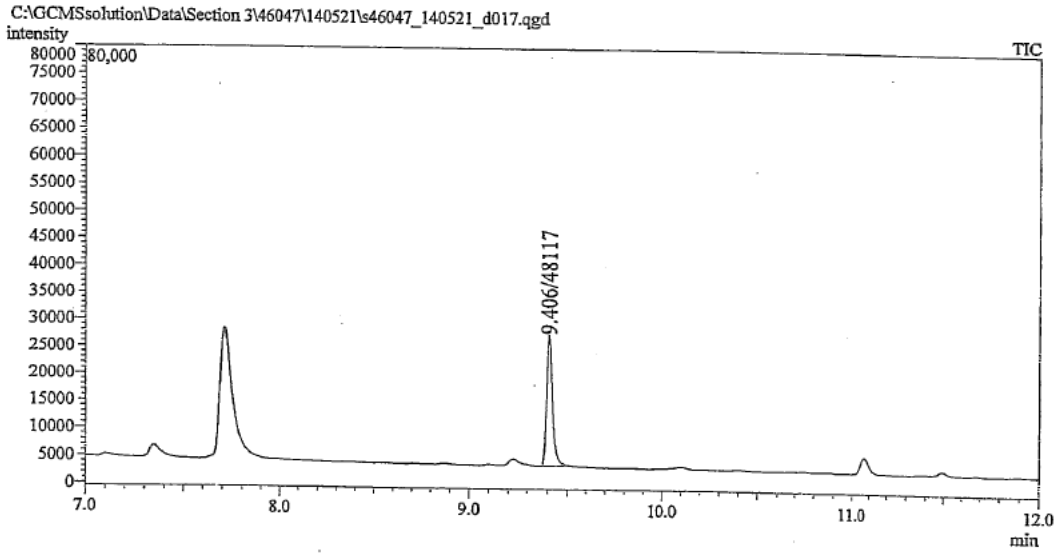
File name : C:\GCMSsolution\Data\Section 3\46047\140515\s46047_140515_d029.qgd



Date : May. 19, 2014 Name :

Fig. 10-2 Chromatograms of GC-MS analysis for test fish (Level 2).

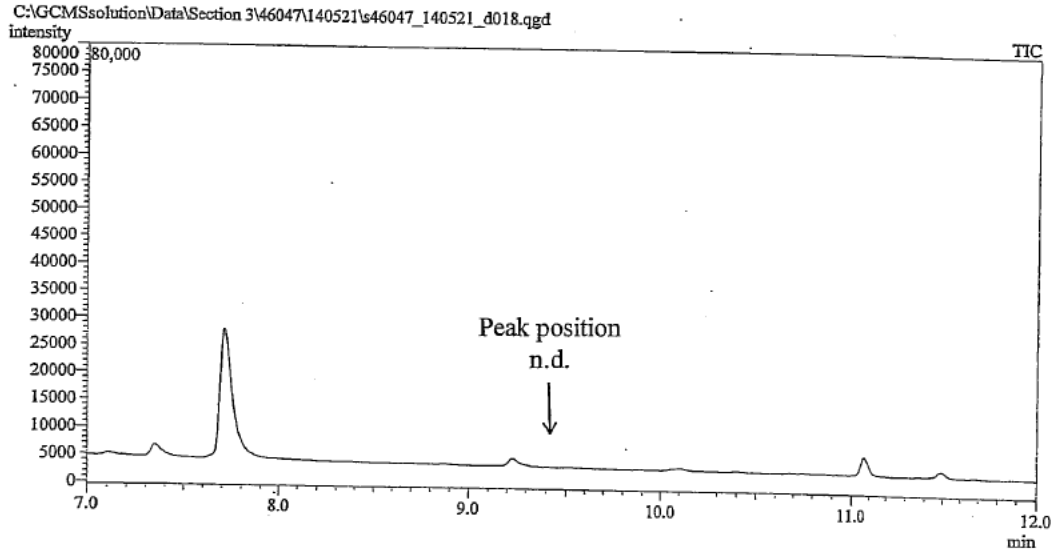
Standard solution 50.0 ug/L (f)
Operating date : May. 22, 2014
File name : C:\GCMSsolution\Data\Section 3\46047\140521\s46047_140521_d017.qgd



Date : May. 22, 2014 Name :

Study No. 46047

Test fish after 19 days (Level 2-a)
Operating date : May. 22, 2014
File name : C:\GCMSsolution\Data\Section 3\46047\140521\s46047_140521_d018.qgd



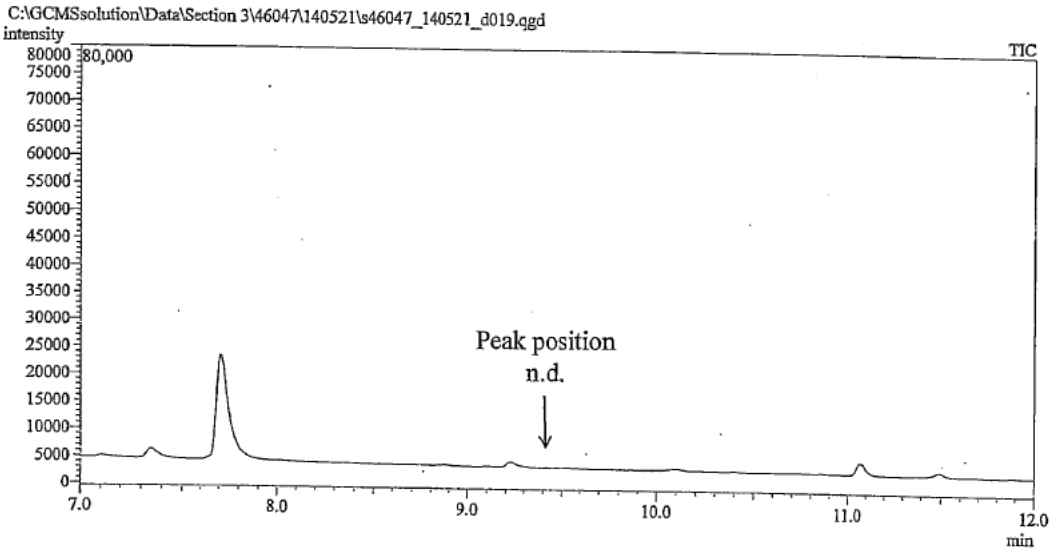
Date : May. 22, 2014 Name :

Fig. 10-3 Chromatograms of GC-MS analysis for test fish (Level 2).

