RESEARCH ARTICLE

A NEW HPLC METHOD FOR VALIDATION AND ESTIMATION OF MOEXIPRIL IN BULK AND ITS PHARMACEUTICAL FORMULATION

V. BHASKARA RAJU[1] and A. LAKSHMANA RAO[*]

[1]Sri Vasavi Institute of Pharmaceutical Sciences, Tadepalligudem, (A.P), India.

[2*]V.V. Institute of Pharmaceutical Sciences, Gudlavalleru, (A.P), India.

ABSTRACT

An accurate and precise HPLC method was developed for the determination of Moexipril. Separation of the drug was achieved on a reverse phase C_{18} column using a mobile phase consisting of phosphate buffer and acetonitrile in the ratio of 35:65 v/v. The flow rate was 0.7 ml/min and the detection wavelength was 210 nm. The linearity was observed in the range of 5-25 µg/ml with a correlation coefficient of 0.9990. The proposed method was validated for its linearity, accuracy, precision and robustness. This method can be employed for routine quality control analysis of Moexipril in tablet dosage forms.

KEYWORDS: *Moexipril, Estimation, RP-HPLC, Validation, Tablets.*

INTRODUCTION

Moexipril is a non-sulfhydryl containing precursor of the active angiotensin-converting enzyme (ACE) inhibitor moexiprilat. It is used to treat high blood pressure (hypertension). It works by relaxing blood vessels, causing them to widen. Lowering high blood pressure helps prevent strokes, heart attacks and kidney problems. Moexipril is a prodrug for moexiprilat, which inhibits ACE in humans and animals. The mechanism through which moexiprilat lowers blood pressure is believed to be primarily inhibition of ACE activity. ACE is a peptidyl dipeptidase that catalyzes the conversion of the inactive decapeptide angiotensin I to the vasoconstrictor substance angiotensin II. Angiotensin II is a potent peripheral vasoconstrictor that also stimulates aldosterone secretion by the adrenal cortex and provides negative feedback on renin secretion. ACE is identical to kininase II, an enzyme that degrades bradykinin, an endothelium-dependent vasodilator. Moexiprilat is about 1000 times as potent as moexipril in inhibiting ACE and kininase II.

Chemically moexiprilat is described as (3S) -2-[(2S)-2-{[(2S)-1-ethoxy-1- oxo- 4- phenylbutan-2-yl]amino}propanoyl]-6,7-dimethoxy-1,2,3,4-tetra hydroisoquinoline-3-carboxylic acid[1] (Figure 1). A few spectroscopic[2], Voltametric[3], TLC[4], HPLC[5] and LC-MS[6-8] methods were reported earlier for the determination of moexipril in bulk and pharmaceutical dosage forms. In the present study the authors report a rapid, sensitive, accurate and precise HPLC method for the estimation of moexipril in bulk samples and in tablet dosage forms.

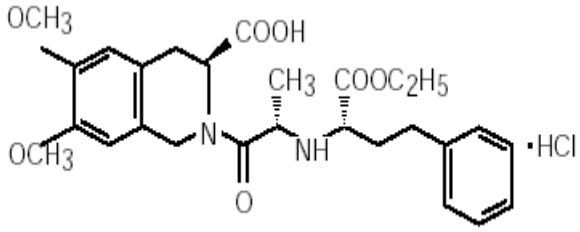

Figure 1: Chemical structure of moexipril

MATERIALS AND METHODS

Chromatographic Conditions

The analysis of the drug was carried out on a Waters HPLC system equipped with a reverse phase Xterra C_{18} column (100 mmx4.6mm; 3.5µm), a 2695 binary pump, a 20 µl injection loop and a 2487 dual absorbance detector and running on Waters Empower software.

Chemicals and Solvents

The reference sample of moexipril was supplied by Sun Pharmaceutical Industries Ltd., Baroda. HPLC grade water and acetonitrile were purchased from E. Merck (India) Ltd., Mumbai. Potassium dihydrogen phosphate and orthophosphoric acid of AR Grade were obtained from S.D. Fine Chemicals Ltd., Mumbai.

