Accepted number	S01-3771
Study number	43771

FINAL REPORT

Bioconcentration study of $C_{6\text{-}2}$ alcohol in carp

January 31, 2002

Kurume Laboratory
Chemicals Evaluation and Research Institute, Japan

STATEMENT

Kurume Laboratory
Chemicals Evaluation and
Research Institute, Japan

Sponsor ASAHI GLASS CO., LTD.

Title Bioconcentration study of C_{6-2} alcohol in carp

Study number 43771

I, the undersigned, hereby declare that this report provides a correct English translation of the Final Report (Study No.43771, issued on January 31, 2002).

The Study Director was changed from Yurika Mouri to Yoshiyuki Inoue, because Yurika Mouri had been retired.

Date

Study Director

Yoshiyuki Inoue

GLP STATEMENT

Kurume Laboratory
Chemicals Evaluation and
Research Institute, Japan

Sponsor ASAHI GLASS CO., LTD.

Title Bioconcentration study of C_{6-2} alcohol in carp

Study number 43771

This test was conducted in compliance with Good Laboratory Practice Standards for industrial chemicals, "Basic standards to be observed by testing facilities in conducting tests stipulated in article 4 of the Order Prescribing Those Items of the Test Relating to the New Chemical Substances and Study on Harmful Effects of Designated Chemical Substances" (March 31, 1984, Revised March 1, 2000, Kanpogyo No.39, Planning and Coordination Bureau, Environment Agency, Yakuhatu No.229, Pharmaceutical Affairs Bureau, Ministry of Health and Welfare, and 59 Kikyoku No.85, Basic Industries Bureau, Ministry of International Trade and Industry, Japan) and "OECD Principles of Good Laboratory Practice" (November 26, 1997).

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

Date January 31, 2002

Study Director Signed in original

Yurika Mouri

QUALITY ASSURANCE STATEMENT

Kurume Laboratory
Chemicals Evaluation and
Research Institute, Japan

Sponsor ASAHI GLASS CO., LTD.

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The inspections of this study were carried out and the results were reported to the test facility management and the Study Director by Quality Assurance Unit of Kurume Laboratory, Chemicals Evaluation and Research Institute, Japan as follows.

Item of audit or inspection	Date of audit or inspection	Date of report to study director	Date of report to test facility management
Study plan	December 13, 2001	December 17, 2001	December 17, 2001
	December 21, 2001	December 21, 2001	December 21, 2001
	January 9, 2002	January 9, 2002	January 9, 2002
Test conduct	December 18, 2001	December 18, 2001	December 18, 2001
	January 11, 2002	January 17, 2002	January 17, 2002
	January 15, 2002	January 17, 2002	January 17, 2002
	January 17, 2002	January 17, 2002	January 17, 2002
	January 25, 2002	January 29, 2002	January 29, 2002
	January 28, 2002	January 29, 2002	January 29, 2002
	January 29, 2002	January 29, 2002	January 29, 2002
Raw data and final report	January 31, 2002	January 31, 2002	January 31, 2002

It has been assured that the final report describes accurately the test method used, that details in the report are in compliance with the study plan and Standard Operating Procedures and that the final report reflects the raw data accurately.

Date January 31, 2002

Quality Assurance Unit, Head Signed in original

Kyoshiro Hori

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Title

Bioconcentration study of C_{6-2} alcohol in carp

Sponsor

ASAHI GLASS CO., LTD.
1-12-1,Yurakucho, Chiyoda-ku, Tokyo 100-8405 JAPAN

Test facility

Kurume Laboratory
Chemicals Evaluation and Research Institute, Japan
19-14 Chuo-machi, Kurume-shi, Fukuoka 830-0023, Japan

Objective

This study was performed to evaluate the bioconcentration potential of C_{6-2} alcohol in carp.

Test method

This test was conducted according to the "Method for Testing the Degree of Accumulation of Chemical Substances in Fish Body" stipulated in the "Testing Methods for New Chemical Substances" (July 13, 1974, Revised October 8, 1998, Kanpogyo No.5, Planning and Coordination Bureau, Environment Agency, Yakuhatu No.615, Pharmaceutical Affairs Bureau, Ministry of Health and Welfare, and 49 Kikyoku No.392, Basic Industries Bureau, Ministry of International Trade and Industry, Japan), and "Bioconcentration : Flow-through Fish Test (Guideline 305, June 14, 1996)" in the OECD Guidelines for Testing of Chemicals.

Applied GLP

(1) Chemical GLP

This test complied with "Basic standards to be observed by testing facilities in conducting tests stipulated in article 4 of the Order Prescribing Those Items of the Test Relating to the New Chemical Substances and Study on Harmful Effects of Designated Chemical Substances (hereafter referred to as "GLP standards")" (March 31, 1984, Revised March 1, 2000, Kanpogyo No.39, Planning and Coordination Bureau, Environment Agency, Yakuhatu No.229, Pharmaceutical Affairs Bureau, Ministry of Health and Welfare, and 59 Kikyoku No.85, Basic Industries Bureau, Ministry of International Trade and Industry, Japan).

(2) OECD-GLP

This test complied with "OECD Principles of Good Laboratory Practice" (November 26, 1997).

Dates

Study initiation date	December 13, 2001
Experimental starting date	December 27, 2001
Experimental completion date	January 24, 2002
Study completion date	January 31, 2002

Storage of test item, raw data, etc.

(1) Test item

About 5 g of the item supplied by the sponsor is sealed in a store vessel and stored in a archive in this laboratory for ten years after receipt of notification that the test item belong to No.1, No.2 or No.3 in Clause 1, Article 4 of "Law Concerning Examination and Regulation of Manufacture, etc. of Chemical Substances". Treatment of the item supplied by the sponsor after the storage period is discussed with sponsor. If it is not stable for the storage period, it is stored while it is kept stable and it is disposed with approval of sponsor.

(2) Raw data and materials, etc.

Raw data, the study plan, documents concerning the study presented by the sponsor, the final report and necessary materials are stored in archives in this laboratory for ten years after the receipt of the notice specified under Clause 1, Clause 2 or Clause 3 in Article 4 of "Law Concerning Examination and Regulation of Manufacture, etc. of Chemical Substances". Treatment of raw data and materials, etc. after the storage period is discussed with the sponsor.

Personnel

Study Director	Yurika Mouri (2nd Chemical Safety Section)
Study personnel (Operation of bioconcentration test)	Yoshiyuki Inoue Yuka Kida Yasuro Kawashima Chitose Fukuda
Staff for fish care	Yasuro Kawashima
Person to conduct of fish acute toxicity test	Yasuro Kawashima Tadayoshi Tonai

Approval of final report

Study Director	Date	January 31, 2002
	Signature	Signed in original
		Yurika Mouri

SUMMARY

Title

Bioconcentration study of C_{6-2} alcohol in carp

Test conditions

Acute toxicity test

(1) Test fish	Orange-red killifish (*Oryzias latipes*)
(2) Duration of exposure	96 hours
(3) Exposure method	Semi static system (renewal of test water, at every 8 - 16 hours)

Bioconcentration test

(1) Test fish	Carp (*Cyprinus carpio*)	
(2) Nominal concentrations of test item		
	High exposure level (Level 1)	10 μg/L
	Low exposure level (Level 2)	1 μg/L
(3) Duration of exposure	28 days	
(4) Exposure method	Continuous flow system	
(5) Analytical method		
Test item	Gas chromatography-mass spectrometry	
Carboxylic acid	Liquid chromatography-tandem mass spectrometry	

Results

(1) 96-hour LC50 value	10.4 mg/L	
(2) Bioconcentration factors at a steady state		
Test item	Level 1	46
	Level 2	≦36
Carboxylic acid	Level 1	≦ 1.1
	Level 2	≦12

1. Test item

In this report, $C_{6\text{-}2}$ alcohol has the following chemical name, etc.

1.1 Chemical name*1 3,3,4,4,5,5,6,6,7,7,8,8,8-Tridecafluoro-1-octanol

1.2 Chemical structure, etc.

Structural formula*1

$$C_6F_{13}C_2H_4OH$$

Molecular formula $C_8H_5F_{13}O$

Molecular weight 364.10

*1 Information supplied by the sponsor

2. Item supplied by sponsor

2.1 Supplier and lot number*1

(1) Supplier ASAHI GLASS CO., LTD.

(2) Lot number Lot 2

2.2 Purity*1

Test item 99.8 wt%

The test item was treated as 100 % in purity.

2.3 Confirmation of test item

Two infrared (IR) spectra of the test item provided by the sponsor and measured at this laboratory were confirmed to be identical (see Fig. 22 and Reference 2).

2.4 Physicochemical properties*1

Appearance	Colorless and transparent liquid
Vapor pressure	36 mmHg (91 - 93 °C)
Density	1.678 g/cm^3 (20°C)

*1 Information supplied by the sponsor

2.5 Storage and stability

(1) Storage condition

Room temperature

(2) Stability

The test item was stable under the storage condition as shown by the finding that IR spectra of the test item before and after the experiment were identical (see Fig. 22).

2.6 Stability under testing conditions

Prior to the bioconcentration test, a stability of the test item under the testing conditions was confirmed by a preliminary test.

3. Performance of acute toxicity test

3.1 Test method

The test was performed in accordance with Japanese Industrial Standard (JIS K 0102-1998-71.), "Testing methods for industrial waste water, Acute toxicity test with fish".

3.2 Test fish

(1) Species Orange-red killifish (*Oryzias latipes*)
Reason for selection : This species is similar in sensitivity to carp and readily available as test fish.

(2) Supplier Ogawa shoten
(Address : 181 Ooishi-machi Kurume-shi, Fukuoka 830-0049, Japan)

(3) Conditions for fish care before acclimatization

Period

The fish were checked visually at receipt and those with any abnormalities were removed. The remainder was reared for 57 days in a flow-through system after the external disinfection for sick prevention and parasitic extermination.

External disinfection

The external disinfection for sick prevention was carried out in an aqueous solution containing 20 mg/L ELBAZIU and 7 g/L sodium chloride for 24 hours. The external disinfection for parasitic extermination was carried out two times in an aqueous solution containing 30 μL/L formalin for 24 hours.

(4) Conditions of acclimatization

Period

After rearing, the fish were transferred to an acclimatizing aquarium and acclimatized there after the external disinfection. The fish showing any abnormalities during this period were removed and the remainder was reared for 26 days in a flow-through system at the temperature of 25 ± 2 °C. The fish were checked for health conditions and reared for 25 days after the external disinfection.

External disinfection

The first external disinfection was carried out in an aqueous solution containing 20 mg/L ELBAZIU and 7 g/L sodium chloride for 24 hours. The second external disinfection was carried out in an aqueous solution containing 20 mg/L ELBAZIU and 7 g/L sodium chloride for 24 hours.

(5) Weight average 0.35 g

(6) Length average 3.3 cm

(7) Certification

The 48-hour LC50 value of the reference substance*2 for the fish of the same lot (TFO-011022) was 0.704 mg/L.

*2 PCP-Na (pentachlorophenol sodium salt, Tokyo Kasei Kogyo Co., Ltd.)

3.3 Dilution water for test

(1) Origin

Groundwater from the premises of Kurume Laboratory.

(2) Water quality assessment

The dilution water for test was taken out on September 3, 2001, and it was analyzed and measured (once every six months in this laboratory). The results are shown in Reference 1.

It was confirmed that the dilution water met the ministerial ordinance of the Ministry of Health and Welfare (December 21, 1992), water quality criteria for fisheries (Shadanhozin Nihon Suisansigen Hogokyokai, March 1983), OECD Guidelines for Testing of Chemicals, "Fish, Early-life Stage Toxicity Test" (Guideline 210, July 17, 1992) and environmental quality standards for water pollutants No.14 (Revised February 22, 1999, Environment Agency) or OECD Guidelines for Testing of Chemicals, "Bioconcentration: Flow-through Fish Test (Guideline 305, June 14, 1996)".

3.4 Test conditions

(1) Test tank	Glass gallon vessel
(2) Volume of test water	3.85 L × 2 / level
(3) Temperature of test water	
At initial exposure	24.0 °C
Before renewal of test water	24.0 °C
(4) Concentration of dissolved oxygen in test water	
At initial exposure	8.1 mg/L
Before renewal of test water	7.0 - 7.7 mg/L

(5) pH of test water

At initial exposure	8.5
Before renewal of test water	8.2

(6) Number of fish 10 / level

(7) Duration of exposure 96 hours

(8) Exposure method Semi static system
(renewal of test water, at every 8 - 16 hours)

3.5 Preparation of stock solution

(1) Dispersant
HCO-20 (Hydrogenated castor oil)

(2) Preparation
The item supplied by the sponsor and HCO-20 (20 times amount of it) were mixed and kneaded. And ion-exchanged water was added to the mixture to prepare 1000 mg/L stock solution.

3.6 Performance of test

(1) Place 214 LC50 room

(2) Date December 17, 2001 - December 21, 2001

3.7 Estimation of 96-hour LC50 value
The 96-hour LC50 value was estimated by the Doudoroff method.

3.8 Result of test
96-hour LC50 value 10.4 mg/L (see Fig. 3)

4. Performance of bioconcentration test

4.1 Test fish

(1) Species 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.

(2) Supplier Sugishima fish farm
(Address : 123-2 Gunchiku Ichibancho, Yatsushiro-shi, Kumamoto 866-0024, Japan)

Date received October 15, 2001

(3) Conditions for fish care before acclimatization
Period
The fish were checked visually at receipt and those showing any abnormalities were removed. The remainder was reared for 13 days in a flow-through system after the external disinfection for sick prevention and parasitic extermination.

External disinfection
The external disinfection for sick prevention was carried out in an aqueous solution containing 50 mg/L OTC (Oxytetracycline hydrochloride) for fisheries and 7 g/L sodium chloride for 24 hours. The external disinfection for parasitic extermination was carried out in an aqueous solution containing 30 μL/L formalin for 24 hours.

(4) Conditions for acclimatization
Period
After rearing, the fish were transferred to an acclimatizing aquarium and acclimatized there after the external disinfection. The fish showing any abnormalities during this period were removed and the remainder was reared for 15 days in a flow-through system at the temperature of 25 ± 2 °C. The fish were checked for health conditions and transferred to test tanks. Thereafter the fish were reared at the same temperature in a flow-through system for 41 days, following an external disinfection.

External disinfection
The external disinfection in the acclimatizing aquarium was carried out in an aqueous solution containing 50 mg/L OTC for fisheries and 7 g/L sodium chloride for 24 hours. The external disinfection in test tanks was carried out in an aqueous solution containing 20 mg/L ELBAZIU and 7 g/L sodium chloride for 24 hours.

(5) Length 6.6 - 8.6 cm

(6) Lot No. TFC-011015

(7) Age Yearling fish

(8) Feeding

Feed	Feed for fry of carp
Composition	Proteins content ≧ 43.0 %
	Lipid content ≧ 3.0 %
Manufacturer	Nippon Formula Feed Mfg. Co., Ltd.

Feeding amount and interval

Amount corresponding to about 2 % of total body weight was fed twice a day in halves.

The fish were starved for 24 hours before sampling.

4.2 Dilution water for test

The same described in Section 3.3.

4.3 Conditions of test and circumstances

(1) Supply of test water

Flow-through system assembled at this laboratory was used.

(2) Test tank

Level 1 and 2	100-L glass tank for volatile item
Control	100-L glass tank

(3) Flow rate of test water

Level 1 and 2

0.02 mL/min for stock solution and 1600 mL/min for dilution water, 2304 L/day of test water, were supplied.

Control

2 mL/min for stock solution and 1600 mL/min for dilution water, 2307 L/day of test water, were supplied.

(4) Stock solution bottle

Level 1 and 2	500-mL glass bottle (Cooling in the refrigerator)	
	(Frequency of renewal	1 time/week)
Level 1 and 2	25-L glass bottle	
	(Frequency of renewal	1 time/week)

(5) Temperature of test water

Level 1	24.6 - 25.1 °C
Level 2	25.1 - 25.9 °C
Control	25.1 - 25.4 °C

(6) Concentrations of dissolved oxygen in test water

Level 1	7.0 - 7.7 mg/L (see Fig. 19)
Level 2	7.0 - 7.7 mg/L (see Fig. 20)
Control	7.4 - 8.0 mg/L (see Fig. 21)

(7) pH of test water

Level 1	7.6 - 7.8
Level 2	7.6 - 7.8
Control	7.7 - 7.9

(8) Time of irradiation with light

Artificial light of white fluorescent lamp (14 hours/day)

(9) Number of fish (at the beginning of exposure)

Level 1 and 2	29
Control	12

(10) Duration of exposure 28 days

Reason : The time to reach a steady-state was estimated to be within 28 days from preliminary test results.

(11) Place 213 Aquatron room

4.4 Preparation of stock solutions

(1) Dispersant

The same as described in Section 3.5 (1).

(2) Preparation

- Level 1

  800 mg/L stock solution was prepared in the same way as described in Section 3.5 (2).

- Level 2

  80 mg/L stock solution was prepared in the same way as described in Section 3.5 (2).

- Control

  HCO-20 was dissolved in ion-exchanged water to prepare 160 mg/L stock solution.

4.5 Test concentrations

Based on preliminary test results for the 96-hour LC50 value and analytical detection limits, test concentrations of the test item were decided as follows. The control was set as a blank test.

Level 1	10 μg/L
Level 2	1 μg/L

4.6 Observation, measurement and cleaning of test tank

(1) Observation of test fish

Condition of test fish was observed visually twice a day.

(2) Flow rate of test water

Flow rate of stock solution and dilution water were measured with graduated cylinder and recorded once a day.

(3) Temperature of test water

Temperature of test water was measured with alcohol thermometer and recorded once a day.

(4) Concentration of dissolved oxygen in test water

Concentration of dissolved oxygen in test water was measured with dissolved oxygen probe and recorded twice a week.

(5) pH of test water

pH of test water was measured with pH meter once a week or more.

(6) Cleaning of test tank

In experimental period, excreta of carp, dirt on test tank, etc. were removed about once a day.

4.7 Analysis of test water and fish

Analysis of test item in test water and test fish was performed with gas chromatography-mass spectrometry (GC-MS) analysis.

If the test item was bioaccumulated in the test fish, the test item had a possibility to be metabolized in the fish body. Therefore, $C_5F_{11}COOH$ (hereafter mentioned as "carboxylic acid") which was an estimated metabolite of the test item in the test fish was analyzed at the same time. Analysis of the carboxylic acid in the test fish was performed with the liquid chromatography - tandem mass spectrometry (LC/MS/MS).

4.7.1 Frequency of analysis

(1) Test water analysis

The test water of each level was analyzed once before first analysis of test fish and at the same time as analysis of test fish thereafter. The number of each sample was one.

(2) Test fish analysis

The test fish of Level 1 and 2 was analyzed five times in duration of exposure. Four fish were taken out at each sampling time and divided into two groups, then both were analyzed individually*3.

The control fish was analyzed before the experimental starting and after the experimental completion. Four fish were taken out at each sampling time and divided into two groups, and then each was analyzed individually. In addition, two fish were taken out and three groups (two fish per group) were used for measurement of lipid contents.

*3 Because one fish was too small to take out the stored sample for the measurement of lipid content, two fish a group were employed.

4.7.2 Pretreatment for analysis

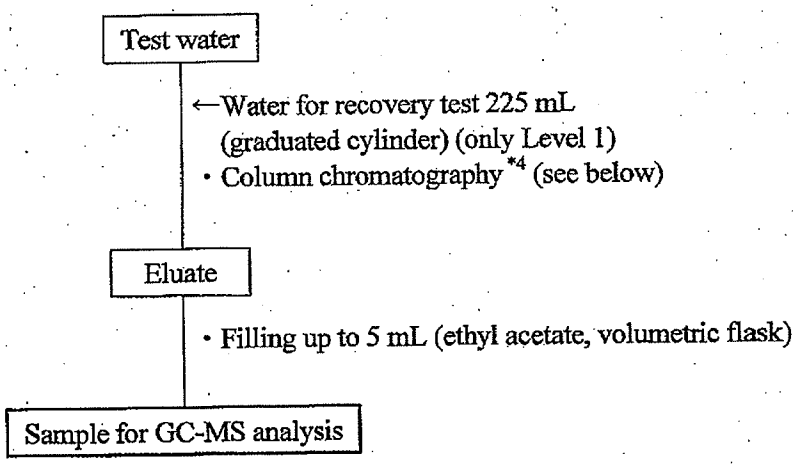
(1) Test water

An aliquot of the test water,

Level 1 25 mL

Level 2 250 mL

was taken from each test tank, and pretreated for gas chromatography-mass spectrometry (GC-MS) analysis as follows :



*4 Conditions of column chromatograph

Mega Bond Elut C8

Conditionings	Ethyl acetate	10 mL
	Methanol	10 mL
	Water*5	10 mL
Loading	Whole volume of the solution was loaded.	
Elution	Eluent 1 Water*5	5 mL
	Nitrogen purge was conducted after the first elution, then the cartridge for dehydration*6 was connected.	
	Eluent 2 Ethyl acetate	4.5 mL
	Test item was eluted with eluent 2.	

*5 City water was treated by Ultra pure water system.

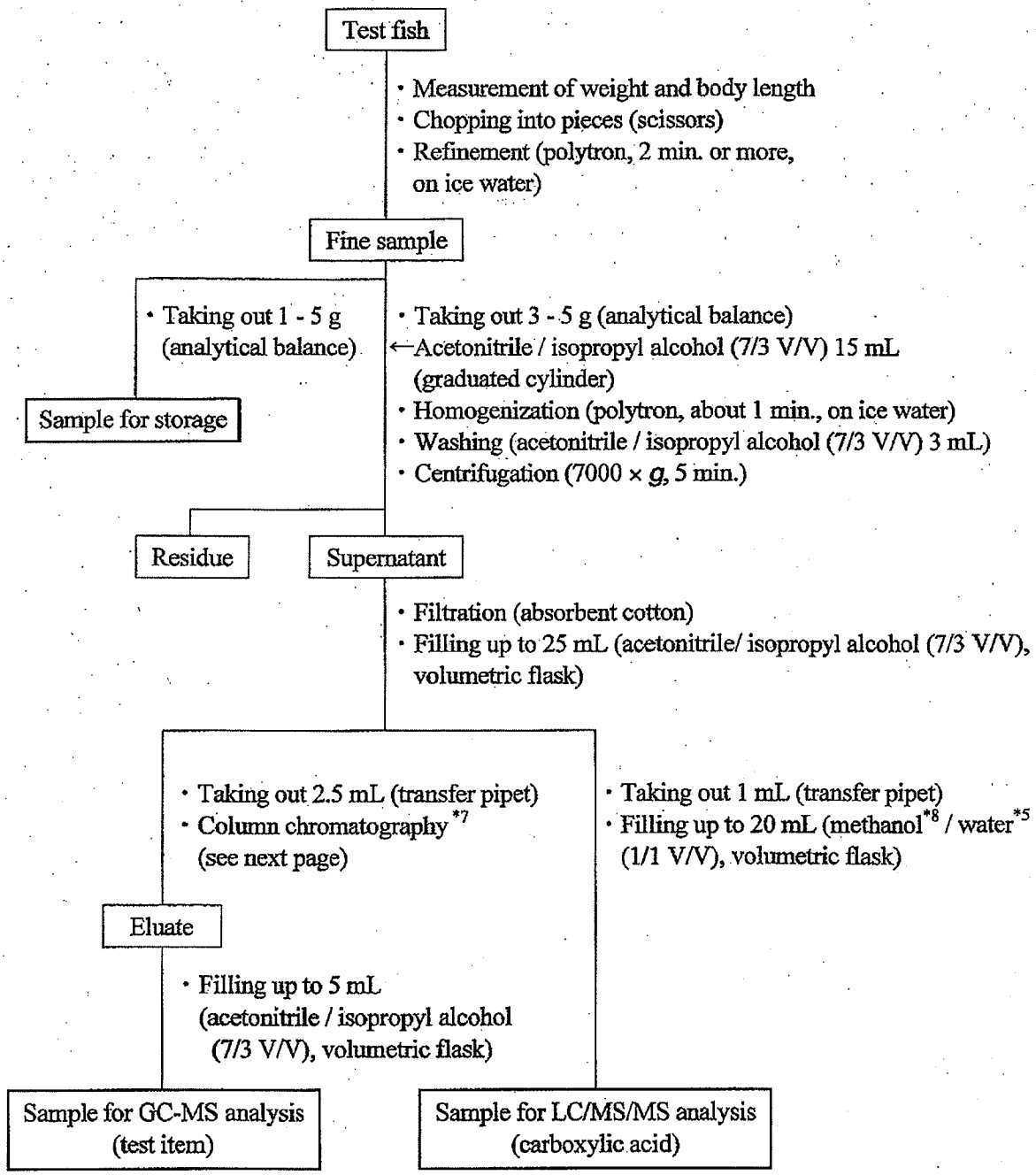
*6 Conditions of the cartridge for dehydration

Sep-Pak Dry

Conditionings	Ethyl acetate	5 mL

(2) Test fish (test item and carboxylic acid)

Test fish were taken from each test tank and pretreated for gas chromatography-mass spectrometry (GC-MS) analysis (test item) and liquid chromatography-tandem mass spectrometry (LC/MS/MS) analysis (carboxylic acid) as follows :



*7 Conditions of column chromatograph

Sep-Pak Silica

Conditionings	Acetonitrile / isopropyl alcohol (7/3 V/V) 5 mL
Loading	Whole volume of the solution was loaded.
Elution	Eluent Acetonitrile / isopropyl alcohol (7/3 V/V) 2 mL

Test item was eluted with the loaded solution and eluent.

*8 Containing 10 mmol/L di-*n*-butylammonium acetate.

4.7.3 Quantitative analysis for test item and carboxylic acid

(1) Analysis for test item

The samples for GC-MS analysis in pretreatment were analyzed by gas chromatography-mass spectrometry under the following analytical conditions. The concentration of the test item in each sample solution was determined on the basis of a comparison of the peak area on the mass fragmentogram of the sample solution with that of a standard solution (see Tables-5, 6, Fig. 6 and Tables-8, 9, 10, Figs. 9, 10, 11).

(a) Analytical conditions

Instrument	Gas chromatograph-mass spectrometer Shimadzu Corporation type QP-5000

Conditions of gas chromatograph

Column	INNOWAX 30 m × 0.25 mm I.D. film thickness 0.25μm
Column temp.	Test water 35 °C (2 min.) $\xrightarrow{①}$ 150 °C (2 min.) $\xrightarrow{②}$ 200 °C (2 min.) Test fish 35 °C (2 min.) $\xrightarrow{①}$ 150 °C (2 min.) $\xrightarrow{②}$ 200 °C (3 min.)
Temp. rate	① 20 °C/min. ② 35 °C/min.
Injection temp.	200 °C
Carrier gas	Helium
Pressure	50 kPa
Total flow	10 mL/min.
Injection method	Splitless
Sample size	3 μL

Conditions of mass spectrometer

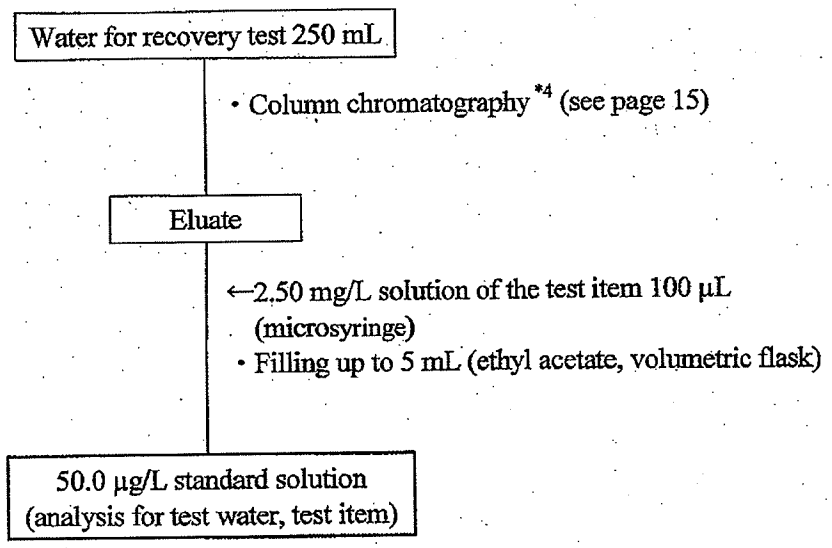
Interface temp.	250 °C
Ionization mode	Electron ionization (EI)
Ionization voltage	70 eV
Monitoring m/z	94.95

(b) Preparation of standard solution

The standard solution to determine the concentration of the test item in samples was prepared as follows.

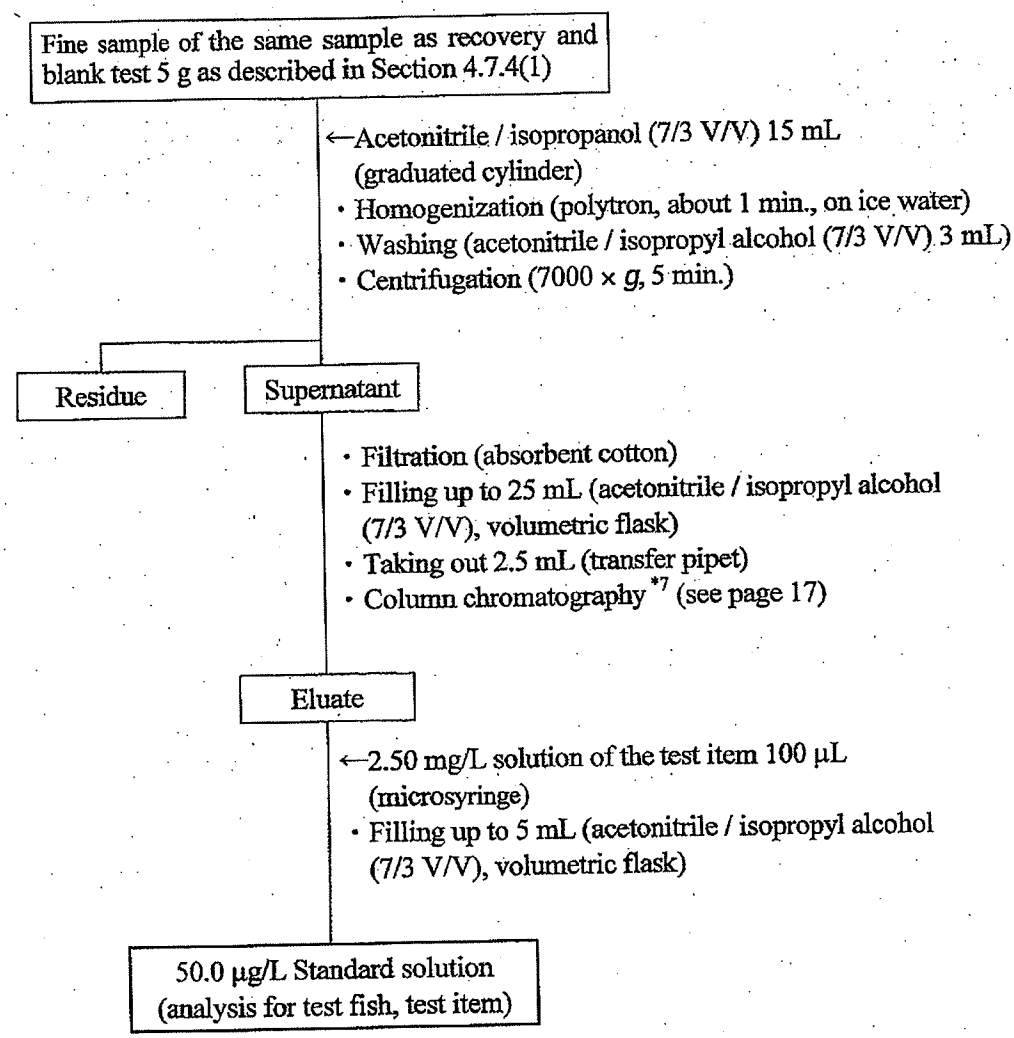
① Test water

100 mg of the item supplied by the sponsor was dissolved in ethyl acetate to prepare 1000 mg/L solution of the test item. 2.50 mg/L solution of the test item was then prepared from this solution by dilution with ethyl acetate. This solution was pretreated as follows to prepare 50.0 μg/L standard solution.



② Test fish

100 mg of the item supplied by the sponsor was dissolved in acetonitrile to prepare 1000 mg/L solution of the test item. 2.50 mg/L solution of the test item was then prepared from this solution by dilution with acetonitrile / isopropyl alcohol (7/3 V/V). This solution was pretreated as follows to prepare 50.0 μg/L standard solution.



(c) Calibration curve

① Test water

25.0, 50.0 and 100 μg/L standard solutions were prepared by the same method as described in (b)①. These standard solutions were analyzed according to the analytical conditions described in (a). A calibration curve was drawn on the basis of the relation between the peak area on the mass fragmentograms and the respective concentrations.

In consideration of the noise level, the lowest detectable peak area of the test item was regarded as 150, which corresponded to 2.5 μg/L of the test item concentration (see Fig. 4).

② Test fish

25.0, 50.0 and 100 μg/L standard solutions were prepared by the same method as described in (b)②. These standard solutions were analyzed according to the analytical conditions described in (a). A calibration curve was drawn on the basis of the relation between the peak area on the mass fragmentograms and the respective concentrations.

In consideration of the noise level, the lowest detectable peak area of the test item was regarded as 150, which corresponded to 2.2 μg/L of the test item concentration (see Fig. 7).

(2) Analysis for carboxylic acid

The samples for LC/MS/MS analysis in pretreatment were analyzed by liquid chromatography- tandem mass spectrometry under the following analytical conditions. The concentration of the carboxylic acid in each sample solution was determined on the basis of a comparison of the peak area on the chromatogram of the sample solution with that of a standard solution (see Tables-12, 13, 14, Figs. 16, 17, 18).

