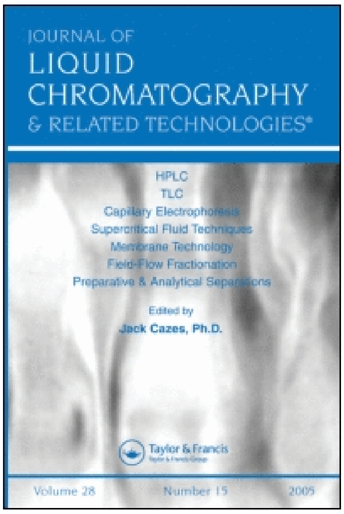

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HIGH PERFORMANCE LIQUID CHROMATOGRAPHY OF NEW 5-ARYLOXY-METHYL-2-AMINO-2-OXAZOLINES: A COMPARATIVE STUDY OF THEIR LIPOPHILICITY

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ABSTRACT

The capacity factors (log k') for a series of bioactive 5-aryloxymethyl-2-amino-2-oxazolines were determined by a high-performance liquid chromatography method using methanol-water as a mobile phase and ODS column as stationary phase. The influence of mobile phase composition was examined, allowing the determination of log k'_w values through extrapolation to 100% water from capacity factors data. The influence of the apparent pH of the mobile phase was studied and discussed in terms of solute ionization. The partition coefficients (log $P_{o/w}$) and ionization constants (pK_a) of all the compounds were measured by classical methods permitting to correlate the different lipophilic indexes.

INTRODUCTION

The hydrophobic property of bioactive compounds, expressed by the 1-octanol/water partition coefficient ($P_{o/w}$), is one of the most important factors to be considered in quantitative structure-activity relationship

(QSAR) studies (1,2). For a number of years, chromatographic methods especially RP-HPLC have been proposed to determine the lipophilicity of drugs (3-6). An interrelationship between the partition coefficient P and the chromatographic column capacity factor k' in RP-HPLC has been established in a Collander-type equation (7).

We recently developed such a chromatographic approach of the lipophilicity applied to new series of 2-amino-2-oxazolines bearing different substituents on the heterocyclic ring (8,9).

The aim of the present study was to study the lipophilicity of new bioactive 5-aryloxymethyl-2-amino-2-oxazolines by mean of a RP-HPLC technique. The reliability of this methodology is checked by correlation of the k' data with the parameter P measured by the classical "shake-flask" method. The influence of the pH eluent on the capacity factor was studied by working at different pH values. The observed variations were related to the pK_a's values of 5-aryloxymethyl-2-amino-2-oxazolines determined by a potentiometric method.

MATERIALS AND METHODS

Apparatus and chromatographic conditions.

Chromatography was performed with a Waters Assoc. apparatus equipped with a Model 501 pump, a Model 455 ultraviolet detector operating at 254 nm and an U_6K manuel injector. The compounds were chromatographed on an Inertsil ODS2 column (250 mm x 4.6 mm, 5 µm particle size)(Interchim). The mobile phase composition ranged from 30 to 50 % (v/v) methanol with 0.06 M phosphate buffer at various pH (7.4 ; 8 ; 9). The flow rate was 1 ml/min. The detector output was recorded on a Model 746 data Module integrator

Standards and reagents

New 5-aryloxymethyl-2-amino-2-oxazolines were synthesized by condensation of monosodium cyanamide with the corresponding 1-aryloxymethyl-2,3-epoxypropanes (10). Their structures were supported by elemental analysis, IR, ^1H and ^{13}C NMR spectral data. Stock solutions containing 1mg/ml of each drug were prepared in methanol and stored at -20°C.

HPLC-grade methanol (Prolabo) was used without further purification to prepare the mobile phase and water was glass-distilled deionized. To prepare the phosphate buffer solutions potassium dihydrogen phosphate and dipotassium hydrogen phosphate trihydrate (Merck) were used. Before use for HPLC, the mobile phase was filtered through a 0.45 µm membrane filter.

