e-ISSN: 2687-6698 **Research Article**

Turkish Journal of Analytical Chemistry
www.turkjac.org
2019
TurkJAC

Development and validation of RPLC method for the simultaneous analysis of ACE inhibitors in tablet formulations

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Abstract

This study aimed to develop and validate an HPLC method for the determination of ACE inhibitors benazepril, cilazapril and quinapril in tablet formulation. To determine the optimum liquid chromatographic separation condition, a systematic approach based on the relationship between mobile phase pH and retention time was used. ACE inhibitors were separated on a YMC Triart C18 (3 µm, 150 x 4.6 mm I.D.) column in an acetonitrile-water binary mixture containing 45% (v/v) acetonitrile adjusted to pH 3. Flow rate 0.5 mL/min, column temperature 37 °C and UV detector wavelength 210 nm were determined as optimum chromatographic conditions for the study. The method showed excellent linearity in the concentration range of 5 – 35 µg/mL for benazepril and cilazapril and 0.5 – 85 µg/mL for quinapril. Mean recovery values were found to be 98.663 ± 1.203 for cilazapril, 99.404 ± 0.864 for benazepril and 99.264 ± 0.626 for quinapril. The proposed method is suitable for the simultaneous separation and quantitative determination of drugs.

Keywords: Hypertension, ACE inhibitors, RPLC, pK_a

1. Introduction

Hypertension can be defined as an increase in arterial blood pressure above normal limits. When this condition is not well controlled, it seriously affects the structure and functions of many organs in the body. In hypertensive patients who are effectively treated, significant reductions in the risk of stroke, heart failure, and myocardial infarction occur [1]. Many drug groups are used in the treatment of hypertension. However, the most preferred groups of these drugs are angiotensin-converting enzyme (ACE) inhibitors [2]. ACE inhibitors can be divided and classified into three broad groups based on chemical structure: (1) ACE inhibitors that are structurally sulfhydryl-containing (e.g. captopril) (2) ACE inhibitors that are dicarboxylic-containing (e.g. cilazapril, quinapril, lisinopril, moexipril, benazepril, ramipril, trandolapril, perindopril) and (3) ACE inhibitors that are phosphorus-containing (e.g. fosinopril) [3–5]. Benazepril, cilazapril, and quinapril selected for this study are ACE inhibitors containing carboxyl groups (Fig. 1).

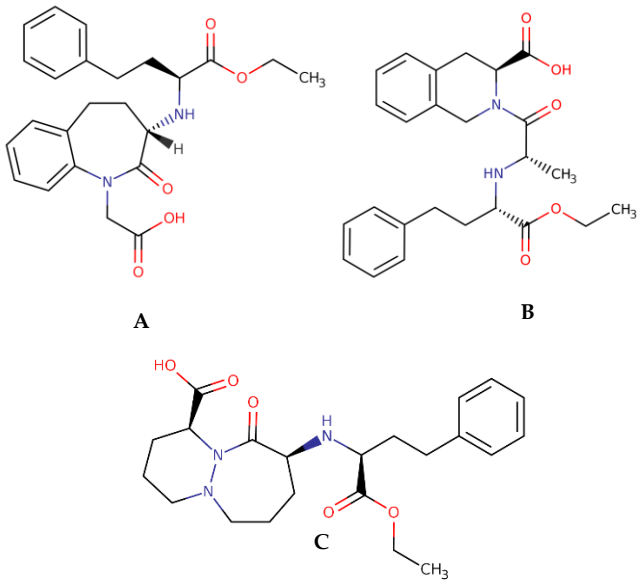

Figure 1. Chemical structure of studied compounds (A) benazepril, (B) quinapril, and (C) cilazapril

Citation: E. Kuzucanli, E.Ç. Demiralay, Y.D. Daldal, Z. Üstün, İ. Konçe, G. Alsancak, Development and validation of RPLC method for the simultaneous analysis of ACE inhibitors in tablet formulations, Turk J Anal Chem, 4(2), 2022, 103–110.
https://doi.org/10.51435/turkjac.1176649

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Received: September 17, 2022
Accepted: November 11, 2022

Benazepril is a potent ACE inhibitor in its group that is converted to benazeprilat with cleavage of the ester group by hepatic esterase. Quinapril is a prodrug that is converted to quinaprilat. This drug is almost as potent as benazeprilat by liver esterase with cleavage of the ester groups. Cilazapril is a new ACE inhibitor that does not contain thiol and sulfhydryl groups in the same group as these two compounds [3].

The discovery and design of a compound used as a drug in pharmaceutical chemistry, and the development of effective analytical methods for chemical analysis and quality control are important. Capillary electrophoresis (CE), high performance liquid chromatography (HPLC), and electrochemical methods are widely used for the analysis of compounds in drug formulations and biological fluids [6]. Among these methods, reverse phase liquid chromatography (RPLC) is more preferred due to its advantages such as accuracy, precision, and reproducibility of the measurements [7–10]. The primary purpose of RPLC studies is to ensure that the studied compounds are separated from each other as soon as possible or to make simultaneous determinations provided that certain validation conditions ICH parameters are met [11,12].

In the RPLC method, the parameters known to affect the retention factor (k) values of the compounds are changed individually or randomly to determine the optimum separation condition in most studies. While this situation causes unnecessary time and material loss, in some cases, it is insufficient in determining the separation condition [13–17]. To determine the chromatographic working conditions, it is necessary to optimize the chromatographic conditions (column temperature, mobile phase pH and mobile phase organic modifier concentration, etc.) in the developed method instead of this trial-and-error method [14–18].

