DETERMINATION OF RAMIPRIL IN PHARMACEUTICAL PREPARATIONS BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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ABSTRACT

The aim of the present study was to develop a procedure based on high-performance liquid chromatography (HPLC) for determination of ramipril in pharmaceutical preparations. Separation of ramipril was achieved on a Ace C_{18} column (5 µm, 250×4.6 mm i.d.) using UV detection with λ=208 nm. The mobile phase consisted of 20 mM phosphate buffer (pH 2.5) containing 0.1% trifluoroacetic acid (TFA)-acetonitrile (50:50, *v/v*). The analysis was performed in less than 5 min with a flow rate of 1.0 mL min^{-1}. Calibration curve was linear over the concentration range of 0.25-7.5 µg mL^{-1}. Intra- and inter-day precision values for ramipril were less than 4.95, and accuracy (relative error) was better than 4.00%. The mean recovery of ramipril were 99.7% for pharmaceutical preparations. The limits of detection (LOD) and quantification (LOQ) were 0.10 and 0.25 µg mL^{-1}, respectively. Also, the method was successfully applied for the quality control of commercial ramipril dosage forms to quantify the drug and to check the formulation content uniformity.

Keywords: Ramipril, Pharmaceutical preparation, HPLC, Validation.

INTRODUCTION

The angiotensin converting enzyme (ACE) inhibitors are one of the first choices of drugs in all grades of hypertension. Most patients require relatively low doses (2.5-10 mg/day) which are well tolerated. When used alone, they control hypertension in 50-60% of patients. When combined with a β blocker/diuretic their therapeutic efficacy extends to 90% because of supraadditive/synergistic effect. Inhibition of ACE lowers blood pressure by decreasing vasoconstriction.

Ramipril belongs to the class of ACE inhibitors. Ramipril, 2-[*N*-[(*S*)-1-ethoxycarbonyl-3-phenylpropyl]-L-alanyl]-(l*S*, 3*S*, 5*S*)-2-azabicyclo [3,3,0]-octane-3-carboxylic acid, is a prodrug. It is used as a drug for treatment of hypertension and related cardiovascular diseases [1]. The chemical structure of ramipril ise shown in Figure 1. It can be seen that ramipril's structure is similar to that of a proline-containing natural peptide.

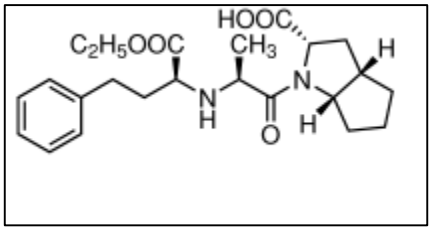

Figure 1. Chemical structure of ramipril.

In previous studies, gas chromatography-mass spectrometry (GC-MS) [2,3], high-performance liquid chromatography (HPLC) [4,5], voltammetry [6], radioimmunoassay [7], potentiometry [8,9], flow-injection analysis [10] and spectrophotometry [11] were reported for quantitative determination of ramipril in pharmaceutical preparations and biological fluids.

The purpose of this investigation was to develop and validate a method using a simple, rapid, sensitive, precise, accurate and specific reversed phase HPLC assay. The method uses a simple mobile phase composition and the rapid run time of 5 min. Hence, this method can be used for the analysis of large number of samples.

MATERIALS AND METHODS

Chemicals and reagents

Ramipril standard was kindly donated from Sigma-Aldrich (St. Louis, MO, USA). Blokace and Delix tablets containing 10 mg of ramipril were obtained from the pharmacy (Erzurum, Turkey). HPLC-grade organic solvents were purchased from Merck (Darmstadt, Germany). All chemicals were of analytical grade. Distilled water was prepared as required by using aquaMAX™ ultra, Young instrument (Korea) ultrawater purification system.

Instrumentation

A Hewlett-Packard series 200a HPLC system equipped with an HP 1046A programmable UV detector and ChemStation software package was used (Hewlett-Packard, Wilmington, DE, USA). The HPLC mobile phase was composed of 20 mM phosphate buffer (pH 2.5) containing 0.1% TFA-acetonitrile (50:50, *v/v*). Separation was achieved using an Ace C_{18} column (5 µm, 4.6×250 mm i.d.) with a guard column (4 mm × 3 mm i.d., Phenomenex) packed with the same material at a flow rate of 1.0 mL min^{-1}. The eluent was monitored by UV detection at 208 nm.

Preparation of the standard and quality control solutions

The stock standard solution of ramipril was prepared with methanol to a concentration of 100 µg mL^{-1} and stored at 4 ^{0}C under refrigeration. The six standard solutions from 0.25 to 7.5 µg mL^{-1} (0.25, 0.5, 1.0, 2.5, 5.0, 7.5 µg mL^{-1}) in methanol were made by a serial dilution. Three quality control (QC) samples at the concentrations of 0.75, 3.0 and 6.0 µg mL^{-1} were prepared from the stock standard solution.