Preparation of phosphate buffer

Seven grams of KH_2PO_4 was weighed into a 1000 ml beaker, dissolved and diluted to 1000 ml with HPLC water. 2 ml of Triethyl amine was added and pH adjusted to 3.0 with orthophosporic acid.

Preparation of mobile phase and diluents

350 ml of the phosphate buffer was mixed with 650 ml of acetonitrile. The solution was degassed in an ultrasonic water bath for 5 minutes and filtered through 0.45µ filter under vacuum.

PROCEDURE

A mixture of buffer and acetonitrile in the ratio of 35:65 v/v was found to be the most suitable mobile phase for ideal separation of moexipril. The solvent mixture was filtered through a 0.45µ membrane filter and sonicated before use. It was pumped through the column at a flow rate of 0.7 ml/min. The column was maintained at ambient temperature. The pump pressure was set at 800 psi. The column was equilibrated by pumping the mobile phase through the column for at least 30 min prior to the injection of the drug solution. The detection of the drug was monitored at 210 nm. The run time was set at 5 min. Under these optimized chromatographic conditions the retention time obtained for the drug was 2.269 min. A typical chromatogram showing the separation of the drug is given in Figure 2.

Calibration Plot

About 10 mg of moexipril was weighed accurately, transferred into a 100 ml volumetric flask and dissolved in 25 ml of a 35:65 v/v mixture of phosphate buffer and acetonitrile. The solution was sonicated for 15 min and the volume made up to the mark with a further quantity of the diluent to get a 100 µg/ml solution. From this, a working standard solution of the drug (15µg/ml) was prepared by diluting 1.5 ml of the above solution to 10 ml in a volumetric flask. Further dilutions ranging from 5-25µg/ml were prepared from the solution in 10 ml volumetric flasks using the above diluent. 20 µl of

each dilution was injected six times into the column at a flow rate of 0.7 ml/min and the corresponding chromatograms were obtained. From these chromatograms, the average area under the peak of each dilution was computed. The calibration graph constructed by plotting concentration of the drug against peak area was found to be linear in the concentration range of 5-25 µg/ml of the drug. The relevant data are furnished in Table-1. The regression equation of this curve was computed. This regression equation was later used to estimate the amount of moexipril in tablet dosage forms.

Figure 2: Typical chromatogram of moexipril

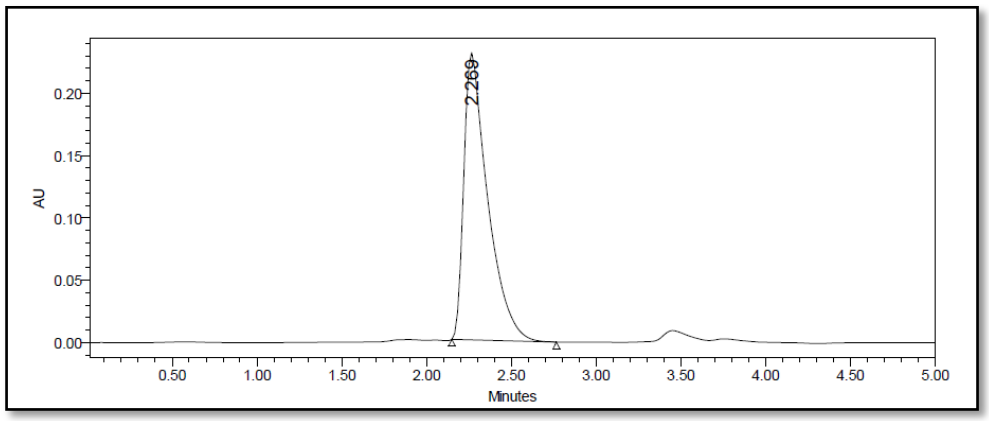

Table-1: Calibration data of the method

Concentration (µg/ml)	Mean peak area (n=5)
5	772714
10	1510226
15	2262917
20	3007400
25	3596241

