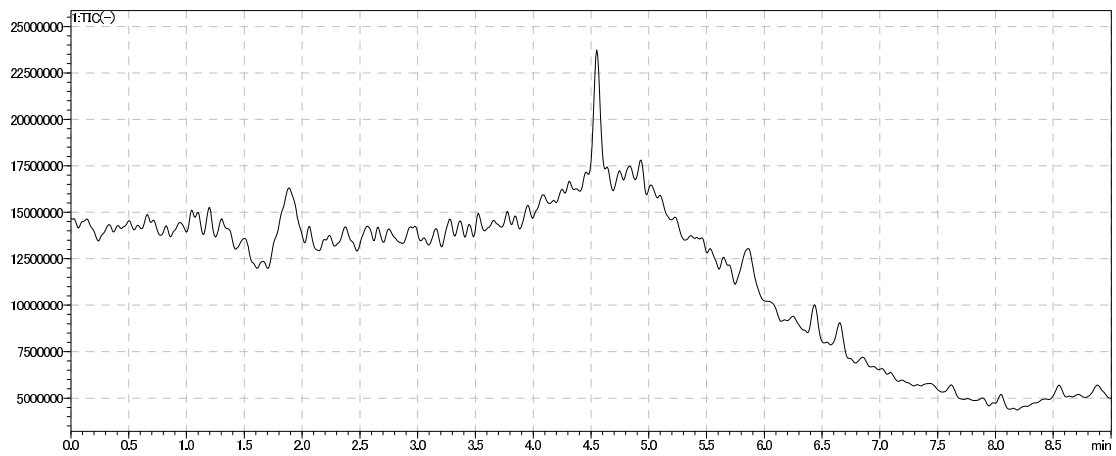
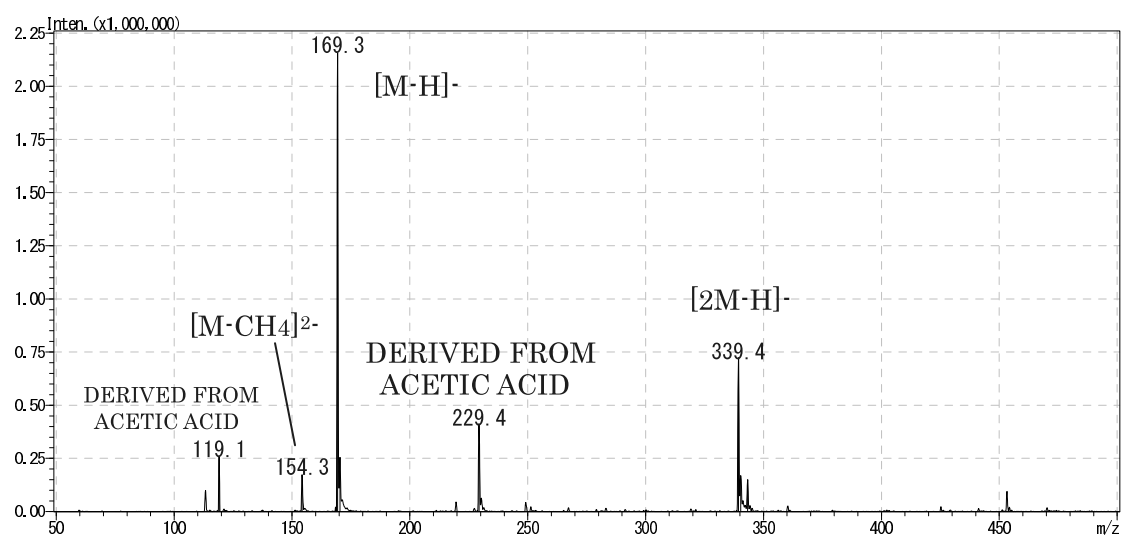