Solvent optimization in the RPLC method is commenced by selecting a binary mobile phase of the correct solvent strength to elute the compound with an acceptable range of k values ($1 < k < 10$) and selectivity factor ($\alpha \geq 1.15$). Solvents such as methanol, acetonitrile, and tetrahydrofuran, which are commonly used in RPLC, are mixed with water at different ratios to ensure sufficient retention and chromatographic separation can be easily estimated. At very low water content the properties of the mobile phase depend largely on the properties of the organic modifier. Thus, the k value can be adjusted to the desired value by changing the mobile phase composition or solvent strength. The change in mobile phase pH also affects the selectivity in the separation of compounds. If the compounds are acidic or basic, the change in selectivity can be easily predicted. While temperature change has a minor effect on the retention of neutral compounds, it has a significant effect on the retention factor for ionizable compounds. In finding the best separation condition, the temperature change acts as an organic solvent [19].

In this study, the selected ACE inhibitors cilazapril, benazepril, and quinapril were determined by the RPLC method alone or simultaneously [18–23]. In addition, there are few studies on the determination of optimum conditions of compounds with the experimental design method related to ACE inhibitors [24–26] In this study, the change in k values depending on the pH of the mobile phase and the organic modifier concentration in the mobile phase was investigated at two different column temperatures (25 – 37 °C) to determine the optimum separation conditions of the selected compounds. With this study, the simultaneous determination of the compounds was made without trial and error. In addition, the method developed was validated according to the International Conference on Harmonization (ICH) and Association of Official Analytical Chemists (AOAC) parameters [12,27] and then quantitative determinations in drug formulations were performed.

2. Materials and methods

2.1. Apparatus

The Shimadzu HPLC system (Shimadzu Technologies, Kyoto, Japan) was used for the liquid chromatographic study. The system consists of a pump (LC-20AD), UV detector (SPD-20A), column oven (CTO-20A), and degassing unit (DGU-20A3). pH measurements of the RPLC mobile phase were performed using Mettler Toledo MA 235 pH/Ion analyzer (Schwerzenbach, Switzerland) and InLab 413 Ag/AgCl combined glass electrode.

2.2. Chemicals

In this study, benazepril, cilazapril, quinapril, pravastatin, and uracil were purchased from Sigma-Aldrich (USA). Acetonitrile was used as an organic solvent in the preparation of the mobile phase, o-phosphoric acid and sodium hydroxide were used as buffer components in the mobile phase, and potassium hydrogen phthalate was used as the primary standard reference in electrode calibration was supplied from Merck (Darmstadt, Germany). All chemicals used in the study are of analytical purity.

2.3. Chromatographic study

In this study, the acetonitrile-water binary mixture containing 45% (v/v) acetonitrile was prepared as a mobile phase for the chromatographic determination of the compounds. o-phosphoric acid (85%, w/w) was added to the mobile phase medium at 25 mM and 1 M

NaOH solution was added to reach the desired mobile pH. Six mobile phases with pH ranging between 2.5 and 5.0 were prepared. The mobile phases were used after degasification in an ultrasonic bath. Chromatographic separation was carried out in a YMC Triart C18 column (3µm, 150 × 4.6mm I.D.). The column oven temperature was set at 37 °C, the flow rate was 0.5 mL/min, and the injection volume was 20 µL. The UV detector was set at 210 nm wavelength.

2.4. Preparation of standard solutions

Stock solutions of compounds were prepared by dissolving in the mobile phase at a concentration of 100 µg/mL for qualitative analysis and 50 µg/mL for calibration. The internal standard (IS) pravastatin (20 µg/mL) was prepared in the same way. For the calibration study, the stock solutions prepared for the working concentration range of each compound were diluted with the mobile phase. The IS concentration was kept constant at 0.5 µg/mL throughout the study. Benazepril and cilazapril were prepared in a concentration range of 1 – 15 µg/mL and quinapril 0.5 – 8 µg/mL.

2.5. Robustness test

The robustness of the method was evaluated according to the system suitability parameter data by analyzing the studied compounds after changing the flow rate (± 0.2), organic modifier content (± 0.5), pH of the mobile phase (± 0.5), and column temperature.

2.6. Analysis of tablets

For quantitative determination of benazepril, cilazapril, and quinapril tablet analysis was performed. In this method, ten tablets were finely powdered and weighted in an equivalent amount to 1 tablet. Then, the powder in the amount of one tablet was put into the volumetric flask and by adding the mobile phase, its volume was made up to 100 mL. To dissolve the active ingredients of the drugs determined in the prepared sample solutions, the solutions were kept in an ultrasonic bath for 20 minutes. The insoluble part in the prepared solution was removed by filtration. Finally, the solution was prepared at different dilution ratios according to the concentration in the calibration range specified for each compound.

2.7. Recovery experiment

A recovery study was conducted to determine the reliability and suitability of the proposed method. Both sample and recovery processes were performed in five replications. This study was carried out by adding a known amount of pure standard and selected internal standard to the tablet sample containing the analyzed active substance. Recovery percentages were calculated using the obtained data.