(a) Analytical conditions

Instrument	Liquid chromatograph-mass spectrometer	
Liquid chromatograph	Agilent	type HP-1100
Mass spectrometer	Micromass	type Quattro Ultima

Conditions of liquid chromatograph

Column	L-column ODS
	15 cm × 2.1 mm I.D. stainless steel
Column temp.	25 °C
Eluent	A (40 %): Water*5,9
	B (60 %): Methanol*9
Flow rate	0.2 mL/min.
Injection volume	20 μL

Conditions of mass spectrometer

Ionization mode	Electrospray
Detection ion	Negative
Detection mode	Selected reaction monitoring
Precursor ion	m/z 313
Product ion	m/z 269
Ion source temp.	130 °C
Desolution temp.	400 °C
Cone voltage	20 V
Collision energy	10 eV

*9 Containing 5 mmol/L di-*n*-butylammonium acetate.

(b) Preparation of standard solution

The standard solution to determine the concentration of the carboxylic acid in the sample solutions was prepared as follows.

100 mg of the carboxylic acid sample was accurately weighed and dissolved in methanol to prepare 1000 mg/L solution of the carboxylic acid. 1.00 μg/L standard solution was then prepared from this solution by dilution with methanol[*8] / water[*5] (1/1 V/V).

(c) Calibration curve

0.50, 1.00 and 2.00 μg/L standard solutions were prepared by the same method as described in (b). These solutions were analyzed according to the analytical conditions described in (a). A calibration curve was drawn on the basis of the relation between the peak area on the chromatograms and the respective concentrations.

In consideration of the noise level, the lowest detectable peak area of the carboxylic acid was regarded as 1000, which corresponded to 0.091 μg/L of the carboxylic acid concentration (see Fig. 14).

4.7.4 Recovery and blank test

(1) Test item

(a) Method

Water and fine sample of two fish (about 10 g) were spiked a specified amount of the test item and prepared in the same way as described in Section 4.7.2 for the recovery tests. The blank tests were also performed in the same manner without the test item. All the recovery and blank tests were performed in duplicate.

(b) Results of recovery test

In the blank tests, the mass fragmentogram 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-4, 7 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.

For analysis of test water (250 ng test item added)
81.0 %, 82.9 % average 82.0 %

For analysis of test fish (5000 ng test item added)
74.2 %, 74.5 % average 74.3 %

(2) Carboxylic acid

(a) Method

Fine sample of two fish (about 10 g) spiked a specified amount of the carboxylic acid for the recovery test was prepared in the same way as described in Section 4.7.2(2). The blank test was also performed in the same manner without the carboxylic acid. All the recovery and blank tests were performed in duplicate.

(b) Results of recovery test

In the blank tests, the chromatogram of LC/MS/MS had no peaks interfering with determination of the carboxylic acid concentration. The duplicate recovery rates and the average of them in the pretreatment are shown below (see Table-11 and Fig. 15). The average recovery rate was used as correction factors for the determination of the carboxylic acid concentrations in the analytical samples.

For analysis of test fish (1000 ng carboxylic acid added)

94.4 %, 93.2 % average 93.8 %

4.7.5 Lipid content in test fish

Lipid contents in the sample for storage of the control test fish were determined with gravimetric analysis after chloroform-methanol extraction.

4.7.6 Calculation of the test item and carboxylic acid concentration in sample and minimum limit of determination

(1) Calculation of the test item concentration in test water

The equations in Tables-5 and 6 were used to obtain the concentrations, and they were rounded to 3 figures.

(2) Determination limit of the test item in test water

The determination limit*10 of the test item in test water was calculated on the basis of that obtained from the calibration curve in Section 4.7.3(1)(c)①as follows.

Level 1	0.61	μg/L
Level 2	0.061	μg/L

(3) Calculation of the test item and carboxylic acid concentration in test fish

(a) Test item

The equations in Tables-8, 9 and 10 were used to obtain the concentrations, and they were rounded to 3 figures.

(b) Carboxylic acid

If the carboxylic acid was concentrated into the test fish, the equations in Tables-12, 13 and 14 were used to obtain the concentrations. However, the measured concentrations of carboxylic acid were not more than the determination limit.

(4) Determination limit of the test item and carboxylic acid in test fish

Assuming the fine sample of fish to be 5 g, the determination limit*10 of the test item and carboxylic acid in test fish were calculated on the basis of that obtained from the calibration curve in Section 4.7.3(1)(c)② and 4.7.3(2)(c).

(a) Test item	30	ng/g
(b) Carboxylic acid	9.7	ng/g

*10 Minimum determination limit of the test item and carboxylic acid (μg/L or ng/g)

$$= \frac{A}{\frac{B}{100} \times \frac{C \times E}{D}}$$

where

A : Minimum determination limit of the test item and carboxylic acid on the calibration curve (μg/L)

B : Recovery rate (%)

C : Sampling volume of test water (mL) or fine sample of 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.

4.7.7 Calculation of average concentration of the test item in test water (duration of exposure)

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

where

$\overline{Cwt}$	:	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)

4.7.8 Calculation of bioconcentration factor (BCF)

Bioconcentration factor (BCF) of the test item and carboxylic acid were calculated as follows.

(1) Calculation of average concentration of the test item in test water for calculating BCF

$$\overline{Cw} = \{ Cw(n-1) + Cw(n) \} / 2 \quad \text{(only 1st analysis of test fish)}$$
$$\overline{Cw} = \{ Cw(n-2) + Cw(n-1) + Cw(n) \} / 3 \quad \text{(from 2nd analysis of test fish)}$$

where

$\overline{Cw}$	:	The average concentration of the test item in test water for calculating BCF (μg/L)
Cw(n)	:	Concentration of the test item in n-th analysis of test water (μg/L)

(2) Calculation of bioconcentration factor of the test item and carboxylic acid

$$BCF = \frac{Cf}{\overline{Cw}}$$

where

BCF	:	Bioconcentration factor of the test item or carboxylic acid
Cf	:	Concentration of the test item or carboxylic acid in test fish (ng/g)
$\overline{Cw}$	:	The average concentration of the test item in test water for calculating BCF (μg/L)

4.7.9 Calculable BCF

On the basis of the minimum determination limit of the test item and carboxylic acid in Section 4.7.6 (4), 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.

(1) Test item	Level 1	3.3
	Level 2	36
(2) Carboxylic acid	Level 1	1.1
	Level 2	12

4.7.10 Calculation of lipid content

Lipid contents were calculated with the following equation.

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

where

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)

4.8 Treatment of numerical values

Values were rounded in accordance with JIS Z 8401:1999 rule B. The each value used for calculation was used without rounding on the way of the calculation.

The concentration values of the test item in test water, the concentration values of the test item and carboxylic acid in test fish were rounded to 3 figures. BCFs values were rounded to 2 figures.

5. Factors possibly affecting accuracy

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

6. Results

6.1 Concentration of the test item in test water

The measured concentrations of the test item in test water are shown in Table-1. Each concentration of the test item was maintained at more than 81 % of each nominated concentration. The variation of the concentrations of the test item was within ± 20 % of the average of the measured concentrations.

Table-1 Measured concentrations of the test item in test water

(Unit : μg/L)

Level	After 1 day	After 11 days	After 15 days	After 19 days	After 22 days	After 28 days	Average (Standard deviation)	Table	Fig.
1	8.94	9.30	9.10	9.43	9.24	8.65	9.11 (0.283)	5	6
2	0.823	0.846	0.842	0.821	0.811	0.870	0.835 (0.0214)	6	

6.2 Bioconcentration factors

BCFs are shown in Table-2 and Table-3.

BCFs in Table-2 plotted against the duration of exposure are shown in Figs. 1, 2 and Figs. 12, 13.

BCFs of the test item were following.

(1) Test item	Level 1	15 – 49
	Level 2	≦36
(2) Carboxylic acid	Level 1	≦ 1.1
	Level 2	≦12

Table-2 BCFs (test item)

( ) : average value

Level	After 11 days	After 15 days	After 19 days	After 22 days	After 28 days	Table	Fig.
1	42 48 (45)	33 32 (32)	27 29 (28)	17 15 (16)	44 49 (46)	8	9
2	≦36 ≦36	≦36 ≦36	≦36 ≦36	≦36 ≦36	≦36 ≦36	9	10

Table-3 BCFs (carboxylic acid)

( ) : average value

Level	After 11 days	After 15 days	After 19 days	After 22 days	After 28 days	Table	Fig.
1	≦1.1 ≦1.1	≦1.1 ≦1.1	≦1.1 ≦1.1	≦1.1 ≦1.1	≦1.1 ≦1.1	12	16
2	≦12 ≦12	≦12 ≦12	≦12 ≦12	≦12 ≦12	≦12 ≦12	13	17

6.3 BCFs at a steady-state (BCFss)

(1) Test item

Because all BCFs were less than 100 (see 6.2), it was evaluated that a steady-state was reached within 28th day. Therefore BCFss was the average of BCFs after 28days (Level 1 46, Level 2 ≦ 36).

(2) Carboxylic acid

Because all BCFs were less than 100 (see 6.2), it was evaluated that a steady-state was reached within 28th day. Therefore BCFss was the average of BCFs after 28days (Level 1 ≦1.1, Level 2 ≦12).

6.4 Lipid content in test fish

The measured lipid contents in the test fish are shown as follows.

Before initiation of experiment	2.95 %
After termination of experiment	2.26 %

6.5 Results of test fish observation

No abnormality in behavior or appearance was noted.

7. Remarks

Instruments, apparatus, special apparatus and reagents, etc. for the test

(1) Instruments for fish care

Micro quantitative pump for supplying stock solution :
Tokyo Rika Kikai Co., Ltd. type GMW

Instrument for measuring concentration of dissolved oxygen :
Iijima Electronics Co., Ltd. type F-102

pH meter Toa Electronics Ltd. type HM-14P

(2) Instruments, apparatus, special apparatus and reagents

Instruments and apparatus

Gas chromatograph-mass spectrometer :
see page 18

Liquid chromatograph-mass spectrometer :
see page 22

Electronic analytical balance :	Sartorius AG type LP4200S
	Shimadzu Corporation type AEX-200B
	Sartorius AG type BP301S
	Metler Toledo type PB602
Infrared spectrophotometer :	Shimadzu Corporation type FTIR-8200PC
Homogenizer (polytron) :	Kinematica type PT3000
	Kinematica type PT3100
Centrifuge :	Hitachi Koki Co., Ltd. type CR21G

Special apparatus

Sep-Pak Silica :	Nihon Waters K. K.
Sep-Pak Dry :	Nihon Waters K. K.
Mega Bond Elut C_8	Varian

Reagents

Acetonitrile (HPLC grade) :	Wako Pure Chemical Industries, Ltd.
Methanol (HPLC grade) :	Wako Pure Chemical Industries, Ltd.
Ethyl acetate(extra pure) :	Kanto Chemical Co., Inc.
Isopropyl alcohol (extra pure) :	NACALAI TESQUE, INC.
Di-*n*-butylammonium acetate (IPC grade) :	Tokyo Kasei Kogyo Co., Ltd.
HCO-20 :	Nikko Chemicals Co., Ltd.

(3) Instruments, apparatus and reagents for gravimetric analysis of lipid content in test fish

Instruments and apparatus

Electronic analytical balance :	Sartorius AG type BP301S
Rotary evaporator :	Tokyo Rika Kikai Co., Ltd. type N-1
Homogenizer (polytron) :	Kinematica type PT3000
	Kinematica type PT3100
Homogenizer (autocellmaster) :	Iuchiseieido Co., Ltd. type CM-200
Vacuum pump :	Sinku Kiko Co., Ltd. type DA-20D
	Sinku Kiko Co., Ltd. type DAH-20C
Vacuum desiccator :	Iuchiseieido Co., Ltd. type VL

Reagents

Purified water :	Takasugi Pharmaceutical Co., Ltd.
Methanol (extra pure) :	Wako Pure Chemical Industries, Ltd.
Chloroform (guaranteed reagent) :	Wako Pure Chemical Industries, Ltd.
Anhydrous sodium sulfate (extra pure) :	Katayama Chemical Industries Co., Ltd.

Table-4 Calculation table for recovery and blank test
(Analysis of test water, test item)

Study No. 43771

Sample description	A	B	C	D	E	F
Standard 50.0μg/L	2909					
Recovery a	2356	1	5	-	202	81.0 %
Recovery b	2412	1	5	-	207	82.9 %
						Average
						82.0 %
Standard 50.0μg/L	3061					
Blank a	n.d.	1	5	-	-	
Blank b	n.d.	1	5	-	-	

(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

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Table-5 Calculation table for analysis of test water
(Level 1, test item)

Study No. 43771

Sample description	A	I
Standard 50.0μg/L	2845	
Test water after 1 day	2085	8.94
Standard 50.0μg/L	3723	
Test water after 11 days	2838	9.30
Standard 50.0μg/L	2811	
Test water after 15 days	2096	9.10
Standard 50.0μg/L	3078	
Test water after 19 days	2379	9.43
Standard 50.0μg/L	2886	
Test water after 22 days	2185	9.24
Standard 50.0μg/L	2927	
Test water after 28 days	2074	8.65
Average concentration of test item in test water	9.11	(S.D. 0.283)

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 82.0%

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 (μg/L)

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

n : Number of test water analyses (n = 6)

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

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Table-6 Calculation table for analysis of test water
(Level 2, test item)

Study No. 43771

Sample description	A	I
Standard 50.0μg/L	2845	
Test water after 1 day	1918	0.823
Standard 50.0μg/L	3723	
Test water after 11 days	2580	0.846
Standard 50.0μg/L	2811	
Test water after 15 days	1940	0.842
Standard 50.0μg/L	3078	
Test water after 19 days	2071	0.821
Standard 50.0μg/L	2886	
Test water after 22 days	1918	0.811
Standard 50.0μg/L	2927	
Test water after 28 days	2086	0.870
Average concentration of test item in test water	0.835	(S.D. 0.0214)

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 82.0%

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 (μg/L)

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

n : Number of test water analyses (n = 6)

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

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Table-7 Calculation table for recovery and blank test
(Analysis of test fish, test item)

Study No. 43771

Sample description	A	C	D	E	F	G
Standard 50.0μg/L	2895					
Recovery a	2148	2.5/25	5	-	3710	74.2 %
Recovery b	2156	2.5/25	5	-	3720	74.5 %
						Average
						74.3 %
Standard 50.0μg/L	2853					
Blank a	n.d.	2.5/25	5	-	-	
Blank b	n.d.	2.5/25	5	-	-	

(a, b : individual sample)

A : Peak area

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

B : Ratio of portion used for analysis (fish homogenate) 5/10

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

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Table-8 Calculation table for analysis of test fish
(Level 1,test item)

Study No. 43771

Sample description	A	G	K	H	J
Standard 50.0μg/L	2922				
Test fish after 11 days a	1678	5.00	386	9.12	42
Test fish after 11 days b	1884	5.00	434	9.12	48
Standard 50.0μg/L	2844				
Test fish after 15 days a	760	3.00	299	9.11	33
Test fish after 15 days b	733	3.00	289	9.11	32
Standard 50.0μg/L	2898				
Test fish after 19 days a	1079	5.00	250	9.28	27
Test fish after 19 days b	1168	5.00	271	9.28	29
Standard 50.0μg/L	2986				
Test fish after 22 days a	685	5.00	154	9.26	17
Test fish after 22 days b	607	5.00	137	9.26	15
Standard 50.0μg/L	3119				
Test fish after 28 days a	1860	5.00	401	9.11	44
Test fish after 28 days b	2049	5.00	442	9.11	49

(a, b : individual sample)

A : Peak area
A(std) : Standard solution A(t) : Sample
B : Ratio of portion used for analysis 2.5/25
C : Final volume 5mL
D : Dilution factor 1
E : Average concentration of blank in analysis of control 0ng/g
F : Recovery rate 74.3%
G : Weight of fine sample (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)
$H = \{ I(n-2) + I(n-1) + I(n) \} / m$; n : Number of test water analyses ; m = 2 when n = 2, m = 3 when n ≧3
I : Concentration of test item in test water (μg/L)
J : BCF
$J = K / H$
P : Concentration of test item in standard solution 50.0μg/L
See Fig. 9

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Table-9 Calculation table for analysis of test fish
(Level 2, test item)

Study No. 43771

Sample description	A	G	K	H	J
Standard 50.0μg/L	3025				
Test fish after 11 days a	n.d.	5.00	-	0.834	-
Test fish after 11 days b	n.d.	5.00	-	0.834	-
Standard 50.0μg/L	3005				
Test fish after 15 days a	n.d.	3.00	-	0.837	-
Test fish after 15 days b	n.d.	3.00	-	0.837	-
Standard 50.0μg/L	2808				
Test fish after 19 days a	n.d.	5.00	-	0.836	-
Test fish after 19 days b	n.d.	5.00	-	0.836	-
Standard 50.0μg/L	3065				
Test fish after 22 days a	n.d.	5.00	-	0.825	-
Test fish after 22 days b	n.d.	5.00	-	0.825	-
Standard 50.0μg/L	2927				
Test fish after 28 days a	n.d.	5.00	-	0.834	-
Test fish after 28 days b	n.d.	5.00	-	0.834	-

(a, b : individual sample)

A : Peak area
A(std) : Standard solution A(t) : Sample
B : Ratio of portion used for analysis 2.5/25
C : Final volume 5mL
D : Dilution factor 1
E : Average concentration of blank in analysis of control 0ng/g
F : Recovery rate 74.3%
G : Weight of fine sample (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)
$H = \{ I(n-2) + I(n-1) + I(n) \} / m$; n : Number of test water analyses ; m = 2 when n = 2, m = 3 when n ≧3
I : Concentration of test item in test water (μg/L)
J : BCF
$J = K / H$
P : Concentration of test item in standard solution 50.0μg/L
See Fig. 10

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Table-10 Calculation table for analysis of test fish
(Control, test item)

Study No. 43771

Sample description	A	E	G	I
Standard 50.0μg/L	2598			
Before the experimental start a	n.d.	-	5.00	-
Before the experimental start b	n.d.	-	5.00	-
Standard 50.0μg/L	2867			
After the experimental completion a	n.d.	-	5.00	-
After the experimental completion b	n.d.	-	5.00	-

(a, b : individual sample)

A : Peak area

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

B : Ratio of portion used for analysis 2.5/25

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 fine sample (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

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Table-11 Calculation table for recovery and blank test
(Analysis of test fish, carboxylic acid)

Study No. 43771

Sample description	A	C	D	E	F	G
Standard 1.00μg/L	10021					
Recovery a	9455	1/25	20	-	944	94.4 %
Recovery b	9341	1/25	20	-	932	93.2 %
						Average
						93.8 %
Standard 1.00μg/L	10006					
Blank a	n.d.	1/25	20	-	-	
Blank b	n.d.	1/25	20	-	-	

(a, b : individual sample)

A : Peak area

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

B : Ratio of portion used for analysis (fish homogenate) 5/10

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

D : Final volume (mL)

E : Amount of blank in test fish (ng)

F : Amount of carboxylic acid 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 carboxylic acid in standard solution 1.00μg/L

Q : Amount of carboxylic acid added (1000ng)

See Fig. 15

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Table-12 Calculation table for analysis of test fish
(Level 1, carboxylic acid)

Study No. 43771

Sample description	A	G	K	H	J
Standard 1.00μg/L	10090				
Test fish after 11 days a	n.d.	5.00	-	9.12	-
Test fish after 11 days b	n.d.	5.00	-	9.12	-
Standard 1.00μg/L	10304				
Test fish after 15 days a	n.d.	3.00	-	9.11	-
Test fish after 15 days b	n.d.	3.00	-	9.11	-
Standard 1.00μg/L	10859				
Test fish after 19 days a	n.d.	5.00	-	9.28	-
Test fish after 19 days b	n.d.	5.00	-	9.28	-
Standard 1.00μg/L	10429				
Test fish after 22 days a	n.d.	5.00	-	9.26	-
Test fish after 22 days b	n.d.	5.00	-	9.26	-
Standard 1.00μg/L	10143				
Test fish after 28 days a	n.d.	5.00	-	9.11	-
Test fish after 28 days b	n.d.	5.00	-	9.11	-

(a, b : individual sample)

A : Peak area
A(std) : Standard solution A(t) : Sample
B : Ratio of portion used for analysis 1/25
C : Final volume 20mL
D : Dilution factor 1
E : Average concentration of blank in analysis of control 0ng/g
F : Recovery rate 93.8%
G : Weight of fine sample (g)
K : Concentration of carboxylic acid 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)
$H = \{ I(n-2) + I(n-1) + I(n) \} / m$; n : Number of test water analyses ; m = 2 when n = 2, m = 3 when n ≧3
I : Concentration of test item in test water (μg/L)
J : BCF
$J = K / H$
P : Concentration of carboxylic acid in standard solution 1.00μg/L
See Fig. 16

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Table-13 Calculation table for analysis of test fish
(Level 2, carboxylic acid)

Study No. 43771

Sample description	A	G	K	H	J
Standard 1.00μg/L	10196				
Test fish after 11 days a	n.d.	5.00	-	0.834	-
Test fish after 11 days b	n.d.	5.00	-	0.834	-
Standard 1.00μg/L	10317				
Test fish after 15 days a	n.d.	3.00	-	0.837	-
Test fish after 15 days b	n.d.	3.00	-	0.837	-
Standard 1.00μg/L	10630				
Test fish after 19 days a	n.d.	5.00	-	0.836	-
Test fish after 19 days b	n.d.	5.00	-	0.836	-
Standard 1.00μg/L	10488				
Test fish after 22 days a	n.d.	5.00	-	0.825	-
Test fish after 22 days b	n.d.	5.00	-	0.825	-
Standard 1.00μg/L	10611				
Test fish after 28 days a	n.d.	5.00	-	0.834	-
Test fish after 28 days b	n.d.	5.00	-	0.834	-

(a, b : individual sample)

A : Peak area
A(std) : Standard solution A(t) : Sample
B : Ratio of portion used for analysis 1/25
C : Final volume 20mL
D : Dilution factor 1
E : Average concentration of blank in analysis of control 0ng/g
F : Recovery rate 93.8%
G : Weight of fine sample (g)
K : Concentration of carboxylic acid 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)
$H = \{ I(n-2) + I(n-1) + I(n) \} / m$; n : Number of test water analyses ; m = 2 when n = 2, m = 3 when n ≧3
I : Concentration of test item in test water (μg/L)
J : BCF
$J = K / H$
P : Concentration of carboxylic acid in standard solution 1.00μg/L
See Fig. 17

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Table-14 Calculation table for analysis of test fish
(Control, carboxylic acid)

Study No. 43771

Sample description	A	E	G	I
Standard 1.00μg/L	11762			
Before the experimental start a	n.d.	-	5.00	-
Before the experimental start b	n.d.	-	5.00	-
Standard 1.00μg/L	10113			
After the experimental completion a	n.d.	-	5.00	-
After the experimental completion b	n.d.	-	5.00	-

(a, b : individual sample)

A : Peak area

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

B : Ratio of portion used for analysis 1/25

C : Final volume 20mL

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

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

G : Weight of fine sample (g)

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

$I = E / G$

P : Concentration of carboxylic acid in standard solution 1.00μg/L

See Fig. 18

January 29, 2002 Name S. Yoshi

Reference 1 Analytical results of dilution water

Sampling date September 3, 2001

Item	Unit	Measured value	Standard value	Detection limit
Total hardness (Ca, Mg)	mg/L	58.6	< 300 *1	
Suspended solid	mg/L	< 1	< 20 *2	1
pH	—	8.3	6.5 ~ 8.5 *3	
Total organic carbon	mg/L	1.6	< 2 *2	0.1
Chemical oxygen demand	mg/L	0.1	< 5 *3	0.1
Residual chlorine	mg/L	< 0.01	< 0.02 *3	0.01
Ammonia nitrogen	mg/L	0.25	< 1 *3	0.01
Total cyan	mg/L	< 0.01	n.d. *3	0.01
Alkalinity	mg/L	198	—	
Electric conductivity	μs/cm	521	—	
Organic phosphorus	mg/L	< 0.1	n.d. *3	0.1
Alkylmercury	mg/L	< 0.0005	n.d. *3	0.0005
Mercury	mg/L	< 0.0005	< 0.0005 *3	0.0005
Cadmium	mg/L	< 0.001	< 0.01 *3	0.001
Cr^{6+}	mg/L	< 0.02	< 0.05 *3	0.02
Lead	mg/L	< 0.005	< 0.1 *3	0.005
Arsenic	mg/L	< 0.002	< 0.05 *3	0.002
Iron	mg/L	0.02	< 1.0 *3	0.01
Copper	mg/L	< 0.005	< 0.005 *3	0.005
Cobalt	mg/L	< 0.001	< 0.001 *5	0.001
Manganese	mg/L	< 0.01	< 0.05 *1	0.01
Zinc	mg/L	< 0.01	< 1.0 *1	0.01
Aluminium	mg/L	0.014	< 0.2 *1	0.001
Nickel	mg/L	< 0.001	< 0.01 *1	0.001
Silver	mg/L	< 0.0001	< 0.0001 *5	0.0001
Organochlorine pesticides				
1,2-Dichloropropane	mg/L	< 0.0001	< 0.06 *4	0.0001
Chlorothalonil	mg/L	< 0.0002	< 0.04 *4	0.0002
Propyzamide	mg/L	< 0.0002	< 0.008 *4	0.0002
Chlornitrofen	mg/L	< 0.0001	< 0.0001 *1	0.0001
Simazine	mg/L	< 0.0003	< 0.003 *4	0.0003
Thiobencarb	mg/L	< 0.001	< 0.02 *4	0.001
Organophosphorous pesticides				
Diazinon	mg/L	< 0.0002	< 0.005 *4	0.0002
Isoxathion	mg/L	< 0.0002	< 0.008 *4	0.0002
Fenitrothion	mg/L	< 0.0002	< 0.003 *4	0.0002
EPN	mg/L	< 0.0002	< 0.006 *4	0.0002
Dichlorvos	mg/L	< 0.0002	< 0.01 *4	0.0002
Iprobenfos	mg/L	< 0.0002	< 0.008 *4	0.0002
PCB	mg/L	< 0.0005	n.d. *4	0.0005

*1 Ministerial ordinance of the Ministry of Health and Welfare No.69 (Revised December 21, 1992)

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

*3 Water quality criteria for fisheries (Shadanhozin Nihon Suisansigen Hogokyokai, March 1983)

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

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

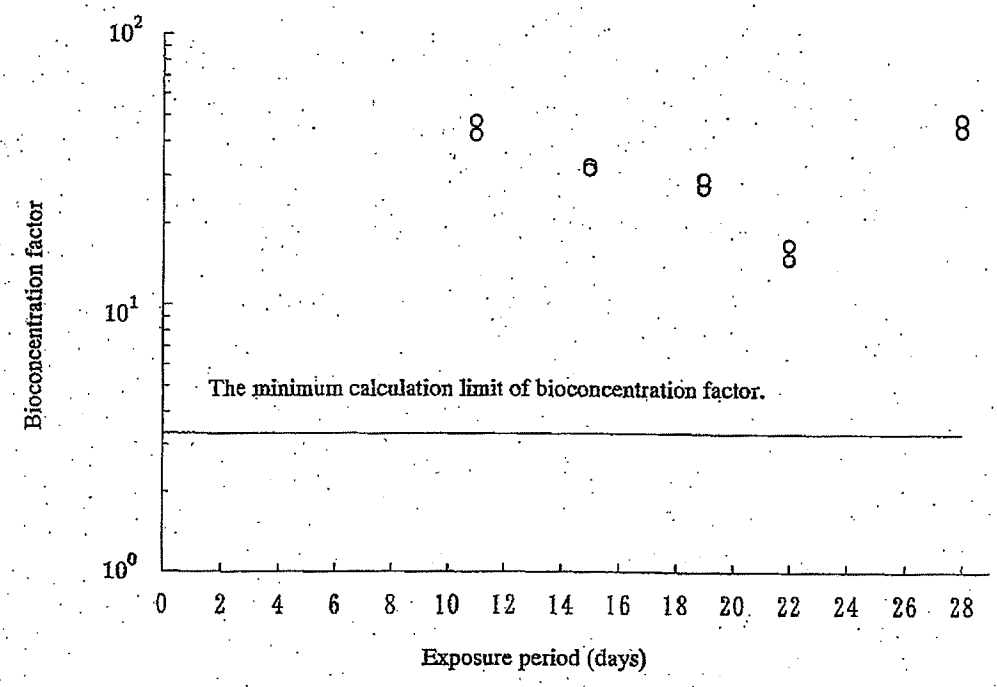


Fig. 1 Correlation between exposure period and bioconcentration factor (Level 1).

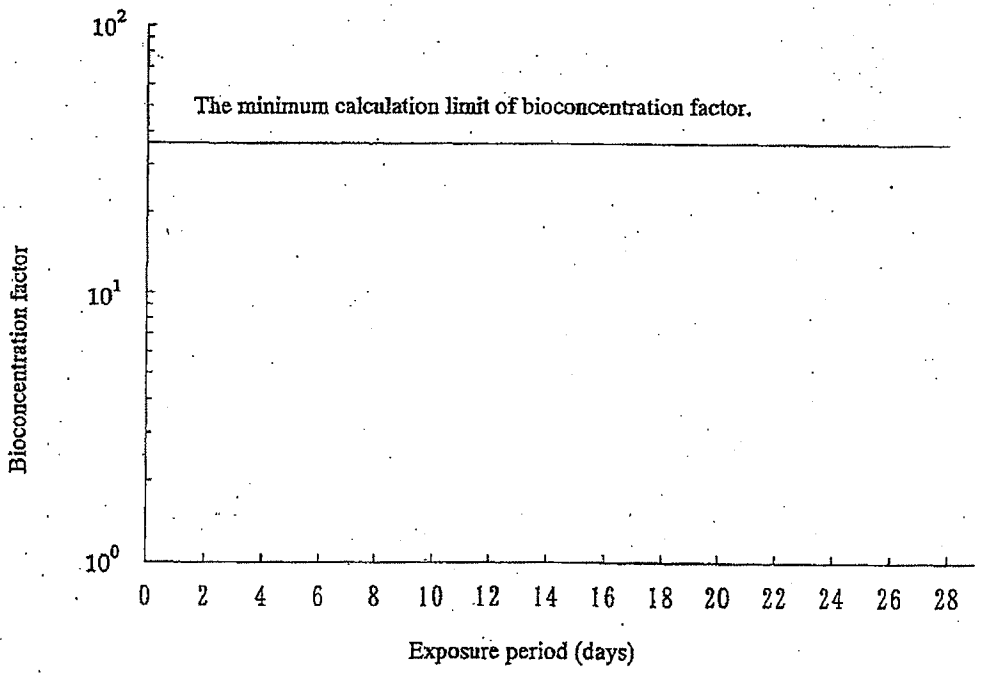
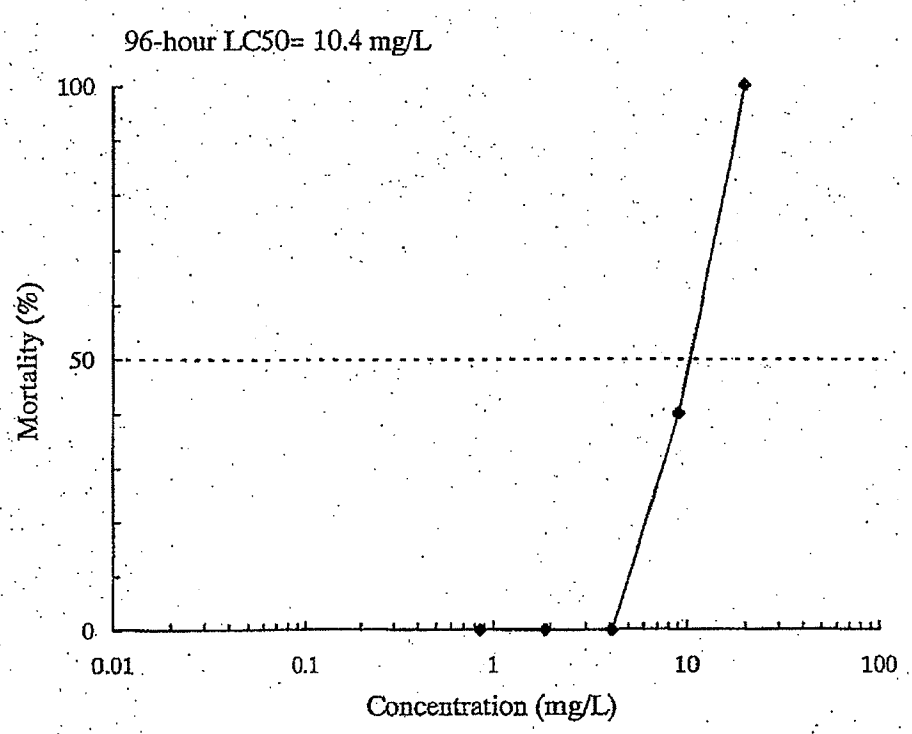


Fig. 2 Correlation between exposure period and bioconcentration factor (Level 2).
Ten data after 11, 15, 19, 22 and 28 days were lower than detection limit.

January 31, 2002 Name L. Goshi



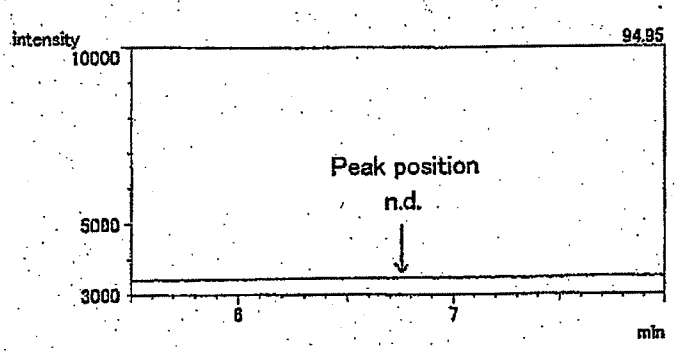
Concentration (mg/L)	Cumulative Mortality (%)			
	24 hours	48 hours	72 hours	96 hours
Control	0	0	0	0
0.854	0	0	0	0
1.88	0	0	0	0
4.13	0	0	0	0
9.09	0	0	20	40
20.0	20	80	100	100

Fig. 3 Concentration - Mortality Curve.

