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Study Report

Study of [redacted] Accumulation in Carp

September 22, 2003

Chemicals Evaluation and Research Institute, Japan
--Kurume Laboratory--

Figures

Title:	Study of [redacted] Accumulation in Carp
Commissioned by:	DuPont KK 1-8-1 Shimo-meguro, Meguro-ku, Tokyo 153-0064
Testing Facility:	Kurume Laboratory Chemicals Evaluation and Research Institute, Japan (CERI) 19-14 Chuo-machi, Kurume-shi, Fukuoka Prefecture 830-0023
Purpose of Study:	To obtain findings on the accumulation of [redacted] in carp.
Personnel:	Study Coordinator: Naoaki YAGATA,* Testing Section 2 Researchers: Yoshitaka KOGA Masanori WATASHIMA

Approval of Study Report: [signed] Naoaki YAGATA
Study Coordinator
September 22, 2003

* Translator's Note: Spellings of personal names may need to be verified.

1. Test Substance

Name: [redacted]

Lot No.: [redacted]

Purity: [redacted]

2. Preparation of Stock Solution

A stock solution having a test substance concentration of 500 mg/L was prepared by first grinding together in a mortar the test substance and a 20-fold amount of crystal sugar, subsequently adding a 20-fold amount (relative to the test substance) of Megafac F-142D and again grinding the ingredients together in a mortar, then adding ion-exchanged water.

3. Preliminary Test of Acute Toxicity

3.1 Test Conditions

(1) Test fish:	Himedaka* (*Oryzias latipes*)
(2) Number of test fish:	2 fish per concentration group
(3) Period of exposure:	96 hours
(4) Method of exposure:	semi-stagnant water method (change of water every 8 to 16 hours)
(5) Preliminary test results:	preliminary LC_{50} over 96 hours was >30.0 mg/L

* TN: Japanese name of fish. English name is not known.

4. Investigation of Analysis Conditions

Based on the preliminary LC_{50} value of the test substance over 96 hours and the sensitivity of analysis, the test concentrations were set as follows

First concentration group: 50 μg/L

Second concentration group: 5 μg/L,

and an examination of the analysis conditions was carried out.

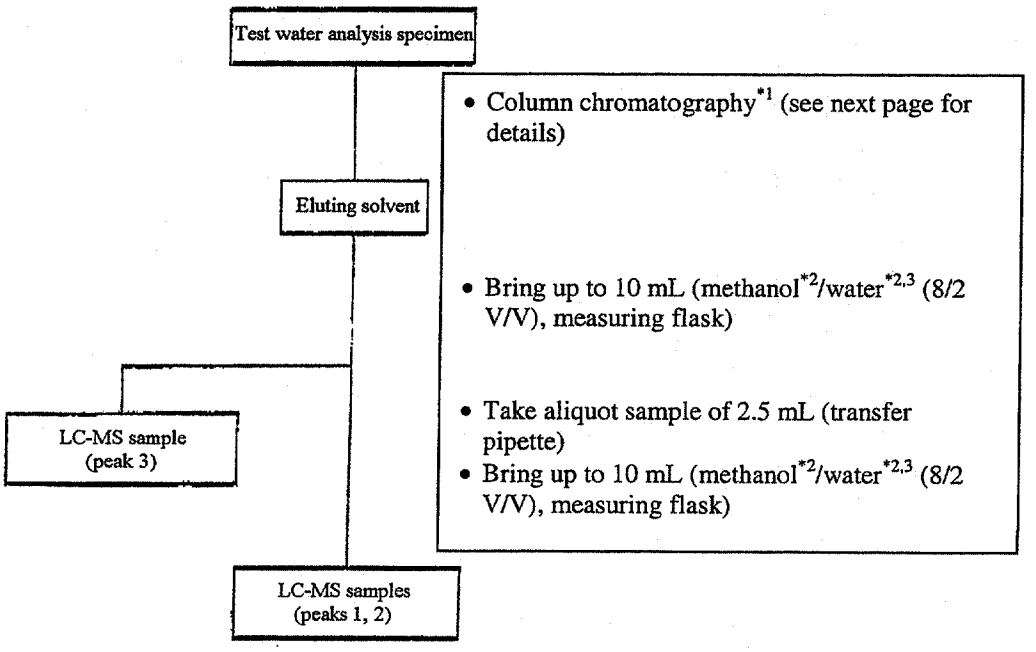
4.1 Test Substance Analysis in Test Water and Test Fish

When the test substance was analyzed by liquid chromatography-mass spectrometry (LC/MS), three peaks were detected. Quantitative determinations were thus carried out on each of the peaks. The respective peak concentrations are indicated as the test substance concentrations shown in section 4.2.2 ("Preparation of Standard Solution"), regardless of the constituent compositions. The peaks were named Peak 1, Peak 2 and Peak 3, in the order of elution.

4.1.1 Investigation of Method for Pretreating Test Water Analysis Specimens

Stock solution (containing 2 μg of the test substance) was added to 400 mL of test water for the recovery test, and pretreatment operations were carried out according to the flow scheme shown below to give a liquid chromatography-mass spectrometry (LC-MS) specimen.

Flow Scheme



*1: Column chromatography conditions:

SepPack C_{18}		
(Cleaning method:	10 mL each of methanol[*2]/water[*2,3] (8/2 V/V) and water[*3])	
Loading method:	Entire amount was loaded.	
Elution method:	First eluting solvent: water[*3]	5 mL
	Second eluting solvent: methanol[*2]/water[*2,3] (8/2 V/V)	8 mL
	The second eluting solvent was furnished for analysis.	

