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Physico-chemical characterization of some beta blockers and anti-diabetic drugs - potentiometric and spectrophotometric p*K*a determination in different co-solvents

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ABSTRACT

In the present study we have determined the fundamental physico-chemical properties such as, ionization constant (p*K*a) and lipophilicity (log *P*) of some β-blockers and anti-diabetic drugs. The apparent ionization constant (ps*K*a) of selected drugs were determined using potentiometric titration in various co solvent-water mixtures (methanol, ethanol, acetonitrile and dioxane) at different temperatures (25 to 45 °C) and ionic strengths (0.15 to 0.5 M). Effect of temperature, ionic strength and dielectric constant on dissociation constant has been compared. The aqueous p*K*a values were then obtained by Yasuda-Shedlovsky extrapolation. In the case of water-soluble drugs (Amiloride hydrochloride, metoprolol tartrate and propranolol hydrochloride), the extrapolated results were in good agreement with that of p*K*a values measured in aqueous solutions under the same experimental conditions, while for the water insoluble drugs (atenolol, amlodipine besylate, gliclazide, glipizide, glibenclamide and pioglitazone), the extrapolated results were in good agreement with the literature values. For few of the selected drugs, the ps*K*a was determined spectrophotometrically and the results were compared with that of potentiometry in various co solvent mixtures. The measured log P values of selected drugs showed acceptable range to that of literature values.

1. Introduction

There are different methods by which dissociation constant of weak acids and bases can be determined. The potentiometric methods are widely used, since they are fast and easy to study ionic equilibria in aqueous and non-aqueous solvents, while other methods such as spectrophotometric and conductometric are laborious, time consuming but they are very accurate [1-3].

The p*K*a value is a key parameter to predict the ionization state of a molecule with respect to pH. The p*K*a of a molecule is the pH at which the molecule is 50 % protonated. The p*K*a of a molecule predicts the degree of ionisation the molecule will have at a particular pH. Since most of the drug compounds have acidic and/or basic functionalities, their ionization state is controlled by both solution pH and acidic dissociation constants (i.e. p*K*a values). These different chemical species (cationic, neutral, or anionic) often have vastly different properties with respect to water solubility, volatility, UV absorption, and reactivity with chemical oxidants. The ionized form is usually more water soluble, while the neutral form is more lipophilic and has higher membrane permeability. The extent of ionization is one of several cardinal properties used to estimate the absorption, distribution, metabolism and excretion of compounds in biological systems and the environment. From dissociation constants, the major species of pharmaceuticals present in the environment (usually in neutral pH range) can be estimated [4,5].

Knowledge of p*K*a values as a function of solvent composition is useful in liquid chromatography (LC) or capillary electrophoresis (CE) for the separation of ionizable compounds. The chromatographic retention and electrophoretic behavior of ionizable compounds strongly depend on the p*K*a of the compound and the mobile-phase pH [6,7]. Satisfactory knowledge of the acid–base behavior of substances in hydro-organic media is therefore essential to optimize analytical procedures for the separation of ionizable compounds by LC [8,9] and CE [10]. Moreover, the acid–base property of a drug molecule is the key parameter for drug development because it governs the solubility, absorption, distribution, metabolism and elimination, particularly for developing new active pharmaceutical ingredients. The transport of drugs into cells and across other membranes is a function of physicochemical properties, p*K*a of the drugs [11].

The ionization constant describes the proportion of different ionic species in which the substance is divided at different pH. The ionic species differ in physical and biological properties [12]. The survey of literature shows that the ionization constant data in aqueous and non-aqueous solvents at different temperatures are not frequently available [13,14]. The p*K*a values are dependent on temperature and solvent concentration and hence these results are very important in pharmaceutical industries, in spectroscopy and in biology [15].

Remarkable developments have been observed in the automation of standard p*K*a determination methods using both potentiometry (GLp*K*a) and spectroscopy (GLp*K*a + D-PAS) [16,17]. Karl *et al.*, [18] had developed a new method and instrument (ProfilerSGA) to address the need for high throughput measurements of p*K*a. Spectral gradient analysis

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(SGA) is suitable for measurements of large number of compounds using small amounts of samples, a typical requirement of early phase drug discovery.

Evaluation of aqueous dissociation constants is an unavoidable requirement in routine drug development. However, many drugs are sparingly soluble in water and any experimental p*K*a determination requires the use of an organic or hydro organic solvent. The mixed-solvent procedure mainly using methanol–water mixtures provides a good alternative for sparingly soluble compounds [19-21]. Here, the cosolvent ionization constants (ps*K*a) in different ratios of methanol–water mixtures are measured and the aqueous p*K*a is obtained by extrapolation. A critical evaluation of different extrapolation approaches for compounds poorly soluble in water should be very useful in drug screening technology.

To overcome the difficulty and improve the solubility of drug compounds, G. Volgyi *et al*. [22] have developed and validated a new single, multicomponent cosolvent mixture consisting of equal volumes of methanol, acetonitrile and dioxane for an efficient p*K*a measurement. However, none of these literature surveys have shown any systematic study of temperature, ionic strength and dielectric constant effect on the dissociation constant of drug compounds.

In this work, we performed a systematic validation to evaluate the p*K*a values to understand the effect of dielectric constant in different co-solvents (methanol, ethanol, 1,4-dioxane and acetonitrile), temperature and ionic strength using some of beta-blockers and antidiabetic drug compounds. The p*K*a of selected drugs were determined by potentiometric titration between 6 and 60% of selected solvents at 25 °C and in 0.15 M ionic strength using KCl. Temperature effect on selected drugs is studied between 25 °C and 45 °C while ionic strength effect is studied between 0.15 M and 0.50 M. Spectrophotometric technique was also applied under similar conditions on those compounds which has a pH-dependent UV spectrum (amiloride hydrochloride, atenolol, propranolol hydrochloride and gliclazide).

The main objective of our present work was to study the effect of co-solvents at various temperatures and ionic strength on some of beta blockers (amiloride, propranolol, atenolol, metoprolol and amlodipine) and anti-diabetic drugs (gliclazide, glipizide and pioglitazone) and compare the extrapolated dissociation constants (p*K*a) results with those determined in aqueous medium or with literature values. The present study also includes the determination of partition co-efficient (log *P*) of all the selected drugs (except that pioglitazone was replaced with glibenclamide) in octanol/water system of all the selected drugs using automated potentiometric titrator (GLp*K*a) and compares the available percentage species at physiological pH. This paper deals with the comparison of psKa of drug compounds determined by pH metry and spectrophotometry methods.

2. Experimental

2.1. Instrumentation

2.1.1. Potentiometric pKa determination

GLp*K*a automated p*K*a analyser (Sirius Analytical Instruments Ltd., Forest Row, UK) fitted with combination Ag/AgCl pH electrode was used for determination of dissociation constants. The p*K*a and ps*K*a values were calculated by RefinementPro™ software (Sirius Analytical Instruments Ltd., Forest Row, UK).