Test fish after 19 days (Level 2-b)

Operating date : May. 22, 2014

File name : C:\GCMSsolution\Data\Section 3\46047\140521\s46047_140521_d019.qgd

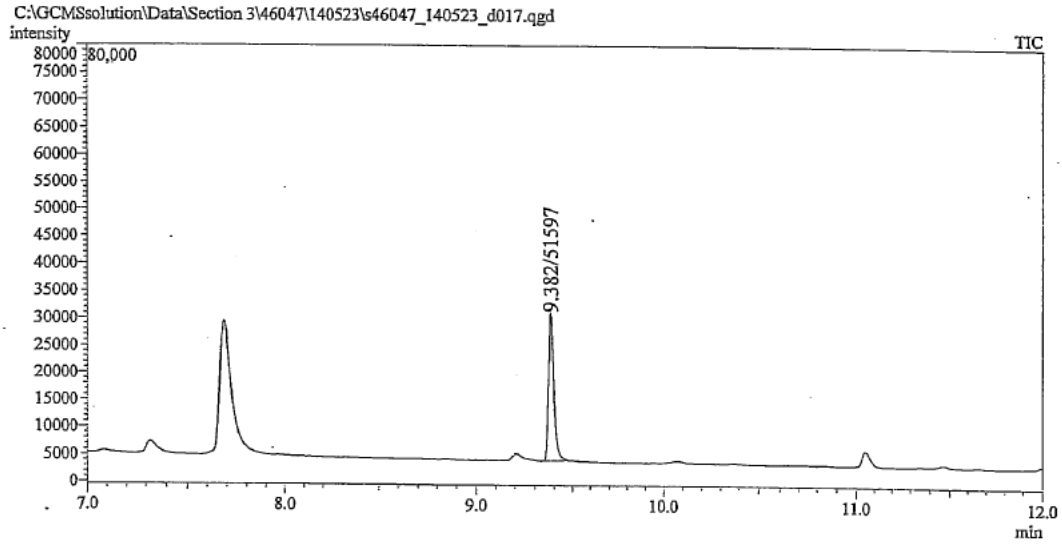


Date : May. 22, 2014 Name :

Fig. 10-4 Chromatograms of GC-MS analysis for test fish (Level 2).

Standard solution 50.0 ug/L (f)

Operating date : May. 24, 2014
File name : C:\GCMSsolution\Data\Section 3\46047\140523\s46047_140523_d017.qgd

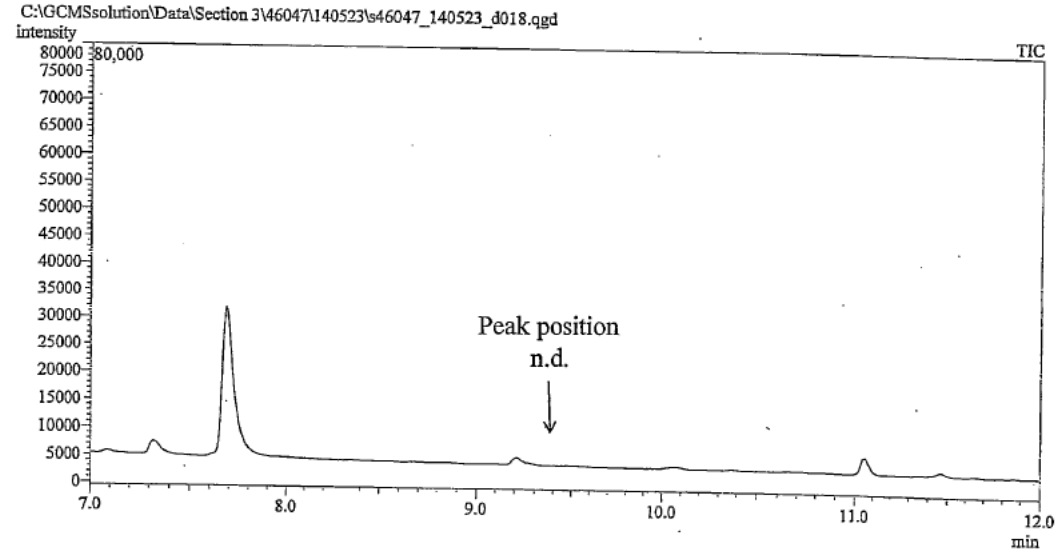


Date : May. 26, 2014 Name :

Study No. 46047

Test fish after 21 days (Level 2-a)

Operating date : May. 24, 2014
File name : C:\GCMSsolution\Data\Section 3\46047\140523\s46047_140523_d018.qgd



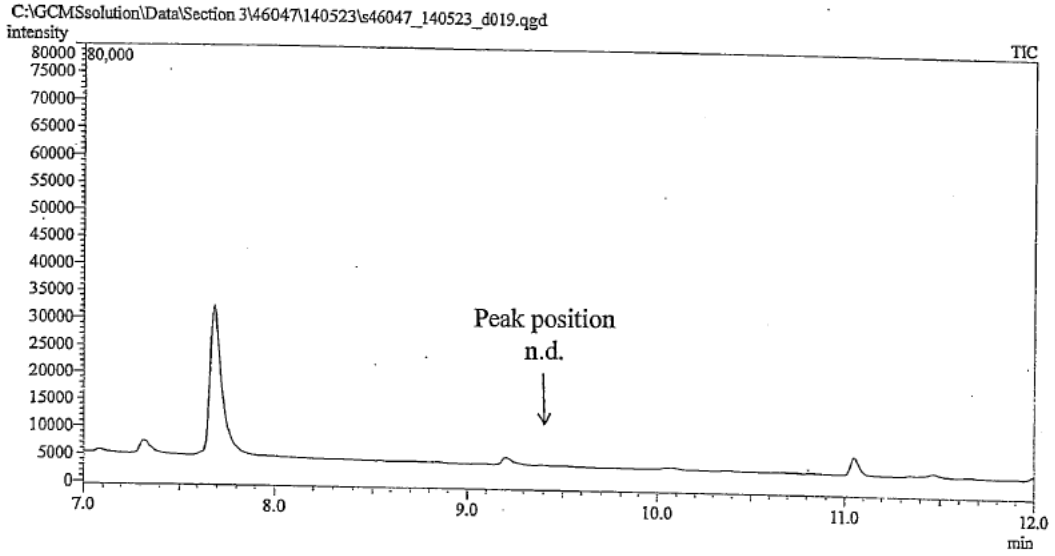
Date : May. 26, 2014 Name :

Fig. 10-5 Chromatograms of GC-MS analysis for test fish (Level 2).

Test fish after 21 days (Level 2-b)

Operating date : May. 24, 2014

File name : C:\GCMSsolution\Data\Section 3\46047\140523\s46047_140523_d019.qgd

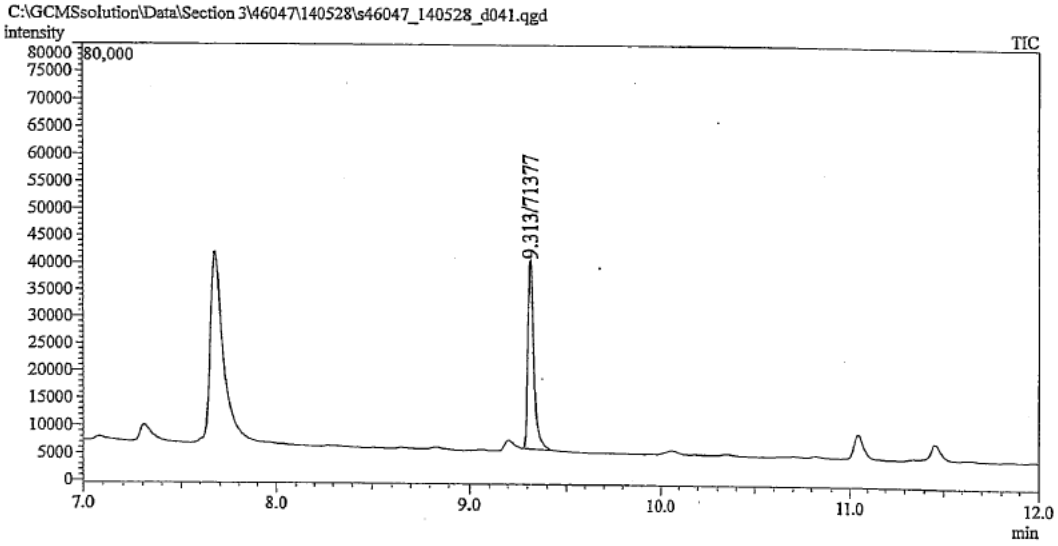


Date : May. 26, 2014 Name :

Fig. 10-6 Chromatograms of GC-MS analysis for test fish (Level 2).

