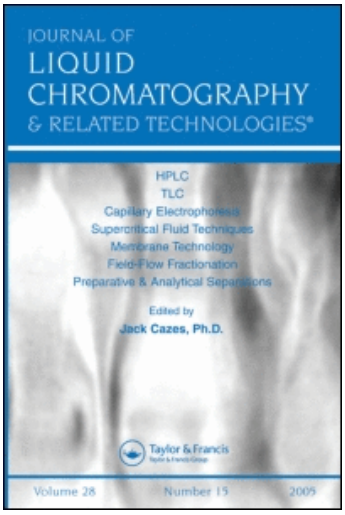


Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

A MICROSCALE HPLC METHOD FOR THE EVALUATION OF OCTANOL-WATER PARTITION COEFFICIENTS IN A SERIES OF NEW 2-AMINO-2-OXAZOLINES

F. Péhourcq[a]; J. Thomas[b]; C. Jarry[b]
[a] Department of Pharmacology, University Victor Segalen, Bordeaux Cedex, France [b] Department of Physical Chemistry, University Victor Segalen, Bordeaux Cedex, France

Online publication date: 18 January 2000

To cite this Article Péhourcq, F. , Thomas, J. and Jarry, C.(2000) 'A MICROSCALE HPLC METHOD FOR THE EVALUATION OF OCTANOL-WATER PARTITION COEFFICIENTS IN A SERIES OF NEW 2-AMINO-2-OXAZOLINES', Journal of Liquid Chromatography & Related Technologies, 23: 3, 443 — 453
To link to this Article: DOI: 10.1081/JLC-100101463
URL: http://dx.doi.org/10.1081/JLC-100101463

J. LIQ. CHROM. & REL. TECHNOL., 23(3), 443–453 (2000)

A MICROSCALE HPLC METHOD FOR THE EVALUATION OF OCTANOL-WATER PARTITION COEFFICIENTS IN A SERIES OF NEW 2-AMINO-2-OXAZOLINES

F. Péhourcq,[1] J. Thomas,[2] C. Jarry[2]

[1] Department of Pharmacology
University Victor Segalen Bordeaux 2
33076 Bordeaux Cedex, France

[2] Department of Physical Chemistry
University Victor Segalen Bordeaux 2
33076 Bordeaux Cedex, France

ABSTRACT

A rapid and simple microscale method for the determination of octanol-water partition coefficients has been developed and evaluated for a series of twenty-six bioactive 2-amino-2-oxazolines. Solutes were equilibrated between n-octanol and water by using the shake-flask approach in a single partitioning. The concentration of each compound was measured in the aqueous phase before and after partitioning directly by RP-HPLC, and the peak areas ratio was used to calculate the partition coefficient log P.

Log P values ranging from -0.22 to 3.69 have been determined with a precision better than ± 0.06 log unit. They were compared with the measured shake-flask octanol-water partition coefficient and with the calculated incremental Clog P values.

The linear regression analyses between these lipophilic parameters show significant correlations, allowing validation of this chromatographic approach for the log P determination.

INTRODUCTION

Lipophilicity is one of the inherent properties of chemical compounds, affecting their biological activity. The logarithm of the octanol-water partition coefficient log P, is the most frequently used parameter for defining the lipophilic character of drugs. It is traditionally obtained by the shake-flask technique, using n-octanol and water as a biphasic liquid system.[1] This technique is sensitive to solute and solvent impurities and needs several mg of analyte to replicate determinations and to obtain accurate values.[2,3] In order to overcome these technical difficulties, some authors have proposed to combine the classical shake-flask technique and the reversed-phase HPLC.[4-8] In these studies, HPLC was used as an analytical tool for the measurement of solute concentrations in both phases, and the P values were obtained from the ratio of peak areas from the octanol and buffer phases, respectively.

