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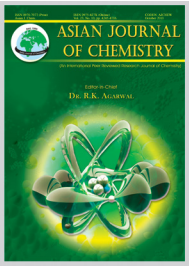

Simultaneous Estimation of Moexipril and Hydrochlorthiazide in Tablet Dosage Form by RP-HPLC Method

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A simple, reproducible and efficient reverse phase high performance liquid chromatographic method was developed for simultaneous estimation of moexipril and hydrochlorthiazide in tablets. A column having 150 mm × 4.6 mm i.d. in isocratic mode with mobile phase containing acetonitrile:phosphate buffer (45:55; adjusted to pH 3) was used. The flow rate was 0.6 mL/min and effluent was monitored at 215 nm. The retention time (min) and linearity range (µg/mL) for moexipril and hydrochlorthiazide were (4.294, 3.368) and (20-60, 20-60), respectively. The developed method was found to be accurate, precise and selective for simultaneous determination of moexipril and hydrochlorthiazide in tablets.

Key Words: Moexipril, Hydrochlorthiazide, RP-HPLC, Determination.

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 angiotensin-converting enzyme in humans and animals[1,2]. Chemically described as (3S)-2-[(2S)-2-{[(2S)-1-ethoxy-1-oxo-4-phenylbutan-2-yl]amino}-propanoyl]-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid. Hydrochlorthiazide is a diuretic of the class of benzothiadiazines widely used in antihypertensive pharmaceutical formulations, alone or in combination with other drugs, which decreases active sodium reabsorption and reduces peripheral vascular resistance. It is chemically 6-chloro-3,4-dihydro-2*H*-1,2,4-benzothiadiazine-7-sulfonamide-1,1-dioxide and was successfully used as one content in association with other drugs in the treatment of hypertension[1,2]. There are few methods appearing in the literature for the simultaneous determination of moexipril and hydrochlorthiazide in tablets. These methods were based on HPLC[3] and UV-derivative spectrophotometry[4]. Literature survey reveals that individual HPLC methods for moexipril[5] and hydrochlorthiazide[6-9] have been developed. In this communication, a simple, precise, reproducible and accurate reverse-phase high performance liquid chromatographic method to estimate moexipril and hydrochlorthiazide in tablet dosage form is reported.

EXPERIMENTAL

The reference sample of moexipril and hydrochlorthiazide was supplied by Torrent Pharmaceutical Industries Ltd., Ahmedabad. 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.

Chromatographic conditions: The analysis of the drug was carried out on a Waters HPLC system equipped with a reverse phase Xterra C_{18} column (150 mm × 4.6 mm; 5 µm), a 2695 binary pump, a 20 µL injection loop and a 2487 dual absorbance detector and running on Waters Empower software. The UV spectrum of the drugs was taken using an Elico SL-159 UV-Visible spectrophotometer.

Preparation of phosphate buffer (pH 3): 7 g of KH_2PO_4 was weighed into a 1000 mL beaker, dissolved and diluted to 1000 mL with HPLC water and pH adjusted to 3 with orthophosporic acid.

Preparation of mobile phase and diluents: 550 mL of the phosphate buffer was mixed with 450 mL of acetonitrile. The solution was degassed in an ultrasonic water bath for 5 min and filtered through 0.45 µ filter under vacuum.

Procedure: A mixture of buffer and acetonitrile in the ratio of 55:45 v/v was found to be the most suitable mobile

phase for ideal separation of moexipril and hydrochlorthiazide. The solvent mixture was filtered through a 0.45 µ membrane filter and sonicated before use. It was pumped through the column at a flow rate of 0.6 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 0.5 h prior to the injection of the drug solution. The detection of the drug was monitored at 215 nm. The run time was set at 8 min. Under these optimized chromatographic conditions the retention time obtained for the drugs moexipril and hydrochlorthiazide was 4.294 min and 3.368 min, respectively. A typical chromatogram showing the separation of the drug is given in Fig. 1.

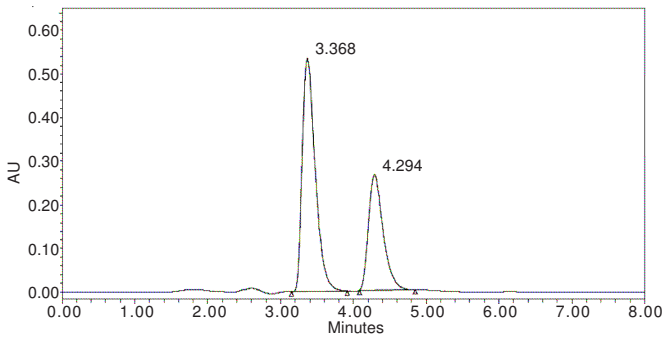

Fig. 1. Typical chromatogram of moexipril and hydrochlorthiazide

Calibration plot: About 100 mg of moexipril and 100 mg of hydrochlorthiazide was weighed accurately, transferred into a 100 mL volumetric flask and dissolved in 25 mL of a 55:45 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 1000 µg/mL solution. From this, a working standard solution of the drugs (40 µg/mL for moexipril and 40 µg/mL for hydrochlorthiazide) was prepared by diluting the above solution to 10 mL in a volumetric flask. Further dilutions ranging from 20-60 µg/mL for moexipril and 20-60 µg/mL for hydrochlorthiazide 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 0.6 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 20-60 µg/mL for moexipril and 20-60 µg/mL for hydrochlorthiazide. The relevant data are furnished in Tables 1 and 2. The regression equations of this curves was computed. This regression equation was later used to estimate the amount of moexipril and hydrochlorthiazide in tablets dosage forms.