Standard solution 50.0 ug/L (f)

Operating date : May. 30, 2014
File name : C:\GCMSsolution\Data\Section 3\46047\140528\s46047_140528_d041.qgd

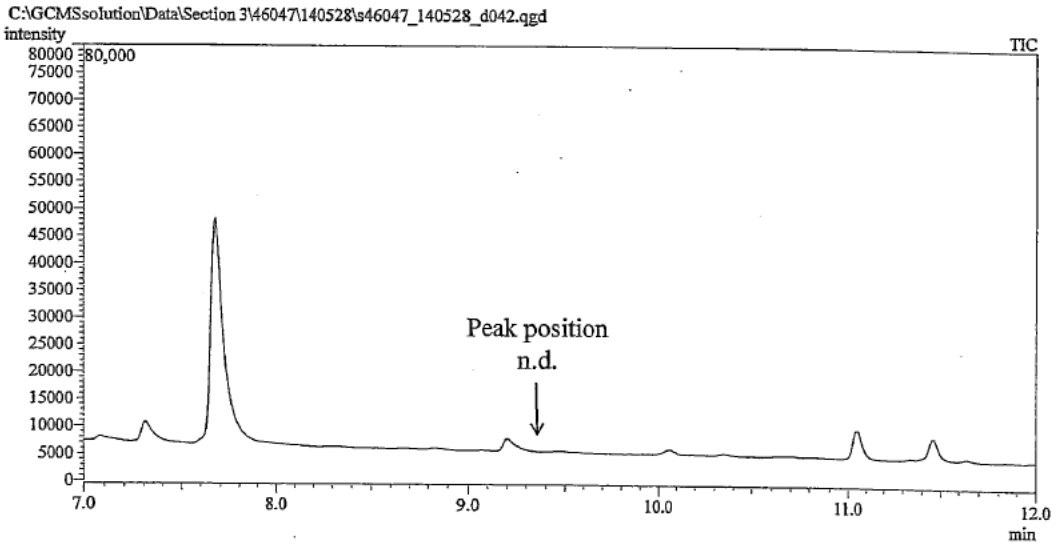


Date : May. 30, 2014 Name :

Study No. 46047

Test fish after 26 days (Level 2-a)

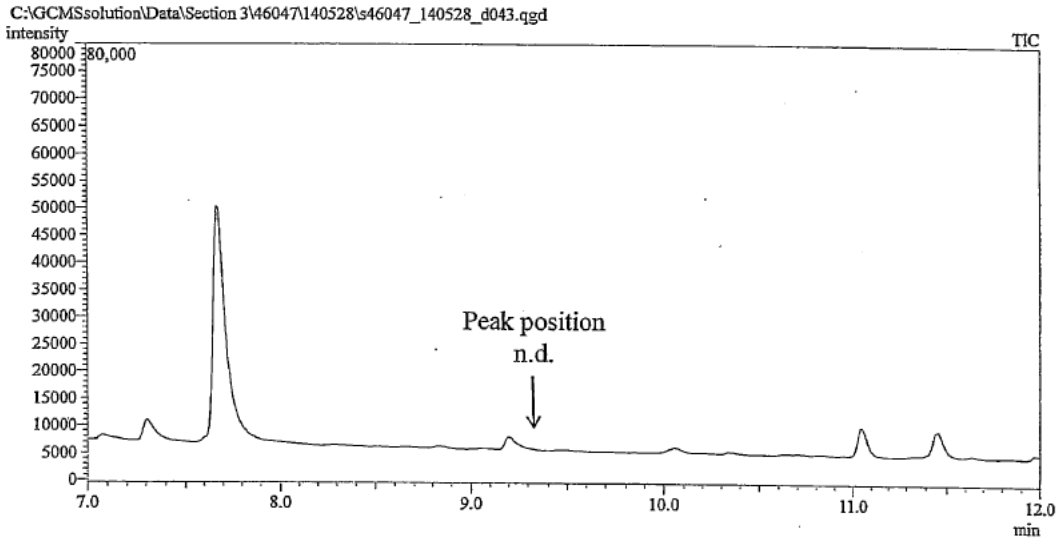
Operating date : May. 30, 2014
File name : C:\GCMSsolution\Data\Section 3\46047\140528\s46047_140528_d042.qgd



Date : May. 30, 2014 Name :

Fig. 10-7 Chromatograms of GC-MS analysis for test fish (Level 2).

Test fish after 26 days (Level 2-b)
Operating date : May. 30, 2014
File name : C:\GCMSsolution\Data\Section 3\46047\140528\s46047_140528_d043.qgd

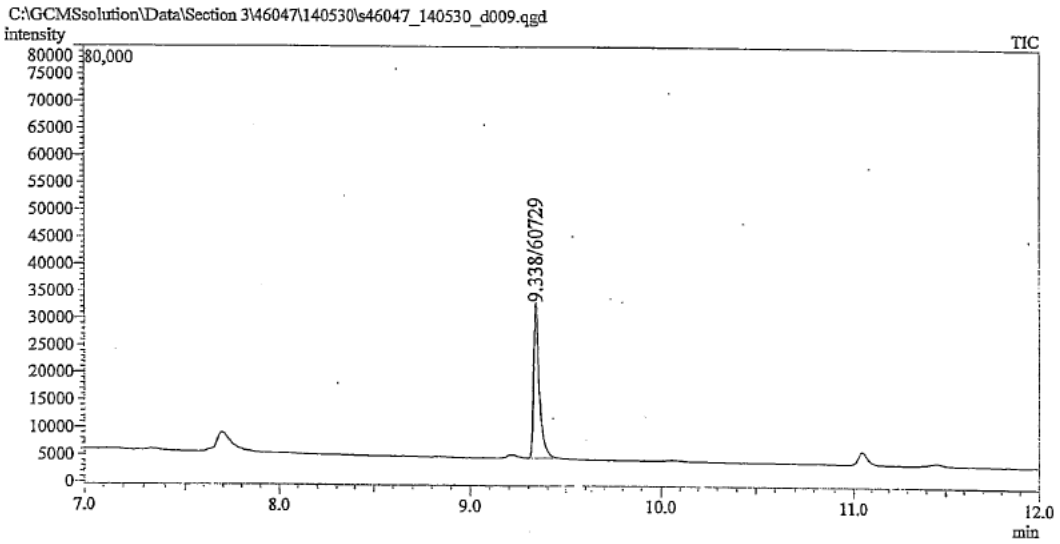


Date : May. 30, 2014 Name :

Fig. 10-8 Chromatograms of GC-MS analysis for test fish (Level 2).