Sample preparation

The average tablet mass was calculated from the mass of tablets of Blokace and Delix (10 mg ramipril tablet, which was composed of ramipril and some excipients). They

were then finely ground, homogenized and portion of the powder was weighed accurately, transferred into a 100 mL brown measuring flask and diluted to scale with methanol. The mixture was sonicated for at least 15 min to aid dissolution and then filtered through a Whatman no 42 paper. An appropriate volume of filtrate was diluted further with methanol so that the concentration of ramipril in the final solution was within the working range and then analyzed by HPLC.

RESULTS AND DISCUSSION

System suitability

A system suitability test of the chromatography system was performed before each validation run. Five replicate injections of a system suitability/calibration standard and one injection of a check standard were made. Area relative standard deviation, tailing factor and efficiency for the five suitability injections were determined. The check standard was quantified against the average of the five suitability injections. For all sample analyses, the tailing factor was ≤ 1.08, efficiency ≥ 1835 and %RSD ≤1.92%.

Linearity

Calibration curve was constructed for ramipril standard by plotting the concentration of compound versus peak area response. Standard solutions containing 0.25, 0.5, 1.0, 2.5, 5.0, 7.5 μg mL^{-1} of ramipril were prepared and 10 μL was injected into the HPLC column (Figure 2).

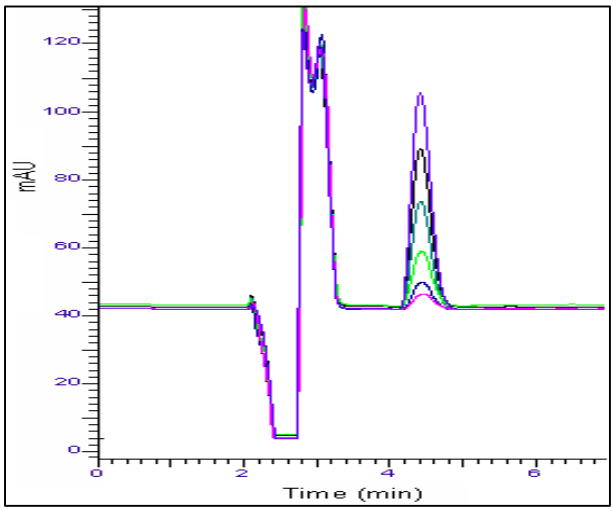

Figure 2. HPLC-UV chromatograms of ramipril (0.25, 0,5, 1.0, 2.5, 5.0, 7.5 μg mL^{-1}).

The linearity was evaluated by linear regression analysis, which was calculated by the least square regression method. The regression equations were calculated from the calibration graphs, along with the standard deviations of the slope (Sb) and intercept (Sa) on the ordinate (Table 1).

Table 1. Linearity of of ramipril by HPLC method.

Method	Range (μg mL^{-1})	LR[a]	Sa	Sb	R	LOD (μg mL^{-1})	LOQ (μg mL^{-1})
HPLC	0.25-7.5	Y = 7164.2x+152.2	15.21	23.42	0.9998	0.10	0.25

[a]Based on three calibration curves, LR:Linear regression, Sa: Standard deviation of intercept of regression line, Sb:Standard deviation of slope of regression line, R: Coefficient of correlation, y: peak area, x: ramipril concentration, LOD: Limit of detection, LOQ: Limit of quantification

Accuracy and precision

Accuracy of the assay method was determined for both intra-day and inter-day variations using the six times analysis of the QC samples. Precision of the assay was determined by repeatability (intra-day) and intermediate precision (interday). Repeatability refers to the use of the analytical procedure within a laboratory over a short period of time that was evaluated by assaying the QC samples during the same day. Intermediate precision was assessed by comparing the assays on different days (3 days). Accuracy and precision calculated for the QC samples during the intra- and inter-day run are given in Table 2. The accuracy ranged from -0.83% to -4.00% and precision from 2.74% to 4.95%. All the values were within the acceptance criteria of 5.0 %.

Table 2. Precision and accuracy of ramipril by HPLC method.

Added	Intra-day			Inter-day		
(μg mL^{-1})	Found ± SD	Accuracy	Precision (RSD%[a])	Found ± SD	Accuracy	Precision (RSD%[a])
0.75	0.72 ± 0.021	-4.00	2.92	0.76 ± 0.029	1.33	3.82
3	2.95 ± 0.081	-1.67	2.75	3.11 ± 0.154	3.67	4.95
6	5.81 ± 0.159	-3.17	2.74	5.95 ± 0.241	-0.83	4.05

SD: Standard deviation of six replicate determinations, RSD: Relative standard deviation,
[a]Average of six replicate determinations, Accuracy: (%relative error) (found-added)/addedx100

Sensitivity

Limits of detection (LOD) and quantification (LOQ) were estimated from the signal-to-noise ratio. The detection limit was defined as the lowest concentration level resulting in a peak height of three times the baseline noise. The quantitation limit was defined as the lowest concentration level that provided a peak height with a signal-to-noise ratio higher than 10, with precision (% RSD) and accuracy (% bias) within ±10%. LOD and LOQ values of HPLC method were determined to be 0.10 and 0.25 μg mL^{-1}, respectively (Table 1).

