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Article

Effect of Organic Solvent Composition on Dissociation Constants of Some Reversible Acetylcholinesterase Inhibitors

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Reversible acetylcholinesterase inhibitors are an important group of drug compounds that are used medicinally to treat Alzheimer's disease. In this study, dissociation constant values (pK_a) of some reversible acetylcholinesterase inhibitors (donepezil, rivastigmine and galantamine) having different functional groups were determined in different percentages of acetonitrile (MeCN)-water and methanol (MeOH)-water binary mixtures using reversed phase liquid chromatography (RPLC) method. In this way, a complete description of the retention behavior of each solute in the space defined by the pH and organic modifier percentages variables was obtained. The linear relationships established between retention factors of the species and the polarity parameter of the mobile phase (E_T^N) was proved to predict accurately retention in liquid chromatography (LC) as a function of the acetonitrile and methanol content. In result, the estimated aqueous pK_a values of studied basic compounds were assessed by comparing them with literature data.

Keywords: reversible acetylcholinesterase inhibitors, dissociation constant, nonlinear regression, polarity parameter, RPLC

Introduction

Alzheimer's disease (AD) is a progressive neurological disorder, the most common form of dementia, characterized by memory loss and other intellectual abilities serious enough to interfere with daily life.[1] Reversible acetylcholinesterase (AChE) inhibitors are drugs used for treatment of Alzheimer's disease. These inhibitors include compounds with different ionizable functional groups. Donepezil, rivastigmine and galantamine as reversible AChE inhibitors are basic and water-insoluble compounds.

The dissociation constant (pK_a) of drugs is of main importance for their absorption, distribution, metabolism, and excretion (ADME). This physicochemical parameter 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. Hence, it is important to calculate properly the drug dissociation constant value of a pharmaceutical compound.[2]

Various experimental modalities have been used for the determination of the dissociation constants, comprising potentiometric titration,[3] spectrophotometry,[4] high performance liquid chromatography (HPLC)[5-7] and capillary electrophoresis (CE) methodologies.[8] Among these techniques, most drugs can be analyzed by reversed phase liquid chromatography (RPLC) technique because of 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.[9]

Analysis of the change in retention time of the analyte *vs.* the change in pH of the mobile phase gives an indirect measure of the dissociation constant. The theory for studying the pH dependence of chromatographic retention for ionizable compounds in liquid chromatography was proposed by Horváth *et al.*[10] with the equation

$$k = \frac{k_{BH^+} + k_B \dfrac{K_a \gamma_{BH_m^+}}{a_{H_m^+}}}{1 + \dfrac{K_a \gamma_{BH_m^+}}{a_{H_m^+}}} \quad (1)$$

where K_a is defined as the dissociation constant of the protonated base (BH^+) in the hydroorganic mixture

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used as mobile phase, and k_{BH^+} and k_B are the limiting retention factors of the protonated and undissociated base, respectively. $a_{H_m^+}$ is the hydrogen ion activity in the mobile phase. $\gamma_{BH_m^+}$ is the activity coefficient of the dissociated base in the mobile phase that can be calculated by the classical Debye-Huckel equation.[11]

For any chemical process occurring in solution, the polarity of the solvent plays a crucial role in determining the outcome.[12] A widely employed measure of solvent polarity is Dimroth and Reichardt's $E_T(30)$.[13] $E_T(30)$ polarity of organic solvent-water binary mixtures mobile phases used in RPLC were 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 *vs.* φ are also satisfactory for most solutes for narrow ranges of organic solvent. However, Johnson *et al.*[12] demonstrated linear relationships in a wide range of organic solvents between the solute log k and E_T^N.[14] E_T^N polarity parameters have been used to predict the chromatographic behavior of ionizable compounds.