Fig.1



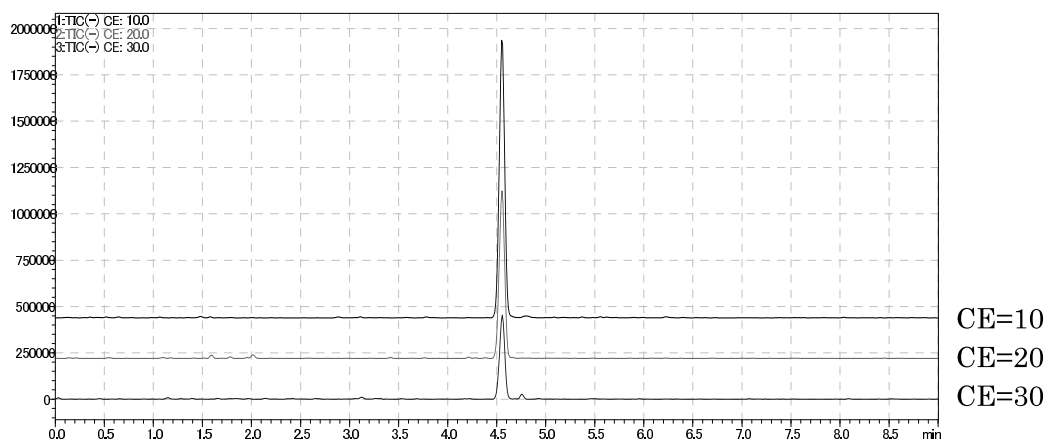
MS CHROMATOGRAM OF E6 STANDARD SPECIMEN OF 10,000 ng/ml (Q1 scan)

Fig.2



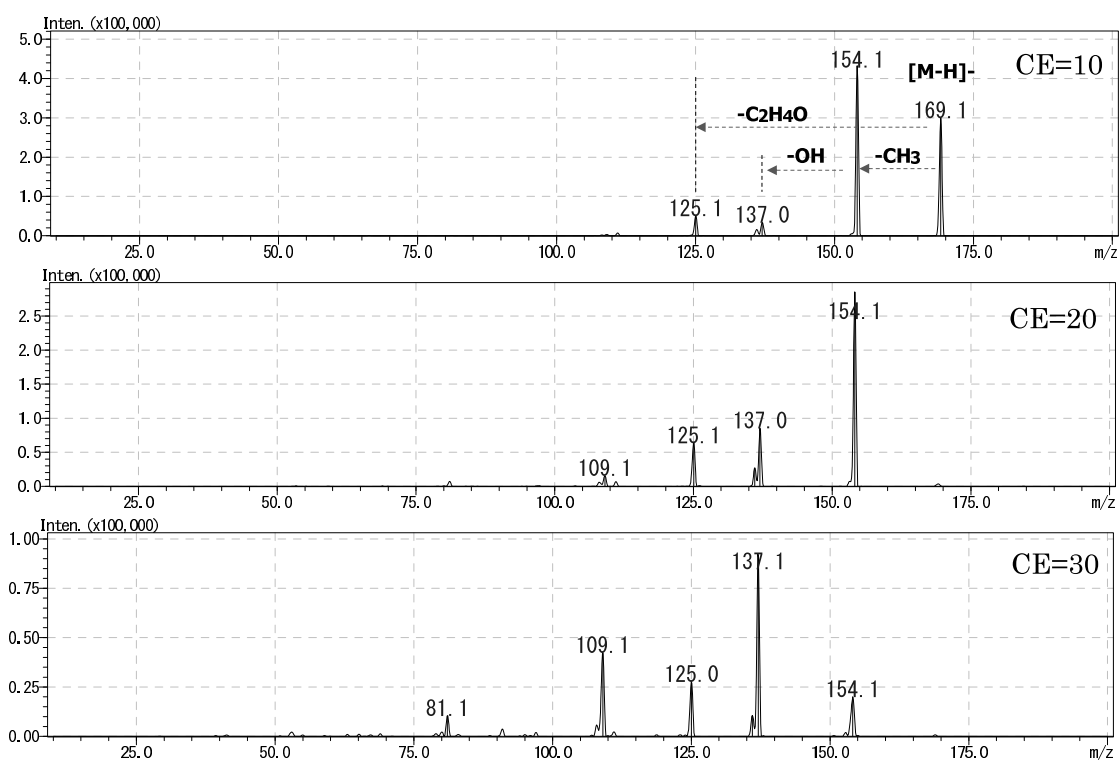
SPECTRUM OF E6 STANDARD SPECIMEN OF 10,000 ng/ml (Q1 scan)

