CERI

Receipt number	652-17-P-5533
Study number	85533

TEST REPORT

Measurement of 1-octanol/water partition coefficient for PFHxA

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

Date March 15, 2018

Study Director

March, 2018

Chemicals Evaluation and Research Institute, Japan, Kurume

CONTENTS

1. Title

   Measurement of 1-octanol/water partition coefficient for PFHxA

2. Sponsor

   Name DAIKIN INDUSTRIES, LTD.

   Address 1-1 Nishihitotsuya, Settsu-shi, Osaka 566-8585, Japan

3. Test facility

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

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

4. Objective

   This study was performed to obtain the 1-octanol/water partition coefficient of PFHxA.

5. Test method

   OECD Guidelines for Testing of Chemicals, No. 117, April 13, 2004, "Partition Coefficient (n-octanol/water), High Performance Liquid Chromatography (HPLC) Method"

6. Dates

   Study initiation date January 29, 2018

   Study completion date March 15, 2018

7. Personnel

   Study Director

   Study personnel

8. Approval of test report

   Date March 15, 2018

   Study Director

9. Summary

Test item

PFHxA

Objective

This study was performed to obtain the 1-octanol/water partition coefficient of PFHxA.

Test method

OECD Guidelines for Testing of Chemicals, No. 117, April 13, 2004, "Partition Coefficient (n-octanol/water), High Performance Liquid Chromatography (HPLC) Method"

Test conditions

Test equipment	High-performance liquid chromatograph (HPLC)
Eluent	Analysis for undissociated form: Methanol/buffer solution (pH2.0)*1 (7/3 v/v)
	Analysis for dissociated form : Methanol/buffer solution (pH7.4)*1 (7/3 v/v)
Test temperature	25±1°C

*1 The test item is a dissociative substance (pKa=3.29). Therefore, this test was performed at pH2.0 and pH7.4. The test item has an undissociated form in pH2.0 and an dissociated form in pH7.4.

Results

Partition coefficient	log Pow=2.1	(25°C, undissociated form at pH2.0)
	log Pow=1.5	(25°C, dissociated form at pH7.4)

10. Test materials

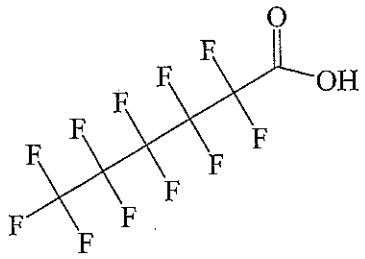
10.1 Test item

a) Chemical name etc.

Chemical name	2,2,3,3,4,4,5,5,6,6,6-undecafluorohexanoic acid
Another name	PFHxA
CAS number	307-24-4

b) Chemical structure etc.

Structural formula



Molecular formula	$C_6HF_{11}O_2$
Molecular weight	314.05

c) Test sample

Purity of test item	99.8%
Impurity	Water 0.2%
Supplier	DAIKIN INDUSTRIES, LTD.
Lot number	T1221

d) Physicochemical property

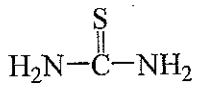
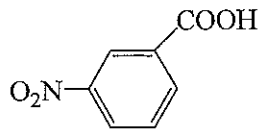
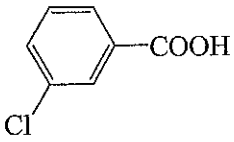
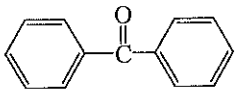
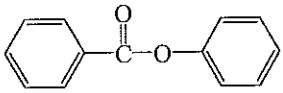
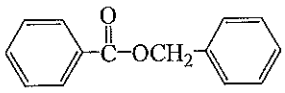
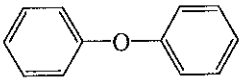
Appearance	White powder

e) Storage conditions

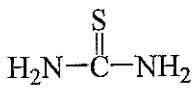
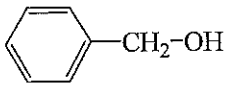
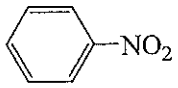
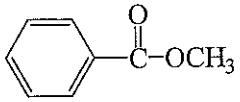
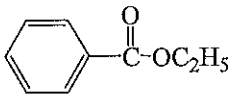
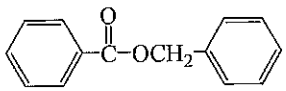
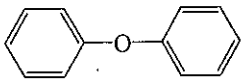
The test sample was stored in a dark storage place at room temperature.

10.2 Reference item

a) Analysis for undissociated form (pH2.0)

Name and CAS number	Structural formula	Supplier and grade	Purity (%)	log Pow
Thiourea (for dead time: t_0) 62-56-6		Wako Pure Chemical Industries JIS special grade	≥98.0	-
3-Nitrobenzoic acid 121-92-6		Tokyo Chemical Industry TCI-GR	>99.0	1.8
3-Chlorobenzoic acid 535-80-8		Tokyo Chemical Industry TCI-EP	>99.0	2.7
Benzophenone 119-61-9		Wako Pure Chemical Industries Wako special grade	≥98.0	3.2
Phenyl benzoate 93-99-2		Nacali tesque Guaranteed reagent	99	3.6
Benzyl benzoate 120-51-4		Wako Pure Chemical Industries Wako special grade	≥99.0	4.0
Diphenyl ether 101-84-8		Wako Pure Chemical Industries Wako special grade	≥99.0	4.2

b) Analysis for dissociated form (pH7.4)

Name and CAS number	Structural formula	Supplier and grade	Purity (%)	log Pow
Thiourea (for dead time: t0) 62-56-6		Wako Pure Chemical Industries JIS special grade	≥98.0	-
Benzyl alcohol 100-51-6		Wako Pure Chemical Industries Wako special grade	≥99.0	1.1
Nitrobenzene 98-95-3		Wako Pure Chemical Industries JIS special grade	≥99.5	1.9
Methyl benzoate 93-58-3		Wako Pure Chemical Industries Wako special grade	≥98.0	2.1
Ethyl benzoate 93-89-0		Wako Pure Chemical Industries Wako special grade	≥98.0	2.6
Benzyl benzoate 120-51-4		Wako Pure Chemical Industries Wako special grade	≥99.0	4.0
Diphenyl ether 101-84-8		Wako Pure Chemical Industries Wako special grade	≥99.0	4.2

11. Performance of test

The test item is a dissociative substance (pKa = 3.29) [refer to the test report "Measurement of dissociation constants in water for PFHxA" (Study No. 85531)]. Therefore, this test was performed at pH2.0 and pH7.4. The test item has an undissociated form in pH2.0 and an dissociated form in pH7.4.