Stability

Stability studies indicated that the samples were stable when kept at room temperature, 4 and -20 ^{0}C refrigeration temperature for 8 h (short-term) and refrigerated at 4 and -20 ^{0}C for 72 h (long-term). The results of stability studies were given in Table 3 and no significant degradation was observed.

Recovery

Recovery studies by spiking different concentrations of pure drug in the preanalyzed tablet samples within the analytical concentration range of the proposed method. The added quantities of the individual drugs were estimated by above method. The results of recovery studies were fond to be satisfactory and the results are presented in Table 4.

Comparison of the methods

The method was validated according to either USP 26 [12] or the ICH guidelines [13] for validation of analytical procedures in order to determine the linearity, sensitivity, precision and accuracy for the analyte. The proposed method is very effective for the assay of ramipril in tablets. The validity of the proposed method was presented by recovery studies using the standard addition method. For this purpose, a known amount of reference drug was spiked to formulated tablets and the nominal value of drug was estimated by the proposed methods. Each level was repeated six times. The results (Table 4) were reproducible with low SD and RSD. No interference from the common excipients was observed.

Table 3. Stability of ramipril in solution.

Stability (%)	Room temperature stability (Recovery % ± RSD)		Refrigerator stability, +4 °C (Recovery % ± RSD)		Frozen stability, - 20 °C (Recovery % ± RSD)	
Added (μg mL^{-1})	8 h	24 h	24 h	72 h	24 h	72 h
0.5	99.3 ± 3.84	99.5 ± 4.17	101.5 ± 5.49	99.3 ± 5.72	99.5 ± 3.84	101.2 ± 3.75
2.5	101.2 ± 4.16	101.4 ± 3.87	98.2 ± 4.62	96.4 ± 4.35	98.2 ± 4.90	98.7 ± 4.28
7.5	99.8 ± 3.28	99.2 ± 4.21	101.4 ± 4.47	101.2 ± 3.54	102.3 ± 5.62	102.4 ± 5.26

RSD: Standard deviation of six replicate determinations

Table 4. Recovery of ramipril in pharmaceutical preparations by HPLC method.

Pharmaceutical preparation	Added (μg mL^{-1})	Intra-day			Inter-day		
		Found ± SD	Recovery (%)	RSD [a] (%)	Found ± SD	Recovery (%)	RSD [a] (%)
Blokace tablet (1.5 μg mL^{-1})	1	1.02 ± 0.047	102	4.61	1.02 ± 0.048	102	4.71
	2.5	2.52 ± 0.128	100.8	5.08	2.51 ± 0.129	100.4	5.14
	6	5.92 ± 0.192	98.7	3.24	6.04 ± 0.276	100.7	4.57
Delix tablet (1.5 μg mL^{-1})	1	0.98 ± 0.028	98	2.86	0.99 ± 0.026	99	2.63
	2.5	2.44 ± 0.081	97.6	3.32	2.47 ± 0.083	98.8	3.36
	6	6.06 ± 0.243	101	4.01	5.89 ± 0.187	98.2	3.17

SD: Standard deviation of six replicate determinations, RSD: Relative standard deviation, [a]Average of six replicate determinations, Accuracy: (%relative error) (found-added)/addedx100

Table 5. Determination of ramipril in pharmaceutical preparations containing ramipril.

Commercial preparation	Method	n	% Recovery ± SD	Confidence recovery	P value	F-test
-	Official method (potentiometric titration)	5	99.83 ± 0.921	-		
Ramipro 5 tablet (2.5 mg)	Spectrophotometry	3	99.02 ± 0.0124	97.2-100.9	0.342	F_c=1.49
Blokace tablet (10 mg)	HPLC	6	100.5 ± 0.123	98.7-102.0		F_t=3.00
Delix tablet (10 mg)	HPLC	6	98.87 ± 0.117	97.6-101.0		

n: number of determination, SD: Standard deviation of six replicate determinations, RSD[a]: Relative standard deviation, F_c: calculated F-value, F_t: tabulated F-value, Ho hypothesis: no statistically significant difference exists between four methods, $F_t > F_c$: Ho hypothesis is accepted (P > 0.05)

The proposed method was compared with spectrophotometry [11] and the official method [14]. The results obtained showed that the calculated *F*-values did not exceed the theoretical values (Table 5) from which we can conclude that the proposed method do not differ significantly from spectrophotometry and the official method.

CONCLUSION

A rapid and simple isocratic HPLC method for determination of ramipril has been developed and validated. This chromatographic assay fulfilled all the requirements to be identified as a reliable and feasible method, including accuracy, linearity, recovery and precision. The chromatographic run time of 5 min allows the analysis of a large number of samples in a short period of time. Therefore, the method is also suitable for analysis of sample during accelerated stability studies, routine analysis of formulations and raw materials.

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