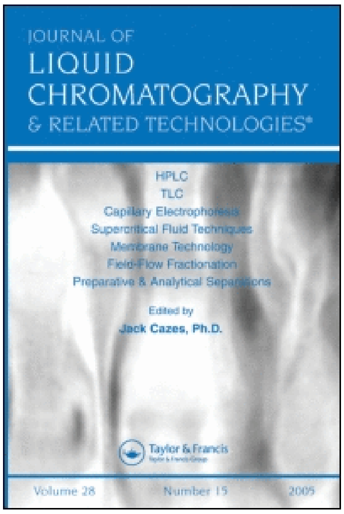

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F. Péhourcq[a]; J. Thomas[b]; C. Jarry[b]
[a] Laboratoire de Pharmacologie, [b] Laboratoire de Chimie, Physique Université Victor Ségalen, Bordeaux, Cedex, France

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RELATIONSHIP STUDY BETWEEN REVERSED-PHASE HPLC RETENTION DATA AND THE pKa VALUES IN A SERIES OF 2-AMINO-2-OXAZOLINES

F. Péhourcq,[†] J. Thomas,[‡] C. Jarry[‡]

[†]Laboratoire de Pharmacologie
[‡]Laboratoire de Chimie Physique
Université Victor Ségalen
146 rue Léo-Saignat
33076 Bordeaux Cedex, France

ABSTRACT

In reversed-phase liquid chromatography, the retention behaviour of ionogenic compounds is strongly related to the pH mobile phase. An application is presented for three basic 5-substituted 2-amino-2-oxazolines. The pH dependence of their capacity factor values, expressed as log k'_W, was investigated in a RP-HPLC procedure using a column packed with poly-methacrylate. The plot of capacity factor *vs* pH was described by a sigmoidal curve, leading us to approach the ionization constants of the molecules. Finally, the pKa values have been discussed in regard to chromatographic parameters and to experimental conditions.

INTRODUCTION

At different pH values, the drugs containing ionogenic functions may exist as a mixture of dissociated and undissociated forms. Their retention behaviour, expressed as the capacity factor, k', in a reversed-phase high-performance liquid chromatographic system (RP-HPLC), is dependent on both the ionized and the unionized species.[1] Consequently, the mobile phase pH is an important tool to accomplish a complete separation between the different solutes in terms of selectivity and of column efficiency.[2,3] In RP-HPLC, as the eluent is a mixture organic modifier/buffer, the aqueous phase pH is chosen in regard to the degree of ionization (pKa) of the studied compounds. If the solute is a weak base, at pH < pKa, the molecule is completely ionized and the retention is low. Conversely, at pH > pKa, the ionization is suppressed and the retention is enhanced.

In the present study, the dependence of the capacity factor (expressed as log k'_W) on the aqueous phase pH was investigated for three potentially active basic drugs using a RP-HPLC procedure (Table 1). This experiment may lead to an approach to the measurement of pKa.

The theoretical basis of the pKa determination using liquid chromatographic retention data was first proposed by Horvath *et al.*[1] Then, others studies related to acidic compounds showed the ability of curve fitting to measure pKa values from capacity factors.[4,5]

In many ways, the RP-HPLC methods, using an octadecyl-modified silica column (ODS), have been found useful only for neutral and acidic compounds, since column degradation occurs with mobile phases of pH above 8.[6] Consequently, in order to investigate the pH dependence of the capacity factor values for basic 2-amino-2-oxazolines, we chose a column packed with hydrophilic polymethacrylate.

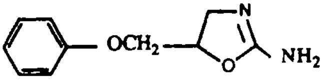
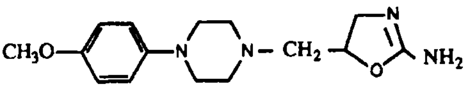
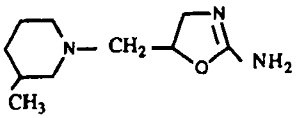
EXPERIMENTAL

Apparatus and Chromatographic Conditions

Chromatography was performed with a Waters Assoc. apparatus equipped with a Model 590 pump, a WISP Model 717 *plus* automatic injector and a Lambda Max Model 480 ultraviolet detector operating at 214 nm.

Table 1

Chemical Structures of Studied 2-Amino-2-oxazolines

Compound No.	Structure
1	
2	
3	

The compounds were chromatographed on a Shodex Pak polymeric column (RS pak D18-613, 151 mm X 6 mm i.d., Asahi). The mobile phase composition ranged from 25% to 60% acetonitrile with 0.067M phosphate buffer (v/v) at various pH values (from 4 to 9). The 9.5 pH value was obtained from an appropriate dilution of sodium hydroxide.

The flow rate was 2.5 mL/min. The detector output was recorded on a Data Jet integrator (Spectra Physics, France).

