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Study number	14738

FINAL REPORT

Biodegradation study of 13F-SFA by microorganisms

February 1, 2007

Kurume Laboratory
Chemicals Evaluation and Research Institute, Japan

STATEMENT

Kurume Laboratory
Chemicals Evaluation and
Research Institute, Japan

Sponsor DAIKIN INDUSTRIES, LTD.

Title Biodegradation study of 13F-SFA by microorganisms

Study number 14738

I, the undersigned, hereby declare that this report provides a correct English translation of the Final Report (Study No. 14738, issued on February 1, 2007).

Date August 20, 2009

Translator

GLP STATEMENT

Kurume Laboratory
Chemicals Evaluation and
Research Institute, Japan

Sponsor DAIKIN INDUSTRIES, LTD.

Title Biodegradation study of 13F-SFA by microorganisms

Study number 14738

The study described in this report was conducted in compliance with the following GLP principles:

(1) "Standard Concerning Testing Facility Relating to New Chemical Substances" (November 21, 2003; No. 1121003, Pharmaceutical and Food Safety Bureau, Ministry of Health, Labour and Welfare; November 17, 2003, No. 3, Manufacturing Industries Bureau, Ministry of Economy, Trade and Industry; No. 031121004, Environmental Policy Bureau, Ministry of the Environment)

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

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

Date February 1, 2007

Study Director Signed in original

QUALITY ASSURANCE STATEMENT

Kurume Laboratory
Chemicals Evaluation and
Research Institute, Japan

Sponsor DAIKIN INDUSTRIES, LTD.

Title Biodegradation study of 13F-SFA by microorganisms

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

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

Item of inspection / audit	Date of inspection / audit	Date of report to Study Director and Test Facility Management
Study plan draft	November 16, 2006	November 16, 2006
Study plan	November 17, 2006	November 17, 2006
Amendment to study plan	January 15, 2007	January 15, 2007
At the start of cultivation	November 21, 2006	November 22, 2006
At the middle of cultivation	December 5, 2006	December 5, 2006
At the end of cultivation	December 19, 2006	December 20, 2006
	December 20, 2006	December 20, 2006
	January 15, 2007	January 15, 2007
Raw data and final report draft	January 29, 2007	January 29, 2007
Final report	February 1, 2007	February 1, 2007

Date February 1, 2007

Head of Quality Assurance Unit Signed in original

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Title

Biodegradation study of 13F-SFA by microorganisms

Sponsor

DAIKIN INDUSTRIES, LTD.
1-1, Nishi-Hitotsuya, Settsu-shi, Osaka 566-8585, Japan

Test facility

Kurume Laboratory
Chemicals Evaluation and Research Institute, Japan
2-7, 3-chome, Miyanojin, Kurume-shi, Fukuoka 839-0801, Japan

Objective

This study was performed to evaluate the biodegradability of 13F-SFA by microorganisms.

Test method

This study was performed according to the following test methods.

(1) "Method for Testing the Biodegradability of Chemical Substances by Microorganisms" stipulated in the "Testing Methods for New Chemical Substances" (November 21, 2003; No. 1121002, Pharmaceutical and Food Safety Bureau, Ministry of Health, Labour and Welfare; November 13, 2003, No. 2, Manufacturing Industries Bureau, Ministry of Economy, Trade and Industry; No. 031121002, Environmental Policy Bureau, Ministry of the Environment)

(2) "Ready Biodegradability: Modified MITI Test (I) (Guideline 301C, Revised July 17, 1992)" in the OECD Guidelines for Testing of Chemicals

Applied GLP

This study was conducted in compliance with the following GLP principles:

(1) "Standard Concerning Testing Facility Relating to New Chemical Substances" (November 21, 2003; No. 1121003, Pharmaceutical and Food Safety Bureau, Ministry of Health, Labour and Welfare; November 17, 2003, No. 3, Manufacturing Industries Bureau, Ministry of Economy, Trade and Industry; No. 031121004, Environmental Policy Bureau, Ministry of the Environment)

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

Dates

Study initiation date	November 17, 2006
Experimental starting date	November 21, 2006
Experimental completion date	December 19, 2006
Study completion date	February 1, 2007

Storage of test item, raw data, etc.

(1) Test item

The test sample is sealed in a storage vessel and stored in archives in this laboratory for ten years after the receipt of notice specified under Clause 1 or Clause 2 in Article 4, Clause 2 or Clause 3 or Clause 8 in Article 4-2, and Clause 2 in Article 5-4 or Clause 2 in Article 24 or Clause 2 in Article 25-3 of "Law Concerning Examination and Regulation of Manufacture, etc. of Chemical Substances". If it is not stable for the storage period, it is stored as long while it is kept stable. Treatment of the test sample after the storage period will be discussed with 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 or Clause 2 in Article 4, Clause 2 or Clause 3 or Clause 8 in Article 4-2, and Clause 2 in Article 5-4 or Clause 2 in Article 24 or Clause 2 in Article 25-3 of "Law Concerning Examination and Regulation of Manufacture, etc. of Chemical Substances". Treatment of raw data and materials, etc. after the storage period will be discussed with sponsor.

Personnel

Study Director

(1^{st} Chemical Safety Section)

Study personnel
(Operation of biodegradation test)

Staff for cultivation of activated sludge

Approval of final report

Study Director	Date	February 1, 2007
	Signature	Signed in original

SUMMARY

Title

Biodegradation study of 13F-SFA by microorganisms

Conditions of cultivation

(1) Concentration of test item	100 mg/L
(2) Concentration of activated sludge (as the concentration of suspended solid)	30 mg/L
(3) Volume of test solution	300 mL
(4) Cultivation temperature	25±1°C
(5) Cultivation duration (under the conditions of darkness)	28 days

Measurement and analysis for calculation of percentage biodegradation

(1) Measurement of biochemical oxygen demand (BOD) with a closed system oxygen consumption measuring apparatus
(2) Determination of test item by gas chromatography (GC)

Other analysis

(1) Determination of 2-(perfluorohexyl)ethanol by gas chromatography (GC)
(2) Determination of 3,3,4,4,5,5,6,6,7,7,8,8,8-tridecafluorooctanoic acid by liquid chromatography-mass spectrometry (LC-MS)
(3) Determination of acrylic acid by high-performance liquid chromatography (HPLC)

Results

(1) Percentage biodegradation by BOD	6%,	13%,	12%	average 10%
(2) Percentage biodegradation of test item (GC)	11%,	13%,	8%	average 11%

Conclusion

Some of the test item was converted into acrylic acid, 2-(perfluorohexyl)ethanol and 3,3,4,4,5,5,6,6,7,7,8,8,8-tridecafluorooctanoic acid under the test conditions of this study. It was considered that some of acrylic acid transferred from the test solution to the absorbent for carbon dioxide attached to the test vessel and that the other was biodegraded by microorganisms. The rest of the test item, 2-(perfluorohexyl)ethanol and 3,3,4,4,5,5,6,6,7,7,8,8,8-tridecafluorooctanoic acid remained in the test solution.

1. Test item

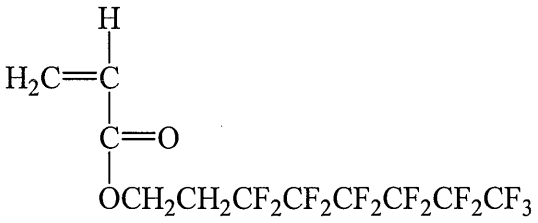
In this report, 13F-SFA has the following chemical name, etc.

1.1 Chemical name*1

3,3,4,4,5,5,6,6,7,7,8,8,8-tridecafluorooctyl acrylate

1.2 Chemical structure, etc. *1

Structural formula



Molecular formula	$C_{11}H_7F_{13}O_2$
Molecular weight	418.15
CAS number	17527-29-6

*1 Information supplied by the sponsor

2. Test sample

2.1 Supplier and lot number*1

Supplier	DAIKIN INDUSTRIES, LTD.
Lot number	6X002

2.2 Purity*1

Test item	99.7%
Impurity	Unknown 0.3%

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. 14 and Reference 3).

2.4 Physicochemical properties[*1]

Appearance	Colorless transparent liquid	
Boiling point	78°C (8 mmHg)	
Density	1.554 g/cm^3 (25°C)	
Solubility	Water	Insoluble
	Dimethylsulfoxide	Soluble (miscible in all proportions)
	Acetone	Soluble (miscible in all proportions)

*1 Information supplied by the sponsor

2.5 Storage and stability

Storage condition

Dark storage place at room temperature

Stability

The test item was stable under the storage conditions, as shown by the finding that IR spectra of the test item before the experimental start and after the experimental completion were identical (see Fig. 14).

3. Activated sludge

3.1 Preparation of activated sludge

Activated sludge used in the present test was prepared as follows.

(1) Sampling sites

On-site sludge sampling was carried out at the following ten locations in Japan.

Fushikogawa city sewage plant (Sapporo-shi, Hokkaido)
Fukashiba industrial sewage plant (Kamisu-shi, Ibaraki)
Nakahama city sewage plant (Osaka-shi, Osaka)
Ochiai city sewage plant (Shinjuku-ku, Tokyo)
Kitakami River (Ishinomaki-shi, Miyagi)
Shinano River (Niigata-shi, Niigata)
Yoshino River (Tokushima-shi, Tokushima)
Lake Biwa (Otsu-shi, Shiga)
Hiroshima Bay (Hiroshima-shi, Hiroshima)
Dokai Bay (Kitakyushu-shi, Fukuoka)

(2) Sampling method

Sewage plant

Return sludge was collected.

Rivers, lake and sea

Surface water and surface soil which was in contact with the atmosphere were collected.

(3) Sampling date

September, 2006

(4) Preparation method of activated sludge

Activated sludge was prepared as follows to maintain its uniformity.

The mixed filtrate (5 L) of the supernatant of the sludge collected at sampling sites was mixed with the filtrate (5 L) of the supernatant of the activated sludge*2 previously cultivated for about 3 months. The mixed filtrate (10 L) was aerated*3 after the pH value of the mixture was adjusted to 7.0±1.0.

*2 Activated sludge cultivated the mixed filtrate (10 L) of the supernatant of the sludge collected at sampling sites according to Section 3.2.

*3 Prefiltered open air was used.

3.2 Cultivation

After ceasing aeration of the sludge mixture for approximately 30 minutes, supernatant corresponding to about one third of the whole volume was removed. Dechlorinated water was added to the remaining portion so that the total volume reached 10 L. This mixture was aerated for 30 minutes or more, and then a predetermined amount of synthetic sewage*4 was added to the mixture so that the concentration of the synthetic sewage was 0.1% in the volume of dechlorinated water added. This procedure was repeated once every day. Cultivation was carried out at 25±2°C.

*4 Synthetic sewage was prepared as follows:

Glucose, peptone and potassium dihydrogenphosphate were dissolved in purified water to obtain 50 g/L of the solution for each component. The pH of the solution was adjusted to 7.0±1.0 with sodium hydroxide.

3.3 Control and use

During cultivation, the appearance of the supernatant, sedimentation of the sludge, formation of flock, pH, dissolved oxygen concentration in the solution and temperature were checked to maintain a normal state of sludge. It was confirmed that these were within the scope of the control standard stipulated in the "Testing Methods for New Chemical Substances", and these results were stored as raw data. Microflora in the activated sludge was microscopically observed and sludge with no abnormal symptoms was used for the test. The activated sludge, which was cultivated for 19 hours after it had been added the synthetic sewage, was used.

3.4 Inspection of activity and date of initiation of use of activated sludge

(1) Inspection of activity

Activity of the sludge was assessed using standard items before initiation of use.

(2) Date of initiation of use

October 17, 2006

4. Performance of biodegradation test

4.1 Preparations for test

(1) Measurement of concentration of suspended solid in activated sludge

The concentration of suspended solid was measured to determine the amount of activated sludge to add.

Method	In accordance with Japanese Industrial Standards (JIS) K 0102-1998 Section 14.1
Date	November 20, 2006
Result	Concentration of suspended solid in the activated sludge was 3780 mg/L.

(2) Preparation of basal culture medium

Each 3 mL of solutions A, B, C and D, which are prescribed in JIS K 0102-1998 Section 21, were made up to 1000 mL with purified water (Japanese Pharmacopeia, Takasugi Pharmaceutical Co., Ltd.), and then the pH of this solution was adjusted to 7.0.

(3) Reference item

Aniline (reagent grade, Showa Chemicals Co., Ltd. Lot No. SR-2626U) was used as a reference item to confirm that the sludge was sufficiently active.

4.2 Preparation of test solutions

The following test solutions were prepared and cultivated under the conditions described in Section 4.3.

(1) Addition of test item or aniline

(a) Test solution (water + test item) (n=1, Vessel No. 1)

In one test vessel, 19.5 μL [30.3 mg = 19.5 μL × 1.554 g/cm^3 (density)] of the test sample was taken out by microsyringe and added to 300 mL of purified water, so that the concentration of the test item reached 100 mg/L.

(b) Test solution (sludge + test item) (n=3, Vessel Nos. 2, 3 and 4)

In each test vessel, 19.5 μL [30.3 mg = 19.5 μL × 1.554 g/cm^3 (density)] of the test sample was taken out by microsyringe and added to the basal culture medium [the volume was less than 300 mL by the volume (2.38 mL) of activated sludge inoculated], so that the concentration of the test item reached 100 mg/L.

(c) Test solution (sludge + aniline) (n=1, Vessel No. 6)

In one test vessel, 29.5 μL [30 mg = 29.5 μL × 1.022 g/cm^3 (density)] of aniline was taken out by microsyringe and added to the basal culture medium [the volume was less than 300 mL by the volume (2.38 mL) of activated sludge inoculated], so that the concentration of aniline reached 100 mg/L.

(d) Test solution (control blank) (n=1, Vessel No. 5)

In one test vessel, nothing was added to the basal culture medium [the volume was less than 300 mL by the volume (2.38 mL) of activated sludge inoculated].

(2) Inoculation of activated sludge

The activated sludge cultivated under the conditions described in Section 3 was added to each test vessel, (b), (c) and (d), so that the concentration of the suspended solid reached 30 mg/L.

4.3 Instruments and conditions of cultivation

(1) Instruments for cultivation

Closed system oxygen consumption measuring apparatus
(Temperature controlled bath and measuring unit : Ohkura Electric Co., Ltd.)
(Data sampler : Asahi Techneion Co., Ltd.)
Vessel 300 mL in volume
Test solutions described in Section 4.2 (a), (b) and (d) : Improved type for volatile substance
Test solution described in Section 4.2 (c) : Improved type
Absorbent for carbon dioxide
Soda lime No.1 (for absorption of carbon dioxide, Wako Pure Chemical Industries, Ltd.)

The test vessels described in Section 4.2 (a), (b) and (d) were connected to the measuring unit via tube with cock.

(2) Conditions of cultivation

Cultivation temperature	25±1°C
Cultivation duration	28 days (under the conditions of darkness)
Stirring method	Each test solution was stirred by a magnetic stirrer.

(3) Room

Apparatus room A

4.4 Observation and measurement of test conditions

(1) Observation of test solution

During the cultivation, the appearance of the test solution was observed once a day and conditions of the instruments were checked properly.

(2) Measurement of biochemical oxygen demand (BOD)

During the cultivation period, the change in BOD of the test solutions was measured by autorecording using a data sampler. Cultivation temperature was measured and recorded once a day.

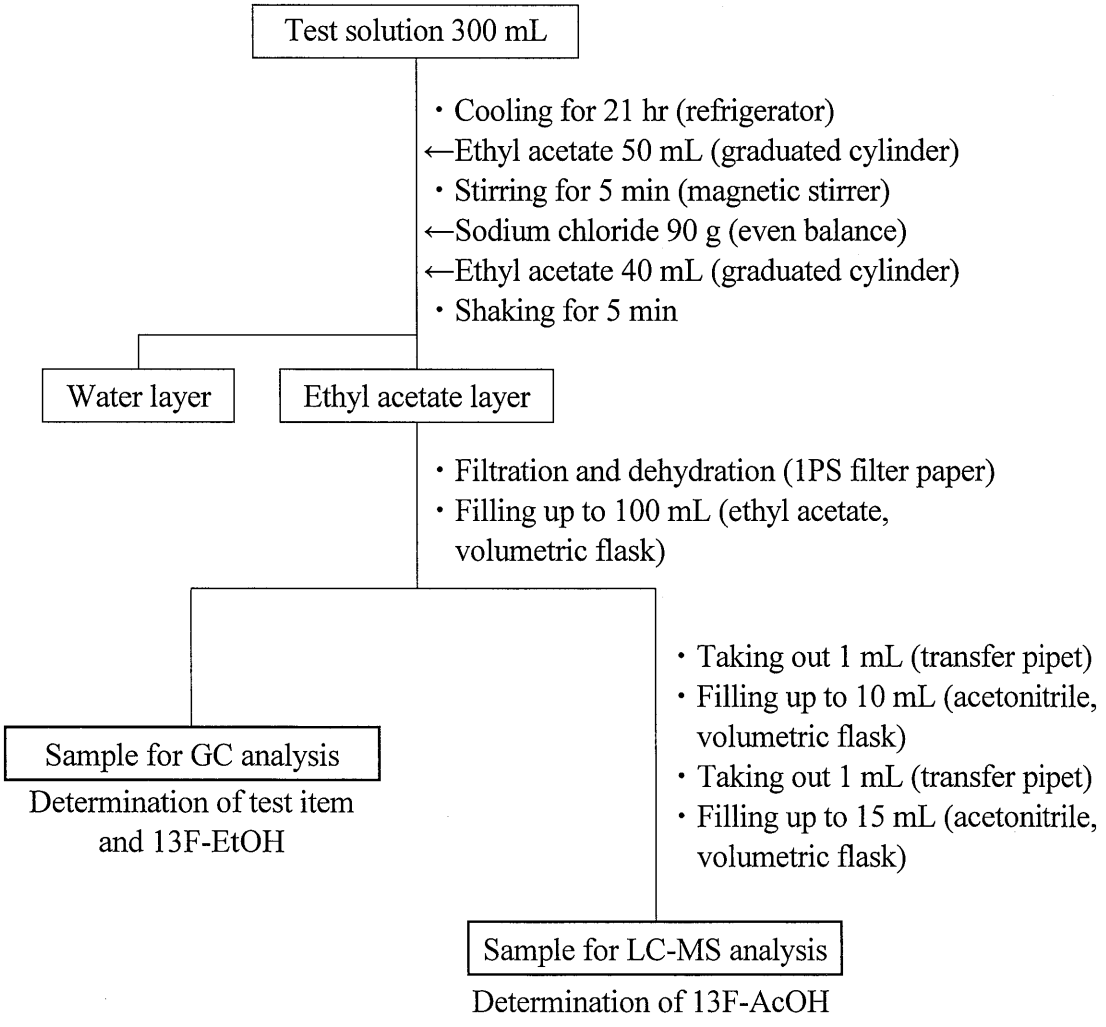
4.5 Analysis of test solution

After the end of the cultivation, the test item and 2-(perfluorohexyl)ethanol (hereinafter referred to as 13F-EtOH), which was a converted product expected from results of the preliminary test prior to this study, in the test solutions were determined. Production of other converted product was suggested from results of the determinations of the test item and 13F-EtOH. Therefore, 3,3,4,4,5,5,6,6,7,7,8,8,8-tridecafluorooctanoic acid (hereinafter referred to as 13F-AcOH), which was also expected to be produced, in the test solutions was determined. The pH of the test solutions was not measured because the test item was thought to be a volatile compound.

Acrylic acid [METI number (2)-984, ready biodegradability] was expected to be produced simultaneously with the production of 13F-EtOH in the test solutions. However, the loss of the test item and 13F-EtOH due to their volatilization in the pretreatment for analysis of acrylic acid was considered. Therefore, additional test solutions were prepared besides the test solutions described in Section 4.2 and were used for the determination of acrylic acid (see Section 4.6).

4.5.1 Pretreatment of test solutions for analysis

After the end of the cultivation, the test solution (water + test item), the test solutions (sludge + test item) and the test solution (control blank) were pretreated to prepare samples for gas chromatography (GC) analysis of the test item and 13F-EtOH, and liquid chromatography-mass spectrometry (LC-MS) of 13F-AcOH as follows.



4.5.2 Quantitative analysis

(1) Determinations of test item and 13F-EtOH

The samples for GC analysis were analyzed under the following conditions. The concentration of the test item in the sample for GC analysis was proportionally calculated by comparing the peak area on the chromatogram of the sample for GC analysis with that on the chromatogram of 303 mg/L standard solution (see Table-5 and Fig. 9). The concentration of 13F-EtOH in the sample for GC analysis was proportionally calculated by comparing the peak area on the chromatogram of the sample for GC analysis with that on the chromatogram of 252 mg/L standard solution (see Table-6 and Fig. 9).