11.1 Test conditions

a) Test equipment

1) Analysis for undissociated form

Instrument	High-performance liquid chromatograph (No. LC-137) LC-2010AHT (Built-in ultraviolet and visible spectrophotometer) (Shimadzu)
Column	L-column ODS (150 mm × 2.1 mm I.D., particle size 5 μm, Chemicals Evaluation and Research Institute, Japan)
Column temperature	25°C
Eluent	Methanol/buffer solution (pH2.0) *2 (7/3 v/v)
Flow rate	0.2 mL/min
Measurement wavelength	210 nm
Injection volume	2 μL

*2 10 mmol/L potassium dihydrogen phosphate solution was adjusted to pH 2.0 by phosphoric acid.

2) Analysis for undissociated form

Instrument	High-performance liquid chromatograph (No. LC-138) LC-2010CHT (Built-in ultraviolet and visible spectrophotometer) (Shimadzu)
Column	L-column ODS (150 mm × 2.1 mm I.D., particle size 5 μm, Chemicals Evaluation and Research Institute, Japan)
Column temperature	25°C
Eluent	Methanol/buffer solution (pH7.4) *3 (7/3 v/v)
Flow rate	0.2 mL/min
Measurement wavelength	210 nm
Injection volume	2 μL

*3 10 mmol/L potassium dihydrogen phosphate solution was adjusted to pH 7.4 by 1 mol/L sodium hydroxide solution.

b) Test temperature

25±1°C

11.2 Test procedures

a) Preparation of reference item solution

1) Analysis for undissociated form (pH2.0)

The reference item solution was prepared as follows.

Reference items	Weight (mg)	Volume (mL)	Solvent	Volume added (mL)	Final volume (mL)	Solvent
Thiourea (for dead time)	About 10	10	Methanol	1	200	Eluent (pH2.0) (see Section 11.1)
3-Nitrobenzoic acid	About 10	10	Methanol	1		
3-Chlorobenzoic acid	About 10	10	Methanol	1		
Benzophenone	About 10	10	Methanol	1		
Phenyl benzoate	About 10	10	Methanol	1		
Benzyl benzoate	About 10	10	Methanol	1		
Diphenyl ether	About 10	10	Methanol	1		

2) Analysis for dissociated form (pH7.4)

The reference item solution was prepared as follows.

Reference items	Weight (mg)	Volume (mL)	Solvent	Volume added (mL)	Final volume (mL)	Solvent
Thiourea (for dead time)	About 20	20	Methanol	0.5	100	Eluent (pH7.4) (see Section 11.1)
Benzyl alcohol	About 20	20	Methanol	0.5		
Nitrobenzene	About 20	20	Methanol	0.5		
Methyl benzoate	About 20	20	Methanol	0.5		
Ethyl benzoate	About 20	20	Methanol	0.5		
Benzyl benzoate	About 10	10	Methanol	0.5		
Diphenyl ether	About 10	10	Methanol	0.5		

b) Preparation for test item solution

1) Analysis for undissociated form (pH2.0)

The test sample (about 10 mg) was weighed with an electronic analytical balance and dissolved in eluent (pH2.0) for HPLC analysis to obtain about 1000 mg/L test item solution. The eluent (pH2.0) was used as a solvent blank.

2) Analysis for dissociated form (pH7.4)

The test sample (about 10 mg) was weighed with an electronic analytical balance and dissolved in eluent (pH7.4) for HPLC analysis to obtain about 500 mg/L test item solution. The eluent (pH7.4) was used as a solvent blank.

c) Measurement of retention times for reference items, and making of regression line

The reference item solution was injected twice to the test equipment, and the retention times of the reference items were measured.

A regression line was made by the method of least squares using the partition coefficient and the logarithmic values of the capacity factors for reference items. The capacity factor (k), the slope of regression equation (a) and the intercept of regression equation (b) were rounded off to three decimal places.

$$k = \frac{t_R - t_0}{t_0}$$

k : Capacity factor

t_R : Retention time of reference item (min)

t_0 : Dead time (min) (average of two measured values)

$$\log Pow = a \times \log k + b$$

a : Slope of regression equation

b : Intercept of regression equation

d) Measurement of retention time for test item

The test item solution was injected twice to the test equipment and the retention time of the test item was measured. The solvent blank was injected once to the test equipment.

11.3 Calculation of partition coefficient

The capacity factor was calculated from the retention time of test item. The partition coefficient of test item was then calculated using the regression equation of regression line and was given as the average value of two measured values.

11.4 Treatment of numerical values

The partition coefficient was represented as logarithm and rounded off to one decimal place.

Values were treated in accordance with Japanese Industrial Standards (JIS) Z 8401:1999 rule B.

12. Results and discussion

12.1 Partition coefficient of test item

The test results are shown as follows.

	log Pow			Table
	Measured value		Average	
Undissociated form (pH2.0)	2.1	2.1	2.1	1
Dissociated form (pH7.4)	1.5	1.5	1.5	2

12.2 Discussion

The average of measured log Pow values was 2.1 (pH 2.0) and 1.5 (pH7.4). It is judged that the test results are valid because the difference between two measured values was within ±0.1.