Chemicals

The synthesis of the 5-substituted 2-amino-2-oxazolines, characterized from spectral data (IR, ^1H and ^{13}C NMR), is described elsewhere.[7,8]

All chemicals and solvents were of analytical or HPLC grade. Acetonitrile HPLC gradient was purchased from SDS (Peypin, France). Water was deionized, doubly glass-distilled. 1-Octanol was obtained from Merck (Darmstadt, Germany). Buffer solutions in pH range 4 - 9 were prepared by mixing the proper volume of 0.067 M aqueous solution of potassium dihydrogen phosphate and di-sodium hydrogen phosphate (KH_2PO_4; Na_2HPO_4 12 H_2O, Prolabo, France). Their pH's were tested by pH-metry.

Measurement of Log k'_w

Stock solutions containing 1mg/mL of 2-amino-2-oxazolines in methanol were diluted with water to the final injected concentrations (50 µg/mL). According to their chromatographic behaviour, the retention time of each compound was determined in triplicate at six different acetonitrile-phosphate buffer mixtures ranging from 25% to 60%. For each compound, the log k'_w value was obtained by a regression analysis of the log k' data, expressed from the retention time, t_r, through the formula : $k' = (t_r - t_0 / t_0)$, and extrapolation to 0% acetonitrile content. 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 each molecule in water. The correlation/regression analyses were carried out with a statistical program (StatView II) on a Power Macintosh computer. This method was previously described for other 2-amino-2-oxazolines.[9,10]

Calculation of pKa Values

The plot of log k'_w *vs* pH is a sigmoidal curve with a midpoint pH corresponding to the pKa value of the tested compound.[11] These graphical measurements were compared to pKa values obtained using a classical potentiometric method.[12]

In the same way, the pKa at different acetonitrile concentrations was determined by plotting the log k' *vs* pH.

RESULTS AND DISCUSSION

The studied compounds are weak bases with different H-accepting sites. A previously reported X-ray crystallographic study showed that the *endo* nitrogen atom of the amidine moiety represents the first protonation site (pKa_1).[13]

For compounds **2** and **3**, a second basic center (pKa_2) was found on a piperazine nitrogen atom and on the piperidine nitrogen atom, respectively. The pKa values, determined by a classical potentiometric technique, are listed in Table 2.

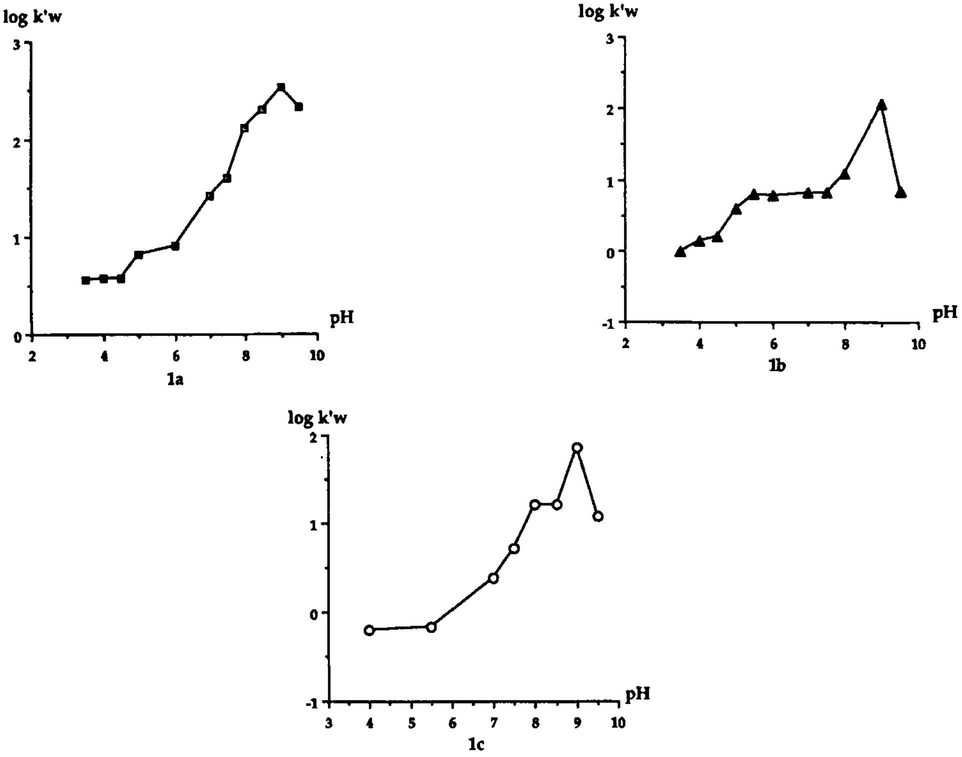


Figure 1. Plots of the capacity factors (expressed as log k'_W) *vs* the aqueous phase pH of the eluent. (1a: compound **1**; 1b: compound **2**; 1c: compound **3**).