The lowest detectable peak area of the test item and 13F-EtOH was regarded as 1500 μV・sec considering the noise level, which corresponded to the test item concentration of 3.0 mg/L and 13F-EtOH concentration of 3.8 mg/L.

(a) Analytical conditions

Instrument	Gas chromatograph Hewlett-Packard Company type HP6890 Series
Detector	Flame ionization detector (FID)
Column	DB-17 film thickness 0.25 μm (Agilent Technologies, Inc.) 30 m × 0.25 mm I.D. fused silica
Column temp.	40°C (3 min) → 140°C (0 min)
Temp. rate	15°C/min
Injection temp.	200°C
Carrier gas	Helium
Column flow rate	1.0 mL/min
Hydrogen	40 mL/min
Air	400 mL/min
Sample size	1 μL
Injection method	Split
Split ratio	5:1
Detector	
Temp.	200°C
Sensitivity	Range 2^0

(b) Preparation of standard solution

The standard solutions to determine the concentration of the test item and 13F-EtOH in the sample for GC analysis were prepared as follows.

① Test item

19.5 μL [30.3 mg = 19.5 μL × 1.554 g/cm^3 (density)] of the test sample was taken out by microsyringe and dissolved in ethyl acetate to obtain 1010 mg/L solution of the test item. 303 mg/L standard solution was then prepared from this solution by dilution with ethyl acetate.

② 13F-EtOH

15.0 μL [25.2 mg = 15.0 μL × 1.678 g/cm^3 (density)] of authentic sample of 13F-EtOH (supplied by the sponsor)[*5] was taken out by microsyringe and dissolved in ethyl acetate to obtain 839 mg/L solution of the 13F-EtOH. 252 mg/L standard solution was then prepared from this solution by dilution with ethyl acetate.

*5 Purity 99.8%
Lot number 180804
13F-EtOH was treated as 100 % in purity.

(c) Calibration curve

① Test item

Standard solutions of 75.8, 152 and 303 mg/L were prepared by the same method as described in (b)①. These solutions were analyzed according to the analytical conditions described in (a). A calibration curve was drawn based on the relation between the peak area on the chromatograms and the respective concentrations (see Fig. 2).

② 13F-EtOH

Standard solutions of 62.9, 126 and 252 mg/L were prepared by the same method as described in (b)②. These solutions were analyzed according to the analytical conditions described in (a). A calibration curve was drawn based on the relation between the peak area on the chromatograms and the respective concentrations (see Fig. 3).

(2) Determination of 13F-AcOH

The samples for LC-MS analysis were analyzed under the following conditions. 13F-AcOH was detected as the negative ions of *m/z* = 376.9 and 754.8 in the LC-MS analysis. Therefore, these two ions were selected as measurement ions. The concentration of 13F-AcOH in the sample for LC-MS analysis was calculated proportionally by comparing the peak area on the total ion chromatogram of the sample for LC-MS analysis with that on the total ion chromatogram of 2.00 mg/L standard solution (see Table-7 and Fig. 10).

The lowest detectable peak area of 13F-AcOH was regarded as 800 considering the noise level, which corresponded to 13F-AcOH concentration of 0.019 mg/L.

(a) Analytical conditions

Instrument	Liquid chromatograph-mass spectrometer
HPLC system	Waters Corporation type Alliance2690
Mass spectrometer	Waters Corporation type ZMD

LC conditions

Column	L-column ODS (15 cm × 2.1 mm I.D., Chemicals Evaluation and Research Institute)
Column temp.	40°C
Eluent	A (80%) : Acetonitrile / formic acid (500/0.25 v/v) B (20%) : Water[*6] / formic acid (500/0.25 v/v)
Flow rate	0.2 mL/min
Sample size	1 μL

*6 City water was treated with ultrapure water system.

Mass conditions

Ionization mode	Electrospray ionization (ESI)
Detection ion	Negative
Detection mode	Selected ion monitoring (SIM)
Measurement ion (*m/z*)	376.9 and 754.8 (see Fig. 13)
Ion source temp.	120°C
Desolvation temp.	350°C
Cone voltage	20 V

(b) Preparation of standard solution

The standard solution to determine the concentration of 13F-AcOH in the sample for LC-MS analysis was prepared as follows.

100 mg of authentic sample of 13F-AcOH (supplied by the sponsor)[*7] was accurately weighed and dissolved in acetonitrile to obtain 1000 mg/L solution of 13F-AcOH. 2.00 mg/L standard solution was then prepared from this solution by dilution with acetonitrile.

*7 Purity 99.4%
Lot number S6X01
13F-AcOH was treated as 100% in purity.

(c) Calibration curve

Standard solutions of 0.500, 1.00 and 2.00 mg/L 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 based on the relation between the peak area on the total ion chromatograms and the respective concentrations (see Fig. 4).

4.5.3 Recovery test and blank test

Each two test solutions (water + test item), two test solutions (sludge + test item), two test solutions (water + 13F-EtOH) and two test solutions (sludge + 13F-EtOH) , two test solutions (water + 13F-AcOH) and two test solutions (sludge + 13F-AcOH) for recovery tests were prepared according to the methods described in Section 4.2. The test solutions were pretreated in accordance with the method described in Section 4.5.1, then analyzed according to the procedures and analytical conditions described in Section 4.5.2.

Each test solution for blank tests was prepared according to the method described in Section 4.2. The test solutions for blank tests were analyzed in the same way as the recovery tests. As for the blank tests, no peak appeared around the peaks of the test item, 13F-EtOH and 13F-AcOH on the chromatograms.

Two individual recovery rates and their averages of the test item, 13F-EtOH and 13F-AcOH on the analytical procedure are shown below. The average recovery rates were used as correction factors, for the determination of the test item, 13F-EtOH and 13F-AcOH in the analytical samples.

(1) Test item (see Table-2 and Fig. 6)

30.3 mg of the test sample was added in the recovery test.

Recovery rate in the test solutions (water + test item)
96.0%, 96.6% average 96.3%

Recovery rate in the test solutions (sludge + test item)
95.6%, 96.1% average 95.8%

(2) 13F-EtOH (see Table-3 and Fig. 7)

25.2 mg of authentic sample of 13F-EtOH was added in the recovery test.

Recovery rate in the test solutions (water + 13F-EtOH)
96.6%, 96.9% average 96.7%

Recovery rate in the test solutions (sludge + 13F-EtOH)
97.3%, 96.2% average 96.7%

(3) 13F-AcOH (see Table-4 and Fig. 8)

30 mg of authentic sample of 13F-AcOH was added in the recovery test.

Recovery rate in the test solutions (water + 13F-AcOH)
97.1%, 97.2% average 97.1%

Recovery rate in the test solutions (sludge + 13F-AcOH)
97.3%, 95.7% average 96.5%

4.6 Preparation and analysis of test solutions for determination of acrylic acid

Besides the test solutions described in Section 4.2, the test solution (water + test item) and the test solution (sludge + test item) were prepared according to Section 4.2 and were cultivated for determination of acrylic acid which was expected to be produced. During the cultivation, the appearance of the test solution was observed according to Section 4.4(1). After the end of the cultivation, acrylic acid in the test solutions was determined.

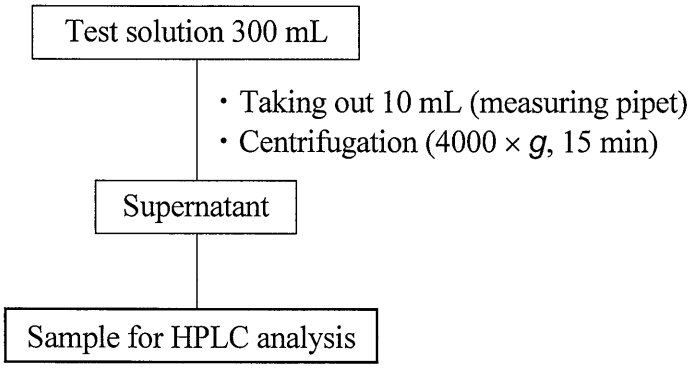
(1) Conditions of cultivation

Cultivation method	Test vessel was the improved type for volatile substance whose volume was 300 mL. Each test solution was cultivated under the closed system performed by connecting the test vessel with air tank via tube. Absorbent for carbon dioxide described in Section 4.3(1) was attached to each test vessel.
Cultivation temperature	Approximately 25°C
Cultivation duration	28 days (under conditions of darkness)[*8]
Stirring method	Each test solution was stirred by a magnetic stirrer.
Room	Environmentally controlled room

*8 Cultivated from the experimental starting date to the experimental completion date described in page 2.

(2) Pretreatment of test solutions for analysis

The test solutions for the determination of acrylic acid were pretreated to prepare samples for high-performance liquid chromatography (HPLC) analysis of acrylic acid as follows.



(3) Determination of acrylic acid

The samples for HPLC analysis were analyzed under the following conditions. The concentration of acrylic acid in the sample for HPLC analysis was calculated proportionally by comparing the peak area on the chromatogram of the sample for HPLC analysis with that on the chromatogram of 20.0 mg/L standard solution (see Table-8 and Fig. 11).

The lowest detectable peak area of acrylic acid was regarded as 14000 μV·sec considering the noise level, which corresponded to acrylic acid concentration of 0.20 mg/L.

(a) Analytical conditions

Instrument	High-performance liquid chromatograph
Pump	Shimadzu Corporation type LC-10ADvp
Detector	Shimadzu Corporation type SPD-10AVvp
Column oven	Shimadzu Corporation type CTO-10ACvp
Auto injector	Shimadzu Corporation type SIL-10ADvp
System controller	Shimadzu Corporation type SCL-10Avp
Degasser	Shimadzu Corporation type DGU-14AM
Column	L-column ODS (15 cm × 2.1 mm I.D., Chemicals Evaluation and Research Institute)
Column temp.	35 °C
Eluent	A (10%) : Acetonitrile / phosphoric acid (1000/1 v/v) B (90%) : Water[*6] / phosphoric acid (1000/1 v/v)
Flow rate	0.2 mL/min
Measurement wavelength	205 nm (see Fig. 12)
Sample size	5 μL
Detector output	1 V/AU

(b) Preparation of standard solution

The standard solution to determine the concentration of acrylic acid in the sample for HPLC analysis was prepared as follows.

100 mg of authentic sample of acrylic acid (Wako Pure Chemical Industries, Ltd., reagent grade)[*9] was accurately weighed and dissolved in purified water to obtain 1000 mg/L solution of acrylic acid. 20.0 mg/L standard solution was then prepared from this solution by dilution with purified water.

*9 Acrylic acid was treated as 100% in purity.

(c) Calibration curve

Standard solutions of 5.00, 10.0 and 20.0 mg/L 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 based on the relation between the peak area on the chromatograms and the respective concentrations (see Fig. 5).

4.7 Calculation of percentage biodegradation

The percentage biodegradations were calculated by the following equations and rounded off to the whole number.

(1) Percentage biodegradation by BOD

$$\text{Percentage biodegradation (\%)} = \frac{\text{BOD - B}}{\text{TOD}^{*10}} \times 100$$

BOD : Biochemical oxygen demand in the test solution (sludge + test item) (experimental) (mg)

B : Biochemical oxygen demand in the control blank (experimental) (mg)

TOD^{*10} : Theoretical oxygen demand required when the test item was completely oxidized (theoretical) (mg)

*10 The purity was regarded as 100%.

(2) Percentage biodegradation of test item

$$\text{Percentage biodegradation (\%)} = \frac{\text{Sw - Ss}}{\text{Sw}} \times 100$$

Ss : Residual amount of the test item in the test solution (sludge + test item) (experimental) (mg)

Sw : Residual amount of the test item in the test solution (water + test item) (experimental) (mg)

4.7 Treatment of numerical values

Values were rounded off in accordance with JIS Z 8401:1999 rule B.

5. Validity of test conditions

The validity criteria of the test and the values in this test are shown in the following table. This test was valid because all of the values in this test met the criteria.

		Value in present test	Value of criterion	See
Difference of extremes of replicate values of percentage biodegradation	Percentage biodegradation by BOD	7%	< 20%	Section 7.3 Percentage biodegradation
	Percentage Biodegradation of test item	5%		
Percentage biodegradation of aniline by BOD	After 7 days	56%	≥ 40%	Table-1 Fig.1
	After 14 days	70%	≥ 65%	
BOD value of control blank	After 28 days	5.8 mg	< 18 mg (< 60 mg/L)	Table-1 Fig. 1

6. Factors that affected reliability of test

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

7. Results

7.1 Appearances of test solutions

Appearances of test media in cultivation vessels were as follows.

	Test solution	Appearance	pH
At the start of cultivation	Water + test item	The test item was not dissolved. The test solution was colorless.	-
	Sludge + test item	The test item was not dissolved. The test solution was colorless.	-
At the end of cultivation	Water + test item	Insoluble compound was observed. The test solution was colorless.	-*11
	Sludge + test item	Presence of insoluble compound except the sludge could not be judged. Growth of the sludge could not be judged. The test solution was colorless.	-*11

*11 After the end of the cultivation, the pH of the test solution (water + test item) and the test solutions (sludge + test item) was not measured because the test item was thought to be a volatile compound.

7.2 Analytical results of test solutions

Analytical results of the test solutions for the biodegradation test described in Section 4.2 after 28 days were as follows.

		Water + test item	Sludge + test item			Theoretical amount	Table	Fig.
		Vessel -1	Vessel -2	Vessel -3	Vessel -4			
BOD*12	mg	0.3	1.6	3.6	3.2	27.3	1	1
Residual amount and percentage residue of test item (GC)	mg	27.9	24.9	24.2	25.6	30.3	5	9
	%①	92	82	80	85	-		
Produced amount and percentage production of 13F-EtOH (GC)	mg	1.6	3.6	3.2	2.8	26.4	6	9
	%②	6	13	12	11	-		
Produced amount and percentage production of 13F-AcOH (LC-MS)	mg	0	0.9	0.8	0.8	27.4	7	10
	%③	0	3	3	3	-		
Confirmation of production of acrylic acid	-	Considering the loss of the test item and 13F-EtOH due to their volatilization in the pretreatment for analysis of acrylic acid, acrylic acid was not determined.				-	-	-
Mass balance of alkyl chain part (①+②+③)	%	98	98	95	99	-	-	-

*12 The value of control blank was subtracted from the values of the test solutions (sludge + test item).

Analytical results of the additional test solutions for the determination of acrylic acid described in Section 4.6 after 28 days were as follows.

		Water + test item	Sludge + test item	Theoretical amount	Table	Fig.
Produced amount and percentage production of acrylic acid (HPLC)	mg	0	0	5.2	8	11
	%	0	0	-		

7.3 Percentage biodegradation

Percentage biodegradations of the test solution for the biodegradation test after 28 days were as follows.

		Sludge + test item				Table
		Vessel -2	Vessel -3	Vessel -4	Average	
Percentage biodegradation by BOD	%	6	13	12	10	1
Percentage biodegradation of test item (GC)	%	11	13	8	11	5

7.4 Discussion

(1) Analytical results

The percentage residue of the test item was as low as 92% and 80-85% in the test solution (water + test item) and the test solutions (sludge + test item), respectively. On the basis of these results, 2-(perfluorohexyl)ethanol (hereinafter referred to as 13F-EtOH), which was expected from results of the preliminary test prior to this study, in the test solutions was determined. As a result, 13F-EtOH was detected in the test solution (water + test item) and the test solutions (sludge + test item) (see Fig. 9). Mass balance of alkyl chain part of the test item (see next page) calculated by sum of the percentage residue of the test item and the percentage production of 13F-EtOH (① +② in Section 7.2) was 98% and 92-96% in the test solution (water + test item) and the test solutions (sludge + test item), respectively. Production of other converted product was suggested because the mass balance in the test solutions (sludge + test item) was slightly low. Therefore, 3,3,4,4,5,5,6,6,7,7,8,8,8-tridecafluorooctanoic acid (hereinafter referred to as 13F-AcOH), which was also expected to be produced, in the test solutions was determined. As a result, the percentage production of 13F-AcOH was 3% in the test solutions (sludge + test item) (see Fig. 10). Mass balance of alkyl chain part of the test item recalculated by sum of the percentage residue of the test item and the percentage productions of 13F-EtOH and 13F-AcOH (①+②+③ in Section 7.2) was as high as 98%, 95% and 99% in the test solutions (sludge + test item). Therefore, it was considered that residues that had the alkyl chain part were the test item, 13F-EtOH and 13F-AcOH.

Considering the loss of the test item due to its volatilization in the pretreatment of the test solution for the determination of acrylic acid [METI number (2)-984, ready biodegradability], which was also expected to be produced, the additional test solutions were cultivated and were used for the determination of acrylic acid in the test solutions. However, acrylic acid was not detected in these test solutions (see Fig. 11). Then, a0crylic acid in the absorbents for carbon dioxide of the additional test solutions was determined because it was considered that acrylic acid transferred from the test solution to the absorbent attached to the test vessel. As a result, acrylic acid was detected in the absorbents, i.e. it was produced by hydrolysis of the test item (see Section 8.1).

As the result of the determination of acrylic acid in the absorbents of the test solution (water + test item) for the biodegradation test, the percentage detection of acrylic acid was 6% and was the same as the percentage production of 13F-EtOH (see Sections 7.2 and 8.1). Therefore, it was considered that all acrylic acid transferred to the absorbent was recovered in the pretreatment described in Section 8.1. The percentage detection of acrylic acid (3%, 5% and 3%, average 4%) was lower than sum of the percentage productions of 13F-EtOH and 13F-AcOH (16%, 15% and 14%, average 15%) in the test solutions (sludge + test item) for the biodegradation test (see Sections 7.2 and 8.1). These results suggested that some of the produced acrylic acid was biodegraded in these test solutions. However, it was difficult to evaluate whether acrylic acid was biodegraded from the percentage biodegradation by BOD, because the percentage biodegradation by BOD corresponding to difference between the percentage detection of acrylic acid and the sum of the percentage productions of 13F-EtOH and 13F-AcOH (11%) was as low as 3%.

From the above discussions, it was considered that some of the test item was converted into acrylic acid, 13F-EtOH and 13F-AcOH and that the rest of the test item, 13F-EtOH and 13F-AcOH remained in the test solution.

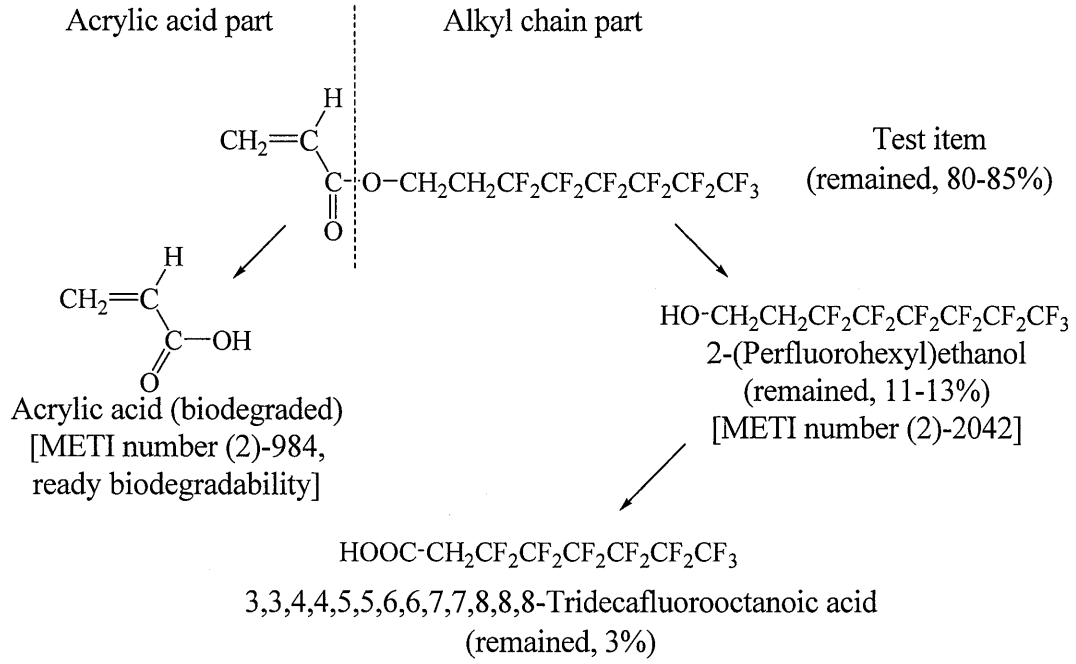
*13 Percentage biodegradation by BOD when 11% of the theoretical amount of acrylic acid was biodegraded.