TABLE-1
CALIBRATION DATA OF MOEXIPRIL

Concentration (µg/mL)	Mean peak area (n=5)
20	1897607
30	2888418
40	3771086
50	4581081
60	5393931

TABLE-2
CALIBRATION DATA OF HYDROCHLORTHIAZIDE

Concentration (µg/mL)	Mean peak area (n=5)
20	3406362
30	5039610
40	6592780
50	8209079
60	9470662

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 and hydrochlorthiazide. Solution containing 40 µg/mL for moexipril and 40 µg/mL for hydrochlorthiazide was subjected to the proposed HPLC analysis to check intra-day and inter-day variation of the method and the results are furnished in Tables 3 and 4. The accuracy of the HPLC method was assessed by analyzing solutions of moexipril and hydrochlorthiazide at 50, 100 and 150 % concentrated levels by the proposed method. The results are furnished in Tables 5 and 6. The system suitability parameters are given in Table-7.

TABLE-3
PRECISION STUDIES FOR MOEXIPRIL

Concentration of moexipril (40 µg/mL)	Peak area	
	Intra-day	Inter-day
Injection-1	3926738	3793313
Injection-2	3882456	3795436
Injection-3	3880132	3748819
Injection-4	3884781	3782548
Injection-5	3892866	3765014
Average	3893395	3777026
Standard deviation	19247.2	19840.8
RSD (%)	0.49	0.53

TABLE-4
PRECESION STUDIES FOR HYDROCHLORTHIAZIDE

Concentration of hydrochlorthiazide (40 µg/mL)	Peak area	
	Intra-day	Inter-day
Injection-1	6706303	6721125
Injection-2	6723787	6700008
Injection-3	6746877	6643842
Injection-4	6722180	6666971
Injection-5	6705041	6662322
Average	6720838	6678854
Standard deviation	16951.0	31123.4
%RSD	0.25	0.47

TABLE-5
ACCURACY STUDIES FOR MOEXIPRIL

Concentration (%)	Amount added (mg)	Amount found (mg)	Recovery (%)	Mean recovery (%)
50	20.08	20.173	100.46	
100	40.00	40.158	100.39	100.22
150	59.60	59.496	99.82	

Estimation of moexipril and hydrochlorthiazide in tablet dosage forms: Two commercial brands of tablets were chosen for testing the suitability of the proposed method to

TABLE-6
ACCURACY STUDIES FOR HYDROCHLORTHIAZIDE

Concentration (%)	Amount added (mg)	Amount found (mg)	Recovery (%)	Mean recovery (%)
50	20.10	20.072	99.86	
100	39.80	39.923	100.31	100.12
150	59.0	59.111	100.18	

TABLE-7
SYSTEM SUITABILITY PARAMETERS

Parameter	Moexipril	Hydrochlorthiazide
Linearity (µg/mL)	20-60	20-60
Correlation coefficient	0.9990	0.9990
Theoretical plates (N)	2076	2634
Tailing factor	1.40	1.50
LOD (µg/mL)	0.02	0.01
LOQ (µg/mL)	0.072	0.036

estimate moexipril and hydrochlorthiazide in tablet formulations. Twenty tablets were weighed and powdered. An accurately weighed portion of this powder equivalent to 100 mg of moexipril and 100 mg of hydrochlorthiazide was transferred into a 100 mL volumetric flask and dissolved in 25 mL of a 55:45 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 was further diluted to get the required concentrations. The solution containing 40 µg/mL was injected into the column six times. The average peak area of the drugs 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 Tables 8 and 9.

TABLE-8
ASSAY AND RECOVERY STUDIES FOR MOEXIPRIL

Formulation	Label claim (mg)	Amount found (mg)	Amount found (%)
Brand-1	40	40.48	101.20
Brand-2	40	39.45	98.62

TABLE-9
ASSAY AND RECOVERY STUDIES FOR HYDROCHLORTHIAZIDE

Formulation	Label claim (mg)	Amount found (mg)	Amount found (%)
Brand-1	40	40.45	101.05
Brand-2	40	39.34	98.35

RESULTS AND DISCUSSION

In the proposed method, the retention time of moexipril and hydrochlorthiazide was found to be 4.294 min and 3.368 min, respectively. Quantification was linear in the concentration range of 20-60 µg/mL for moexipril and 20-60 µg/mL for hydrochlorthiazide. The regression equation of the linearity plot of concentration of moexipril and hydrochlorthiazide over its peak area was found to be $Y = 232300.2 + 86853.11X$ ($r^2 = 0.999$) for moexipril and $Y = 424471 + 152980.69X$ ($r^2 = 0.999$) for hydrochlorthiazide, where X is the concentration of moexipril and hydrochlorthiazide (µg/mL) and Y is the corresponding peak area. The number of theoretical plates calculated was 2076 for moexipril and 2634 for hydrochlorthiazide, which indicates efficient performance of the column. The limit of detection and limit of quantification for moexipril were found to be 0.02 and 0.072 µg/mL and for hydrochlorthiazide were found to be 0.01 and 0.036 µg/mL respectively, which indicate the sensitivity of the method. The use of phosphate buffer and acetonitrile in the ratio of 55:45 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.

Conclusion

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

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