SHIMADZU

Simplified Measurement of Partition Coefficient by Means of the Pretreatment Function in the Auto-sampler

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Introduction

A partition coefficient is a parameter reflecting hydrophobicity of chemical regents and is commonly assessed as the characteristic factor which affects the bio-availability of the chemical compound in the pharmaceutical area. The parameter typically used is the partition coefficient of octanol versus water (*Pow*), which is expressed in common logarithms of concentration ratio (Log*Pow*). However, since there are some technological consideration and requirement of experienced skills for the operation in the existing methods, it is to be desired that an automated methodology for determination of Log*Pow* with high reliability and efficiency is developed.

In this presentation, we demonstrate the applicability of the developed method for determination of partition coefficient by the automated injection from the water or octanol phase separately using the auto-sampler. The results indicate that the Log*Pow* was correctly obtained with reliability and high throughput determination.

System configuration

The Shimadzu Nexera system constructed of an LC-30AD solvent delivery unit, a DGU-20A5 degassing unit, a SIL-30AC auto-sampler equipped with the pretreatment functions, a CTO-30A temperature column oven and a SPD-M20A Photo Diode Array detector with a temperature-controlled flow cell. Data was collected and processed by a LabSolutions LC/GC chromatographic data system. Deep-well plates were used as sample vials.

Analytical conditions

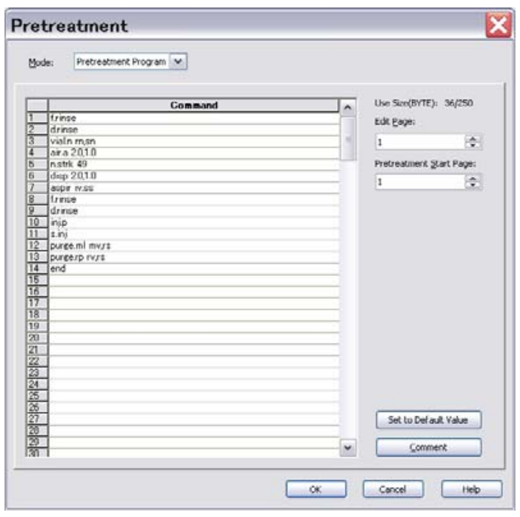
Column	: Gemini 4.6 mmID x 30 mm, 3 µm (Phenomenex, Inc.)
Mobile phase	: A; Water / Acetonitrile / TFA = 95 / 5 / 0.05 (v/v/v)
	B; Water / Acetonitrile / TFA = 5 / 95 / 0.05 (v/v/v)
Gradient	: 2 % (0 - 2 min), 2% to 100% (2 min – 2.9 min), 100 % (2.9 - 4.2 min)
Flow rate	: 1.2 mL/min
Column oven	: 30 ºC
Injection vol.	: 10 µL
Detector	: UV 254 nm, 220 nm
Samples	: 10 mM sample* (5 µL)+Octanol (400 µL) +Water (400 µL) in a well
	: *Antipyrine, Lidocaine, Carbamazepine, Desipramine, Bifonazole, Chlorpromazine, Propranolol and Trimethoprim

Automated pretreatment function

SIL-30AC has a major two types of its pretreatment modes; one is a standardized mode and the other is a pretreatment program mode. In the standardized mode, simple dilution can be performed by just setting the dilution factor, "Reagent" and other a few parameters shown in Fig.1, whereas a detailed program including dilution can be programmed in the pretreatment program mode.

Fig.1 pretreatment setting (Dilution mode)

The optimized program consisting of 14 lines commands was utilized in order to perform the automated determination of partition coefficient as details described in Fig. 2. In the 5th line in the table of Fig. 2, the needle depth was set, as the needle stroke, 49 mm for water layer and 37 mm for octanol layer. Air was dispensed from the needle in the water layer to prevent from contamination. In addition, the needle was rinsed using internal and external solvent before the pretreatment.