$C_3H_4O_2 + 3O_2 \rightarrow 3CO_2 + 2H_2O$ $\quad 3O_2 / C_3H_4O_2 = 1.33$

Percentage biodegradation by BOD

$= \{30.0 \times C_3H_4O_2 / C_{11}H_7F_{13}O_2 \times 11 / 100 \times 1.33\} / 27.3 \times 100 = 3(\%)$

Conversion of test item in test solutions (sludge + test item) (presumption)



(2) Percentage biodegradation by BOD

As described in (1), the percentage biodegradation by BOD corresponding to the biodegraded amount of acrylic acid was considered to be as low as 3%. Therefore, the main reason why the percentage biodegradation by BOD was 6%, 13% and 12% was considered to be the variation of the amount of background respiration of the activated sludge among the test solutions.

7.5 Conclusion

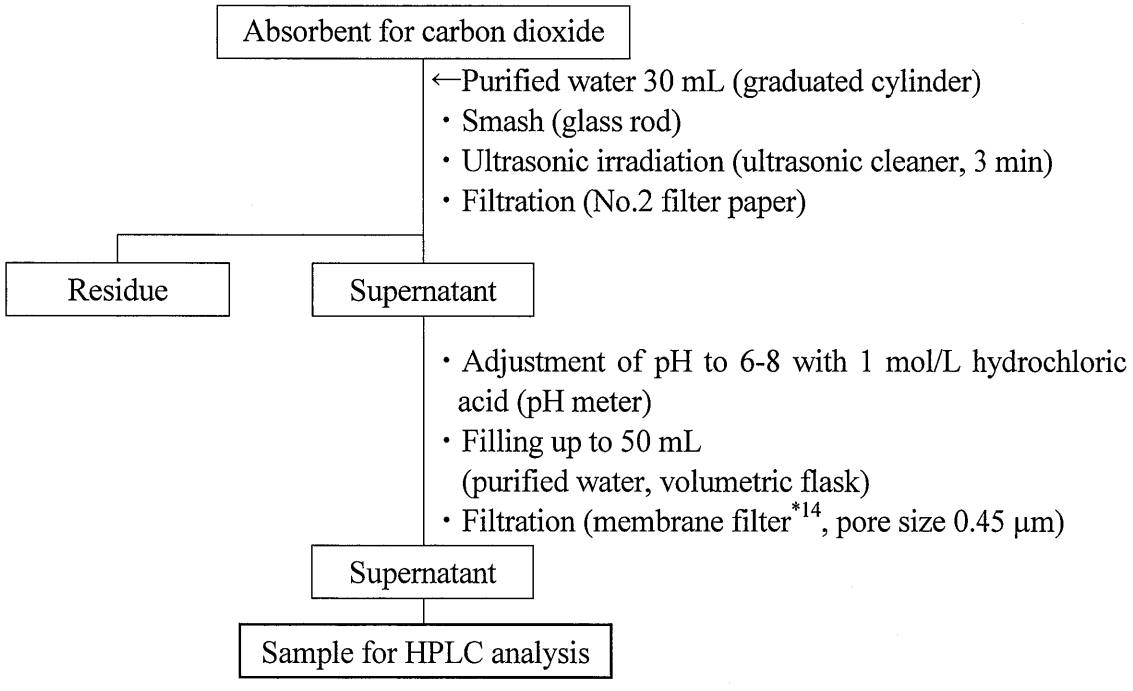
Some of the test item was converted into acrylic acid, 2-(perfluorohexyl)ethanol and 3,3,4,4,5,5,6,6,7,7,8,8,8-tridecafluorooctanoic acid under the test conditions of this study. It was considered that some of acrylic acid transferred from the test solution to the absorbents for carbon dioxide attached to the test vessel and that the other was biodegraded by microorganisms. The rest of the test item, 2-(perfluorohexyl)ethanol and 3,3,4,4,5,5,6,6,7,7,8,8,8-tridecafluorooctanoic acid remained in the test solution.

8. Remarks

8.1 Determination of acrylic acid in absorbents for carbon dioxide

Acrylic acid was not detected in the additional test solutions in Section 4.6 and it was considered that the produced acrylic acid transferred from the test solution to the absorbent for carbon dioxide attached to the test vessel. The absorbents of the test solutions for the determination of acrylic acid were pretreated as follows and acrylic acid was determined. Moreover, acrylic acid in the absorbents of the test solutions for the biodegradation test was similarly determined because acrylic acid was detected in the absorbents of the test solutions for the determination of acrylic acid.

(1) Pretreatment of the absorbents for carbon dioxide



*14 Nuclepore Syrfil-MF

(2) Determination of acrylic acid

See Section 4.6(3).

(3) Analytical results

Analytical results of the absorbents for carbon dioxide of the test solutions for the determination of acrylic acid were as follows.

		Water + test item	Sludge + test item	Theoretical amount	Reference
Detected amount and percentage detection of acrylic acid (HPLC)	mg	0.3	0.3	5.2	1, 4
	%	6	5	-	

Analytical results of the absorbents for carbon dioxide of the test solutions for the biodegradation test were as follows.

		Water + test item	Sludge + test item			Theoretical amount	Reference
		Vessel-1	Vessel-2	Vessel-3	Vessel-4		
Detected amount and percentage detection of acrylic acid (HPLC)	mg	0.3	0.1	0.2	0.2	5.2	2, 5
	%③	6	3	5	3	-	

8.2 Instruments used for test

Fourier transform infrared spectrophotometer :
Shimadzu Corporation type IRPrestige-21

Closed system oxygen consumption measuring apparatus :
see page 9

Gas chromatograph : see page 11

Liquid chromatograph-mass spectrometer
see page 13

High-performance liquid chromatograph :
see page 16

Electronic analytical balance : Sartorius AG type BP210S
Mettler-Toledo International Inc. type AE-163

Ultraviolet and visible spectrophotometer :
JASCO Corporation type V-660

Refrigerated centrifuge : KUBOTA Manufacturing Corporation type 5922

Shaker : Taitec Corporation type SR-2w

pH meter : Toa Electronics Ltd. type HM-50G

Ultrasonic cleaner: Yamato Scientific Co., Ltd type B-32H

8.3 Reagents used for analysis

Acetonitrile (HPLC grade) : Wako Pure Chemical Industries, Ltd.
Purified water (Japanese Pharmacopeia) :
Takasugi Pharmaceutical Co., Ltd.
Ethyl acetate (reagent grade) : Kanto Chemical Co., Inc.
Sodium chloride (reagent grade) : MANAC Incorporated
Phosphoric acid (reagent grade) : Wako Pure Chemical Industries, Ltd.
Formic acid (reagent grade) : Wako Pure Chemical Industries, Ltd.
Acrylic acid (reagent grade) : Wako Pure Chemical Industries, Ltd.
1 mol/L Hydrochloric acid (for volumetric analysis) :
Wako Pure Chemical Industries, Ltd.
2-(Perfluorohexyl)ethanol Supplied by the sponsor
3,3,4,4,5,5,6,6,7,7,8,8,8-Tridecafluorooctanoic acid
Supplied by the sponsor

Table-1 Calculation table for percentage biodegradation by BOD

Study No. 14738 Duration of cultivation: 28 days

Vessel No.	7th day		14th day		21st day		28th day		Mean Deg. (%)
	BOD (mg)	Deg. (%)	BOD (mg)	Deg. (%)	BOD (mg)	Deg. (%)	BOD (mg)	Deg. (%)	
[6]	53.9	56	66.9	70	73.5	76	74.6	76	
[5]	3.5	-	4.1	-	5.1	-	5.8	-	
[2]	3.4	0	3.9	-1	7.0	7	7.4	6	10
[3]	4.0	2	4.6	2	7.9	10	9.4	13	
[4]	4.0	2	4.7	2	7.6	9	9.0	12	
[1]	0.2	-	0.3	-	0.3	-	0.3	-	

Deg. : Percentage biodegradation

Vessel No. [6] : Sludge + aniline
Vessel No. [5] : Control blank [B]
Vessel No. [2] [3] [4] : Sludge + test item
Vessel No. [1] : Water + test item

Test item of 30.3 mg was added.

Chart of BOD : Fig. 1

Deg. = [BOD - B] / [TOD] × 100 (%)

TOD of test item : 27.3 (mg)

$C_{11}H_7F_{13}O_2 + 11.75\ O_2 \rightarrow 11\ CO_2 + 3.5\ H_2O + 13\ F$

$11.75\ O_2 / C_{11}H_7F_{13}O_2 = 375.99 / 418.15 = 0.90$

TOD = 30.3 × 0.90 = 27.3 (mg)

TOD of aniline : 90.3 (mg)

$C_6H_7N + 8.75\ O_2 \rightarrow 6\ CO_2 + 3.5\ H_2O + NO_2$

$8.75\ O_2 / C_6H_7N = 279.99 / 93.13 = 3.01$

TOD = 30 × 3.01 = 90.3 (mg)

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Table-2 Calculation table for recovery rate of test item

Study No. 14738

Sample description	A	D	E	F
Standard solution 303mg/L	159493			
Water + test item -1	153173	29.1	96.0	96.3
Water + test item -2	154010	29.3	96.6	
Sludge + test item -1	152434	29.0	95.6	95.8
Sludge + test item -2	153266	29.1	96.1	
Control blank	n.d.			

Amount of test item added : 30.3 (mg)

A : Peak area (μV·sec)

B : Final volume : 100 (mL)

C : Ratio of portion used for analysis : 300/300

D : Recovery amount (mg)

$Dw = G \times (A(\text{Water + test item}) / A(\text{Standard})) \times (B / C) / 1000$

$Ds = G \times \{ (A(\text{Sludge + test item}) - A(\text{Control blank})) / A(\text{Standard}) \} \times (B / C) / 1000$

E : Recovery rate (%)

$E = D / 30.3 \text{ (mg)} \times 100$

F : Average recovery rate (%)

G : Concentration of standard solution : 303 (mg/L)

See Fig. 6

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Table-3 Calculation table for recovery rate of 13F-EtOH

Study No. 14738

Sample description	A	D	E	F
Standard solution 252mg/L	102026			
Water + 13F-EtOH -1	98539	24.3	96.6	96.7
Water + 13F-EtOH -2	98881	24.4	96.9	
Sludge + 13F-EtOH -1	99265	24.5	97.3	96.7
Sludge + 13F-EtOH -2	98130	24.2	96.2	
Control blank	n.d.			

Amount of 13F-EtOH added : 25.2 (mg)

A : Peak area (μV·sec)

B : Final volume : 100 (mL)

C : Ratio of portion used for analysis : 300/300

D : Recovery amount (mg)

$Dw = G \times (A(\text{Water + 13F-EtOH}) / A(\text{Standard})) \times (B / C) / 1000$

$Ds = G \times \{ (A(\text{Sludge + 13F-EtOH}) - A(\text{Control blank})) / A(\text{Standard}) \} \times (B / C) / 1000$

E : Recovery rate (%)

$E = D / 25.2 \text{ (mg)} \times 100$

F : Average recovery rate (%)

G : Concentration of standard solution : 252 (mg/L)

See Fig. 7

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Table-4 Calculation table for recovery rate of 13F-AcOH

Study No. 14738

Sample description	A	D	E	F
Standard solution 2.00mg/L	91497			
Water + 13F-AcOH -1	88806	29.1	97.1	97.1
Water + 13F-AcOH -2	88958	29.2	97.2	
Sludge + 13F-AcOH -1	89011	29.2	97.3	96.5
Sludge + 13F-AcOH -2	87573	28.7	95.7	
Control blank	n.d.			

Amount of 13F-AcOH : 30 (mg)

A : Peak area (-)

B : Final volume : 15 (mL)

C : Ratio of portion used for analysis : 1/10×1/100×300/300

D : Recovery amount (mg)

$Dw = G \times (A(\text{Water + 13F-AcOH}) / A(\text{Standard})) \times (B / C) / 1000$

$Ds = G \times \{ (A(\text{Sludge + 13F-AcOH}) - A(\text{Control blank})) / A(\text{Standard}) \} \times (B / C) / 1000$

E : Recovery rate (%)

$E = D / 30 \text{ (mg)} \times 100$

F : Average recovery rate (%)

G : Concentration of standard solution : 2.00 (mg/L)

See Fig. 8

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Table-5 Calculation table for percentage biodegradation of test item

Study No. 14738

Sample description	A	E	F	G	H
Standard solution 303mg/L	152003				
[1] Water + test item	134661	27.9	92		
[2] Sludge + test item	119869	24.9	82	11	
[3] Sludge + test item	116166	24.2	80	13	11
[4] Sludge + test item	123174	25.6	85	8	
[5] Control blank	n.d.				

Amount of test item added : 30.3 (mg)

A : Peak area (μV·sec)

B : Final volume : 100 (mL)

C : Ratio of portion used for analysis : 300/300

D : Recovery rate : 96.3 (%) (Water + test item)
95.8 (%) (Sludge + test item)

E : Residual amount of test item (mg)

$Ew = I \times (A(\text{Water + test item}) / A(\text{Standard})) \times (B / C) / (D / 100) / 1000$

$Es = I \times \{ (A(\text{Sludge + test item}) - A(\text{Control blank})) / A(\text{Standard}) \} \times (B / C) / (D / 100) / 1000$

F : Percentage residue (%)

$F = E / 30.3 \text{ (mg)} \times 100$

G : Percentage biodegradation (%)

$G = \{ (E(\text{Water + test item}) - E(\text{Sludge + test item})) / E(\text{Water + test item}) \} \times 100$

H : Average percentage biodegradation (%)

I : Concentration of standard solution : 303 (mg/L)

See Fig. 9

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Table-6 Calculation table for percentage production of 13F-EtOH

Study No. 14738

Sample description	A	E	F	G
Standard solution 252mg/L	100126			
[1] Water + test item	6001	1.6	6	6
[2] Sludge + test item	13646	3.6	13	
[3] Sludge + test item	12454	3.2	12	12
[4] Sludge + test item	10709	2.8	11	
[5] Control blank	n.d.			

Amount of test item added : 30.3 (mg)

Theoretical amount of 13F-EtOH : 26.4 (mg)

$(30.3) \times (C_8H_5F_{13}O / C_{11}H_7F_{13}O_2)$

A : Peak area (μV·sec)

B : Final volume : 100 (mL)

C : Ratio of portion used for analysis : 300/300

D : Recovery rate : 96.7 (%) (Water + test item)

96.7 (%) (Sludge + test item)

E : Amount of 13F-EtOH (mg)

Ew = H × (A(Water + test item) / A(Standard)) × (B / C) / (D / 100) / 1000

Es = H × { (A(Sludge + test item) - A(Control blank)) / A(Standard) }

× (B / C) / (D / 100) / 1000

F : Percentage production (%)

F = E / 26.4 (mg) × 100

G : Average percentage production (%)

H : Concentration of standard solution : 252 (mg/L)

See Fig. 9

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Table-7 Calculation table for percentage production of 13F-AcOH

Study No. 14738

Sample description	A	E	F	G
Standard solution 2.00mg/L	82583			
[1] Water + test item	n.d.	0	0	0
[2] Sludge + test item	2380	0.9	3	
[3] Sludge + test item	2154	0.8	3	3
[4] Sludge + test item	2042	0.8	3	
[5] Control blank	n.d.			

Amount of test item added : 30.3 (mg)

Theoretical amount of 13F-AcOH : 27.4 (mg)

$(30.3) \times (C_8H_3F_{13}O_2 / C_{11}H_7F_{13}O_2)$

A : Peak area (-)

B : Final volume : 15 (mL)

C : Ratio of portion used for analysis : 1/10×1/100×300/300

D : Recovery rate : 97.1 (%) (Water + test item)

96.5 (%) (Sludge + test item)

E : Amount of 13F-AcOH (mg)

Ew = H × (A(Water + test item) / A(Standard)) × (B / C) / (D / 100) / 1000

Es = H × { (A(Sludge + test item) - A(Control blank)) / A(Standard) }

× (B / C) / (D / 100) / 1000

F : Percentage production (%)

F = E / 27.4 (mg) × 100

G : Average percentage production (%)

H : Concentration of standard solution : 2.00 (mg/L)

See Fig. 10

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Table-8 Calculation table for percentage production of acrylic acid (test solution for analysis of acrylic acid)

Study No. 14738

Sample description	A	D	E
Standard solution 20.0mg/L	1427740		
Water + test item	n.d.	0	0
Sludge + test item	n.d.	0	0
Control blank	n.d.		

Amount of test item added : 30.3 (mg)

Theoretical amount of acrylic acid : 5.2 (mg)

$(30.3) \times (C_3H_4O_2 / C_{11}H_7F_{13}O_2)$

A : Peak area (μV·sec)

B : Final volume : 10 (mL)

C : Ratio of portion used for analysis : 10/300

D : Amount of acrylic acid (mg)

Dw = F × (A(Water + test item) / A(Standard)) × (B / C) / 1000

Ds = F × { (A(Sludge + test item) - A(Control blank)) / A(Standard) } × (B / C) / 1000

E : Percentage production (%)

E = D / 5.2 (mg) × 100

F : Concentration of standard solution : 20.0 (mg/L)

See Fig. 11

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Reference 1 Calculation table for percentage detection of acrylic acid
(CO_2 absorbent, test solution for analysis of acrylic acid)

Study No. 14738

Sample description	A	D	E
Standard solution 20.0mg/L	1425431		
Water + test item	471153	0.3	6
Sludge + test item	403814	0.3	5
Control blank	n.d.		

Amount of test item added : 30.3 (mg)

Theoretical amount of acrylic acid : 5.2 (mg)

$(30.3) \times (C_3H_4O_2 / C_{11}H_7F_{13}O_2)$

A : Peak area (μV·sec)

B : Final volume : 50 (mL)

C : Ratio of portion used for analysis : 1

D : Amount of acrylic acid (mg)

Dw = F × (A(Water + test item) / A(Standard)) × (B / C) / 1000

Ds = F × { (A(Sludge + test item) - A(Control blank)) / A(Standard) } × (B / C) / 1000

E : Percentage detection (%)

E = D / 5.2 (mg) × 100

F : Concentration of standard solution : 20.0 (mg/L)

See Reference 4

January 29, 2007 Name _____

Reference 2 Calculation table for percentage detection of acrylic acid (CO_2 absorbent)

Study No. 14738

Sample description	A	D	E	F
Standard solution 20.0mg/L	1433100			
[1] Water + test item	443994	0.3	6	6
[2] Sludge + test item	210699	0.1	3	
[3] Sludge + test item	338425	0.2	5	4
[4] Sludge + test item	249622	0.2	3	
[5] Control blank	n.d.			