Fig.3



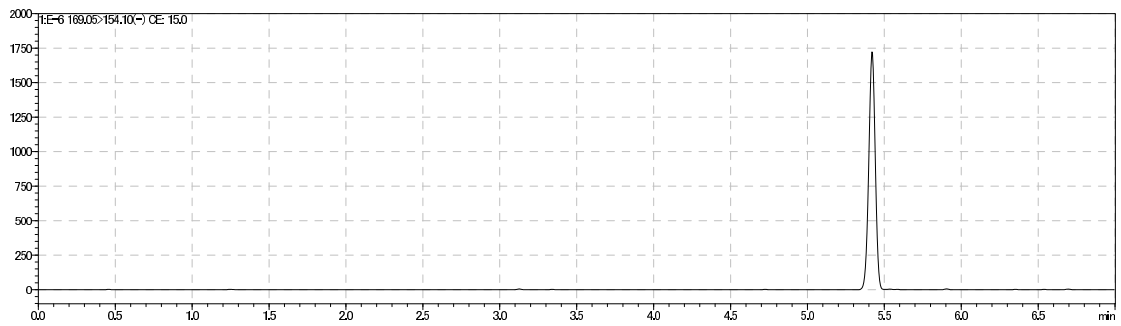
MS CHROMATOGRAM OF E6 STANDARD SPECIMEN OF 10,000 ng/ml (product ion scan)

Fig.4



PRODUCT ION OF E6 STANDARD SPECIMEN OF 10,000 ng/ml (precursor ion : m/z 169.10)