Measurement of log k'

The column dead-time of the system (t_0) was measured as the time from injection to the first distortion of the baseline after injection of pure water. Consequently, the stock solutions of tested compounds were diluted with water to the final injected concentrations of 50 µg/ml. According to their chromatographic behaviour, the retention time (t_r) of each compound was determined at six different methanol-phosphate buffer mixtures ranged from 30 to 50%. The compounds were injected separate from each other three times and the mean value of the retention time was retained. The log k'_w value for each compound was obtained by regression analysis of log k' data, expressed from the retention times t_r, through the formula : $k' = (t_r - t_0) / t_0$, and extrapolation to 0% methanol content.

The correlation/regression analysis were carried out with a statistical program on a Vectra computer (Hewlett Packard).

Measurement of pK_a

The pK_a's determinations were performed using a classical potentiometric method described elsewhere (11).

Determination of log P

The octanol-water partition coefficients ($P_{o/w}$) were determined by the classical "shake-flask" technique using a conventional methodology. Samples in a weight range of 5-10 mg were partitioned between 5 ml of n-octanol saturated with water and 50 ml of water saturated with n-octanol. The pH of the water phase was adjusted at 11, ensuring that all compounds were more than 99% unionized.

RESULTS AND DISCUSSION

The chemical formulae of the tested 5-aryloxymethyl-2-amino-2-oxazolines are given in Table 1.

Determination of log k'_W and S (slope of the regression analysis)

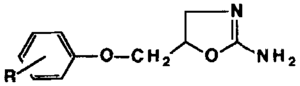
In this study we have chosen to measure the log k' value extrapolated to 0% of the organic modifier in the mobile phase (log k'_W). For some authors (3,12), this technique (polycratic method) allows more adequate evaluation of the hydrophobic nature of the solute and provides a scale of lipophilic parameters normalized to one set of conditions more closely related to log $P_{o/w}$. In these conditions, the test compounds were chromatographed under a variety of conditions in which the percentage of methanol varied from 30 to 50%. Experiments with lower percentages of methanol than 30% afforded experimental retention times too long to be measured, mainly at pH 9. For all compounds, linear relationships ($r >$ 0,98) were proved to exist between the log k' values and methanol concentrations, allowing the calculation of log k'_W and S through extrapolation (Table 1). The slopes S for the equations were mostly constant. Statistically significant correlations were noted between log k'_W and S at each pH ($r = 0.919$, 0.877 and 0.917 - pH = 7.4, 8 and 9). These results may be related to the structural similarity of all tested compounds in regard to their partition behaviour in RP-HPLC.

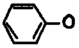
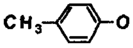
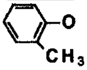
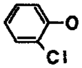
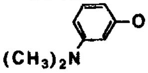
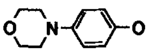
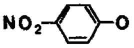
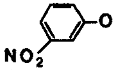
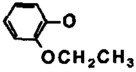
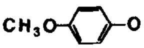
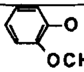
Effect of the pH eluent on log k'_W

The influence of solute ionization on RP-HPLC determination of capacity factors has been often discussed (4,5,13,14). In general, for the determination of hydrophobicity, the unionized form of solutes is taken as the reference state.

In order to study the variation of log k'_W in terms of ionization, the pK_a 's determinations have been performed (Table 1). 5-aryloxymethyl-2-amino-2-oxazolines are basic molecules ($pK_a = 8.49 \pm 0.16$, variation coefficient 2.6%). These pK_a values are comparable with those of other 5-substituted-2-amino-2-oxazolines, indicating a slight influence of the

