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Development and Validation of a Liquid Chromatographic Method for Concurrent Assay of Weakly Basic Drug Verapamil and Amphoteric Drug Trandolapril in Pharmaceutical Formulations

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ABSTRACT

The analysis of weakly basic drugs such as verapamil by reverse-phase liquid chromatography remains a problem, particularly when present in combination with other drugs such as amphoteric compounds like trandolapril. In this study, the simple, accurate, precise and fully validated RP-LC method for the simultaneous determination of verapamil and trandolapril in combined dosage forms has been developed. The LC method allowed quantitation over the ranges of 0.50-18.00 μg/mL and 0.05-1.00 μg/mL for verapamil and trandolapril, respectively. The detection limits were found to be 0.008 μg/mL and 0.018 μg/mL for verapamil and trandolapril, respectively. Moreover, pK_a values of verapamil and trandolapril were determined via the dependence of the retention factor on the pH of the mobile phase for ionizable substances. The effect of the mobile phase composition on the ionization constant was studied by measuring the pK_a at different methanol-water mixtures, ranging 50-65% (v/v). It was shown that RP-HPLC was suitable for the high throughput analysis of the combination of verapamil and trandolapril. The method also allows a number of cost and time saving benefits and can be readily employed for the analysis of pharmaceutical formulations. The method has been verified, without any interference from excipients, for the concurrent analysis of these compounds in tablets.

Key words: verapamil, trandolapril, ramipril, pK_a, HPLC, simultaneous determination

INTRODUCTION

The inhibitors of the angiotensin-converting enzyme (ACE inhibitors) are widely used for the treatment of mild to moderate hypertension and heart failure, either alone or in conjunction with other drugs[(1)]. Trandolapril (TRA, (2S, 3aR, 7aS)-1-[(S)-N-[(S)-1-(ethoxycarbonyl)-3-phenyl propyl] alanyl] hexahydro-2-indolinecarboxylic acid), is a long acting, highly lipophilic non-peptide, ACE inhibitor with a carboxyl group but without sulphydry1 group[(2)] (Figure 1A). It is used for the management of hypertension and for the stable patients who have evidence of left ventricular systolic dysfunction or symptoms of heart failure within the first 2 days after acute myocardial infarction[(3,4)]. Tablet dosage form contains 1, 2 or 4 mg TRA.

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Verapamil hydrochloride (VER, (5-[3,4-dimethoxyphenethyl) methylamino]-2-(3,4-dimethoxyphenyl)-2-isopropylvaleronitrile hydrochloride), a slow calcium channel antagonist, inhibits the trans membrane influx of calcium ions into the heart and vascular smooth muscle cells (Figure 1B). It also enhances myocardial blood flow due to the calcium antagonistic effect on the smooth vascular muscles of coronaries. Therefore, it contributes to the anti-ischemic and anti-anginal efficiency in all types of coronary artery diseases and is also used as anti-hypertensive and anti-arrhythmic[(5)]. The film-coated tablet dosage form contains 40 mg, 80 mg, or 120 mg of VER for oral administration.

The dissociation constant (pK_a) of a drug molecule is a key parameter in absorption, distribution, metabolism, excretion and toxicity researches because it governs solubility, absorption, distribution and elimination of substances[(6)]. Also, the pK_a values constitute important data for thorough

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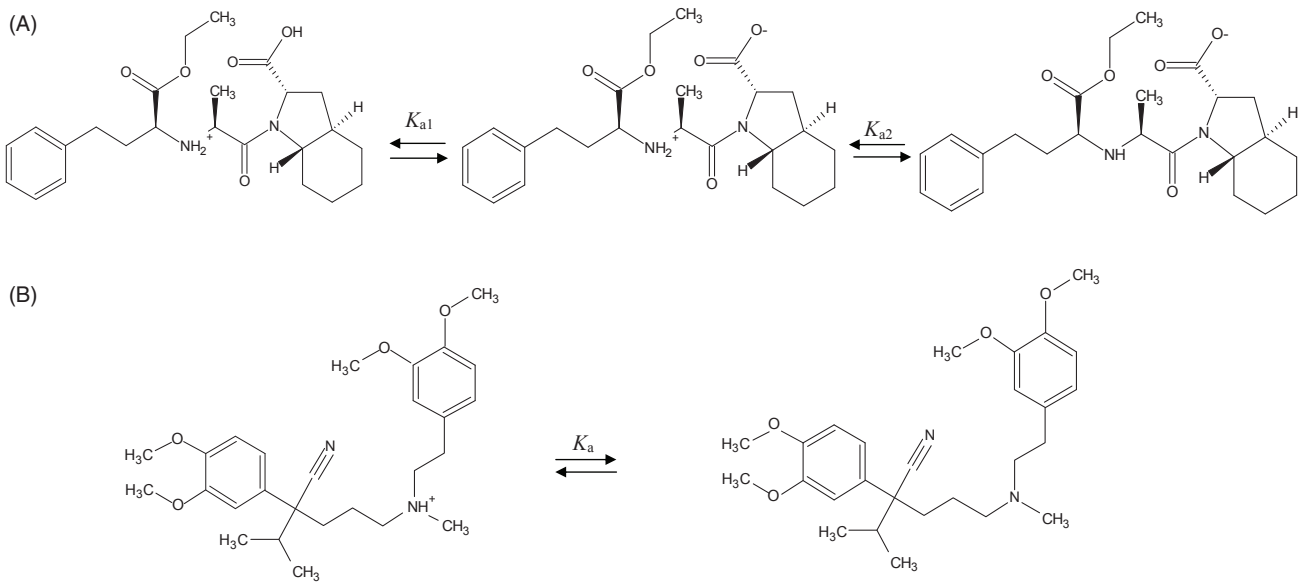