Amount of test item added : 30.3 (mg)

Theoretical amount of acrylic acid : 5.2 (mg)

$(30.3) \times (C_3H_4O_2 / C_{11}H_7F_{13}O_2)$

A : Peak area (μV·sec)

B : Final volume : 50 (mL)

C : Ratio of portion used for analysis : 1

D : Amount of acrylic acid (mg)

Dw = G × (A(Water + test item) / A(Standard)) × (B / C) / 1000

Ds = G × { (A(Sludge + test item) - A(Control blank)) / A(Standard) } × (B / C) / 1000

E : Percentage detection (%)

E = D / 5.2 (mg) × 100

F : Average percentage detection (%)

G : Concentration of standard solution : 20.0 (mg/L)

See Reference 5

January 22, 2007 Name _____

Study No. 14738 (Test item 13F-SFA)

Cultivating conditions:

Concentration

Test item	100 (mg/L)
Reference item (aniline)	100 (mg/L)
Activated sludge	30 (mg/L)
Temperature	25 ± 1 ℃
Duration	28 days (Nov.21,2006 - Dec.19,2006)

Note: —

Vessel No.	Sample Description	BOD (mg)			
		7th day	14th day	21st day	28th day
[1]	Water + test item	0.2	0.3	0.3	0.3
[2]	Sludge + test item	3.4	3.9	7.0	7.4
[3]	Sludge + test item	4.0	4.6	7.9	9.4
[4]	Sludge + test item	4.0	4.7	7.6	9.0
[5]	Control blank [B]	3.5	4.1	5.1	5.8
[6]	Sludge + aniline	53.9	66.9	73.5	74.6

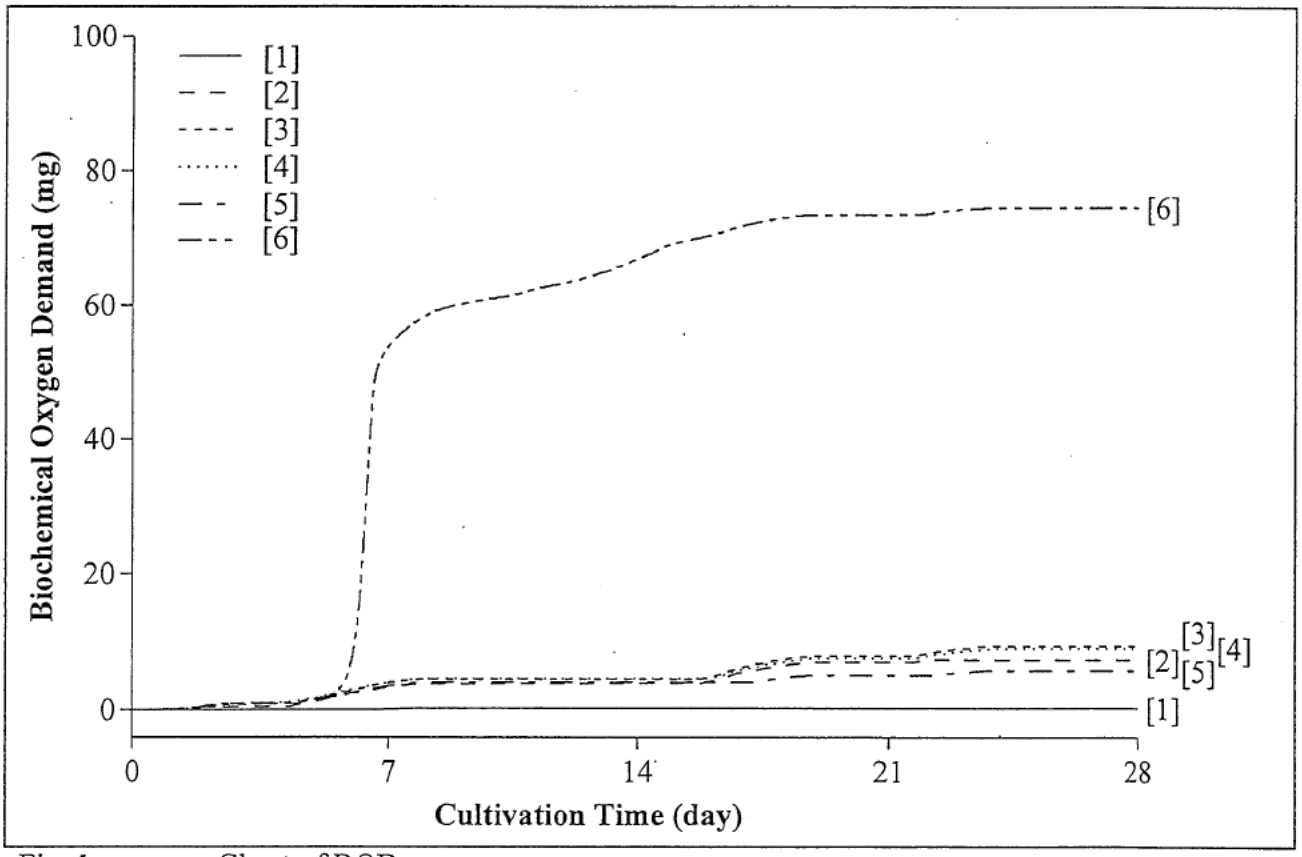


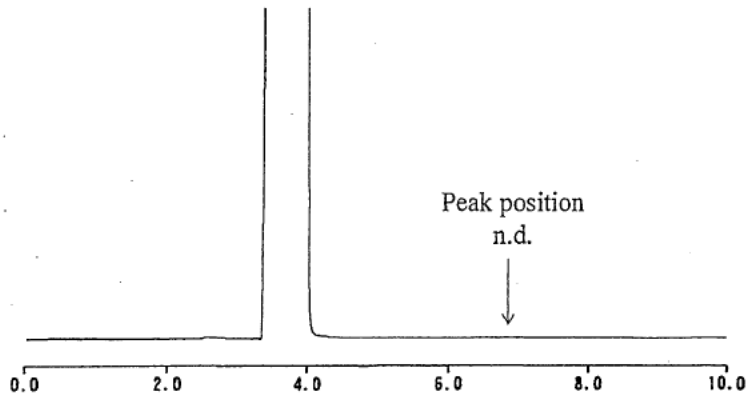
Fig. 1 Chart of BOD.

Dec.19,2006 Name _____

Solvent blank

Operating Date : Dec.01,2006

PKNO	Time (min)	Area (μV·sec)	Height (μV)	Conc. (%)

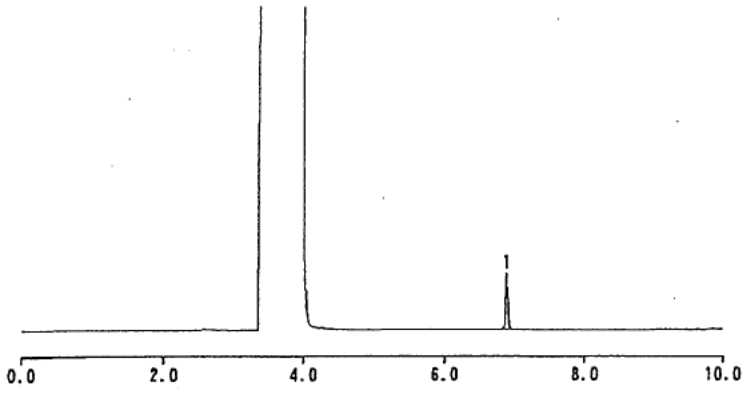


Operator

Standard solution 75.8 mg/L

Operating Date : Dec.01,2006

PKNO	Time (min)	Area (μV·sec)	Height (μV)	Conc. (%)
1	6.87	39474	16923	100.00
	Total	39474		100.00

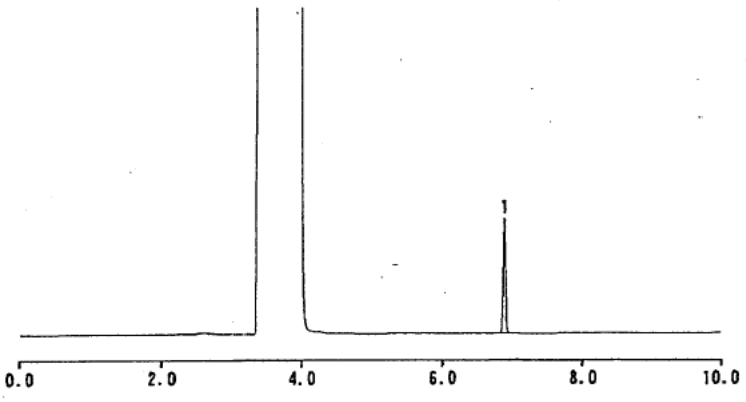


Operator :

Standard solution 152 mg/L

Operating Date : Dec.01,2006

PKNO	Time (min)	Area (μV·sec)	Height (μV)	Conc. (%)
1	6.87	78397	34101	100.00
	Total	78397		100.00

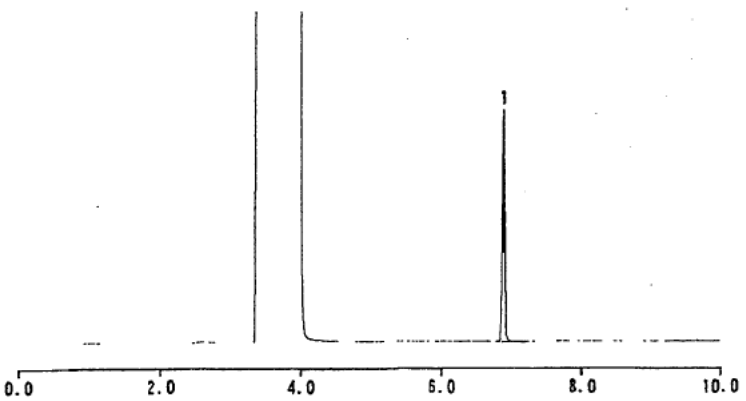


Operator :

Standard solution 303 mg/L

Operating Date : Dec.01,2006

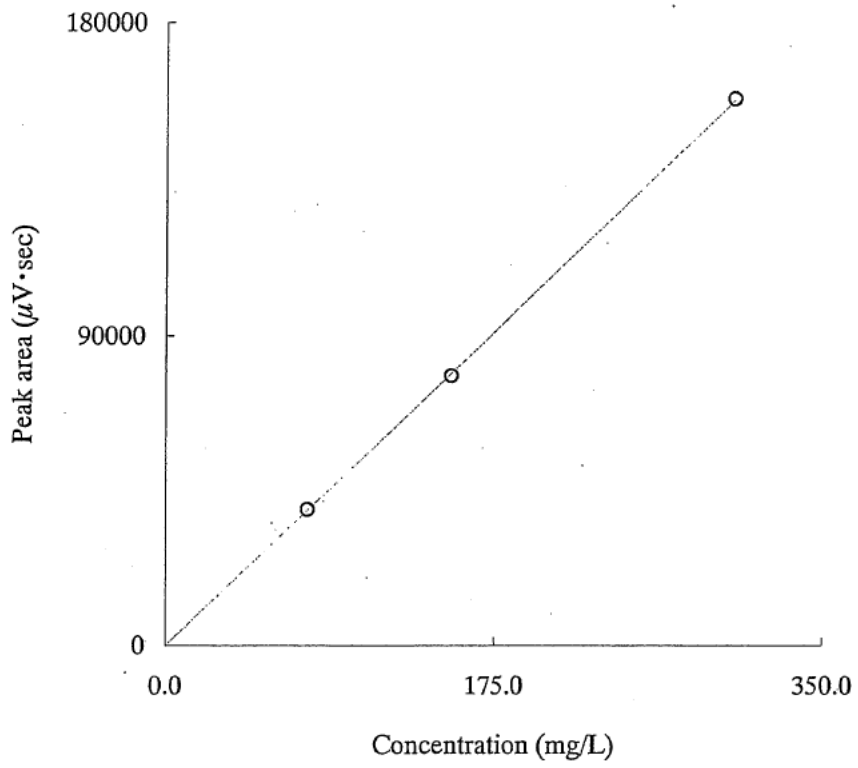
PKNO	Time (min)	Area (μV·sec)	Height (μV)	Conc. (%)
1	6.88	158146	67630	100.00
	Total	158146		100.00



Operator :

Fig. 2 - 1 Chromatograms of GC analysis for calibration curve (test item).

Date : Dec.5,2006 Name : _____



y = 521x
r = 1.00

Concentration (mg/L)	Peak area (μV·sec)
75.8	39474
152	78397
303	158146

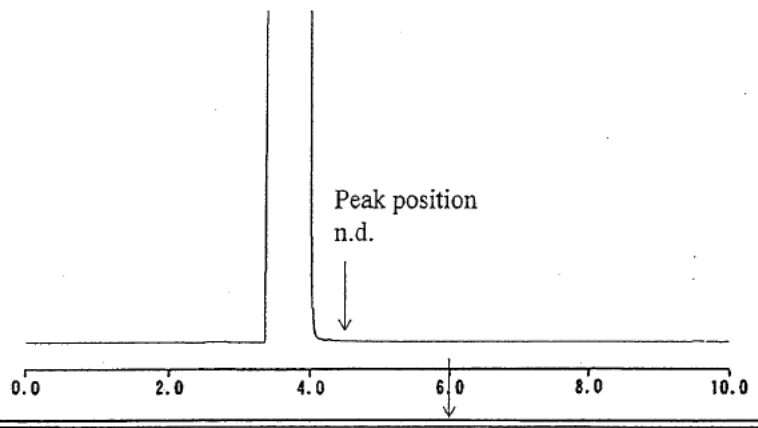
Fig. 2 - 2 Calibration curve of test item.

December 6, 2006 Name _____

Solvent blank

Operating Date : Dec.01,2006

PKNO	Time (min)	Area (μV·sec)	Height (μV)	Conc. (%)

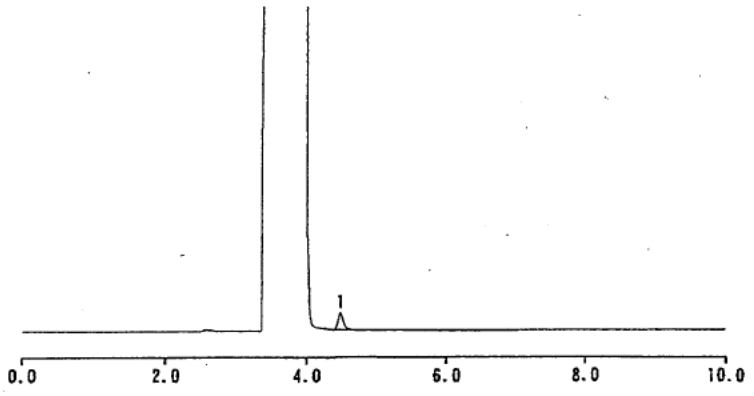


Operator :

Standard solution 62.9 mg/L

Operating Date : Dec.01,2006

PKNO	Time (min)	Area (μV·sec)	Height (μV)	Conc. (%)
1	4.48	24668	4997	100.00
	Total	24668		100.00

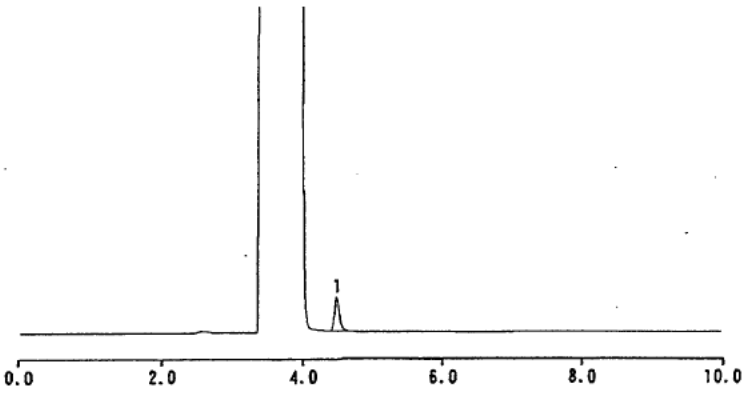


Operator :

Standard solution 126 mg/L

Operating Date : Dec.01,2006

PKNO	Time (min)	Area (μV·sec)	Height (μV)	Conc. (%)
1	4.48	50407	10315	100.00
	Total	50407		100.00

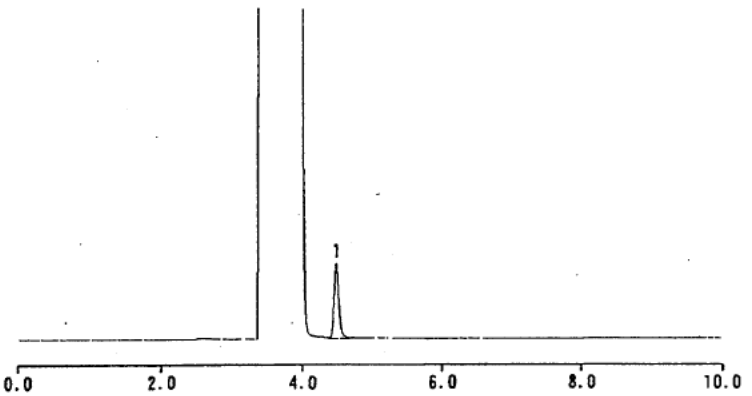


Operator :

Standard solution 252 mg/L

Operating Date : Dec.01,2006

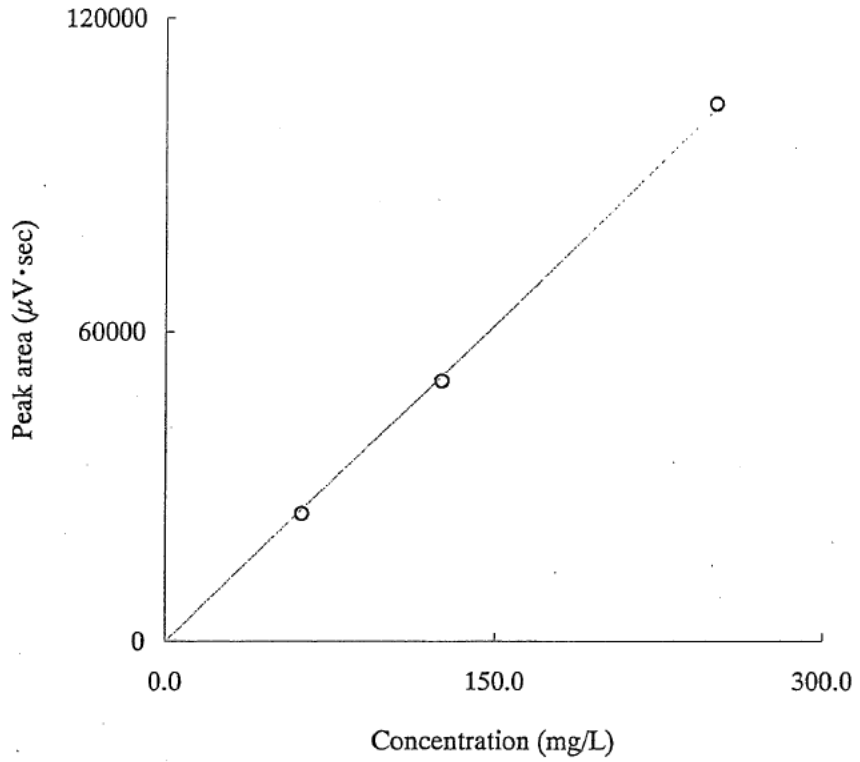
PKNO	Time (min)	Area (μV·sec)	Height (μV)	Conc. (%)
1	4.48	103654	22133	100.00
	Total	103654		100.00



Operator :

Fig. 3 - 1 Chromatograms of GC analysis for calibration curve (13F-EtOH).

Date : Dec. 1, 2006 Name :



y = 408x
r = 1.00

Concentration (mg/L)	Peak area (μV·sec)
62.9	24668
126	50407
252	103654

Fig. 3 - 2 Calibration curve of 13F-EtOH.

December 1, 2006 Name _____

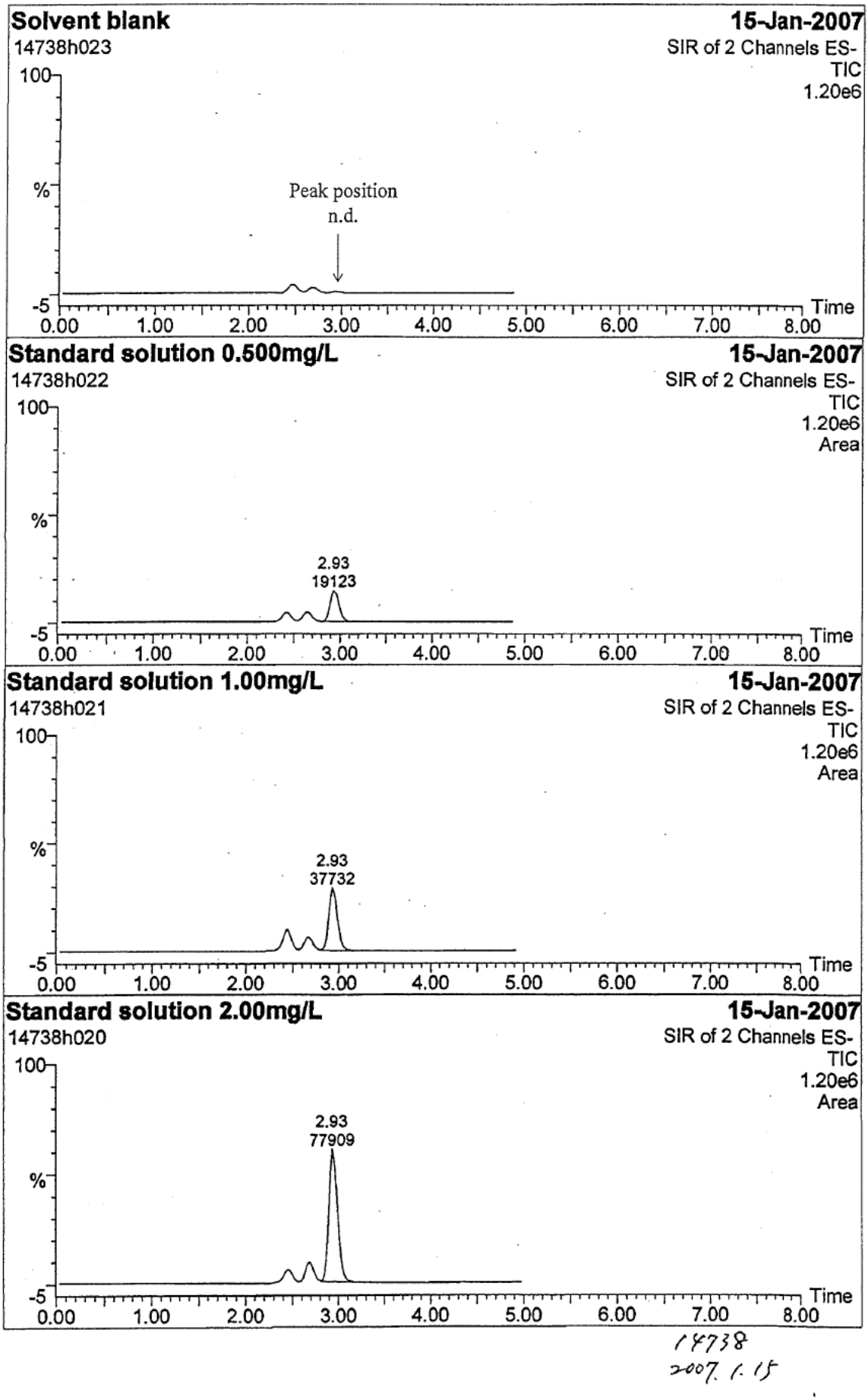
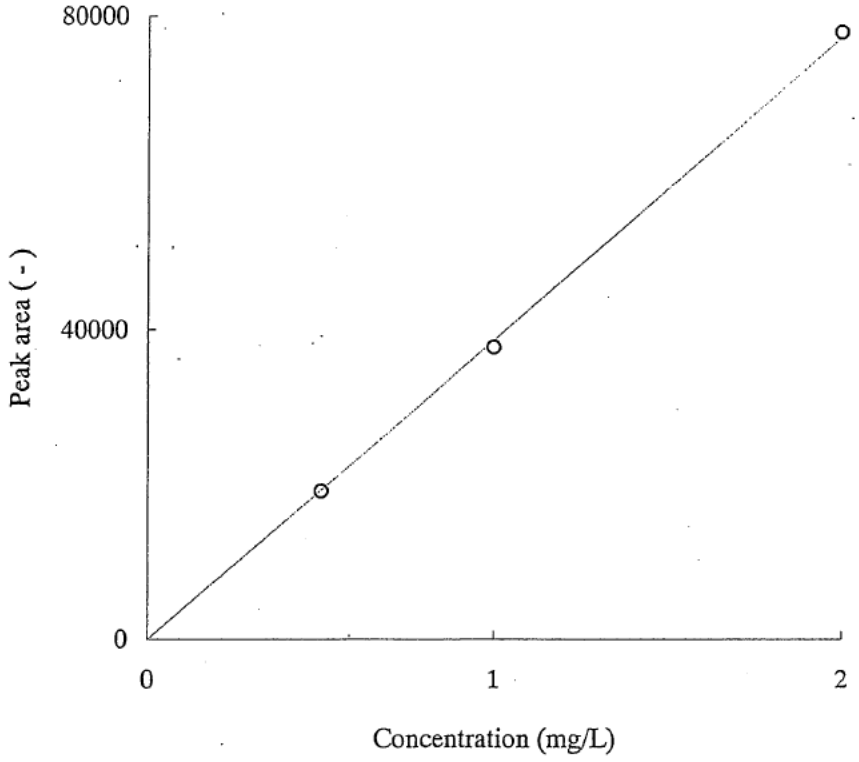


Fig. 4 - 1 Total ion chromatograms of LC-MS analysis for calibration curve (13F-AcOH).



y = 38688x
r = 1.00

Concentration (mg/L)	Peak area (-)
0.500	19123
1.00	37732
2.00	77909

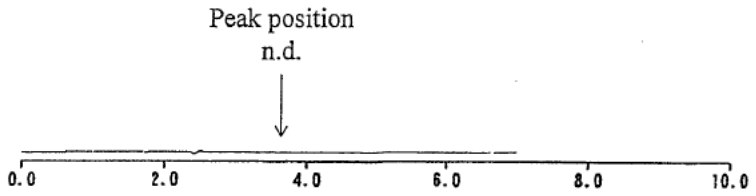
Fig. 4 - 2 Calibration curve of 13F-AcOH.