Standard solution 50.0 ug/L (f)

Operating date : May. 31, 2014
File name : C:\GCMSsolution\Data\Section 3\46047\140530\s46047_140530_d009.qgd

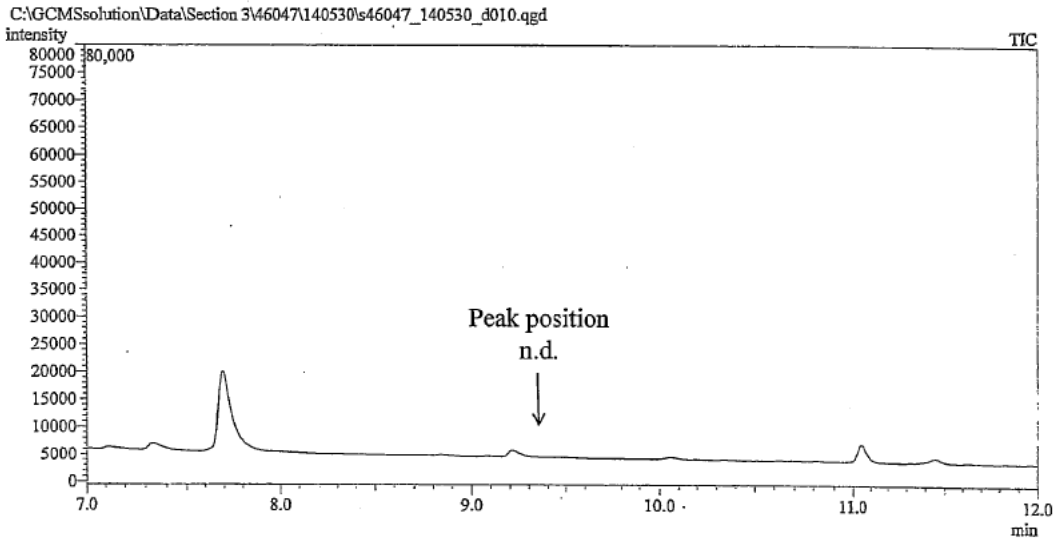


Date : Jun. 03, 2014 Name :

Study No. 46047

Test fish after 28 days (Level 2-a)

Operating date : May. 31, 2014
File name : C:\GCMSsolution\Data\Section 3\46047\140530\s46047_140530_d010.qgd



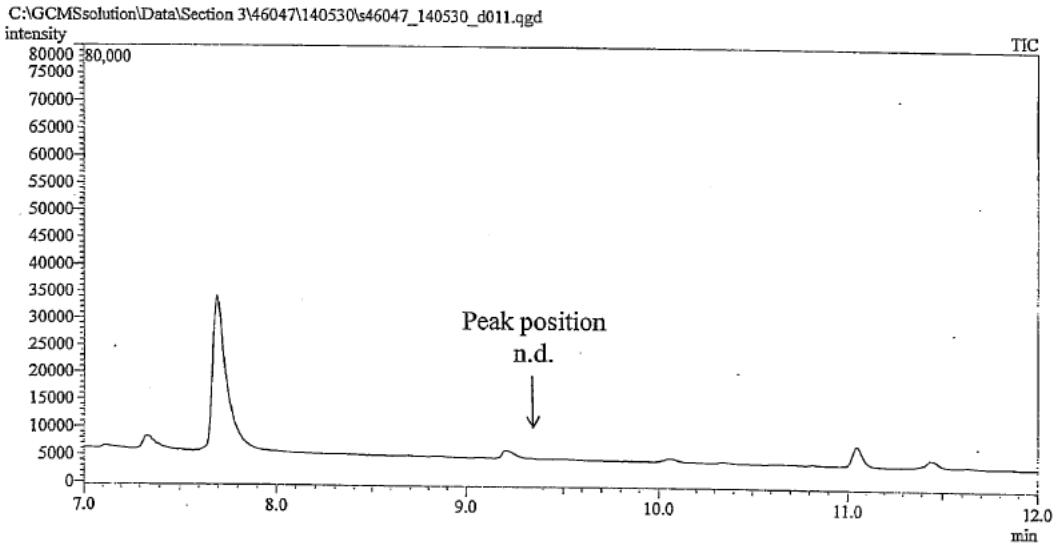
Date : Jun. 03, 2014 Name :

Fig. 10-9 Chromatograms of GC-MS analysis for test fish (Level 2).

Test fish after 28 days (Level 2-b)

Operating date : May. 31, 2014

File name : C:\GCMSsolution\Data\Section 3\46047\140530\s46047_140530_d011.qgd

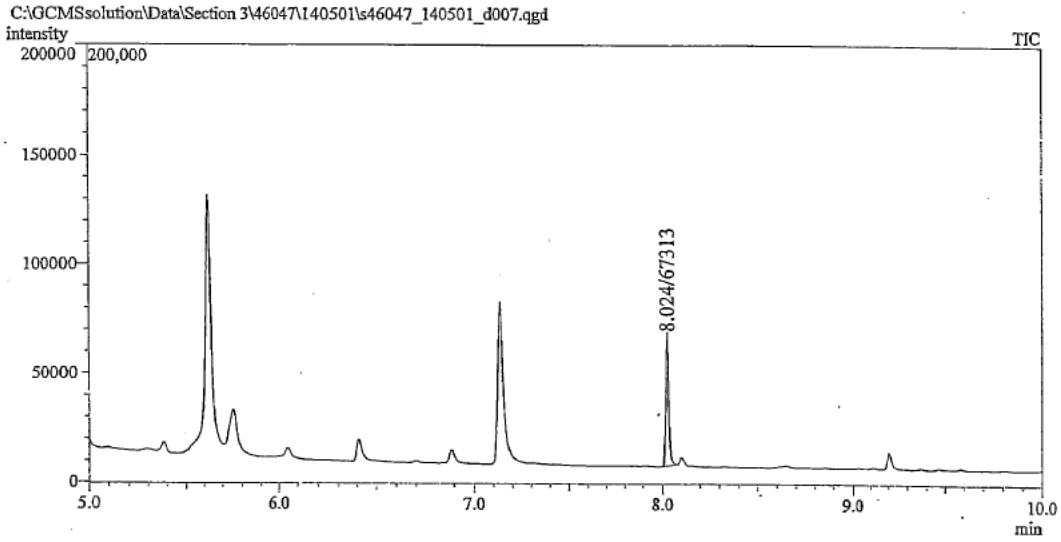


Date : Jun. 03, 2014 Name :

Fig. 10-10 Chromatograms of GC-MS analysis for test fish (Level 2).