Date : December 21, 2001 Name Tadayoshi Tonai

Solvent (w)
Date : 01/11/17
File name : C:¥GCMSsolution¥Data¥試験第2課¥43771¥43771a78.qgd

Study No. 43771

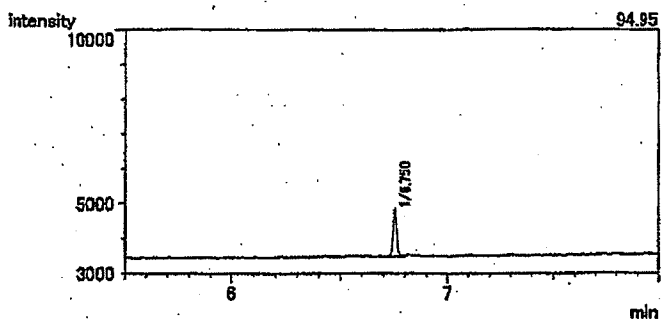


Peak No.	Time(min)	m/z	Area
1	–	94.95	–

Nov. 19, 2001 Name: K. Yuka

Standard solution 25.0ug/L (w)
Date : 01/11/17
File name : C:¥GCMSsolution¥Data¥試験第2課¥43771¥43771a79.qgd

Study No. 43771



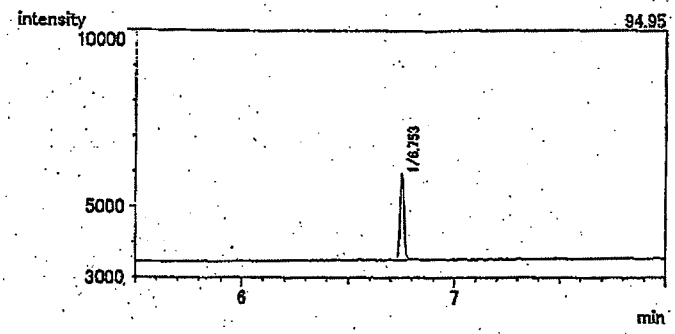
Peak No.	Time(min)	m/z	Area
1	6.75	94.95	1607

Nov. 19, 2001 Name: K. Yuka

Fig. 4-1 (1/2) Mass fragmentogram of GC/MS analysis for calibration curve (test water, test item)

Standard solution 50.0ug/L (w)
Date : 01/11/17
File name : C:¥GCMSsolution¥Data¥試験第2課¥43771¥43771a80.qgd

Study No. 43771

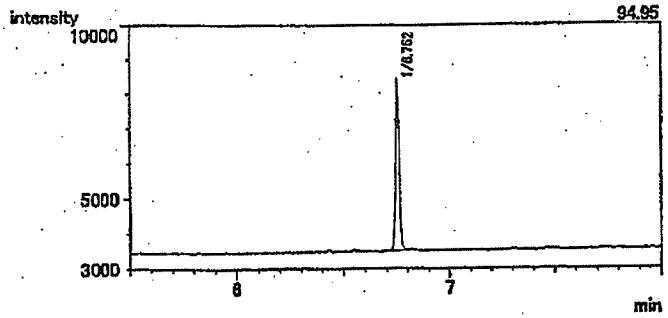


Peak No.	Time(min)	m/z	Area
1	6.75	94.95	3181

Nov. 19, 2001 Name: K. Yuka

Standard solution 100ug/L (w)
Date : 01/11/17
File name : C:¥GCMSsolution¥Data¥試験第2課¥43771¥43771a81.qgd

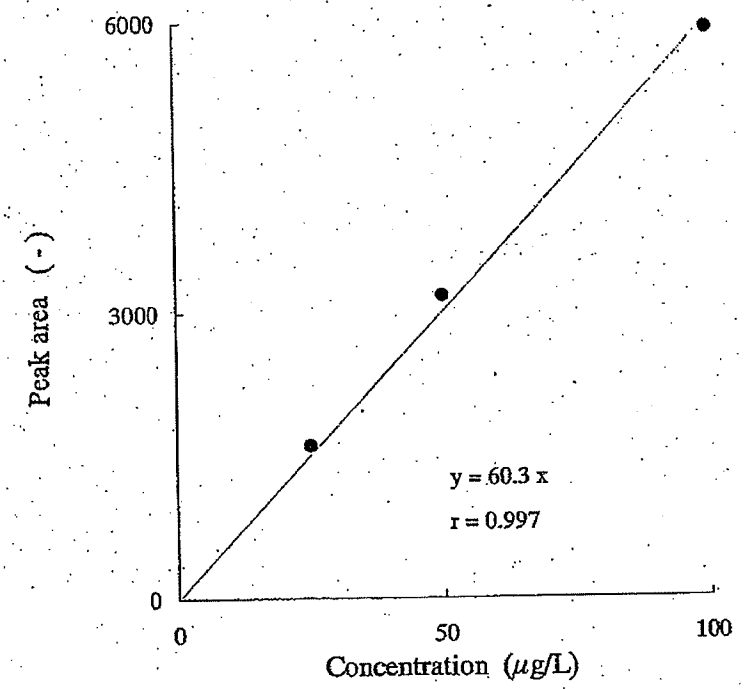
Study No. 43771



Peak No.	Time(min)	m/z	Area
1	6.75	94.95	5922

Nov. 19, 2001 Name: K. Yuka

Fig. 4-1 (2/2) Mass fragmentogram of GC/MS analysis for calibration curve (test water, test item)



Concentration (μg/L)	Peak area (-)
25.0	1607
50.0	3181
100	5922

Conditions of GC-MS analysis

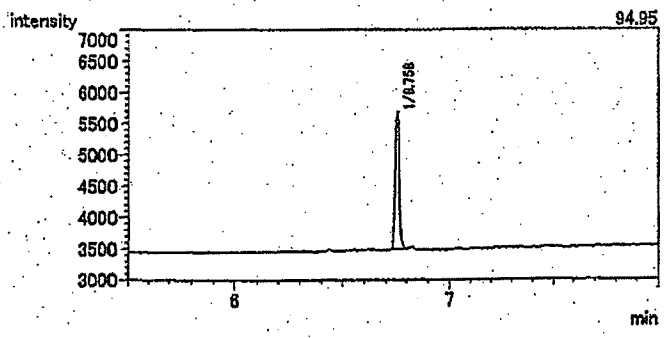
Instrument	:	Shimadzu QP5000
Sample	:	C_{6-2}アルコール
Solvent	:	Ethyl acetate
GC Conditions		
Injection vol.	:	3μL
Column (Size)	:	INNOWAX (30m × 0.25mm I.D.) Film thickness 0.25μm
Col. temp.	:	35℃(2min.) →①→ 150℃(2min.) →②→ 200℃(2min.)
Rate	:	①20℃/min., ②35℃/min.
Injection Temp.	:	200℃
Carrier Gas	:	He (Pressure 50kPa, Total flow rate 10mL/min.)
Inlet mode	:	Splitless
MS Conditions		
Ionization mode	:	EI
Interface temp.	:	250℃
Ionization vol.	:	70eV
Monitoring ion	:	m/z = 94.95

Fig. 4 - 2 Calibration curve and conditions of GC/MS analysis for C_{6-2}アルコール (test water).

January 29, 2002 Name I. Yoshi

Standard solution 50.0ug/L (w)
Date : 01/11/17
File name : C:¥GCMSsolution¥Data¥試験第2課¥43771¥43771a72.qgd

Study No. 43771

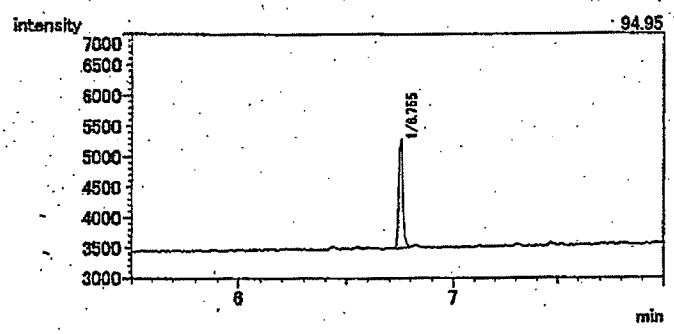


Peak No.	Time(min)	m/z	Area
1	6.76	94.95	2909

Nov. 19, 2001 Name: K. Yuka

Recovery test from test water a
Date : 01/11/17
File name : C:¥GCMSsolution¥Data¥試験第2課¥43771¥43771a73.qgd

Study No. 43771

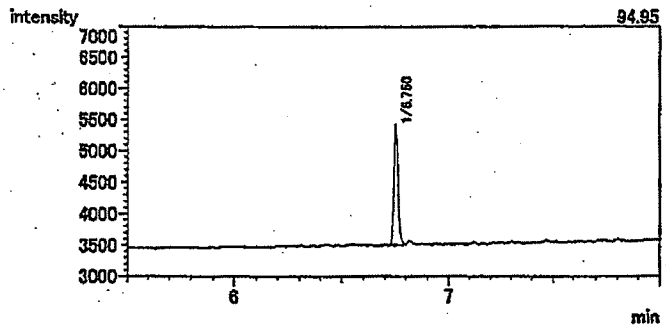


Peak No.	Time(min)	m/z	Area
1	6.76	94.95	2356

Nov. 19, 2001 Name: K. Yuka

Recovery test from test water b
Date : 01/11/17
File name : C:¥GCMSsolution¥Data¥試験第2課¥43771¥43771a74.qgd

Study No. 43771



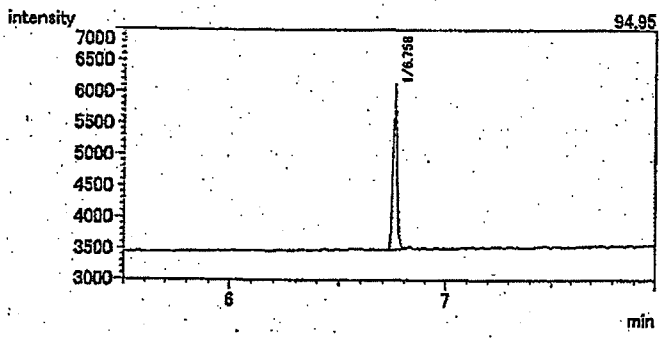
Peak No.	Time(min)	m/z	Area
1	6.75	94.95	2412

Nov. 19, 2001 Name: K. Yuka

Fig. 5-1 Mass fragmentogram of GC/MS analysis for recovery and blank test (analysis of test water, test item)

Standard solution 50.0ug/L (w)
Date : 01/11/17
File name : C:¥GCMSsolution¥Data¥試験第2課¥43771¥43771a75.qgd

Study No. 43771

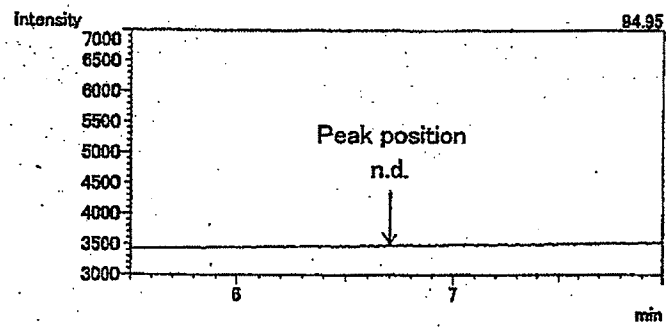


Peak No.	Time(min)	m/z	Area
1	6.76	94.95	3061

Nov. 19, 2001 Name: K. Yuka

Blank test of test water a
Date : 01/11/17
File name : C:¥GCMSsolution¥Data¥試験第2課¥43771¥43771a76.qgd

Study No. 43771

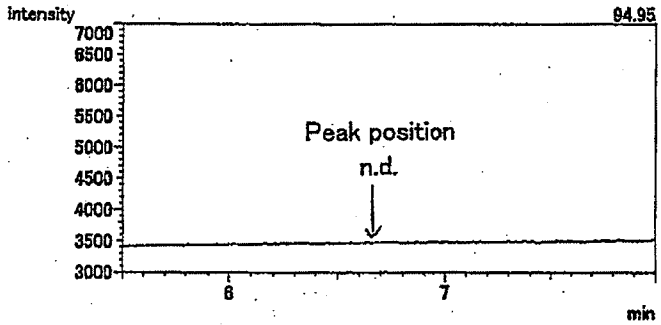


Peak No.	Time(min)	m/z	Area
1	-	94.95	-

Nov. 19, 2001 Name: K. Yuka

Blank test of test water b
Date : 01/11/17
File name : C:¥GCMSsolution¥Data¥試験第2課¥43771¥43771a77.qgd

Study No. 43771



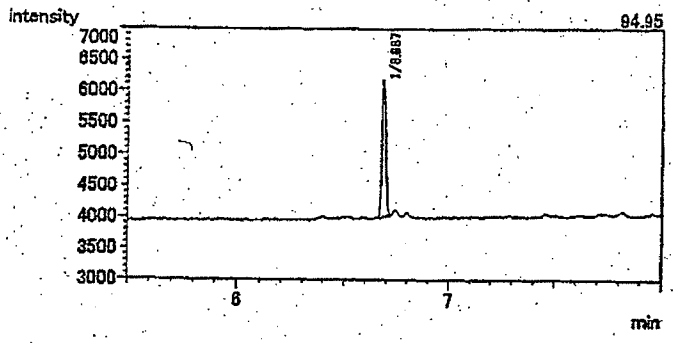
Peak No.	Time(min)	m/z	Area
1	-	94.95	-

Nov. 19, 2001 Name: K. Yuka

Fig. 5-2 Mass fragmentogram of GC/MS analysis for recovery and blank test (analysis of test water, test item)

Standard solution 50.0 μg/L (W)
Date : 01/12/28
File name : C:¥GCMSsolution¥Data¥試験第2課¥43771¥43771w4.qgd

Study No. 43771

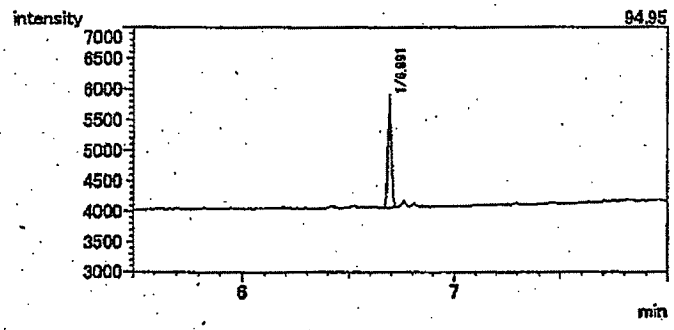


Peak No.	Time(min)	m/z	Area
1	6.68	94.95	2845

Dec. 28, 2001 Name: d. goshi

Test water after 1 day (Level1)
Date : 01/12/28
File name : C:¥GCMSsolution¥Data¥試験第2課¥43771¥43771w5.qgd

Study No. 43771

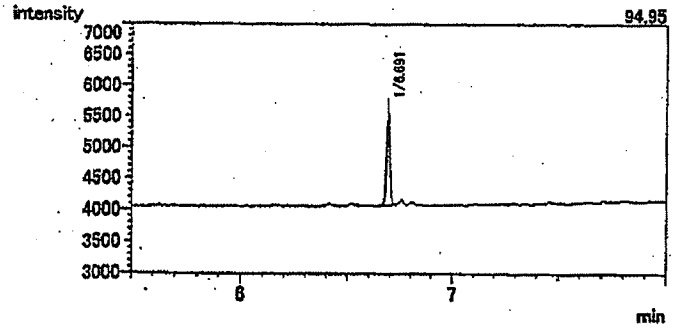


Peak No.	Time(min)	m/z	Area
1	6.69	94.95	2085

Dec. 28, 2001 Name: d. goshi

Test water after 1 day (Level2)
Date : 01/12/28
File name : C:¥GCMSsolution¥Data¥試験第2課¥43771¥43771w6.qgd

Study No. 43771



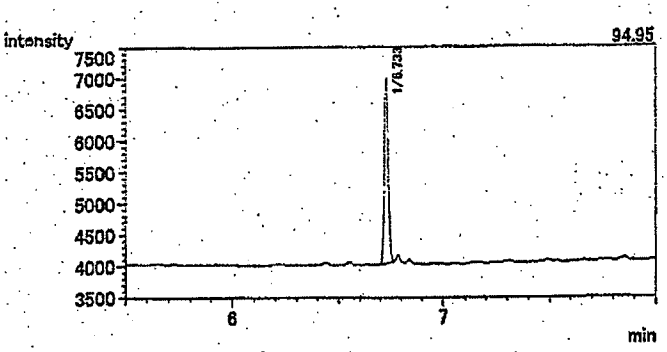
Peak No.	Time(min)	m/z	Area
1	6.69	94.95	1918

Dec. 28, 2001 Name: d. goshi

Fig. 6-1 Mass fragmentogram of GC/MS analysis for test water (test item)

Standard solution 50.0ug/L (W)
Date : 02/01/07
File name : C:¥GCMSsolution¥Data¥試験第2課¥43771¥43771w10.qgd

Study No. 43771

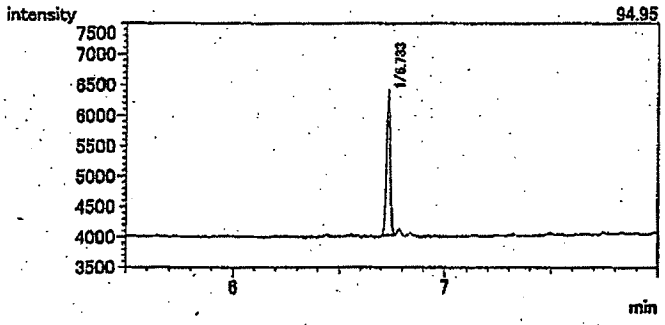


Peak No.	Time(min)	m/z	Area
1	6.73	94.95	3723

Jan. 07, 2002 Name: l. goshi

Test water after 11days (Level1)
Date : 02/01/07
File name : C:¥GCMSsolution¥Data¥試験第2課¥43771¥43771w8.qgd

Study No. 43771



Peak No.	Time(min)	m/z	Area
1	6.73	94.95	2838

Jan. 07, 2002 Name: l. goshi

Test water after 11days (Level2)
Date : 02/01/07
File name : C:¥GCMSsolution¥Data¥試験第2課¥43771¥43771w9.qgd

Study No. 43771

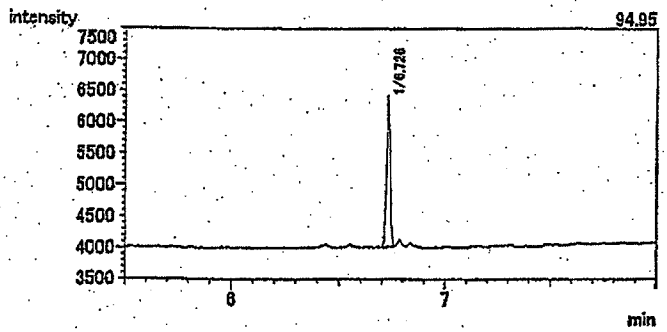
Peak No.	Time(min)	m/z	Area
1	6.73	94.95	2580

Jan. 07, 2002 Name: l. goshi

Fig. 6-2 Mass fragmentogram of GC/MS analysis for test water (test item)

Standard solution 50.0ug/L (W)
Date : 02/01/11
File name : C:¥GCMSsolution¥Data¥試験第2課¥43771¥43771w25.qgd

Study No. 43771

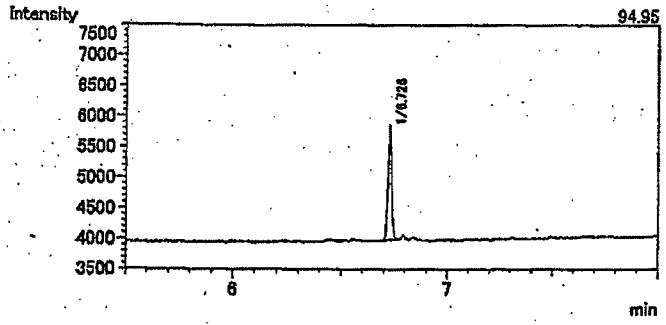


Peak No.	Time(min)	m/z	Area
1	6.73	94.95	2811

Jan. 11, 2002 Name: S. Goshi

Test water after 15 days (Level1)
Date : 02/01/11
File name : C:¥GCMSsolution¥Data¥試験第2課¥43771¥43771w26.qgd

Study No. 43771

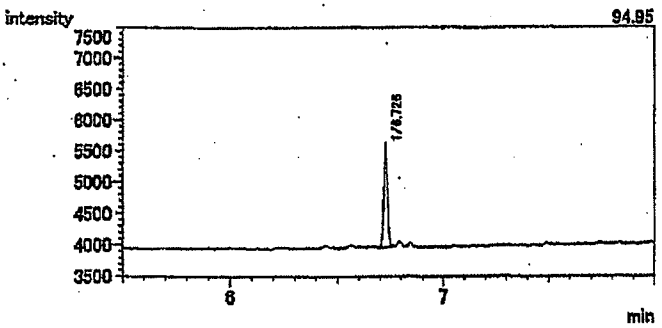


Peak No.	Time(min)	m/z	Area
1	6.73	94.95	2096

Jan. 11, 2002 Name: S. Goshi

Test water after 15 days (Level2)
Date : 02/01/11
File name : C:¥GCMSsolution¥Data¥試験第2課¥43771¥43771w27.qgd

Study No. 43771



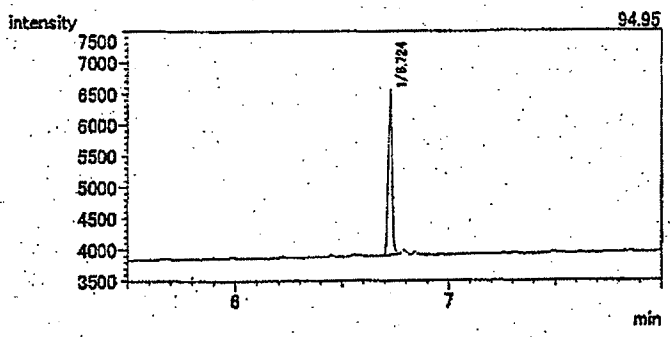
Peak No.	Time(min)	m/z	Area
1	6.73	94.95	1940

Jan. 11, 2002 Name: S. Goshi

Fig. 6-3 Mass fragmentogram of GC/MS analysis for test water (test item)

Standard solution 50.0ug/L (W)
Date : 02/01/15
File name : C:¥GCMSsolution¥Data¥試験第2課¥43771¥43771w31.qgd

Study No. 43771

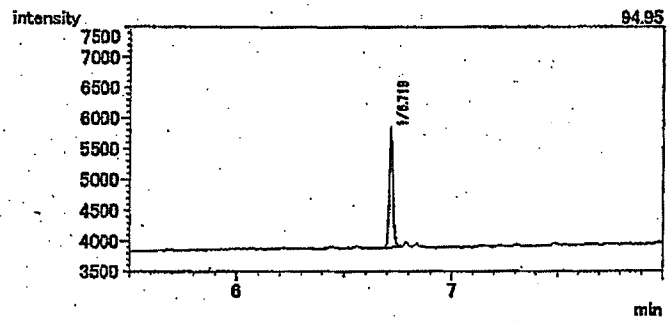


Peak No.	Time(min)	m/z	Area
1	6.73	94.95	3078

Jan. 15, 2002 Name: I. Goshi

Test water after 19 days (Level1)
Date : 02/01/15
File name : C:¥GCMSsolution¥Data¥試験第2課¥43771¥43771w29.qgd

Study No. 43771

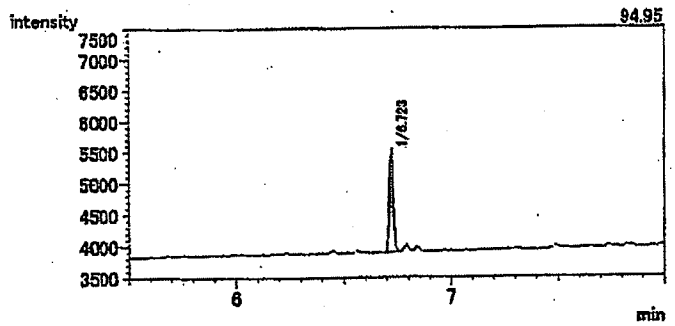


Peak No.	Time(min)	m/z	Area
1	6.72	94.95	2379

Jan. 15, 2002 Name: I. Goshi

Test water after 19 days (Level2)
Date : 02/01/15
File name : C:¥GCMSsolution¥Data¥試験第2課¥43771¥43771w30.qgd

Study No. 43771



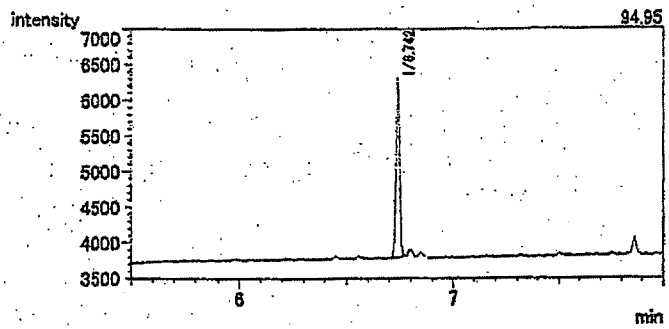
Peak No.	Time(min)	m/z	Area
1	6.73	94.95	2071

Jan. 15, 2002 Name: I. Goshi

Fig. 6-4 Mass fragmentogram of GC/MS analysis for test water (test item)

Standard solution 50.0μg/L (W)
Date : 02/01/18
File name : C:¥GCMSsolution¥Data¥試験第2課¥43771¥43771w37.qgd

Study No. 43771



Peak No.	Time(min)	m/z	Area
1	6.74	94.95	2886

Jan. 18, 2002 Name: K. Yuka

Test water after 22 days (level1)
Date : 02/01/18
File name : C:¥GCMSsolution¥Data¥試験第2課¥43771¥43771w35.qgd

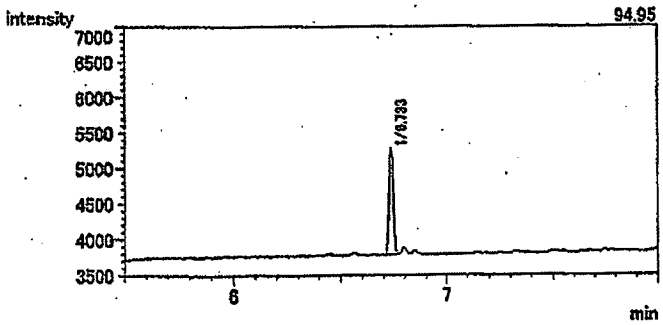
Study No. 43771

Peak No.	Time(min)	m/z	Area
1	6.74	94.95	2185

Jan. 18, 2002 Name: K. Yuka

Test water after 22 days (level2)
Date : 02/01/18
File name : C:¥GCMSsolution¥Data¥試験第2課¥43771¥43771w36.qgd

Study No. 43771



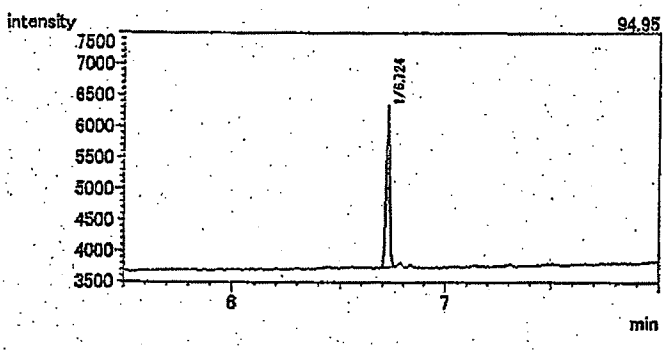
Peak No.	Time(min)	m/z	Area
1	6.73	94.95	1918

Jan. 18, 2002 Name: K. Yuka

Fig. 6-5 Mass fragmentogram of GC/MS analysis for test water (test item)

Standard solution 50.0ug/L (W)
Date : 02/01/24
File name : C:¥GCMSsolution¥Data¥試験第2課¥43771¥43771w38.qgd

Study No. 43771

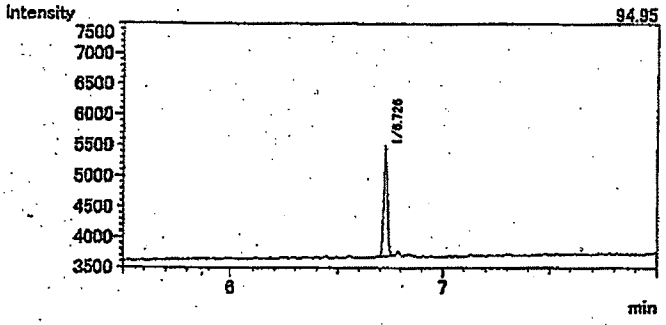


Peak No.	Time(min)	m/z	Area
1	6.73	94.95	2927

Jan. 24, 2002 Name: I. yoshi

Test water after 28 days (Level1)
Date : 02/01/24
File name : C:¥GCMSsolution¥Data¥試験第2課¥43771¥43771w39.qgd

Study No. 43771

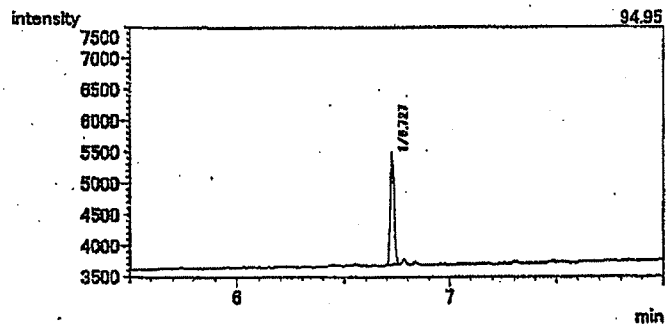


Peak No.	Time(min)	m/z	Area
1	6.73	94.95	2074

Jan. 24, 2002 Name: I. yoshi

Test water after 28 days (Level2)
Date : 02/01/24
File name : C:¥GCMSsolution¥Data¥試験第2課¥43771¥43771w40.qgd

Study No. 43771



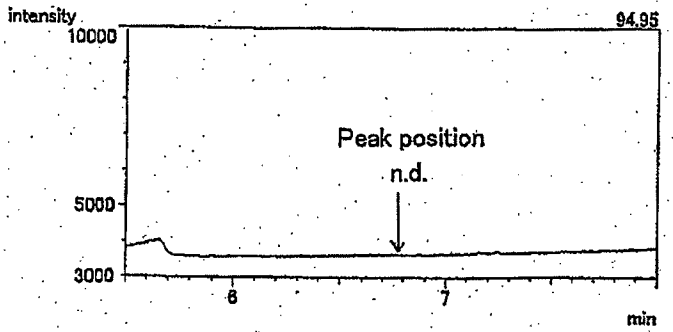
Peak No.	Time(min)	m/z	Area
1	6.73	94.95	2086

Jan. 24, 2002 Name: I. yoshi

Fig. 6-6 Mass fragmentogram of GC/MS analysis for test water (test item)

Solvent (F)
Date : 01/11/27
File name : C:¥GCMSsolution¥Data¥試験第2課¥43771¥43771b1.qgd

Study No. 43771

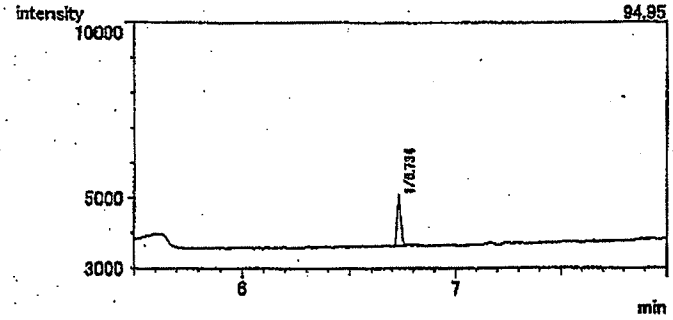


Peak No.	Time(min)	m/z	Area
1	–	94.95	–

Nov. 28, 2001 Name: d. Yoshi

Standard solution 25.0ug/L (F)
Date : 01/11/27
File name : C:¥GCMSsolution¥Data¥試験第2課¥43771¥43771b2.qgd

Study No. 43771



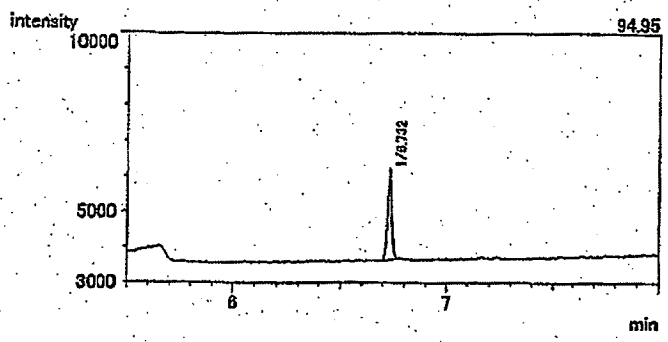
Peak No.	Time(min)	m/z	Area
1	6.73	94.95	1665

Nov. 28, 2001 Name: d. Yoshi

Fig. 7-1 (1/2) Mass fragmentogram of GC/MS analysis for calibration curve (test fish, test item)

Standard solution 50.0μg/L (F)
Date : 01/11/27
File name : C:¥GCMSsolution¥Data¥試験第2課¥43771¥43771b3.qgd

Study No. 43771

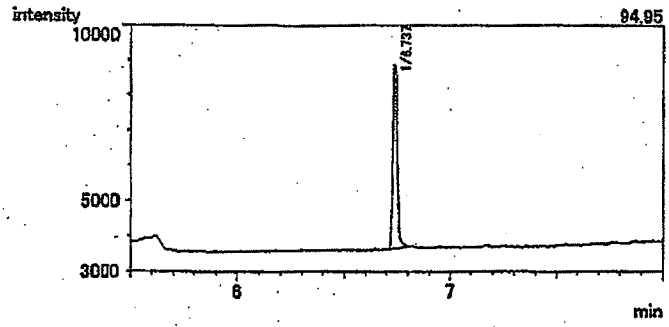


Peak No.	Time(min)	m/z	Area
1	6.73	94.95	3254

Nov. 28, 2001 Name: I. Goshi

Standard solution 100μg/L (F)
Date : 01/11/27
File name : C:¥GCMSsolution¥Data¥試験第2課¥43771¥43771b4.qgd

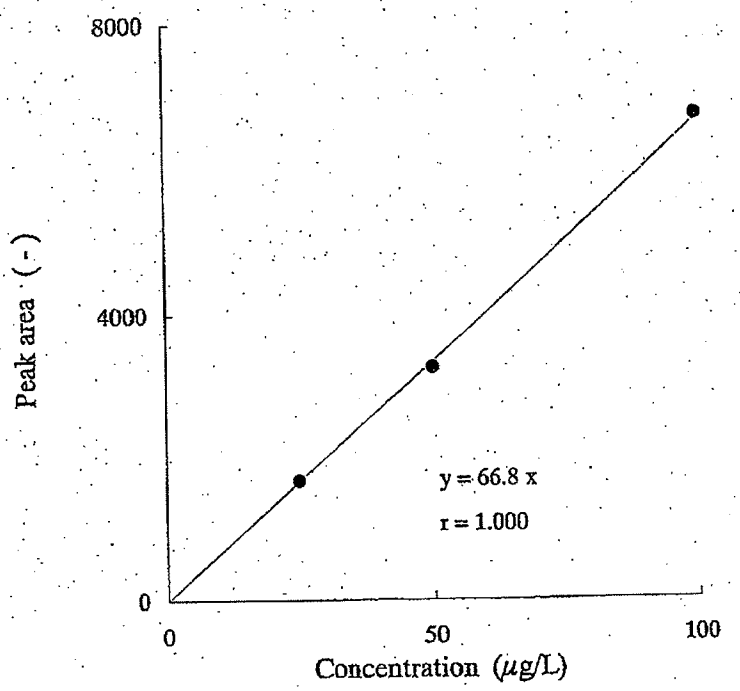
Study No. 43771



Peak No.	Time(min)	m/z	Area
1	6.73	94.95	6727

Nov. 28, 2001 Name: I. Goshi

Fig. 7-1 (2/2) Mass fragmentogram of GC/MS analysis for calibration curve
(test fish, test item)



Concentration (μg/L)	Peak area (-)
25.0	1665
50.0	3254
100	6727

Conditions of GC-MS analysis

Instrument	:	Shimadzu QP5000
Sample	:	$C_{6\text{-}2}$アルコール
Solvent	:	Acetonitrile/Isopropyl alcohol (7/3 V/V)
GC Conditions		
Injection vol.	:	3μL
Column (Size)	:	INNOWAX (30m × 0.25mm I.D.) Film thickness 0.25μm
Col. temp.	:	35℃(2min.) →①→ 150℃(2min.) →②→ 200℃(3min.)
Rate	:	①20℃/min., ②35℃/min.
Injection Temp.	:	200℃
Carrier Gas	:	He (Pressure 50kPa, Total flow rate 10mL/min.)
Inlet mode	:	Splitless
MS Conditions		
Ionization mode	:	EI
Interface temp.	:	250℃
Ionization vol.	:	70eV
Monitoring ion	:	m/z = 94.95

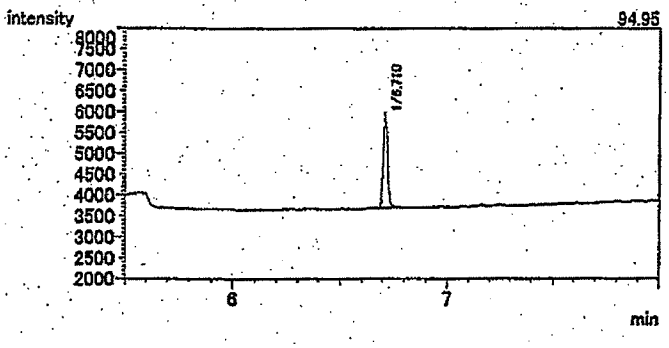
Fig. 7 - 2 Calibration curve and conditions of GC/MS analysis for $C_{6\text{-}2}$アルコール (test fish).