Fig.5



MRM CHROMATOGRAM OF E6 STANDARD SPECIMEN OF 100 ng/ml

Fig.6

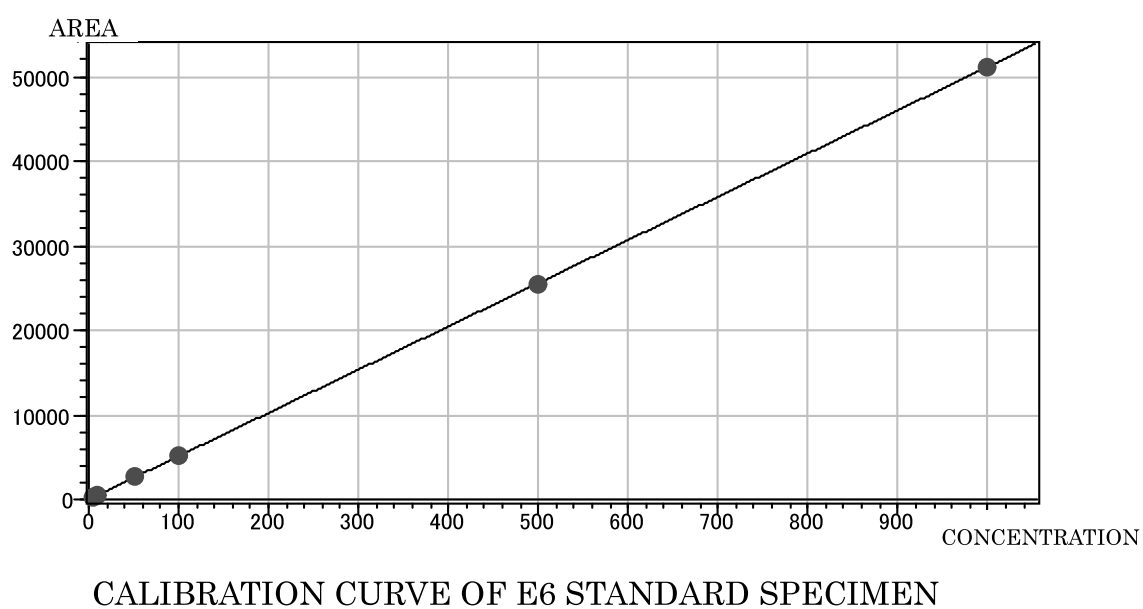
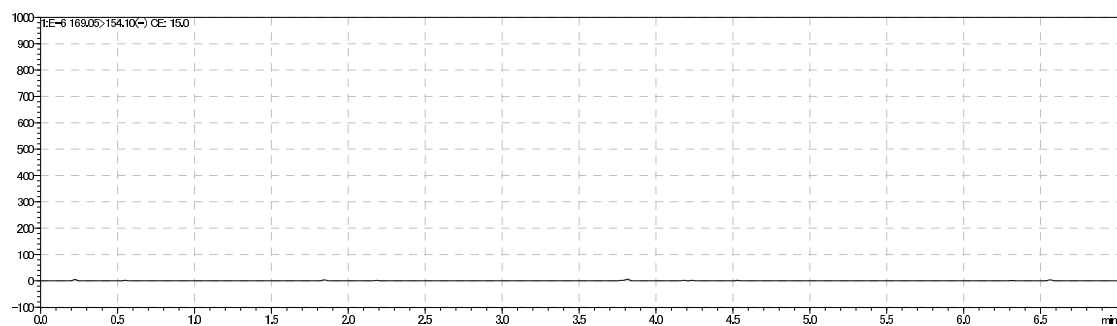
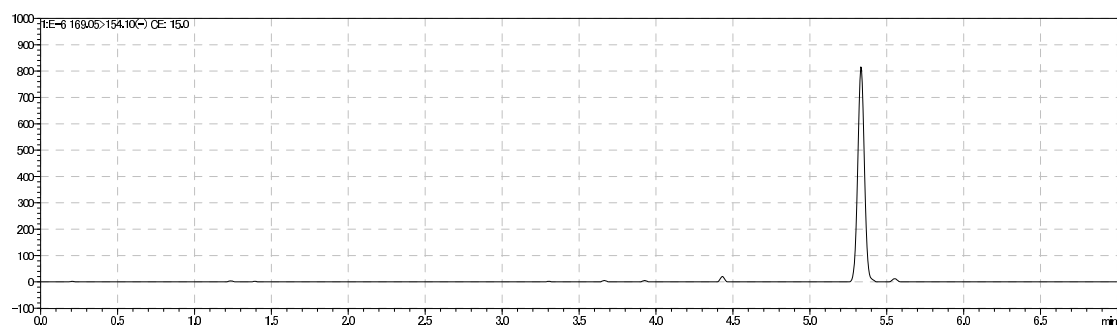


Fig.7



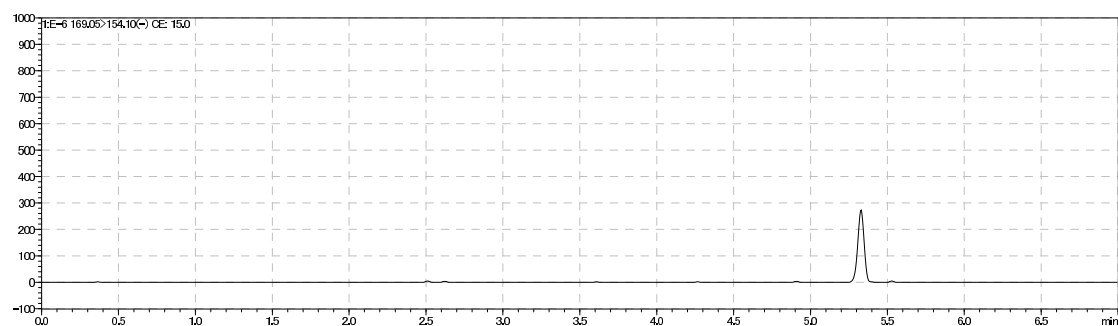
MRM CHROMATOGRAM OF E6 IN SAMPLE HEATED AT STANDARD PRESSURE
FOR TWO HOURS AFTER OYSTER ESSENCE EXTRACTION