Table 1 Calculation table for partition coefficient by HPLC method (pH2.0)

Study No. 85533

		t_R	k	log k	log Pow
Reference item	1-a	1.99	t_0 =	1.99	
	1-b	1.98			
	2-a	2.98	0.501	-0.300	1.8
	2-b	2.98	0.501	-0.300	1.8
	3-a	4.13	1.081	0.034	2.7
	3-b	4.13	1.081	0.034	2.7
	4-a	6.60	2.325	0.366	3.2
	4-b	6.60	2.325	0.366	3.2
	5-a	9.48	3.776	0.577	3.6
	5-b	9.49	3.781	0.578	3.6
	6-a	12.51	5.302	0.724	4.0
	6-b	12.53	5.312	0.725	4.0
	7-a	14.53	6.320	0.801	4.2
	7-b	14.56	6.335	0.802	4.2
Test item	a	3.30	0.662	-0.179	2.1
	b	3.30	0.662	-0.179	2.1
	(a,b : individual sample)			Average	2.1

$k = (t_R - t_0) / t_0$

t_0 : Dead time (average of two measured values) (min)

t_R : Retention time (min)

$\log Pow = 2.078 \times \log k + 2.487$

$r = 0.995$

Reference item

1	Thiourea
2	3-Nitrobenzoic acid
3	3-Chlorobenzoic acid
4	Benzophenone
5	Phenyl benzoate
6	Benzyl benzoate
7	Diphenyl ether

See Figs. 1,2

March 1, 2018 Name

Table 2 Calculation table for partition coefficient by HPLC method (pH7.4)

Study No. 85533

		t_R	k	log k	log Pow
Reference item	1-a	1.99	t_0 =	1.99	
	1-b	1.99			
	2-a	2.68	0.347	-0.460	1.1
	2-b	2.68	0.347	-0.460	1.1
	3-a	4.00	1.010	0.004	1.9
	3-b	4.00	1.010	0.004	1.9
	4-a	4.48	1.251	0.097	2.1
	4-b	4.48	1.251	0.097	2.1
	5-a	5.95	1.990	0.299	2.6
	5-b	5.95	1.990	0.299	2.6
	6-a	12.76	5.412	0.733	4.0
	6-b	12.76	5.412	0.733	4.0
	7-a	14.97	6.523	0.814	4.2
	7-b	14.97	6.523	0.814	4.2
Test item	a	3.18	0.598	-0.223	1.5
	b	3.18	0.598	-0.223	1.5
	(a,b : individual sample)			Average	1.5

$k = (t_R - t_0) / t_0$

t_0 : Dead time (average of two measured values) (min)

t_R : Retention time (min)

$\log Pow = 2.533 \times \log k + 2.022$

$r = 0.989$

Reference item

1	Thiourea
2	Benzyl alcohol
3	Nitrobenzene
4	Methyl benzoate
5	Ethyl benzoate
6	Benzyl benzoate
7	Diphenyl ether

See Figs. 3,4

March 14, 2018 Name

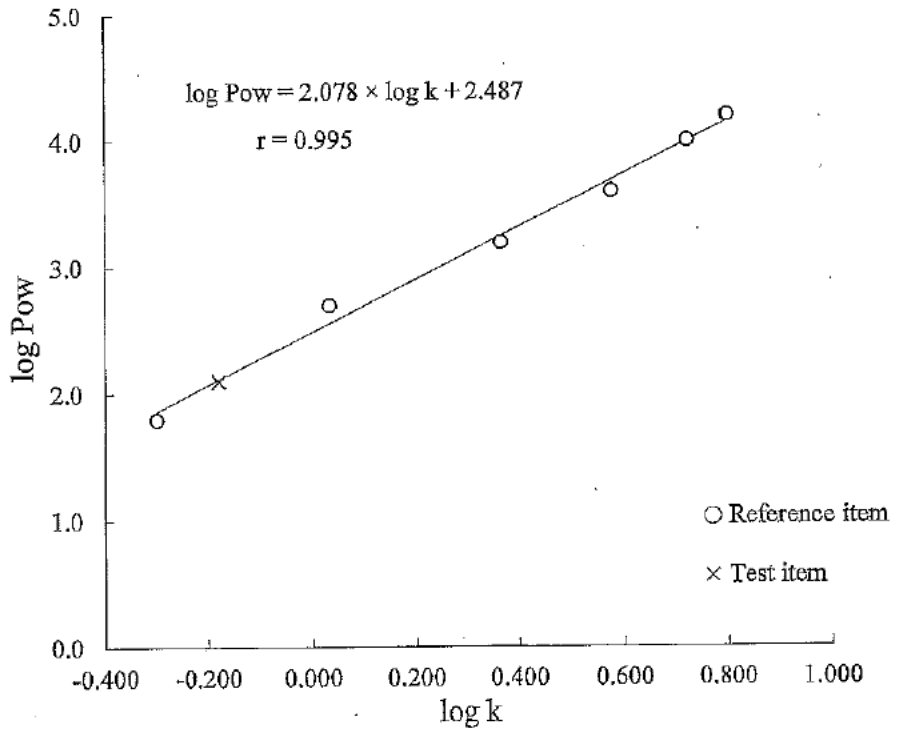
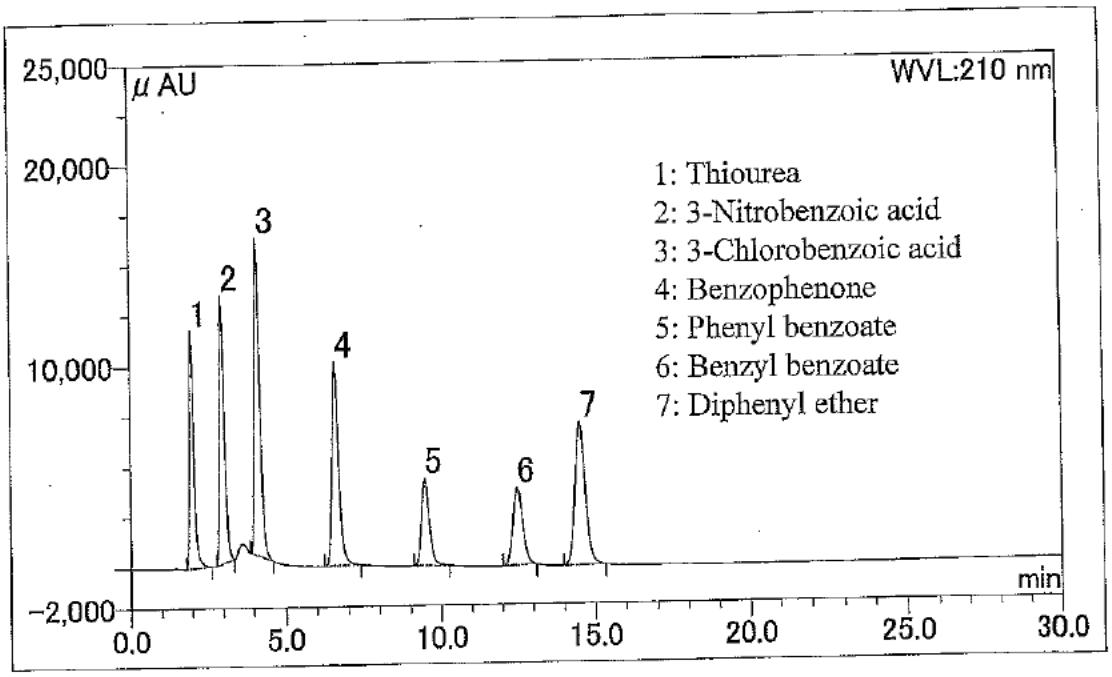


Fig. 1 Calibration curve for partition coefficient by HPLC method(pH2.0).