3. Results and discussion

Benazepril, cilazapril, and quinapril are compounds containing both acidic and basic functional groups. In the chemical structure of these compounds, there is a carboxylic acid as an acidic functional group and a secondary amine as a basic functional group. The retention of compounds with ionizable functional groups in the HPLC column varies according to the pH value of the mobile phase. Determination of ionization/protonation constant (pK_a) values is necessary to predict the ionization of the compound at a given pH [28]. Lipophilicity expressed as logP, must be known in chromatographic analyses. The increase in this value is a result of the compound's high affinity in the RPLC column [19]. The calculated pK_a and logP values for the studied compounds are given in Table 1.

In this study, a mobile phase optimization study was performed to determine the optimum separation condition in the quantitative determination of cilazapril, benazepril, and quinapril used in the treatment of hypertension by the RPLC method. With knowing the pK_a values of the compounds, it is possible to determine the pH values at which they are in molecular or ionized form. For this, pH values above and below 1.5 units of pK_a value are determined as working pH ranges. For this, the effects of column temperature, acetonitrile concentration of the mobile phase, and pH change on the retention factors of the compounds were investigated by keeping the chromatographic conditions constant. The t_0 value used in the calculation of the k value was determined using the standard uracil solution used as the non-retained species in the column. The k values at each pH value (2.5 – 5.0) studied were calculated by using the t_R and t_0 values of the compounds in the acetonitrile water binary mixture containing %40 (v/v), 45% (v/v) and %50 (v/v) acetonitrile. Compounds were highly retained on the HPLC column in 40% acetonitrile medium at 37 °C. The k values are higher than the 45% acetonitrile medium. In a 50% acetonitrile medium, k values of cilazapril were calculated below 1 in the pH range studied. The k values at 25 °C are greater than at 37 °C. In liquid chromatographic studies, it is aimed to complete the analyses as soon as possible. Also, the k value must be ≥ 1.

Table 1. pK_a and logP values of compounds [29]

Compounds	pK_a		logP
Benazepril	pK_{a1}: 3.04	pK_{a2}: 4.74	1.11
Quinapril	pK_{a1}: 3.71	pK_{a2}: 5.12	1.38
Cilazapril	pK_{a1}: 3.26	pK_{a2}: 4.49	-0.23

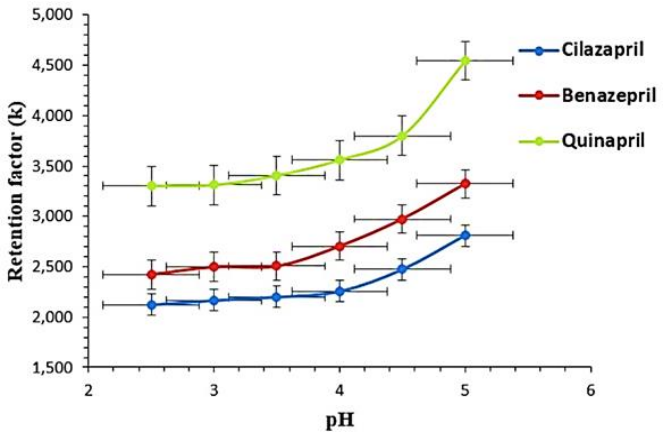

Figure 2. Sigmoidal behavior showing the relationship between mobile phase pH and *k* values of compounds

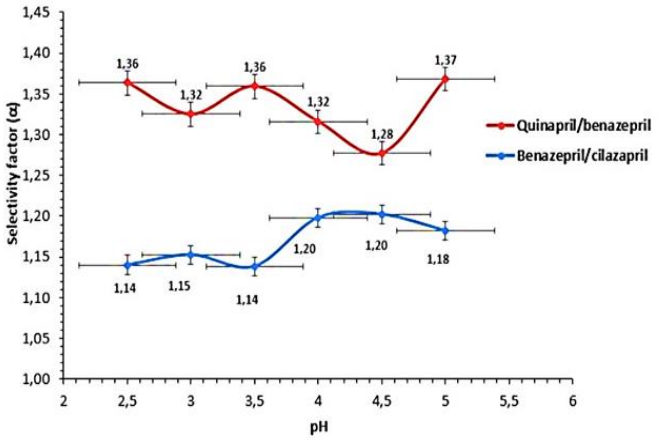

Figure 3. Variation in α values for compound pairs with mobile phase pH

For this purpose, according to the results of acetonitrile concentration in three different concentrations, the analyzes performed at 40% (v/v) acetonitrile and 37 °C were chosen as the appropriate conditions. In this condition, the sigmoidal behavior of the basic functional group was observed when the k values of the Compounds were plotted against the mobile phase pH values (Fig. 2).

Mid-point of sigmoidal curves gives the pK_a value of the compound in the water-acetonitrile binary mixture studied. The situation where pH = pK_a is not the optimum condition for separation. At this pH value, tailing is observed in the peaks of the compounds. This is undesirable for quantitative determination [7]. At pH 2.5, where ionization takes place, the compounds are retained little in the HPLC column. Therefore, pH values of the mobile phase are preferred for separation. In addition, optimum chromatographic separation occurs if the k values of the compounds are in the range of $1 \leq k \leq 10$, the selectivity factor (α) is greater than 1.15 and the peak resolution (R_s) value is greater than 1.5. The selectivity factor is calculated by dividing the retention factor (k_2) of the second peak by the retention factor of the first peak (k_1). When Fig. 3 is examined, the α value is below 1.15 at pH 2.5 and 3.5. Benazepril and cilazapril did not differ from each other at these pH values. Separation should occur as soon as possible in a chromatographic assay. For this, an acetonitrile-water binary mixture containing 45% (v/v) acetonitrile adjusted to pH 3.0 was determined as the condition in which the specified chromatographic parameters were met.