January 15, 2007 Name _____ _____

Solvent blank

Operating Date : Dec.19,2006

PKNO	Time (min)	Area (μV·sec)	Height (μV)	Conc. (%)

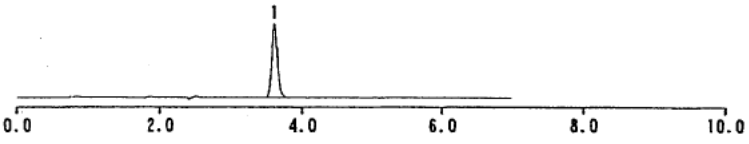


Operator :

Standard solution 5.00 mg/L

Operating Date : Dec.19,2006

PKNO	Time (min)	Area (μV·sec)	Height (μV)	Conc. (%)
1	3.60	362087	63755	100.00
	Total	362087		100.00

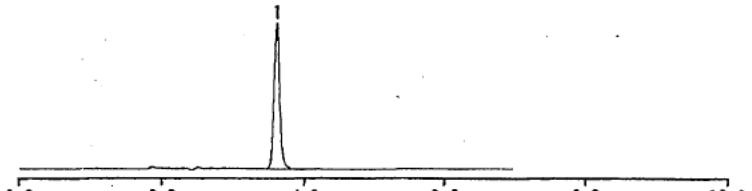


Operator :

Standard solution 10.0 mg/L

Operating Date : Dec.19,2006

PKNO	Time (min)	Area (μV·sec)	Height (μV)	Conc. (%)
1	3.60	718773	126350	100.00
	Total	718773		100.00

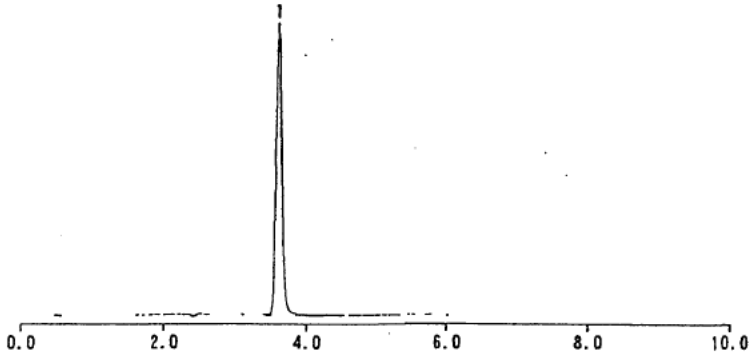


Operator :

Standard solution 20.0 mg/L

Operating Date : Dec.19,2006

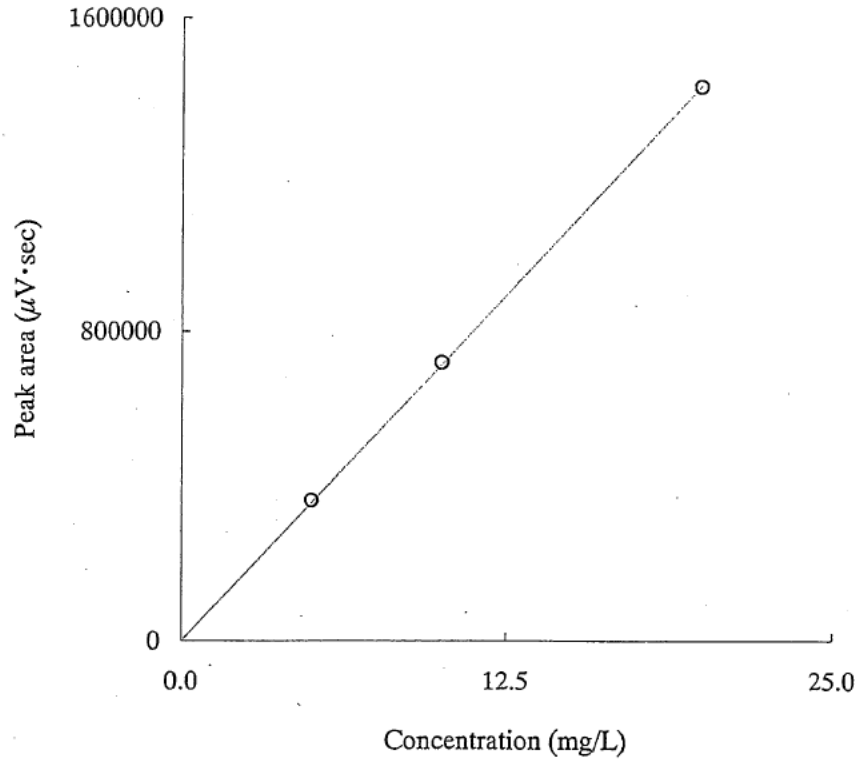
PKNO	Time (min)	Area (μV·sec)	Height (μV)	Conc. (%)
1	3.60	1424652	249572	100.00
	Total	1424652		100.00



Operator :

Fig. 5 - 1 Chromatograms of HPLC analysis for calibration curve (acrylic acid).

Date : Dec.19,2006 Name :



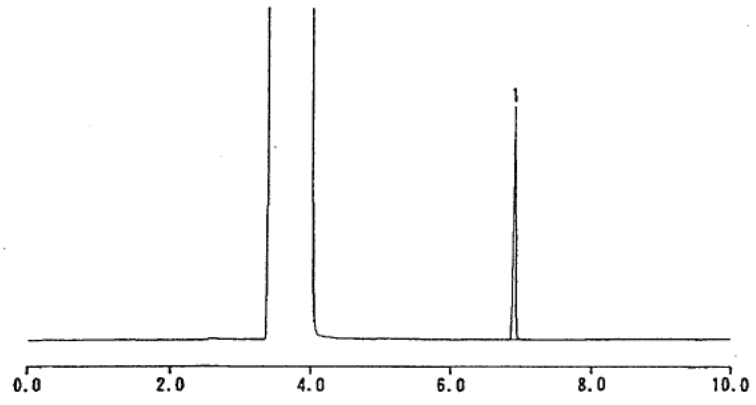
y = 71412x
r = 1.00

Concentration (mg/L)	Peak area (μV·sec)
5.00	362087
10.0	718773
20.0	1424652

Fig. 5 - 2 Calibration curve of acrylic acid.

January 17, 2007 Name _____

Standard solution 303 mg/L

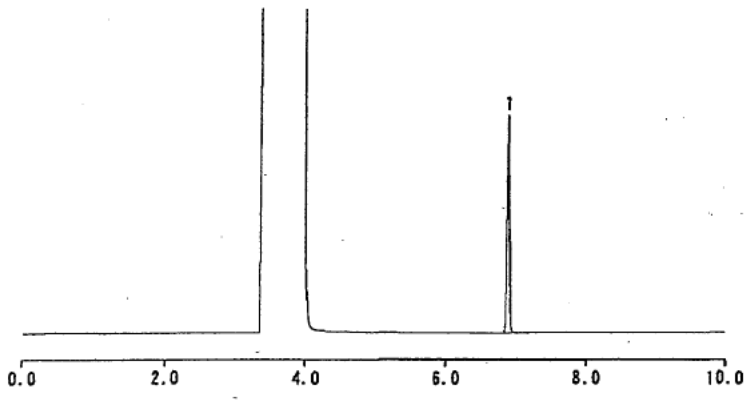


Operating Date : Dec.01,2006

PKNO	Time (min)	Area (μV·sec)	Height (μV)	Conc. (%)
1	6.88	159493	68172	100.00
Total		159493		100.00

Operator :

Water + test item - 1

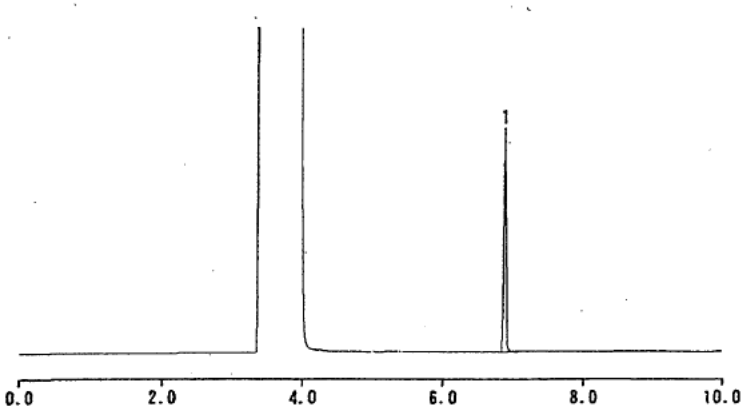


Operating Date : Dec.01,2006

PKNO	Time (min)	Area (μV·sec)	Height (μV)	Conc. (%)
1	6.88	153173	65412	100.00
Total		153173		100.00

Operator :

Water + test item - 2



Operating Date : Dec.02,2006

PKNO	Time (min)	Area (μV·sec)	Height (μV)	Conc. (%)
1	6.88	154010	66451	100.00
Total		154010		100.00

Operator :

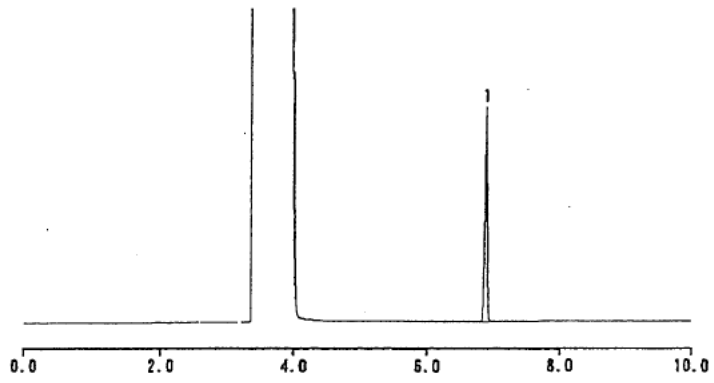
Fig. 6 - 1 Chromatograms of GC analysis for recovery test (test item).

Date : Dec.5,2006 Name : _____

Sludge + test item - 1

Operating Date : Dec.02,2006

PKNO	Time (min)	Area (μV·sec)	Height (μV)	Conc. (%)
1	6.88	152434	66117	100.00
	Total	152434		100.00

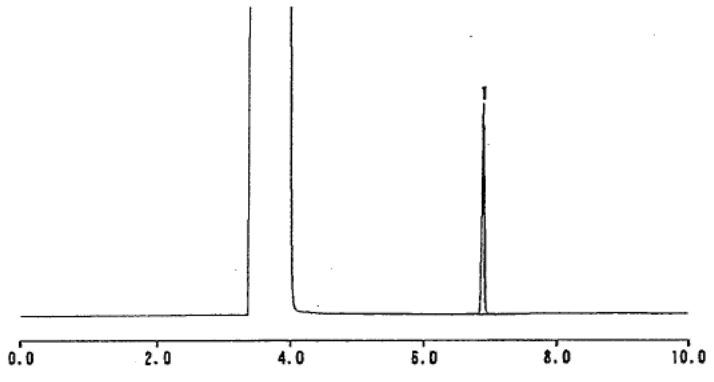


Operator

Sludge + test item - 2

Operating Date : Dec.02,2006

PKNO	Time (min)	Area (μV·sec)	Height (μV)	Conc. (%)
1	6.88	153266	66132	100.00
	Total	153266		100.00

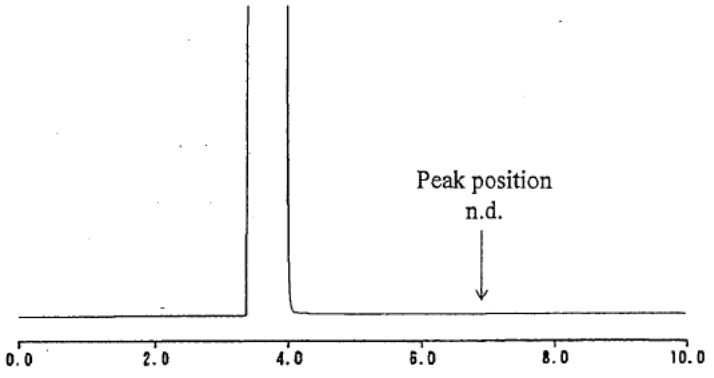


Operator :

Control blank

Operating Date : Dec.02,2006

PKNO	Time (min)	Area (μV·sec)	Height (μV)	Conc. (%)



Operator :

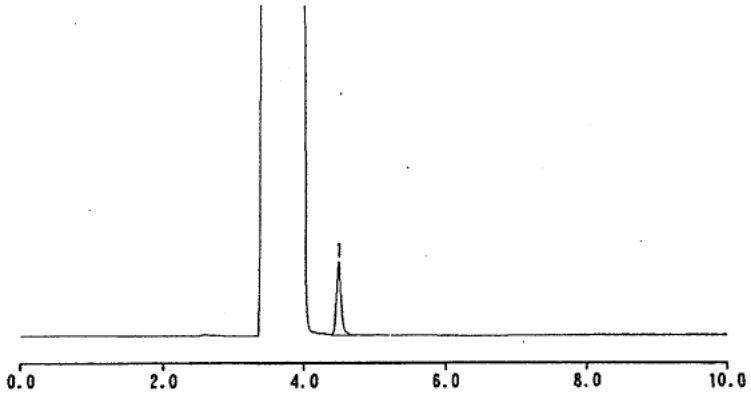
Fig. 6 - 2 Chromatograms of GC analysis for recovery test (test item).

Date : Dec. 5, 2006 Name : _____

Standard solution 252 mg/L

Operating Date : Dec.01,2006

PKNO	Time (min)	Area (μV·sec)	Height (μV)	Conc. (%)
1	4.48	102026	21373	100.00
	Total	102026		100.00

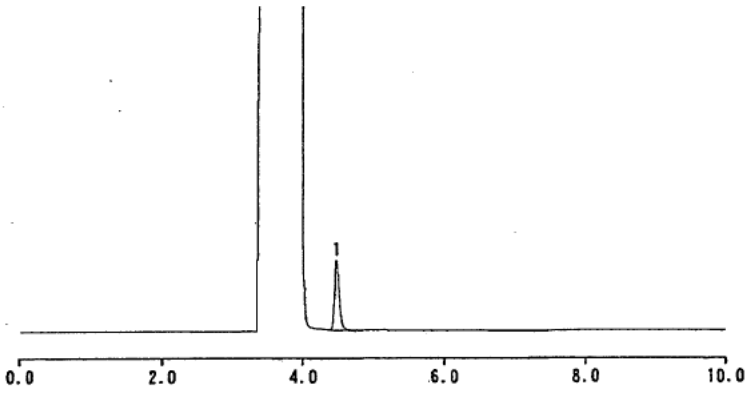


Operator :

Water + 13F-EtOH - 1

Operating Date : Dec.01,2006

PKNO	Time (min)	Area (μV·sec)	Height (μV)	Conc. (%)
1	4.48	98539	20888	100.00
	Total	98539		100.00

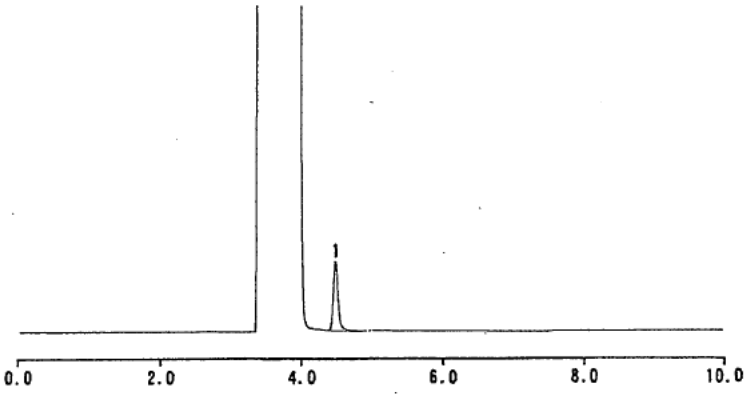


Operator :

Water + 13F-EtOH - 2

Operating Date : Dec.01,2006

PKNO	Time (min)	Area (μV·sec)	Height (μV)	Conc. (%)
1	4.48	98881	20606	100.00
	Total	98881		100.00



Operator :

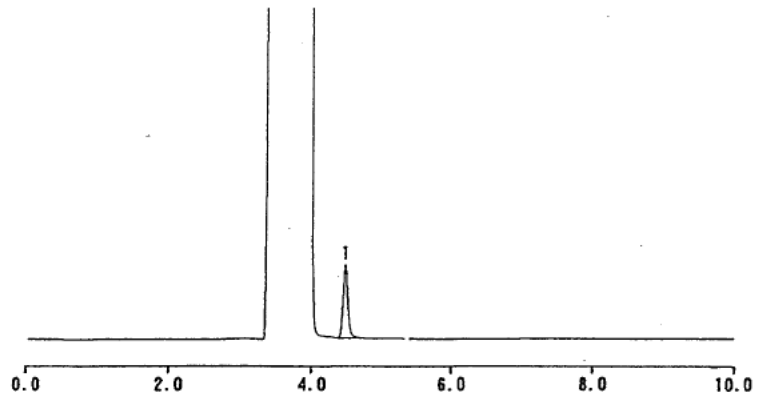
Fig.7 - 1 Chromatograms of GC analysis for recovery test (13F-EtOH).