Variation of the retention factor of neutral form of a base (k_B) and the retention factor of protonated form of a base (k_{BH^+}) with percentage of organic modifier in the mobile phase is represented by the normalized Dimroth and Reichardt polarity parameter (equations 2 and 3).

$$\log k_B = C_B + e_B E_T^N \tag{2}$$

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

Substituting equations 2 and 3 into equation 1 the theoretical expression describing the dependence of the retention factor for basic solutes as a combined function of pH and E_T^N may be expressed as follows:

$$k = \frac{10^{(C_{BH^+} + e_{BH^+} E_T^N)} + 10^{(C_B + e_B E_T^N)} (K_a \gamma_{BH_m^+} / a_{H_m^+})}{1 + (K_a \gamma_{BH_m^+} / a_{H_m^+})} \tag{4}$$

where C_{BH^+} and C_B are intercept values of the protonated and neutral species, respectively. Similarly, e_{BH^+} and e_B are slope values of these species.[15]

Aqueous dissociation constants are a relevant technological property to know stability and solubility of drug. A critical evaluation of different extrapolation approaches for compounds poorly soluble in water should be very useful in drug development.[16] Many drugs are poorly soluble in water and therefore literature proposes several different approaches for their aqueous pK_a estimation. In this work, the chromatographic dissociation constant values of donepezil, rivastigmine and galantamine were determined in various MeCN-water and MeOH-water binary mixtures. pK_a values of each compounds were measured in five different organic solvent-water mixtures. To obtain the best aqueous pK_a value from pK_a data, two different extrapolation methods have been tried. In the first approach, pK_a values were plotted against MeCN and MeOH mole fraction (X of organic solvents).[17] This method was applied using the following equation:

$$ {}_s^s pK_a = aX + b \tag{5}$$

where the intercepts of these linear equations obtained from this approach were the aqueous pK_a values of studied compounds

In second approach, Yasuda-Shedlovsky equation[18,19] was used in order to predict aqueous pK_a values from the pK_a values. This extrapolation method establishes a correlation with the dielectric constant. The following equation has been adopted:

$$pK_a + \log[H_2O] = a_\varepsilon \varepsilon^{-1} + b_\varepsilon \tag{6}$$

where $\log[H_2O]$ is the molar water concentration of the given solvent mixture, ε is the dielectric constant of the mixture and a and b are the slope and intercept, respectively. This method is the most widely used procedure in organic solvent-water binary mixtures.

When the pK_a value is not directly available, it can often be estimated from the chemical structure of the compound. Marvin Sketch program[9] was used for this aim. For a given structure, it estimates not only pK_a values but also other physicochemical parameters.

Experimental

Chemicals and reagents

All chemicals were used directly without any further purification. Donepezil hydrochloride monohydrate and galantamine hydrobromide were purchased from Sigma-Aldrich (St. Louis, USA). Rivastigmine tartrate was kindly provided by Novartis Pharm. Inc. (Istanbul, Turkey). Orthophosphoric acid (min. 85%) was obtained from Riedel-de-Haen (Darmstadt, Germany); other chemicals (acetonitrile, methanol, sodium hydroxide, ammonia, ammonium bicarbonate, potassium hydrogen phthalate) employed were of analytical grade (Merck, Darmstadt, Germany). Double distilled water was used for the preparation of the mobile phase.

Stock solution of the investigated compounds (100 μg mL^{-1}) were prepared in mobile phase, stored at 4 °C, and then further diluted with mobile phase to the desired concentration. All solutions were protected from light and were used within 24 h to avoid decomposition.

Apparatus

The chromatographic hardware used comprised of a Shimadzu HPLC system (Shimadzu Technologies, Kyoto, Japan) equipped with a pump (LC-20AD), a UV-Vis detector (SPD-20A), a column oven (CTO-20A) and a degasser system (DGU-$20A_3$). pH measurements of the mobile phase were performed with a Mettler Toledo MA 235 pH/ion analyzer (Schwerzenbach, Switzerland) using M-T assembly pH electrode. For the standardization of potentiometric system according to the IUPAC rules,[20] potassium hydrogen phthalate solution (0.05 mol kg^{-1}) was used.