The Purnell equation (Eq. 1) shows the relationship between the α, R_s, and k values.

$$R_S = \frac{1}{4}\sqrt{N}\left[\frac{(\alpha-1)}{\alpha}\right]\left[\frac{k_2}{(1+k_2)}\right] \qquad (1)$$

For this reason, the R_s value between the two peaks must be calculated using this equation in the qualitative determination. The values calculated according to the Purnell equation under this mobile phase condition are given in Table 2.

Since cilazapril is a more hydrophilic compound compared to quinapril and benazepril (Table 1), it cannot be determined simultaneously with hydrophobic compounds in conventional C18 and C8 columns. YMC Triart C18 column (3 µm, 150 x 4.6 mm I.D.) chosen in this study is a column with moderate hydrogen bonding capacity and provides simultaneous separation of both hydrophobic and hydrophilic compounds. In addition, it is a column that has a better peak shape than conventional C18 columns [30].

After the optimization of the liquid chromatographic method developed in the study, method validation was performed for the quantitative determination of the compounds. A widely used technique of quantitation involves the addition of an internal standard (IS) to compensate for errors in the analytical measurements [31,32]. The IS method is preferred to exclude systematic and random errors such as additives in drug formulations and volume errors during sample injection. When the internal standard is selected, it must be chromatographically separated from the compounds determined under optimal separation conditions. In this study, pravastatin was selected as the IS. Under the selected optimal separation conditions, pravastatin could be retained in this column because it was present in its molecular form. The chromatogram obtained under the optimal separation conditions is shown in Fig. 4.

Table 2. Calculated data of compounds at optimum separation condition

Compounds	k_2	α	k_2/k_2+1	$(\alpha-1)/\alpha$	$(\frac{1}{4})\sqrt{N}$	R_s
Pravastatin (I.S)	1.009					
Cilazapril	2.168	2.149	0.684	0.535	14.703	5.379
Benazepril	2.499	1.153	0.714	0.132	18.668	1.766
Quinapril	3.311	1.325	0.768	0.245	14.389	2.710

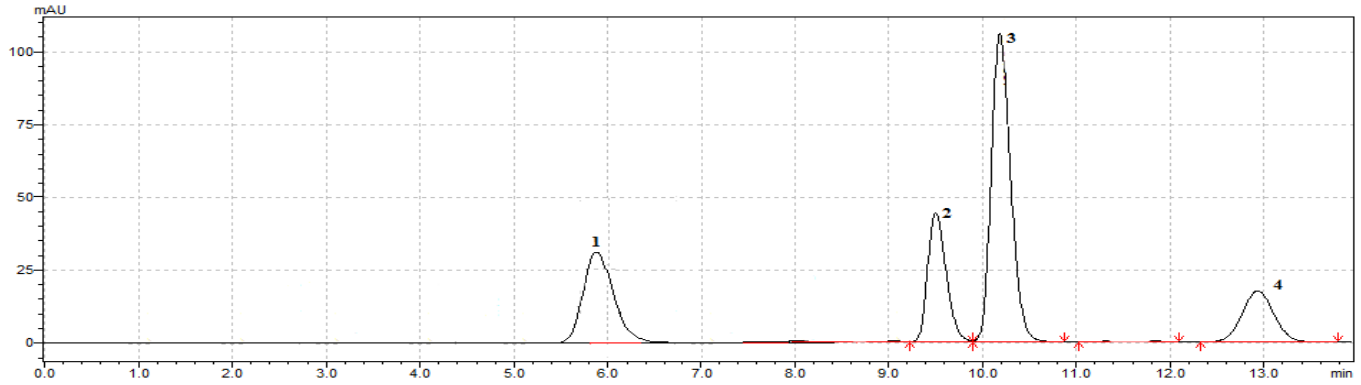

Figure 4. The chromatogram obtained according to the optimum separation condition determined: (1) pravastatin (I.S), (2) cilazapril, (3) benazepril, (4) quinapril

Table 3. System suitability parameters for compounds

Parameters	P (I.S)	C	B	Q	R.V.
t_R	6.017	9.503	10.186	12.934	—
Tailing factor (T_f)	1.183	1.113	1.159	1.051	≤ 2
Retention factor (k)	1.009	2.116	2.373	3.241	≥ 1
Peak resolution (R_s)	—	5.221	2.109	2.548	≥ 2
Theoretical plates (N)	6540	10630	11398	6821	≥ 2000
Separation factor (α)	—	2.097	1.188	1.289	>1
RSD% (t_R, for retention time)	0.992	0.126	0.160	0.189	≤ 1
RSD% (for peak area)	0.537	0.294	0.398	0.245	≤ 1

P: Pravastatin, C: Cilazapril, B: Benazepril, Q: Quinapril, R.V.: Recommended value

Table 4. Calibration curve parameters for the analysis of compounds

Parameters	Cilazapril	Benazepril	Quinapril
Regression Equation	y = 1.848x - 0.376	y = 4.498x - 0.855	y = 3.244x - 0.093
Standard error of slope	0.013	0.033	0.022
Standard error of intercept	0.111	0.293	0.084
Correlation Coefficient (r)	0.999	0.999	0.999
Linearity Range (µg/mL)	1 – 15	1 – 15	0.5 – 8
Limit of Detection (LOD) (µg/mL)	0.277	0.299	0.158
Limit of Quantification (LOQ) (µg/mL)	0.838	0.906	0.478

Once the optimal separation conditions were determined, the suitability of the chromatographic system was determined according to the U.S. Pharmacopoeia 24th (USP) and AOAC guidelines [12,33]. For this purpose, chromatographic parameters were calculated by injecting the compounds into the HPLC system (Table 3).