Date : Dec.5,2006 Name : _____

Sludge + 13F-EtOH - 1

Operating Date : Dec.01,2006

PKNO	Time (min)	Area (μV·sec)	Height (μV)	Conc. (%)
1	4.48	99265	21276	100.00
	Total	99265		100.00

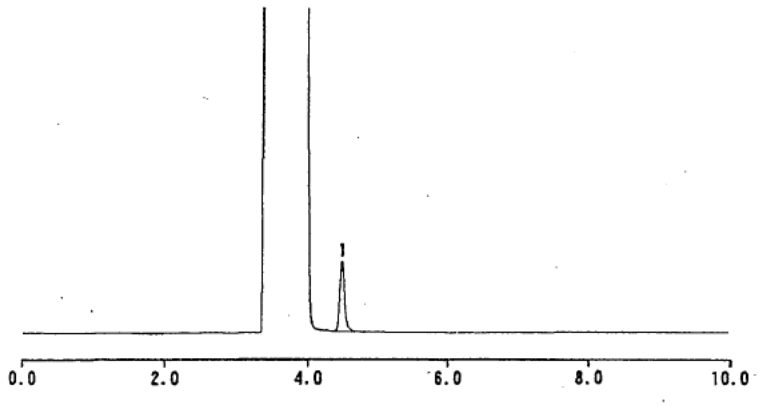


Operator :

Sludge + 13F-EtOH - 2

Operating Date : Dec.01,2006

PKNO	Time (min)	Area (μV·sec)	Height (μV)	Conc. (%)
1	4.47	98130	20779	100.00
	Total	98130		100.00

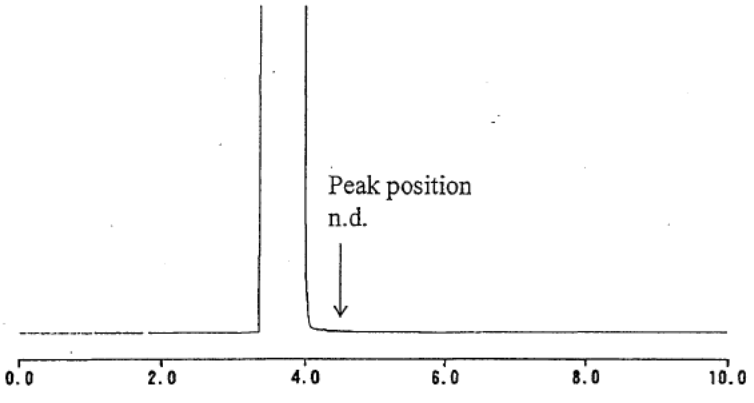


Operator :

Control blank

Operating Date : Dec.01,2006

PKNO	Time (min)	Area (μV·sec)	Height (μV)	Conc. (%)



Operator :

Fig. 7 - 2 Chromatograms of GC analysis for recovery test (13F-EtOH).

Date : Dec. 5, 2006 Name :

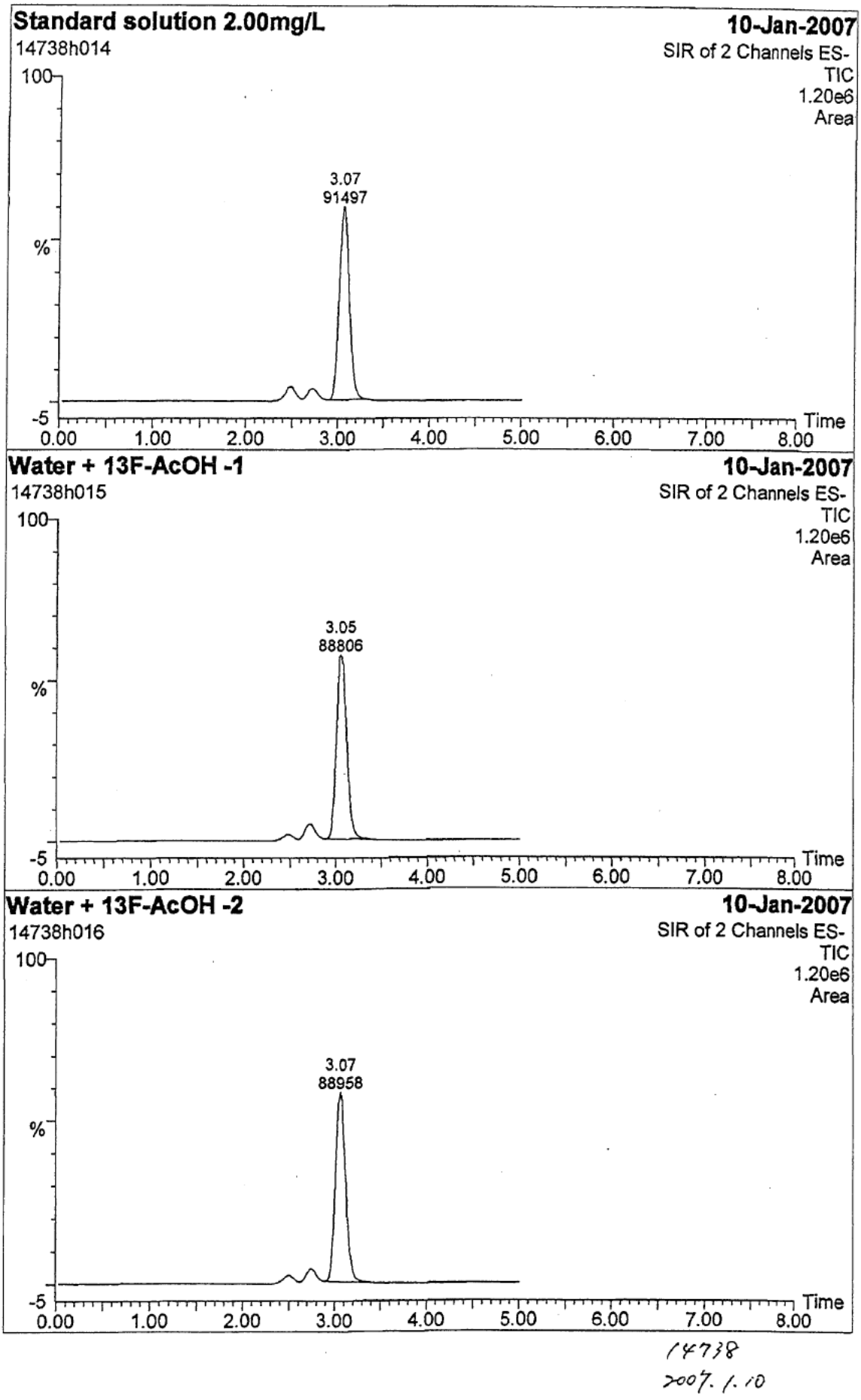


Fig. 8 - 1 Total ion chromatograms of LC-MS analysis for recovery test (13F-AcOH).

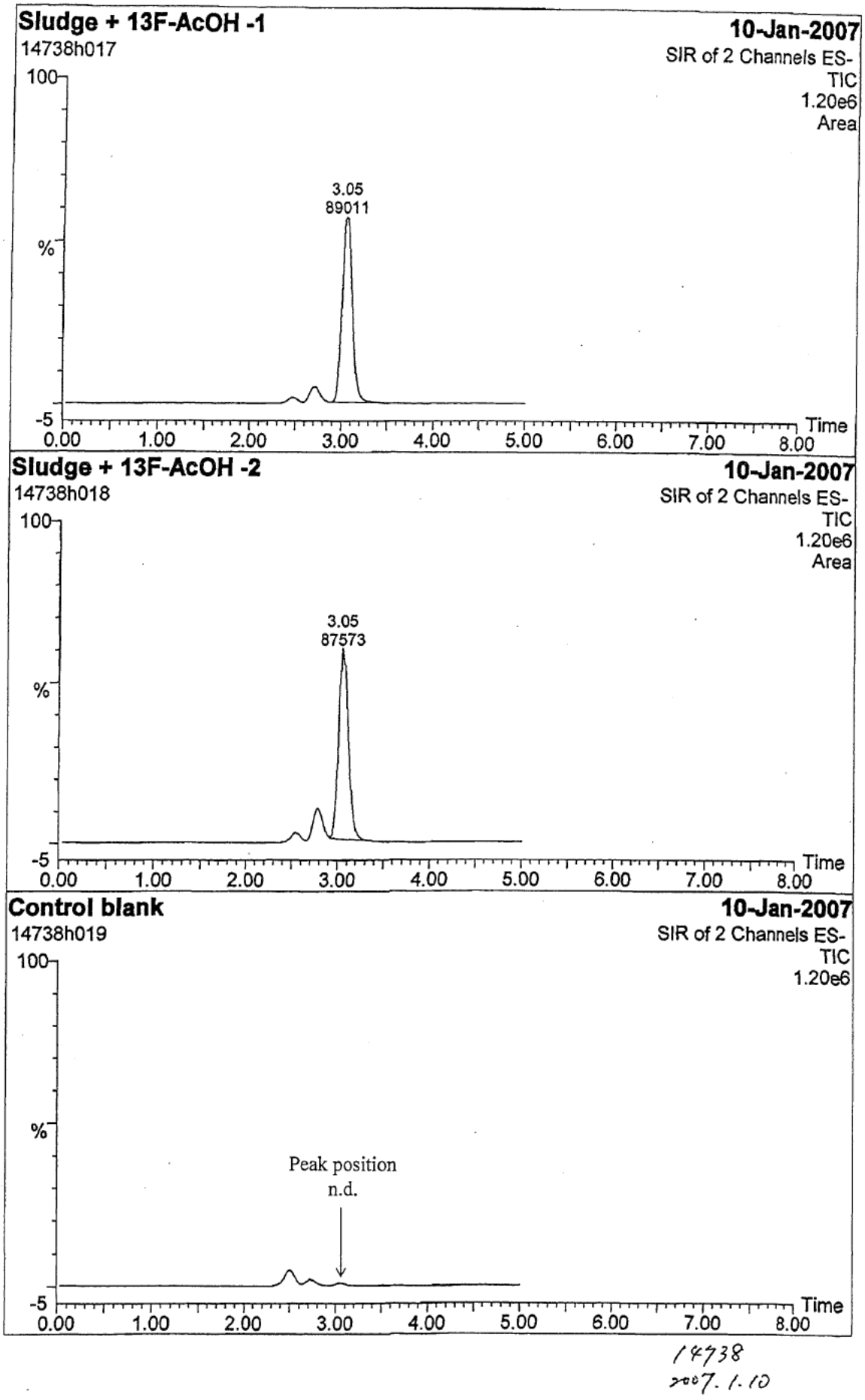
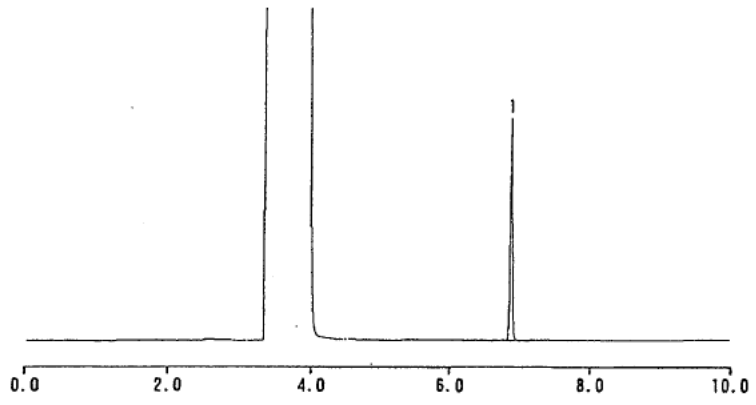


Fig. 8 - 2 Total ion chromatograms of LC-MS analysis for recovery test (13F-AcOH).

Standard solution 303 mg/L

Operating Date : Dec.20,2006

PKNO	Time (min)	Area (μV·sec)	Height (μV)	Conc. (%)
1	6.87	152003	65068	100.00
	Total	152003		100.00

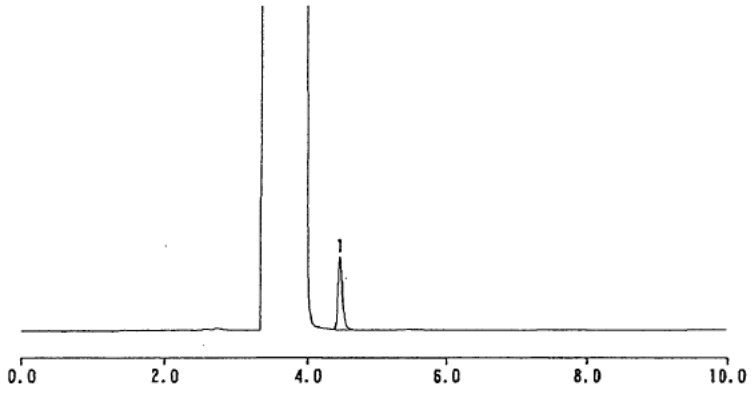


Operator :

Standard solution 252 mg/L (13F-EtOH)

Operating Date : Dec.20,2006

PKNO	Time (min)	Area (μV·sec)	Height (μV)	Conc. (%)
1	4.47	100126	21580	100.00
	Total	100126		100.00

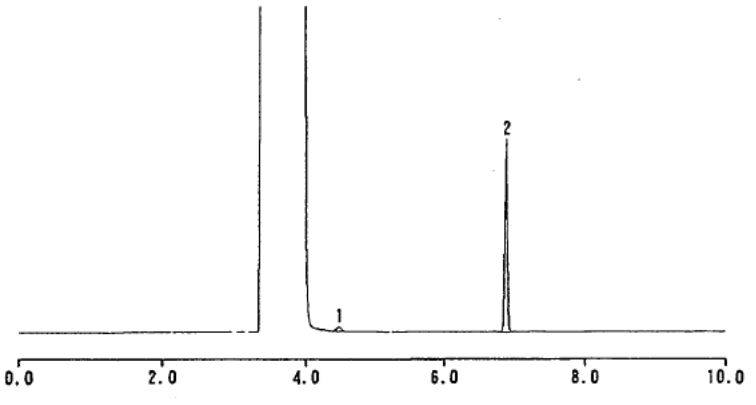


Operator :

[1] Water + test item

Operating Date : Dec.20,2006

PKNO	Time (min)	Area (μV·sec)	Height (μV)	Conc. (%)
1	4.47	6001	1182	4.27
2	6.87	134661	57558	95.73
	Total	140662		100.00

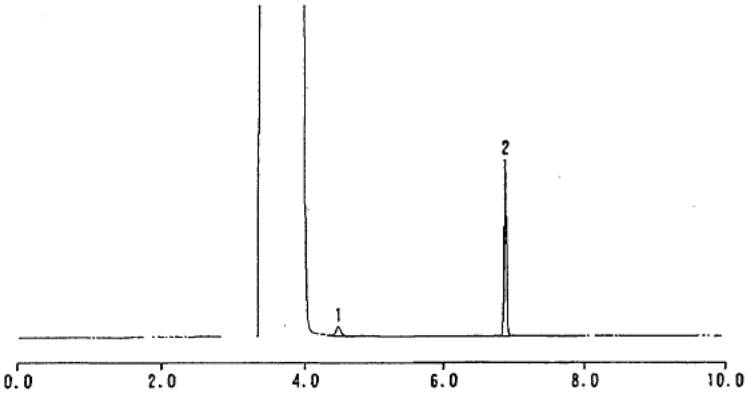


Operator :

[2] Sludge + test item

Operating Date : Dec.20,2006

PKNO	Time (min)	Area (μV·sec)	Height (μV)	Conc. (%)
1	4.47	13646	2775	10.22
2	6.87	119869	51718	89.78
	Total	133515		100.00



Operator :

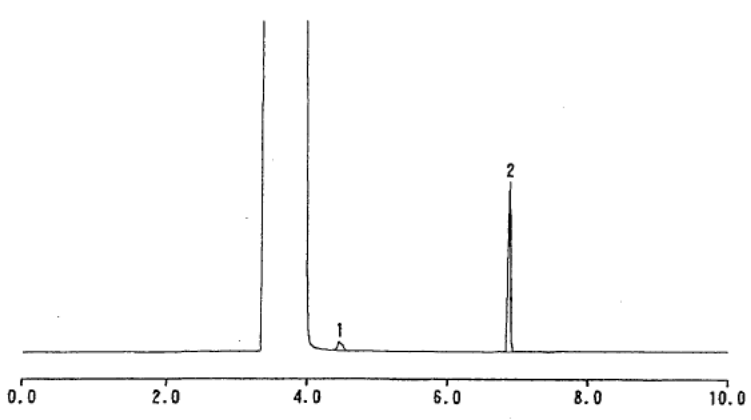
Fig. 9 - 1 Chromatograms of GC analysis for test solution (test item and 13F-EtOH)

Date : Dec.20,2006 Name :

[3] Sludge + test item

Operating Date : Dec.20,2006

PKNO	Time (min)	Area (μV·sec)	Height (μV)	Conc. (%)
1	4.47	12454	2531	9.68
2	6.87	116166	49519	90.32
	Total	128620		100.00

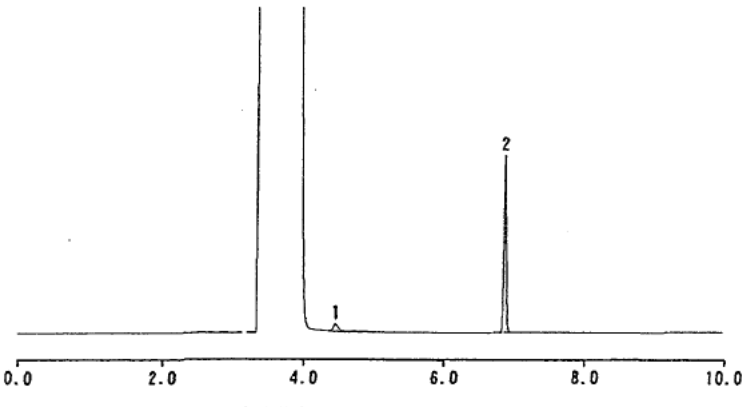


Operator :

[4] Sludge + test item

Operating Date : Dec.20,2006

PKNO	Time (min)	Area (μV·sec)	Height (μV)	Conc. (%)
1	4.47	10709	2174	8.00
2	6.87	123174	53138	92.00
	Total	133883		100.00

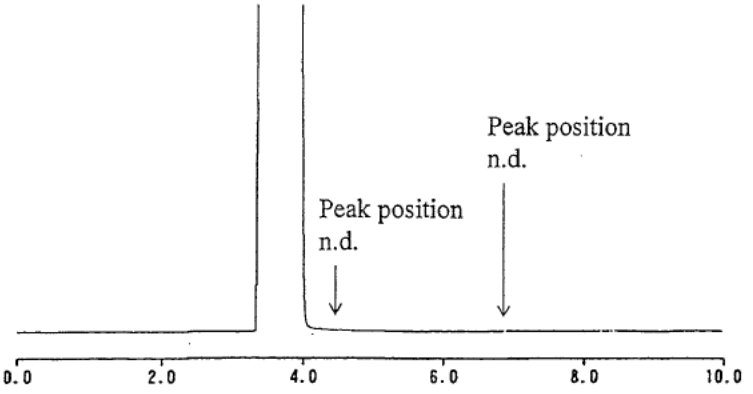


Operator :

[5] Control blank

Operating Date : Dec.20,2006

PKNO	Time (min)	Area (μV·sec)	Height (μV)	Conc. (%)



Operator :

Fig. 9 - 2 Chromatograms of GC analysis for test solution (test item and 13F-EtOH)

Date : Dec.20,2006 Name :

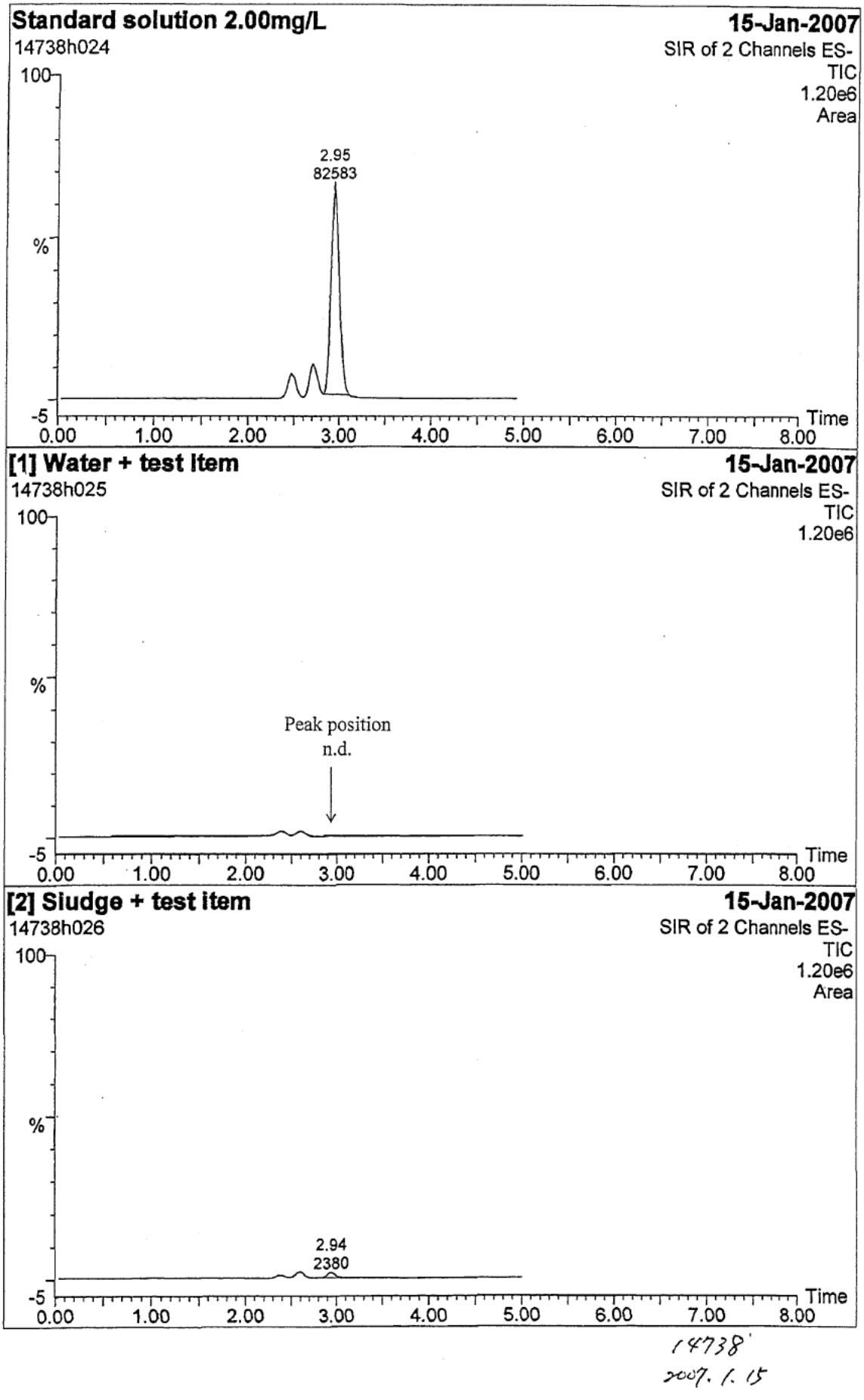


Fig. 10 - 1 Total ion chromatograms of LC-MS analysis for test solution (13F-AcOH).