*2: Contains 10 mmol/L of ammonium acetate

*3: Water obtained by treating tap water using an ultrapure water production system.

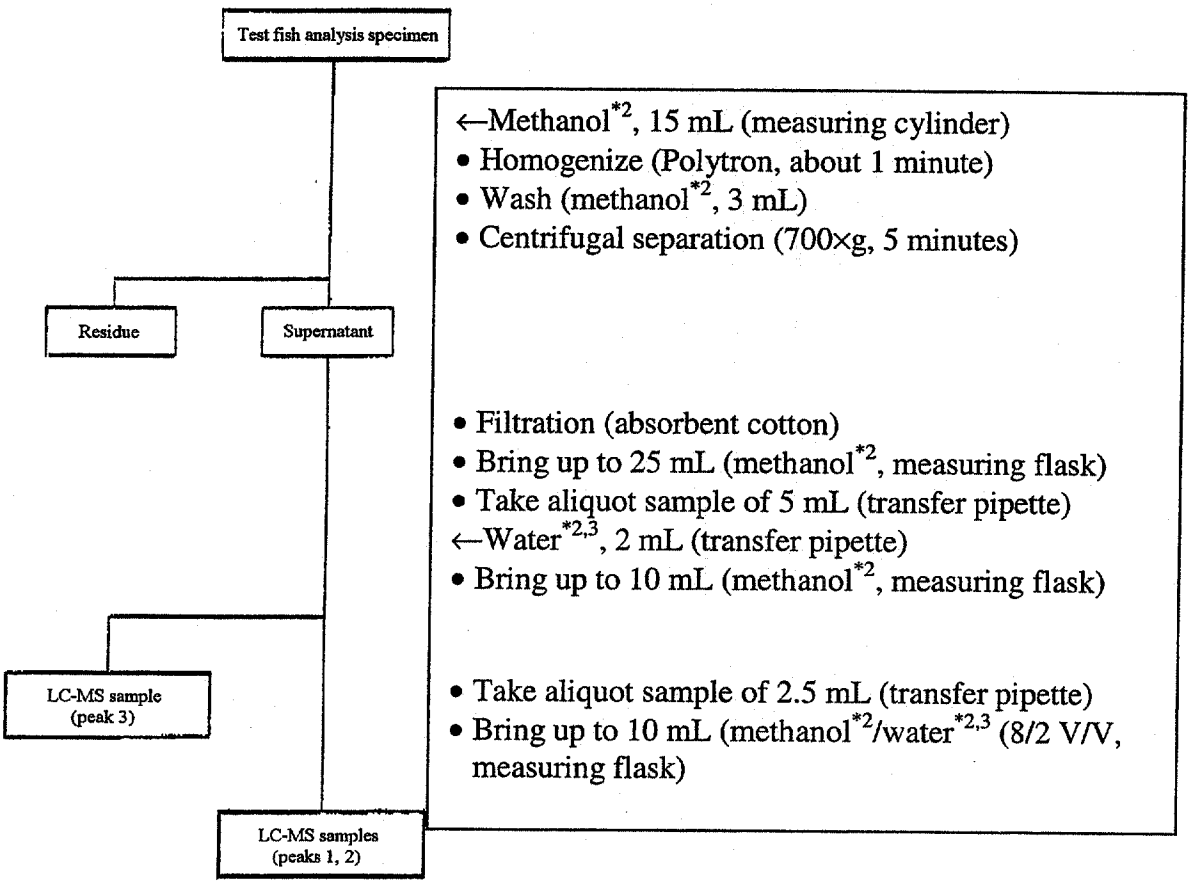
The percent recovery in each of the above operations are given below (see Fig. 1).

Percent recovery (n = 1)	Peak 1:	85.1%
	Peak 2:	90.5%
	Peak 3:	74.2%
Blank (n = 1)	Peak 1:	not detected
	Peak 2:	not detected
	Peak 3:	not detected

4.1.2 Investigation of Method for Pretreating Test Fish Analysis Specimens

Stock solution (containing 5 μg of the test substance) was added to 5 g of a finely ground sample of the test fish for the recovery test, and pretreatment operations were carried out according to the flow scheme shown below to give a liquid chromatography-mass spectrometry (LC-MS) specimen.

Flow Scheme



The percent recovery in each of the above operations are given below (see Fig. 2).

Percent recovery (n = 1)	Peak 1:	104%
	Peak 2:	119%
	Peak 3:	(not measurable due to insufficient LC-MS sensitivity)
Blank (n = 1)	Peak 1:	not detected
	Peak 2:	not detected
	Peak 3:	not detected

4.2 Quantitative Analysis

4.2.1 Quantitative Determination Conditions:

Apparatus:	Liquid chromatograph-mass spectrometer
Pump:	PU-980 (JASCO)
Autosampler:	AS-950 (JASCO)
Mass Spectrometer:	Quattro II (Micromass)

Liquid Chromatography Conditions

Column:	L-column ODS (CERI); 15 cm × 2.1 mm I.D.
Column temperature:	35°C
Eluting solvent:	A (80%): methanol[*2]
	B (20%): water[*2,3]
Flow rate:	0.2 mL/min
Injection rate:	20 μL

Mass Spectrometer Conditions

Ionization method:	Electrospray (ESI)
Detected ions:	Positive ions
Detection method:	Selected ion monitoring (SIM)
Selected ions:	Peak 1 m/z 571
	Peak 2 m/z 671
	Peak 3 m/z 771
Ion source temperature:	120°C
Cone voltage:	50 V