Chromatographic procedure

In the present work, the effect of solvent composition in mobile phases was analyzed at five solvent levels. At each composition, different pH values were studied, spread over the pH range from 5.0 to 10.5. In this study, mobile phases used were different proportions of MeCN (25 to 55 vol%) and MeOH (35 to 65 vol%), respectively. *o*-Phosphoric acid and ammonium bicarbonate were used as buffer components because of their appropriate pK_a values and the symmetrical peak shape of studied compounds in these buffer solutions. The concentration of the buffer systems was in all cases 30 mmol L^{-1} (concentration after mixing the organic solvent-water binary mixtures). Chromatographic pK_a determination was carried out using a X Terra C18 column (250 mm × 4.6 mm i.d., 5 μm, Waters, Milford, Massachusetts, USA). Chromatographic measurements were done at 25 °C with an eluent flow rate of 1 mL min^{-1}. The volume of solution injected into the column was 20 μL for each run. The compounds studied had different optimal wavelengths (for donepezil and galantamine: 230 nm; for rivastigmine: 225 nm; for uracil: 254 nm).

Results and Discussion

In the present study, the retention factors for the investigated compounds were obtained at different percentages of acetonitrile/methanol in the mobile phase and at different mobile phase pH values. Selected organic modifier concentration in MeCN-water binary mixtures is much smaller than that in MeOH-water binary mixtures, because MeCN is a stronger solvent than MeOH. Therefore, concentrations of these two solvents are not the same.

The pK_a values, as well as the retention factors of the studied compounds, were obtained by fitting the retention factors-pH data to equation 1, usually by non-linear regression. Nowadays, there are many commercial computer programs to perform these fittings, including ORIGIN Pro 8 software. The pK_a values and the retention factors of investigated compounds in different MeCN and MeOH ratios calculated by NLREG[21] and ORIGIN[22] programs are given in Tables 1 and 2, together with respective standard deviations. As expected, dissociation constant values decrease with the increase of MeCN and MeOH contents, according to the general behavior of weak basic compounds. The reason is that MeCN is less basic than methanol. Hence, bases are stronger in these solvents.

From the data given in Tables 1 and 2, it is immediately obvious that the nature of solvent plays a fundamental role in the base equilibria. As shown in Tables 1 and 2, all drugs studied have one pK_a value corresponding to base functional group. pK_a values obtained for donepezil are consistent with the dissociation equilibrium of *N*-benzylpiperidine ring (Figure 1). The pK_a of rivastigmine is similar to the value of donepezil and can be assigned to the dissociation of the secondary amine group. Galantamine has an azepine moiety and pK_a value of this compound can be associated with nitrogen atom in azepine (Figure 1).

The retention factors were obtained over a pH range of 5.0-10.5, in order to determine pK_a of these acetylcholinesterase inhibitors using RPLC method. In Figures 2 and 3, data pairs of *k* and pH for investigated compounds in different percentages of MeCN-water and MeOH-water binary mixtures are shown, together with the corresponding experimental and calculated retention factors. It can be concluded that plots of sigmoidal curves of retention factor (*k*) *vs.* pH of the mobile phase are related to the influence of an organic modifier on the dissociation of basic solute.

Variation of the mobile phase pH is a key parameter to enhance the chromatographic selectivity and retention time for ionization compounds. Figure 4 shows the typical chromatograms of drugs eluted by different percentages of MeCN/MeOH in the mobile phase at different pH environment. It provided that the retention times (t_R) of donepezil, rivastigmine and galantamine increased as the pH value increased.

In this work, equation 4 was evaluated statistically for these drugs at different conditions. In this way, a complete description of the retention behavior of each solute in the space defined by the pH of the mobile phase and E_T^N variables was obtained. The coefficients describing a retention behavior of these solutes using equation 4 are

Table 1. Retention factors of undissociated (k_B) and protonated (k_{BH^+}) bases and the dissociation constant values of investigated compounds in various acetonitrile/water ratios calculated by NLREG and ORIGIN programs