March 1, 2018 Name

Reference solution –a (pH2.0)

Operator:	Kumiko Tanaka
Operating date:	28/Feb/2018
Sample ID:	85533_180228_1
Program:	85533,85546
Vial No.:	1_4
Channel:	UV_VIS_1



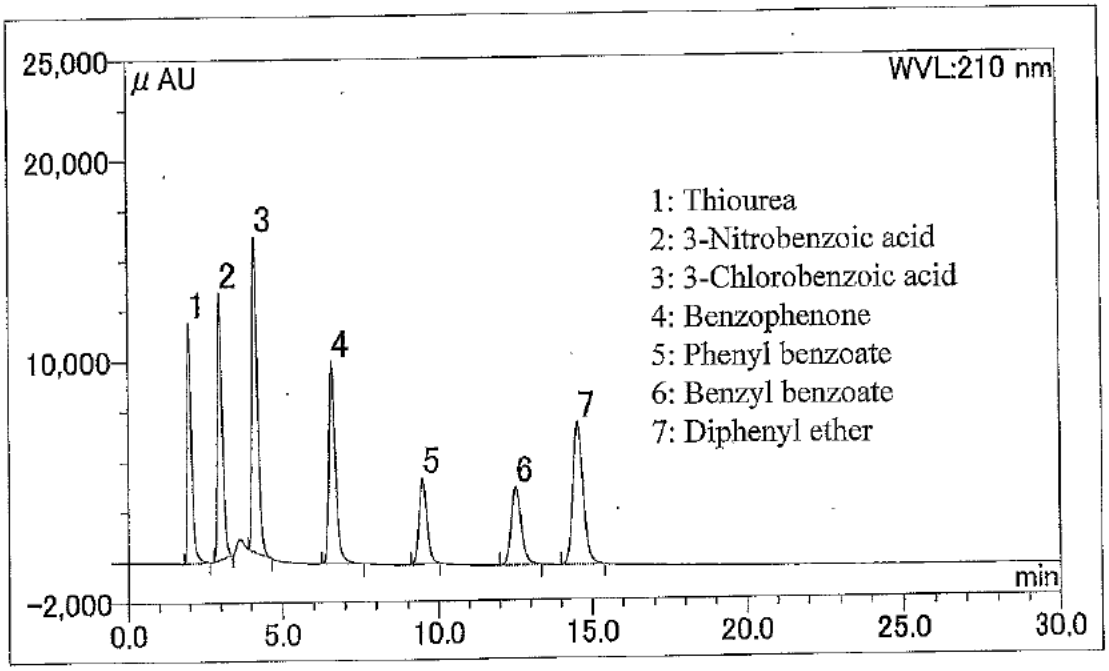
Peak No.	Time (min)	Height (μ AU)	Area (μ AU·sec)	Area (%)
1	1.99	11777	119846	12.83
2	2.98	13336	151815	16.26
3	4.13	15769	188952	20.23
4	6.60	10087	148443	15.90
5	9.48	4308	77407	8.29
6	12.51	3852	83667	8.96
7	14.53	7098	163724	17.53
Total	–	–	933854	100.00

2018. 3. 1

Fig. 2 - 1 Chromatogram of HPLC analysis for partition coefficient by HPLC method (pH2.0).

Reference solution –b (pH2.0)

Operator:	Kumiko Tanaka
Operating date:	28/Feb/2018
Sample ID:	85533_180228_2
Program:	85533,85546
Vial No.:	1_4
Channel:	UV_VIS_1



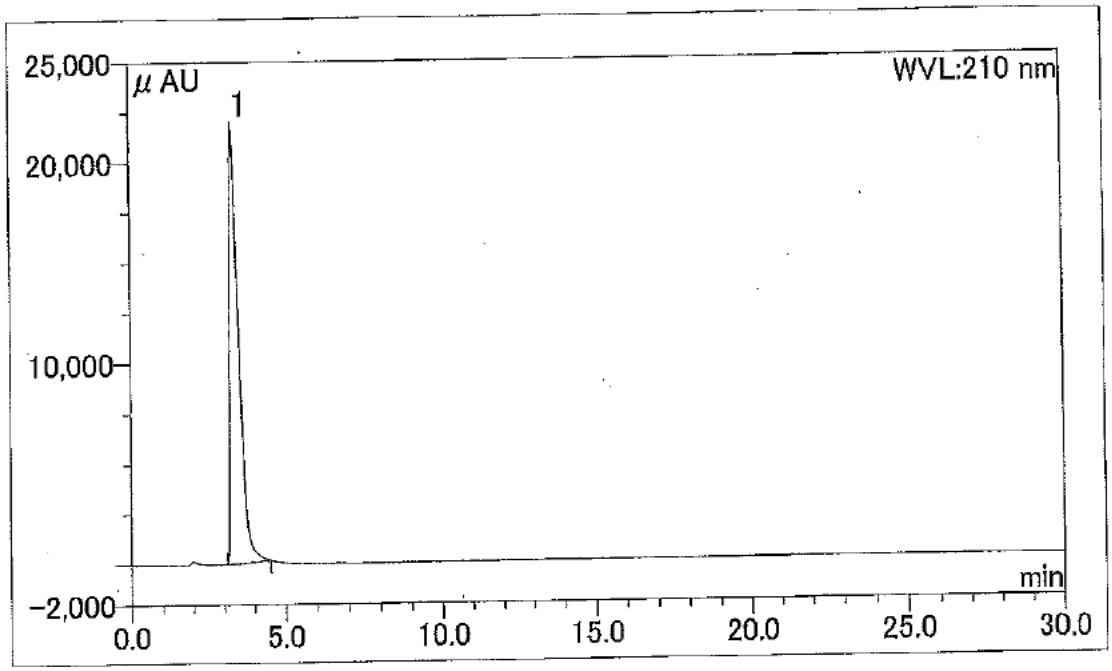
Peak No.	Time (min)	Height (μ AU)	Area (μ AU·sec)	Area (%)
1	1.98	11949	122434	13.05
2	2.98	13271	151936	16.19
3	4.13	15638	188732	20.12
4	6.60	10040	149364	15.92
5	9.49	4250	75487	8.05
6	12.53	3850	86260	9.19
7	14.56	7053	164029	17.48
Total	–	–	938243	100.00

2018. 3. 1

Fig. 2 - 2 Chromatogram of HPLC analysis for partition coefficient by HPLC method (pH2.0).