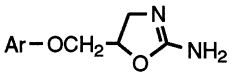
We report here the evaluation of the logarithm of the octanol-water partition coefficient (designed as log P*) for a series of 26 bioactive 2-amino-2-oxazolines, achieved by a rapid, reliable, and simple microscale HPLC method. To avoid the peak distortion following cumulative n-octanol injections already noticed[7] and in order to enhance the stability of 2-amino-2-oxazolines, we chose to evaluate log P* by comparison of the areas of the appropriate integrated peak from the aqueous phase before and after partitioning.

For fourteen 2-amino-2-oxazolines, log P was determined by the classical shake-flask method by measurement of relative concentrations using UV absorption. A significant linear regression was obtained between log P* and log P, allowing for validation of our method. Furthermore, we studied the behaviour of the weakly soluble compounds in relation with the influence of methanol on octanol-water partitioning. Finally, for all 2-amino-2-oxazolines the measured log P* values were correlated with those calculated through an incremental method (Clog P).

EXPERIMENTAL

Apparatus and Chromatographic Conditions

The chemical structures of compounds evaluated in partitioning experiments are depicted in Figures 1 and 2. The 5-dialkylaminomethyl- and the



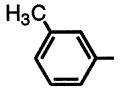
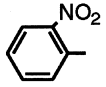
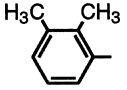
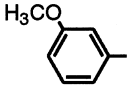
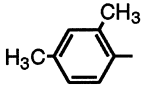
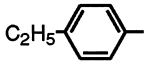
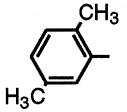
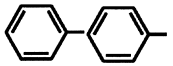
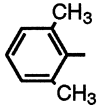
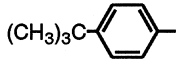
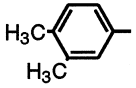
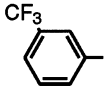
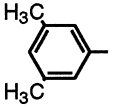
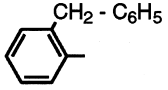
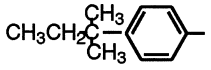
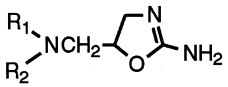
n°	Ar	n°	Ar
1		9	
2		10	
3		11	
4		12	
5		13	
6		14	
7		15	
8		16	

Figure 1. Structural formulae of 5-aryloxymethyl-2-amino-2-oxazolines.

5-aryloxymethyl-2-amino-2-oxazolines were synthesized according to already reported methods.[9,10] Their structures were supported by elemental analysis, IR, ^{1}H and ^{13}C NMR spectral data. 2-Amino-2-oxazolines are basic compounds with pK_a values ranging from 8.23 to 9.75.



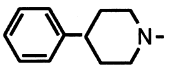
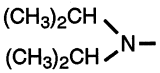
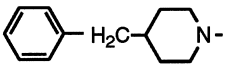
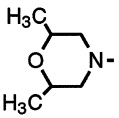
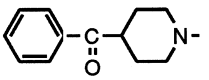
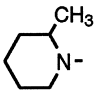
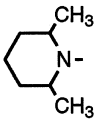
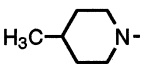
n°		n°	
17		22	
18		23	
19		24	
20		25	
21		26	

Figure 2. Structural formulae of 5-dialkylaminomethyl-2-amino-2-oxazolines.

All the chemicals used were of analytical or HPLC grade. n-Octanol, disodium hydrogen phosphate and di-potassium hydrogen phosphate were purchased from Prolabo (Paris, France). Methanol was supplied from Farmitalia Carlo Erba (Milan, Italy). Water was deionized and doubly-glass distilled.

All chromatographic measurements were performed at room temperature and at a flow rate of 1.5 mL/min on a chromatograph equipped with a Waters Model 590 pump, an autosampler Waters 717 plus (Waters Associates, Milford, MA, USA). The compounds were chromatographed on a C_{18} Novapak column (3.9 mm x 150 mm, 4 µm particle size, Waters). The mobile phase composition was a mixture of phosphate buffer M/15, pH 7.4 - methanol. The percentage of methanol was chosen in order to achieve retention times ranging from 2.5 to 6 minutes, for each compound. An UV1000 spectrophotometer (Thermo Quest, San Jose, CA, USA) set to the wavelength maximum of each compound was used for sample analysis.