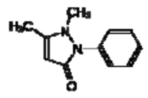


#	Description
1	Rinsing the outside of the needle using the external rinsing pump.
2	Rinsing the outside of the needle.
3	Needle move to the sample well "rn" is a rack number defined in the batch sequence file. "sn" is a sample number also defined in the batch sequence file.
4	Aspirating air.
5	Moving down the needle into water or octanol layer. 49 mm is a needle stroke for water layer (in the left figure).
6	Dispensing air from the needle.
7	Aspirating water layer (10 µL). "iv" is a injection volume defined in the batch sequence file. "ss" is a sampling speed set in the method file.
8	Rinsing the outside of the needle using the external rinsing pump.
9	Rinsing the outside of the needle.
10	Needle move to the injection port
11	Injecting and Starting analysis
12	Wash the measuring line by rinse solution 0
13	Exchange the solution in the rinse port
14	End of the pretreatment program

Fig. 2 pretreatment settings (pretreatment program mode)

Experimental results

Octanol and water was mixed well prior to adding the target compound. The sample solution was vortexed followed by a centrifugation at 5000 rpm in 3 min. All samples were analyzed in the water layer at first followed by the octanol layer. The obtained chromatograms of eight model compounds are shown in Fig. 3 to 10, respectively. The blank test was performed before and after each of the analyzed layer in order to check the carryover. The results of these blank test are shown as overlapped (red colored) on each chromatogram. Calculated Log*POW* based on the obtained peak area of water or octanol layer are listed in Table. 1.



Antipyrine

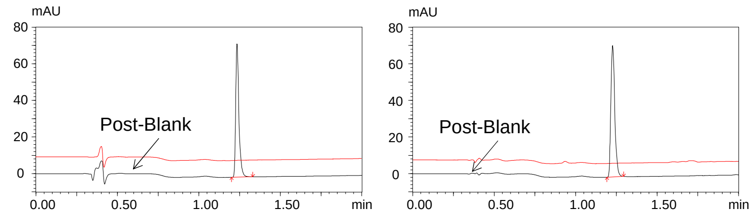
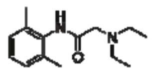


Fig. 3 Chromatogram of the Antipyrine (Left: water layer, Right: octanol layer)



Lidocaine

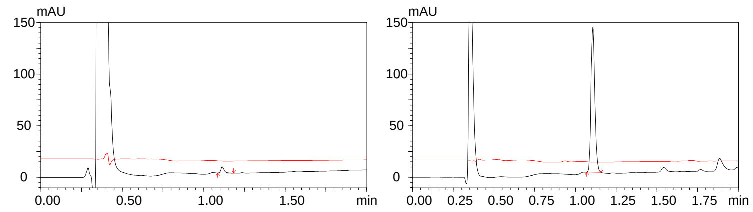
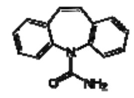


Fig. 4 Chromatogram of the Lidocaine (Left: water layer, Right: octanol layer)



Carbamazepine

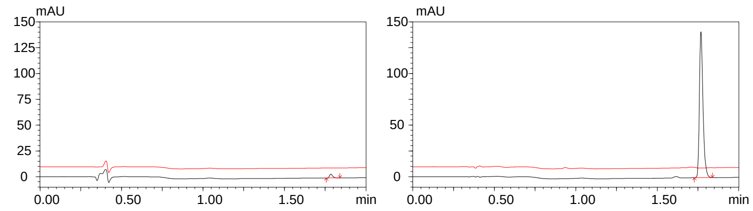
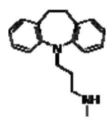


Fig. 5 Chromatogram of the Carbamazepine (Left: water layer, Right: octanol layer)



Desipramine

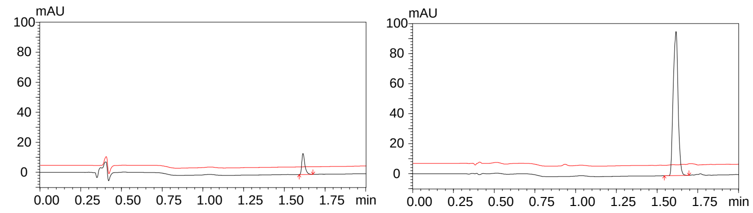
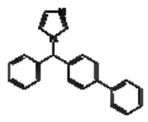


Fig. 6 Chromatogram of the Desipramine (Left: water layer, Right: octanol layer)