MeCN / vol%	Rivastigmine				Donepezil				MeCN / vol%	Galantamine			
	NLREG		ORIGIN		NLREG		ORIGIN			NLREG		ORIGIN	
35	k_{BH^+}	0.355 (0.052)	k_{BH^+}	0.206 (0.069)	k_{BH^+}	1.699 (0.158)	k_{BH^+}	1.231 (0.378)	25	k_{BH^+}	0.187 (0.017)	k_{BH^+}	0.157 (0.055)
	k_B	6.494 (0.078)	k_B	6.651 (0.069)	k_B	27.882 (0.226)	k_B	28.267 (0.341)		k_B	2.150 (0.019)	k_B	2.173 (0.030)
	pK_a	8.500 (0.030)	pK_a	8.505 (0.022)	pK_a	8.420 (0.0219	pK_a	8.418 (0.027)		pK_a	8.010 (0.026)	pK_a	8.007 (0.043)
40	k_{BH^+}	0.307 (0.008)	k_{BH^+}	0.294 (0.019)	k_{BH^+}	1.216 (0.063)	k_{BH^+}	1.049 (0.156)	30	k_{BH^+}	0.131 (0.011)	k_{BH^+}	0.123 (0.012)
	k_B	3.156 (0.011)	k_B	3.206 (0.017)	k_B	14.170 (0.089)	k_B	14.693 (0.139)		k_B	1.242 (0.124)	k_B	1.221 (0.006)
	pK_a	8.388 (0.009)	pK_a	8.390 (0.012)	pK_a	8.399 (0.016)	pK_a	8.400 (0.022)		pK_a	7.875 (0.012)	pK_a	7.870 (0.016)
45	k_{BH^+}	0.257 (0.013)	k_{BH^+}	0.204 (0.028)	k_{BH^+}	0.839 (0.039)	k_{BH^+}	0.706 (0.050)	35	k_{BH^+}	0.090 (0.057)	k_{BH^+}	0.044 (0.041)
	k_B	1.867 (0.017)	k_B	1.898 (0.021)	k_B	8.408 (0.055)	k_B	8.541 (0.043)		k_B	0.831 (0.016)	k_B	0.900 (0.010)
	pK_a	8.273 (0.026)	pK_a	8.256 (0.029)	pK_a	8.378 (0.018)	pK_a	8.379 (0.012)		pK_a	7.738 (0.057)	pK_a	7.526 (0.069)
50	k_{BH^+}	0.225 (0.005)	k_{BH^+}	0.243 (0.014)	k_{BH^+}	0.615 (0.015)	k_{BH^+}	0.614 (0.042)	40	k_{BH^+}	0.065 (0.008)	k_{BH^+}	0.063 (0.037)
	k_B	1.008 (0.007)	k_B	0.948 (0.010)	k_B	4.898 (0.021)	k_B	4.873 (0.036)		k_B	0.539 (0.007)	k_B	0.582 (0.007)
	pK_a	8.156 (0.024)	pK_a	8.175 (0.034)	pK_a	8.346 (0.012)	pK_a	8.347 (0.018)		pK_a	7.580 (0.041)	pK_a	7.405 (0.081)
55	k_{BH^+}	0.197 (0.005)	k_{BH^+}	0.208 (0.005)	k_{BH^+}	0.453 (0.020)	k_{BH^+}	0.538 (0.050)	45	k_{BH^+}	0.048 (0.009)	k_{BH^+}	0.013 (0.005)
	k_B	0.591 (0.006)	k_B	0.585 (0.006)	k_B	2.999 (0.027)	k_B	3.089 (0.041)		k_B	0.365 (0.007)	k_B	0.406 (0.003)
	pK_a	8.024 (0.044)	pK_a	7.998 (0.035)	pK_a	8.314 (0.026)	pK_a	8.317 (0.036)		pK_a	7.411 (0.059)	pK_a	7.368 (0.027)

Standard deviation values in parentheses.

