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High performance thin-layer chromatography determination of temocapril in pharmaceutical formulation

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ABSTRACT

A simple, economic, fast and precise High performance thin layer chromatographic method has been developed for determination of temocapril from the tablet formulation. Amlodipine besylate is used as internal standard. Temocapril is a novel prodrug of an angiotensin-converting enzyme (ACE) inhibitor. The separation was performed on Silica gel $60F_{254}$ HPTLC plates with Ethyl acetate: Acetonitrile: Acetic acid in the proportion (8.0 : 2.0 : 0.1) v/v/v, as a solvent system. The determination was carried out using the densitometric absorbance mode at 225nm. The linearity range for temocapril was 12 to 32 µg/mL. The HPTLC method was evaluated in terms of sensitivity linearity, accuracy, precision and reproducibility.

KEYWORDS

HPTLC;
Temocapril;
Pharmaceutical formulation.

INTRODUCTION

Temocapril is a novel prodrug of an angiotensin-converting enzyme (ACE) inhibitor. Recently, it was reported that treatment with an ACE inhibitor, Temocapril Hydrochlorothiazide, improved forearm vasodilatory response to reactive hyperemia, suggesting a beneficial effect on endothelia Unlike many other ACE inhibitors, Temocapril is rapidly hydrolyzed at its 2-ethyl ester group to be converted into the pharmacologically active diacid metabolite Temocaprilat, which is a potent inhibitor of ACE[1] l function. It is excreted predominantly in bile. Data indicated that, unlike many other ACE inhibitors, Temocapril is excreted predominantly in the feces in both humans and other animals. In humans temocapril has been found to improve insulin resistance partly by increasing adiponectin levels. Cardiac remodeling was improved by Temocapril in humans. It improves renal function and as well as in decreases urinary albumin excretion in diabetics hypertensive patients.

The structures of this drug is shown in Figure 1[2]. Literature survey reveals that several clinical studies are going on Temocapril and there are very few analytical and bioanalytical methods are reported to determine Temocapril in different matrices like formulation, plasma, serum, urine, and cerebrospinal fluids[3-9]. It also reveals that there are no methods reported for complete analysis of Temocapril from bulk, formulation on HPTLC. The method described is economic, simple, fast, precise and accurate for determination of temocapril from pharmaceutical formulation.

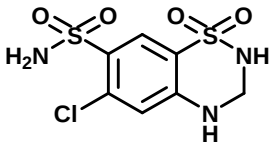

Figure 1 : The structure of temocapril

EXPERIMENTAL

Chemical and reagents

Working standards of Temocapril and Amlodipine besylate were obtained from reputed firms with valid certificates of analysis. All the chemicals used were of analytical grade purchased from Qualigens Fine chemicals (India). All dilutions were performed in standard volumetric flasks.

Instrumentation

A camag, Linomat IV sample applicator was used. Camag Twin trough glass chamber (20x10cm) was used for development of plates. And Camag TLC scanner II equipped with cats 3 Version software was used for interpretation of data.

Preparation of working standard solutions

Temocapril standard (25 mg) was accurately weighed into a 25-mL volumetric flask, dissolved in a minimum quantity of methanol, and diluted to volume with the same solvent to furnish a solution of concentration 1000 μg mL^{-1}. This was used as stock solution; further dilutions were prepared using this stock solution.

Preparation of internal standard solution

Accurately weighed 25 mg of standard Amlodipine besylate (99.82%) was taken in a 25 ml volumetric flask. This was dissolved in minimum quantity of methanol and made up to volume to get a concentration of 1000 μg mL^{-1}.

Sample preparation

Twenty tablets were weighed and the average weight was calculated. The tablets were then powdered and an amount equivalent to one tablet was dissolved in a minimum volume of methanol. This solution was filtered through a Whatman no. 41 paper and the filtrate was collected in a 200-mL volumetric flask and diluted to volume with methanol. This solution was then diluted tenfold with methanol to furnish a solution containing 20μg mL^{-1} Temocapril. Amlodipine (IS) solutions of concentration 70 μg mL^{-1} is added to this solution

Chromatographic condition

The experiment was performed on silica gel $60F_{254}$ HPTLC plates using mobile phase comprising of Ethyl acetate: Acetonitrile: Acetic acid in the volume ratio (8.0 : 2.0 : 0.1) v/v/v. The plate was prewashed by methanol and activated in an oven at 110^0C for 1 hour before use. The sample solutions were applied on the HPTLC plate as sharp bands of 7 mm width with the help of Camag Linomat IV sample applicator at the distance of 15 mm from the edge of the HPTLC plate with the speed of 10 sec/μl. Ascending development to distance of 8cm was performed in saturated 20cm x 10cm camag twin trough chamber for 15min at room temperature. The developed TLC plate was air dried and then scanned between 200 and 400 nm using Camag TLC Scanner II with Cats 3 version of the software. The wavelength chosen for further quantification was 225 nm. The spectra of Temocapril and HPTLC chromatogram for Temocapril and Amlodipine besylate are shown in Figure 2 and Figure 3 respectively.