Test solution –a (pH2.0)

Operator:	Kumiko Tanaka
Operating date:	28/Feb/2018
Sample ID:	85533_180228_3
Program:	85533,85546
Vial No.:	1_3
Channel:	UV_VIS_1



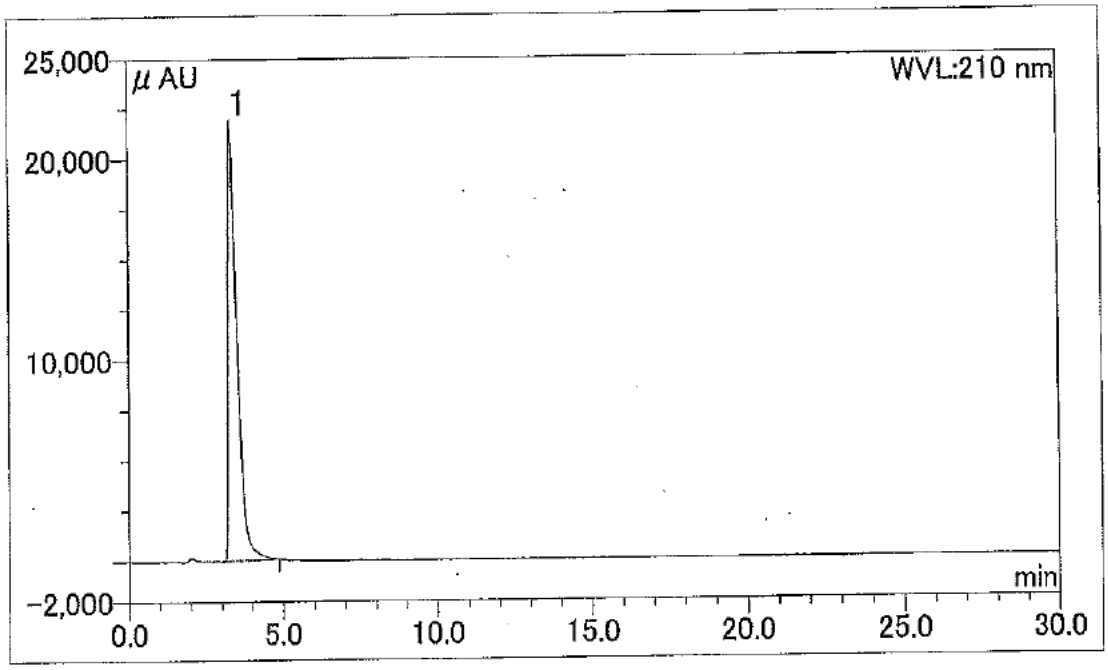
Peak No.	Time (min)	Height (μ AU)	Area (μ AU·sec)	Area (%)
1	3.30	22000	441197	100.00
Total	–	–	441197	100.00

2018. 3. 1

Fig. 2 - 3 Chromatogram of HPLC analysis for partition coefficient by HPLC method (pH2.0).

Test solution –b (pH2.0)

Operator:	Kumiko Tanaka
Operating date:	28/Feb/2018
Sample ID:	85533_180228_4
Program:	85533,85546
Vial No.:	1_3
Channel:	UV_VIS_1



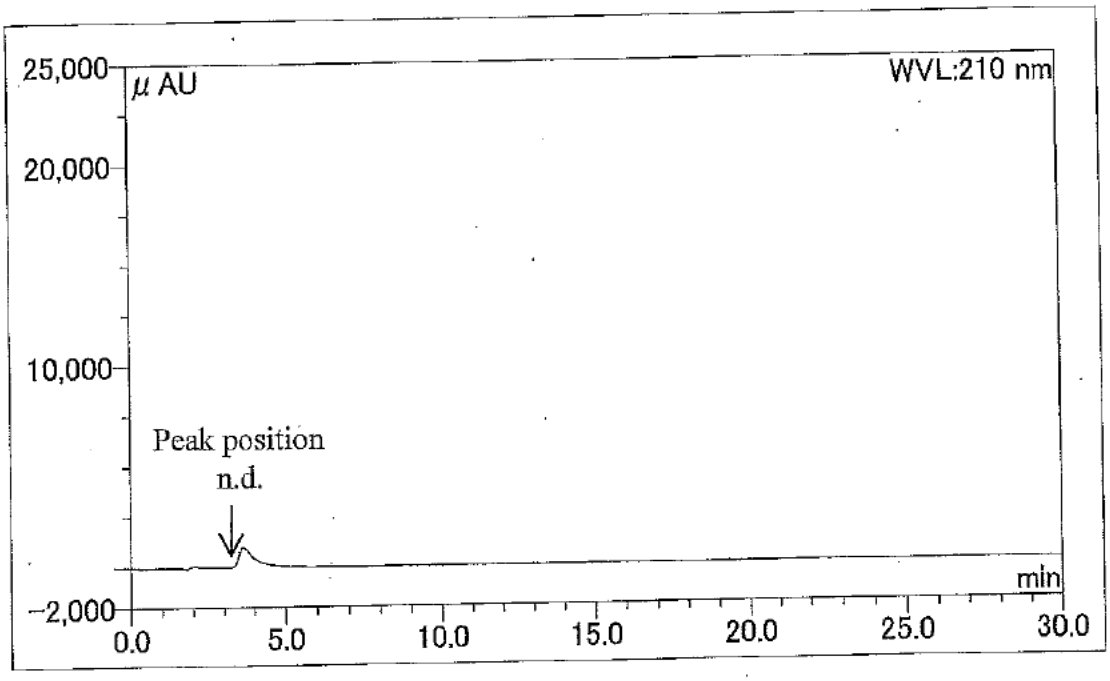
Peak No.	Time (min)	Height (μ AU)	Area (μ AU·sec)	Area (%)
1	3.30	21906	445857	100.00
Total	–	–	445857	100.00

2018. 3. 1

Fig. 2 - 4 Chromatogram of HPLC analysis for partition coefficient by HPLC method (pH2.0).