Standard solution 50.0 ug/L (f)

Operating date : May. 01, 2014
File name : C:\GCMSsolution\Data\Section 3\46047\140501\s46047_140501_d007.qgd

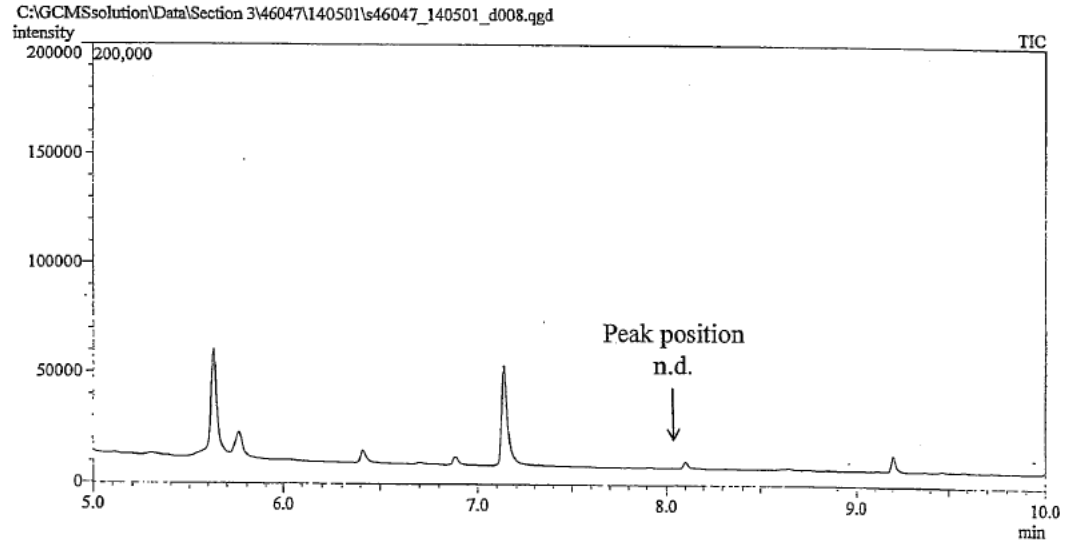


Date : May. 02, 2014 Name :

Study No. 46047

Test fish before experimental start (Control-a)

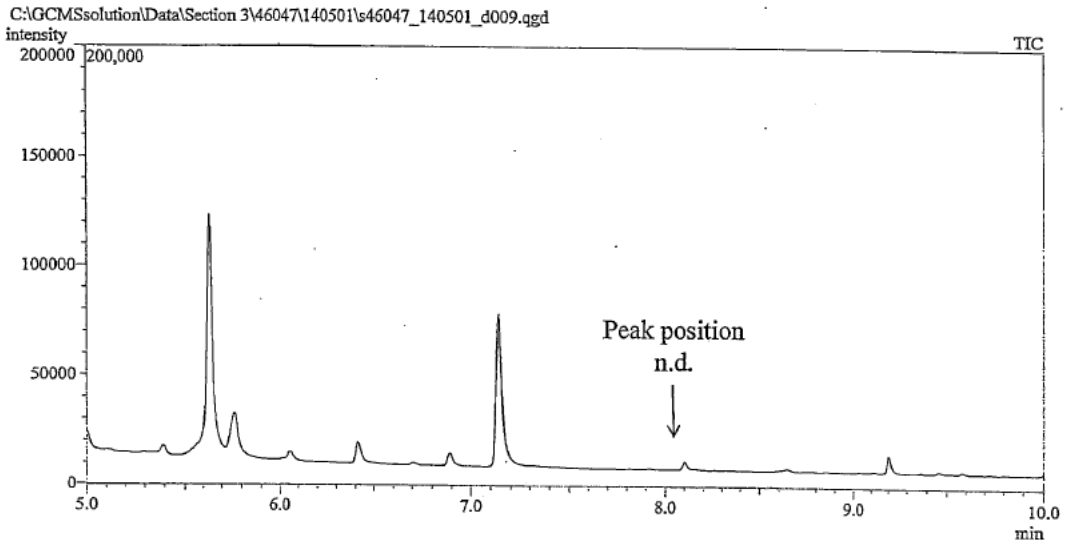
Operating date : May. 01, 2014
File name : C:\GCMSsolution\Data\Section 3\46047\140501\s46047_140501_d008.qgd



Date : May. 02, 2014 Name :

Fig. 11-1 Chromatograms of GC-MS analysis for test fish (Control).

Test fish before experimental start (Control-b)
Operating date : May. 01, 2014
File name : C:\GCMSsolution\Data\Section 3\46047\140501\s46047_140501_d009.qgd

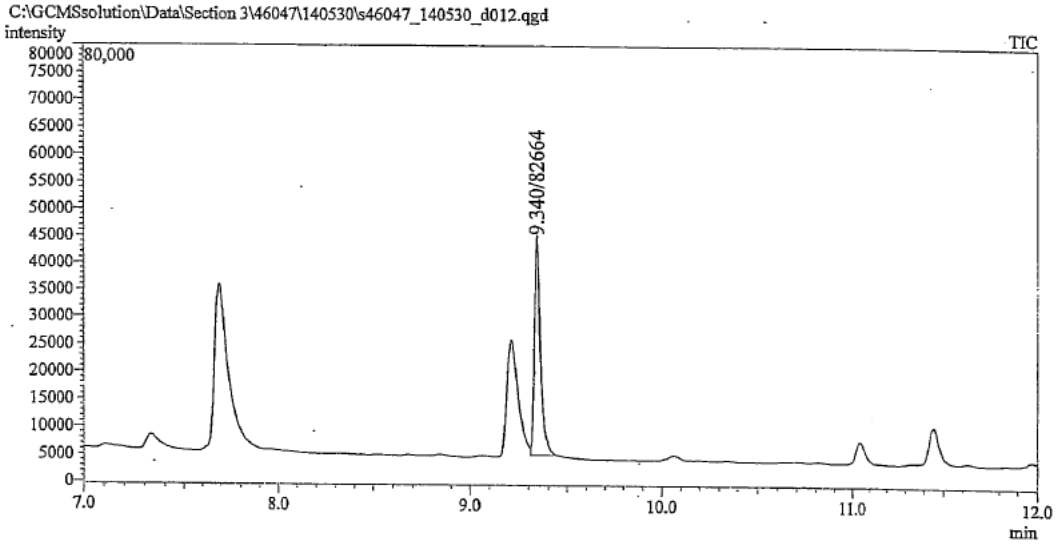


Date : May. 02, 2014 Name :

Fig. 11-2 Chromatograms of GC-MS analysis for test fish (Control).

Standard solution 50.0 ug/L (f)

Operating date : May. 31, 2014
File name : C:\GCMSsolution\Data\Section 3\46047\140530\s46047_140530_d012.qgd

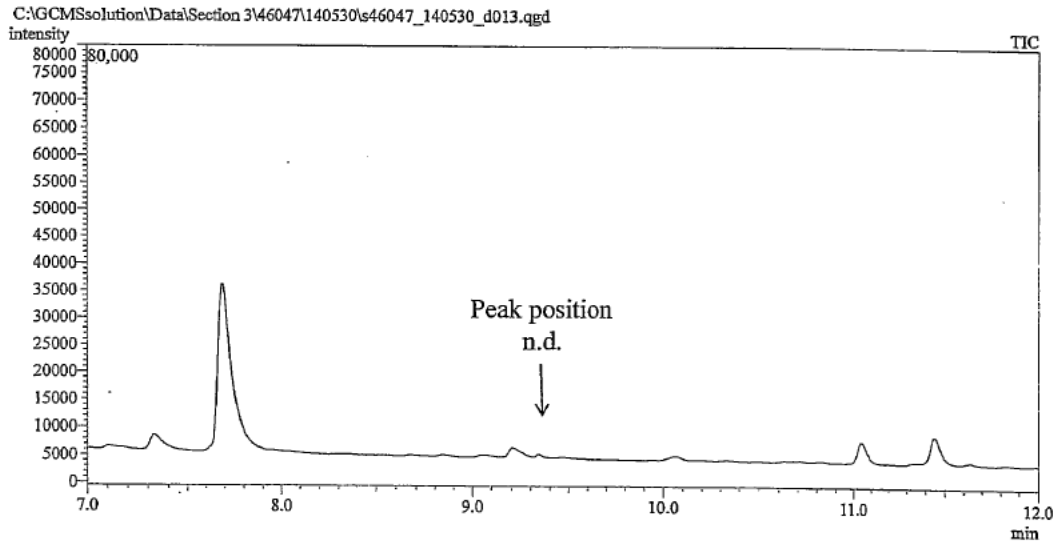


Date : Jun. 03, 2014 Name :