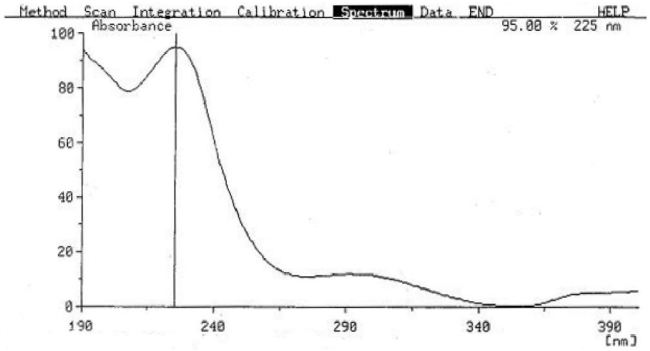

Figure 2 : UV spectra of temocapril

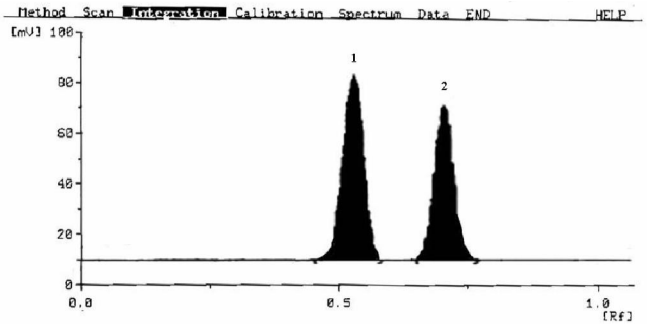

Figure 3 : Typical HPTLC chromatogram 1) Temocapril 2) Amlodipine besylate

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RESULT AND DISCUSSION

Linearity

Linearity was evaluated by analysis of working standard solutions of Temocapril of six different concentrations[10]. The concentration range for each of the pharmaceutical in the working standard solutions was 12 μg mL^{-1} to 32 μg mL^{-1} for Temocapril. The peak area ratio and concentration of drug was subjected to regression analysis to calculate the calibration equations and correlation coefficients. The regression data obtained are represented in TABLE 1. The result shows that with-in the concentration range mentioned above there was an excellent correlation between peak area ratio and concentration of drug.

TABLE 1 : Results of linearity

Analyte	Slope (mean)	Intercept (mean)	Correlation coefficient (n=5)
Temocapril	0.0365	0.0123	0.999

Limit of detection and limits of quantitation

The limit of detection (LOD) and limit of quantitation (LOQ) were established at signal-to-noise ratio of 3:1 and 10:1 respectively. The LOD and LOQ of Temocapril was experimentally determined by six injections of each drug. The LOD of Temocapril found to be 4 μg mL^{-1}. The LOQ of Temocapril was found to be 8 μg mL^{-1}.

Precision

Repeatability was studied by carrying out system precision. System precision was determined from results for seven replicate injections of the standard solutions[10]. The relative standard deviations was less than 2% for the drug. Method precision was determined from results from seven independent determinations at 100% of the test concentrations of Temocapril in the product. The RSD was 0.62%.

Assay

From the above sample solution 10μl was spotted in triplicate along with same concentration of standard solution on to the plate under the optimized chromatographic conditions. The peak area ratio values of Temocapril, was calculated. The amount of Temocapril present in this solution were then estimated using calibration curve method. Results of assay are tabulated in TABLE 2.

TABLE 2 : Result for assay

Drug	Labeled claim (mg)	Drug found in mg (n=7)	% RSD	% Assay
Temocapril	20	19.96	0.62	99.82

Recovery studies

Recovery experiments were carried out to check for the presence of positive or negative interferences from excipients present in the formulation, and to study the accuracy and precision of the method. Recovery experiment was performed by the standard addition method. The recovery of the added standard was studied at two different levels viz 110% and 120% of the estimated amount of the drug[10]. Each set of recovery of added standard was calculated. The results of recovery experiment are tabulated in TABLE 3.

TABLE 3 : Result for recovery

Level	Wt of sample	Amount of drug added in mg	Mean peak area ratio	Amount recovered in mg	% Recovery	Individual recovery (%)
	1020.2	0	0.7591	19.97	99.86	
0	1021.8	0	0.7682	20.18	100.89	
	1018.7	0	0.7560	19.92	99.59	
						100.11
	1020.3	5.03	0.9366	24.64	98.43	
110%	1019.2	5.07	0.9470	24.94	99.48	
	1019.4	5.05	0.9422	24.81	99.03	
						98.98
	1020.2	10.02	1.1343	29.84	99.41	
120%	1019.4	10.05	1.1254	29.63	98.61	
	1018.7	10.08	1.1333	29.86	99.27	
						99.10
				Average Recovery		99.40
				Confidence level		0.140

Robustness

The robustness of the method was studied, during method development, by determining the effects of small variation, of mobile phase composition (±2%), chamber saturation period, development distance and scanning time (10% variation of each). No significant change of Rf or response to drugs was observed, indicating the robustness of the method.

CONCLUSION

The high performance thin layer chromatographic method for the determination of Temocapril from their fixed dosage form was found to be accurate and precise. Thus, the proposed HPTLC method can be successfully applied for the routine quality control analysis of Temocapril from its fixed dosage form.

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