January 30, 2002

Name N. Goshi

Standard solution 50.0ug/L (F)
Date : 01/12/07
File name : C:¥GCMSsolution¥Data¥試験第2課¥43771¥43771c4.qgd

Study No. 43771

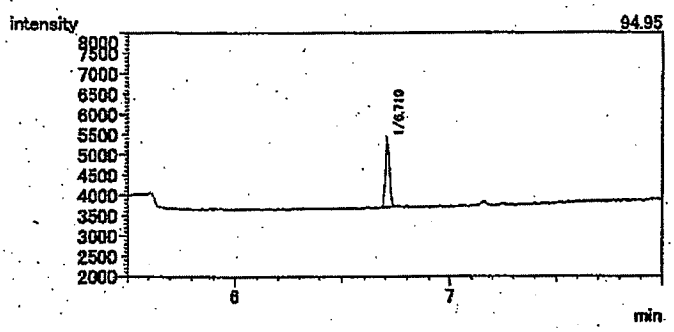


Peak No.	Time(min)	m/z	Area
1	6.71	94.95	2895

Dec. 7, 2001 Name: I. Yoshi

Recovery test from test fish a
Date : 01/12/07
File name : C:¥GCMSsolution¥Data¥試験第2課¥43771¥43771c5.qgd

Study No. 43771



Peak No.	Time(min)	m/z	Area
1	6.71	94.95	2148

Dec. 07, 2001 Name: I. Yoshi

Recovery test from test fish b
Date : 01/12/07
File name : C:¥GCMSsolution¥Data¥試験第2課¥43771¥43771c6.qgd

Study No. 43771

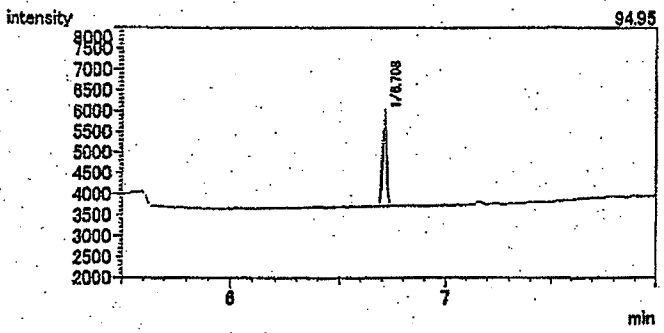
Peak No.	Time(min)	m/z	Area
1	6.71	94.95	2156

Dec. 07, 2001 Name: I. Yoshi

Fig. 8-1 Mass fragmentogram of GC/MS analysis for recovery and blank test (analysis of fish, test item)

Standard solution 50.0μg/L (F)
Date : 01/12/07
File name : C:¥GCMSsolution¥Data¥試験第2課¥43771¥43771c7.qgd

Study No. 43771

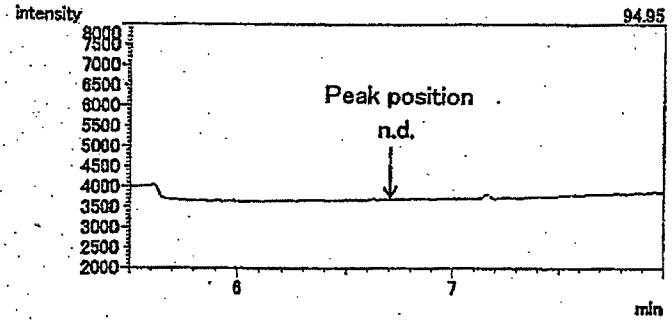


Peak No.	Time(min)	m/z	Area
1	6.71	94.95	2853

Dec. 07, 2001 Name: d. Goshi

Blank test of test fish a
Date : 01/12/07
File name : C:¥GCMSsolution¥Data¥試験第2課¥43771¥43771c8.qgd

Study No. 43771

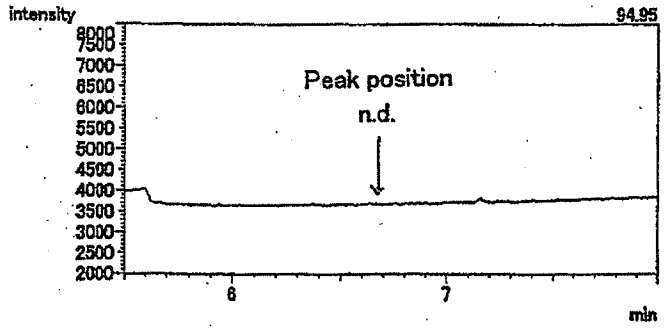


Peak No.	Time(min)	m/z	Area
1	–	94.95	–

Dec. 07, 2001 Name: d. Goshi

Blank test of test fish b
Date : 01/12/07
File name : C:¥GCMSsolution¥Data¥試験第2課¥43771¥43771c9.qgd

Study No. 43771



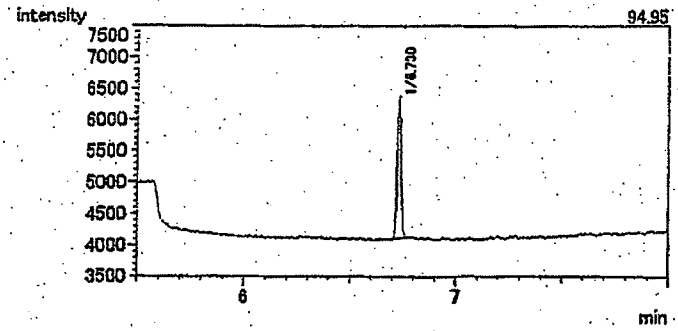
Peak No.	Time(min)	m/z	Area
1	–	94.95	–

Dec. 07, 2001 Name: d. Goshi

Fig. 8-2 Mass fragmentogram of GC/MS analysis for recovery and blank test (analysis of fish, test item)

Standard solution 50.0μg/L (F)
Date : 02/01/07
File name : C:¥GCMSsolution¥Data¥試験第2課¥43771¥43771z4.qgd

Study No. 43771

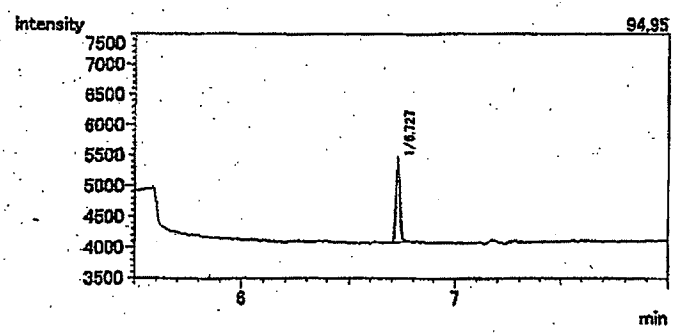


Peak No.	Time(min)	m/z	Area
1	6.73	94.95	2922

Jan. 07, 2002 Name: I. Goshi

Test fish after 11days (Level1-a)
Date : 02/01/07
File name : C:¥GCMSsolution¥Data¥試験第2課¥43771¥43771z5.qgd

Study No. 43771

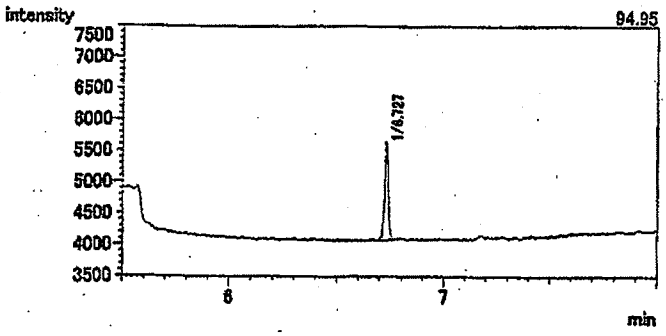


Peak No.	Time(min)	m/z	Area
1	6.73	94.95	1678

Jan. 07, 2002 Name: I. Goshi

Test fish after 11days (Level1-b)
Date : 02/01/07
File name : C:¥GCMSsolution¥Data¥試験第2課¥43771¥43771z6.qgd

Study No. 43771



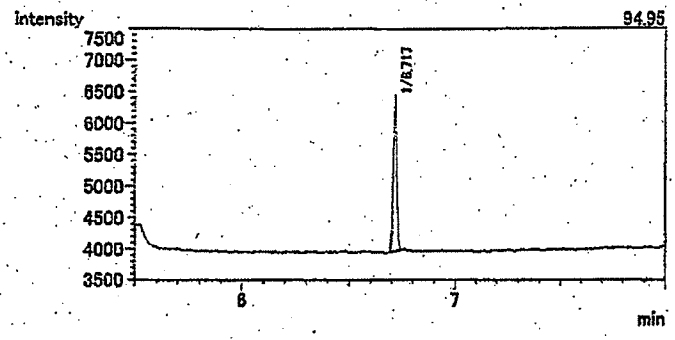
Peak No.	Time(min)	m/z	Area
1	6.73	94.95	1884

Jan. 07, 2002 Name: I. Goshi

Fig. 9-1 Mass fragmentogram of GC/MS analysis for test fish (Level 1, test item)

Standard solution 50.0ug/L (F)
Date : 02/01/11
File name : C:¥GCMSsolution¥Data¥試験第2課¥43771¥43771z18.qgd

Study No. 43771

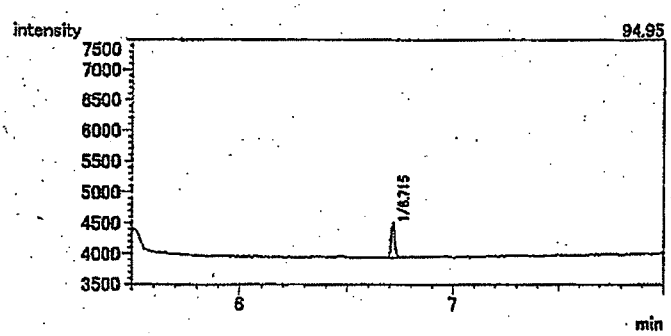


Peak No.	Time(min)	m/z	Area
1	6.72	94.95	2844

Jan. 11, 2002 Name: J. Goshi

Test fish after 15 days (Level 1-a)
Date : 02/01/11
File name : C:¥GCMSsolution¥Data¥試験第2課¥43771¥43771z19.qgd

Study No. 43771

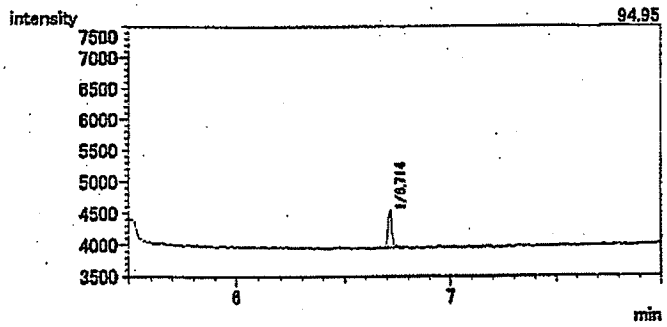


Peak No.	Time(min)	m/z	Area
1	6.72	94.95	760

Jan. 11, 2002 Name: J. Goshi

Test fish after 15 days (Level 1-b)
Date : 02/01/11
File name : C:¥GCMSsolution¥Data¥試験第2課¥43771¥43771z20.qgd

Study No. 43771



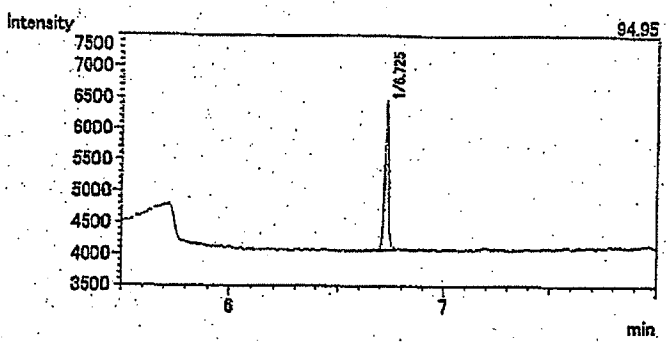
Peak No.	Time(min)	m/z	Area
1	6.72	94.95	733

Jan. 11, 2002 Name: J. Goshi

Fig. 9-2 Mass fragmentogram of GC/MS analysis for test fish (Level 1, test item)

Standard solution 50.0ug/L (F)
Date : 02/01/15
File name : C:¥GCMSsolution¥Data¥試験第2課¥43771¥43771z27.qgd

Study No. 43771

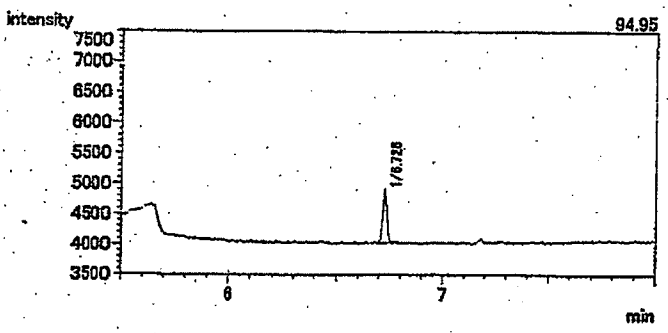


Peak No.	Time(min)	m/z	Area
1	6.73	94.95	2898

Jan. 15, 2002 Name: I. Goshi

Test fish after 19 days (Level1-a)
Date : 02/01/15
File name : C:¥GCMSsolution¥Data¥試験第2課¥43771¥43771z28.qgd

Study No. 43771

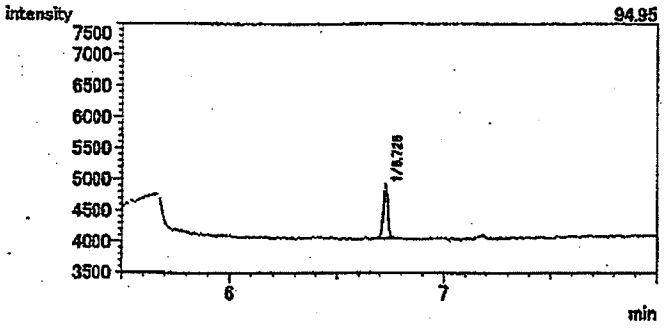


Peak No.	Time(min)	m/z	Area
1	6.73	94.95	1079

Jan. 15, 2002 Name: I. Goshi

Test fish after 19 days (Level1-b)
Date : 02/01/15
File name : C:¥GCMSsolution¥Data¥試験第2課¥43771¥43771z29.qgd

Study No. 43771



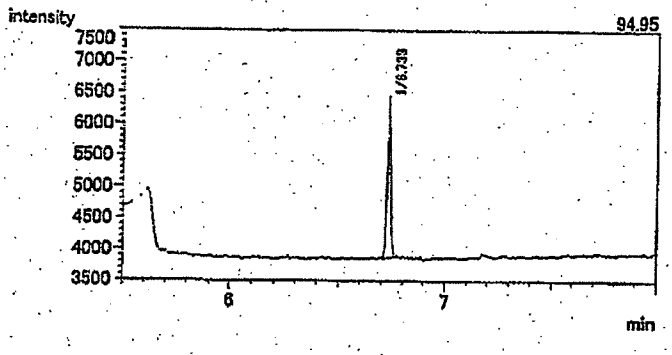
Peak No.	Time(min)	m/z	Area
1	6.73	94.95	1168

Jan. 15, 2002 Name: I. Goshi

Fig. 9-3 Mass fragmentogram of GC/MS analysis for test fish (Level 1, test item)

Standard solution 50.0ug/L (F)
Date : 02/01/18
File name : C:¥GCMSsolution¥Data¥試験第2課¥43771¥43771z33.qgd

Study No. 43771

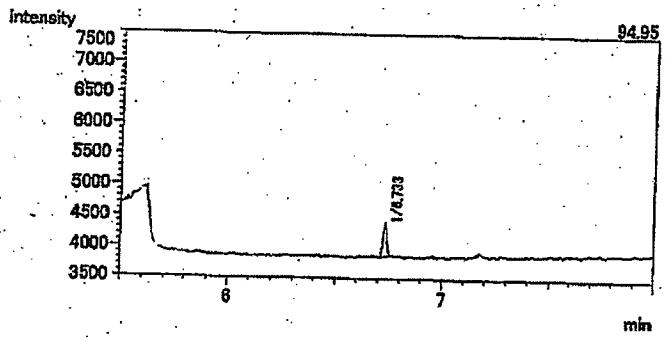


Peak No.	Time(min)	m/z	Area
1	6.73	94.95	2986

Jan. 18, 2002 Name: K. Yuka

Test fish after 22 days (Level 1-a)
Date : 02/01/18
File name : C:¥GCMSsolution¥Data¥試験第2課¥43771¥43771z34.qgd

Study No. 43771

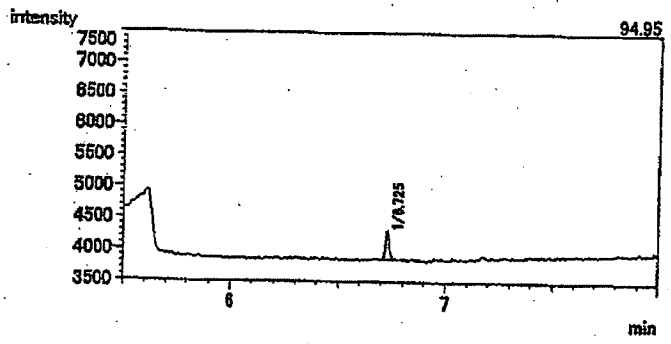


Peak No.	Time(min)	m/z	Area
1	6.73	94.95	685

Jan. 18, 2002 Name: K. Yuka

Test fish after 22 days (Level 1-b)
Date : 02/01/18
File name : C:¥GCMSsolution¥Data¥試験第2課¥43771¥43771z35.qgd

Study No. 43771



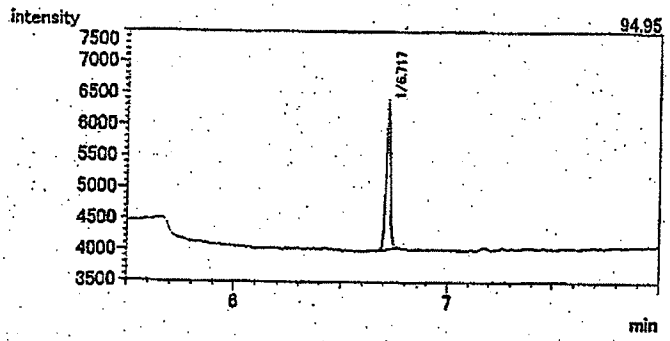
Peak No.	Time(min)	m/z	Area
1	6.73	94.95	607

Jan. 18, 2002 Name: K. Yuka

Fig. 9-4 Mass fragmentogram of GC/MS analysis for test fish (Level 1, test item)

Standard solution 50.0μg/L (F)
Date : 02/01/24
File name : C:¥GCMSsolution¥Data¥試験第2課¥43771¥43771z39.qgd

Study No. 43771

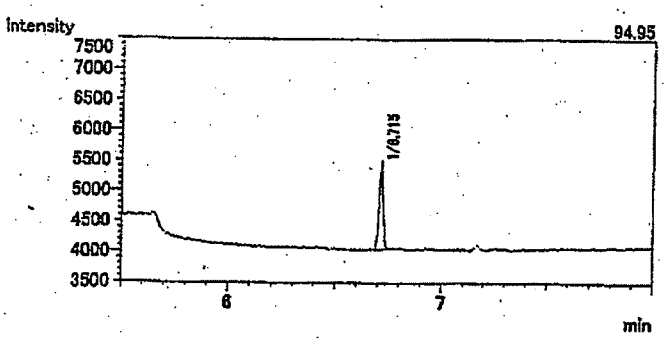


Peak No.	Time(min)	m/z	Area
1	6.72	94.95	3119

Jan. 24, 2002 Name: I. Goshi

Test fish after 28 days (Level1-a)
Date : 02/01/24
File name : C:¥GCMSsolution¥Data¥試験第2課¥43771¥43771z40.qgd

Study No. 43771

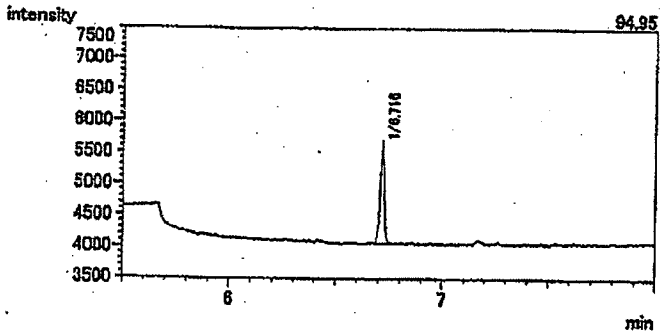


Peak No.	Time(min)	m/z	Area
1	6.72	94.95	1860

Jan. 24, 2002 Name: I. Goshi

Test fish after 28 days (Level1-b)
Date : 02/01/24
File name : C:¥GCMSsolution¥Data¥試験第2課¥43771¥43771z41.qgd

Study No. 43771



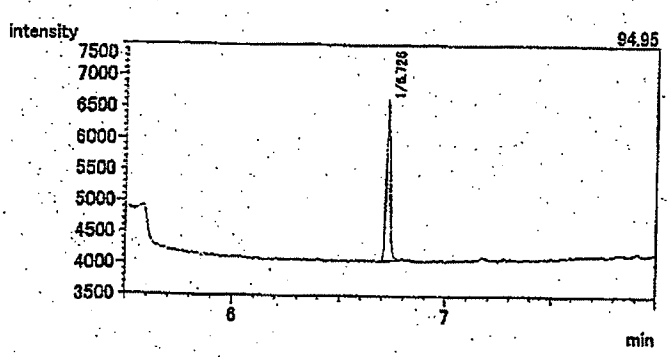
Peak No.	Time(min)	m/z	Area
1	6.72	94.95	2049

Jan. 24, 2002 Name: I. Goshi

Fig. 9-5 Mass fragmentogram of GC/MS analysis for test fish (Level 1, test item)

Standard solution 50.0ug/L (F)
Date : 02/01/07
File name : C:¥GCMSsolution¥Data¥試験第2課¥43771¥43771z7.qgd

Study No. 43771

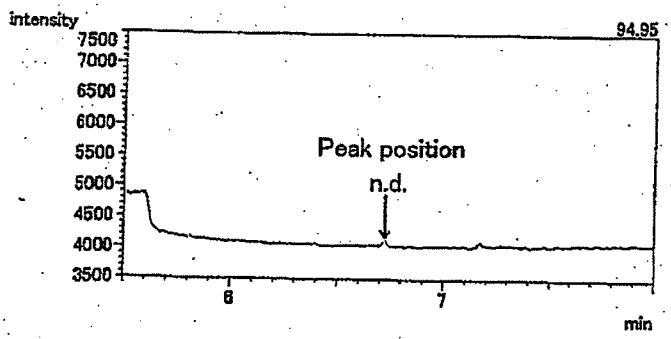


Peak No.	Time(min)	m/z	Area
1	6.73	94.95	3025

Jan. 07, 2002 Name: I. Goshi

Test fish after 11days (Level2-a)
Date : 02/01/07
File name : C:¥GCMSsolution¥Data¥試験第2課¥43771¥43771z8.qgd

Study No. 43771

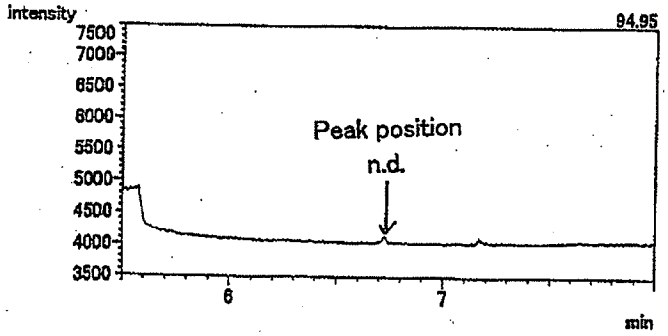


Peak No.	Time(min)	m/z	Area
1	–	94.95	–

Jan. 07, 2002 Name: I. Goshi

Test fish after 11days (Level2-b)
Date : 02/01/07
File name : C:¥GCMSsolution¥Data¥試験第2課¥43771¥43771z9.qgd

Study No. 43771



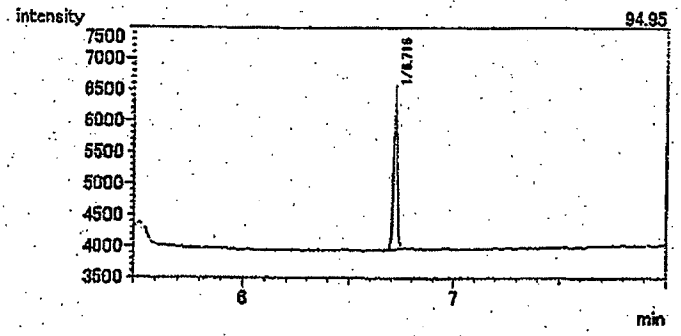
Peak No.	Time(min)	m/z	Area
1	–	94.95	–

Jan. 07, 2002 Name: I. Goshi

Fig. 10-1 Mass fragmentogram of GC/MS analysis for test fish (Level 2, test item)

Standard solution 50.0μg/L (F)
Date : 02/01/11
File name : C:¥GCMSsolution¥Data¥試験第2課¥43771¥43771z15.qgd

Study No. 43771

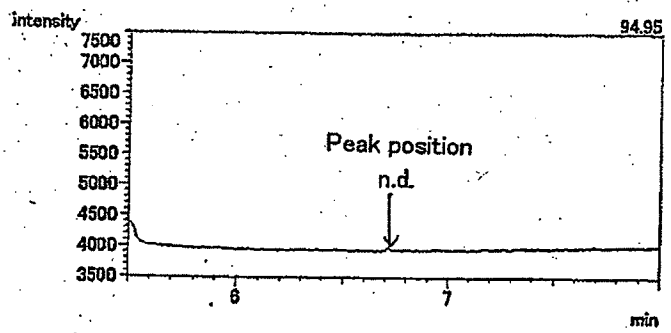


Peak No.	Time(min)	m/z	Area
1	6.72	94.95	3005

Jan. 11, 2002 Name: I. Goshi

Test fish after 15 days (Level 2-a)
Date : 02/01/11
File name : C:¥GCMSsolution¥Data¥試験第2課¥43771¥43771z16.qgd

Study No. 43771

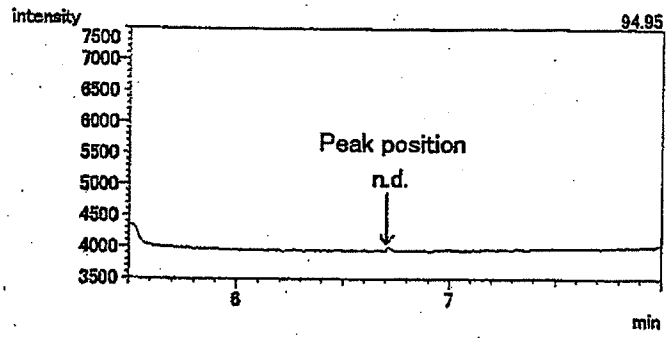


Peak No.	Time(min)	m/z	Area
1	-	94.95	-

Jan. 11, 2002 Name: I. Goshi

Test fish after 15 days (Level 2-b)
Date : 02/01/11
File name : C:¥GCMSsolution¥Data¥試験第2課¥43771¥43771z17.qgd

Study No. 43771



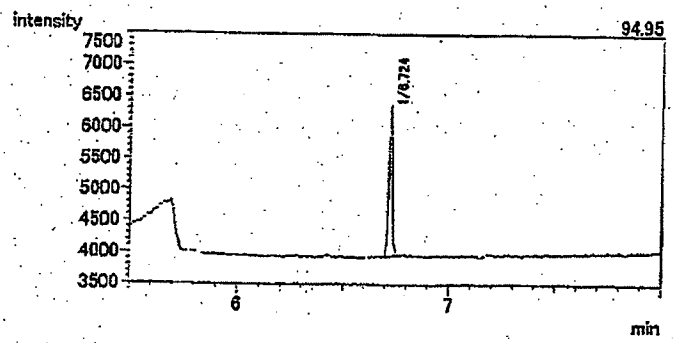
Peak No.	Time(min)	m/z	Area
1	-	94.95	-

Jan. 11, 2002 Name: I. Goshi

Fig. 10-2 Mass fragmentogram of GC/MS analysis for test fish (Level 2, test item)

Standard solution 50.0μg/L (F)
Date : 02/01/15
File name : C:¥GCMSsolution¥Data¥試験第2課¥43771¥43771z21.qgd

Study No. 43771

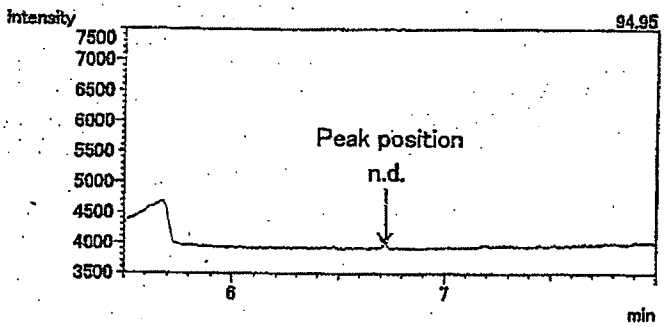


Peak No.	Time(min)	m/z	Area
1	6.73	94.95	2808

Jan. 15, 2002 Name: S. Goshi

Test fish after 19 days (Level2-a)
Date : 02/01/15
File name : C:¥GCMSsolution¥Data¥試験第2課¥43771¥43771z22.qgd

Study No. 43771

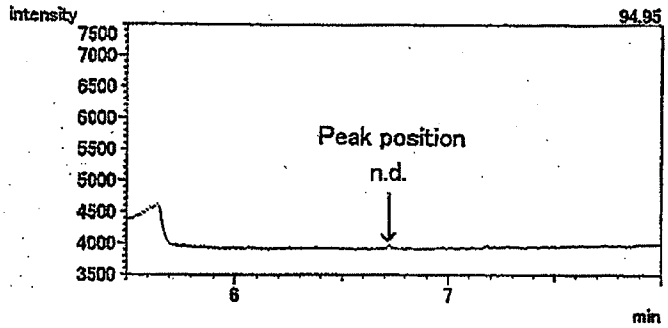


Peak No.	Time(min)	m/z	Area
1	-	94.95	-

Jan. 15, 2002 Name: S. Goshi

Test fish after 19 days (Level2-b)
Date : 02/01/15
File name : C:¥GCMSsolution¥Data¥試験第2課¥43771¥43771z23.qgd

