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Investigation of thermodynamic acidity constants of some statins with RPLC method

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ABSTRACT

In this study, thermodynamic acidity constants (${}^{s}_{s}pK_a$) of atorvastatin, pravastatin, and rosuvastatin using chromatographic data in acetonitrile–water binary mixtures with acetonitrile percentages of 40, 45 and 50% (v/v) were determined with reverse phase liquid chromatographic method (RPLC). pH values were measured in the hydroorganic mixture, which was used as the mobile phase, taking into account the effect of the activity coefficients. The combined effect of the two factors (solvent percentage and pH of the mobile phase) on the chromatographic behavior of these compounds was investigated. ${}^{s}_{s}pK_a$ values and limiting retention factors of these compounds were calculated chromatographically using equations derived. From these values, the aqueous ${}^{w}_{w}pK_a$ of statins was calculated by different approaches. The chromatographic determination was achieved on a Xterra RP18 (250 mm × 4.6 mm, 5 μm I.D.) analytical column.

1. Introduction

The discovery of HMG-CoA (3-hydroxy-3-methylglutaryl-coenzyme A) which acts as inhibitors called statin that was a breakthrough in the prevention of hypercholesterolemia and related diseases [1]. Statins are group of drugs that are used primarily in lowering blood cholesterol. These compounds are generally capable in lowering cholesterol by 20 to 60%. During the past decade, evidence has emerged that statins also have neuroprotective effects. It has been reported that the intake of statins is associated with a decreased incidence of Alzheimer's disease (AD) and Parkinson's disease (PD) [2]. To this class belong 8 drugs: mevastatin and lovastatin, which were the first developed and studied in humans; pravastatin and simvastatin, which can be considered as derivatives of the parental lovastatin; and atorvastatin, fluvastatin, rosuvastatin and pitavastatin, which are distinct synthetic compounds [3].

The chemical structures of statins govern their water solubility, which in turn influences their absorption, distribution, metabolism and excretion. The acidity constant (pK_a) value is a main item in the biophysical characterization of a drug and may be helpful in predicting the behavior of a drug under in vivo conditions. Briefly, the properties of a drug molecule inside the body depend on the acidity constant of the drug. Hence, it is important to calculate properly the drug acidity constant value of a pharmaceutical compound [4].

Acidity constant (pK_a) values established for organic solvent–water binary mixture systems can be extremely useful for the prediction of retention and optimization of chromatographic separations of compounds [5,6]. There are several methods for the determination of acidity constants such as high performance liquid chromatography (HPLC), capillary electrophoresis (CE), potentiometry and UV–vis spectrometry. In most of these methods, a physical property of an analyte is measured as a function of the pH of a solution and resulting data are used for the determination of acidity constants. Among these techniques, most of the drugs can be analyzed by HPLC technique because of the several advantages like rapidity, specificity, accuracy, precision and ease of automation in this method. Moreover, pK_a values can also be predicted by computational methods on the basis of molecular structure [7].

Analysis of the change in retention time of the analyte versus the change in pH of the mobile phase gives an indirect measure of the acidity constant. The theory for studying the pH dependence of chromatographic retention for ionizable compounds in LC was proposed by Horvath et al. [8]. The expression:

$$k = \frac{k_{HA} + k_{A^-}\dfrac{K_a}{a_{H^+_m}\gamma_{A^-_m}}}{1 + \dfrac{K_a}{a_{H^+_m}\gamma_{A^-_m}}} \quad (1)$$

represents the variation of the retention factor for a weak monoprotic acid (HA) with the hydrogen ion activity in the mobile phase, $\left(a_{H^+_m}\right)$. HA and A^- represent undissociated acid and fully dissociated acid, respectively. The acidity constant in the acetonitrile–water mixture used as mobile phase is represented by K_a. $\gamma_{A^-_m}$ is the activity coefficient of the dissociated acid in the mobile phase that can be calculated by the classical Debye–Hückel equation [9]. The observed retention factor (k)

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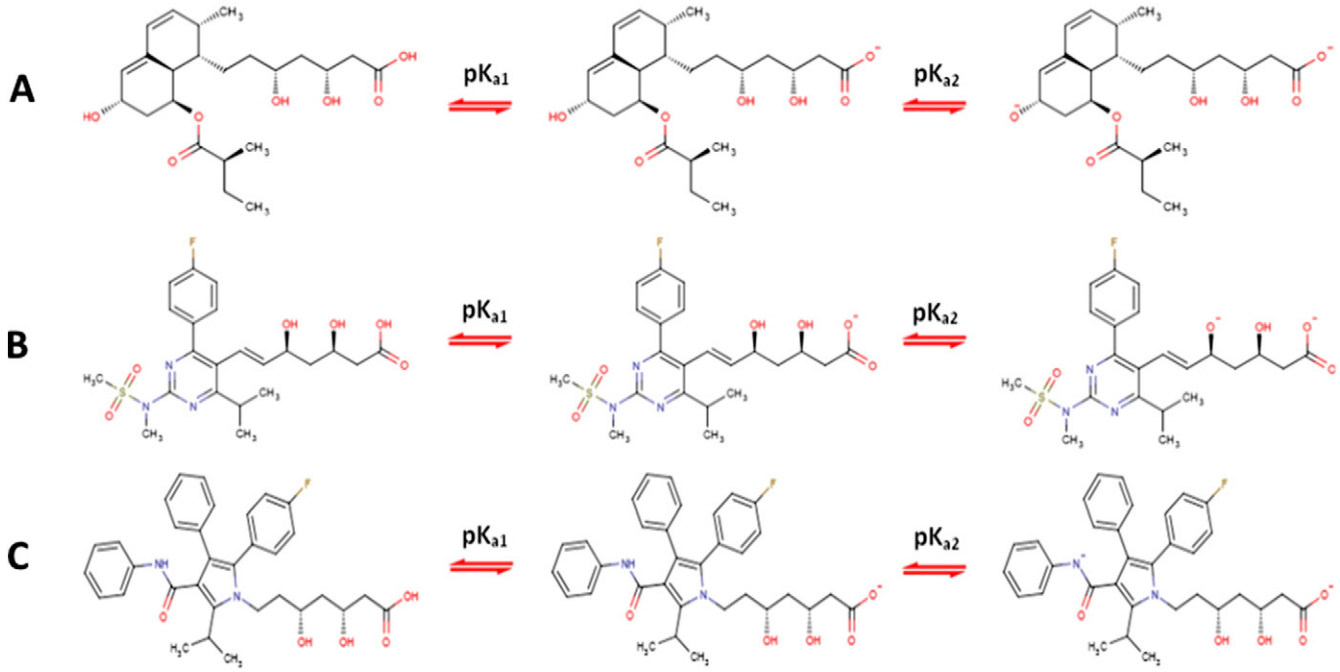