The results of system suitability parameters according to USP (Table 3) showed that the developed chromatographic method was suitable for the analysis and analytical method validation part [33].

In the system suitability test according to the AOAC guideline, the retention times and %RSD of the peak areas of the three compounds are below 2%. This indicates that the change in repeatable injections is small [12]. The tailing factor showing the symmetry of the analyte peak is also below 2%.

A calibration curve was prepared to determine the linearity of the developed method. Linear regression parameters of the peak area ratios versus concentrations of benazepril, cilazapril, and quinapril were presented in Table 4. The limit of detection (LOD) and limit of quantitation (LOQ) were measured for studied compounds. These parameters were determined according to 3.3:1 and 10:1 signal / noise ratios. The described method was linear for the three compounds. The results meet the acceptance criteria according to the ICH and AOAC guidelines, which stated that the coefficient of determination should be > 0.999 [11,12].

Intraday (**repeatability**) and interday (**reproducibility**) precision were determined by injecting two different concentrations at three different times on the same day and these same concentrations on three different days. These results are reported in Table 5. The results are sufficiently accurate and the relative standard deviation (% RSD) values of the results calculated from the analyzes performed with five replicates are below 2% [11].

Table 5. Intraday and interday precision analysis results of the analysis method of compounds

Compounds	Theoretical concentration (µg/mL)	Intraday measured concentration mean (µg/mL)	% RSD	Hor[1]	Interday measured concentration mean (µg/mL)	% RSD	HoR[2]
Cilazapril	2.500	2.571	0.830	0.060	2.787	1.240	0.089
	12.000	12.321	0.560	0.051	12.838	1.553	0.361
Benazepril	2.500	2.515	0.552	0.035	2.678	1.227	0.077
	12.000	12.457	0.799	0.065	12.671	1.017	0.083
Quinapril	1.000	0.892	0.152	0.011	0.932	1.089	0.078
	6.000	6.550	0.938	0.085	6.691	0.754	0.068

[1] Horrat ratio for repeatability
[2] HoRrat ratio for reproducibility

Table 6. Robustness results of the developed method

Parameter	Retention time (t_R)			Peak area (mAu)		
	Cilazapril	Benazepril	Quinapril	Cilazapril (5 μg/mL)	Benazepril (5 μg/mL)	Quinapril (5 μg/mL)
Optimize condition	9.503	10.186	12.934	354856	861931	259040
Increased organic modifier (50:50)	6.211	7.598	9.775	360048	874221	260064
Decreased organic modifier (40:60)	14.225	15.004	17.066	352064	861277	260448
Increased pH of the mobile phase (pH 3.5)	9.749	10.366	13.147	360447	866288	260412
Decreased pH of the mobile phase (pH 2.5)	9.348	9.938	12.251	355200	860882	258136
Increased flow rate (0.8 mL/min)	7.105	8.433	10.004	354926	862006	258611
Decreased temperature (25 °C)	10.245	11.587	14.688	355706	864072	260449

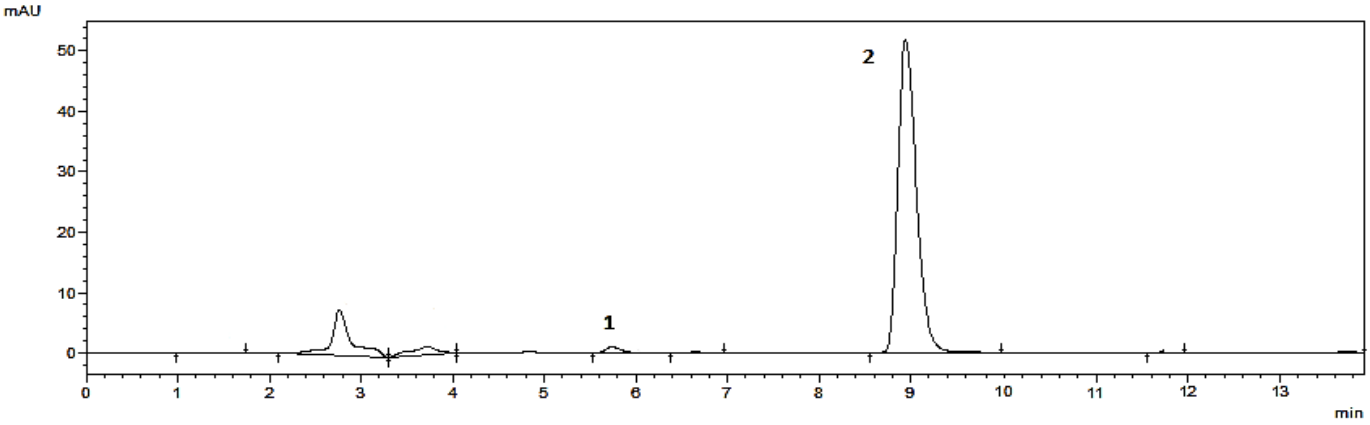

Figure 5. Chromatogram of Inhibace (2.5 mg) tablet sample containing cilazapril. (1) Pravastatin (I.S.) (0.5 μg/mL) and (2) Cilazapril (5 μg/mL)