Fig.8



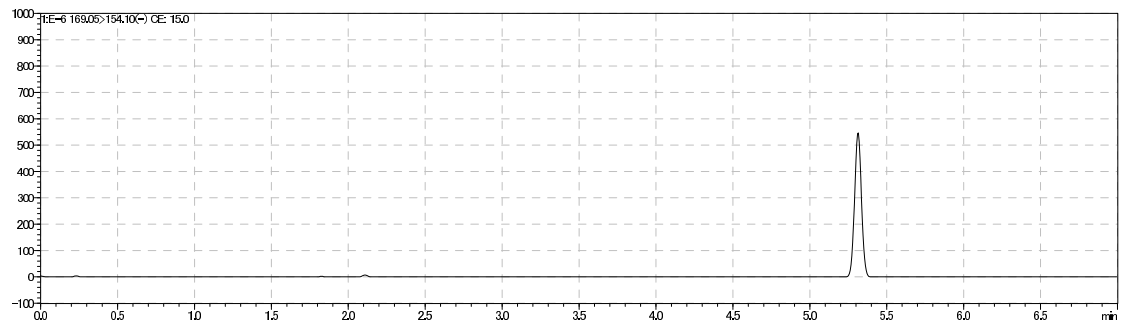
MRM CHROMATOGRAM OF E6 IN SAMPLE HEATED AT STANDARD PRESSURE
FOR 18 HOURS AFTER OYSTER ESSENCE EXTRACTION

Fig.9



MRM CHROMATOGRAM OF E6 IN SAMPLE HEATED AT 3 atm
FOR ONE HOUR AFTER OYSTER ESSENCE EXTRACTION

Fig.10



MRM CHROMATOGRAM OF E6 IN SAMPLE HEATED AT 3 atm
FOR TWO HOURS AFTER OYSTER ESSENCE EXTRACTION

LC-MS/MS ANALYSIS RESULTS OF E6 IN HEATING EXPERIMENT SAMPLE AT STANDARD PRESSURE (TEMPORAL COMPARISON)

RAW OYSTER		OYSTER ESSENCE (1 atm, 1h)	HEATED AT 1 atm FOR TWO HOURS AFTER OYSTER ESSENCE EXTRACTION
TEMPERATURE		92–94°C	92–94°C
LIQUID MEASURE	1 kg	681.34 g	602.07 g
VOLUME PROPORTION		100%	88%
E6 CONCENTRATION µg/mL	N.D.	N.D.	N.D.
TOTAL AMOUNT OF E6 (IN UNITS OF OYSTER 1 kg)	N.D.	N.D.	N.D.
HEATED AT 1 atm FOR FOUR HOURS AFTER OYSTER ESSENCE EXTRACTION		HEATED AT 1 atm FOR SIX HOURS AFTER OYSTER ESSENCE EXTRACTION	HEATED AT 1 atm FOR EIGHT HOURS AFTER OYSTER ESSENCE EXTRACTION
TEMPERATURE		92–94°C	92–94°C
LIQUID MEASURE	568.42 g	529.38 g	481.73 g
VOLUME PROPORTION		78%	71%
E6 CONCENTRATION µg/mL	.09 ± 0.04 (QUANTITATIVE LIMIT OR LESS)	0.12 ± 0.09 (QUANTITATIVE LIMIT OR LESS)	0.29 ± 0.08 (QUANTITATIVE LIMIT OR LESS)
TOTAL AMOUNT OF E6 (IN UNITS OF OYSTER 1 kg)	51.2 ± 22.7 (QUANTITATIVE LIMIT OR LESS)	63.5 ± 47.6 (QUANTITATIVE LIMIT OR LESS)	139.7 ± 38.5 (QUANTITATIVE LIMIT OR LESS)

QUANTITATIVE LIMIT 0.5µg/mL

LC-MS/MS ANALYSIS RESULTS OF E6 IN HEATING EXPERIMENT SAMPLE (TEMPORAL COMPARISON)