Figure 1. Structures and dissociation equilibria of studied compounds (A) TRA, (B) VER.

understanding of certain chemical phenomena such as biological uptake, and the binding of these molecules to environmental matrices and forming chelates with metallic cations. The pK_a values of drugs are applied to estimate the major species of pharmaceuticals present in the environment (usually in neutral pH range) and dosage-form development. A satisfactory knowledge of the acid-base behavior of substances in hydro-organic media such as methanol (MeOH)-water is therefore essential to predict the influence of pH on selectivity and retention during liquid chromatography (LC) and also to optimize analytical conditions for the separation of ionizable compounds by different techniques[7,8]. Although MeOH-water mobile phases have been used in reverse phase LC (RP-LC) separation procedures, the pK_a values of VER and TRA have not yet been established in MeOH-water binary mixtures.

Pharmaceuticals containing the combination of VER and TRA were recently introduced into therapy. The fixed-dose combinations consist of a sustained-release (SR) tablet formulation of VER and an instant-release granulation of TRA. While validated assays have been reported for each drug individually[9-13], there is only one assay method that permits quantification of all the species present, i.e., VER and TRA[13]. Besides, the analysis of weakly basic drugs such as VER by RP-LC remains a problem, particularly when present in combination with other drugs such as amphoteric compounds like TRA. In this study, we propose to develop a simple, accurate, precise and fully validated RP-LC method for the simultaneous determination of VER and TRA in their combined dosage forms. The proposed method will be used to determine the combined dosage forms of TARKA FORTE® tablets (containing 240 mg of VER and 4 mg of TRA).

The pK_a values of studied compounds are either not known accurately or not available at all. Only a limited works related to pK_a values of studied compounds are found in the literatures[14-16]. A further aim and the the novelty of this manuscript was to determine pK_a values of VER and TRA in several MeOH-water binary mixtures, i.e., 50, 55, 60 and 65% v/v, in order to overcome the lack of information related with the acid-base equilibria of this kind of compounds by means of chromatographic measurements.

MATERIALS AND METHODS

I. *Chemicals and Reagents*

All chemicals and solvents were of analytical - reagent grade and used without further purification. Pharmaceutically active compounds used in this study were kindly supplied as follows: VER and TRA from (ABBOTT Pharm. Ind.); ramipril (RAM), an internal standard (IS) during separation studies because of its shorter elution time and similar structure from Nobel Pharm. Ind. TARKA FORTE® tablets (ABBOTT Pharm. Ind.) were obtained from local pharmacy. HPLC grade MeOH and sodium hydroxide were from Merck (Darmstadt, Germany). Ortho-phosphoric acid (min. 85%) was from Riedel (Riedel-de Haen, Germany). Hydrochloric acid (Titrisol) and potassium hydrogen phthalate (dried at 110°C before use, Fluka) were used. All stock solutions of hydrochloric acid, potassium hydroxide and potassium hydrogen phthalate were prepared by water. Water, with conductivity lower than 0.05 m/Scm was obtained with a Zeneer Power I (Human Corp.)

Stock standard solutions of VER, TRA and RAM were prepared in MeOH at 200 μg/mL. Working solutions were diluted with the corresponding mobile phase to 10 μg/mL. All stock and working solutions were protected from light and stored in fridge at about 4°C. The dead time (t_o) was measured by injecting uracil solution [0.01% (v/w), in water]

which was established for each mobile phase composition and pH studied.