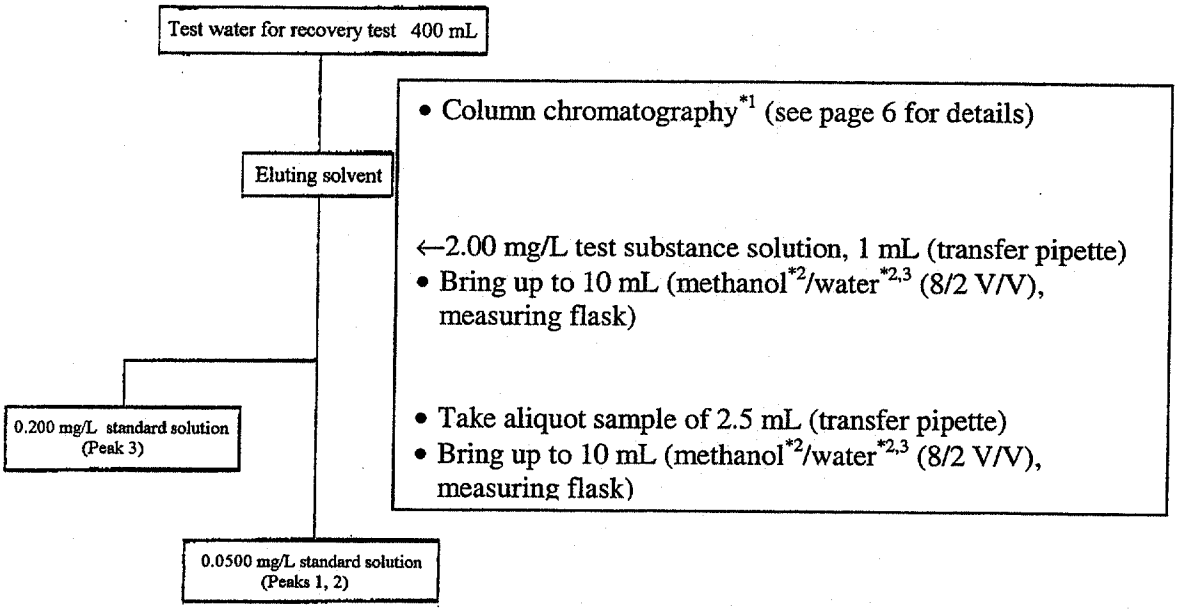
4.2.2 Preparation of Standard Solution:

Standard solutions for determining the test substance concentration in the analytic sample were prepared as described below.

(1) Test water analysis:

One hundred milligrams of the sample furnished was precisely weighed out and dissolved in methanol to form a 1,000 mg/L test substance solution. This solution was then dissolved in methanol[*2]/water[*2,3] (8/2 V/V) to form a 2.00 mg/L solution. Pretreatment operations were carried out on the resulting solution in accordance with the flow scheme shown below, thereby forming 0.0500 mg/L (for Peaks 1 and 2) and 0.200 mg/L (for Peak 3) standard solutions.

Flow Scheme



(2) Test fish analysis:

One hundred milligrams of the sample furnished was precisely weighed out and dissolved in methanol to form a 1,000 mg/L test substance solution. This solution was then diluted with methanol[*2]/water[*2,3] (8/2 V/V) to form 0.0500 mg/L standard solutions for Peaks 1 and 2 and a 0.200 mg/L standard solution for Peak 3.

(3) Preparation of calibration curve:

Following the same procedure as that used to prepare the standard solutions in (2) above, 0.0250, 0.0500 and 0.100 mg/L standard solutions for Peaks 1 and 2, and 0.100, 0.200 and 0.400 mg/L standard solutions for Peak 3 were prepared. These were analyzed under the quantitative determination conditions given in section 4.2.1 above, and a calibration curve was prepared from the respective concentrations and the peak areas on the chromatogram (see Fig. 3).