	HEATED AT 1 atm FOR TEN HOURS AFTER OYSTER ESSENCE EXTRACTION	HEATED AT 1 atm FOR 12 HOURS AFTER OYSTER ESSENCE EXTRACTION	HEATED AT 1 atm FOR 14 HOURS AFTER OYSTER ESSENCE EXTRACTION
TEMPERATURE	92-94℃	92-94℃	92-94℃
LIQUID MEASURE	399.63 g	335.33 g	263.33 g
VOLUME PROPORTION	59%	49%	39%
E6 CONCENTRATION (μg/mL)	0.54 ± 0.04	0.86 ± 0.18	1.42 ± 0.26
TOTAL AMOUNT OF E6 (IN UNITS OF OYSTER 1 kg)	215.8 ± 16.0	288.4 ± 60.4	373.9 ± 68.5

	HEATED AT 1 atm FOR 16 HOURS AFTER OYSTER ESSENCE EXTRACTION	HEATED AT 1 atm FOR 17 HOURS AFTER OYSTER ESSENCE EXTRACTION	HEATED AT 1 atm FOR 18 HOURS AFTER OYSTER ESSENCE EXTRACTION	HEATED AT 1 atm FOR 19 HOURS AFTER OYSTER ESSENCE EXTRACTION
TEMPERATURE	92-94℃	92-94℃	92-94℃	92-94℃
LIQUID MEASURE	181.98 g	151.89 g	125.38 g	89.57 g
VOLUME PROPORTION	27%	22%	18%	13%
E6 CONCENTRATION (μg/mL)	2.63 ± 0.03	3.42 ± 0.30	4.86 ± 0.10	6.49 ± 0.19
TOTAL AMOUNT OF E6 (IN UNITS OF OYSTER 1 kg)	478.6 ± 5.5	519.5 ± 45.6	609.3 ± 12.5	581.3 ± 17.0

QUANTITATIVE LIMIT 0.5μg/mL

LC-MS/MS ANALYSIS RESULTS OF E6 IN PRESSURIZATION/HEATING EXPERIMENT SAMPLE USING PRESSURE COOKER

RAW OYSTER		OYSTER ESSENCE (1 atm, 2h)	AT 3 atm FOR ONE HOUR AFTER OYSTER ESSENCE EXTRACTION			AT 3 atm FOR TWO HOURS AFTER OYSTER ESSENCE EXTRACTION		
TEMPERATURE		92°C	135°C			135°C		
LIQUID MEASURE	20 kg	23 kg	21.2 kg			19.1 kg		
		100%	92%			83%		
E6 CONCENTRATION (µg/mL)	N.D.	N.D.	1.7	±	0.10	3.5	±	0.39
TOTAL AMOUNT OF E6 µg (IN UNITS OF OYSTER 1 kg)	N.D.	N.D.	1802	±	106.0	3342.5	±	372.5

QUANTITATIVE LIMIT 0.5µg/mL

USED DEVICE : OAMVP α-C-08EL (KAJIWARA INC.)

NOTE THAT SPECIFIC GRAVITY OF SAMPLE TO WHICH ETHANOL IS ADDED
SO AS TO BE 70% AFTER EXTRACTING SAMPLE WAS ASSUMED AS 1.
SD NEEDS TO BE RECALCULATED.

Fig.14

2012/7/13

ANALYSIS RESULT

TO WATANABE OYSTER LABORATORY

REQUEST SUMMARY

SPECIMEN ①3,5-dihydroxy-4-methoxybenzyl alcohol
②CONCENTRATED-OYSTER MEAT EXTRACTED ESSENCE

USED
DEVICE SHIMAZU HIGH-PERFORMANCE LIQUID CHROMATOGRAPH LC-2010 SYSTEM

PURPOSE OF
ANALYSIS ANALYSIS OF 3, 5-dihydroxy-4-methoxybenzyl alcohol
IN CONCENTRATED-OYSTER MEAT EXTRACTED ESSENCE