Table 1

Experimental Parameters for Micro Shake-Flask HPLC Analysis

Compound	M (%)	λ_{max} (nm)	A[a] (μg)	P_m[b] (%)
1	50	269	100	2.5
2	50	268	50	2.5
3	50	274	100	2.5
4	50	272	50	2.5
5	50	261	100	2.5
6	50	275	50	2.5
7	50	276	50	2.5
8	50	272	50	2.5
9	45	264	50	2.5
10	45	272	50	2.5
11	50	268	100	2.5
12	55	245	50	2.5
13	55	273	200	5
14	50	273	50	2.5
15	50	210	50	2.5
16	50	220	100	2.5
17	40	208	50	2.5
18	40	208	50	2.5
19	30	208	50	2.5
20	40	208	50	2.5
21	40	208	50	2.5
22	48	208	50	2.5
23	58	208	100	3.3
24	48	208	50	2.5
25	40	208	50	2.5
26	40	208	50	2.5

[a] A: amount of compound used for the partitioning. [b] P_m: percentage of methanol present in octanol-water final mixture.

The Table 1 depicts the individual chromatographic characteristics of each studied 2-amino-2-oxazoline. All compounds were separated using similar chromatographic conditions in a reversed-phase mode. The percentage of methanol varied between 30% and 58%, respectively. The areas were measured using a Data Jet integrator (Thermo Quest).

Determination of Log P by the Micro HPLC Method (Log P*)

All the experiments were performed at ambient temperature (20 - 22°C). The pH of the water phase was 12, ensuring that all compounds were more than 99% unionized.

One milligram of each 2-amino-2-oxazoline was dissolved in the adequate volume of methanol in order to achieve 1 mg/mL stock solutions, except for compounds **1**, **3**, **5**, and **11** (2 mg/mL). Then, an appropriate aliquot of these methanolic solutions was dissolved in water at pH 12, saturated with n-octanol, to obtain final concentrations ranging from 50 µg/ mL to 200 µg/mL. Under the above described chromatographic conditions, 25 µL to 50 µL of this aqueous phase was injected onto the chromatograph, leading to the determination of a peak area before partitioning (W_0).

In screw-capped glass tubes, X mL of the aqueous phase (V_{aq}) was then added to Y mL of n-octanol (V_{oct}), previously saturated with water at pH 12. Except for 3 solutes, all the experiments were performed with $V_{aq} = V_{oct} = 1.0$ mL. To enhance the sensitivity of the final measurement, smaller volumes were used for the compound **23** ($V_{oct} = 0.2$ mL) and for compounds **13** and **22** ($V_{oct} = 0.5$ mL), respectively. The mixture was shaken by mechanical rotation during 30 minutes (rpm: 20). After a 30 minutes temperature-controlled centrifugation achieved at 2000 rpm, a 50 µL to 100 µL aliquot of the lower aqueous phase was injected into the chromatograph column. This led to the determination of a peak area after partitioning (W_1).

The P* value was calculated from the formula:

$$\frac{W_0 - W_1}{W_1} \times \frac{V_{aq}}{V_{oct}}$$

Five independent measurements were performed for each compound leading to a mean log P* value.

Determination of Log P by the "Classical" Shake-Flask Method

The octanol-water partition coefficients of fourteen 5-aryloxymethyl-2-amino-2-oxazolines were determined by the shake-flask technique using a conventional methodology.[1] Briefly, samples in a weight range of 5-10 mg were partitioned between 5 mL of n-octanol saturated with water at pH 12 and 50 mL of water at pH 12 saturated with n-octanol. The water phase absorbance was measured at an appropriate wavelength before and just after the partitioning experiment.[11]

Determination of the Calculated Incremental ClogP Values

For the twenty-six 2-amino-2-oxazolines, the Clog P values were calculated by a fragmental method available in MacLogP.[12]

RESULTS AND DISCUSSION

The log P, log P* and Clog P values of all studied compounds are listed in Table 2.