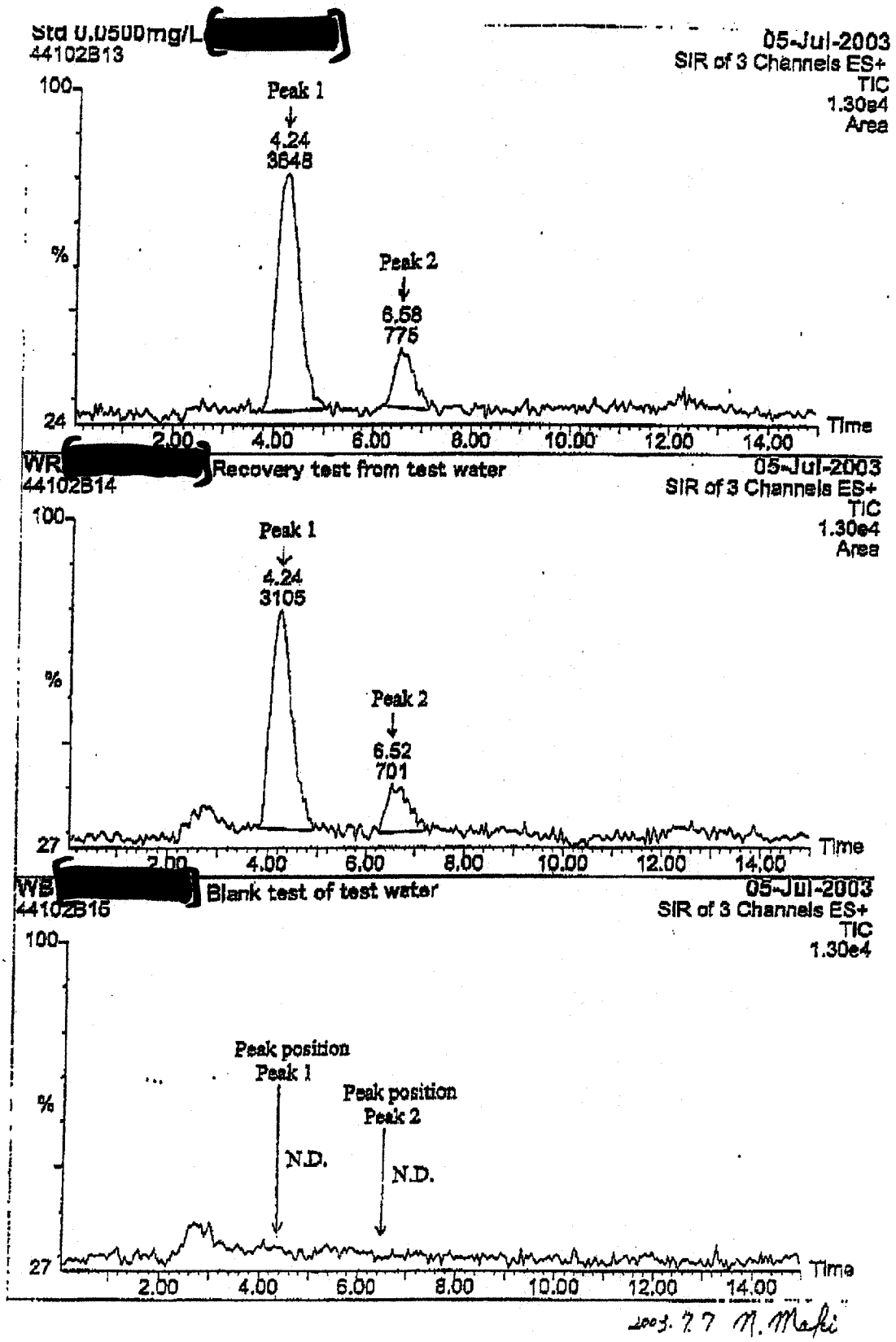


Fig.1-1 Mass fragmentogram of LC-MS analysis for recovery and blank test (analysis of test water)

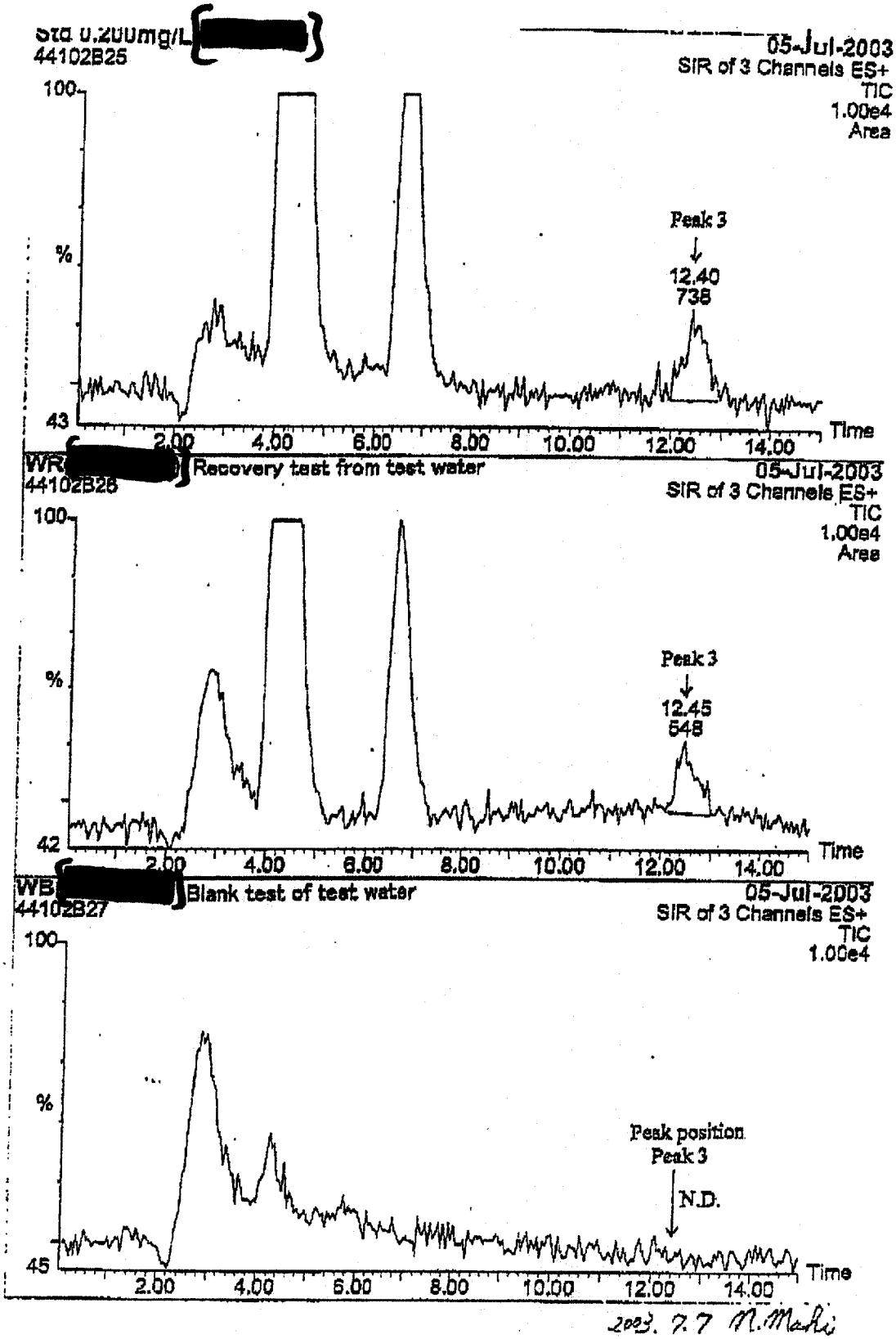


Fig.1-2 Mass fragmentogram of LC-MS analysis for recovery and blank test (analysis of test water)