Solvent blank (pH2.0)

Operator:	Kumiko Tanaka
Operating date:	28/Feb/2018
Sample ID:	85533_180228_5
Program:	85533,85546
Vial No.:	1_1
Channel:	UV_VIS_1



Peak No.	Time (min)	Height (μ AU)	Area (μ AU·sec)	Area (%)
Total	–	–	0	0.00

2018. 3. 1

Fig. 2 - 5 Chromatogram of HPLC analysis for partition coefficient by HPLC method (pH2.0).

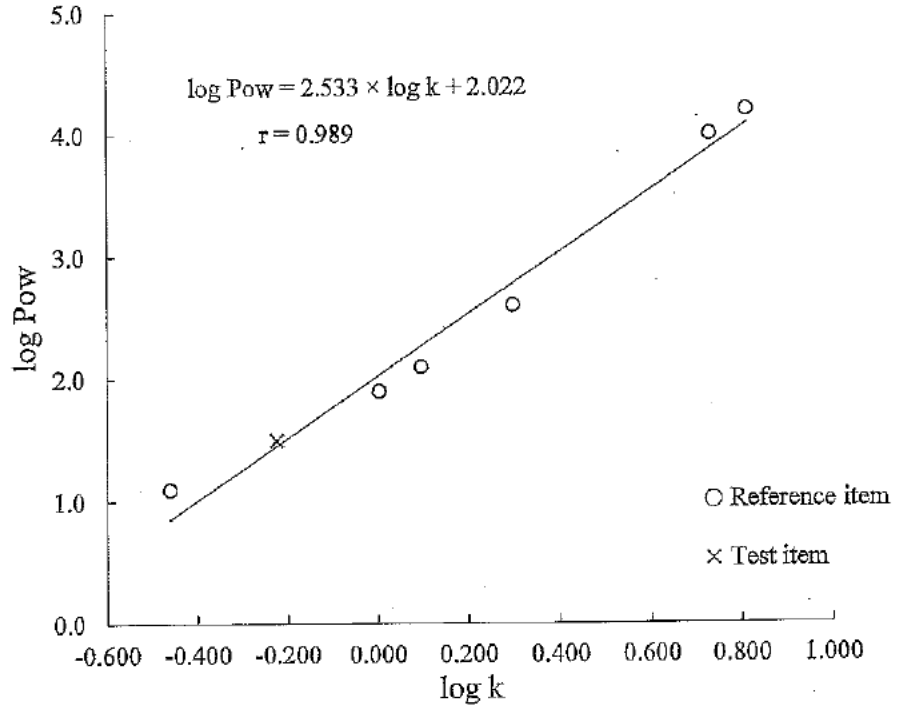
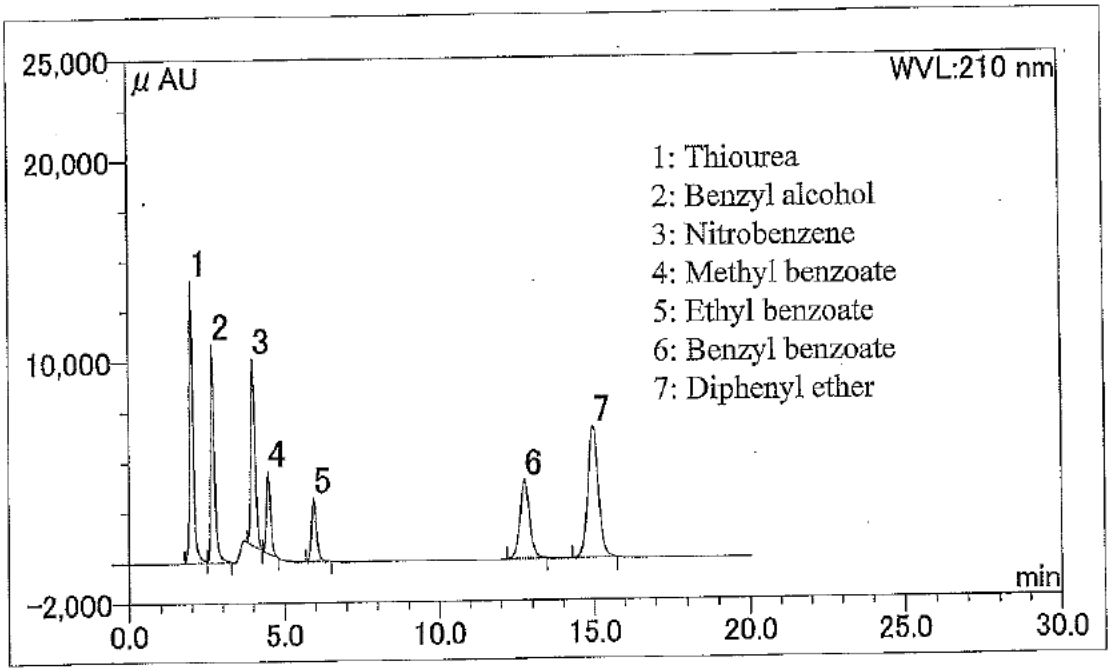


Fig. 3 Calibration curve for partition coefficient by HPLC method (pH7.4).