2.1.2. Electrode calibration

The four-plus parameter technique [19,23,25] was used for electrode calibration in both aqueous medium and co solvent mixtures. HCl solutions of known concentration, containing 6–60 wt% selected co solvents-water mixtures were titrated with standardized KOH at constant ionic strength (I = 0.15 M using KCl) and temperature (25.0±0.5 °C), under argon atmosphere, from pH 1.8 to 12.0 without any sample present ("blank" titration). The operational pH reading was related to the concentration pcH values by a multi parametric equation.

$$\mathrm{pH} = \alpha + S_{\mathrm{P}c}\mathrm{H} + J_{\mathrm{H}}[H^+] + \frac{j_{\mathrm{OH}}K_w}{[H^+]} \quad (1)$$

The parameters are determined by a weighted nonlinear least squares procedure. The intercept parameter 'α' corresponds to '–LogaH^+' at the working temperature and ionic strength. The *j*H term corrects pH readings for the nonlinear pH response due to liquid junction and asymmetry potentials in moderately acidic solutions, while the *j*OH term corrects for high-pH nonlinear effect. Factor 'S' accounts that a particular electrode may not have 100% Nernstian slope, 'Kw' is the ionisation constant of water. These values were obtained from three parallel "blank" titrations. The "goodness-of-fit" (GOF) value of less than 1.5 was observed for all the blank titrations, indicating the statistical significance of the data. These parameters were used to calculate the p*K*a and ps*K*a values.

2.2. Methods

2.2.1. Reagents

The following drugs viz; amiloride hydrochloride (AML), propranolol hydrochloride (PRO), atenolol (ATN), metoprolol tartrate (MT), amlodipine besylate (AB) belonging to betablockers and gliclazide (GLC), pioglitazone (PGL), glipizide (GLZ), glibenclamide (GLB), belonging to anti-diabetic drugs were selected for the present investigation. PRO, ATN, MT, AB, and PGL were obtained from IPCA Laboratories Limited, Mumbai; AML, GLC and GLZ were obtained as gift samples from Bal Pharma Limited, Bangalore. GLB was obtained from Sigma. All the drug compounds used were of pharmaceutical grade.

Of the chemicals used for p*K*a determination, methanol, ethanol, 1,4-dioxane and acetonitrile were of HPLC grade from Merck. Solutions and solvent mixtures were made up of distilled water obtained from Millipore, Milli-Q (Bedford, MA, USA) purification system. Readymade 0.5 M solutions of potassium hydroxide and hydrochloric acid were obtained from Merck. Potassium hydroxide is standardized against primary standard using potassium hydrogen phthalate. Potassium hydrogen phthalate is purchased from Sigma. Di-potassium hydrogen phosphate and potassium chloride were of analytical grade from Sigma. Standards for HPLC log P measurements were procured from Sigma.

2.2.2. Preparation of co-solvent mixtures

For ps*K*a determination of selected drugs, a 80% (v/v) methanol, 60% (v/v) ethanol, 1,4-dioxane and 50% (v/v) of acetonitrile in 0.15 M KCl adjusted water is prepared and used throughout our investigation.

2.2.3. Titration in aqueous medium

For bases, in each experiment, about 2 mg of sample, containing 10 mL of 0.15 M ionic strength water, was pre acidified to pH 1.8–2.0 with 0.5 M HCl and then titrated with 0.5 M KOH to an appropriately high pH, usually 12. In the case of acids, the titration was performed in the opposite direction.

The titrations were performed at 25.0 ± 0.5 °C, under argon atmosphere, at I = 0.15 M ionic strength using KCl. A minimum of three parallel measurements were carried out and the p*K*a values of samples were calculated using RefinementPro™ software.

2.2.4. Titration in co solvent–water mixtures

About 2 mg each of selected drug compound, containing 6-10 mL of 0.15 M semi-aqueous solutions, was titrated under the same conditions as in aqueous titration (Section 2.2.3) but in the presence of co-solvents containing 6 and 60 wt% of methanol, ethanol, acetonitrile and 1,4-dioxane. Each sample was measured in all the four selected co-solvents independently. At each selected co-solvent mixture, measurements were carried out in at least in three different wt% at constant ionic strength (*I* = 0.15 M using KCl) and temperature (25.0±0.5 °C) under argon atmosphere. The effect of ionic strength (I = 0.15 M, 0.3 M and 0.5 M using KCl) at constant temperature (25.0±0.5 °C) and the effect of temperature (25, 35 and 45 °C) at constant ionic strength (*I* = 0.15 M KCl) using methanol as co solvent were carried out under argon atmosphere. The apparent ionization constants in the mixed solvent (ps*K*a) were calculated from the difference (Bjerrum) plot. The results obtained through this difference plot is often found suitable for detecting small errors in titration such as acidity error, concentration error and corrections to these errors has become necessary to achieve the best fit. Difference plots allow the p*K*a values of monoprotic and multiprotic weak acids to be determined rapidly and with good precision [24-25]. To obtain the best aqueous p*K*a value from ps*K*a data the Yasuda-Shedlovsky (YS) procedure was applied to estimate the aqueous p*K*a value. The following equation has been adopted.

$$p_s K_a + \log[H_2O] = \frac{a}{\varepsilon} + b \qquad (2)$$

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 co-solvent techniques [20,21].

2.2.5. UV-pH titration in aqueous medium and in co-solvent–water mixtures

The UV/pH titrations were performed using D-PAS technique (Sirius Analytical Instruments Ltd., Forest Row, UK) attached to a GLp*K*a [26]. The p*K*a and ps*K*a values were calculated using RefinementPro™ software. Spectrophotometric method can only be applied, for p*K*a/ps*K*a measurement, to those compounds which has a chromophore at close proximity of the ionization centre. This would help to have a sufficient change in absorbance as a function of pH. Of all the selected drugs in the present investigation, only AML, PRO, GLC and ATN is found to have chromophores in which ionisation groups were part of the conjugation and produced enough changes in the absorbance with change in the pH.

In each experiment, a 10.0 mM stock solution of the sample was prepared in DMSO. 50 µL aliquot of the stock solution was then diluted to 10 mL with 0.15 M KCl solution containing 0.25 mL of phosphate buffer (di-potassium hydrogen phosphate) to produce the required sample concentration. Blank spectra are collected from reference vial without sample but with 50 µL of DMSO. For bases, in each experiment, the pH of the sample solution was adjusted to pH=2 using 0.5 M HCl and then titrated with 0.5 M KOH to pH=12. For acids, titration is started from high to low pH. Spectral data were recorded in the region of 200–700 nm after each pH measurement. All measurements were performed in all the four selected co-solvents at least in three different wt%, at constant ionic strength (*I* = 0.15 M KCl) and temperature (25.0±0.5 °C) under argon atmosphere. Three parallel measurements were carried out. Sample concentrations of 5–100 µM were used for UV/pH titration. The p*K*a values of samples were calculated by Target Factor Analysis [27].

2.2.6. Potentiometric log P determination (octanol/water system)

The logarithm of octanol/water partition coefficient (log P_{oct}) was determined by the dual-phase potentiometric titration [28,29]. Log *P* (partition coefficient) is a measure of the lipophilicity of a compound. A blank acid-base titration is compared to a titration in the presence of the compound. A difference curve is produced from the volume of KOH required to reach a given pH with and without the compound. The difference curve is converted to a Bjerrum plot. The axes are reversed and the volume difference is converted to the average number of bound protons per molecule (½Á) (Figure 1). Titrations are then performed in the presence of varying amounts of partition solvent, octanol. From the octanol-containing titrations the apparent ionization constants (po*K*a) and then the log *P* values were estimated and refined by a weighted non-linear least squares procedure where the p*K*a values were used as unrefined contributions.