Table 2. Retention factors of undissociated (k_B) and protonated (k_{BH^+}) bases and the dissociation constant values of investigated compounds in various methanol/water ratios calculated by NLREG and ORIGIN programs

MeOH / vol%	Rivastigmine				Donepezil				MeOH / vol%	Galantamine			
	NLREG		ORIGIN		NLREG		ORIGIN			NLREG		ORIGIN	
45	k_{BH^+}	0.493 (0.051)	k_{BH^+}	0.512 (0.064)	k_{BH^+}	1.690 (0.544)	k_{BH^+}	1.856 (0.570)	35	k_{BH^+}	0.166 (0.033)	k_{BH^+}	0.167 (0.046)
	k_B	7.754 (0.094)	k_B	7.500 (0.151)	k_B	65.931 (0.926)	k_B	63.749(1.126)		k_B	3.287 (0.045)	k_B	3.360 (0.071)
	pK_a	8.214 (0.026)	pK_a	8.209 (0.030)	pK_a	8.120 (0.029)	pK_a	8.106 (0.026)		pK_a	7.861 (0.032)	pK_a	7.881 (0.037)
50	k_{BH^+}	0.343 (0.038)	k_{BH^+}	0.390 (0.043)	k_{BH^+}	1.207 (0.255)	k_{BH^+}	1.531 (0.291)	40	k_{BH^+}	0.092 (0.026)	k_{BH^+}	0.122 (0.035)
	k_B	4.157 (0.062)	k_B	4.094 (0.083)	k_B	31.783 (0.409)	k_B	28.127(0.536)		k_B	2.029 (0.031)	k_B	1.993 (0.046)
	pK_a	8.085 (0.034)	pK_a	8.074 (0.033)	pK_a	8.057 (0.031)	pK_a	8.046 (0.030)		pK_a	7.731 (0.040)	pK_a	7.730 (0.043)
55	k_{BH^+}	0.243 (0.019)	k_{BH^+}	0.253 (0.024)	k_{BH^+}	0.857 (0.066)	k_{BH^+}	1.025 (0.078)	45	k_{BH^+}	0.064 (0.023)	k_{BH^+}	0.022 (0.026)
	k_B	2.569 (0.028)	k_B	2.520 (0.041)	k_B	14.160 (0.100)	k_B	14.894(0.137)		k_B	1.345 (0.024)	k_B	1.389 (0.029)
	pK_a	7.969 (0.026)	pK_a	7.963 (0.028)	pK_a	8.002 (0.015)	pK_a	7.997 (0.015)		pK_a	7.577 (0.048)	pK_a	7.576 (0.041)
60	k_{BH^+}	0.202 (0.002)	k_{BH^+}	0.204 (0.002)	k_{BH^+}	0.723 (0.004)	k_{BH^+}	0.724 (0.005)	50	k_{BH^+}	0.046 (0.009)	k_{BH^+}	0.029 (0.012)
	k_B	1.898 (0.003)	k_B	1.902 (0.004)	k_B	9.483 (0.006)	k_B	9.470 (0.008)		k_B	1.029 (0.008)	k_B	0.979 (0.011)
	pK_a	7.831 (0.004)	pK_a	7.832 (0.004)	pK_a	7.935 (0.001)	pK_a	7.934 (0.002)		pK_a	7.399 (0.022)	pK_a	7.399 (0.024)
65	k_{BH^+}	0.176 (0.015)	k_{BH^+}	0.172 (0.008)	k_{BH^+}	0.632 (0.070)	k_{BH^+}	0.553 (0.090)	55	k_{BH^+}	0.037 (0.009)	k_{BH^+}	0.020 (0.010)
	k_B	1.513 (0.017)	k_B	1.573 (0.010)	k_B	6.968 (0.094)	k_B	6.828 (0.134)		k_B	0.839 (0.007)	k_B	0.721 (0.008)
	pK_a	7.686 (0.030)	pK_a	7.706 (0.013)	pK_a	7.865 (0.033)	pK_a	7.861 (0.035)		pK_a	7.232 (0.028)	pK_a	7.238 (0.026)

Standard deviation values in parentheses.

listed in Table 3. The retention behavior of these compounds was accurately modeled as a function of pH and polarity of the mobile phase with the equations proposed. The results shown in Table 3 demonstrate a good performance of the liquid chromatographic method for determination of dissociation constants.