Company Sanitized. Does not contain TSCA CBI

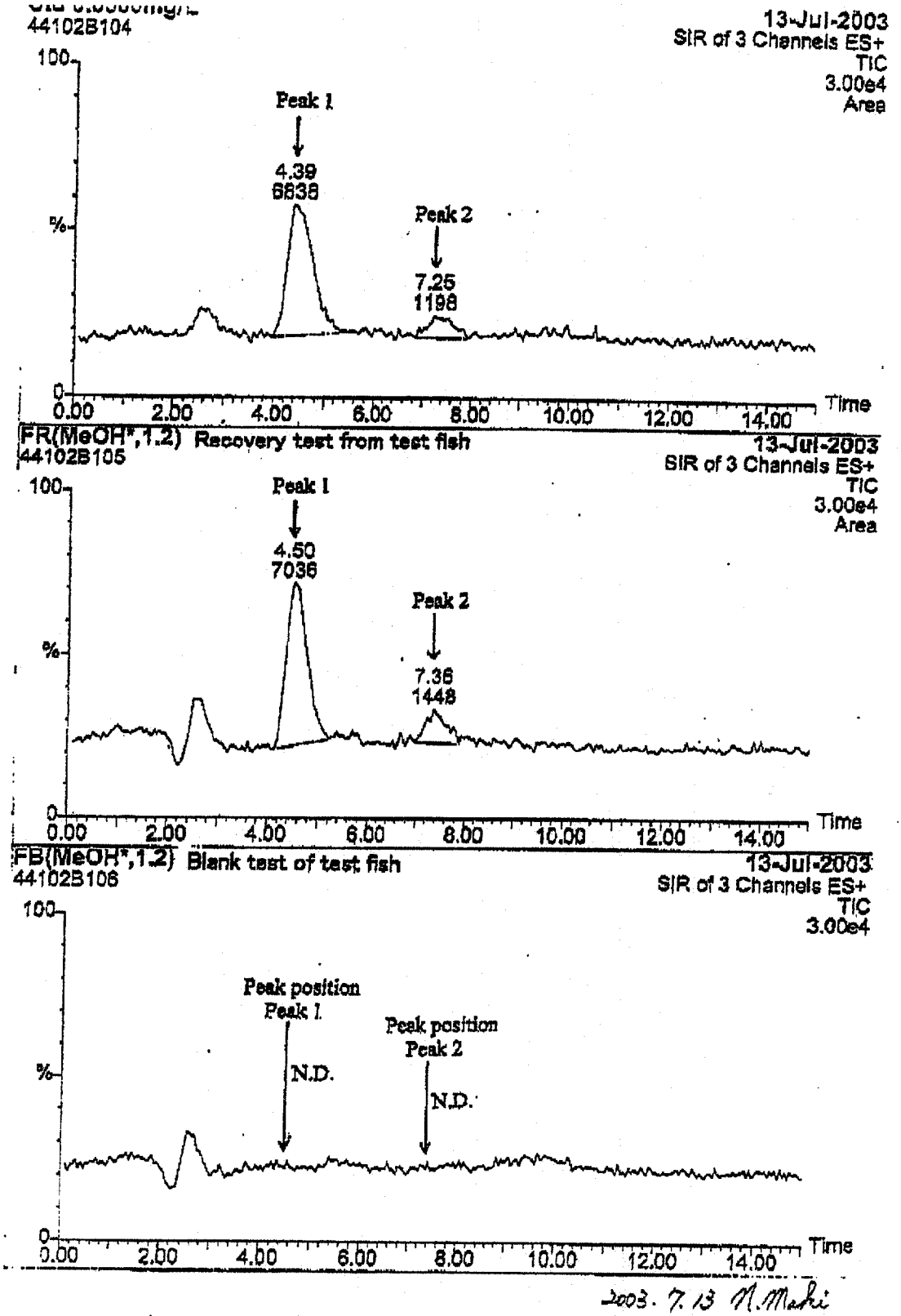


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Fig.2 Mass fragmentogram of LC-MS analysis for recovery and blank test (analysis of test fish)