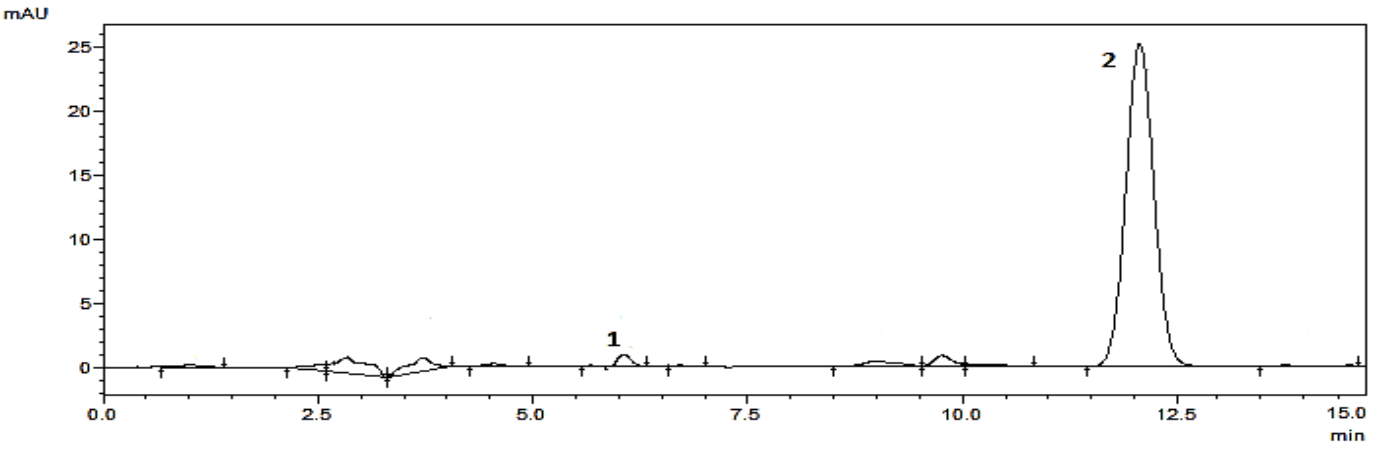

Figure 6. Chromatogram of Cibacen (10 mg) tablet sample containing benazepril. (1) Pravastatin (0.5 μg/mL) (I.S.) and (2) Benazepril (5 μg/mL)

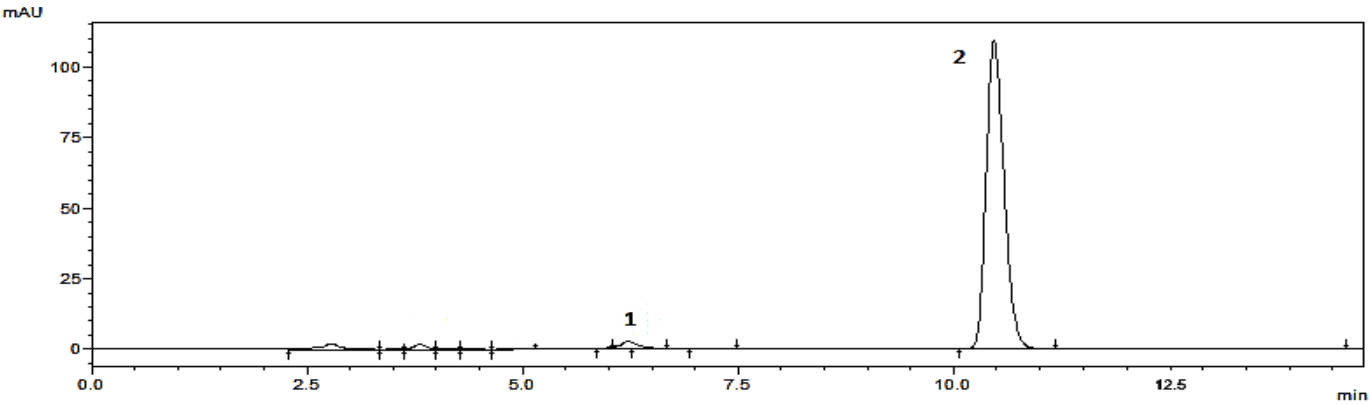

Figure 7. Chromatogram of Cibacen (10 mg) tablet sample containing benazepril. (1) Pravastatin (0.5 μg/mL) (I.S.) and (2) Benazepril (5 μg/mL)

Table 7. Result of assay and the recovery analysis of studied compound in tablet formulations

	Cilazapril		Benazepril		Quinapril	
	Quantity found (mg)	Recovery (%)	Quantity found (mg)	Recovery (%)	Quantity found (mg)	Recovery (%)
1	2.490	99.616	9.999	99.992	4.956	99.124
2	2.437	97.473	9.894	98.940	4.960	99.207
3	2.474	98.942	9.882	98.824	4.951	99.018
4	2.512	99.122	9.905	99.144	5.009	100.030
5	2.488	98.164	9.942	99.404	4.957	99.264
Mean±CI*	2.480± 0.039	98.663 ± 1.203	9.942 ± 0.096	99.404 ± 0.864	4.957 ± 0.050	99.264 ± 0.626
SD	0.028	0.846	0.068	0.608	0.035	0.440
% RSD	1.118	0.857	0.680	0.611	0.704	0.443
% Bias	-0.792	-1.337	-0.580	-0.596	-0.852	-0.736