In the first described approach, equation 5, was used to estimate the aqueous pK_a values of studied reversible AChE

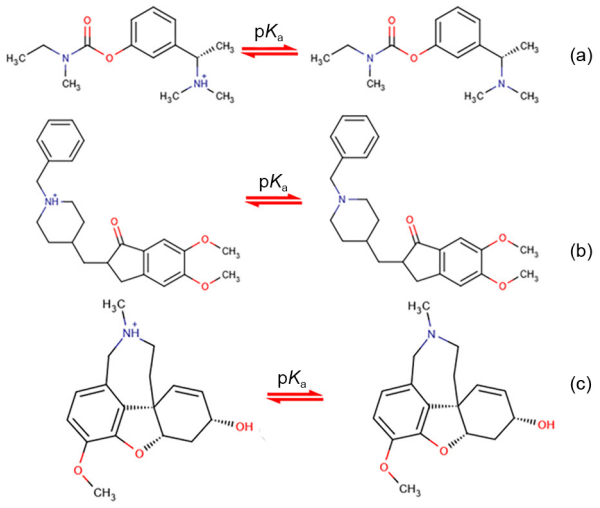

Figure 1. Dissociation behavior of (a) rivastigmine; (b) donepezil and (c) galantamine.

inhibitors. There is actually a linear relationship between pK_a of the rivastigmine, donepezil, galantamine used and mole fraction of methanol and acetonitrile in the binary mixtures. The results of linear equations for compounds used are provided in Table 4. All these straight lines show reasonably satisfactory correlation coefficients and standard deviations.

Then, the following expression, equation 6, was used to estimate the aqueous pK_a of investigated substances. The parameters of the Yasuda-Shedlovsky equations are also summarized in Table 5. It can be seen that basic functional groups have negative slopes. Different functional groups show remarkably different slopes. It is interesting to compare the slopes of a given molecule in MeCN-water and in MeOH-water binary mixtures. The linearity of the plots is characterized by the regression coefficients (r) values which indicate significant linear correlation for the molecules examined. The average of the r values is 0.999.

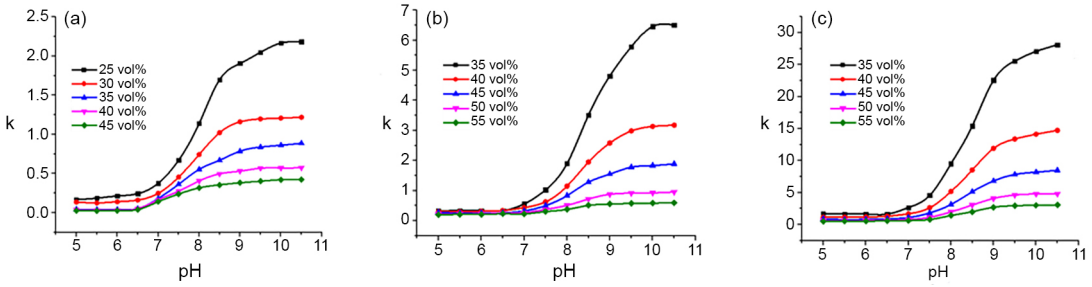

Figure 2. Plots of the calculated retention factors *vs.* the pH of the mobile phase for (a) galantamine; (b) rivastigmine and (c) donepezil in several MeCN-water mobile phases.

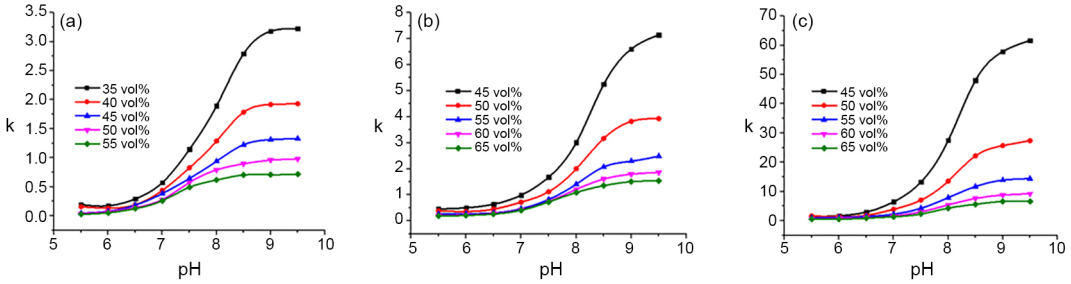

Figure 3. Plots of the calculated retention factors *vs.* the pH of the mobile phase for (a) galantamine; (b) rivastigmine and (c) donepezil in several MeOH-water mobile phases.