II. *Apparatus*

The LC analysis was carried out on a Shimadzu HPLC system with a pump (LC-20 AD), a DAD detector system (SPD-M 20A) and column oven (CTO 20 AC). This equipment has a degasser system (DGU 20 A). The system operates at 202, 206 and 210 nm for VER, TRA and RAM, respectively. An X-Terra RP-18 (250 × 4.60 mm i.d. × 5 μm) column was used as stationary phase at 40°C. Mettler Toledo MA 235 pH/ion analyzer with Hanna HI 1332 Ag/AgCl combined glass electrode was used for pH measurements.

III. *Chromatographic Procedure*

Throughout this study, the mobile phases assayed were MeOH-water mixtures at 50, 55, 60 and 65% v/v, containing 15 mM phosphoric acid. The pH of the mobile phase was adjusted between 2.2 and 10.0 by sodium hydroxide. The flow rate was maintained at 1.2 mL/min and injected volume was 20 μL.

The column was pre-conditioned for at least 1h at low flow-rate (0.5 mL/min) with mobile phase at the corresponding pH before the first injection. For each compound, the retention time values, t_R, were determined from three separate injections for each mobile phase composition and pH considered.

The chromatographic retention of ionizable compounds is strongly dependent on the pH of the mobile phase. Thus it is required to measure and control the mobile phase pH accurately, in many instances, for efficient separations of ionizable compounds by LC[17].

Several procedures can be used to measure the mobile phase pH. The most common procedure is to measurement of the pH in the aqueous buffer before mixing it with the organic modifier. A more rigorous procedure, recommended by the IUPAC, is to measure the pH of the mobile phase after mixing the aqueous buffer and the organic modifier. In this instance, the electrode system used to measure pH can be calibrated either with aqueous buffers, or with buffers prepared in the same solvent composition used as mobile phase. This requires knowledge of the pH value of reference buffers prepared in different aqueous-organic solvent mixtures[18,19].

As pH values have been previously determined in MeOH-water mixtures for the primary standard series of substances proposed by National Institute of Standards and Technology (NIST), in accordance with the IUPAC rules, values in MeOH-water mixtures can thus be measured. In this study, pH of the mobile phase was measured after mixing the aqueous buffer and the organic modifier. Potassium hydrogen phthalate solutions (0.05 mol/kg) dissolved in the appropriate MeOH-water medium were used as primary standard buffer[20-22]. In general, each methanol contents for the chromatographic retention were studied from acid to basic pH. pH measurements were performed in triplicate to ensure stability and reproducibility of the mobile phase system. The pK_a values of studied compounds were determined from *k*/pH data pairs by means of the non-linear regression program NLREG[23].

IV. *Preparation of Standard Solutions and Calibration*

Stock solutions (100 μg/mL) of VER, TRA and RAM (IS) were prepared in MeOH. All solutions were protected from light and used within 24h to avoid decomposition. Standard solutions were prepared separately with mobile phase by varying concentrations of VER and TRA in the range of 0.5-18 μg/mL and 0.05-1.0 μg/mL, respectively and the concentration of IS was maintained at a constant level of 2.0 μg/mL. Triplicate 20 mL injections were made for each solution. The calibration curves for LC analysis were constructed by plotting the peak area ratio of the drug to that of internal standard against the drug concentration. The proposed method was validated as to precision (reported as the relative standard deviation, R.S.D. (%)), linearity (evaluated by regression equations), detection and determination limits and accuracy.

The limit of detection (LOD) and limit of quantitation (LOQ) of the procedure are also obtained, which were calculated according to the 3 s/m and 10 s/m criterions[24,25], respectively, where s, is the standard deviation of the absorbance (n = 4) of the lowest amount of the linearity range and m is the slope of the corresponding calibration curve.

The ruggedness and precision were checked in the same and different days. The R.S.D. (%) values were calculated to check the ruggedness and precision of the methods. Accuracy was determined by recovery studies.

V. *Procedure for Tablets*

Ten tablets labeled to contain 240.0 mg VER and 4.0 mg TRA and excipients were weighed and finely powdered. The powder equivalent to one tablet content was accurately weighed, transferred into a 100 mL calibrated flask, diluted with MeOH, stirred for about 10 min and then completed to volume with the same solution. This solution was filtered and the filtrate was collected in a clean flask. After filtration, appropriate solutions were prepared by taking suitable aliquots of clear filtrate. In order to obtain a final solution, the constant amount of IS was added and these solutions were diluted with mobile phase and MeOH. The amounts of VER and TRA were calculated from the corresponding regression equations.

VI. *Recovery Studies from Tablets and Laboratory Made Mixtures*

In order to demonstrate the applicability of the method, the recovery tests were carried out by analyzing synthetic mixtures of VER and TRA. After five repeated experiments, the recoveries from these synthetic mixtures were calculated for each compound.