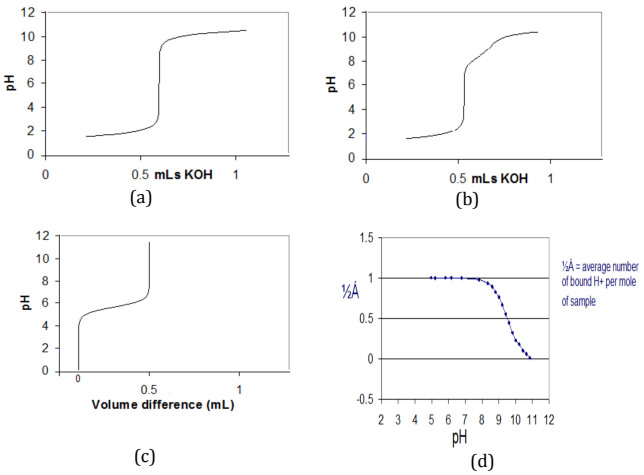

Figure 1. Graphs showing (a) blank titration curve, (b) with sample, (c) volume difference curve and (d) Bjerrum plot.

About 1-2 mg of sample having 10 mL of 0.15 M KCl was titrated under the same conditions as in p*K*a determinations but in the presence of a partitioning solvent, water-saturated octanol. Each sample was analysed in triplicate, with three titrations in each experiment (beaker). Solid sample was weighed into each beaker and specified volume of octanol (Table 1) is added manually at the beginning of first titration and sonicated for 30 to 45 minutes for its dissolution. Titration is then carried out as per Section 2.2.3. In second and third titration, octanol was added automatically by the instrument through the dispenser. The total volume of the beaker is always maintained less than 15 mL (working capacity of each titration vessel).

2.2.7. HPLC log P determination

The distribution coefficient (log D) is determined by correlation of the HPLC retention time of compound under investigation to a calibration curve calculated by linear regression of the retention times of the known log D values at pH=7.4 of selected substances. Calibration of the system is carried out by injecting dilute solutions of a mixture of seven accurately known log *P* "standards" of mixed hydrogen-bond-donor and -acceptor strengths. The compounds used, together

with their corresponding log *P* values in parentheses, are *N*-methyl aniline (1.66), sulphinide (2.07), labetolol (2.65), 4-iodophenol (2.91), diltiazem (3.38), triphenylene (4.37), and chloropromazine (5.35).

Table 1. Different octanol-water phase ratio for log P experimental design.

Beaker	Octanol volume (first addition is always manual in advance and sonicate for 30 to 45 minutes)			Aqueous volume (mL)
1st Beaker	0.5	1.0	3.5	10
2nd beaker	0.25	0.25	0.25	10
3rd Beaker	0.5	0.5	1.0	10

A Serveyor HPLC system (Thermo Fisher, USA) equipped with quaternary gradient pump, auto sampler, column oven and photodiode array detector (PDA) was employed for analysis. Chromatographic data was acquired using ChromQuest 4.2 software.

An X-Terra C18, 100×2.1 mm, 5 µm column was used for separation. The mobile phase consisting of A: buffer (10 mM ammonium acetate pH=7.4 adjusted with ammonium hydroxide) and B: a mixture of 90% acetonitrile and 10% buffer with a timed gradient programme was used. The gradient condition of the mobile phase was: 0 min 4% solvent B, 16.0 min 90% solvent B, 18.2 min 90% solvent B, 18.9 min 4% solvent B and 25 min 4% solvent B. The flow rate of the mobile phase was 0.5 mL/min with detection at 220 nm. The column temperature was kept at 37 °C and the injection volume was 10 µL.

This method is applied for the log P determination of all the selected drugs by HPLC. Six replicate injections were made for standard and samples were injected in duplicate.

3. Results and discussion

3.1. Dissociation constant

3.1.1. Potentiometry

The selected compounds (Figure 2) in the present investigation differ in acid–base properties representing a variety of proton-binding sites in both the type and the strength. There are monovalent bases (all beta-blockers and PGL) with guanidine (AML), isopropyl amine (PRO, ATN and MT), primary amine (AB) and pyridine nitrogen atom(s) (PGL). Compounds GLC and GLZ are acids due to the 'NH group of sulfonyl urea.

Generally, potentiometry in aqueous medium is the method of choice for the p*K*a determination for molecules having solubility higher than 0.8 mM concentration in the whole pH interval of the titration. Out of all selected compounds, only 3 compounds whose p*K*a values could be measured in aqueous medium either by potentiometry (PRO and MT) or spectrophotometric titration (AML). These molecules are considered "water-soluble" and its results are utilised to evaluate the accuracy of the results obtained in various co solvents by extrapolation method.

All other compounds selected in this study did not fulfil the solubility criterion (less than 0.8 µM) and hence, the apparent dissociation constants, ps*K*a values were measured in different methanol–water, ethanol-water, acetonitrile-water and 1,4-dioxane-water mixtures and the corresponding extrapolated p*K*a value were obtained by Yasuda-Shedlovsky (YS) extrapolation to zero co-solvent content. The average ps*K*a values and the standard deviations were calculated from three parallel titrations at each *R*-value.

The ionization ability of the molecules is characterized here by the dissociation constant (p*K*a) measured by potentiometric and spectrophotometric techniques in different co-solvents - water mixtures. The obtained results show the good reproducibility of potentiometric and spectrophotometric p*K*a determination in all the selected co solvent-water mixtures. The standard deviation values varied between 0.01 and 0.15. From the measured ps*K*a values the aqueous p*K*a can be obtained by extrapolation. In order to select the best extrapolation procedure in different co solvents, we compared the Yasuda–Shedlovsky (YS) linear relationships using Eq. 2. Results indicate that this method provide appropriate p*K*a values in the cases of both potentiometric and UV/pH techniques. Table 3 summarizes the different solvent weight percent range used for the titration, dielectric constant (ε), regression coefficient (R^2), the number of titrations (n), extrapolated aqueous p*K*a values and the method of titration.

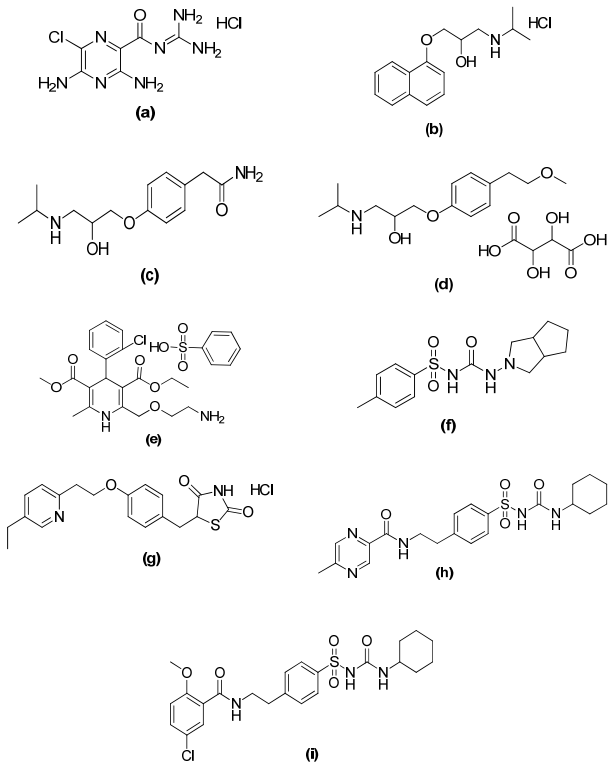

Figure 2. (a) Amiloride hydrochloride (AML); (b) propranolol hydrochloride (PRO); (c) atenolol (ATN); (d) metoprolol tartrate (MT); (e) amlodipine besylate (AB); (f) gliclazide (GLC); (g) pioglitazone (PGL); (h) glipizide (GLZ) and (i) glibenclamide (GLB).