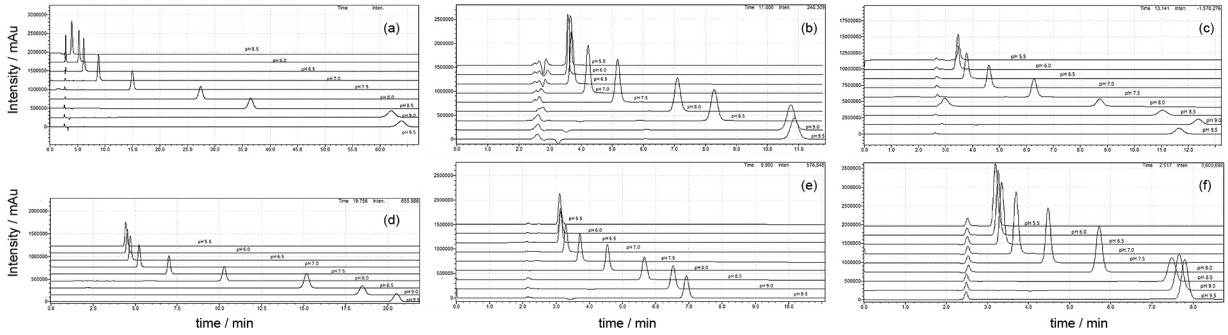

Figure 4. Typical HPLC chromatograms of studied compounds eluted by the different percentages of mobile phase at different pH environment. (a) Donepezil (55 vol% MeOH); (b) rivastigmine (55 vol% MeOH); (c) galantamine (35 vol% MeOH); (d) donepezil (45 vol% MeCN); (e) rivastigmine (45 vol% MeCN); and (f) galantamine (25 vol% MeCN).

Table 3. Results of the application of the retention model proposed for all the experimental retention data available for donepezil, rivastigmine and galantamine (coefficients describe chromatographic retention in terms of equation 4 for basic compounds)

Medium	Compound	C_{BH^+}	e_{BH^+}	C_B	e_B	*SSQ*	*RRMSD*	N
MeCN	donepezil	–8.945	10.780	–13.820	17.920	2.428	3.328	60
	rivastigmine	–4.557	4.830	–15.530	19.190	0.116	3.264	60
	galantamine	–9.228	9.618	–10.570	12.330	0.060	5.301	60
MeOH	donepezil	10.010	–14.420	24.200	–32.990	51.005	6.177	45
	rivastigmine	9.833	–14.950	16.530	–23.080	0.182	2.870	45
	galantamine	6.454	–11.290	7.192	–10.410	0.089	3.892	45

C_{BH^+} and C_B: intercept values of the protonated and neutral species, respectively; e_{BH^+} and e_B: slope values of the protonated and neutral species, respectively; SSQ: sums of squares of the residuals = $\sum(k_{obs.} - k_{pred.})^2$; RRMSD: relative root mean squared differences = $100\sqrt{\sum(k_{obs.} - k_{pred.})^2 / \sum(k_{obs.})^2}$; N: experimental retention data.

Table 4. Linear equations between experimental dissociation constant values and the mole fraction of acetonitrile/methanol in the binary mixtures

Medium	Compound	Equation	R
MeCN	donepezil	pK_a = –3.945 (0.033)x + 9.085 (0.007)	0.999
	rivastigmine	pK_a = –0.886 (0.037)x + 8.555 (0.008)	0.997
	galantamine	pK_a = –5.637 (0.063)x + 8.571 (0.010)	0.999
MeOH	donepezil	pK_a = –2.792 (0.034)x + 8.993 (0.012)	0.999
	rivastigmine	pK_a = –1.347 (0.019)x + 8.481 (0.007)	0.999
	galantamine	pK_a = –3.977 (0.056)x + 8.646 (0.030)	0.999

Standard deviation values in parentheses.