Study No. 43771



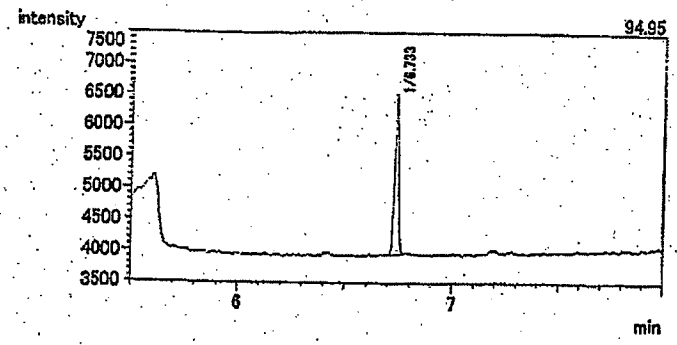
Peak No.	Time(min)	m/z	Area
1	-	94.95	-

Jan. 15, 2002 Name: S. Goshi

Fig. 10-3 Mass fragmentogram of GC/MS analysis for test fish (Level 2, test item)

Standard solution 50.0ug/L (F)
Date : 02/01/18
File name : C:¥GCMSsolution¥Data¥試験第2課¥43771¥43771z30.qgd

Study No. 43771

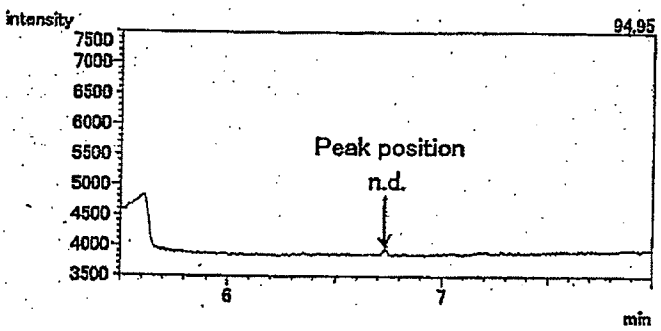


Peak No.	Time(min)	m/z	Area
1	6.73	94.95	3065

Jan. 18, 2002 Name: K. Yuka

Test fish after 22 days (Level 2-a)
Date : 02/01/18
File name : C:¥GCMSsolution¥Data¥試験第2課¥43771¥43771z31.qgd

Study No. 43771

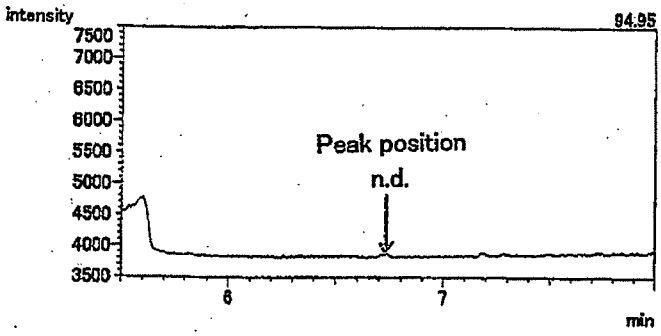


Peak No.	Time(min)	m/z	Area
1	-	94.95	-

Jan. 18, 2002 Name: K. Yuka

Test fish after 22 days (Level 2-b)
Date : 02/01/18
File name : C:¥GCMSsolution¥Data¥試験第2課¥43771¥43771z32.qgd

Study No. 43771



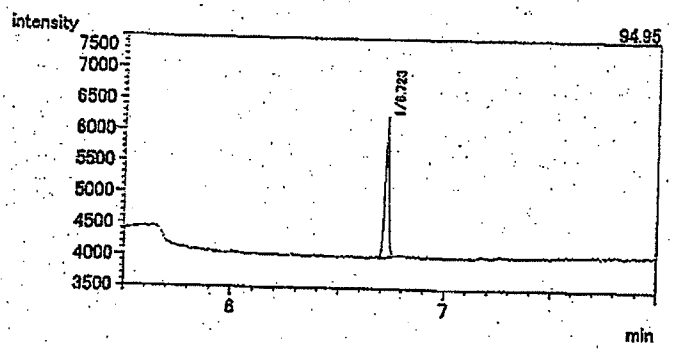
Peak No.	Time(min)	m/z	Area
1	-	94.95	-

Jan. 18, 2002 Name: K. Yuka

Fig. 10-4 Mass fragmentogram of GC/MS analysis for test fish (Level 2, test item)

Standard solution 50.0ug/L (F)
Date : 02/01/24
File name : C:¥GCMSsolution¥Data¥試験第2課¥43771¥43771z36.qgd

Study No. 43771

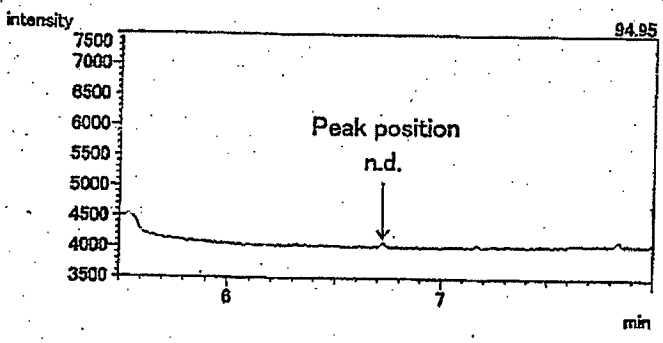


Peak No.	Time(min)	m/z	Area
1	6.73	94.95	2927

Jan. 24, 2002 Name: I. Goshi

Test fish after 28 days (Level2-a)
Date : 02/01/24
File name : C:¥GCMSsolution¥Data¥試験第2課¥43771¥43771z37.qgd

Study No. 43771

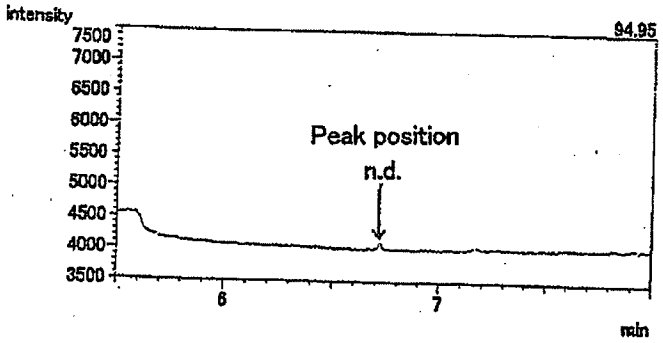


Peak No.	Time(min)	m/z	Area
1	–	94.95	–

Jan. 24, 2002 Name: I. Goshi

Test fish after 28 days (Level2-b)
Date : 02/01/24
File name : C:¥GCMSsolution¥Data¥試験第2課¥43771¥43771z38.qgd

Study No. 43771



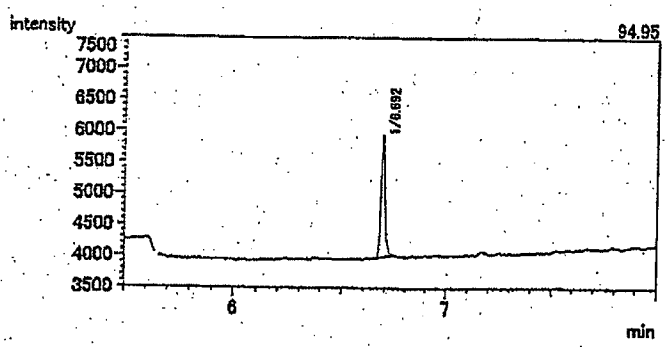
Peak No.	Time(min)	m/z	Area
1	–	94.95	–

Jan. 24, 2002 Name: I. Goshi

Fig. 10-5 Mass fragmentogram of GC/MS analysis for test fish (Level 2, test item)

Standard solution 50.0μg/L (F)
Date : 01/12/27
File name : C:¥GCMSsolution¥Data¥試験第2課¥43771¥43771z1.qgd

Study No. 43771

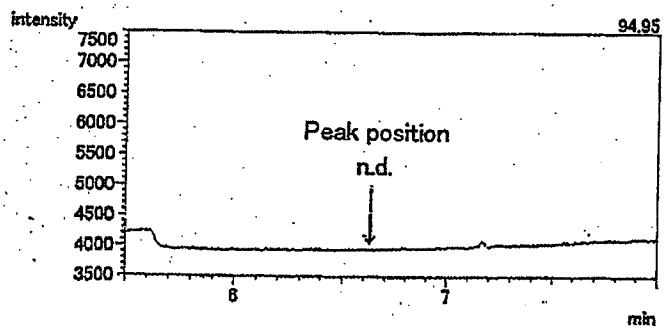


Peak No.	Time(min)	m/z	Area
1	6.69	94.95	2598

Dec. 27, 2001 Name: l. Goshi

Before the experimental start (control-a)
Date : 01/12/27
File name : C:¥GCMSsolution¥Data¥試験第2課¥43771¥43771z2.qgd

Study No. 43771

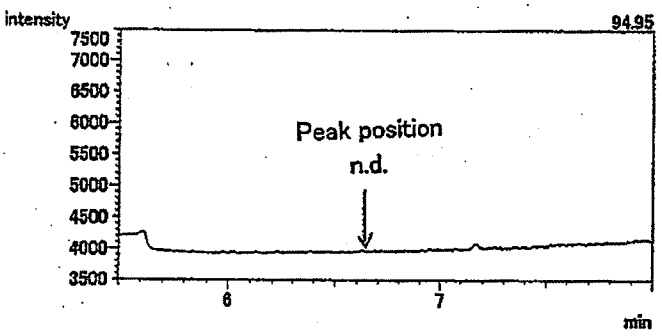


Peak No.	Time(min)	m/z	Area
1	-	94.95	-

Dec. 27, 2001 Name: l. Goshi

Before the experimental start (control-b)
Date : 01/12/27
File name : C:¥GCMSsolution¥Data¥試験第2課¥43771¥43771z3.qgd

Study No. 43771



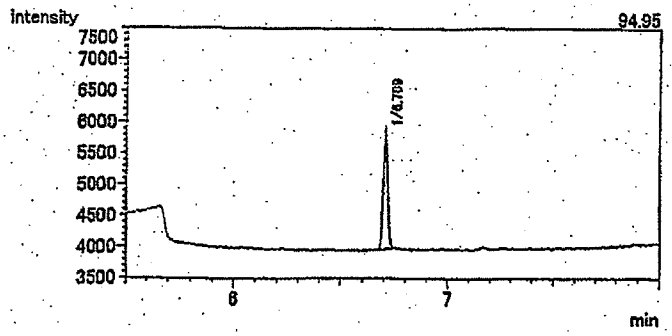
Peak No.	Time(min)	m/z	Area
1	-	94.95	-

Dec. 27, 2001 Name: l. Goshi

Fig. 11-1 Mass fragmentogram of GC/MS analysis for test fish (Control, test item)

Standard solution 50.0μg/L (F)
Date : 02/01/25
File name : C:¥GCMSsolution¥Data¥試験第2課¥43771¥43771z42.qgd

Study No. 43771

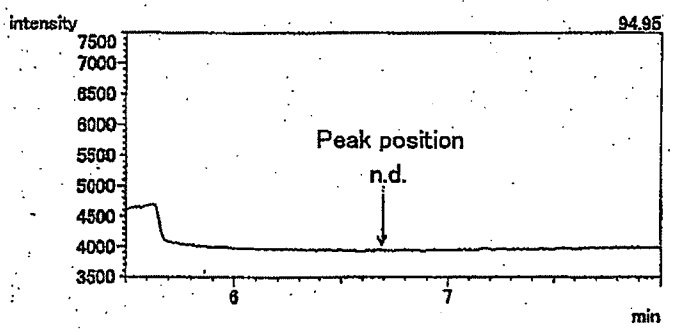


Peak No.	Time(min)	m/z	Area
1	6.71	94.95	2867

Jan. 25, 2002 Name: I. Yoshi

After the experimental completion (Control-a)
Date : 02/01/25
File name : C:¥GCMSsolution¥Data¥試験第2課¥43771¥43771z43.qgd

Study No. 43771

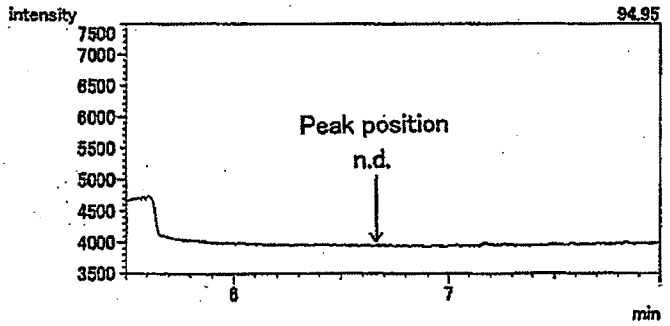


Peak No.	Time(min)	m/z	Area
1	–	94.95	–

Jan. 25, 2002 Name: I. Yoshi

After the experimental completion (Control-b)
Date : 02/01/25
File name : C:¥GCMSsolution¥Data¥試験第2課¥43771¥43771z44.qgd

Study No. 43771



Peak No.	Time(min)	m/z	Area
1	–	94.95	–

Jan. 25, 2002 Name: I. Yoshi

Fig. 11-2 Mass fragmentogram of GC/MS analysis for test fish (Control, test item)

Study No. 43291

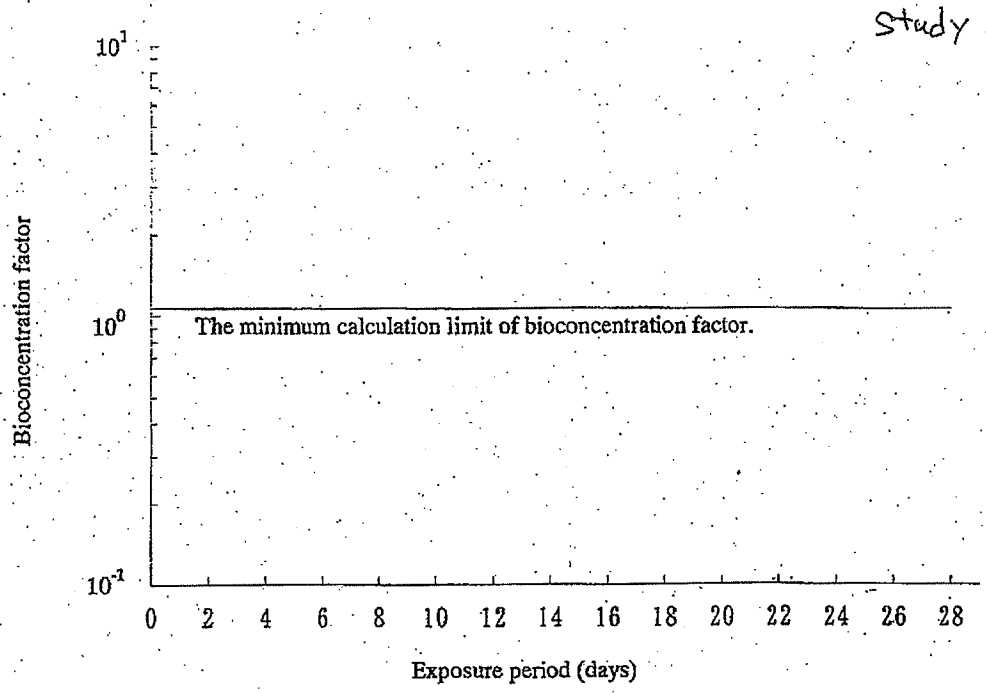


Fig. 12 Correlation between exposure period and bioconcentration factor (Level 1, carboxylic acid).

Ten data after 11, 15, 19, 22 and 28 days were lower than detection limit.

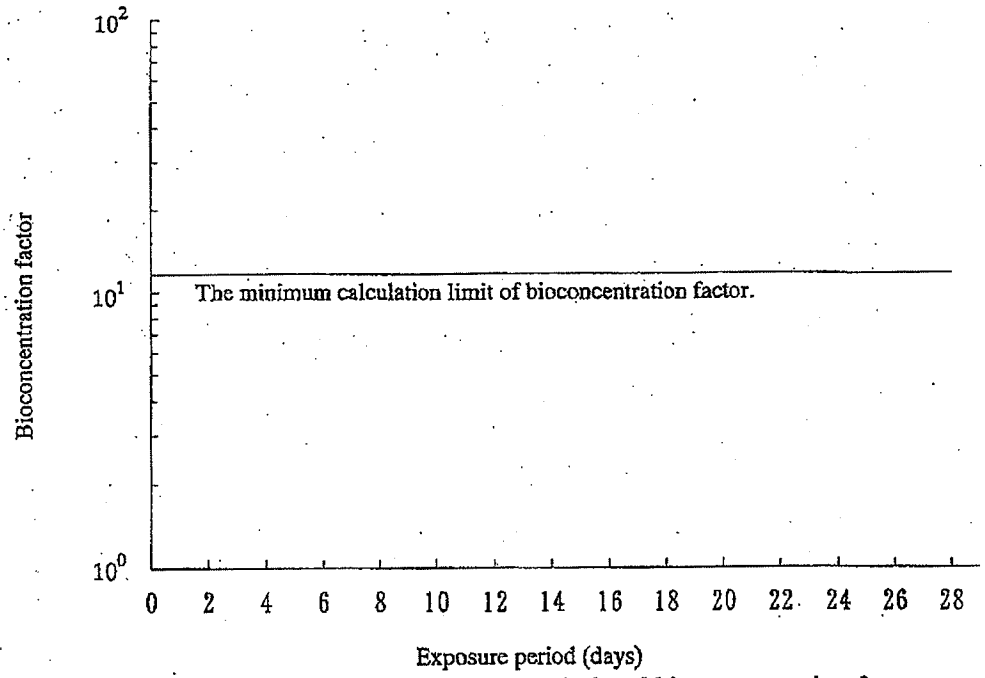
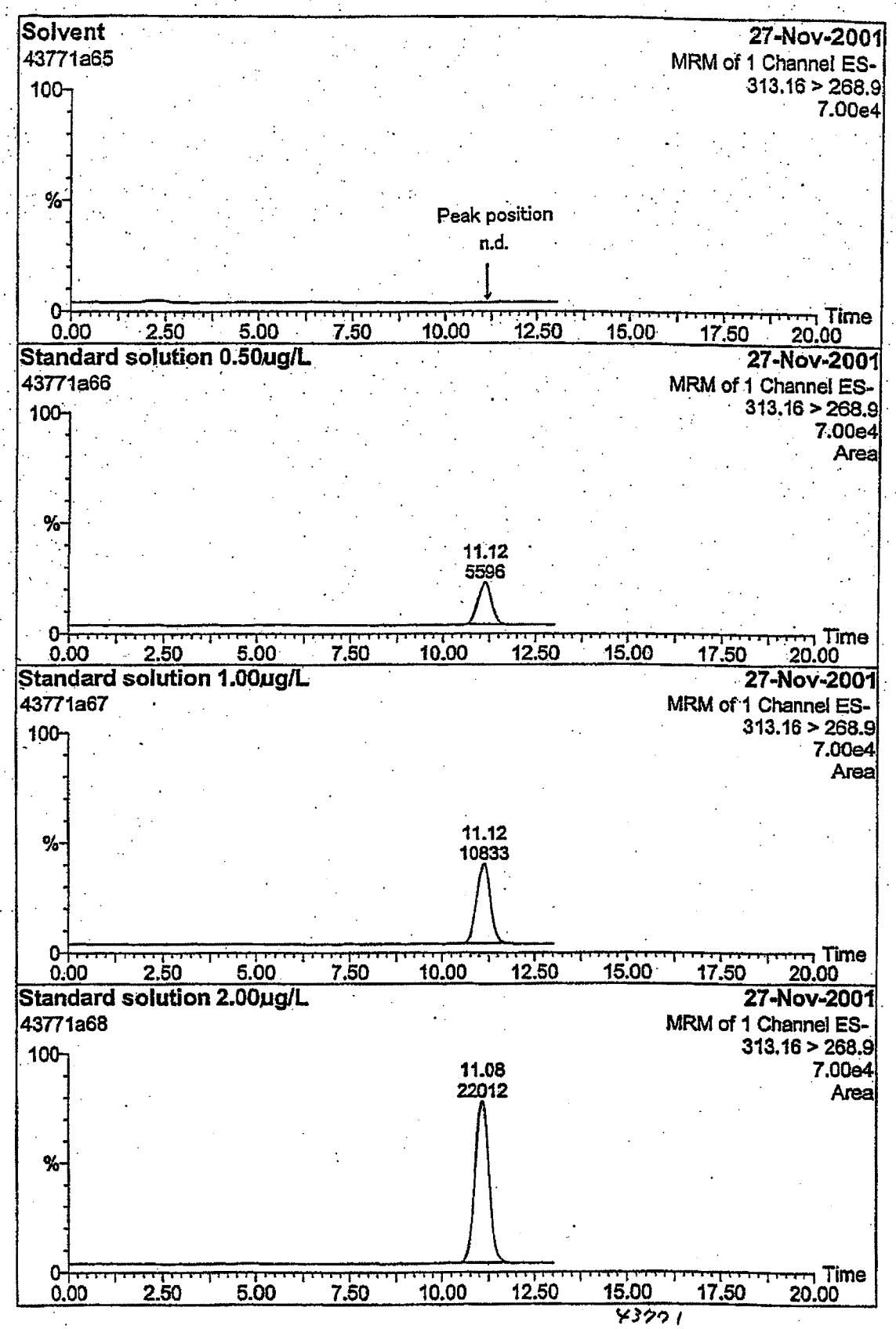


Fig. 13 Correlation between exposure period and bioconcentration factor (Level 2, carboxylic acid).

Ten data after 11, 15, 19, 22 and 28 days were lower than detection limit.

January 31, 2002 Name S. Yoshi



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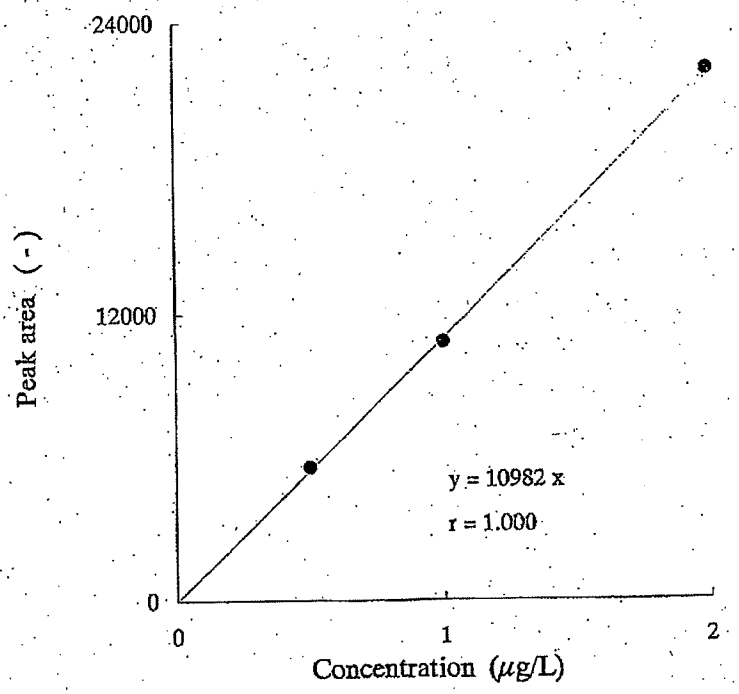
2001. 11. 27.
S. Yoshi

Fig. 14-1 Chromatogram of LC/MS/MS analysis for calibration curve (carboxylic acid)



Concentration (μg/L)	Peak area (-)
0.500	5596
1.000	10833
2.000	22012

Conditions of LC-MS analysis

Instrument	:	Agilent HP-1100, Micromass Quattro Ultima
Sample	:	Carboxylic acid
Solvent	:	Methanol *7 / water *4 (1/1 V/V)
LC Conditions		
Column (Size)	:	L-column ODS (15cm × 2.1mm I.D.)
Col. temp.	:	25℃
Eluent	:	A (40%)=Water *4,8
	:	B (60%)=Methanol *8
Flow rate	:	0.2mL/min.
Injection vol.	:	20μL
MS/MS Conditions		
Ionization mode	:	ESI
Detection ion	:	Negative
Detection mode	:	Selected reaction monitoring
Precursor ion	:	m/z = 313
Product ion	:	m/z = 269
Ion source temp.	:	130℃
Cone voltage	:	20V
Cone voltage	:	10eV

Fig. 14 - 2 Calibration curve and conditions of LC/MS/MS analysis for Carboxylic acid (test fish).

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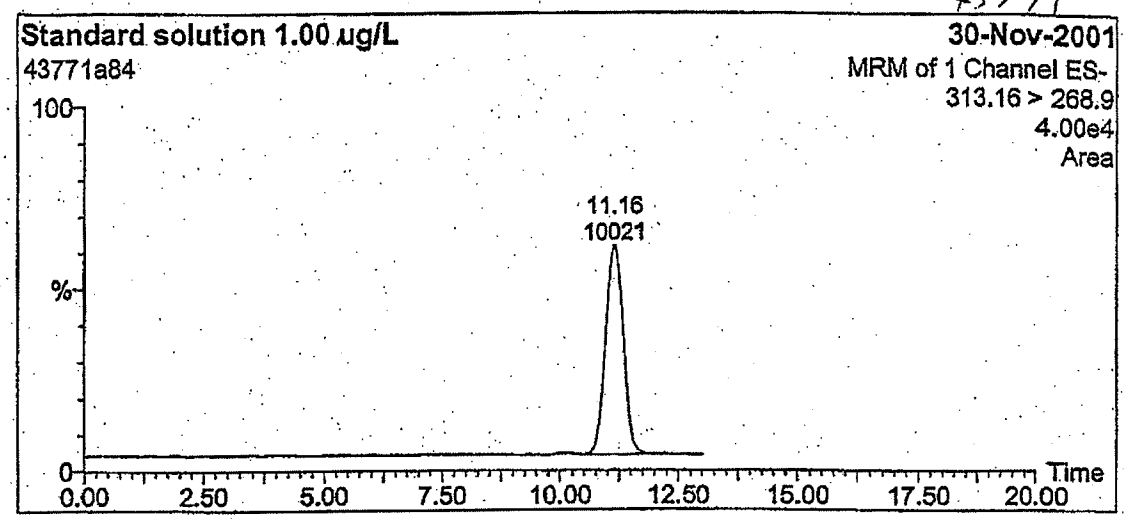
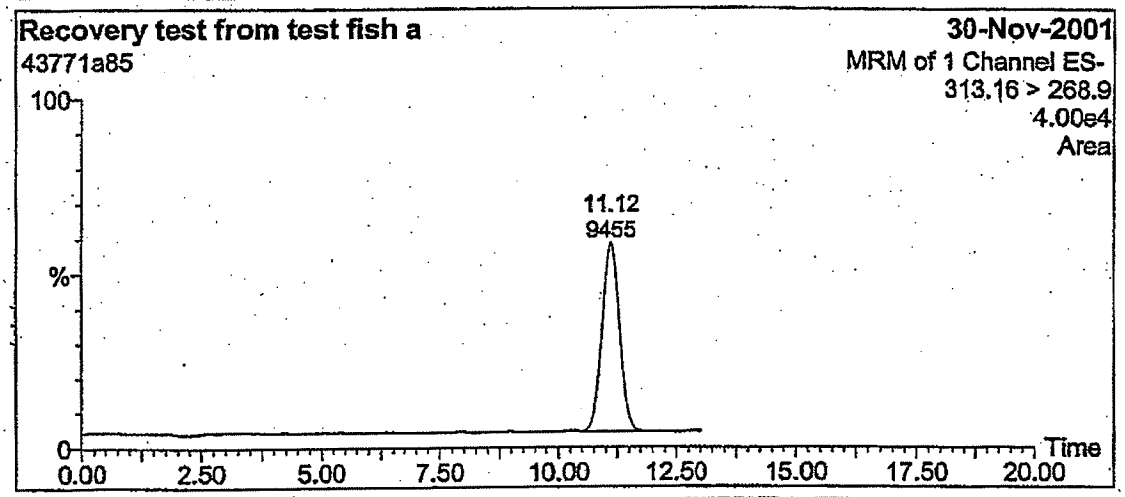
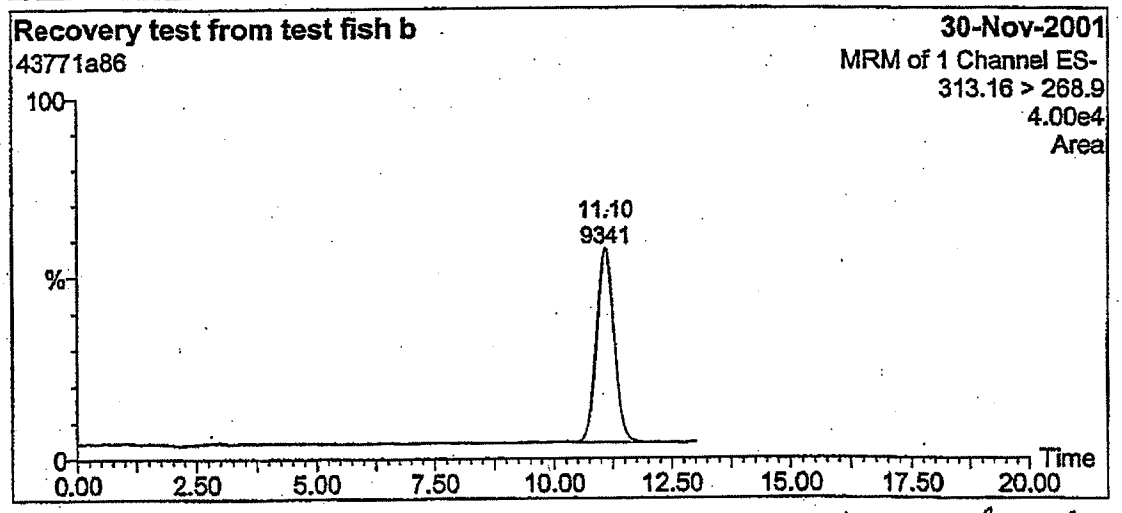


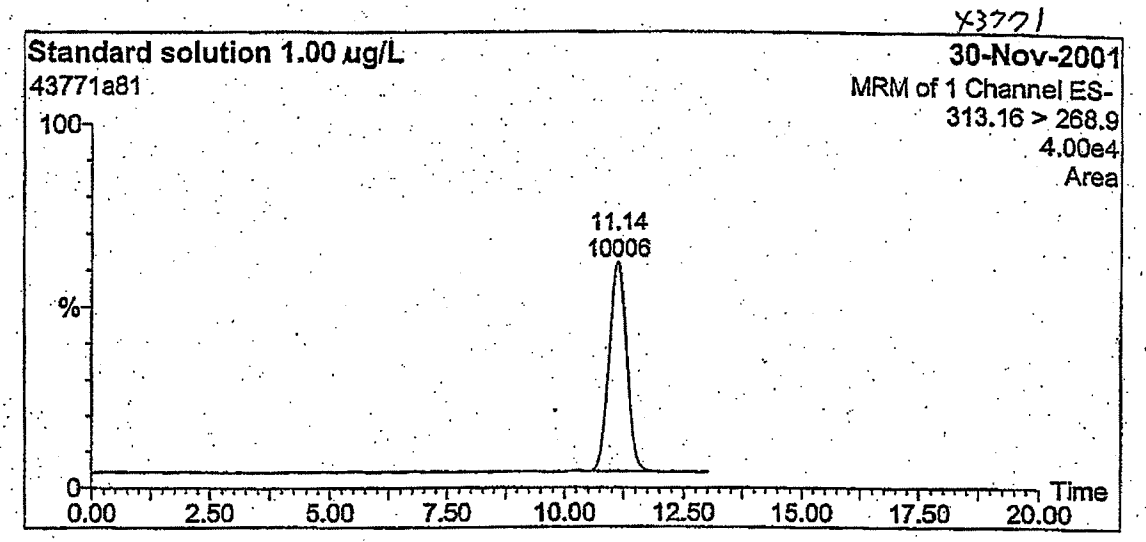


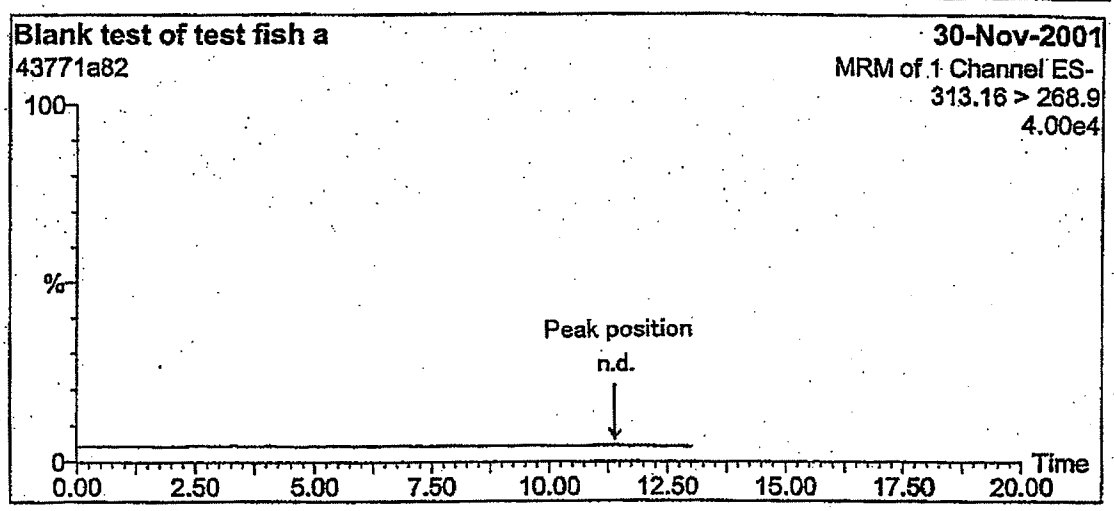


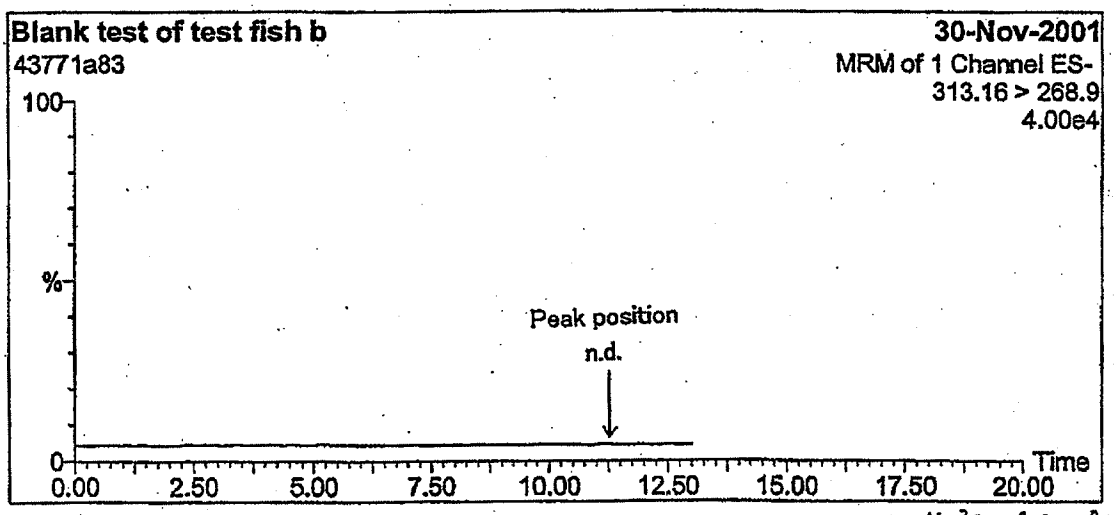
2001. 11. 30. I. yoshi

Fig. 15-1 Chromatogram of LC/MS/MS analysis for recovery and blank test (analysis of test fish, carboxylic acid)