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To verify the accuracy of the method, recovery experiments were performed by adding a known amount of pure drug to pre-analyzed tablets. Known amounts of the pure drug (and at a constant level of IS) were added to VER and TRA tablet formulation and the mixtures were analyzed. The percent recovery was calculated by comparing the concentration obtained from spiked samples with the actual added concentration. Thus, the effect of common excipients in tablet formulation on chromatograms (e.g., tailing, broadening) was investigated. Recovery experiments from tablets also showed the reliability and suitability of the method.

RESULTS AND DISCUSSION

I. *Determination of Dissociation Constants (pK_a)*

The acid base chemistry of pharmaceutically active compounds plays a pivotal role in the development of a new drug. Among all the physicochemical parameters of a chemical entity, the acid dissociation constant (pK_a) is one of the most important ones. It is especially useful in the pharmaceutical industry for drug design, formulation and metabolism. Besides, the solubility and permeability of drug compounds are pK_a dependant. pK_a value also affects the drug-receptor binding. Pharmaceuticals and the determination of useful dosage forms and regimes for drugs depend upon an understanding of drug dissociation and the extent of dissociation that will occur in the body systems.

Most of the ACE inhibitors contain both proton acceptor and proton donor groups, which may be ionized and/or protonated. Their retention on column depends on the percentage of ionized and non-ionized species of each compound. The most dramatic pH-related changes in retention occur at pH values within $pK_a \pm 1.5$. The ionization value helps in selecting the pH of the buffer in the mobile phase. Thus, knowledge of the acid-base dissociation constants of studied compounds in MeOH-water mixtures, which are usually used as the mobile phase, can help to improve the analytical method and can lead to a better understanding of the chromatographic behavior of these compounds.

Dissociation equilibria of VER and TRA are shown in Figure 1. As deduced from Figure 1A, VER is a weak monoprotic base and the ionization process can be written as:

$$BH^+ \overset{K_a}{\rightleftharpoons} B + H^+ \quad (1)$$

The observed retention factors can be described as a function of retention factors of the neural and ionized species and the equation can be given by

$$k_{obs} = \frac{k_0 + k_1 \frac{[H^+]}{K_a}}{1 + \frac{[H^+]}{K_a}} \quad (2)$$

where k_0 and k_1 are the retention factors of the neutral and fully ionized base, respectively.

TRA is an amphoteric compound throughout the working pH range and the overall dissociation process can be given as: K_{a1} and K_{a2} are the dissociation constants of the carboxylic acid and amino groups, respectively.

$$H_2B^+ \overset{K_{a1}}{\rightleftharpoons} HB + H^+ \overset{K_{a2}}{\rightleftharpoons} B^- + 2H^+ \quad (3)$$

The expression of the observed retention factors can be given by

$$k_{obs} = \frac{k_0 + k_{-1} \frac{K_{a1}}{[H^+]} + k_1 \frac{[H^+]}{K_{a2}}}{1 + \frac{K_{a1}}{[H^+]} + \frac{[H^+]}{K_{a2}}} \quad (4)$$

where k_0, k_{-1} and k_1 are the retention factors of the neutral, the anionic, and the cationic forms of the ampholyte and K_{a1} and K_{a2} are the corresponding acid dissociation constants, respectively. The pK_a values of RAM (IS) was studied as details and reported in our previous paper[26].

In this study, the retention factors were determined for each mobile phase composition and pH studied. In Figure 2, data pairs of k/pH for studied compounds in 55% (v/v) MeOH are shown and the correlation between the experimental