* Confidence interval at 95% confidence level

In addition, precision values are calculated from the Horwitz (HorRat) equation [34], which represents an empirical relation between the acceptable precision and the corresponding analyte concentration in the sample. HorRat value was calculated in accordance with AOAC guidelines. HorRat(r), HorRat values for repeatability is calculated as RSD_r/PRSD(R), HorRat(R), HorRat values for reproducibility is calculated as RSD_R/PRSD(R) (PRSD(R) = $2^{(1-0.5\log C)}$, C is the mass concentration expressed in the power of 10, i.e 1 µg/g = 10^{-6}). The AOAC Guidelines suggested that HorRat values < 2. The repeatability and reproducibility results for studied compounds showed HorRatr values of < 2, which complied with the AOAC guidelines.

For the quantification of cilazapril, benazepril, and quinapril in tablet formulations, tablet solutions were prepared as described in the "Material and Method" section, and the ratio of the peak area of the analyzed compounds to the peak area values of pravastatin because of the analysis was evaluated in the corresponding calibration functions. Then, the number of active compounds contained in the tablets was calculated (Table 7). Chromatograms showing the analysis of the tablet samples were given in Fig. 5 for cilazapril, Fig. 6 for benazepril, and Fig. 7 for quinapril.

The robustness of an analytical procedure refers to its ability to remain unaffected by small changes in method parameters and changes in quantitative results. None of the changes caused a significant change in the peak area and tailing factor of the compounds (Table 6). Quantitative determination of compounds is possible, although changes in retention times are more significant. The reproducibility of the results obtained because of the small changes in this study has proven the method to be robust.

The accuracy of an analytical method refers to the closeness of agreement between the accepted reference value and the found value. Accuracy studies were performed by adding the standard determined at a certain concentration. Accuracy is expressed as percent recovery and is calculated from the slope and intercept values of the calibration curve. The calculated data were given in Table 7. The calculated average recovery values show that the accuracy of the method is high and that the excipients in the sample medium have no effect.

The average % recovery of three compounds complied with the AOAC Guidelines, which stated that this value should be between 92 – 105% [12].

Experimental results of the amount of benazepril, cilazapril, and quinapril in the selected commercial tablets, expressed as a percentage of label claims were in good agreement with the label claims. The calculated percent recoveries show that the sample preparation le preparation techniques developed for the quantification of the compounds studied are not affected by interferences.

4. Conclusion

The mobile phase was optimized for the quantitative study under the chromatographic conditions determined in this study. In the studies conducted in the literature, the trial-and-error method has been widely used, apart from the experimental design method. In this study, the mobile phase was optimized for the chromatographic separation of ionizable compounds using the pH-*k* relationship. For this, the retention time of cilazapril, benazepril, and quinapril and the suitability of other chromatographic parameters were determined based on the effect of the percentage of organic solvent and pH of the mobile phase. The developed method was validated, and sufficient results were obtained for all the controlled validation parameters. RSD value was calculated below 2% in determining the precision in quantitative analysis of compounds in tablet formulations by RPLC analysis. The evaluation of the obtained results showed that the developed method is suitable for routine use.

Acknowledgment

This work was supported by Süleyman Demirel University Scientific Research Projects Coordination (Project 4578-YL2-16).

References

[1] I. Maraj, J.N. Makaryus, A. Ashkar, S.I. McFarlane, A.N. Makaryus, Hypertension management in the high cardiovascular risk population, Int. J. Hypertens, 2013, 2013, 1–7.

[2] N.J. Brown, D.E. Vaughan, Angiotensin-converting enzyme inhibitors, Circulation, 97, 1998, 1411–1420.

[3] L. Brunton, B. Chabner, B. Knollman, Goodman and Gilman's The Pharmacological Basis of Therapeutics (9. edition), 1996, New York, McGraw-Hill.

[4] R. Kumar, R. Sharma, K. Bairwa, R. Kumar Roy, A. Kumar, A. Baruwa, Modern development in ACE inhibitors, Der Pharmacia Lettre, 2(3), 2010, 388–419.

[5] B.G. Katzung, Basic & Clinical Pharmacology (12. edition), 2011, San Francisco, The McGraw-Hill Companies.

[6] S.D. Firke, K.R. Bhoi, K.R. Patil, S.D. Jayswal, A.R. Tekade, S.S. Chalikwar, A state-of-the-art review on applications of different analytical techniques for some ace inhibitors, Anal. Chem. Lett., 10(1), 2020, 60–79.

[7] Y. Kazakevich, Y. Lobrutto, HPLC for Pharmaceutical Scientists (1. edition), 2007, Canada, Wiley-Interscience.

[8] V.R. Meyer, Practical High-Performance Liquid Chromatography (16. edition), 2010, United Kingdom, John Wiley and Sons, Ltd.

[9] Y.D. Daldal, E.Ç. Demiralay, S.A. Ozkan, Effect of organic solvent composition on dissociation constants of some reversible acetylcholinesterase inhibitors, J. Braz. Chem. Soc., 27(3), 2016, 493–499.

[10] Y.D. Daldal, E.Ç. Demiralay, Chromatographic and UV–visible spectrophotometric p*K*a determination of some purine antimetabolites, J. Mol. Liq., 317, 2020, 1–8.