X3771



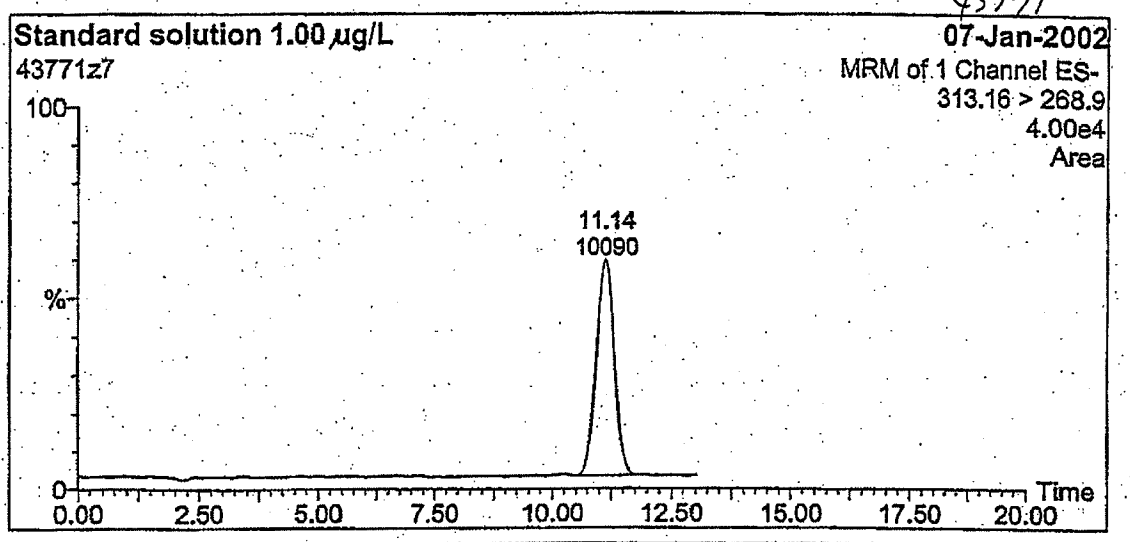


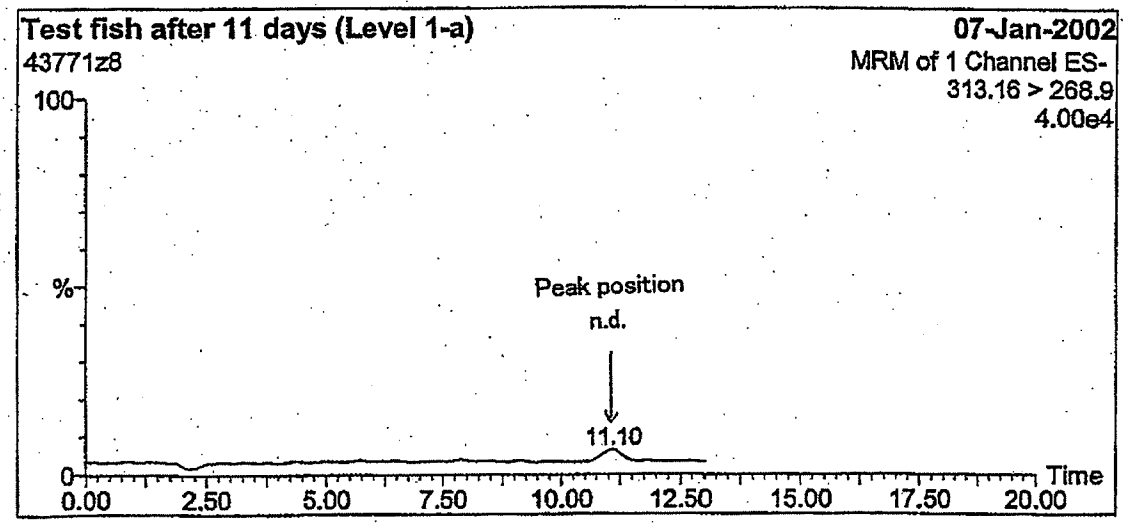


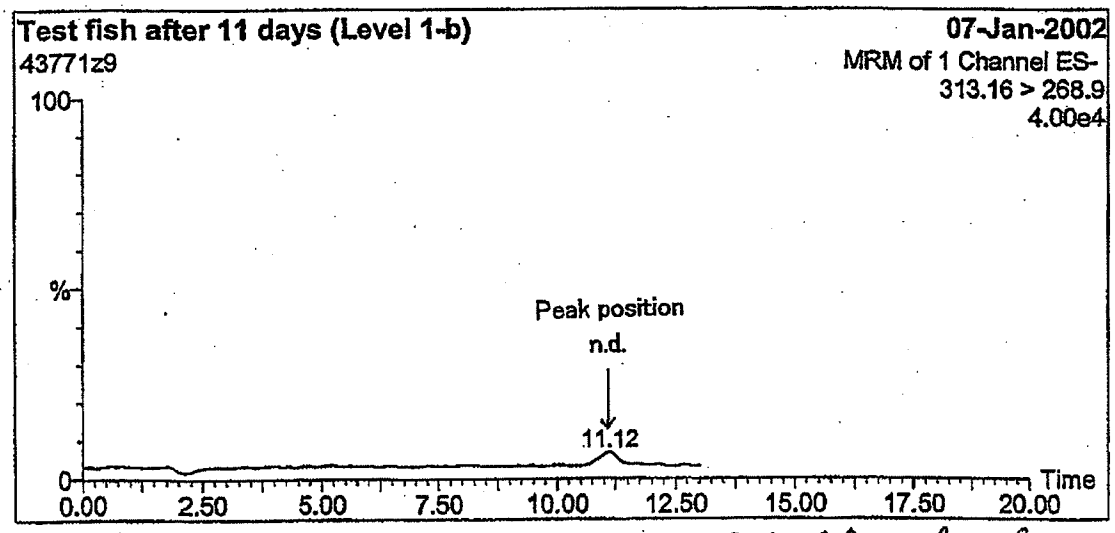
2001. 11. 30. L. Goshi

Fig. 15-2 Chromatogram of LC/MS/MS analysis for recovery and blank test (analysis of test fish, carboxylic acid)

43771







2002. 1. 7 I. Yoshi

Fig. 16-1 Chromatogram of LC/MS/MS analysis for test fish (Level 1, carboxylic acid)

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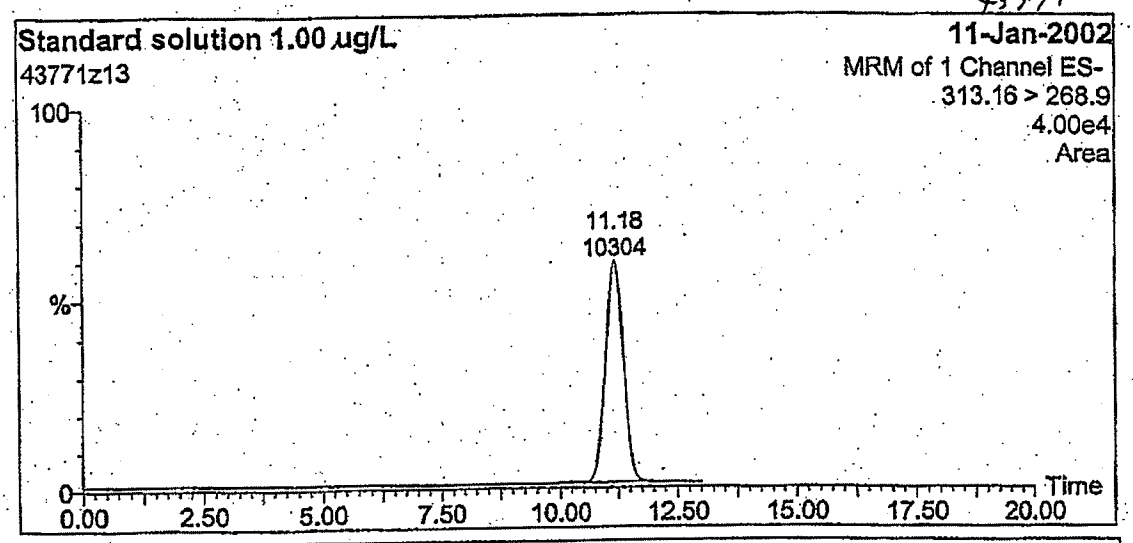
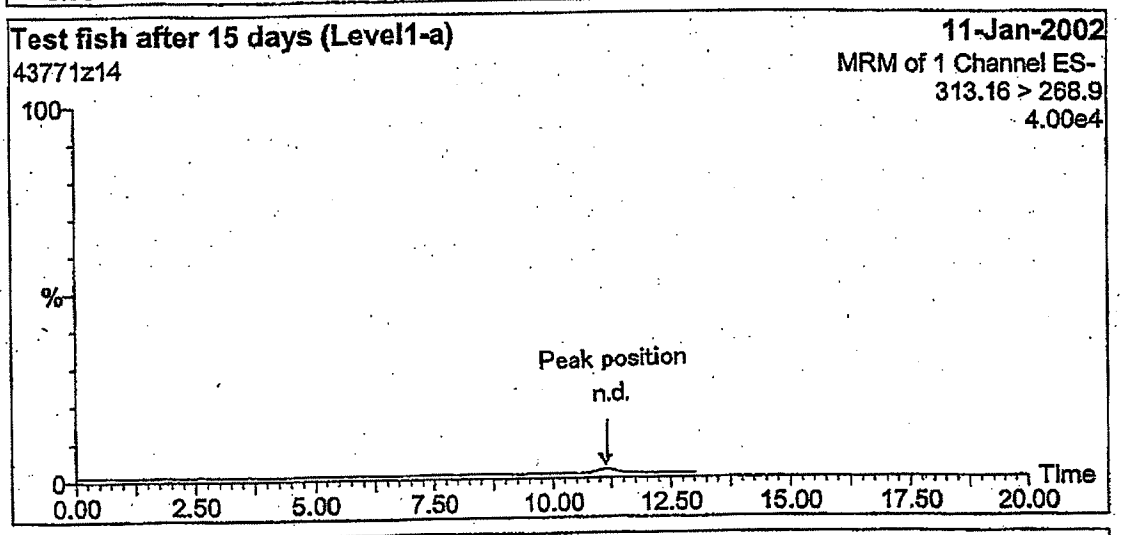
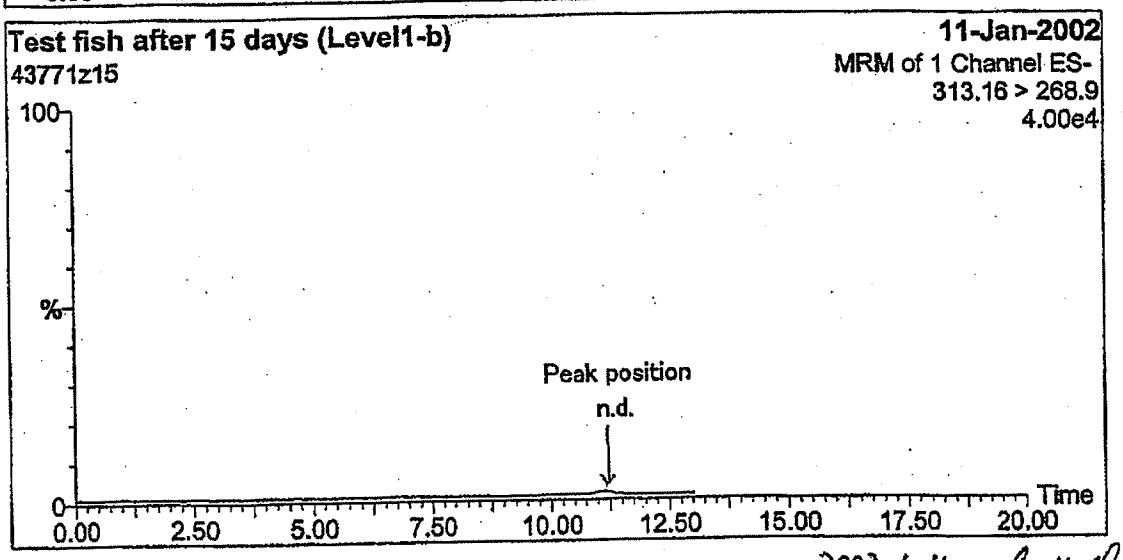


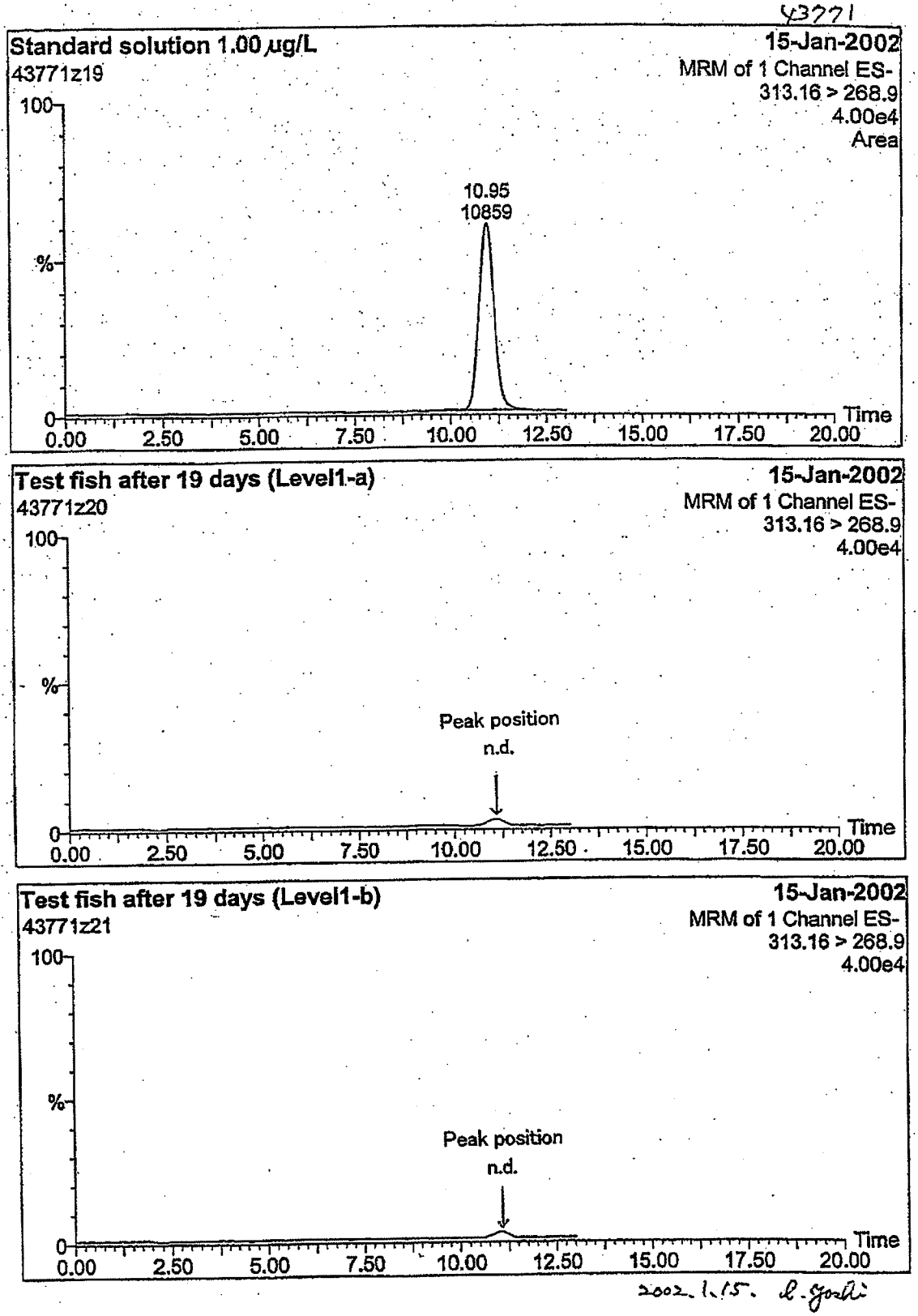




2002.1.11. d. yoshi

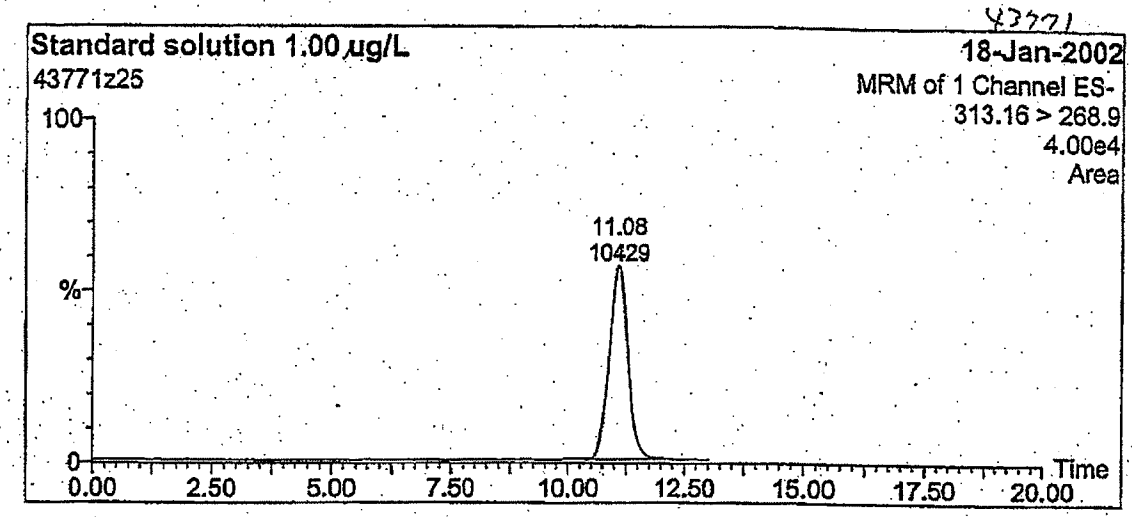
Fig. 16-2 Chromatogram of LC/MS/MS analysis for test fish (Level 1, carboxylic acid)

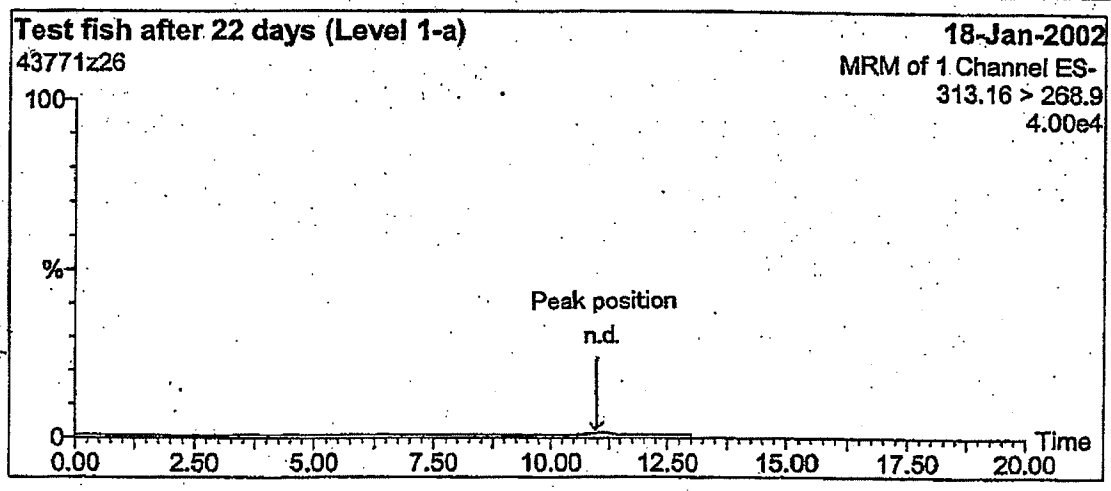
43771

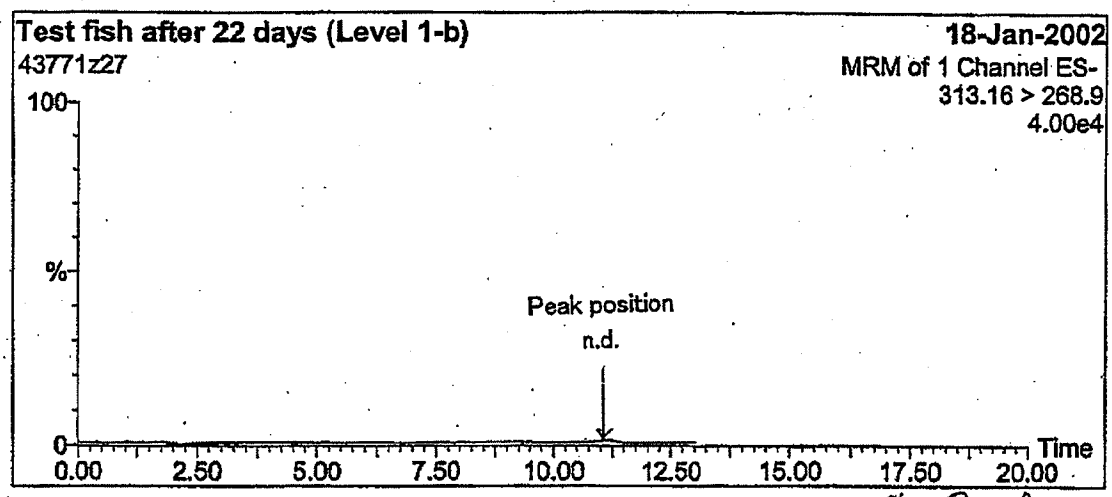


2002. 1. 15. 8. Goshi

Fig. 16-3 Chromatogram of LC/MS/MS analysis for test fish (Level 1, carboxylic acid)



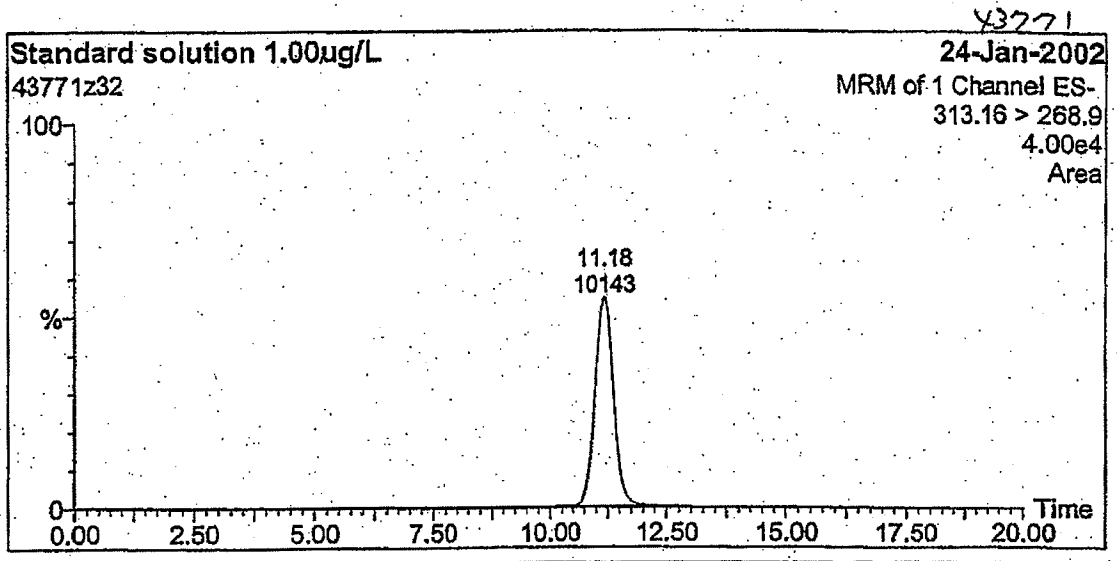


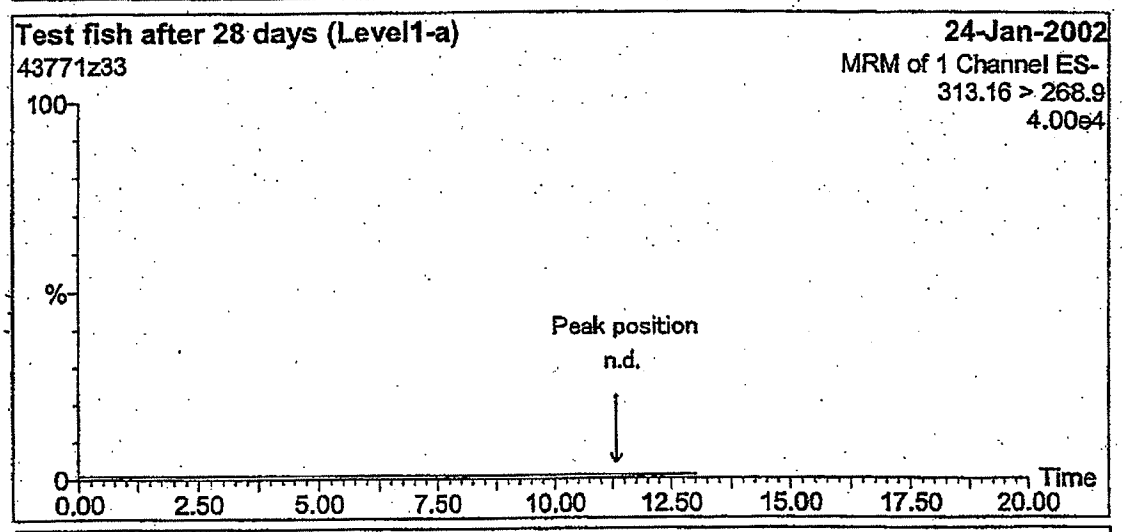


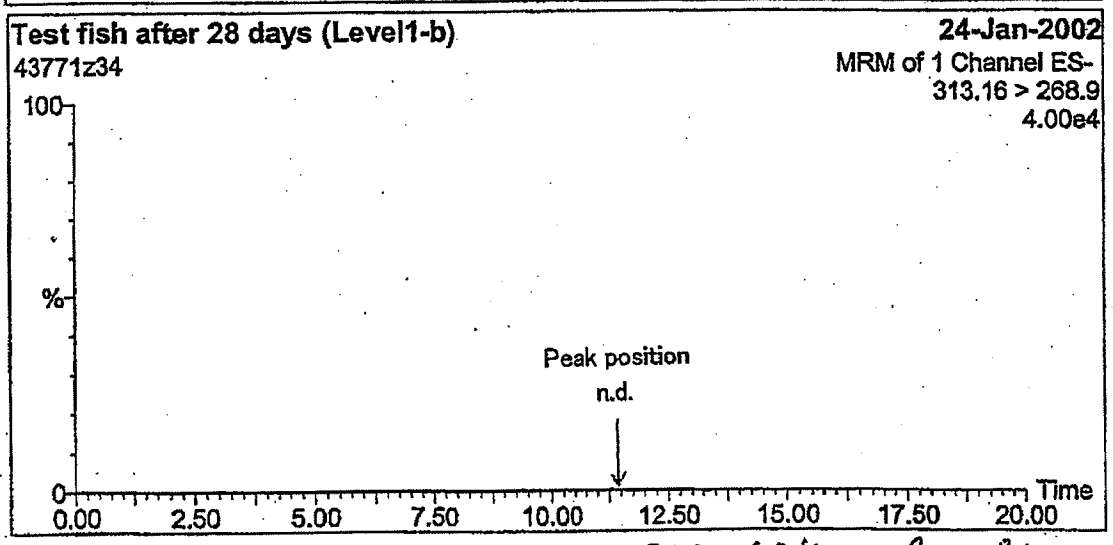
2002. 1. 18 K. Yuka

Fig. 16-4 Chromatogram of LC/MS/MS analysis for test fish (Level 1, carboxylic acid)

43771







2002. 1. 24. L. Joshi

Fig. 16-5 Chromatogram of LC/MS/MS analysis for test fish (Level 1, carboxylic acid)

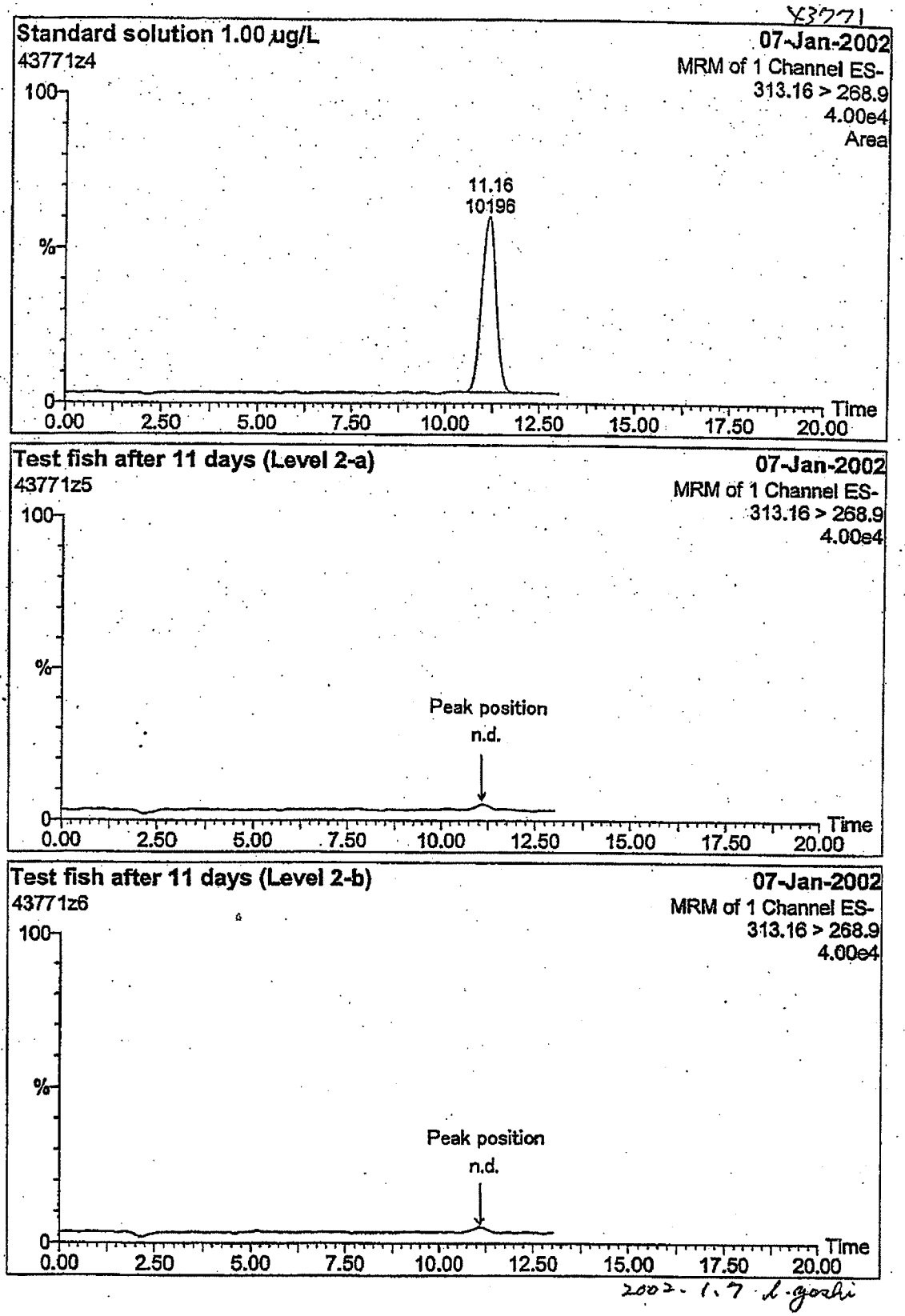
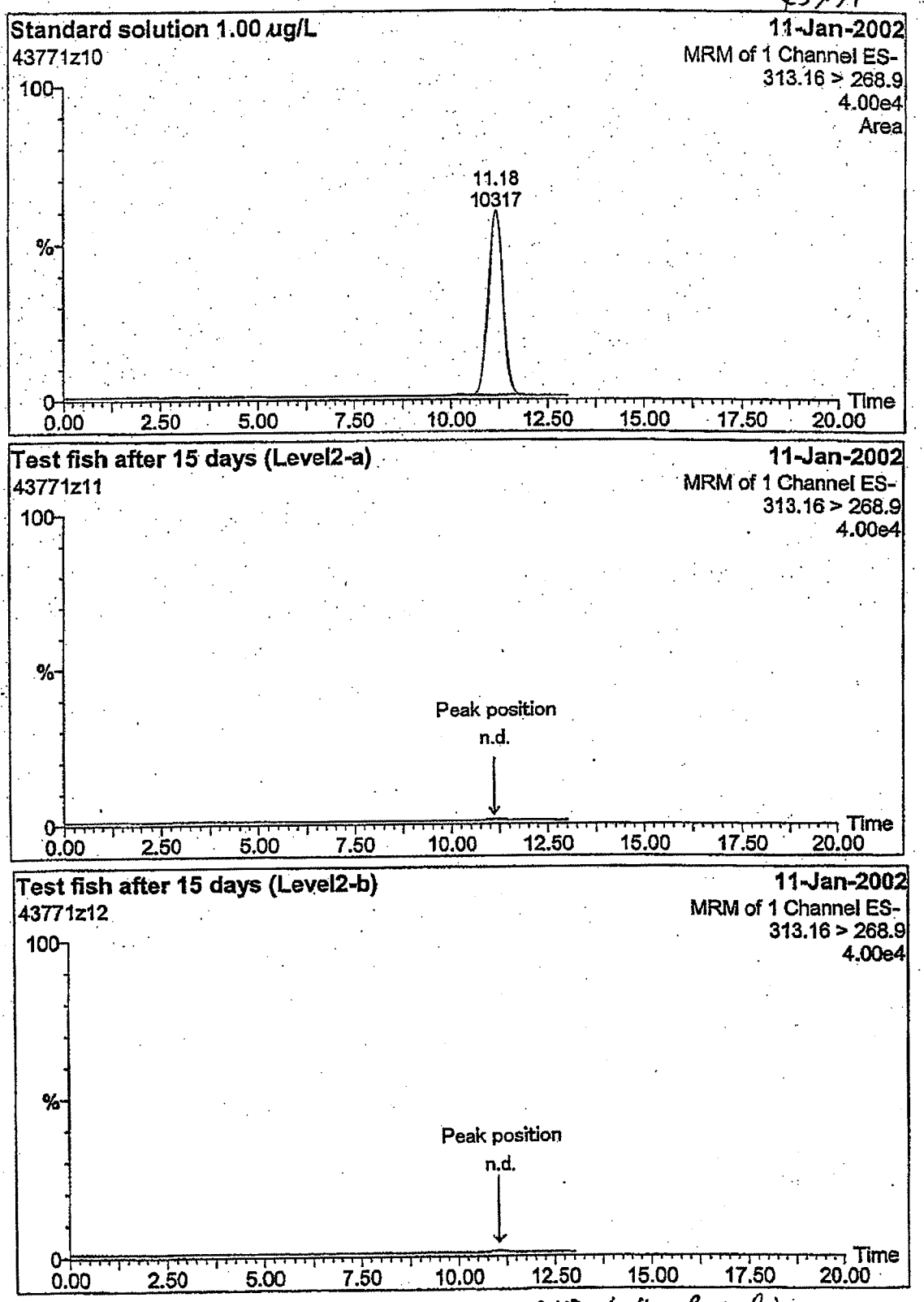