TABLE 1 : ANALYTICAL DATA OF 5-ARYLOXYMETHYL-2-AMINO-2-OXAZOLINES



N°	SUBSTITUENT	pH 7,4 logK'$_w$	Slope	pH 8 logK'$_w$	Slope	pH 9 logK'$_w$	Slope	log $P_{o/w}$	pK_a
1		1.804	-0.029	1.910	-0.026	1.998	-0.031	1.27	8.59
2		2.242	-0.033	2.115	-0.027	2.394	-0.034	1.79	8.52
3		1.977	-0.031	2.020	-0.028	2.512	-0.037	1.69	8.54
4		2.264	-0.032	2.503	-0.033	2.513	-0.035	2.00	8.71
5		2.037	-0.030	2.136	-0.030	2.374	-0.035	1.77	8.67
6		2.106	-0.031	2.318	-0.032	2.427	-0.038	1.40	8.49
7		1.537	-0.026	1.607	-0.027	1.612	-0.028	0.60	8.39
8		1.711	-0.028	1.700	-0.024	2.103	-0.034	1.17	8.35
9		1.695	-0.027	1.784	-0.027	2.030	-0.033	1.23	8.10
10		1.948	-0.030	1.966	-0.028	2.145	-0.033	1.24	8.52
11		1.739	-0.030	1.618	-0.024	1.881	-0.031	1.26	8.42
12		1.545	-0.027	1.587	-0.026	1.825	-0.031	0.85	8.56

nature of the 5-substituted moiety (7,8). For compounds 1-12, no particular influence due to the nature or the position of the substituent on the phenyl ring was observed.

For all compounds the ionization percentage is near 90% at pH 7.4, 75% at pH 8 and below 25% at pH 9. The variations of the log K'_W values with the apparent pH eluent (Table 1) are consistent with the ionization effects. At pH 9 the compounds were less ionized, they showed less hydrophilic properties and thus the capacity factor was maximum. On the contrary the capacity factor should be minimum at lowest pH. Nevertheless between pH 7.4 and 8 the increase of log k'_W was not always regular (i.e. compounds 2, 8, 10), suggesting particular ionization effects.

Correlation between lipophilic indexes

The log k'_W were correlated with log $P_{o/w}$, according to equations I, II and III for the experimental data listed in Table I

I pH 7.4 $\log k'_W = 0.561\ (\pm 0.082) \log P + 1.123\ (\pm 0.116)$
$(n = 12,\ r = 0.907,\ s = 0.110,\ F = 46.60,\ p \leq 0.0001)$

II pH 8 $\log k'_W = 0.606\ (\pm 0.129) \log P + 1.117\ (\pm 0.182)$
$(n = 12,\ r = 0.829,\ s = 0.173,\ F = 22.07,\ 0.0001 \leq p \leq 0.005)$

III pH 9 $\log k'_W = 0.668\ (\pm 0.093) \log P + 1.245\ (\pm 0.131)$
$(n = 12,\ r = 0.915,\ s = 0.168,\ F = 51.85,\ p \leq 0.0001)$

where n is the number of data, r is the correlation coefficient, s is the standard error of the estimate, F is a measure of the significance of the correlation and p is the probability level.

A definite correlation was established among the hydrophobic parameters ; it was better when log k'_W measured at pH 9 was used.

The apparent partition coefficient D has been calculated at the eluent pH, based on the knowledge of the acid dissociation constants :

$$D = \frac{P}{1 + [(H^+)/Ka_1]}$$

In order to take into account the basic behaviour of the molecules, the correlations between log k'$_W$ and log D were established. The substitution of log D instead of log P did not improve the correlation, especially at pH 7.4 and 8 (r = 0.828, 0.750 and 0.914 for pH 7.4, 8 and 9 respectively). This result could be related to the ionization effects already noticed during the experiment.

CONCLUSION

The influence of solute ionization on RP-HPLC determination of log P is obvious. In general, for the determination of hydrophobicity, the unionized form is taken as the reference state. This can be difficult to obtain retention data for acids and bases in their uncharged state due to the limited pH operating range of silica bonded phases.

In order to study the ionization effects of 5-aryloxymethyl-2-amino-2-oxazolines we determined the log k'$_W$ at pH operating range near the ionization constant value (pK$_a$). We chose an ODS column with a stationary phase claimed to be stable up to pH 9 (with a phosphate buffer/methanol eluent).

The results indicated that the increase of log k'$_W$ with the apparent pH eluent was consistent with the expected ionization effects for these basic compounds. However it should be noticed that the studied amines were all structurally related, and had nearly identical pK$_a$ values.

From the octanol/water partition coefficients, satisfactory correlations among the hydrophobic parameters have been established. In particular the correlation coefficients calculated for the experiments at pH 7.4 and pH 9 were not greatly different ; thus it should be tempting to use the log k'$_W$ values measured at physiologic pH for our further QSAR studies.

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