Figure 3 and 4 represents the Yasuda-Shedlovsky trend charts for beta blockers and anti-diabetic compounds selected in this study. It can be seen that basic functional groups have negative slopes and produce straight lines in the total interval. The acids have positive slopes. The linearity of the plots is characterized by the regression coefficients values which indicate significant linear correlation for the molecules examined. The average of the R^2 values is 0.9945.

The p*K*a results in Table 2 allow interpretation of the structure–property relationships (SPR) between the basicity and the chemical structure of the molecules. In compound AML, the central element of the structure is a guanidine, the N atom of which is the proton-binding site which is an integral part of the conjugated double bond, while in compounds PRO, ATN and MT, the N atom of isopropyl amino group is the proton-binding site which is not a part of conjugation. Isopropyl group increases electron density on N atom due to +I (inductive) effect and hence imparts the basicity. In case of compounds 'AB' and 'PIO', primary amine and pyridine group, respectively, makes the molecule comparatively weak base.

Table 2. p*K*a values by Yasuda-Shedlovsky extrapolation in different co-solvents.

Compound	Co-solvent	Wt %	ε	$pK_a + \log [H_2O] = a/\varepsilon + b$		R^2	n	p*K*a ± S.D. (YS)	Literature values[a]	Method
				a	b					
Amiloride hydrochloride	Methanol	20-50	56-70	-20.7	10.7	0.9989	4	8.72 ± 0.05	8.7±0.1	Potentiometric
		20-50	56-70	-61.1	11.4	0.9883	4	8.82 ± 0.02		Spectrophotometry
	Ethanol	30-60	43-60	-25.8	10.5	0.9797	4	8.47 ± 0.05		Potentiometric
		30-60	43-60	-21.1	10.6	0.9836	4	8.55 ± 0.01		Spectrophotometry
	Acetonitrile	20-60	51-70	41.9	9.7	0.9999	3	8.51 ± 0.07		Potentiometric
		10-60	51-74	69.2	9.4	0.9835	4	8.55 ± 0.03		Spectrophotometry
	1,4-Dioxane	30-50	34-52	10.2	10.2	0.9880	3	8.64 ± 0.02		Potentiometric
		10-50	34-70	19.3	10.2	0.9920	4	8.70 ± 0.03		Spectrophotometry
							3	8.71 ± 0.004		UV-pH (aqueous)
Proopranolol hydrochloride	Methanol	10-50	56-74	-146.3	12.9	0.9982	5	9.31 ± 0.05	9.53±0.1	Potentiometric
		10-48	57-74	-188.1	13.6	0.9919	5	9.41 ± 0.03		Spectrophotometry
	Ethanol	10-50	49-73	-179.9	13.5	0.9985	5	9.45 ± 0.08		Potentiometric
		10-60	43-72	-115.4	12.5	0.9999	3	9.28 ± 0.20		Spectrophotometry
	Acetonitrile	10-35	63-75	-223.7	14.0	0.9993	4	9.42 ± 0.03		Potentiometric
		10-49	55-74	-107.1	12.4	0.9960	3	9.26 ± 0.12		Spectrophotometry
	1,4-Dioxane	10-45	38-70	-41.1	11.7	0.9968	4	9.44 ± 0.04		Potentiometric
		10-60	25-69	-12.7	11.2	0.9891	5	9.30 ± 0.03		Spectrophotometry
							3	9.46 ± 0.02		pH metry - aqueous
Atenolol	Methanol	20-60	52-70	-138.6	12.9	0.9990	5	9.37 ± 0.04	9.48±0.1	Potentiometric
		20-60	52-70	-184.8	13.6	0.9970	5	9.51 ± 0.07		Spectrophotometry
	Ethanol	20-43	53-67	-207.1	14.0	0.9996	5	9.63 ± 0.06		Potentiometric
		10-49	50-73	-114.4	12.6	0.9808	4	9.41 ± 0.05		Spectrophotometry
	Acetonitrile	20-35	63-71	-222.5	14.1	0.9948	4	9.50 ± 0.03		Potentiometric
		10-60	51-75	-57.7	12.0	0.9745	4	9.52 ± 0.03		Spectrophotometry
	1,4-Dioxane	20-50	34-61	-30.3	11.6	0.9996	5	9.43 ± 0.04		Potentiometric
		10-60	26-70	-13.3	11.4	0.9815	5	9.53 ± 0.02		Spectrophotometry
Metoprolol tartrate	Methanol	10-40	61-74	-163.2	13.2	0.9986	4	9.35 ± 0.04	9.51±0.1	Potentiometric
	Ethanol	7-50	49-74	-183.7	13.6	0.9994	5	9.53 ± 0.09		Potentiometric
	Acetonitrile	6-35	63-76	-216.6	13.9	0.9989	4	9.43 ± 0.01		Potentiometric
	1,4-Dioxane	10-45	38-70	-7.7	11.2	0.9637	4	9.36 ± 0.01		Potentiometric
							3	9.51 ± 0.02		pH metry - aqueous
Amlodipine besylate	Methanol	21-62	50-70	-108.7	12.3	0.9993	5	9.13 ± 0.02	9.2±0.2	Potentiometric
	Ethanol	21-39	55-66	-199.4	13.6	0.9988	3	9.29 ± 0.03		Potentiometric
	Acetonitrile	21-31	65-70	-387.7	16.2	0.9914	3	9.48 ± 0.01		Potentiometric
	1,4-Dioxane	21-51	32-60	-34.6	11.5	0.9993	4	9.30 ± 0.03		Potentiometric
Pioglitazone	Methanol	37-59	53-63	-174.7	9.3	0.9981	4	5.37 ± 0.04	5.59±0.2	Potentiometric
	Ethanol	34-56	46-59	-192.1	9.7	0.9989	3	5.56 ± 0.05		Potentiometric
	Acetonitrile	31-52	55-66	-131.0	8.8	0.9949	3	5.38 ± 0.04		Potentiometric
	1,4-Dioxane	30-50	34-52	-19.4	6.6	0.9971	3	4.74 ± 0.06		Potentiometric
Gliclazide	Methanol	20-60	52-70	25.8	7.0	0.9996	4	5.58 ± 0.01	5.54±0.2	Potentiometric
		10-53	56-75	32.8	7.0	0.9909	5	5.63 ± 0.01		Spectrophotometry
	Ethanol	20-60	43-67	11.8	7.2	0.9996	4	5.63 ± 0.01		Potentiometric
		10-53	48-73	28.3	7.1	0.9997	3	5.70 ± 0.01		Spectrophotometry
	Acetonitrile	11-60	51-74	177.8	5.0	0.9980	4	5.58 ± 0.06		Potentiometric
		21-53	54-70	111.3	6.0	0.9728	3	5.64 ± 0.08		Spectrophotometry
	1,4-Dioxane	20-60	25-61	50.6	6.6	0.9882	5	5.48 ± 0.05		Potentiometric
		10-52	32-70	69.9	6.4	0.9956	5	5.58 ± 0.07		Spectrophotometry
Glipizide	Methanol	20-60	52-70	110.2	5.5	0.9985	4	5.19 ± 0.01	5.16±0.3	Potentiometric
	Ethanol	30-60	43-60	36.3	6.8	0.9961	3	5.49 ± 0.05		Potentiometric
	Acetonitrile	12-50	55-74	110.0	5.7	0.9853	4	5.42 ± 0.03		Potentiometric
	1,4-Dioxane	27-50	34-55	94.3	5.4	0.9988	4	4.89 ± 0.15		Potentiometric

[a] Ref [30].