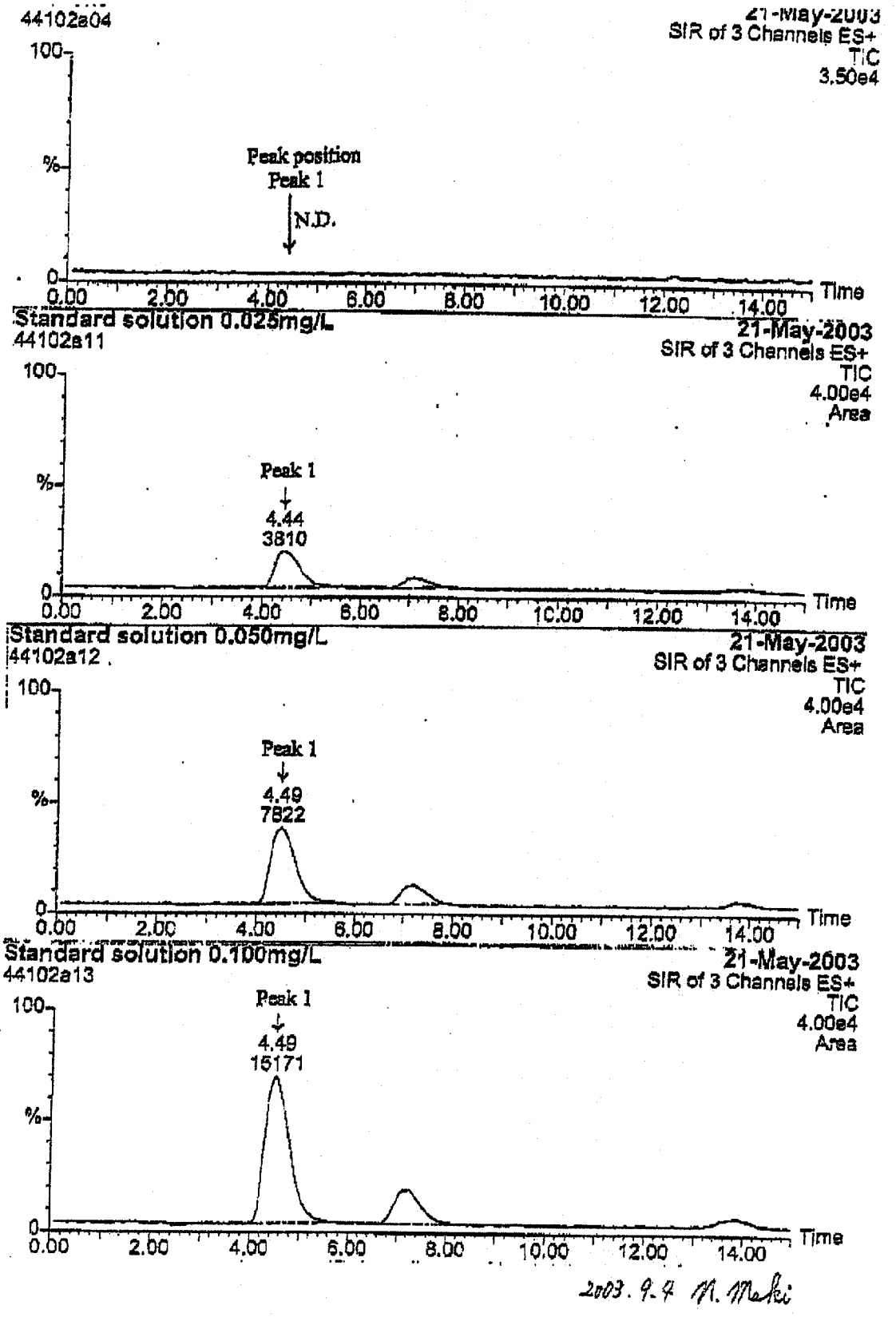
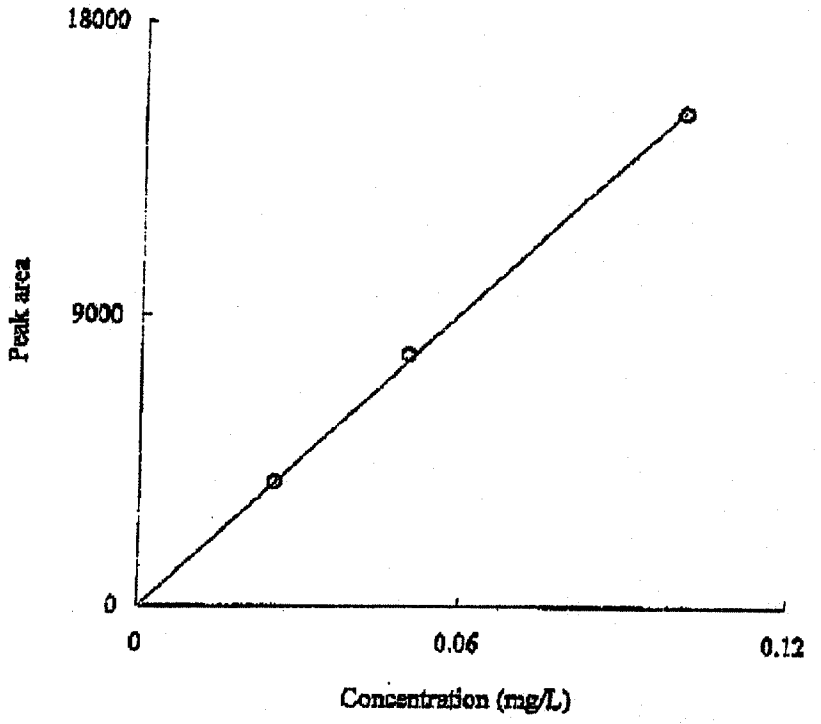


Fig.3-1-1 Mass fragmentogram of LC-MS analysis for calibration curve (Peak 1)



y = 152491x
r = 1.00

Concentration (mg/L)	Peak area
0.0250	3810
0.0500	7782
0.100	15171

Fig. 3 - 1 - 2 Calibration curve of LC-MS analysis (Peak 1).