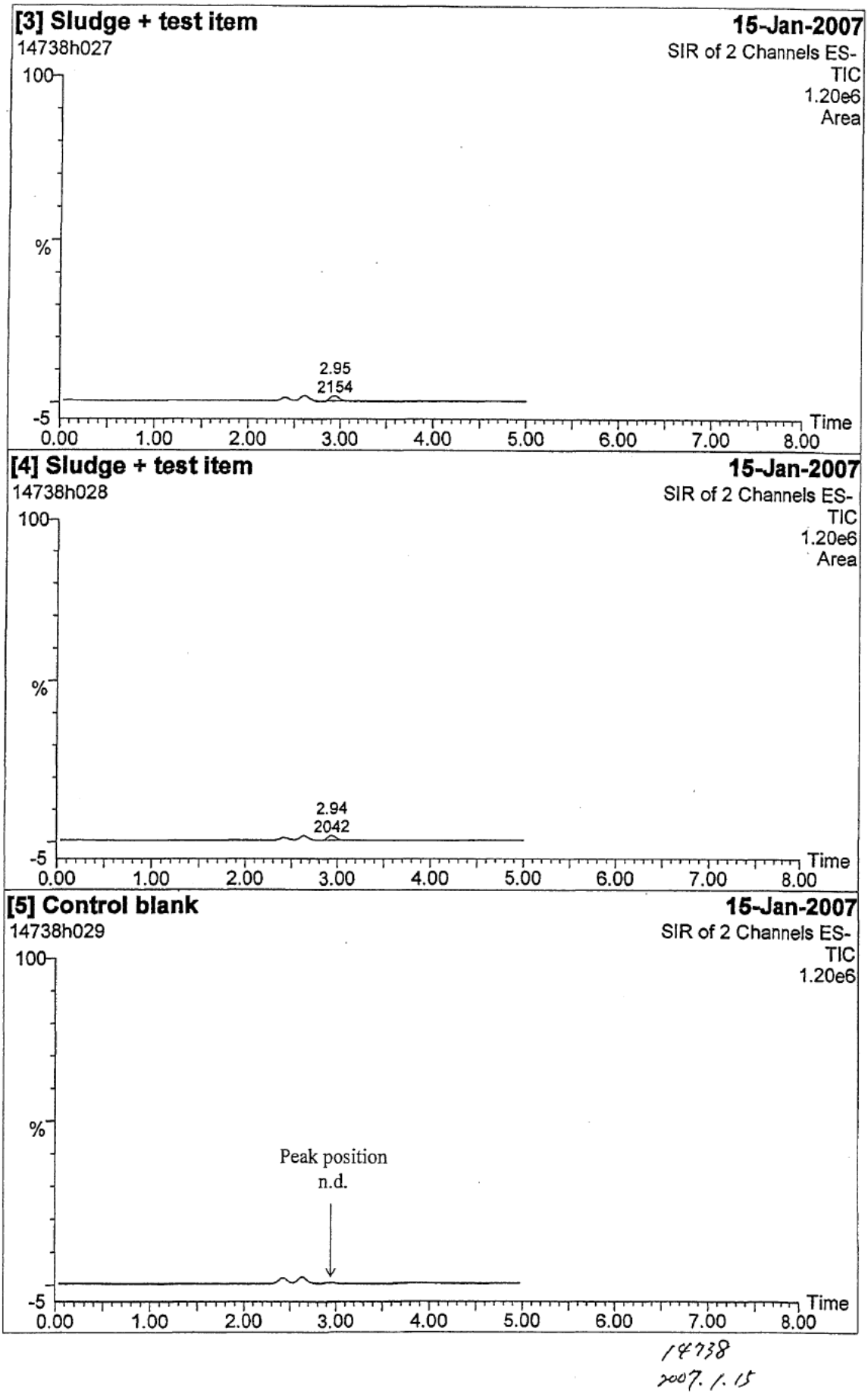
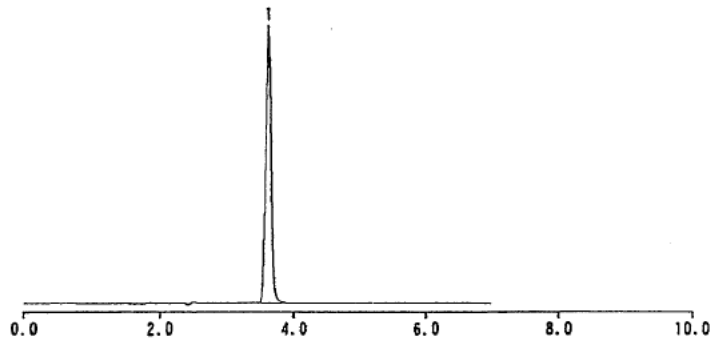


Fig. 10 - 2 Total ion chromatograms of LC-MS analysis for test solution (13F-AcOH).

Standard solution 20.0 mg/L

Operating Date : Dec.19,2006

PKNO	Time (min)	Area (μV·sec)	Height (μV)	Conc. (%)
1	3.60	1427740	249595	100.00
	Total	1427740		100.00



Operator :

Water + test item

Operating Date : Dec.19,2006

PKNO	Time (min)	Area (μV·sec)	Height (μV)	Conc. (%)

Peak position
n.d.

Operator :

Sludge + test item

Operating Date : Dec.19,2006

PKNO	Time (min)	Area (μV·sec)	Height (μV)	Conc. (%)

Peak position
n.d.

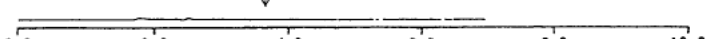
Operator :

Control blank

Operating Date : Dec.19,2006

PKNO	Time (min)	Area (μV·sec)	Height (μV)	Conc. (%)

Peak position
n.d.



Operator :

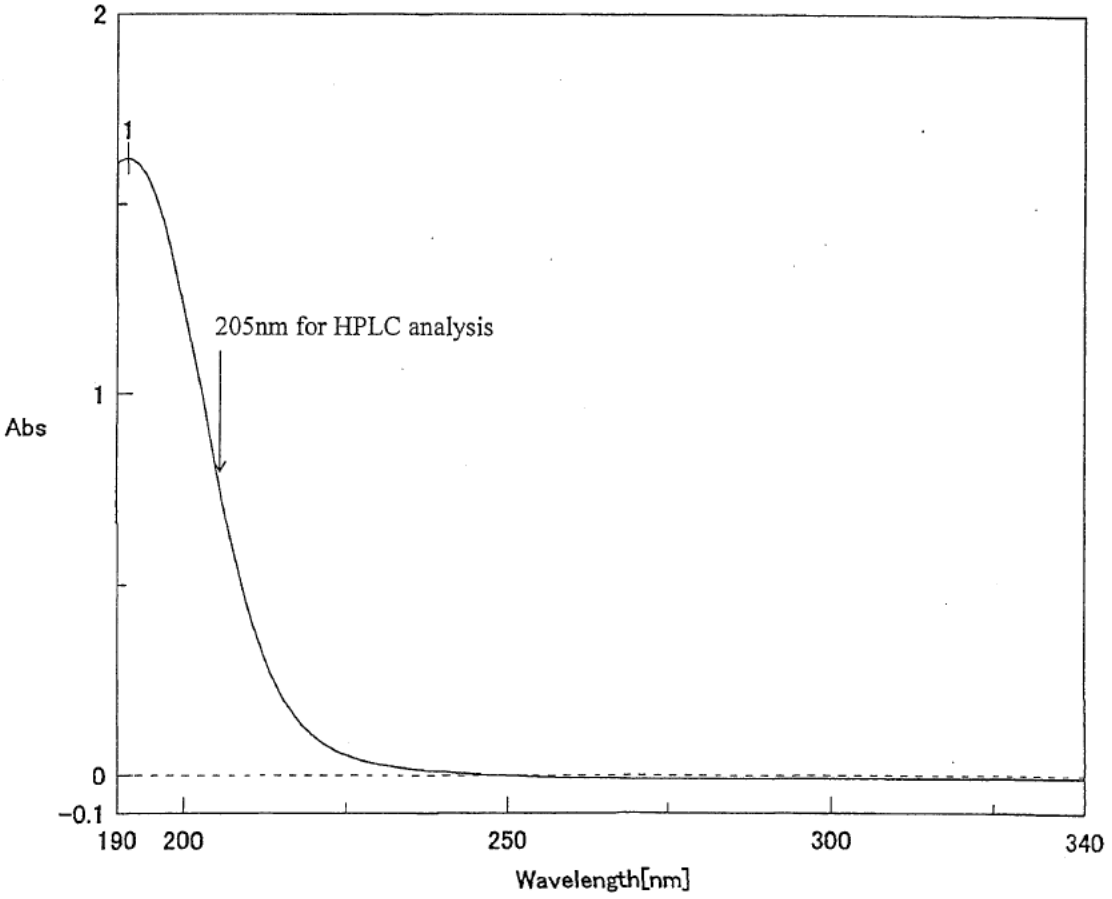
Fig. 11 Chromatograms of HPLC analysis for test solution for analysis of acrylic acid (acrylic acid).

Date : Jan.29,2007 Name :

入力日 2006、11、21

StudyNo.	14738	Wavelength	190.00 - 340.00
Date	Nov. 21, 2006	Scale Limit	~~2.0000~~ - -0.1000
Sample	Acrylic acid	Slit Width	(UV) 2.0nm
Solvent	Purified water	Scan Speed	200nm/min
Reference	-	Sampling Pitch	0.200000
Cell	10mm x 10mm, quartz	Analyst	
Instrument	JASCO	Note	10.0 mg/L
Photometric Mode	Abs		

Chemicals Evaluation and Research Institute, Japan Kurume Laboratory



Peak List

1 : 191.60 (1.6193)

Fig. 12 **UV spectrum of** acrylic acid .

Study No. 14738

Analytical conditions

Instrument MS : Waters ZMD, LC : Waters Alliance2690

Sample 30.0mg/L 13F-AcOH solution

LC Conditions

Inlet system Column

Column L-column ODS Column size 15 cm x 2.1 mm I.D.

Column temp. 40℃

Eluent A (80%) : Acetonitrile / formic acid (500/0.25 V/V)

B (20%) : Water / formic acid (500/0.25 V/V)

Flow rate 0.2 mL/min

Sample size 2 μL (Solvent Acetonitrile)

MS Conditions

Ionization mode ESI Detection mode Negative

Function SCAN

Mass range (m/z) 200 - 1000

Probe Capillary 3.0 kV Desolvation temp. 350 ℃ Desolvation gas 400 L/hr

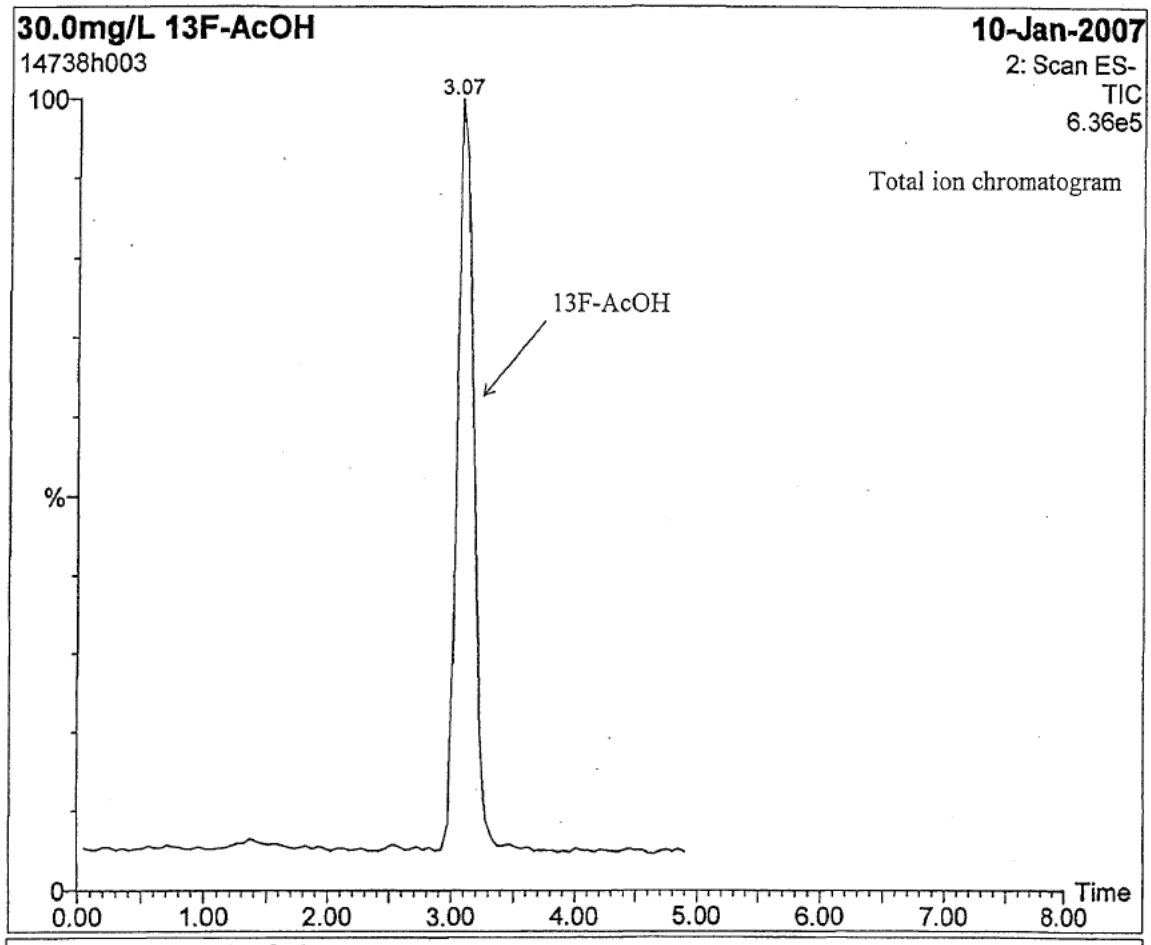
Source Cone 20 V, Extractor 2 V, RF Lens 0.2 V

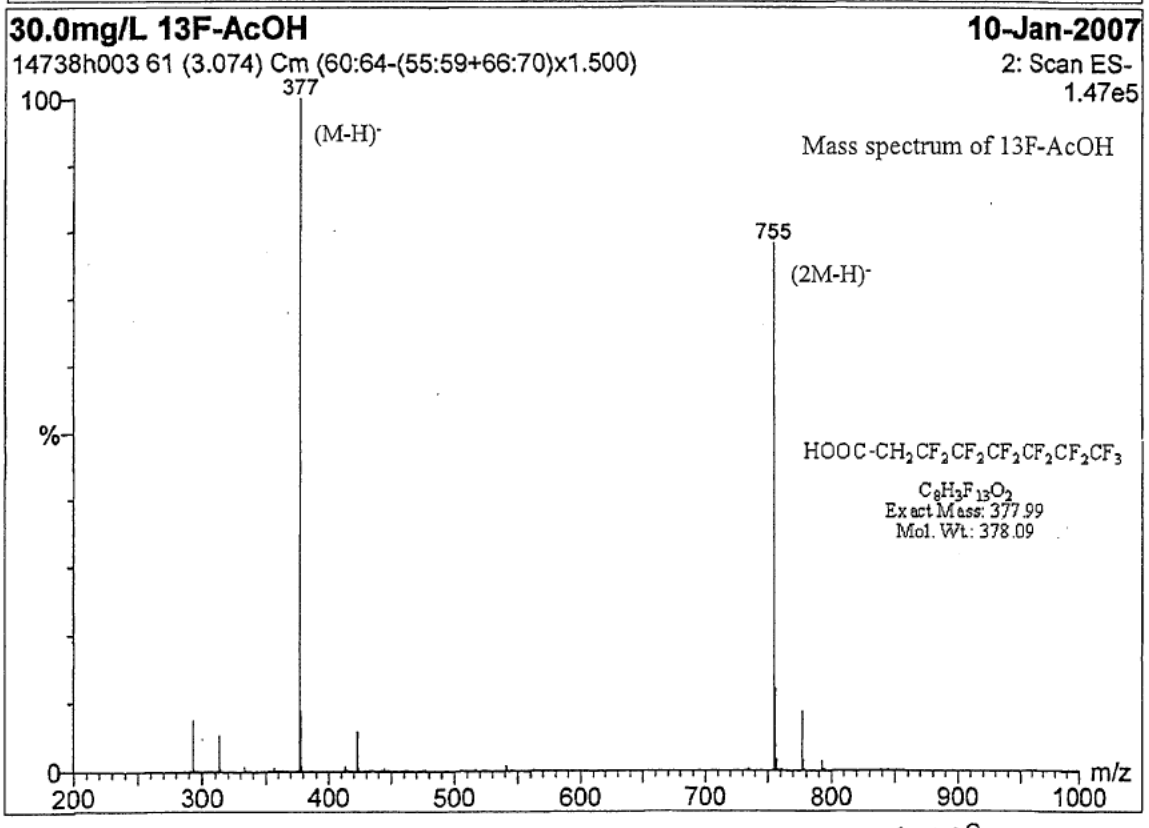
Source block temp. 120 ℃

Operator

Kurume Laboratory, Chemicals Evaluation and Research Institute, Japan

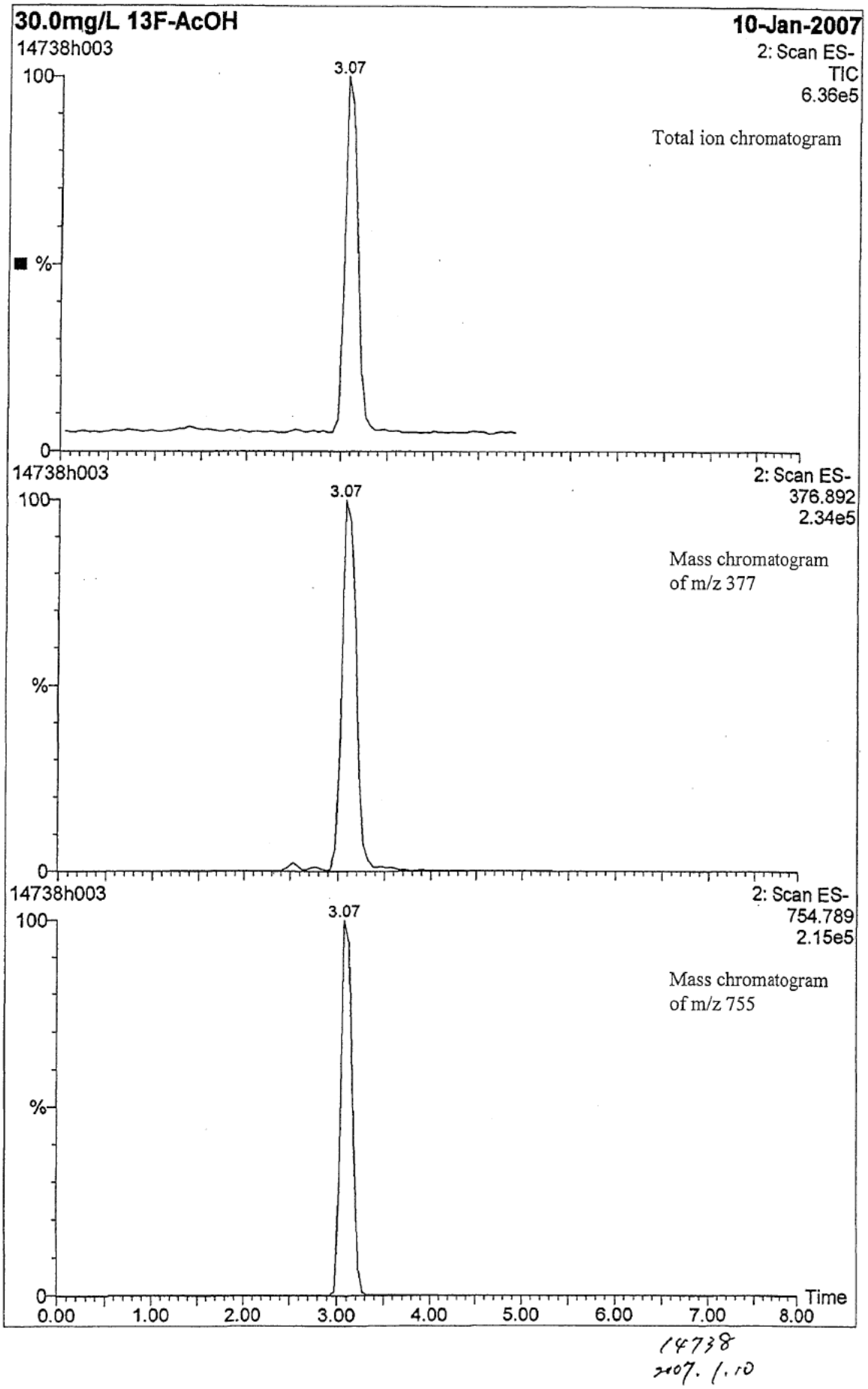
Fig. 13 - 1 Mass spectrum of 13F-AcOH.