Table 5. Aqueous dissociation constant values by Yasuda-Shedlovsky extrapolation in different co-solvents

Co-solvents	Compound	Vol%	ε	$pK_a + \log[H_2O] = a_\varepsilon\varepsilon^{-1} + b_\varepsilon$		r	n	pK_a
				a	b			
MeCN	rivastigmine	35-55	64.563-55.760	–197.366	13.284	1.000	5	9.019
	donepezil	35-55	64.563-55.760	–47.17	10.88	1.000	5	8.534
	galantamine	25-45	68.941-60.109	–284.421	13.869	1.000	5	8.493
MeOH	rivastigmine	45-55	63.019-55.875	–262.930	14.107	1.000	5	9.005
	donepezil	45-55	63.019-55.875	–129.192	11.893	0.999	5	8.499
	galantamine	35-45	66.531-59.195	–343.996	14.763	0.999	5	8.626

ε: Dielectric constant of the mixture; log[H_2O]: molar water concentration of the given solvent mixture; a: slope; b: intercept; r: regression coefficients; n: number of studied organic modifier concentration; pK_a: dissociation constant.

On the other hand, the Marvin Sketch program[9] was used to compute the drugs' aqueous pK_a. The pK_a values reported in the literature and predicted by this program[9] are presented in Table 6. In literature reports, different pK_a values for the same compound are calculated. In the present study, calculated pK_a values for galantamine are not in full consistency with that reported by Meloun *et al.*[23] In that study,[23] galantamine was determined in different ionic strengths and temperatures. For rivastigmine, pK_a values were predicted by Hsieh *et al.*,[24] and Luan *et al.*[25] In these studies, there are no experimental values. There is only one study in the literature for donepezil.[26] This study was carried out by capillary electrophoresis method. Therefore, calculated pK_a values for donepezil are not in full consistency with that reported by Ishihama *et al.*[26] Table 6 shows that despite the use of different equipment and determination by different persons on different days using different starting drug concentrations, close values were obtained for dissociation constants of the determinations. A comparison with representative dissociation constants available in the literature shows that either method gives satisfactory values.

Conclusions

This work represents the first study dealing with the chromatographic determination of pK_a values of some

Table 6. Aqueous pK_a values of some acetylcholinesterase inhibitors obtained from different methodologies

Compound	MeOH		MeCN		Literature values	Marvin Sketch program
	pK_a-X	Yasuda-Shedlovsky	pK_a-X	Yasuda-Shedlovsky		
Rivastigmine	8.993	9.005	9.035	9.019	8.90;[24] 8.99[25]	8.89
Donepezil	8.481	8.499	8.555	8.534	9.1[26]	8.62
Galantamine	8.646	8.626	8.571	8.493	8.21[23]	8.91

pK_a: Dissociation constant.

reversible acetylcholinesterase inhibitors at different proportions of MeCN-water and MeOH-water binary mixtures. The combined effect of the two factors (solvent percentage and pH of the mobile phase) on the retention behavior of donepezil, rivastigmine, and galantamine was investigated. Dissociation constant values and limiting retention factors of these compounds were calculated chromatographically using derived equations. Mole fraction and Yasuda-Shedlovsky extrapolation methods were proposed to obtain the aqueous pK_a values. The results obtained agree very well with those obtained by calculation by means of the Marvin Sketch program, which is based on the analysis of the chemical structure relative to the dissociation of the selected basic group. The important data extracted from this work can be used for pharmacokinetic and pharmacological studies of these drugs. Moreover, the knowledge of the chromatographic behavior of the drug compounds in different water-organic solvent media is a useful parameter to optimize analytical procedures for the separation of ionizable compounds by liquid chromatography.

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