As previously reported,[2,3] the determination of the partition coefficient by the classical UV spectroscopy shake-flask method is encumbered by some difficulties in measuring a solute concentration in the aqueous phase accurately. Due to the high sensitivity of the HPLC equipment available today, the described microscale method can be used to measure the octanol-water partition coefficients for compounds with a poor absorption in the UV mode, e.g., 5-dialkylaminomethyl-2-amino-2-oxazolines (**17**-**26**). Moreover, the UV detection at the λ_{max} for each compound affords sufficient sensitivity to measure directly more than 1000-fold relative concentration differences in aqueous phase before and after partitioning. So, the log P value of very lipophilic compounds such as **15** and **16** can be determined accurately after a single partitioning.

Consequently, the partition coefficients of all studied compounds have been evaluated by this HPLC microscale method (logP* values ranged from 0.22 to 3.69). In order to validate this microscale method, the log P* were correlated with the shake-flask log P for compounds **1** - **14**.

The equation and the statistical parameters of the linear regression analysis were established as:

log P* = 1.027 (± 0.060) log P - 0.052(± 0.129)
(n = 14; s= 0.114; r= 0.98; F = 294.15, p=0.0001)

A significant correlation was obtained between log P and log P*. The slope of the linear regression was close to 1. This result is a check on the validity of the chosen procedure for the partition coefficient determination of structurally related 2-amino-2-oxazolines.

Furthermore, this validation permitted to correlate log P* and Clog P values for 25 compounds according to the following equation.

log P* = 0.960 (± 0.046) Clog P - 0.012 (± 0.096)
(n = 25; s= 0.222; r= 0.975; F = 443.17, p=0.0001)

Table 2

The log P, log P*, and Clog P Values for 2-Amino-2-Oxazolines

5-Aryloxymethyl-2-Amino-2-Oxazolines

Compound	Log P[b]	Log P* (Mean ± SD)[a]	Clog P
1	1.75	1.61 ± 0.02	1.74
2	2.25	2.38 ± 0.06	2.19
3	2.23	2.24 ± 0.06	2.24
4	2.29	2.37 ± 0.01	2.24
5	2	1.76 ± 0.04	2.24
6	2.1	2.30 ± 0.04	2.19
7	2.25	2.24 ± 0.02	2.24
8	2.09	2.19 ± 0.03	2.09
9	0.80	0.80 ± 0.01	ND
10	1.34	1.31 ± 0.01	1.33
11	2.27	2.27 ± 0.03	2.27
12	2.80	2.72 ± 0.06	3.13
13	2.84	2.87 ± 0.02	3.07
14	2.35	2.36 ± 0.01	2.37
15	ND[c]	3.05 ± 0.01	3.31
16	ND[c]	3.69 ± 0.05	3.59

5-Dialkylaminomethyl-2-Amino-2-Oxazolines

Compound	Log P* (Mean ± SD)	Clog P
17	0.19 ± 0.02	0.35
18	0.98 ± 0.06	1.28
19	0.16 ± 0.05	0.35
20	0.73 ± 0.06	0.99
21	0.94 ± 0.04	1.51
22	1.96 ± 0.04	2.04
23	2.38 ± 0.05	2.57
24	1.05 ± 0.01	0.89
25	-0.22 ± 0.02	-0.59
26	0.91 ± 0.05	0.99

[a] Log P* were measured by the microscale HPLC method (n=5).
[b] Thomas et al. (1997). [c] ND: not determined.