Fig. 1. Scheme of (A) pravastatin (B) rosuvastatin (C) atorvastatin molecule dissociations.

is an average of the retention factors of the acid (k_{HA}) and basic forms (k_{A^-}). It is known that these factors are very useful to explain the differences between the affinity of the ionized and neutral forms to the stationary phase.

For any chemical process occurring in solution, the polarity of the solvent plays a crucial role in determining the outcome [10]. A widely employed measure of solvent polarity is Dimroth and Reichardt's $E_T(30)$ [11]. $E_T(30)$ polarity of acetonitrile–water binary mixture mobile phases used in RPLC was measured and compared with chromatographic retention and selectivity. This polarity index is often used (also in this study) in its normalized, dimensionless form, the so called empirical parameter of solvent polarity, E_T^N. Linear plots of log k versus the φ are also satisfactory for most solutes for narrow ranges of organic solvent. However, Johnson et al. [10] demonstrated linear relationships in a wide range of organic solvents between the solute log k and E_T^N [12]. E_T^N polarity parameter has been used to predict the chromatographic behavior of ionizable compounds.

Variation of the retention factor of neutral form of an acid (k_{HA}) and the retention factor of anionic form of an acid (k_{A^-}) with percentage of acetonitrile in the mobile phase is represented by the normalized Dimroth and Reichardt polarity parameter (Eqs. (2), (3)).

$$\log k_{HA} = C_{HA} + e_{HA}E_T^N \tag{2}$$

$$\log k_{A^-} = C_{A^-} + e_{A^-}E_T^N \tag{3}$$

Substituting Eqs. (2) and (3) into Eq. (1) the theoretical expression describing the dependence of the retention factor for acidic solutes as a combined function of pH and E_T^N may be expressed as follows:

$$k = \frac{10^{\left(C_{HA}+e_{HA}E_T^N\right)} + 10^{\left(C_{A^-}+e_{A^-}E_T^N\right)}\left(K_a/a_{H_m^+}\gamma_{A_m^-}\right)}{1+\left(K_a/a_{H_m^+}\gamma_{A_m^-}\right)} \tag{4}$$

where $a_{H_m^+}$ is the thermodynamic activity of the indicated species in the binary mixture. C_{HA} and C_{A^-} are intercept values of the neutral and anionic species, respectively. Similarly, e_{HA} and e_{A^-} are slope values of these species [13].

To obtain measurable RPLC retention parameters, an addition of organic modifier to the mobile phase is normally necessary, especially in the case of water–insoluble substances [14]. This work was undertaken to calculate the thermodynamic acidity constants of atorvastatin, pravastatin, and rosuvastatin in various acetonitrile–water binary mixtures in order to overcome the lack of information related with the dissociation equilibria of compounds by means of RPLC method. In a living system, drugs are assumed to encounter a mainly aqueous environment. Therefore, thermodynamic aqueous acidity constant values ($^w_wpK_a$) of investigated drugs were calculated by means of Yasuda–Shedlovsky equation [15,16] and linear relationship between the mole fraction of acetonitrile and the $^s_spK_a$ values. Yasuda–Shedlovsky

Table 1
The retention factors of neutral (k_{HA}) and anionic (k_{A^-}) species and the thermodynamic acidity constant values of investigated compounds in various acetonitrile/water ratios calculated by NLREG program.

Compounds	40% ACN				45% ACN				50% ACN			
Pravastatin	k_{HA}	1.107	pK_{a1}	5.516	k_{HA}	0.718	pK_{a1}	5.705	k_{HA}	0.490	pK_{a1}	6.000
	k_{A^-}	0.139			k_{A^-}	0.106			k_{A^-}	0.086		
Rosuvastatin	k_{HA}	3.782	pK_{a1}	5.446	k_{HA}	2.234	pK_{a1}	5.727	k_{HA}	1.352	pK_{a1}	5.945
	k_{A^-}	0.666			k_{A^-}	0.397			k_{A^-}	0.272		
Atorvastatin	k_{HA}	14.645	pK_{a1}	5.474	k_{HA}	6.954	pK_{a1}	5.661	k_{HA}	3.529	pK_{a1}	5.871
	k_{A^-}	2.920			k_{A^-}	1.403			k_{A^-}	0.807		

The values between parentheses are the standard deviations.