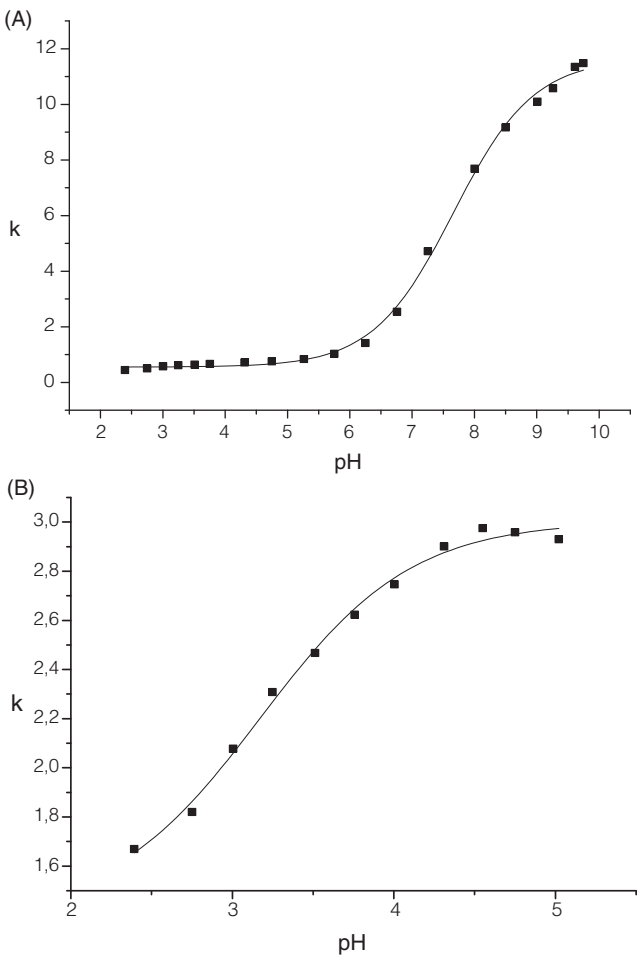

Figure 2. Plot of chromatographic retention factor, k, vs. the pH of mobile phase with 55% (v/v) of MeOH. (A) VER, (B) TRA.

capacity factors of the compounds studied over the whole experimental pH range was good.

Typical sigmoid shape curve was obtained for VER in Figure 2A, showing the dependence of the analyte retention factors upon the pH of the mobile phase, so did an ampholytic compound shown in Figure 2B. In general, the retention of the investigated analytes increased as the pH increased.

The dissociation constant values determined for the equilibria involved for VER and TRA in 50, 55, 60 and 65% v/v MeOH-water mixtures at 40.0 ± 0.1°C are shown in Table 1, together with respective standard deviations. The Table 1 also gives the pK_a values reported in the literatures, together with those predicted using the program ACD/pK_a DB[16]. ACD/pK_a DB is a software program that calculates accurate acid-base ionization constants under 25°C and zero ionic strength in aqueous solutions for almost any organic structure. This program uses fragment methods to build a large number of equations with experimental or calculated electronic constants to predict aqueous pK_a values.

As deduced in Table 1, the pK_{a1} values of TRA associated with the carboxylic group in the proline moiety were smaller than those generally observed with aromatic derivatives in water. pK_a values of this carboxylic acid (proline) moiety vary in the range of 2.8 to 3.1. On the other hand, the pK_{a2} values correspond to the secondary amine group vary from 3.8 to 4.1 in the studied range of MeOH.

It is well known that one of the most important factors in determining the dissociation constants is the reaction medium. The pK_a values of VER in MeOH-water binary mixtures decrease whereas pK_{a1} values of TRA in MeOH-water binary mixtures increase and pK_{a2} values decrease with percentage of MeOH. These variations could be explained by the fact that there is preferential solvation in these media that is related to the structural features of these binary mixtures. It has been found that in several water-organic co-solvent mixtures, such as MeOH-water mixtures, pK_a values of a given substance show a linear relationship with the mole fraction of organic co-solvent[27]. This is indicated by the following expression:

$$pK_{a,\varphi} = pK_{a,w} + \varphi\Delta pK \quad (5)$$

where p$K_{a,w}$ indicates the dissociation constant in water, φ is the mole fraction of organic cosolvent, ΔpK is the slope of the linear relationship, and p$K_{a,\varphi}$ is the pK_a at the corresponding composition. The intercept, slope and correlation coefficients 9.439, -4.728, 0.991 for VER and 2.129, 2.295, 0.987 for TRA, respectively. These results represent, in general, the assumption of a linear relationship between pK_a values of studied compounds and the mole fraction of MeOH is a good approximation in studied range of MeOH.

II. *Analysis of Tablets*

Drug analysis is undertaken during various phases of pharmaceutical development, such as formulation and stability studies, quality control and pharmacological testing in animals and humans. All these investigations require reliable and validated analytical methods in order to assay drugs in pharmaceutical formulations. Precision and accuracy can often be enhanced by the use of an appropriate internal standard during HPLC method, which also serves to correct for fluctuations in the detector response.

An IS is usually included in the assay when samples require significant pretreatment or preparation. Often, a sample preparation step that includes reaction, filtration, precipitation, extraction, and so on, results in sample losses. When added prior to sample preparation, a properly chosen IS can correct these sample losses. Several compounds were tested as possible ISs: the most suitable was ramipril (RAM). Ideally, an internal standard should display similar physicochemical properties to the analytes. RAM was chosen as the IS because the structure of RAM is quite similar (differ in only one ring) to TRA and it showed shorter retention time with better peak shape and resolution.