In case of GLC and GLZ, the ionisable '-NH' of sulfonyl urea moiety makes the molecule acidic due to – E (electron with drawing) effect of sulfonyl group attached to '-NH' of urea group.

A donor number or DN is a qualitative measure of Lewis basicity. A donor number is defined as the negative enthalpy value for the 1:1 adduct formation between a Lewis base and the standard Lewis acid. The units are kilocalories per mole for historical reasons [31]. The donor number is a measure of the ability of a solvent to solvate cations and Lewis acids. The method was developed by V. Gutmann [32]. Likewise Lewis acids are characterized by acceptor numbers.

The dielectric constants of all the selected co solvents in the present study are lower than that of water, which affects the ionization equilibria. A solvent will be more likely to promote ionization of a dissolved acidic molecule in the following circumstances [33].

- A protic solvent can form hydrogen bonds and will promote ionisation.
- A solvent with a high donor number is a strong Lewis base.
- A solvent with a high dielectric constant will promote ionisation.

For a given acid, p*K*a values will vary depending on the solvent. The degree of dissociation of an acid increases with increase of solvent basicity. On the other hand, dissociation is relatively less for solvents having low dielectric constant. Co-solvent properties used in the present studies are provided in Table 3.

It can be seen from the table that methanol and ethanol is more basic than water, but its dielectric constant is less. Acetonitrile is less basic than water, methanol or ethanol, but its dielectric constant is more than methanol or ethanol. Hence, in general, acids are weaker and bases are stronger in this solvent.

Table 3. Solvent properties at 25 °C.

Solvent	Dielectric constant	Donor number (kcal/mol)
Water	80	18
Acetonitrile	37.5	14.1
Methanol	33	19
Ethanol	24.3	31.5
1,4 - Dioxane	2.3	14.8

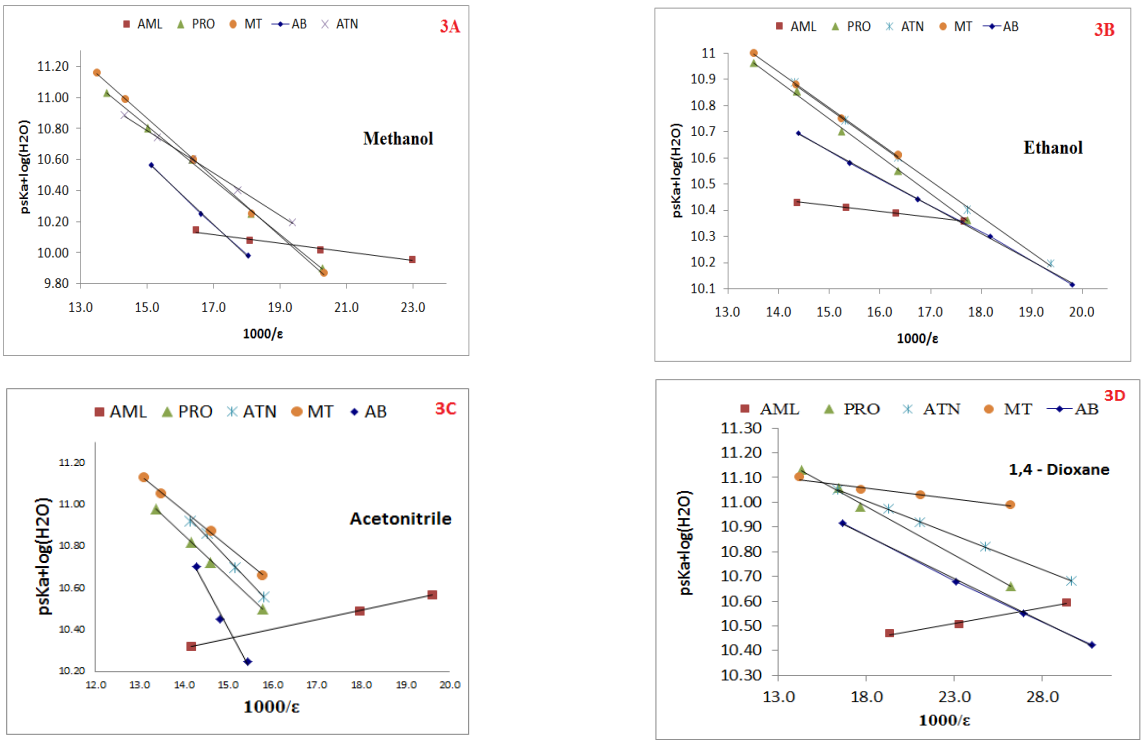

Figure 3. Beta blockers – ps*K*a Trend charts by potentiometry.

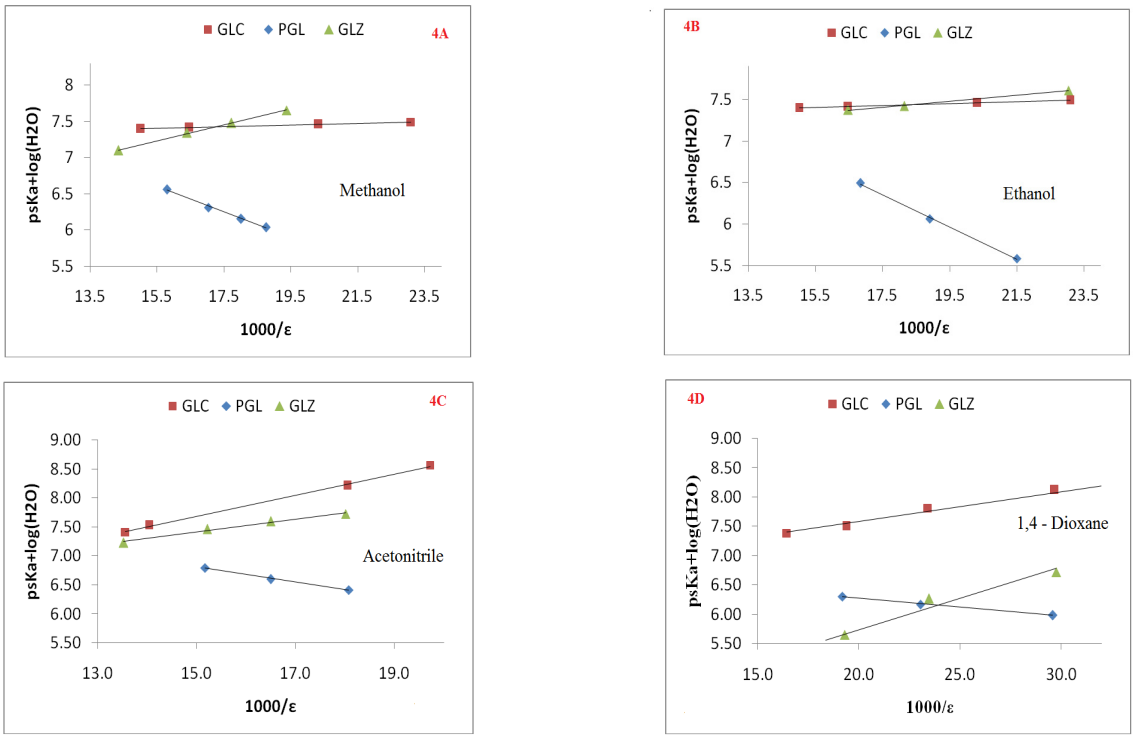

Figure 4. Anti-diabetic – ps*K*a trend charts by potentiometry.