Table 2

pKa Values of Studied 2-Amino-2-oxazolines

Chemicals		pKa Measured by Potentiometry	pKa Estimated by HPLC
Compound **1**	pKa 1:	8.59	7.06
Compound **2**	pKa 1:	8.05	8.24
	pKa 2:	5.22	4.20
Compound **3**	pKa 1:	9.23	not determined
	pKa 2:	7.13	8.76

pH Dependence of the Capacity Factor Values

The pH dependence of the capacity factor values is illustrated in Figure 1a (compound **1**) and in Figures 1b and 1c (compounds **2** and **3**), respectively. For bases, already published studies have shown that the retention variation with pH is typically described by a sigmoidal function.[3,14] The pKa value can be determined at the midpoint of the the plot of log k' *vs* pH. In such experiments, a sufficient number of experimental data are necessary for the measurement of pKa with a good accuracy.

For the compounds **1** and **2**, the curve outlines almost agree with the analytical parameters (one and two H-accepting sites, respectively). But for **3**, as it will be further discussed, only one midpoint was observed.

The pKa values measured from the sigmoidal plots are depicted in Table 2. The observed differences may be related to the nature of the column, to the nature of the organic modifier, to the ionic strength of the buffer, or to the existence of ion-pair interactions.

Reversed-phase packings are the most widely used types of stationary phases in HPLC. Generally, the columns are filled with microparticule, porous silicas treated by means of silanization, using appropriate alkylsilanes. These packings suffer from a limited pH stability, and the measurement of k' *vs* pH is less accurate for basic solutes than for acidic ones.[2,15] This may be due to silanol effects, which play an important role with bases. By using a polymeric column, we avoided the change in the ionization state of the surface silanols, which may otherwise produce potential errors.[16] We chose a column packed with hydrophilic polymethacrylate gel having octadecyl (C_{18}) groups.

Hanson *et al.*[15] have recently reviewed the synthesis and properties of polymer-coated RP stationary phases. On this type of polymer-coated RP column, the retention mechanism differs from that observed with classical silanized silicas. The difference is due to the existence of polar groups in polymethacrylate and, generally, excellent separations of basic compounds have been reported.[15]

Acetonitrile exhibits a more complex behaviour towards the acid-base system than methanol.[16] Acetonitrile is an aprotic solvent with a high dielectric constant and, so, in this medium, bases are not completely dissociated. In this work, the use of acetonitrile leads to sharp peaks, permitting measurement of the capacity factor, k', with a sufficient accuracy.

Table 3

Effect of the Medium on the Acidity Constant

	Compound 1	Compound 2	
Acetonitrile (%)	pKa_1	pKa_1	pKa_2
30	7	8.71	4.63
35	7.05	9.61	4.54
40	7.24	10.29	4.47
45	7.57	10.75	5.45

The deviations observed between the pKa values (Table 2) may be explained in terms of dielectric constant difference between the aqueous/organic mobile phase and the water.[4] As the 2-amino-2-oxazolines are weak bases, they are protonated at pH < pKa. In this ionization state, they could be mixed with the phosphate ions from the buffer in ion-pair interactions. When the 2-amino-2-oxazolines are in an un-ionized state (pH above the pKa's values), these ion-pair interactions are not involved, and the capacity factors diminish. This phenomenon is illustrated by the decrease of the log k'_W values, always observed at pH 9.5 (Figures 1a, 1b and 1c). Consequently, for the compound **3**, we cannot measure the pKa_1 value, this ionization constant being close to 9.5 (Table 2).

Effect of the Mobile Phase Composition on the Acidity Constant

The effect of the mobile phase composition on the ionization equilibrium was studied by measuring the pKa at different acetonitrile concentrations, ranging from 30 to 45%. The ionization constant values obtained for the compounds **1** and **2** are shown in Table 3.

The pKa_1 values increase with increasing the organic modifier percentage in the mobile phase. This effect, already observed by Li *et al.*,[4] could be explained by a change of the mixture dielectric constant. According to Coulomb's law, the attraction force is inversely proportional to the dielectric constant. For acetonitrile, the dielectric constant is 38.8 at 20°C, whereas it is 78.5 for water. Thus, an increase of the acetonitrile concentration leads to a decrease of the dielectric constant of the mixture. Consequently, the acetonitrile percentage and the acidity constant varied in the same way. Our results (Table 3) agree with this observation.

CONCLUSION

In this work, the dependence of the capacity factor (expressed as log k'_W) on the aqueous phase pH was investigated for three 2-amino-2-oxazolines using a RP-HPLC procedure. In order to avoid the change in the ionization state of stationary phase surface silanols, we chose a column packed with hydrophilic polymethacrylate having octadecyl groups.

The acidity constants of these basic compounds were determined from the sigmoidal curve drawn by plotting the pH variation upon the log k'_W, at its midpoint. They were compared to the values measured using a potentiometric method.

In a second part, we studied the effect of the mobile phase composition on the acidity constants, permitting us to observe the relationship between the two parameters in the retained RP-HPLC system.

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