Table 3

Effect of Methanol on log P Determination

Compound	P_m (%)[a]	log P*
	0	0.80 ± 0.01
9	2.5	0.80 ± 0.01
	5	0.77 ± 0.02
	5	2.87 ± 0.02
13	10	2.90 ± 0.04
	2.5	2.36 ± 0.01
14	5	2.38 ± 0.02
	10	2.32 ± 0.02

[a] P_m (%): percentage of methanol present in octanol-water final mixture.

Due to a missing fragment value, Clog P of 9 cannot be calculated. As judged by this linear regression analysis a significant correlation was established between the measured and the calculated log P. The slope was statistically close to 1.

On the other hand, as the 2-amino-2-oxazolines were initially dissolved in methanol before spiking the aqueous phase, we chose to study the effect of methanol on the octanol-water partitioning. The effect of variable percentages of methanol on log P* was evaluated for three compounds (**9**, **13**, and **14**). According to their lipophilicity, different methanol concentrations were retained from 0% (aqueous phase only) to 10% (Table 3). We detected no measurable change in the partition coefficient under the above conditions, since log P* values did not differ by much more than the measurement precision (0.06 unit).

CONCLUSION

In this study, the applicability of a micro shake-flask method for the log P determination of twenty-six basic compounds has been demonstrated. This method uses only few μg of compound in a single partitioning exploiting the potential of HPLC as a microanalysis technique. Furthermore, as already published,[6] the compound purity is not as indispensable as it is when quantitation is accomplished by the UV spectroscopy shake-flask method. HPLC analysis presents also the capability to perform log P determinations for several compounds in a single partitioning experiment.

All the previous studies recommending the use of HPLC for the log P determination needed the measurements of the solute in the aqueous phase and in the octanol phase, respectively. The latter was achieved either by injecting directly the n-octanol phase or by injecting a sample obtained after the n-octanol elimination, i.e. by lyophilization. None of these procedures has been successful with compounds such as the 2-amino-2-oxazolines. Indeed, a peak distortion and an important loss of resolution occurred after cumulative n-octanol injections. Moreover, the 2-amino-2-oxazolines were found unstable during the n-octanol elimination step.

By combination of a derived shake-flask approach and HPLC as a detection technique, we determined the log P of a series of 2-amino-2-oxazolines. This easy and rapid method could be used to obtain log P values for new compounds, especially those with extreme lipophilicity values.

REFERENCES

1. A. Leo, C. Hansch, D. Elkins, Chem. Rev., **71**, 525-554 (1971).

2. J. C. Dearden, G. M. Bresnen, Quant. Struct.-Act. Relat., **7**, 133-144 (1988).

3. R. F. Rekker, R. Mannhold, in **Calculation of Drug Lipophilicity. The Hydrophobic Fragmental Constant Approach**, H.-J. Kraus, ed., VCH Publishers, Inc., New York, 1992.

4. A. Nahum, C. Horvàth, J. Chromatogr., **192**, 315-322 (1980).

5. P. Hairsine, Lab. Practice, **38**, 73-75 (1989).

6. H. Ford, C. L. Merski, J. A. Kelley, J. Liq. Chromatogr.,**14**, 3365-3386 (1991).

7. M. Henczi, J. Nagy, D. F. Weaver, J. Liq. Chromatogr., **17**, 2605-2613 (1994).

8. M. Henczi, J. Nagy, D. F. Weaver, J. Pharm. Pharmacol., **47**, 345-347 (1995).

9. C.Jarry, R. Golse, E. Panconi, M. H. Creuzet, Eur. J. Med. Chem., **21**, 138-142 (1986).

10. F. Demotes-Mainard, J. Tranchot, J. Cambar, C. Jarry, Arch. Pharm., **325**, 193-198 (1991).

11. J. Thomas, O. Adetchessi, F. Péhourcq, P. Dallet , C. Jarry , J. Liq. Chromatogr., **20**, 671-679 (1997).

12. Biobyte Corporation, **MacLogP 2.5**, Claremont, CA, USA, 1998.

Received March 20, 1999
Accepted May 18, 1999
Manuscript 5023