Study No. 46047

After the experimental completion (Control-a)

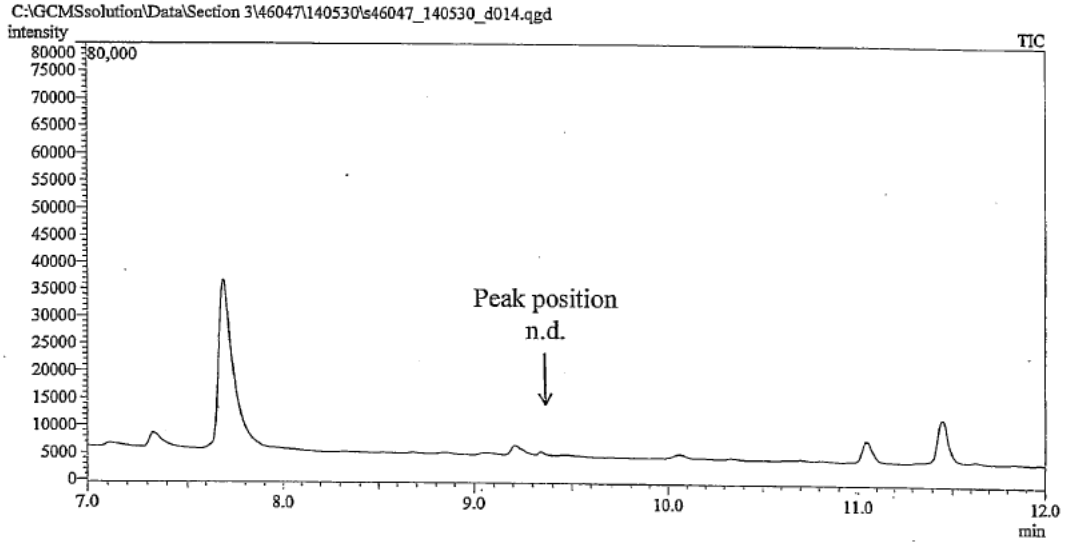
Operating date : May. 31, 2014
File name : C:\GCMSsolution\Data\Section 3\46047\140530\s46047_140530_d013.qgd



Date : Jun. 03, 2014 Name :

Fig. 11-3 Chromatograms of GC-MS analysis for test fish (Control).

After the experimental completion (Control-b)
Operating date : May. 31, 2014
File name : C:\GCMSsolution\Data\Section 3\46047\140530\s46047_140530_d014.qgd



Date : Jun. 03, 2014 Name :

Fig. 11-4 Chromatograms of GC-MS analysis for test fish (Control).

Instrument GCMS-QP2010 (Shimadzu)

Sample Standard solution 10.0 mg/L (test item)

GC Conditions

Column	HP-INNOWAX (30 m × 0.25 mm I.D., film thickness 0.25 μm, Agilent Technologies)
Column temperature	40°C (5 min) $\xrightarrow{①}$ 140°C (0 min) $\xrightarrow{②}$ 240°C (5 min) (Temp. rate ① 20 °C/min ② 50 °C/min)
Carrier gas	He
Control mode	Pressure (54.8 kPa)
Injection temperature	200°C
Injection volume	1 μL (Solvent; Ethyl acetate)
Inlet mode	Splitless
Sampling time	2 min

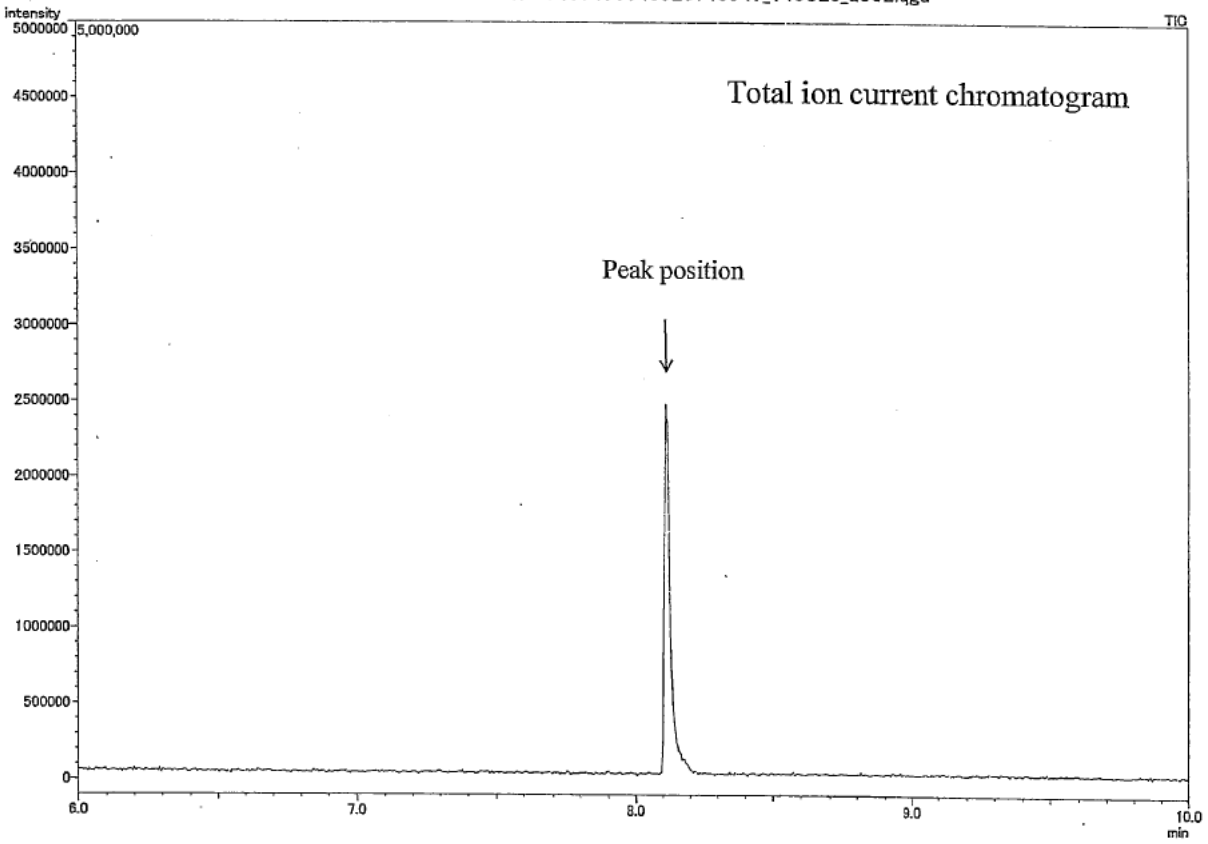
MS Conditions

Ionization mode	EI
Detection mode	Scan
Mass range	*m/z* 50 – 450
Ion source temperature	230°C
Interface temperature	250°C
Ionization voltage	70 V

Date 2014 / 6 / 20 Operator __

Fig. 12-1 Mass spectrum of GC-MS analysis for test item (analytical conditions).