Table 4. Effect of temperature on the ps*K*a values.

Therapeutical class	Compound	Co-solvent	Wt %	ps*K*a			I (M)	Method
				25 °C	35 °C	45 °C		
	AML	Methanol	40	8.91	8.74	8.57	0.15	Potentiometric
	PRO			9.04	8.88	8.73		Potentiometric
Beta blockers	ATN			9.13	8.84	8.57		Potentiometric
	MT			9.10	8.88	8.68		Potentiometric
	AB			8.99	8.73	8.50		Potentiometric
	GLC			5.94	5.86	5.81		Potentiometric
Anti-diabetics	PIO			5.01	4.86	4.67		Potentiometric
	GLZ			5.86	5.81	5.76		Potentiometric

Table 5. Effect of ionic strength on the ps*K*a values.

Therapeutical class	Compound	Co-solvent	Wt %	ps*K*a			Temp °C	Method
				0.15 M	0.3 M	0.5 M		
	AML	Methanol	40	8.91	8.96	9.01	25	Potentiometric
	PRO			9.04	9.08	9.15		Potentiometric
Beta blockers	ATN			9.13	9.18	9.25		Potentiometric
	MT			9.10	9.12	9.15		Potentiometric
	AB			8.99	9.04	9.09		Potentiometric
	GLC			5.94	5.96	5.98		Potentiometric
Anti-diabetics	PIO			5.01	5.06	5.14		Potentiometric
	GLZ			5.86	5.90	5.94		Potentiometric

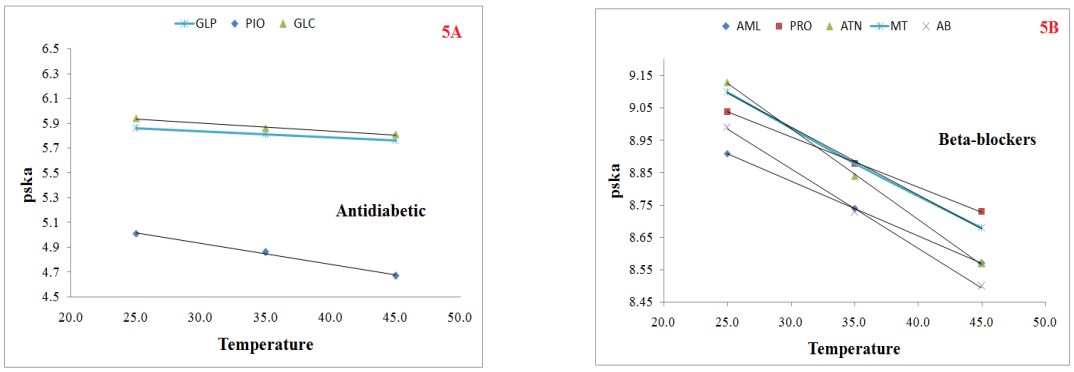

Figure 5. Effect of temperature on ionization constant.

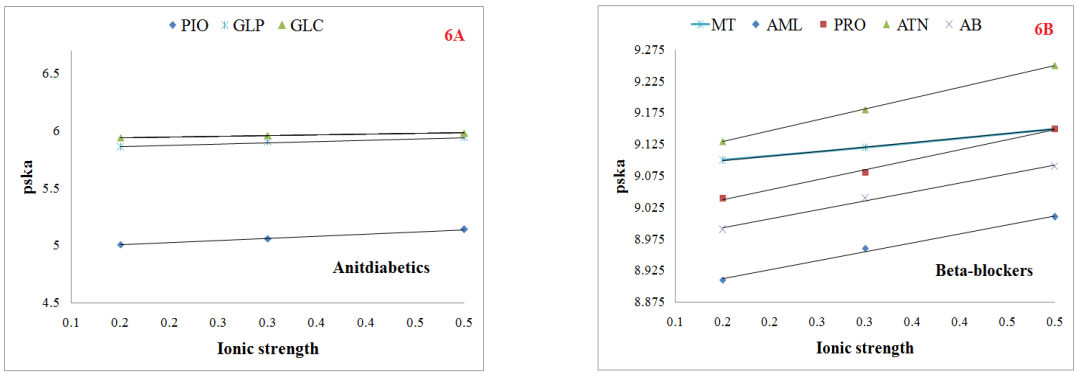

Figure 6. Effect of Ionic strength on ionization constant.

Figure 5 and 6 represent the ps*K*a value trend charts of selected compounds due to temperature and ionic strength effect, respectively. It is evident from these trend charts that the dissociation constants of all the drugs decreased with increasing temperature, while same compounds showed increasing trend with increasing ionic strength. This is in good agreement with the literature findings. The results are presented in Table 4 and 5.

Analysis of the slope of YS equations allows us to have a better insight into the solvation effect of the different co solvent mixtures upon the ionization of different functional groups. This can be better explained in case of AML, where it being a base, positive slope was observed for both acetonitrile and dioxane solvent. This has been validated by both potentiometry and spectrophotometry methods. Comparatively, significant lower slope values in 1,4-dioxane in almost all the selected compounds, with the exception of GLZ, indicate higher ionic diameters of the solvated molecules in the four-component solvent system. In solvents having low dielectric constant, ions tend to associate, which complicates the interpretation of p*K*as. In particular, in aprotic solvents the process of homo-conjugation occurs when the conjugate base forms a hydrogen bond with the parent acid [34].The slope values of all the compounds in different co solvents chosen in the present study having the same ionisation groups are presented in Table 6.

Table 6. Comparison of slope for the compounds having isopropyl amine groups.

Compound	Ionisation group	Slope							
		Methanol		Ethanol		Acetonitrile		1,4 - Dioxane	
		pH metry	UV/pH	pH metry	UV/pH	pH metry	UV/pH	pH metry	UV/pH
AML	Guanidine	-20.7	-61.1	-25.8	-21.1	41.9	69.2	10.2	19.3
PRO	Iso propyl amine	-146.3	-188.1	-179.9	-115.4	-223.7	-107.1	-41.1	-12.7
ATN		-138.6	-184.8	-207.1	-114.4	-222.5	-57.7	-30.3	-13.3
MT		-163.2	NA	-183.7	NA	-216.6	NA	-7.7	NA
AB	Primary amine	-108.7	NA	-199.4	NA	-387.7	NA	-34.6	NA
PIO	Pyridine	-174.7	NA	-192.1	NA	-131.0	NA	-19.4	NA
GLC	Sulfonyl urea	25.8	32.8	11.8	28.3	177.8	111.3	50.6	69.9
GLZ		110.2	NA	36.3	NA	110	NA	94.3	NA

Table 7. The percentage of the species in stomach, gastrointestinal tract and plasma.