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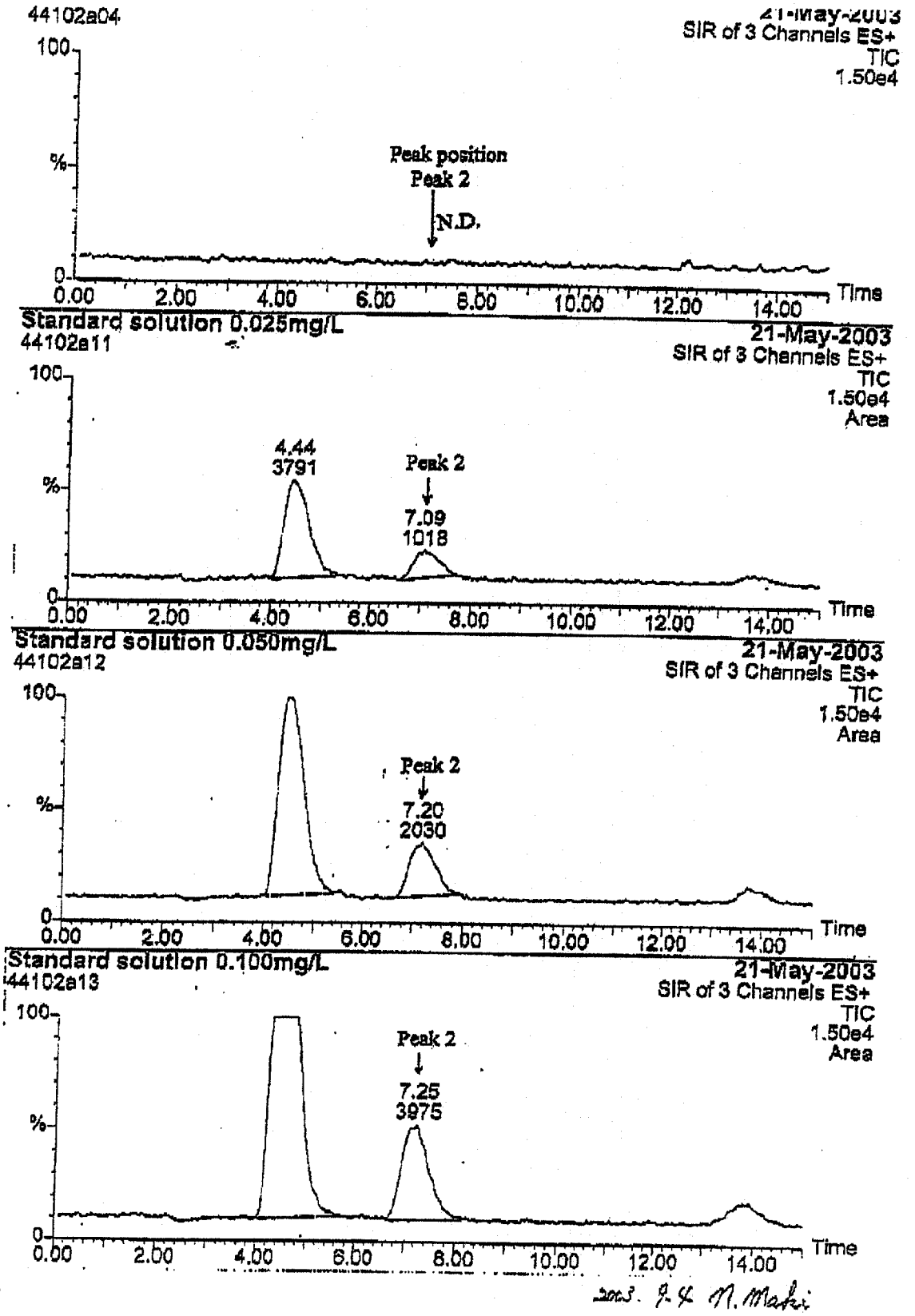
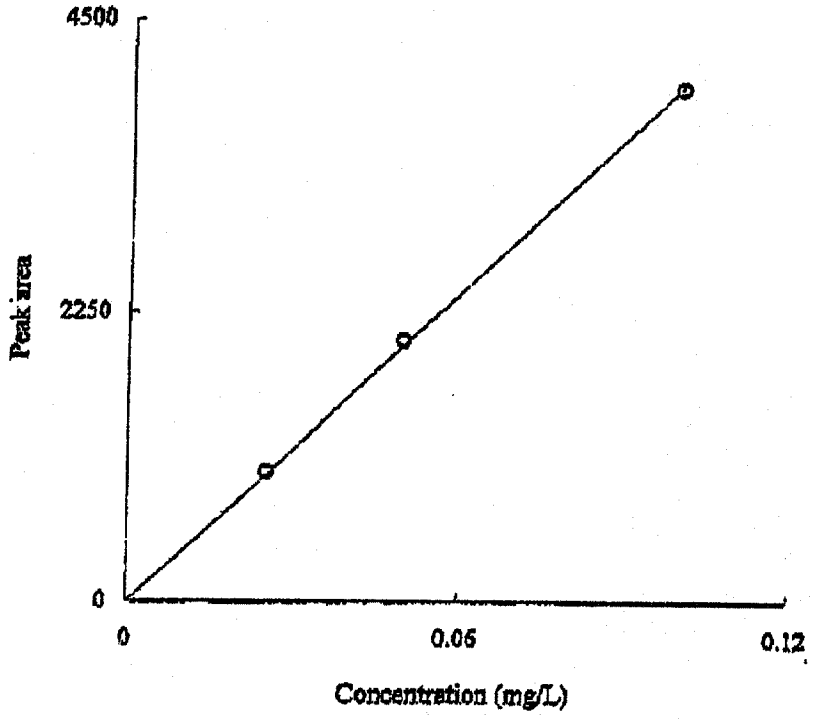


Fig.3-2-1 Mass fragmentogram of LC-MS analysis for calibration curve (Peak 2)



y = 39958x
r = 1.00

Concentration (mg/L)	Peak area
0.0250	1018
0.0500	2030
0.100	3975

Fig. 3 - 2 - 2 Calibration curve of LC-MS analysis (Peak 2).