Bifonazol

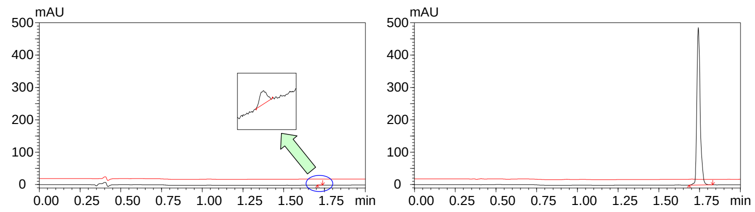
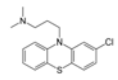


Fig. 7 Chromatogram of the Bifonazol (Left: water layer, Right: octanol layer)



Chlorpromazine

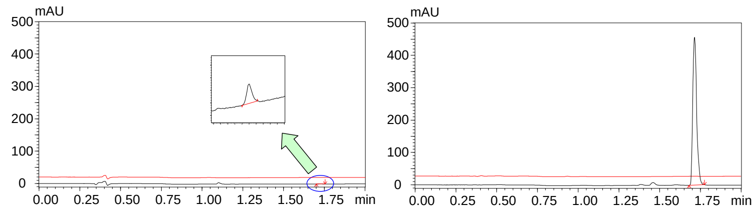
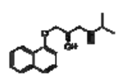


Fig. 8 Chromatogram of the Chlorpromazine (Left: water layer, Right: octanol layer)



Propranolol

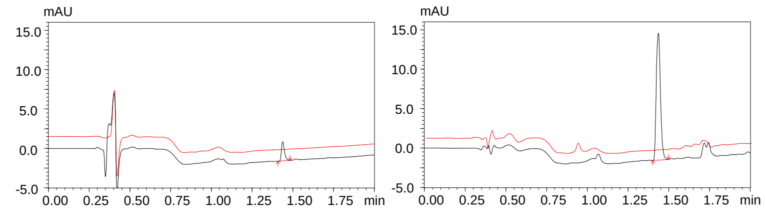
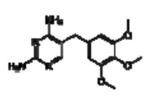


Fig. 9 Chromatogram of the Propranolol (Left: water layer, Right: octanol layer)



Trimethoprim

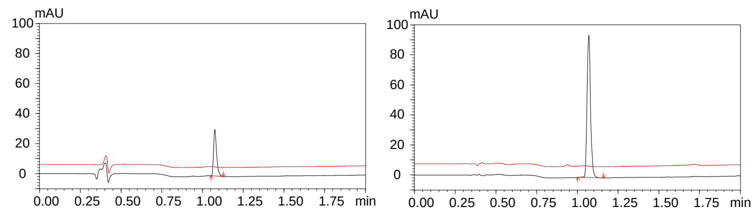


Fig. 10 Chromatogram of the Trimethoprim (Left: water layer, Right: octanol layer)

Discussion

Determined value of each Log*Pow* by the developed method was found to be in good agreement with the description in authentic references such as the MERCK Index.
For example, the obtained Log*Pow* of Bifonazole, which is higher than any others among the compounds in the table, indicates its high lipophilicity as the reference described. While, the obtained Log*Pow* of Antipyrine indicates its hydrophilicity that is in agreement with its high solubility in water described in the reference. In addition, carryover had not been observed in the analysis.

Table. 1 Peak area and Log*Pow*

Sample name	Peak area		Log
	water layer (Cw)	octanol layer (Co)	(Co/Cw)
Antipyrine	98891	120615	0.086
Lidocaine	8478	250793	1.471
Carbamazepine	5158	217252	1.624
Desipramine	18182	199983	1.041
Bifonazole	62	688978	4.046
Chlorpromazine	568	766123	3.13
Propranolol	3134	28869	0.964
Trimethoprim	39395	142481	0.558

Conclusion

These results indicate that the SIL-30AC with pretreatment function is a powerful tool for the automated determination of Log*Pow* without any requirement of experienced skills.

Acknowledgement

We would like to thank Mr. Takahiro TAKEUCHI (TEIJIN PHARMA LIMITED) who gave us useful advices and discussions for this study.