Compound	Percentage concentration					
	Stomach pH 1.5		Small intestine (jejunum) pH 6.5		Plasma pH 7.4	
	B[a]	BH+[a]	B[a]	BH+[a]	B[a]	BH+[a]
AML	0	100	1.21	98.79	8.89	91.11
PRO	0	100	38.01	61.99	17.03	82.97
ATN	0	100	0.29	99.71	2.26	97.74
MT	0	100	8.20	91.80	58.49	41.51
AB	0	100	15.16	84.84	58.68	41.32
Compound	**Percentage concentration**					
	Stomach pH 1.5		**Small intestine (jejunum) pH 6.5**		**Plasma pH 7.4**	
	A-[b]	**AH[b]**	**A-[b]**	**AH[b]**	**A-[b]**	**AH[b]**
GLC	0	100	8.20	91.80	41.52	58.48
GLB	0	100	1.41	98.59	10.21	89.79
GLZ	0	100	6.59	93.41	35.93	64.07

[a]Monovalent bases.
[b]Acid.

Further, we used the p*K*a values to calculate the percentage concentration of protonated species at three relevant pH values in the body. The pH partition hypothesis considers the non-ionized, neutral species (at base: B, at acid: AH) to be favourable for the absorption using passive transport through the lipoid membranes. Though all the basic compounds are predominantly present in ionized (BH+) form in different compartments, 38%, 8.2% and 15.16% of PRO, MT and AB neutral form (B) is present in the jejunum. However, all the basic compounds selected in the present study, neutral form ranging between 2.2% and 58.5% is found to be present in the plasma. The acid compounds are exceptional within the selected compounds because its neutral form (AH) is dominant in all three compartments of the human body, although up to 41% of ionized species is found to be present in plasma. Results are summarized in Table 7.

3.1.2. Spectrophotometry

Out of all the selected drugs of the present study, only AML, PRO, ATN and GLC is found to produce reasonable absorption spectra with the change in pH. Among the selected drugs for the spectrophotometric study, AML has exhibited excellent absorption spectra with the changes in pH, as shown in Figure 7. This can be explained by the fact that, in case of AML, ionisable –NH group of guanidine moiety is part of the system of conjugated double bond. Target factor analysis (TFA) has been applied to deduce the p*K*a values from the multi wavelength UV absorption data recorded at different pH values. Results are shown in Table 2. Results indicate that the extrapolated p*K*a values by YS method is in good agreement with the pH metry-aqueous p*K*a values. The spectrophotometric results containing distribution of species and spectral absorbance change with pH for AML, PRO, ATN and GLC are presented in Figure 8A to 8H.

3.2. Lipophilicity

The log P_{oct} values were measured by dual phase potentiometric titration. This method, being fast, precise and automated, is considered as the "gold standard" of log *P* determination [35], and it can be used for log *P* measurements even for compounds of high lipophilicity. Typically compounds partition to a much greater extent in their neutral form than their ionized form, hence a larger portion of the neutral species will disappear into the octanol phase.

Since high log P_{oct} values were expected, especially for GLB and PRO, small amounts of octanol was applied during the titration. Figure 9 represents the typical shift of Bjerrum curve during the course of titration in presence of octanol for all the compounds studied in the present investigation. From these titrations, the apparent ionization constants (po*K*a) were calculated. The log P values for acids and bases were then calculated using Eq. 3 and 4, respectively.

$$\text{Acid with one pKa : P} = \frac{10^{(\text{pKa} - \text{poKa})} - 1}{r} \quad (3)$$

$$\text{Base with one pKa : P} = \frac{10^{(\text{poKa} - \text{pKa})} - 1}{r} \quad (4)$$

where *r* is the volume ratio of octanol to water. The experimental log *P* values obtained by Potentiometric and HPLC methods are given in Table 8. On the close examination of Bjerrum curves of all the compounds, it is evident that all the compounds belonging to beta blocker class (AML, PRO, ATN, MT and AB) is bases as all the measured apparent p*K*a (po*K*a) values shifted to lower pH values from their aqueous p*K*a values. In the case of GLC, GLB and GLZ (all anti-diabetics), the po*K*a values shifted to higher pH values from their aqueous pKa suggesting that these compounds are acids.

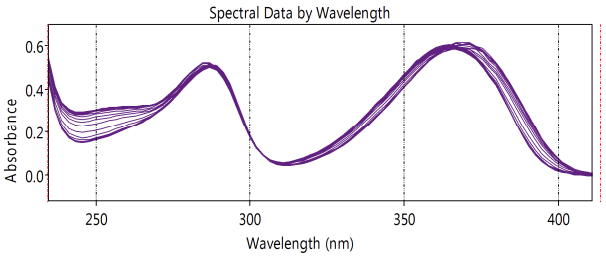

Figure 7. AML – Spectral data with change in pH.