Various mobile phase systems were used to provide an appropriate LC separation (50, 53, 55 and 60% v/v). Four different types of columns such as Synergy Max-RP (5 μm, 150 mm × 4.6 mm i.d.), Symmetry Shield C-8 (5 μm, 150 mm × 3.9 mm i.d.), YMC Pack ODS-AM (5 μm, 150 mm × 4.6 mm) and X Terra C-18 (5 μm, 250 mm × 4.6 mm i.d.) were tested in order to find the best resolution and peak shape of the studied compounds. X Terra C-18 column has been selected and used successfully as a stationary phase for the simultaneous determination and separation of VER, TRA and IS. This stationary phase has extended pH stability and is thermally more stable and more efficient than classical silica-based packing.

In order to find the optimum chromatographic conditions for RP-LC determination of VER, TRA and IS the influence of pH on the mobile phase and column temperature were examined. The pH of the mobile phase has

Table 1. The pK_a values of studied compounds predicted by ACD Lab[16] and obtained by chromatographic method in methanol-water media at 40°C

Compounds	Literature values	ACD Lab	NLREG[20]			
			50% (v/v)MeOH	55% (v/v)MeOH	60% (v/v)MeOH	65% (v/v)MeOH
VER	8.92[14]	8.97 ± 0.50[16]	8.00 ± 0.06	7.83 ± 0.10	7.59 ± 0.07	7.39 ± 0.04
TRA	5.60[15]	3.17 ± 0.20	2.82 ± 0.06	2.92 ± 0.05	3.03 ± 0.05	3.12 ± 0.01
		5.43 ± 0.39	4.11 ± 0.11	4.04 ± 0.10	3.93 ± 0.09	3.84 ± 0.07

always been adjusted to be 2.7 with 15 mM orthophosphoric acid, which is optimum pH with best peak asymmetry and retention values. The column temperature was set between 25 and 40°C. Higher temperature has been shown to minimize the tailing of the basic compounds by accelerating the rate of interaction with the stationary phase[28]. Mobile phases containing the organic modifier methanol have been shown to improve peak performance for basic compounds compared to those based on acetonitrile[29]. 40°C was selected due to shorter analysis time and improved peak shapes. Finally, the mobile phase MeOH: water 55 : 45 (v/v) with 15 mM H_3PO_4 (pH 2.7) at a flow rate of 1.2 mL/min was chosen as the most suitable carrier for LC analysis. The proposed LC method provides a simple procedure for the simultaneous analysis of VER and TRA in drug formulations by DAD detection at 202 and 206 nm, respectively.

Using the conditions described above, a satisfactory chromatographic peak resolution was obtained in a short analysis time (< 7 min.) as seen in Figure 3. For all compounds, sharp and symmetrical single peaks were obtained with good resolution.

System suitability for the proposed method was evaluated. The parameters tested for system suitability included retention time, tailing factor, capacity factor, resolution, theoretical plates, selectivity factor and RSD% of retention time. The results from system suitability tests are presented in Table 2 for each compound. Typically, at least two of these criteria are required to demonstrate system suitability for the proposed method. As shown in Table 2, the presented chromatographic conditions ensured adequate retention of all compounds, since capacity factor values satisfied the conditions (≥ 1.0) except for those of VER and RAM. The proposed method has also enabled good resolution of analytes with values of resolution factors of adjacent peaks greater than 2.0. The results obtained from system suitability tests are in agreement with the USP requirements.

The relationship between drug concentration and the response was linear and the proposed LC method allowed quantitation over the 0.50-18.00 µg/mL and 0.05-1.00 µg/mL range for VER and TRA, respectively. The calibration curves were obtained by the linear least squares regression. Since the correlation coefficient was not acceptable concerning the linearity performance of the analytical procedures alone, the SE of slope and SE of intercept were used to evaluate the precision of the regression. These parameters should be comparable to the RSD values which were obtained in precision studies in the given concentration range. Linearity, range, regression equations, correlation coefficient, LOD and LOQ values and other related validation data are reported in Table 3. The low values of SE of intercept and slope and correlation coefficient > 0.999 in mobile phase, established