METHOD AND RESULTS OF ANALYSIS

- The Shim-pack CLC-ODS (250 mmL × 4.6 mm i.d., 5 μm) WAS USED AS A COLUMN TO SEPARATE THE SPECIMEN. ABSORBANCE WAS DETECTED AT A WAVELENGTH OF 210 nm. FOR DETAILS OF ANALYSIS CONDITIONS, REFER TO <SEPARATE SHEET-1>.
- The kept 3, 5-dihydroxy-4-methoxybenzyl alcohol was dissolved to prepare 30 μg/ml (35% methanol) solution and the solution was injected to the HPLC. The results are shown in Fig.1.
- 5% OF FORMIC ACID WAS ADDED TO THE KEPT CONCENTRATED-OYSTER MEAT EXTRACTED ESSENCE TO MAKE A SPECIMEN SOLUTION AND THE SOLUTION WAS INJECTED TO THE HPLC. THE RESULTS ARE SHOWN IN Fig.2. 3, 5-dihydroxy-4-methoxybenzyl alcohol IN THE CONCENTRATED -OYSTER MEAT EXTRACTED ESSENCE WAS CALCULATED AS APPROXIMATELY 30 μg/ml.

PERSON IN CHARGE OF ANALYSIS KEIKO YAMABE STAMP OF APPROVAL

DOCUMENT NO.42331

Fig.15

2012/7/13

<SEPARATE SHEET-1>

ANALYSIS CONDITION

COLUMN : Shim-pack CLC-ODS(M) (250 mmL.×4.6 mm.i.d., 5 µm)

MOBILE PHASE : A; WATER

B; ACETONITRILE

B.Conc 7.5 % (0-10 min) → 100 % (10.01-15 min) → 7.5 % (15.01-22 min)

FLOW RATE : 1.0 mL / min

MIXER : 0.27 mL

COLUMN TEMPERATURE: 25 °C

AMOUNT OF INJECTION: 10 µL

DETECTION: ABSORBANCE DETECTION

MEASURED : 210 nm

WAVELENGT : D2

LAMP : D2

CELL : STANDARD

DETAILS OF ANNEX

Fig.1 : RESULTS OF STANDARD SOLUTION (30 µg/ml)

Fig.2 : RESULTS OF SPECIMEN

Fig.16

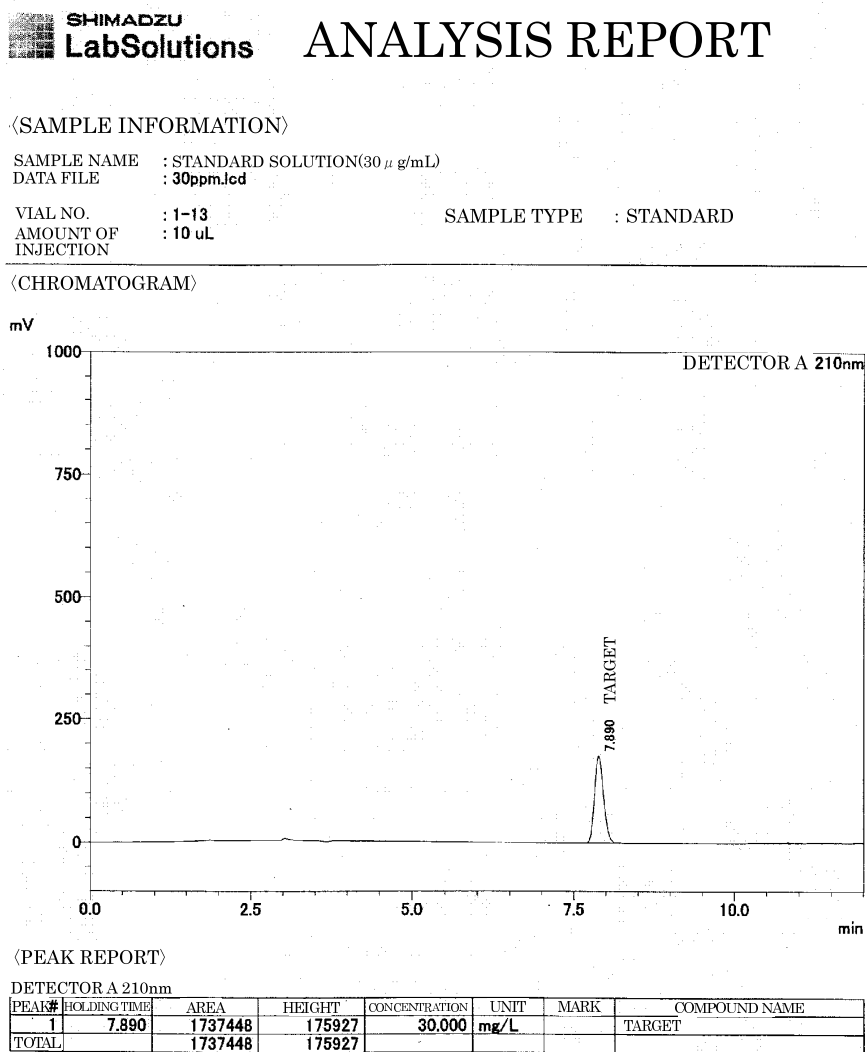
Fig.1 RESULTS OF STANDARD SOLUTION (30 μ g/ml)