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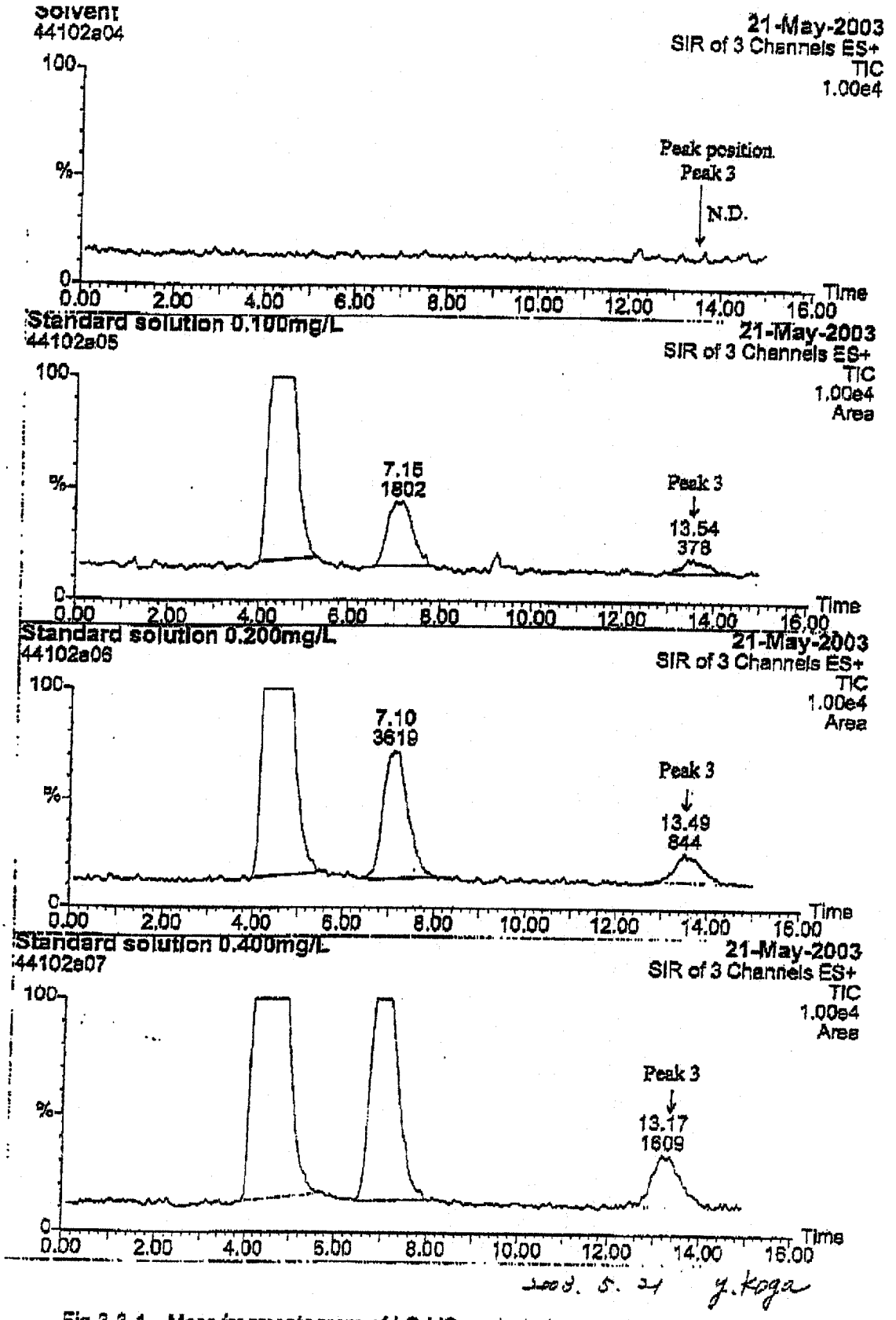
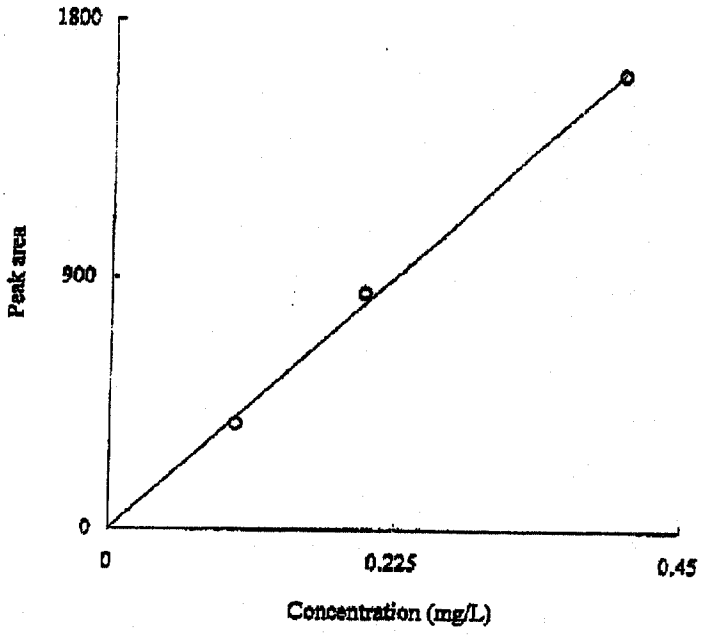


Fig.3-3-1 Mass fragmentogram of LC-MS analysis for calibration curve (Peak 3)



y = 4049x
r = 0.999

Concentration (mg/L)	Peak area
0.100	378
0.200	844
0.400	1609

Fig. 3 - 3 - 2 Calibration curve of LC-MS analysis (Peak 3).

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