March 14, 2018 Name

Reference solution -a (pH7.4)

Operator:	Tomoko Nakayoshi
Operating date:	13/Mar/2018
Sample ID:	85533.85546_180313_7
Program:	85533,85546_180313_20_LC138
Vial No.:	1_7
Channel:	UV_VIS_1



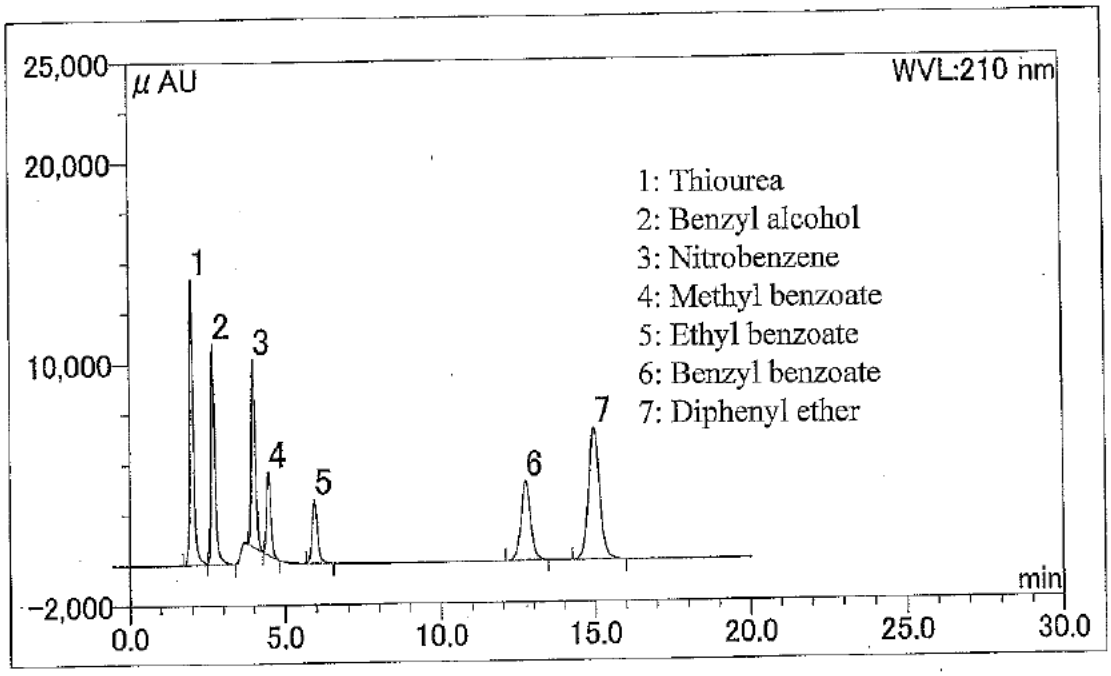
Peak No.	Time (min)	Height (μ AU)	Area (μ AU·sec)	Area (%)
1	1.99	13998	119113	19.37
2	2.68	10827	96003	15.62
3	4.00	9236	82683	13.45
4	4.48	4042	38541	6.27
5	5.95	3132	37653	6.12
6	12.76	3902	84656	13.77
7	14.97	6514	156157	25.40
Total	-	-	614807	100.00

2018. 3. 14

Fig. 4 - 1 Chromatogram of HPLC analysis for partition coefficient by HPLC method (pH7.4).

Reference solution -b (pH7.4)

Operator:	Tomoko Nakayoshi
Operating date:	13/Mar/2018
Sample ID:	85533.85546_180313_8
Program:	85533,85546_180313_20_LC138
Vial No.:	1_7
Channel:	UV_VIS_1



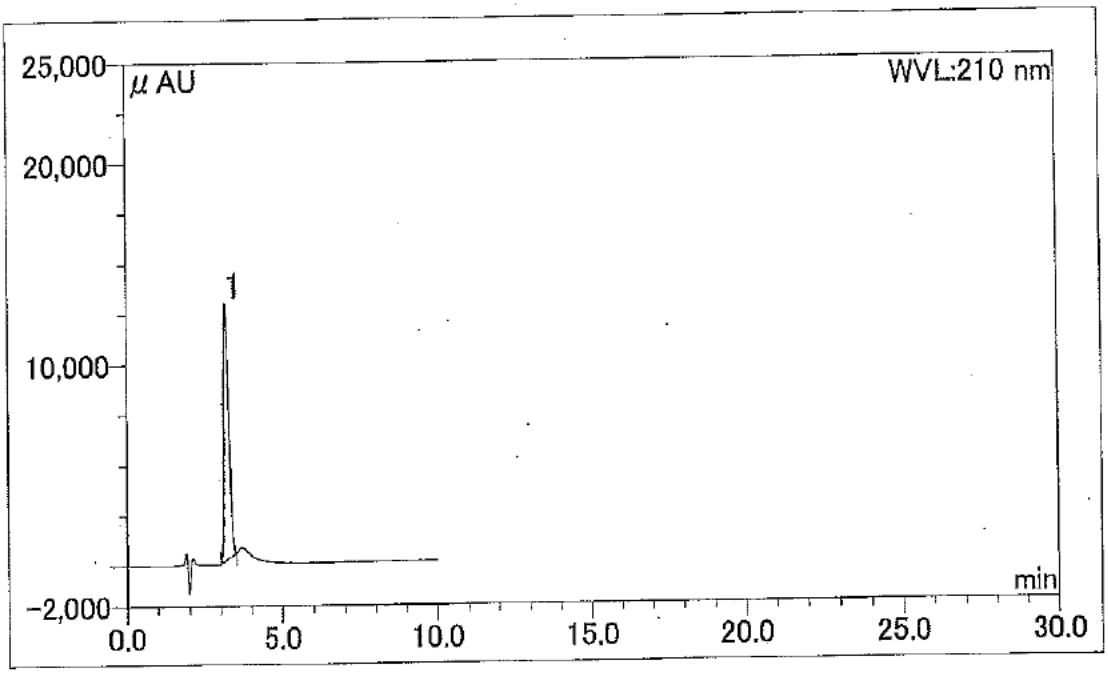
Peak No.	Time (min)	Height (μAU)	Area (μAU·sec)	Area (%)
1	1.99	14151	119626	19.29
2	2.68	10964	96897	15.63
3	4.00	9292	82942	13.38
4	4.48	4082	38723	6.25
5	5.95	3159	37915	6.11
6	12.76	3917	85250	13.75
7	14.97	6549	158702	25.59
Total	-	-	620056	100.00

2018.3.14

Fig. 4 - 2 Chromatogram of HPLC analysis for partition coefficient by HPLC method (pH7.4).