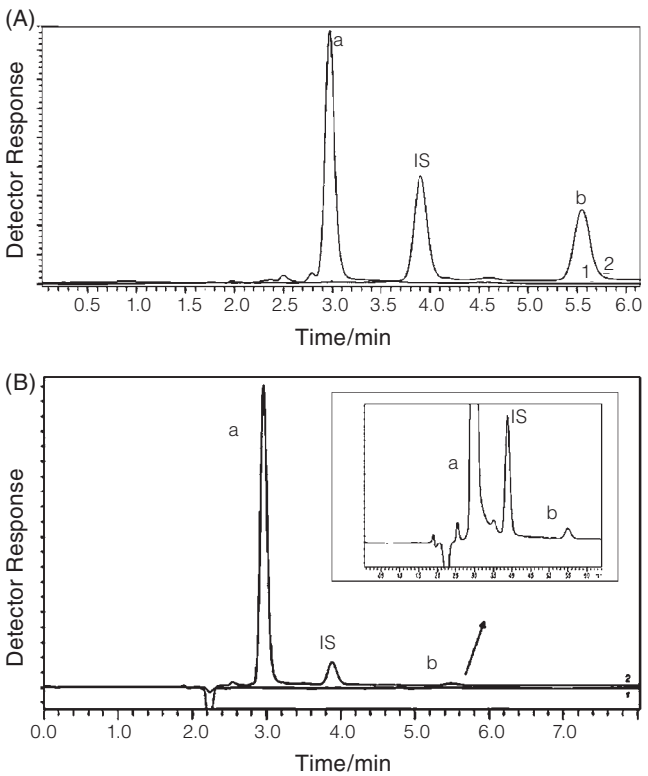

Figure 3. (A) Chromatogram of a standard mixture of studied compounds (1) Mobile phase and (2) mobile phase containing 10 µg/mL VER (a), 25 µg/mL TRA (b) and 15 µg/mL RAM (IS). The eluents were monitored at 202 nm. (B) Chromatogram of tablet dosage forms (Tarka Forte®), (1) Mobile phase and (2) mobile phase containing 12.0 µg/mL VER (a), 0.2 µg/mL TRA (b) and 2.0 µg/mL RAM (IS). Mobile phase consist of MeOH: water (55 : 45, v/v) mixture containing 15 mM H_3PO_4, with the pH adjusted to 2.70 with NaOH.

Table 2. System suitability parameters

	VER	RAM (IS)	TRA	Recommended value
Retention time (min)	2.964	3.910	5.497	
Tailing factor (T)	1.278	1.134	1.116	≤ 2
Capacity factor	0.322	0.744	1.452	≥ 1
Resolution (R)	3.557	-	5.122	> 2
Theoretical plates (*N*)	3400	3457	5035	> 2000
Selectivity factor (α)	2.311	-	1.952	> 1.15
RSD% (for retention time)	0.187	0.183	0.180	≤ 1

Table 3. Statistical evaluation of the calibration data of verapamil and trandolapril by RP-HPLC

	VER	TRA
Linearity range (µg/mL)	0.50-18.00 (n = 8)	0.05-1.00 (n = 7)
Slope	2.179	0.494
Intercept	-0.311	0.002
SE of slope	0.008	0.002
SE of intercept	0.083	0.004
Correlation coefficient (r)	0.9999	0.9999
Detection limit (LOD) (µg/mL)	0.008	0.018
Quantitation limit (LOQ) (µg/mL)	0.025	0.050

SE is standard error.

the precision of the proposed method.

Precision and reproducibility of the method were evaluated by doing replicate analysis of standard solutions in the mobile phase. Repeatability and reproducibility were characterized by mean recovery and RSD and the results are summarized in Table 4. As deduced from Table 4, there was no significant difference for the assay, as tested by within-day and between days. The RSD% values were between 0.088 and 3.443 for VER and 0.029 and 2.672 for TRA for the studied concentrations. These results supported good precision of the method.

In order to show the accuracy, validity and applicability of the proposed method, recovery tests have been performed by analyzing laboratory-made mixtures of VER and TRA which reproduced four different ratios (Table 5).

The mean percentage recoveries obtained after five repeated experiments were between 99.99 and 100.30 for VER and 99.38 and 101.60 for TRA with low level of RSD values at about 0.671 and 1.399 for VER and TRA, respectively. It is concluded, that the proposed method is sufficiently accurate and precise in order to be applied to pharmaceutical dosage forms. High percentage recovery data shows that the method is free from the interferences of the excipients used in the formulations.

The results from the laboratory-made mixtures (Figure 3A), analysis by the proposed LC method were extended to the simultaneous determination of VER and TRA in commercial tablet forms. This method can be used successfully in the presence of each drug without prior separation of the excipients. Each tablet contains the active ingredients of 240 mg of VER as verapamil hydrochloride, 4 mg TRA and the inactive ingredients as the excipients. It was found that the removal of the excipients before analysis was unnecessary (Figure 3B).

Figure 3 shows a chromatogram of the separation of mixtures of VER, TRA and IS in a mobile phase of MeOH-water with 55% (v/v) at pH 2.7. The studied compounds appeared as symmetrical single peaks, forming well shaped, perfectly separated from the solvent front and each other. The peak of TRA is enlarged in the chromatogram because of the quantity of the studied compound in the mixture and in the pharmaceutical dosage form. Additionally, no interfering peak was obtained in the analysis due to tablet excipients. The use of proposed LC method was verified by means of replicate estimations of pharmaceutical preparations and results were analyzed statistically and given in Table 6. It can be concluded that the proposed method can be employed for simultaneous quantitation and routine quality control analysis of this binary mixture in pharmaceuticals. A short analysis time for high-throughput analysis is most desirable and a preliminary review of literatures showed that the currently reported methods were unsuitable for the analysis of the drug combination. High percentage recovery data shows that the proposed LC method is free from the interferences of the excipients used in the formulations.