Validation of the proposed method

The specificity, linearity, precision, accuracy, limit of detection, limit of quantification, robustness and system suitability parameters were studied systematically to validate the proposed HPLC method for the determination of moexipril. Solution containing 15 µg/ml of moexipril was subjected to the proposed HPLC analysis to check intra-day and inter-day variation of the method and the results are furnished in Table-2. The accuracy of the HPLC method was assessed by analyzing solutions of moexipril at 50, 100 and 150% concentrated levels by the proposed method. The results are furnished in Table-3. The system suitability parameters are given in Table-4.

Table-2: Precision of the proposed HPLC method

Concentration of Moexipril(15µg/ml)	Peak area	
	Intra-day	Inter-day
Injection-1	2318936	2285443
Injection-2	2324359	2286891
Injection-3	2312122	2292600
Injection-4	2318277	2289041
Injection-5	2324091	2298544
Average	2319557	1773220
Standard Deviation	5022.7	5240.2
%RSD	0.22	0.23

Estimation of moexipril in tablet dosage forms

Two commercial brands of tablets were chosen for testing the suitability of the proposed method to estimate moexipril in tablet formulations.

Twenty tablets were weighed and powdered. An accurately weighed portion of this powder equivalent to 25 mg of moexipril was transferred into a 100 ml volumetric flask and dissolved in 25 ml of a 35:65 v/v mixture of phosphate buffer and acetonitrile. The contents of the flask were sonicated for 15 min and a further 25 ml of the diluent was added, the flask was shaken continuously for 15 min to ensure complete solubility of the drug. The volume was made up with the diluent and the solution was filtered through a 0.45 µ membrane filter. This solution containing 15 µg/ml of moexipril was injected into the column six times. The average peak area of the drug was computed from the chromatograms and the amount of the drug present in the tablet dosage form was calculated by using the regression equation obtained for the pure drug. The relevant results are furnished in Table-5.

Table-3: Accuracy studies

Concentration	Amount added (mg)	Amount found mg)	% Recovery	% Mean recovery
50%	7.87	7.79	98.4%	98.4%
100%	15.0	14.86	98.6%	
150%	22.80	22.54	98.2%	

RESULTS AND DISCUSSION

In the proposed method, the retention time of moexipril was found to be 2.269 min. Quantification was linear in the concentration range of 5-25 µg/ml. The regression equation of the linearity plot of concentration of moexipril over its peak area was found to be Y=86631.2+142884.56X (r^2=0.9990), where X is the concentration of moexipril (µg/ml) and Y is the corresponding peak area. The number of theoretical plates calculated was 2190, which indicates efficient performance of the column. The limit of detection and limit of quantification were found to be 0.03 µg/ml and 0.09 µg/ml respectively, which indicate the sensitivity of the method. The use of phosphate buffer and acetonitrile in the ratio of 35:65 v/v resulted in peak with good shape and resolution.

The high percentage of recovery indicates that the proposed method is highly accurate. No interfering peaks were found in the chromatogram of the formulation within the run time indicating that excipients used in tablet formulations did not interfere with the estimation of the drug by the proposed HPLC method.

Table-4: System suitability parameters

Parameter	Result
Linearity ((µg/ml)	5-25
Correlation coefficient	0.9990
Theoretical plates (N)	2190
Tailing factor	1.6
LOD (µg/ml)	0.03
LOQ (µg/ml)	0.09

Table-5: Assay and recovery studies

Formulation	Label claim (mg)	Amount found (mg)	% Amount found
Formulation 1	15	15.003	100.0
Formulation 2	15	14.992	99.97

CONCLUSION

The proposed HPLC method is rapid, sensitive, precise and accurate for the determination of Moexipril and can be reliably adopted for routine quality control analysis of Moexipril in its tablet dosage forms.

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ADDRESS FOR CORRESPONDENCE

dralrao@gmail.com