Fig. 17-1 Chromatogram of LC/MS/MS analysis for test fish (Level 2, carboxylic acid)

43771



2002. 1. 11 d. yoshi

Fig. 17-2 Chromatogram of LC/MS/MS analysis for test fish (Level 2, carboxylic acid)

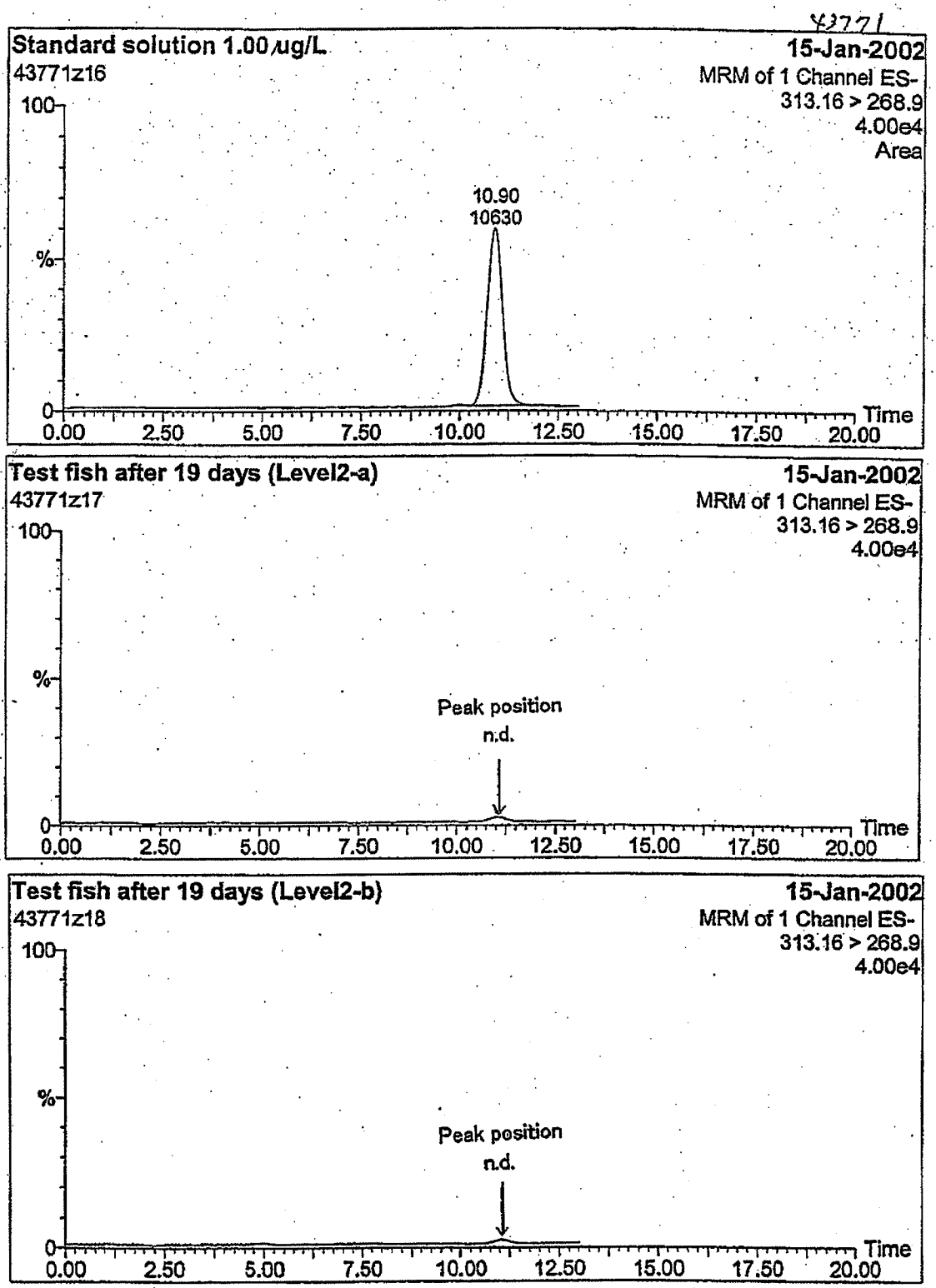


Fig. 17-3 Chromatogram of LC/MS/MS analysis for test fish (Level 2, carboxylic acid)

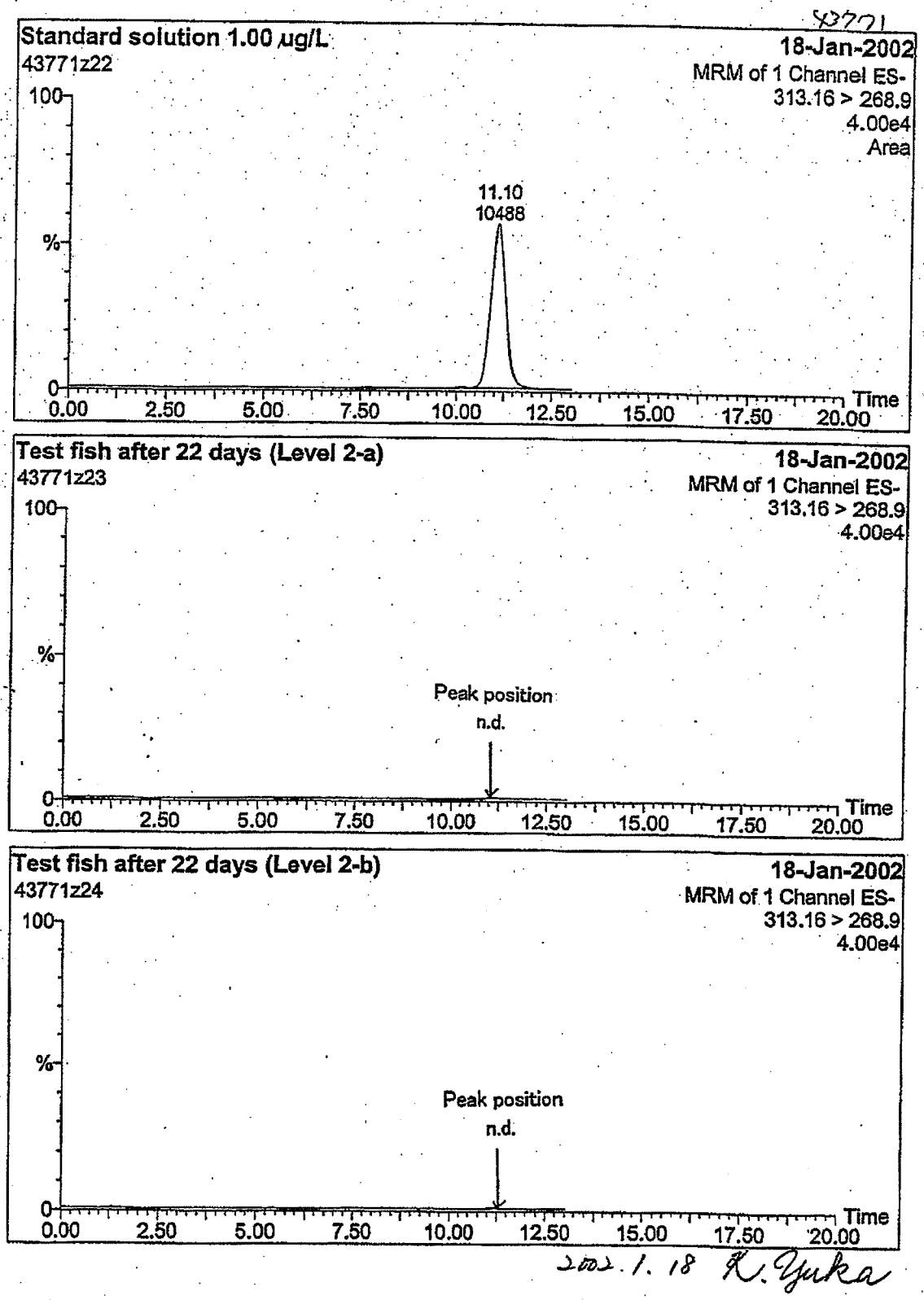
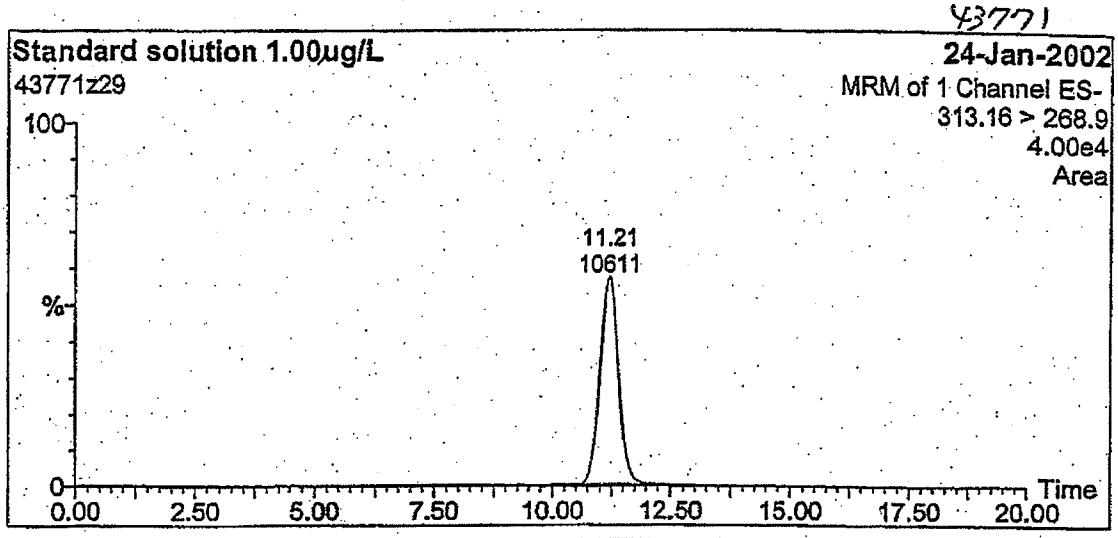
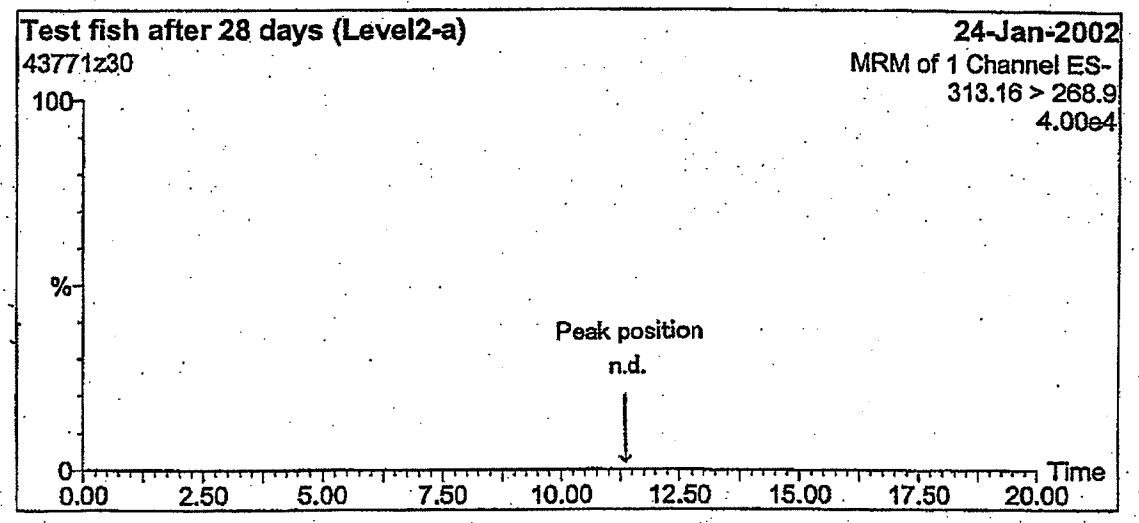
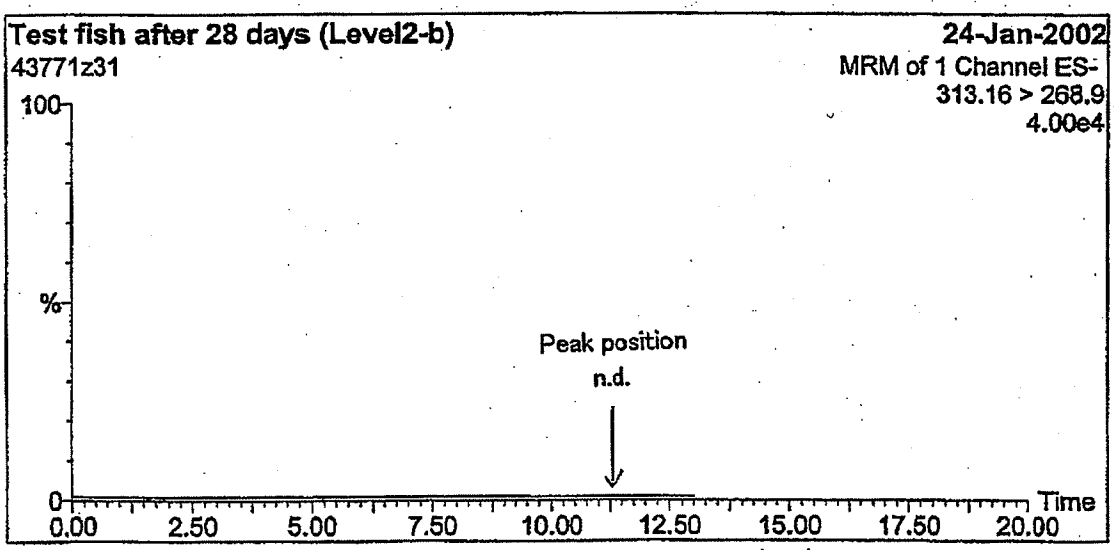


Fig. 17-4 Chromatogram of LC/MS/MS analysis for test fish (Level 2, carboxylic acid)

43771







2002.1.24. L. Goshi

Fig. 17–5 Chromatogram of LC/MS/MS analysis for test fish (Level 2, carboxylic acid)

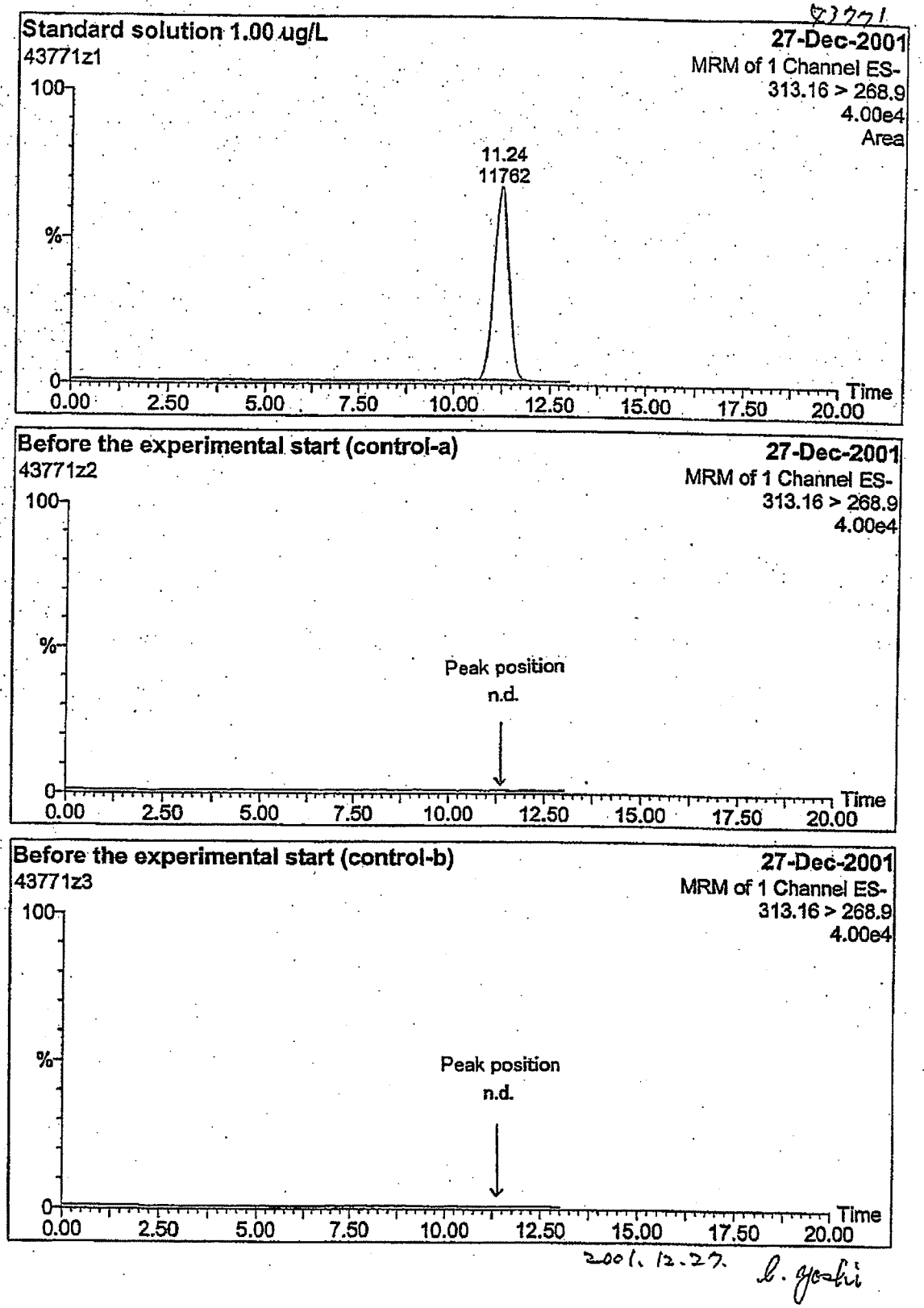


Fig. 18-1 Chromatogram of LC/MS/MS analysis for test fish (Control carboxylic acid)

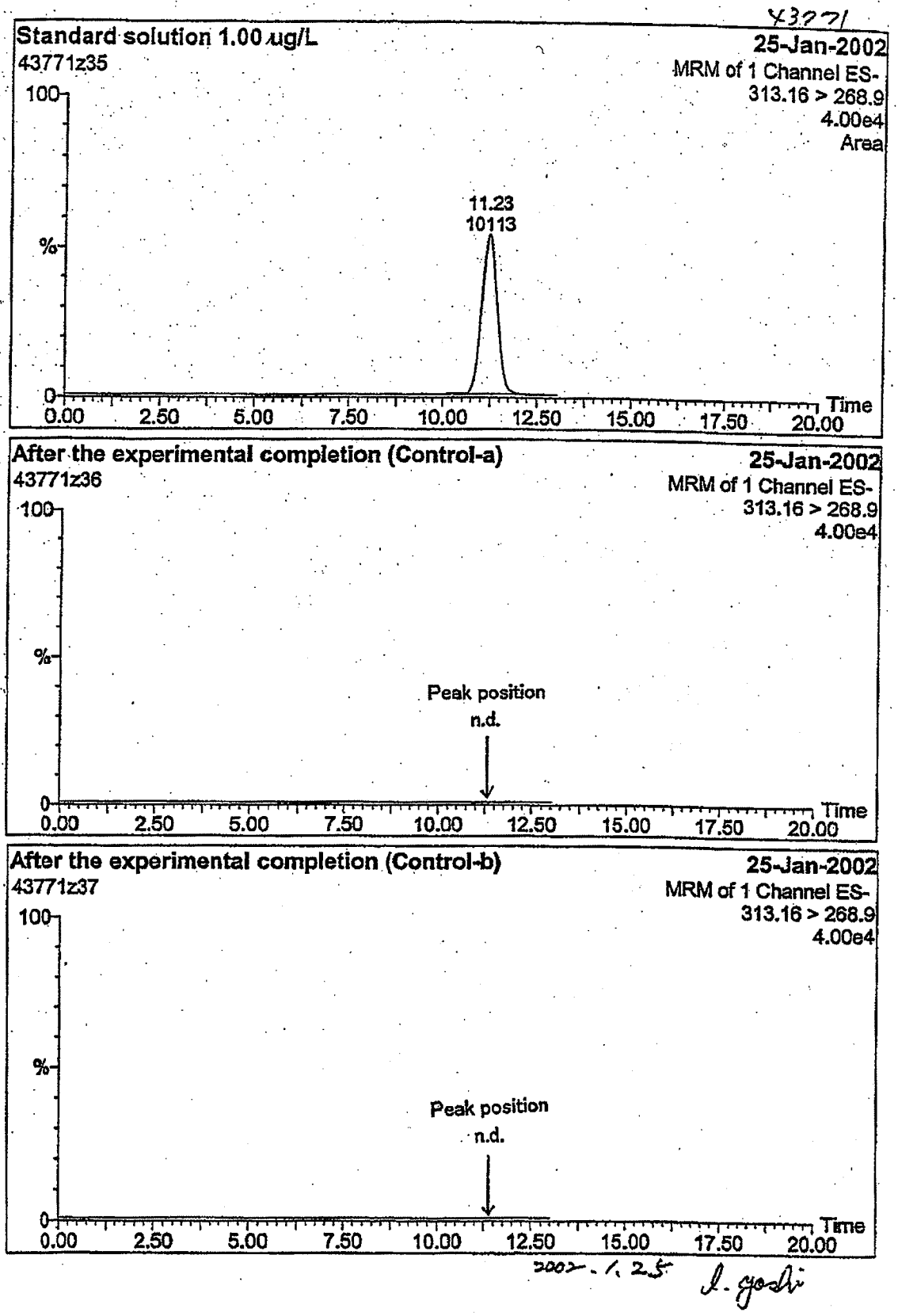


Fig. 18-2 Chromatogram of LC/MS/MS analysis for test fish (Contorl, carboxylic acid)

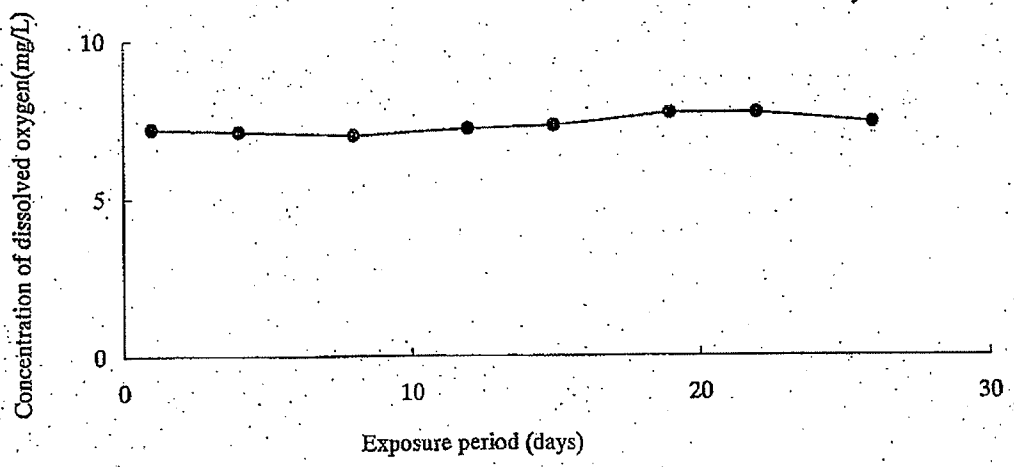


Fig. 19 Concentration of dissolved oxygen (Level 1).

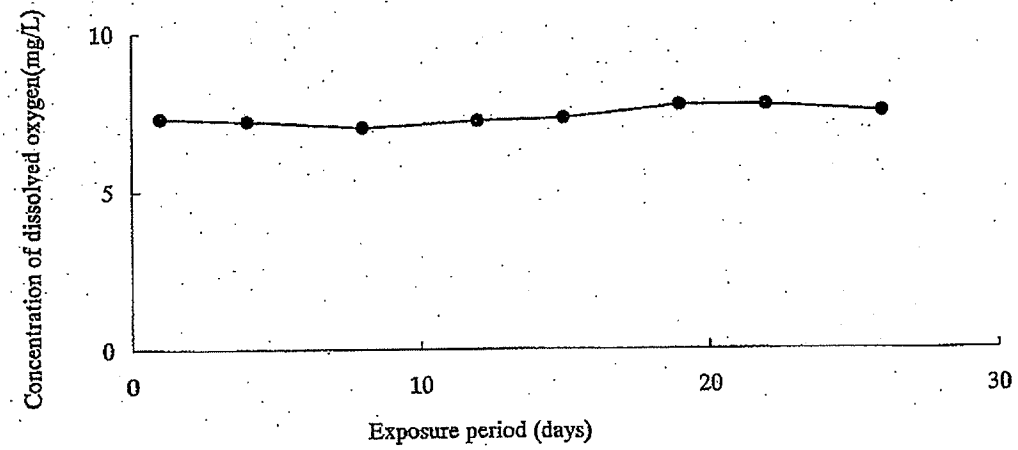


Fig. 20 Concentration of dissolved oxygen (Level 2).

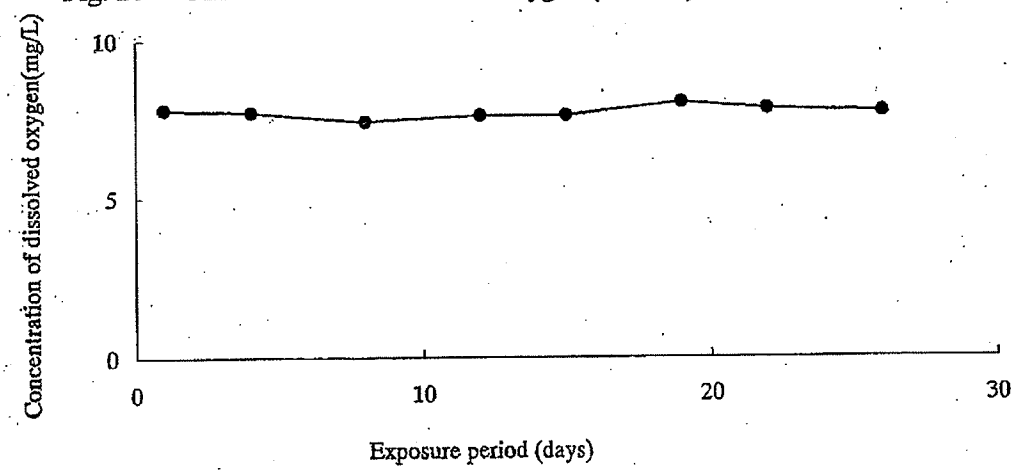


Fig. 21 Concentration of dissolved oxygen (Control).

January 31, 2002 Name J. Joshi

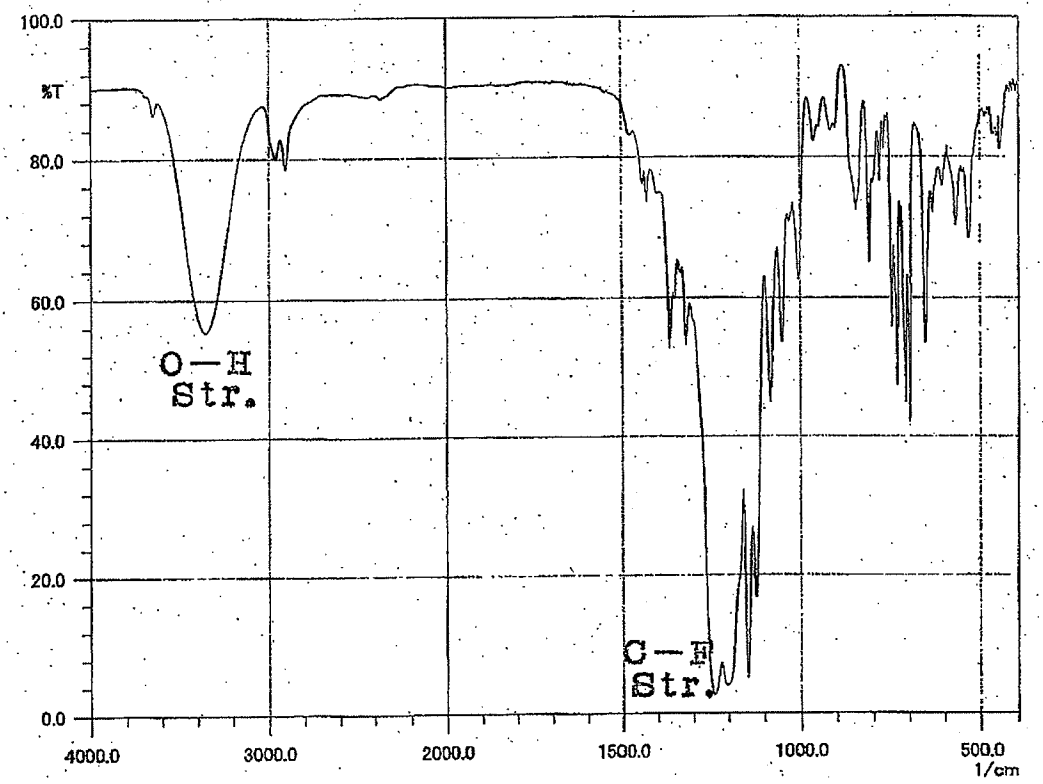


Fig. 22-1 IR spectrum of test item measured before experimental start.

Study No. : 43771
Sample : C6-2 PW2-12
Method : Neat
Date : Dec. 13, 2001
Name : h. yoshi

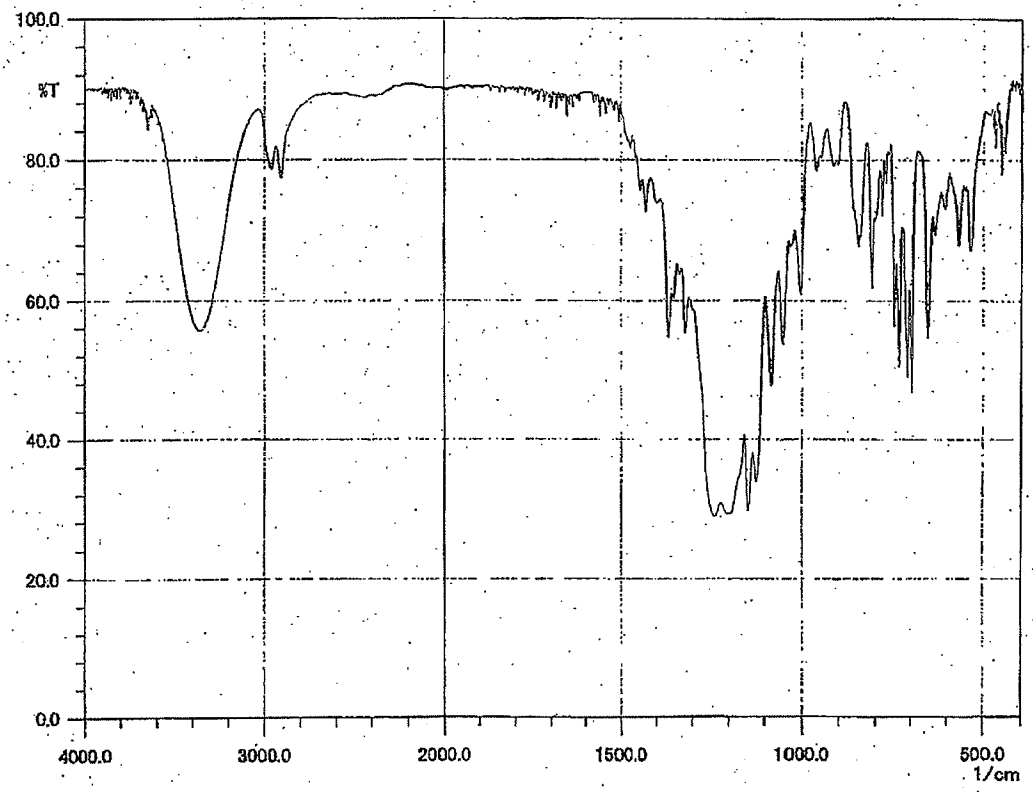


Fig. 22-2 IR spectrum of test item measured after the experimental completion.