Standard solution 10.0 mg/L
Operating date : Mar. 25, 2014
File name : C:¥GCMSsolution¥Data¥Section 3¥46047¥140325¥46047_140325_d002.qgd



Peak No. 1 R.Time:8.11(Scan#:623)
MassPeaks:401 BasePeak:55(238542)
RawMode:Averaged 8.09-8.21(618-644)

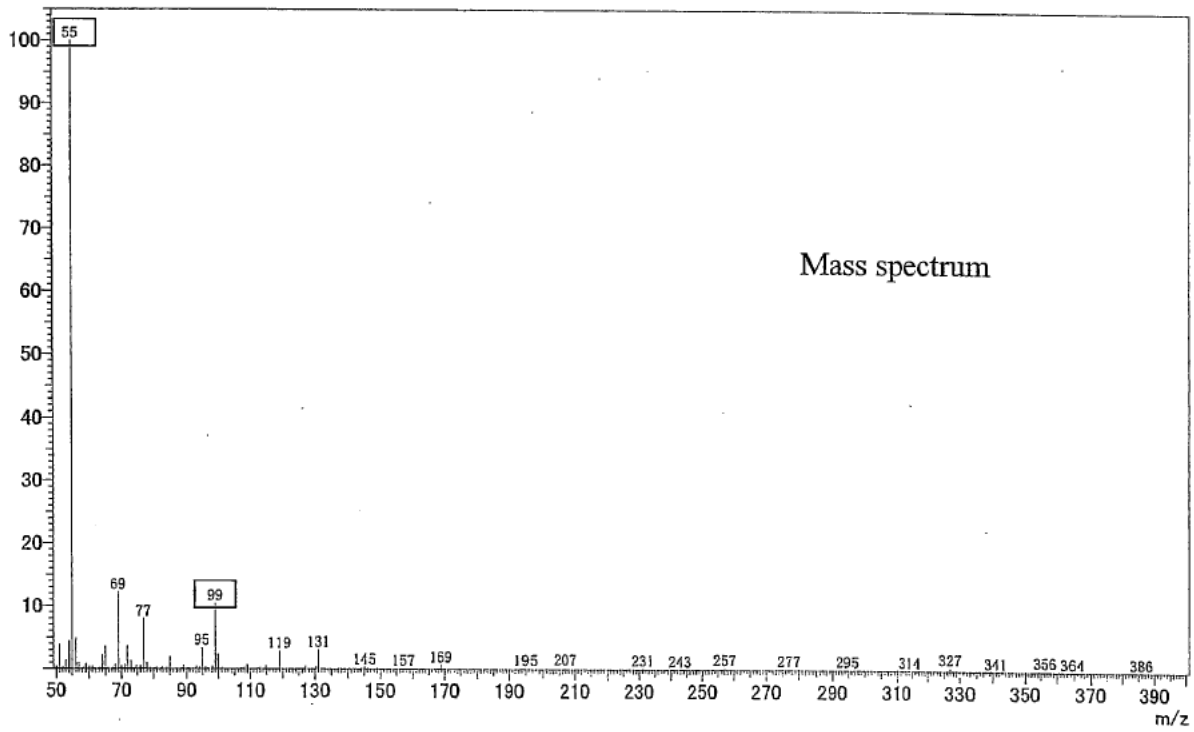
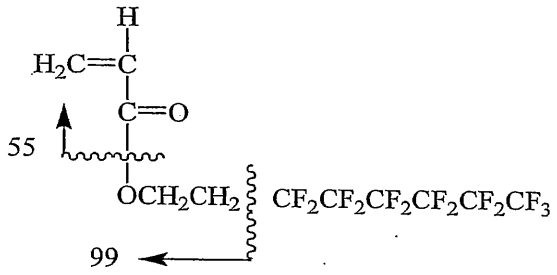


Fig. 12-2 Mass spectrum of GC-MS analysis for test item.

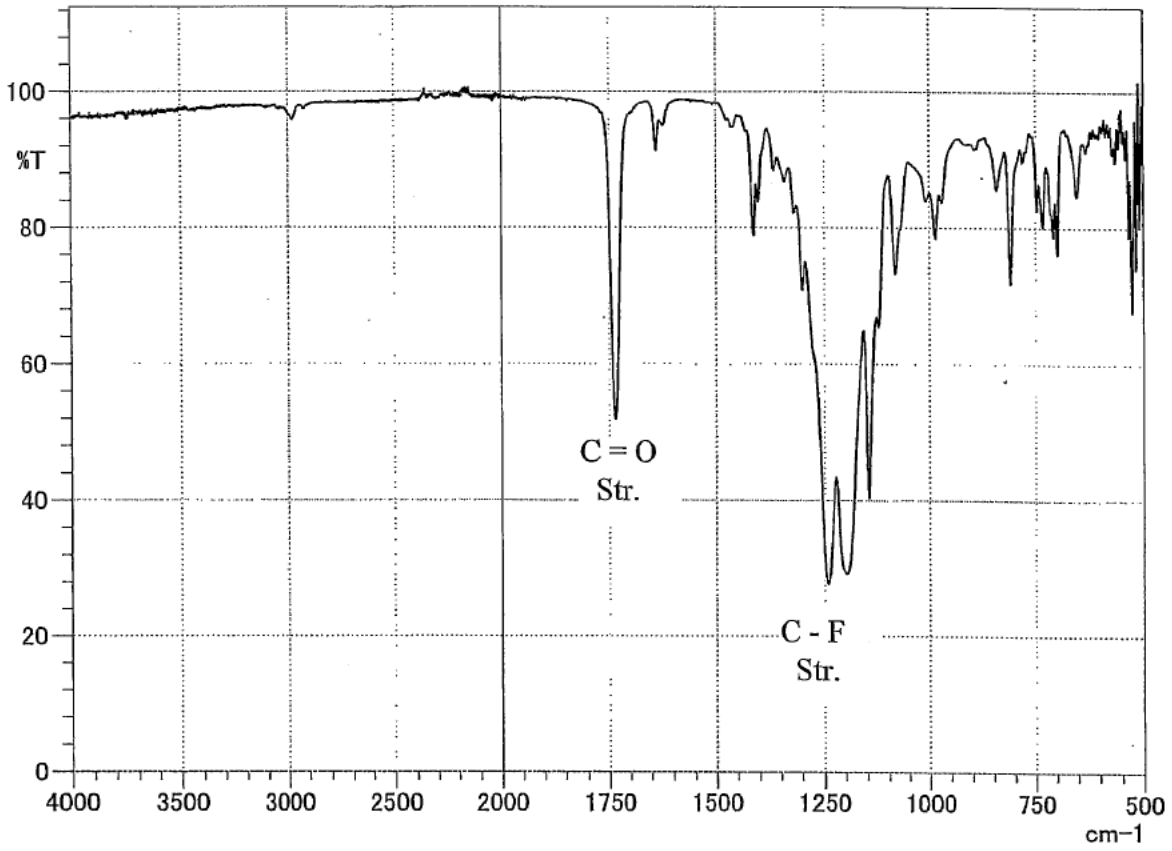
Date : Mar. 25, 2014 Name :