[11] ICH, Validation of Analytical Procedures: Text and Methodology Q2 (R1) Harmonized Tripartite Guideline 2005.

[12] AOAC International (2016) Guidelines for Standard Method Performance Requirements AOAC Official Methods of Analysis. Appendix F, 1-18.

[13] E.Ç. Demiralay, D. Koç, Y.D. Daldal, C. Çakır, Determination of chromatographic and spectrophotometric dissociation constants of some beta lactam antibiotics, J. Pharm. Biomed. Anal, 71, 2012, 139– 143.

[14] E.Ç. Demiralay, Z. Üstün, Y.D. Daldal, Estimation of thermodynamic acidity constants of some penicillinase-resistant penicillins, J Pharm Biomed Anal, 91, 2014, 7– 11.

[15] E.Ç. Demiralay, An experimental design approach to optimization of the liquid chromatographic separation conditions for the determination of metformin and glibenclamide in pharmaceutical formulation, Acta Chim Slov, 59(2), 2012, 307–314.

[16] T. Lundsted, E. Seifert, L. Abramo, B. Thelin, A. Nyström, J. Pettersen, R. Bergamn, Experimental design and optimization, Chemom Intell Lab Syst, 42, 1998, 3–40.

[17] S. Bolton, C. Bon, Pharmaceutical Statistics Practical and Clinical Application (4. edition), 2004, Boca Raton, CRC Press.

[18] D. Bonazzi, R. Gotti, V. Andrisano, V. Cavrini, Analysis of ace inhibitors in pharmaceutical dosage forms by derivative uv spectroscopy and liquid chromatography (hplc), J Pharm Biomed Anal, 16(3), 1997, 431–438.

[19] F. Colin, K. Poole, Chromatography today. Elsevier Science, First Edition, Amsterdam, 1991

[20] A. Gumieniczek, H. Hopkala, High-performance liquid chromatographic assay of quinapril in tablets, Pharm Acta Helv, 73(4), 1998, 183–185.

[21] J.A. Prieto, R.M. Jiménez, R.M. Alonso, Quantitative determination of the angiotensin-converting enzyme inhibitor cilazapril and its active metabolite cilazaprilat in pharmaceuticals and urine by high-performance liquid chromatography with amperometric detection, J Chromatogr B Biomed Appl, 714(2), 1998, 285–292.

[22] L. Manna, L. Valvo, S. Alimonti, A liquid chromatographic lon-pairing method for simultaneous determination of benazepril hydrochloride, fosinopril sodium, ramipril and hydrochlorothiazide in pharmaceutical formulations. Chromatographia, 53(1), 2001, 271–275.

[23] F. Elsebaei, Y. Zhu, Fast gradient high performance liquid chromatography method with uv detection for simultaneous determination of seven angiotensin converting enzyme inhibitors together with hydrochlorothiazide in pharmaceutical dosage forms and spiked human plasma and urine. Talanta, 85(1), 2011, 123–129.

[24] S. Kiran, T.Y. Pasha, M. Asif, M. Kamal, T. Jawaid, M.A Alossaimi, B. Ramesh, M. Majumder, Development and validation of a reverse phase-high performance liquid chromatography method for the assay of benazepril hydrochloride using a quality-by-design approach, Lat Am J Pharm, 41(5), 2022, 931–943.

[25] B. Otašević, J. Šljivić, A. Protić, N. Maljurić, A. Malenović, M. Zečević, Comparison of aqbd and grid point search methodology in the development of micellar hplc method for the analysis of cilazapril and hydrochlorothiazide dosage form stability, Microchem J, 145, 2019, 655–663.

[26] S.M. Auwal, M. Zarei, C.P. Tan, M. Basri, N. Saari. Enhanced physicochemical stability and efficacy of angiotensin i-converting enzyme (ace) - inhibitory biopeptides by chitosan nanoparticles optimized using box- behnken design, Sci Rep, 8, 2018, 1–11.

[27] ICH, International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use, Validation of analytical procedure: Text and Methodology Q2 (R1), 2005

[28] D.T. Manallack. The pK_a distribution of drugs: application to drug, Perspect Medicin Chem, 1, 2007, 25–38.

[29] M. Remko, Acidity, lipophilicity, solubility, absorption, and polar surface area of some ace inhibitors. Chem Zvesti CHEM PAP, 61(2), 2007, 133–141.

[30] Y.D. Daldal, E.Ç. Demiralay, Development of liquid chromatographic and uv-visible spectrophotometric methods for determination of pK_a values of folic acid antimetabolites, J Pharm Biomed Anal, 212, 2022,1–8.

[31] A. Vonaparti, M. Kazanis, I. Panderi, Development and validation of a liquid chromatographic/electrospray ionization mass spectrometric method for the determination of benazepril, benazeprilat and hydrochlorothiazide in human plasma, J Mass Spectrom, 41, 2006, 593–605.

[32] S.K. Paszun, B, Stanisz, W. Pawlowski, Rapid and simple stability indicating hplc method for the determination of cilazapril in pure substance and pharmaceutical formulation in comparison with classic and derivative spectrophotometric methods, Acta Pol Pharm, 69(2), 2012, 193–201.

[33] R. McNally, The United States Pharmacopoeia, 24th revision, Q5 Taunton, M.A, 2000.

[34] W. Horwitz, L.R. Kamps, K.W. Boyer, Quality assurance in the analysis of foods and trace constituents, J Assoc Anal Chem, 63, 1980, 1344–1354.