Study No. : 43771
Sample : C6-2アルコール
Method : Neat
Date : Jan. 29, 2002
Name : I. Yoshi

Study No. 43771

Instrument Shimadzu QP-5000

Sample Test item

GC Conditions

Column INNOWAX

Size 30 m x 0.25 mm I.D., Film thickness 0.25 μm

Temp. 35 °C (2 min) → 150 °C (2 min)

Temp. rate 20 °C/min

Sample size 1 μL (Solvent Ethyl acetate)

Inlet mode (Splitless), Injection temp. 200 °C

Column head pressure 50 kPa, Carrier gas flow (He) 1.0 mL/min

Total flow 10 mL/min , Sampling time 1.0 min

MS Conditions

Ionization Mode EI , Detection Mode Positive

Monitoring ion m/z 50 – 500

Interface temp. 250 °C

Ionization voltage 70 eV

Note –

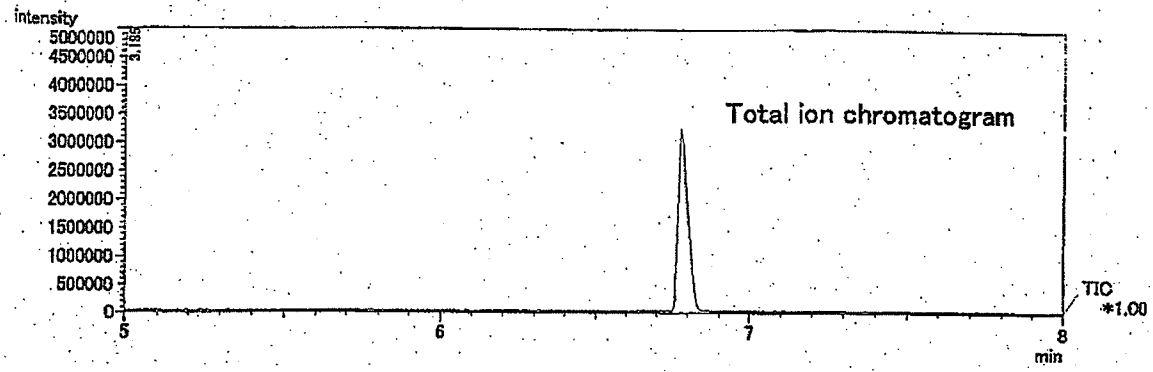
Kurume Laboratory, Chemicals Evaluation and Research Institute, Japan

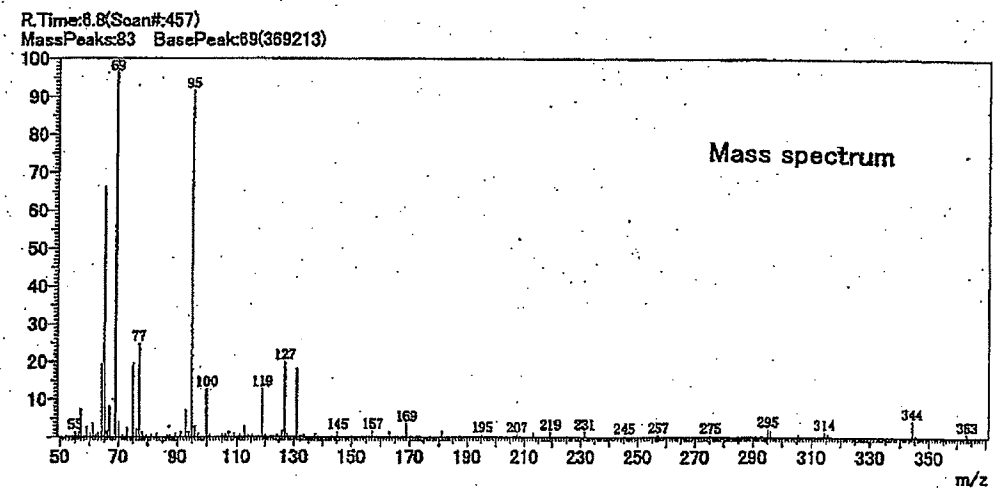
Fig. 23–1 MS spectrum of test item (analytical conditions)

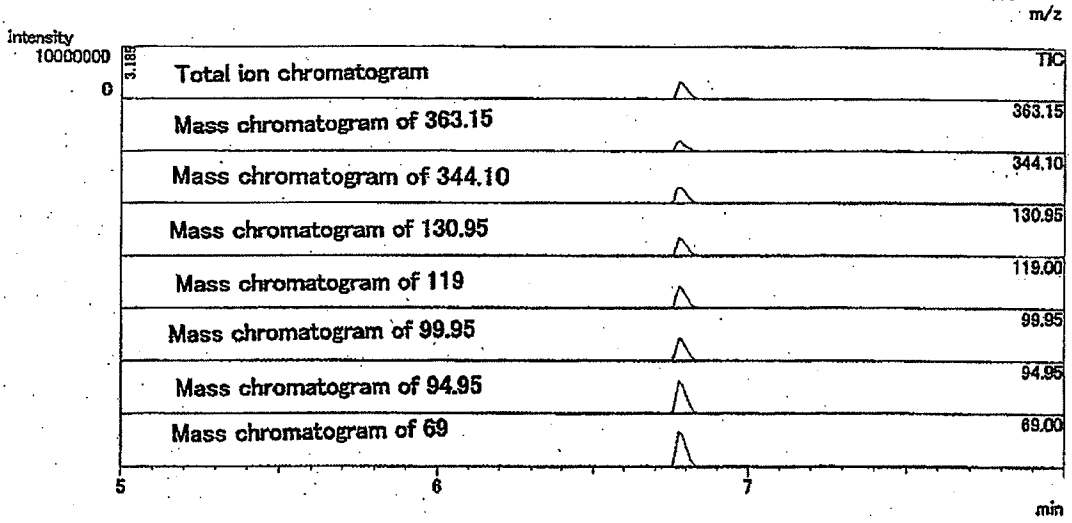
Date 2001 / 11 / 1 Operator L. yoshi

Standard solution 100mg/L (E.A.)
Date : 01/11/01
File Name : C:¥GCMSsolution¥Data¥試験第2課¥43771¥43770a01.qgd

Study No.43771







Nov.01,2001 Name: I. Goshi

Fig. 23-2 MS spectrum of test item (main presumed fragments)

$C_6F_{13}C_2H_4OH$

M.W. 364

m/z	Fragment ion
363	$(M-H)^+$
344	$(M-HF)^{+\cdot}$
131	$(C_3F_5)^+$
119	$(CF_2CF_3)^+$
100	$(C_2F_4)^{+\cdot}$
95	$(CF_2C_2H_4OH)^+$
69	$(CF_3)^+$

Fig. 23-3 MS spectrum of test item (main presumed fragments)

Date 2001/ 11 / 1 Name A. Goshi

Study No. 43771

Instrument MS : Micromass Quattro Ultima

HPLC : Agilent HP-1100

Sample Carboxylic acid

HPLC Conditions

Inlet system Column

Sample size 5 μL

(Solvent Methanol/water (1/1 V/V))

Column L-column ODS (SUS)

Column size 15 cm x 2.1 mm I.D., Column temp. 25 °C

Eluent A (40%) : Water (containing 5mmol/L di-n-butylamine acetate)

B (60%) : Methanol (containing 5mmol/L di-n-butylamine acetate)

Flow rate 0.2 mL/min

MS Conditions

Ionization mode ESI, Detection mode Negative

Probe ESI Capillary 3.5 kV, Desolvation temp. 400 °C, Desolvation gas 650 L/hr

Source Cone 20 V

Source block temp. 120 °C

MS Ion energy -0.2 V, Multiplier 600 V

Monitoring ion m/z 50 – 800

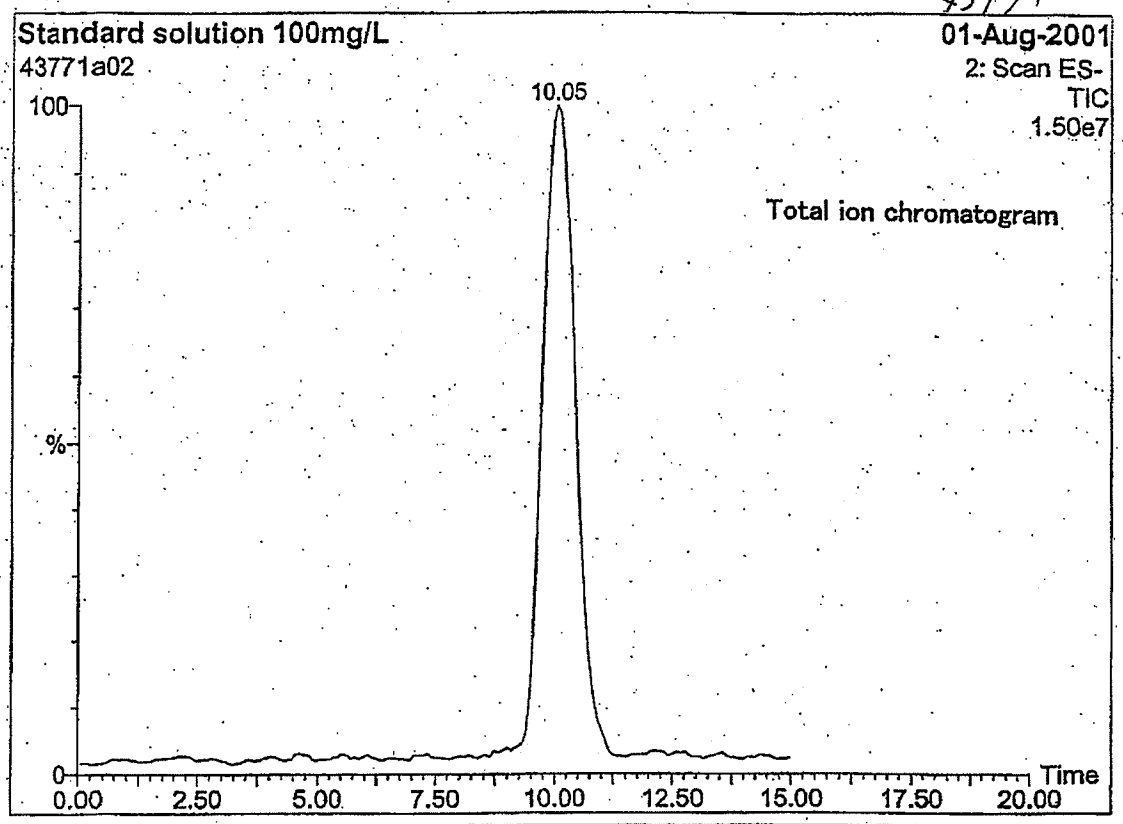
Note -

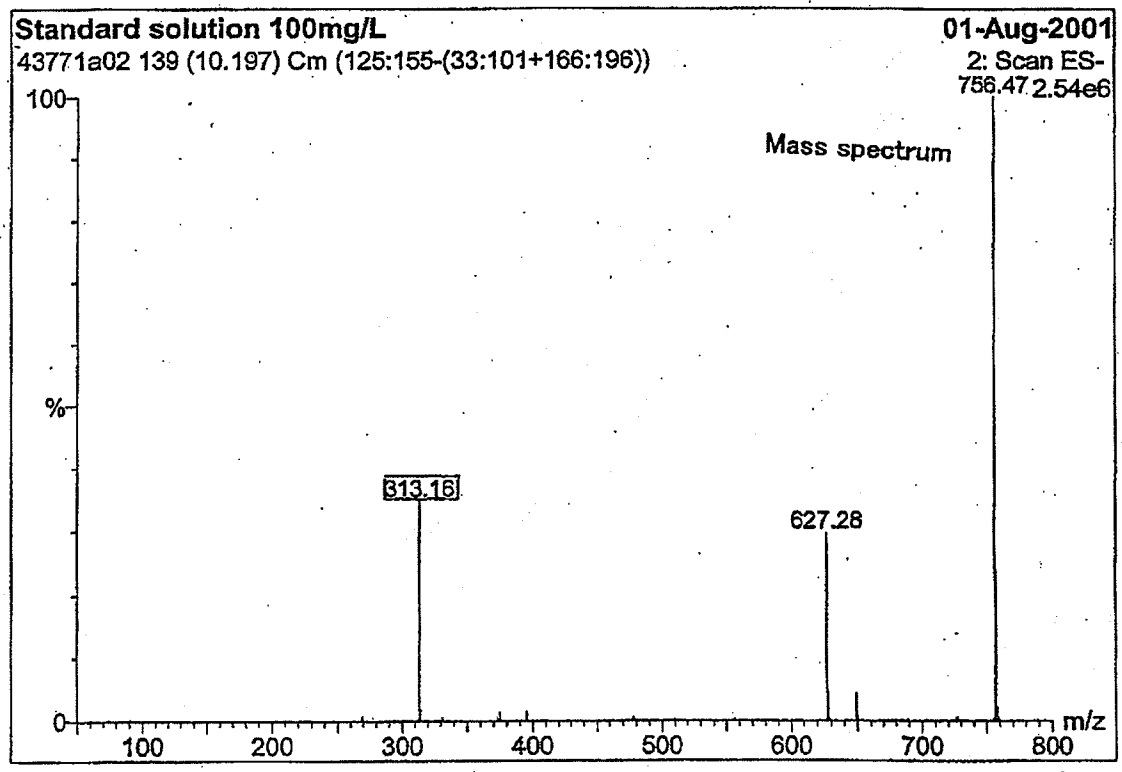
Kurume Laboratory, Chemicals Evaluation and Research Institute, Japan

Fig. 24-1 MS spectrum of carboxylic acid (analytical conditions)

Date 2001 / 8 / 1 Operator I. yoshi

43771





2001. 8. 1 I. yoshi

CONTAINS CONFIDENTIAL BUSINESS INFORMATION

Fig. 24-2 MS spectrum of carboxylic acid

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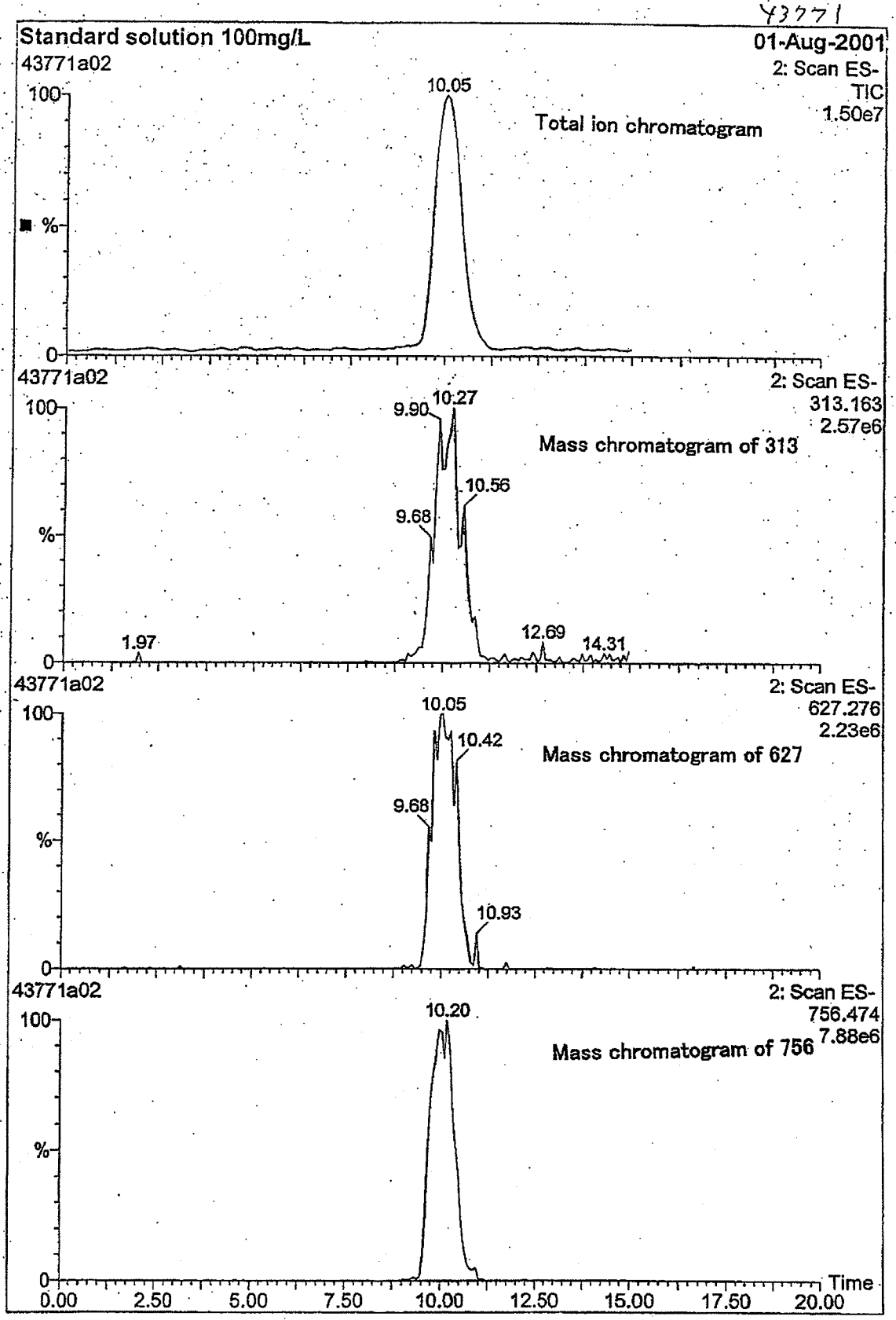


2001. 8. 1 I. Goshi

Fig. 24-3 MS spectrum of carboxylic acid

$F(CF_2)_5COOH$

M.W. 314

m/z	Molecular-related ion
756	$(2M + (C_4H_9)_2NH - H)^-$
627	$(2M - H)^-$
313	$(M - H)^-$

Fig. 24-4 MS spectrum of carboxylic acid

Date 2001/ 8 / 1 Name S. Yoshi

Study No. 43771

Instrument MS : Micromass Quattro Ultima

HPLC : Agilent HP-1100

Sample Carboxylic acid

HPLC Conditions

Inlet system Column

Sample size 5 μL

(Solvent Methanol/water (1/1 V/V))

Column L-column ODS (SUS)

Column size 15 cm x 2.1 mm I.D., Column temp. 25 ℃

Eluent A (40%) : Water (containing 5mmol/L di-n-butylamine acetate)

B (60%) : Methanol (containing 5mmol/L di-n-butylamine acetate)

Flow rate 0.2 mL/min

MS Conditions

Ionization mode ESI, Detection mode Negative

Probe ESI Capillary 3.5 kV, Desolvation temp. 400 ℃, Desolvation gas 650 L/hr

Source Cone 20 V

Source block temp. 120 ℃

MS Ion energy -0.2 V, Multiplier 600 V

Monitoring ion m/z 100 – 600

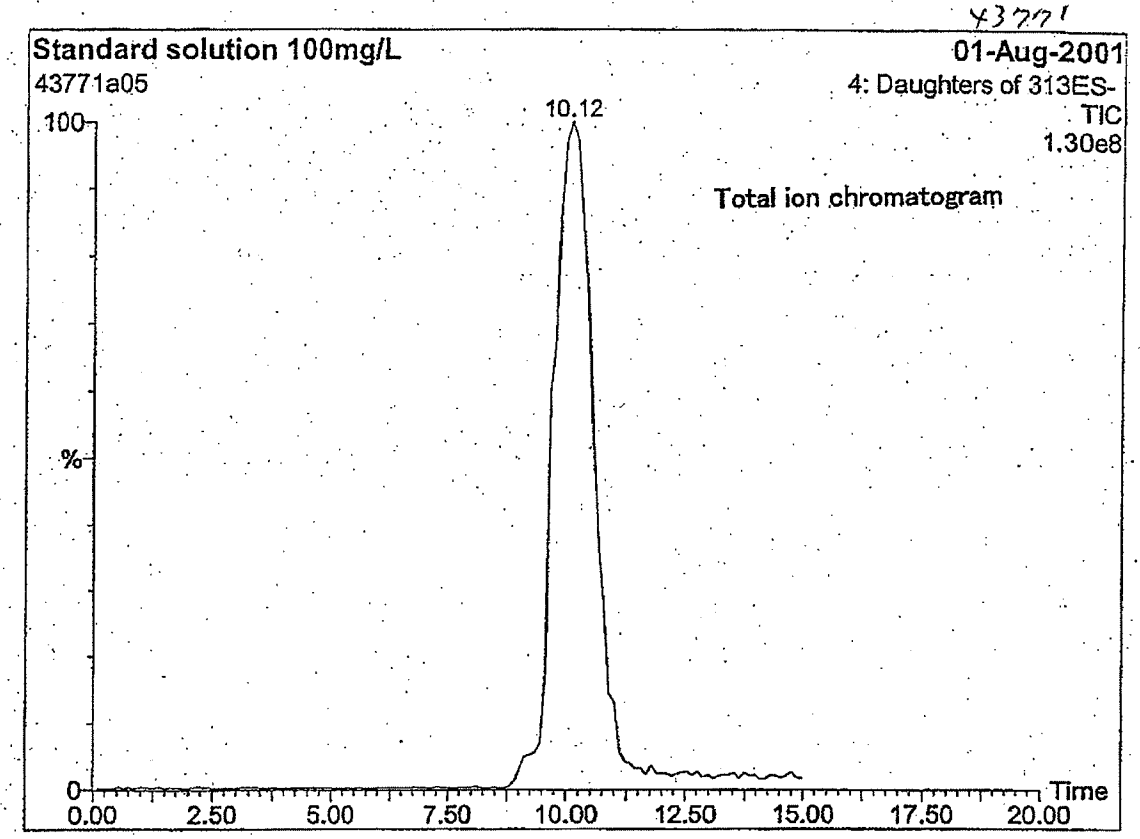
Note –

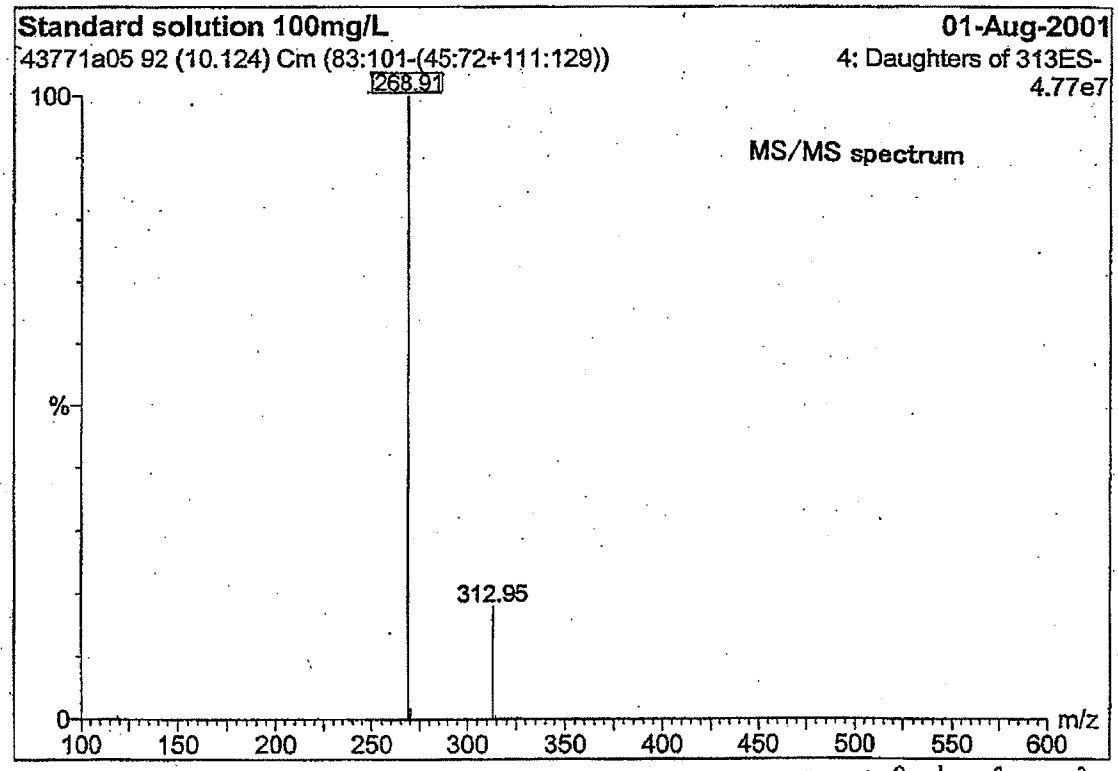
Kurume Laboratory, Chemicals Evaluation and Research Institute, Japan

Fig. 25-1 MS/MS spectrum of carboxylic acid (analytical conditions)

Date 2001/8/1 Operator A. Yoshi

43771





2001.8-1 I. Yoshi

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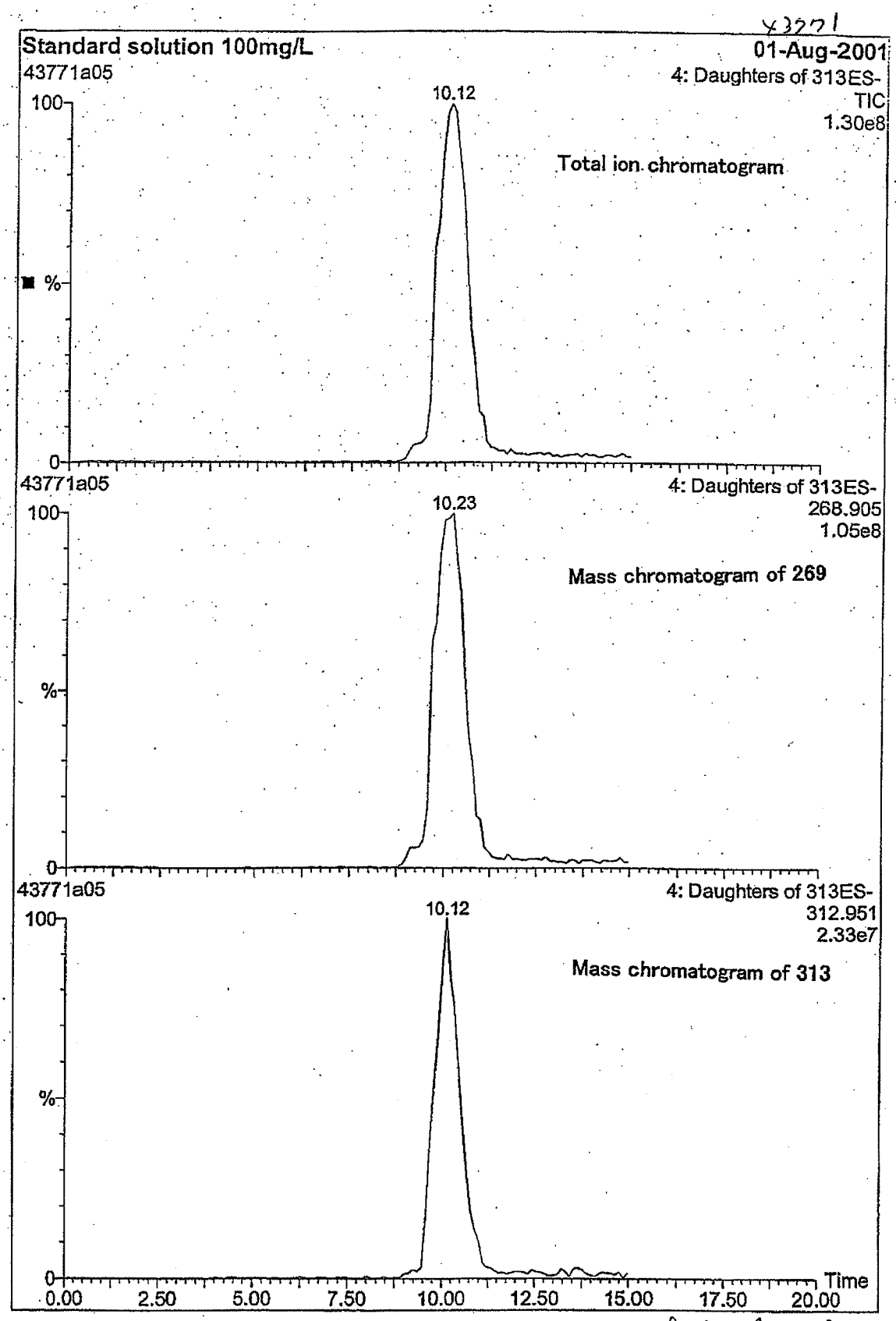
Fig. 25–2 MS/MS spectrum of carboxylic acid



2001. 8. 1 I. Yoshi

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Fig. 25–3 MS/MS spectrum of carboxylic acid

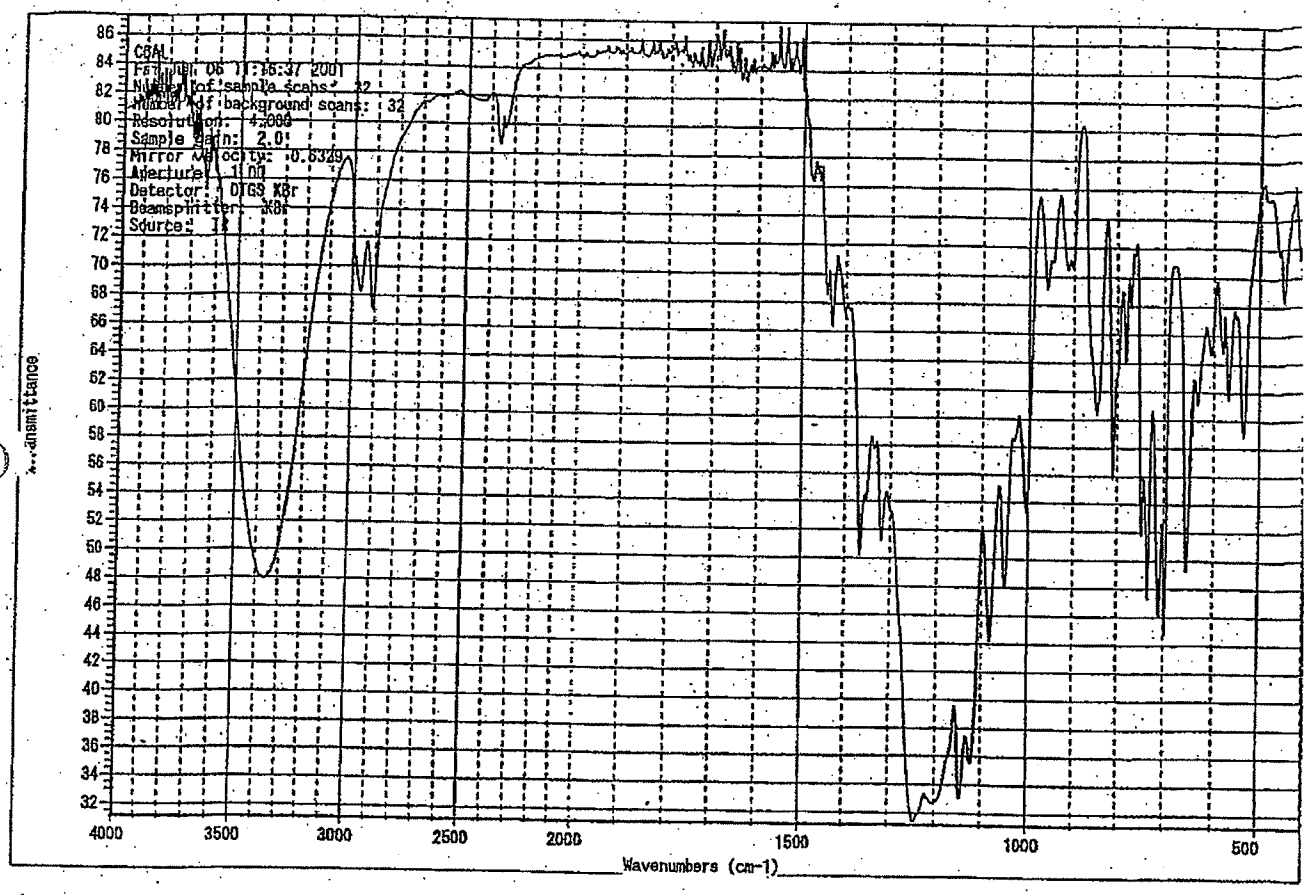
$F(CF_2)_5COOH$

M.W. 314

m/z	Product ion
313	$(M - H)^-$
269	$(M - COOH)^-$

Fig. 25-4 MS/MS spectrum of carboxylic acid

Date 2001/ 8 / 1 Name S. yoshi



Reference 2 IR spectrum supplied by sponsor

確認番号　００１

基準適合試験施設確認書

財団法人化学物質評価研究機構
　理事長　平石　次郎　殿

化学物質の審査及び製造等の規制に関する法律に係る試験施設に関する基準確認実施要領に基づき、下記試験施設については、新規化学物質に係る試験及び指定化学物質に係る有害性の調査の項目等を定める命令第４条に規定する試験施設に関する基準に適合していることを確認します。
なお、確認の有効期限は、本確認書の交付日から起算して３年間とします。

平成１２年１１月１７日

通商産業省基礎産業局長　岡本　巌

記

試験施設の名称	財団法人化学物質評価研究機構 久留米事業所
試験施設の所在地	福岡県久留米市中央町１９番１４号
試験項目	分解度試験、濃縮度試験、分配係数試験