Molecular weight 418.15

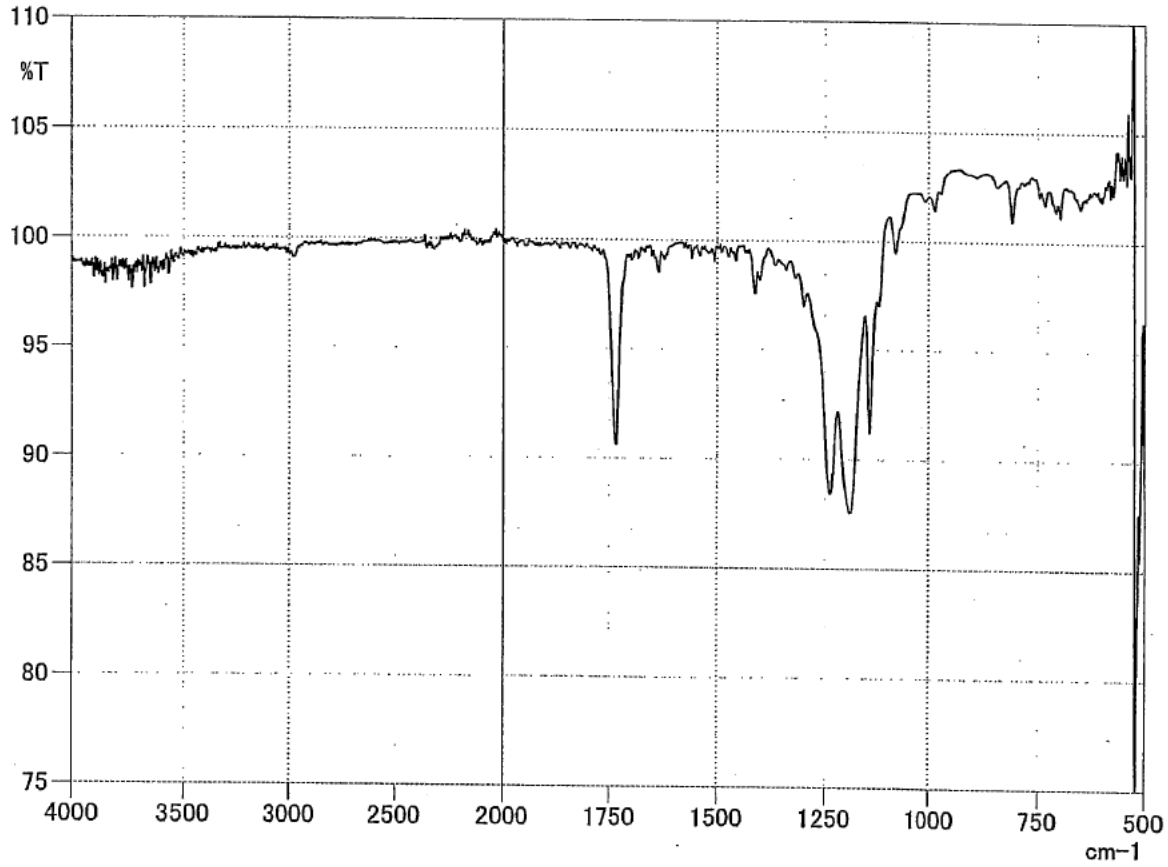
m/z	Detected ion
99	See above
55	See above

Fig. 12-3 Mass spectrum of GC-MS analysis for test item
(The interpretation of the detected ions).



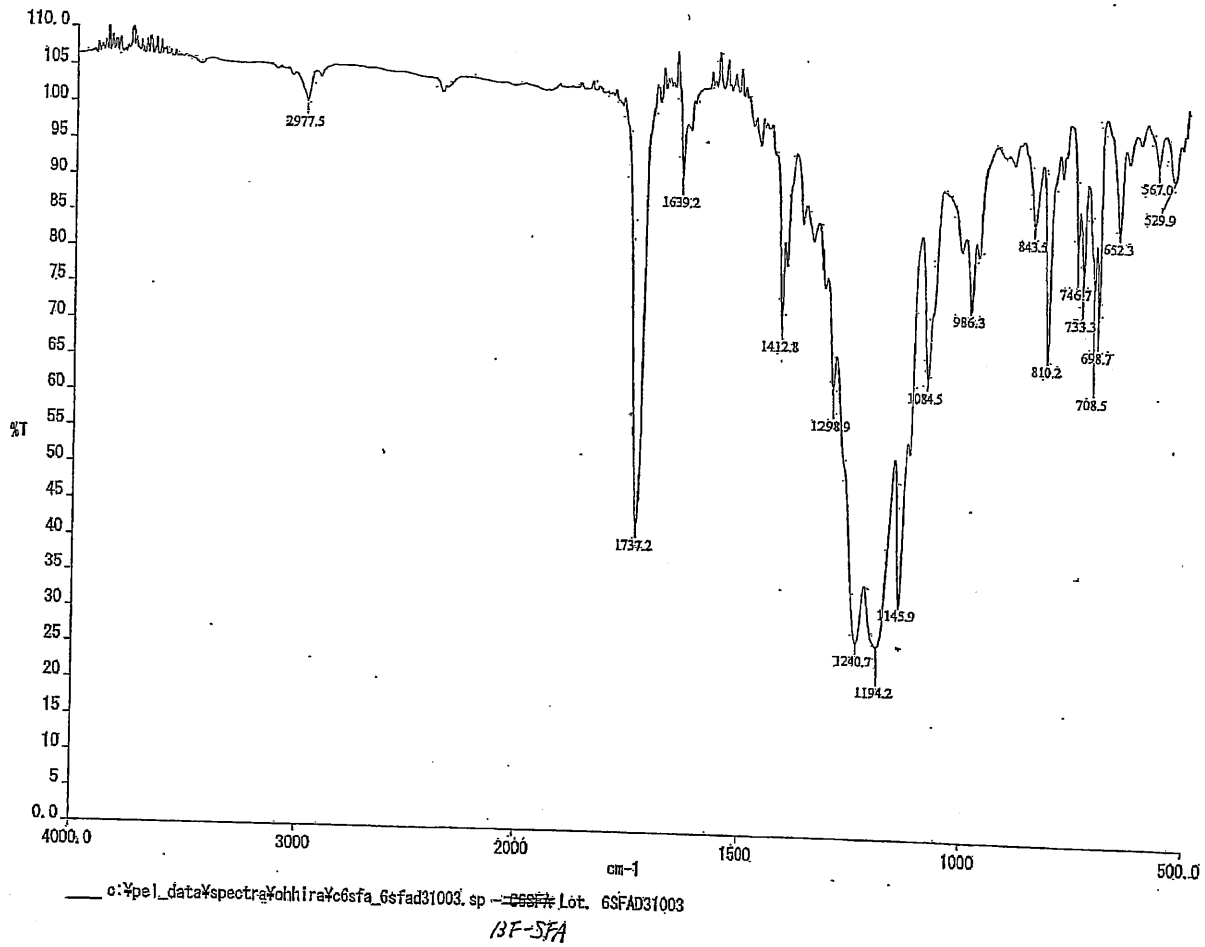
Instrument	: Shimadzu IRAffinity-1S
Study No.	: 46047
Sample	: Test item
Method	: ATR
Date	: March 24, 2014
Name	:

Fig. 13-1 IR spectrum of test item measured before experimental start.



Instrument	: Shimadzu IRAffinity-1S
Study No.	: 46047
Sample	: Test item
Method	: ATR
Date	: June 24,2014
Name	:

Fig. 13 - 2 IR spectrum of test item measured after experimental completion.



BF-SFA

Reference 2 IR spectrum supplied by sponsor.