Test solution –a (pH7.4)

Operator:	Tomoko Nakayoshi
Operating date:	13/Mar/2018
Sample ID:	85533.85546_180313_9
Program:	85533,85546_180313_LC138
Vial No.:	1_2
Channel:	UV_VIS_1



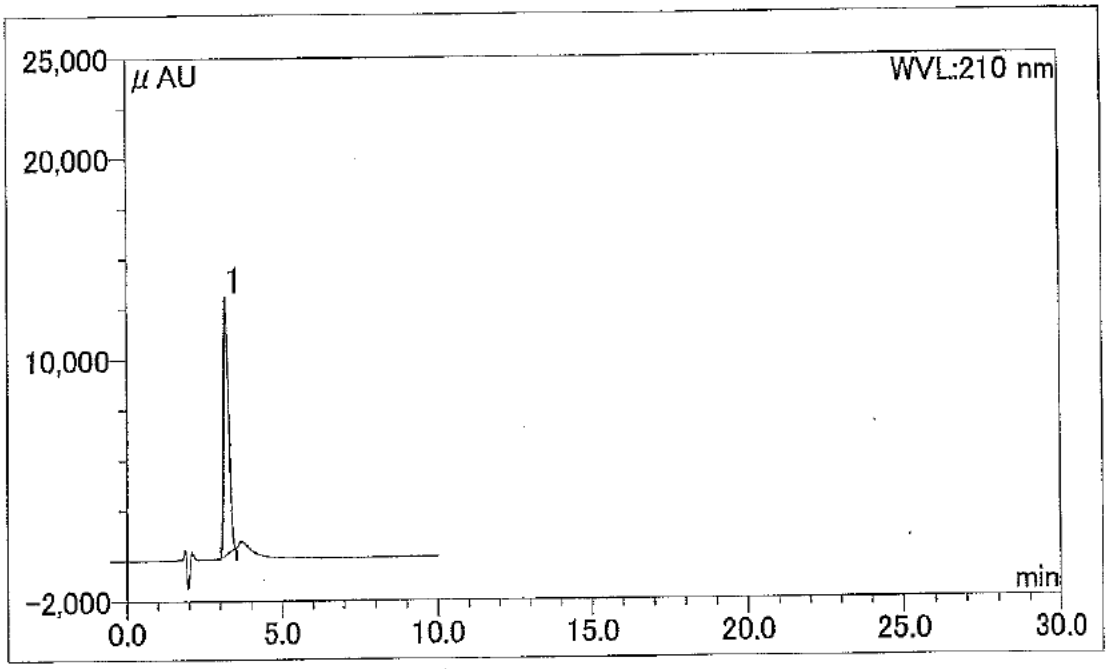
Peak No.	Time (min)	Height (μ AU)	Area (μ AU·sec)	Area (%)
1	3.18	12760	144295	100.00
Total	–	–	144295	100.00

2018. 3. 14

Fig. 4 - 3 Chromatogram of HPLC analysis for partition coefficient by HPLC method (pH7.4).

Test solution –b (pH7.4)

Operator:	Tomoko Nakayoshi
Operating date:	13/Mar/2018
Sample ID:	85533.85546_180313_10
Program:	85533,85546_180313_LC138
Vial No.:	1_2
Channel:	UV_VIS_1



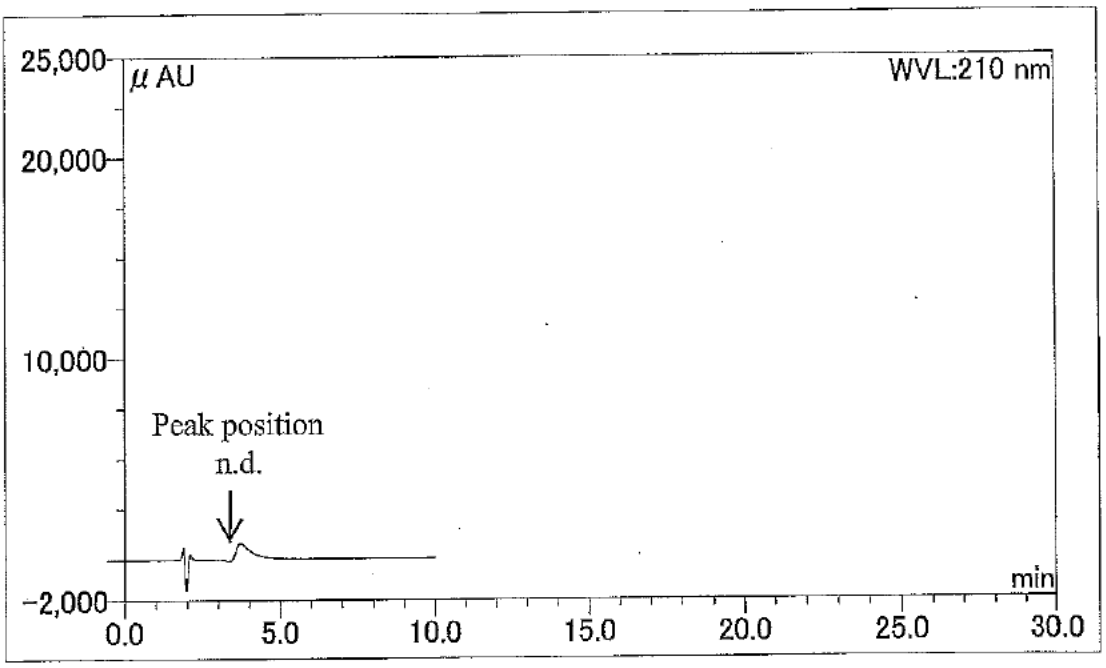
Peak No.	Time (min)	Height (μ AU)	Area (μ AU·sec)	Area (%)
1	3.18	12771	144751	100.00
Total	–	–	144751	100.00

2018. 3. 14

Fig. 4 - 4 Chromatogram of HPLC analysis for partition coefficient by HPLC method (pH7.4).

Solvent blank (pH7.4)

Operator:	Tomoko Nakayoshi
Operating date:	13/Mar/2018
Sample ID:	85533.85546_180313_11
Program:	85533,85546_180313_LC138
Vial No.:	1_4
Channel:	UV_VIS_1



Peak No.	Time (min)	Height (μ AU)	Area (μ AU·sec)	Area (%)
Total	–	–	0	0.00

2018. 3. 14

Fig. 4 - 5 Chromatogram of HPLC analysis for partition coefficient by HPLC method (pH7.4).