Fig.17

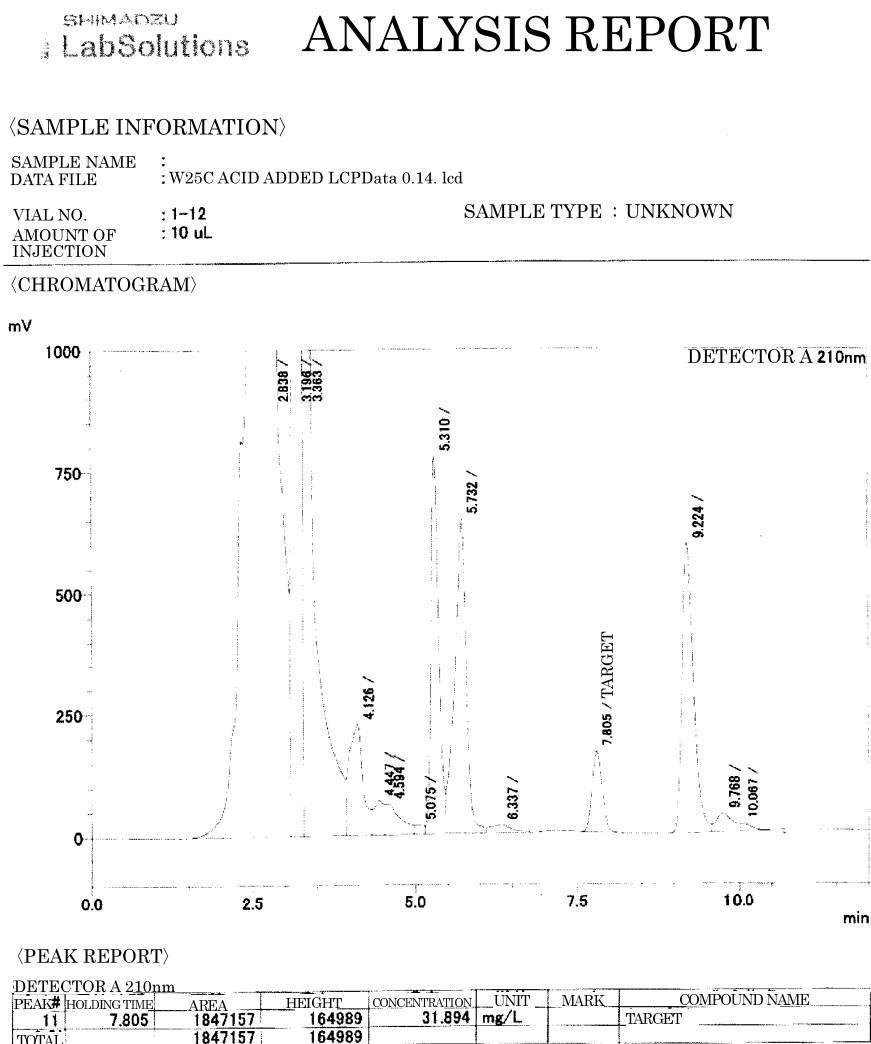


Fig.2 RESULTS OF SPECIMEN SOLUTION