The stability of the reference compounds and sample

Table 4. Summary of repeatability (intra-day) and reproducibility (inter-day) precision data for verapamil and trandolapril

Compound	concentration (μg/mL)	Intra-day	Inter-day
		Mean Recovery* % ± RSD%	Mean Recovery* % ± RSD%
Verapamil	1	99.761 ± 0.545	100.680 ± 3.443
	10	100.164 ± 0.088	99.642 ± 0.205
	15	100.021 ± 0.098	99.978 ± 0.245
Trandolapril	0.1	99.932 ± 0.117	99.879 ± 2.672
	0.4	99.966 ± 0.029	99.555 ± 1.197
	0.8	99.907 ± 0.130	100.116 ± 0.666

*Each value is obtained from five experiments (n = 5).

Table 5. Determination of verapamil and trandolapril in laboratory-made mixtures

Compounds (μg/mL)		Found*		Recovery%		Bias%		RSD%	
VER	TRA	VER	TRA	VER	TRA	VER	TRA	VER	TRA
1	0.2	1.013	0.197	101.30	98.50	-1.3	1.5	2.187	1.943
5	0.2	4.989	0.200	99.78	100.00	0.22	0	0.132	0.559
12	0.2	12.004	0.199	100.03	99.50	-0.03	0.5	0.288	1.700
18	0.2	18.013	0.199	100.07	99.50	-0.07	0.5	0.077	1.188
Mean Recovery				100.30	99.38				
RSD%				0.671	1.348				
12	0.05	11.999	0.050	99.99	100.00	0.01	0	0.340	2.692
12	0.10	12.019	0.103	100.16	103.00	-0.16	-3.0	0.057	0.915
12	0.20	11.985	0.200	99.88	100.00	0.12	0	0.159	1.121
12	1.00	11.991	1.034	99.93	103.40	0.07	-3.4	0.198	0.869
Mean Recovery				99.99	101.60				
RSD%				0.189	1.399				

*The mean of five experiments.

Table 6. Results of the assay and the recovery analysis of verapamil and trandolapril in pharmaceutical dosage forms

	Verapamil	Trandolapril
Labeled claim (mg)	240.00	4.00
Amount found (mg)[a]	239.87	3.96
RSD (%)	0.14	1.56
Bias (%)	0.05	1.05
Added (mg)	240.00	4.00
Found (mg)[a]	240.04	4.01
Recovery (%)	100.02	100.12
RSD% of recovery	0.085	0.35
Bias (%)	-0.016	-0.116

[a] Each value of the mean five experiments.

solutions were examined by analyzing standard solutions of the compounds in the mobile phase aged at +4°C, in the dark against a freshly prepared sample. The results demonstrated that the working reference solutions were stable for up to one week. The obtained peak area ratio for the assay reference solutions did not change considerably over a week period.

CONCLUSIONS

This work presents the first study dealing with the determination of pK_a values of VER and TRA by chromatographic method in MeOH-water binary mixtures. The pK_a values of studied compounds are useful for facilitating occurrence, effects, and control of ACE inhibitors in scientific studies for investigators. Dependence of retention of ACE inhibitors on pH of the mobile phase was determined using LC methodology and the results were applied for the determination of pK_a values.

Using the chromatographic system presented here, the separation of weakly basic and amphoteric compounds has been performed. This was attributed to the careful selection of the column, which decreased the negative interactions between the stationary phase and the charged basic species that can lead to peak broadening. The proposed method gives a good resolution between VER, TRA and internal standard within a short analysis time. The chromatographic system can be used to concurrent analysis of VER and TRA within a short analysis time (< 7 min), without either interfering in the detection or quantitation of the other inactive ingredients or possible interferences. The developed HPLC method was validated by evaluation of the required validation parameters. The LOD and LOQ values, precision, accuracy, resolution, selectivity, tailing, and capacity factors, etc were obtained. The present study proposes a rapid, simple, sufficiently precise, and accurate method for the simultaneous determination of VER, TRA in raw material and pharmaceutical dosage forms. High percentages of recovery show that the method is free from the interferences of the commonly used excipients and additives in the formulations of the drug. The proposed method is more sensitive, rapid and precise than the literature method. The assay method can be used as a versatile analytical tool suitable for the simultaneous analysis of these drugs and would be of interest for quality control and clinical monitoring laboratories to evaluate product performance of a combined tablet dosage forms.

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