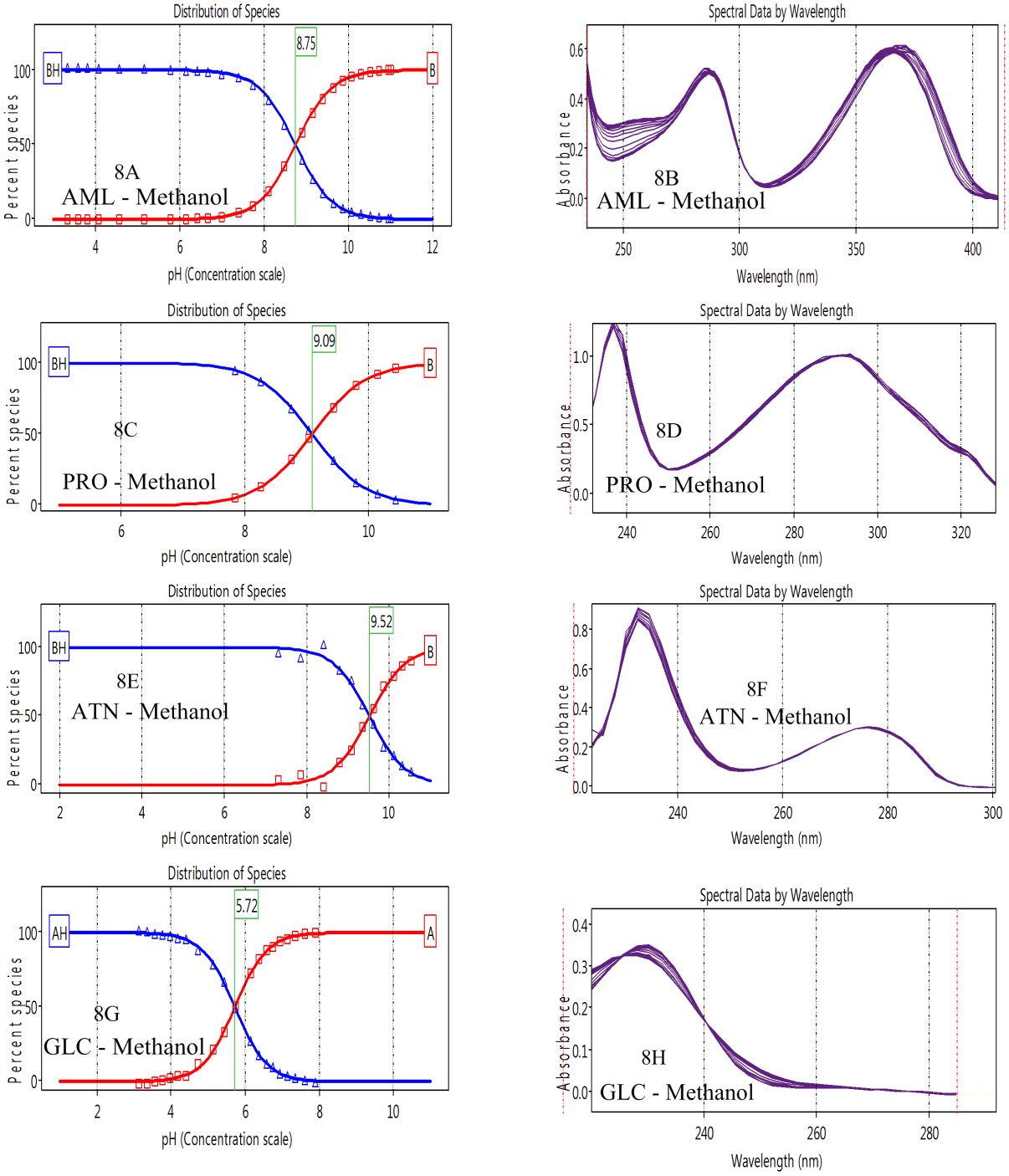

Figure 8. Distribution of species and spectral data of selected drugs.

Table 8. Log *P* values obtained by Potentiometric and HPLC methods.

Therapeutical class	Compound	Potentiometric Log *P* ± RMSD	HPLC log D 7.4	Literature log *P*[a]
Beta blockers	AML	0.04 ± 0.18	0.16	0.09[b]
	PRO	3.48 ± 0.09	3.34	3.00
	ATN	0.28 ± 0.07	0.32	0.50
	MT	1.95 ± 0.07	1.79	1.60
	AB	3.39 ± 0.07	3.28	1.90
Anti-diabetics	GLC	2.07 ± 0.07	2.18	2.60
	GLB	4.20 ± 0.11	4.05	3.80
	GLZ	2.35 ± 0.16	2.27	2.50

[a] Ref. [30].
[b] Phenomenex DMPK guide.

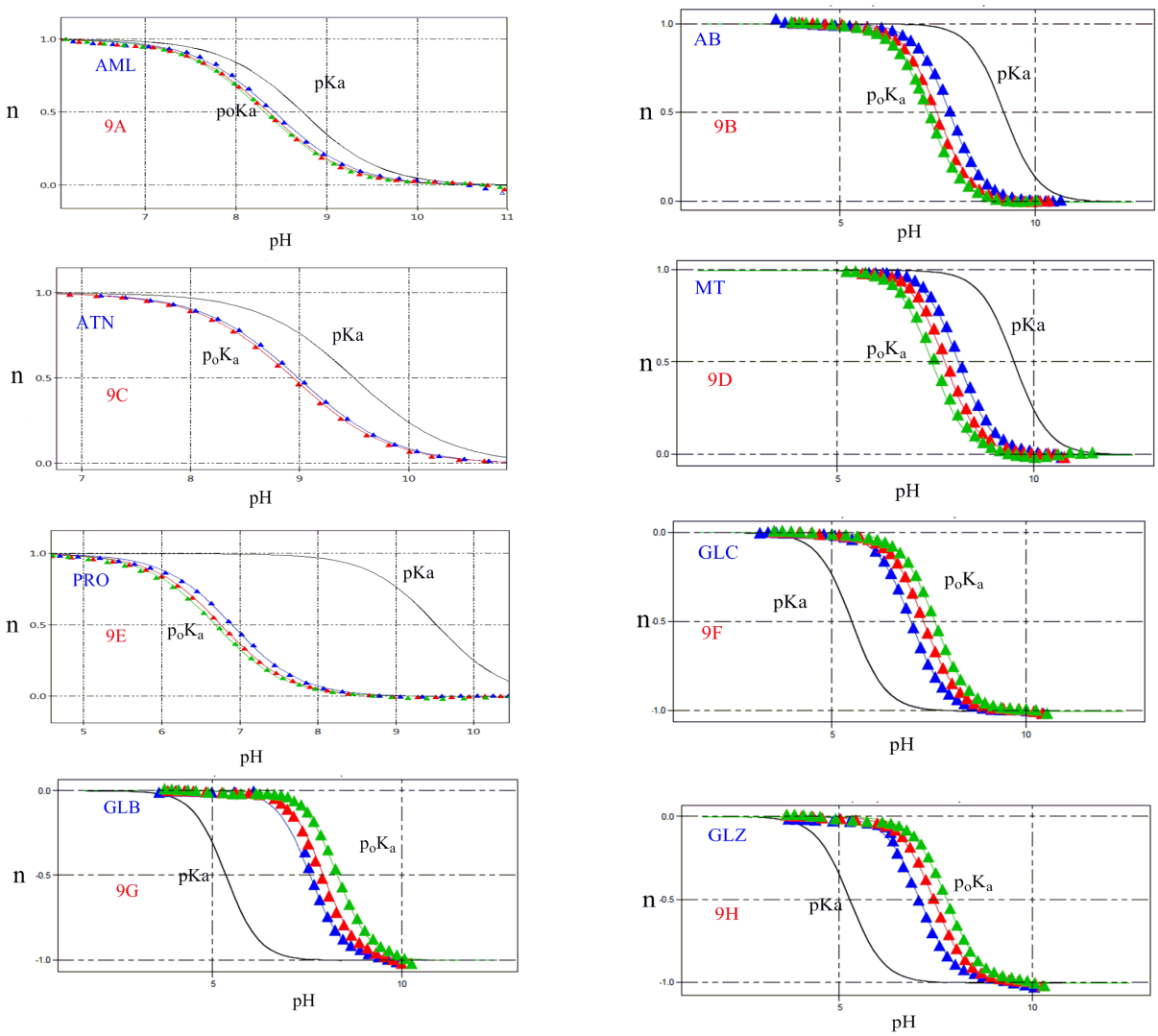

Figure 9. Log *P* - Bjerrum curves for selected compounds.

A plot of log *k'*, the chromatographic capacity factor, against log *P* is linear and the correlation co-efficient for HPLC log *P* measurement is found to be 0.9973. Close look at the log *P* results between potentiometry and partition HPLC, reveals that the values with each technique differ by only 0.2. Since, method adopted for the literature values are unknown, it is obvious that the values differ considerably with our experimental values. Hence our present work is limited only till to the comparison between potentiometry and HPLC methods.

4. Conclusion

The application of co solvent–water mixtures improves the solubility of poorly water soluble drugs thus their ps*K*a values can be measured in lower proportion of organic solvent. The co solvent dissociation constants (ps*K*a) of selected compounds were determined in different co solvent–water mixtures by potentiometric and spectrophotometric methods. The co solvent–water mixtures did not cause large shifts in ps*K*a values and the Yasuda–Shedlovsky extrapolation procedure was proposed to obtain the aqueous p*K*a values. The extrapolated data are in good agreement with p*K*a values measured in water. The average deviation is p*K*a = 0.2. In this way the proposed different co solvents can be applied to those compounds which are not soluble in aqueous solutions and can be easily adopted in drug discovery laboratory for the determination of dissociation constant in a high throughput manner. The knowledge of the acid–base behaviour of the drug compounds, in different hydro-organic media is therefore a useful parameter to optimize analytical procedures for the separation of ionizable compounds by liquid chromatography, especially when these drugs are present in combination with other drugs.

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