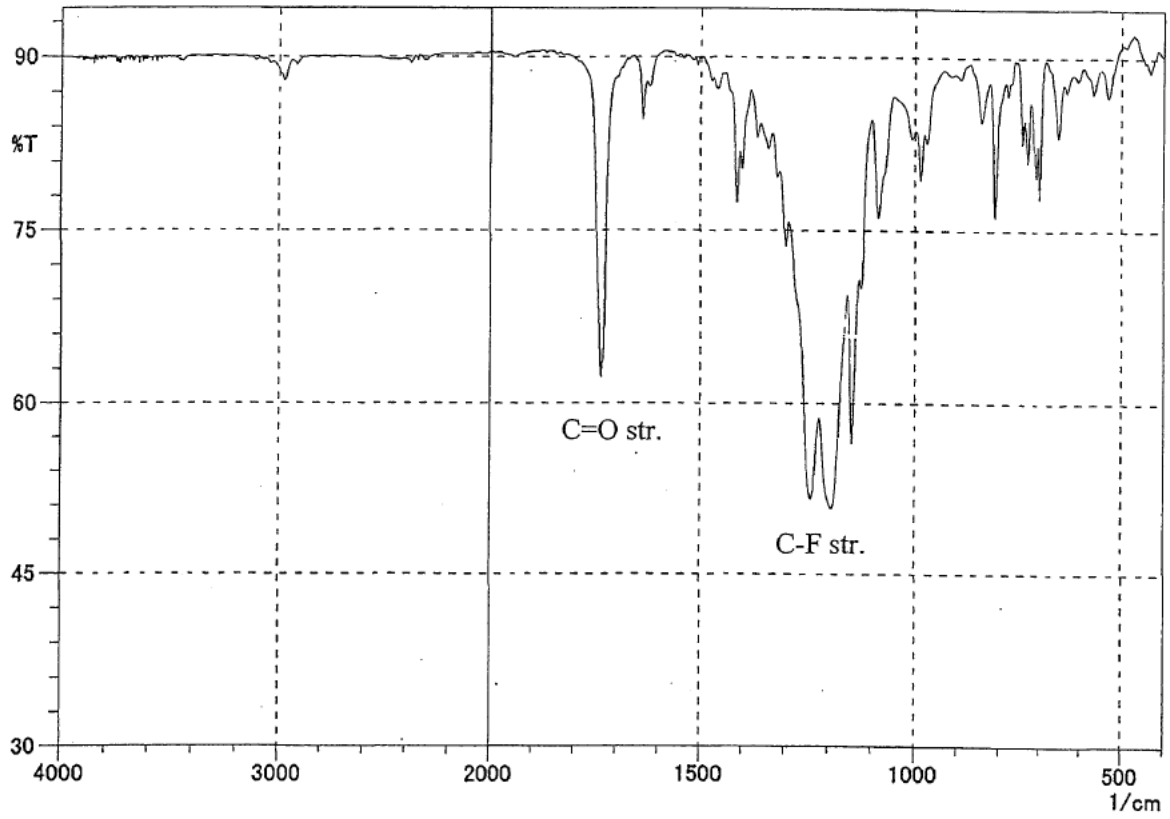
14738
2007. 1. 10

Fig. 13 - 2 Mass spectrum of 13F-AcOH.



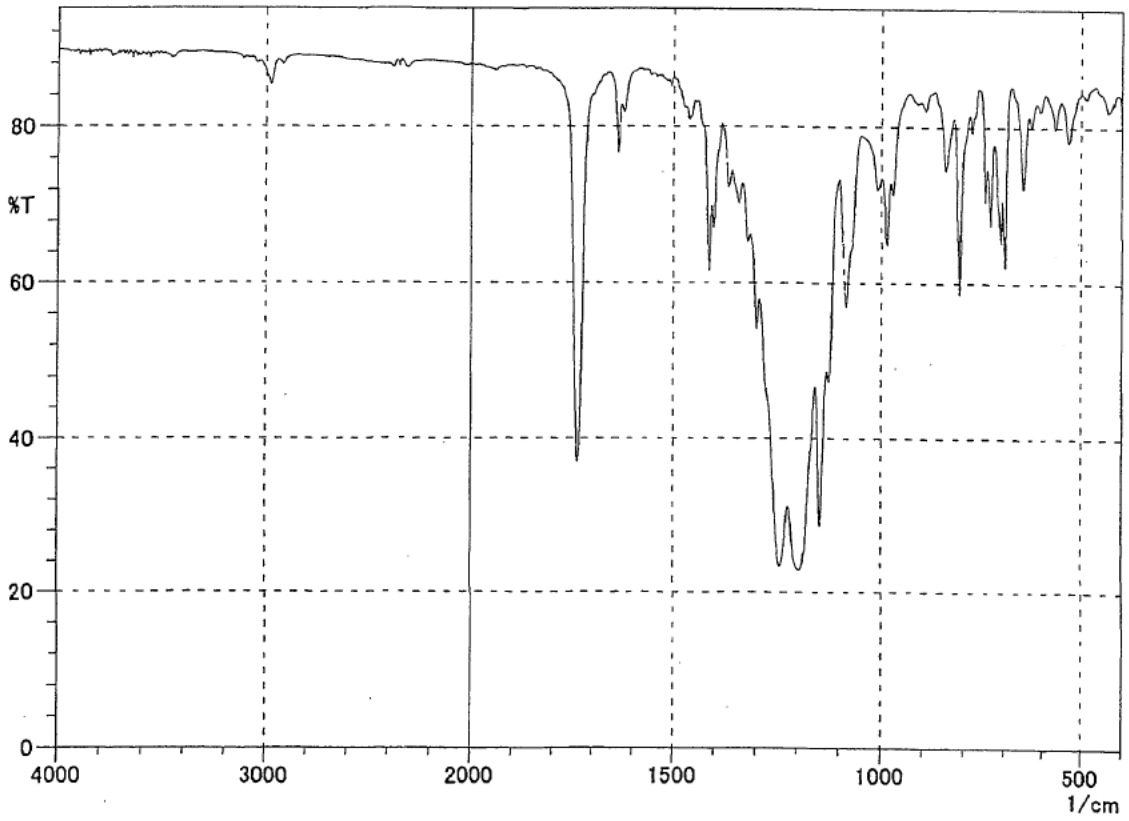
14738
2007. 1.10

Fig. 13 - 3 Mass spectrum of 13F-AcOH.



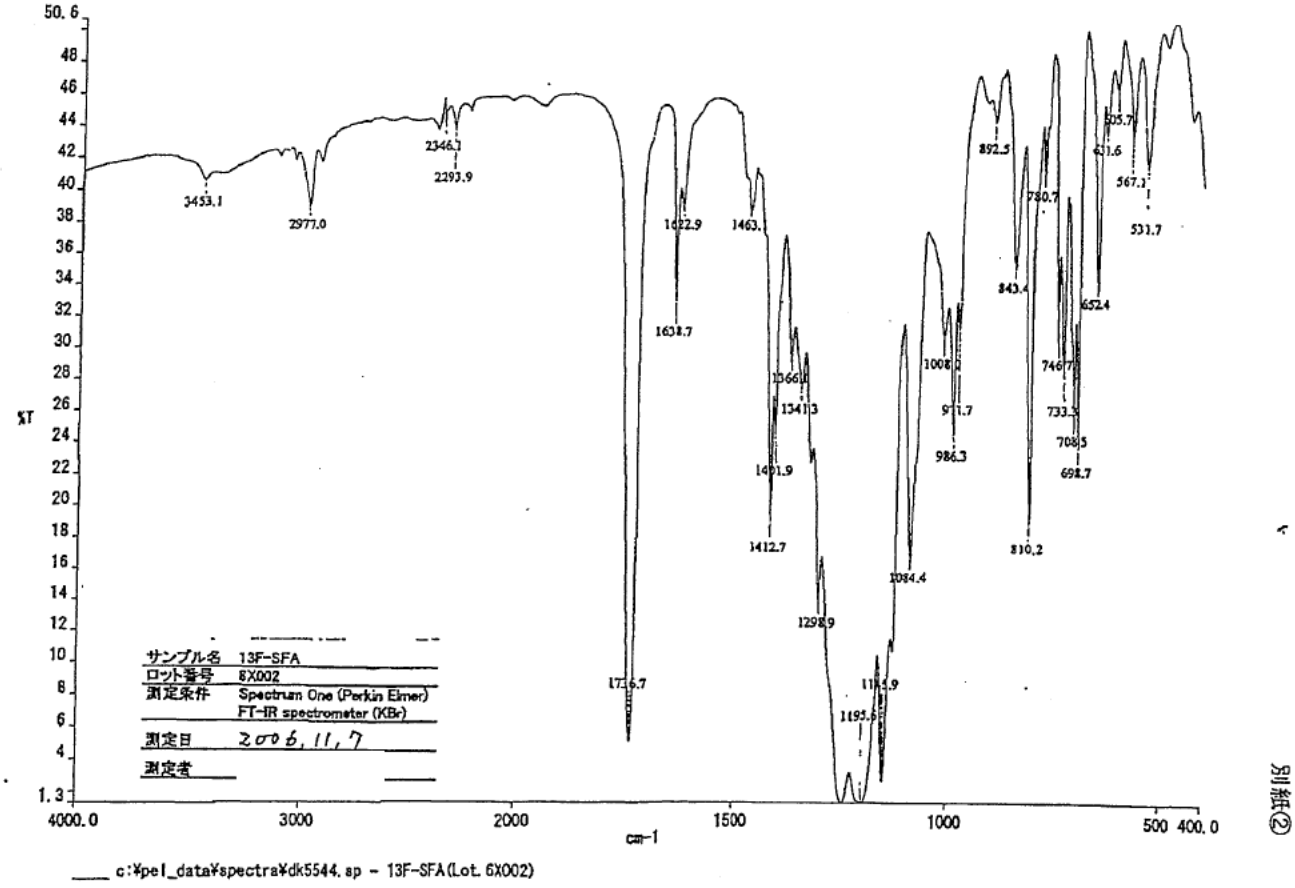
Instrument	: Shimadzu IRPrestige-21
Study No.	: 14738
Sample	: Test item
Method	: Neat
Date	: November 15, 2006
Name	:

Fig.14 - 1 IR spectrum of test item measured before experimental start.



Instrument	: Shimadzu IRPrestige-21
Study No.	: 14738
Sample	: Test item
Method	: Neat
Date	: January 18, 2007
Name	:

Fig.14 - 2 IR spectrum of test item measured after experimental completion.



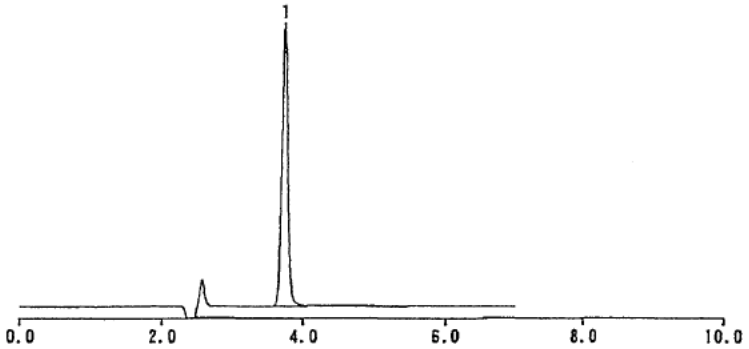
別紙②

Reference 3 IR spectrum supplied by sponsor.

Standard solution 20.0 mg/L

Operating Date : Dec.22,2006

PKNO	Time (min)	Area (μV·sec)	Height (μV)	Conc. (%)
1	3.72	1425431	240828	100.00
	Total	1425431		100.00

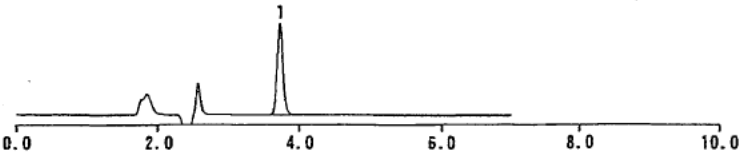


Operator :

Water + test item

Operating Date : Dec.22,2006

PKNO	Time (min)	Area (μV·sec)	Height (μV)	Conc. (%)
1	3.72	471153	79448	100.00
	Total	471153		100.00

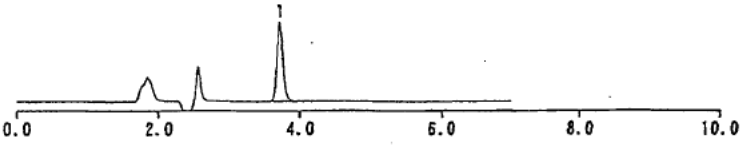


Operator :

Sludge + test item

Operating Date : Dec.22,2006

PKNO	Time (min)	Area (μV·sec)	Height (μV)	Conc. (%)
1	3.70	403814	67875	100.00
	Total	403814		100.00

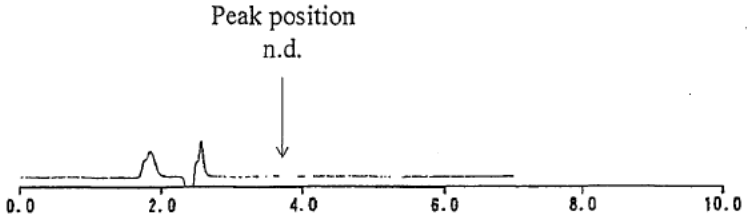


Operator :

Control blank

Operating Date : Dec.22,2006

PKNO	Time (min)	Area (μV·sec)	Height (μV)	Conc. (%)



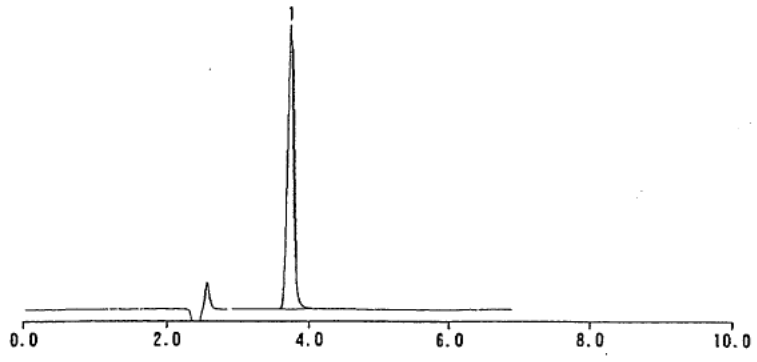
Operator :

Reference 4 Chromatograms of HPLC analysis for CO2 absorbent (acrylic acid, test solution for analysis of acrylic acid). Date : Dec.22,2006 Name :

Standard solution 20.0 mg/L

Operating Date : Dec.22,2006

PKNO	Time (min)	Area (μV·sec)	Height (μV)	Conc. (%)
1	3.72	1433100	240949	100.00
	Total	1433100		100.00

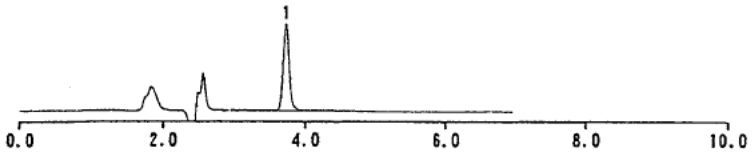


Operator :

[1] Water + test item

Operating Date : Dec.22,2006

PKNO	Time (min)	Area (μV·sec)	Height (μV)	Conc. (%)
1	3.72	443994	75841	100.00
	Total	443994		100.00

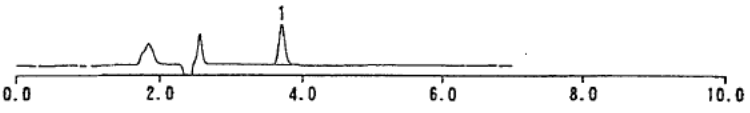


Operator :

[2] Sludge + test item

Operating Date : Dec.22,2006

PKNO	Time (min)	Area (μV·sec)	Height (μV)	Conc. (%)
1	3.70	210699	35587	100.00
	Total	210699		100.00



Operator :

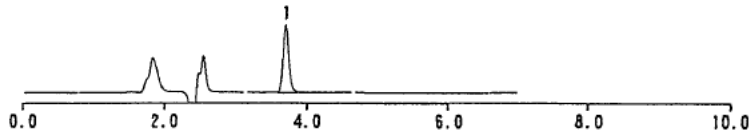
Reference 5 - 1 Chromatograms of HPLC analysis for CO2 absorbent (acrylic acid).

Date : Dec.22,2006 Name :

[3] Sludge + test item

Operating Date : Dec.22,2006

PKNO	Time (min)	Area (μV·sec)	Height (μV)	Conc. (%)
1	3.68	338425	57554	100.00
	Total	338425		100.00

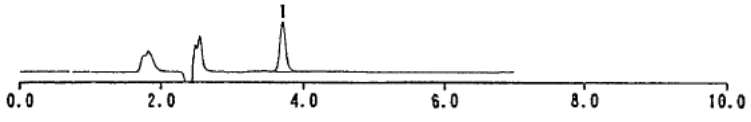


Operator :

[4] Sludge + test item

Operating Date : Dec.22,2006

PKNO	Time (min)	Area (μV·sec)	Height (μV)	Conc. (%)
1	3.68	249622	42627	100.00
	Total	249622		100.00

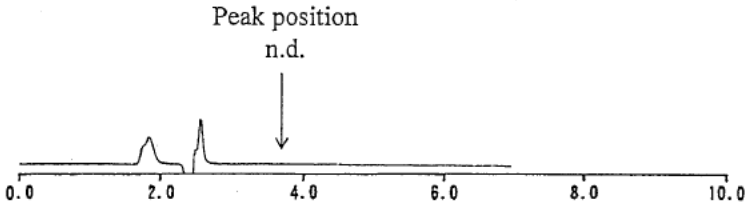


Operator :

[5] Control blank

Operating Date : Dec.22,2006

PKNO	Time (min)	Area (μV·sec)	Height (μV)	Conc. (%)



Operator :

Reference 5 – 2 Chromatograms of HPLC analysis for CO2 absorbent (acrylic acid).

Date : Dec.22,2006 Name :

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　近藤　雅臣　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適合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並びに第一種監視化学物質及び第二種監視化学物質に係る有害性の調査の項目等を定める省令第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、確認の有効期間は、平成１９年１２月２２日から起算して３年間とします。

平成１９年１２月２１日

経済産業省製造産業局長　細野　哲弘

記

試験施設の名称	財団法人化学物質評価研究機構 ①久留米事業所及び②東京事業所
試験施設の所在地	①福岡県久留米市宮ノ陣三丁目２番７号 ②埼玉県北葛飾郡杉戸町大字下高野１６００
試験項目	分解度試験、濃縮度試験及び分配係数試験