



SIMULTANEOUS ESTIMATION OF VALSARTAN AND HYDROCHLOROTHIAZIDE IN TABLETS BY RP-HPLC METHOD

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Abstract

A simple, reproducible and efficient reverse phase high performance liquid chromatographic method was developed for simultaneous determination of valsartan and hydrochlorothiazide in tablets. A column having 100mmx4.6mm i.d. in isocratic mode with mobile phase containing Acetonitrile: Phosphate buffer (50:50; adjusted to pH 3.0) was used. The flow rate was 0.8 ml/min and effluent was monitored at 225 nm. The retention time (min) and linearity range ($\mu\text{g/ml}$) for valsartan and hydrochlorothiazide were (5.59, 2.36) and (10-50, 10-50), respectively. The developed method was found to be accurate, precise and selective for simultaneous determination of valsartan and hydrochlorothiazide in tablets.

Key words: Valsartan, Hydrochlorothiazide, RP-HPLC.

Introduction

Valsartan, (S)-N-(1-Oxopentyl)-N-[[2'-(1H-tetrazol-5-yl)[1,1'-biphenyl]-4-yl]methyl]-L-valine, is an orally active specific angiotensin II receptor blocker effective in lowering blood pressure in hypertensive patients¹. Hydrochlorothiazide 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-2H-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 hypertension². Simultaneous determination of both drugs is highly desirable as this would allow more efficient generation of clinical data and could be more cost-effective than separate assays. There are very few methods appearing in the literature for the simultaneous determination of valsartan and hydrochlorothiazide in tablets.

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Since these methods were based on HPLC³⁻⁶, GC-MS⁷, capillary electrophoresis⁸ and UV-derivative spectrophotometry⁹, the procedure was inconvenient for determination and the run times were rather long. The aim of this study was to develop a simple, precise and accurate reverse-phase high performance liquid chromatographic method to estimate valsartan and hydrochlorothiazide in tablets. This method was simple and rapid and provides accurate and precise results, as compared with other methods which have been reported. Criteria employed for assessing the suitability of said solvent system were cost-effectiveness in terms of time required for analysis, solvent noise and preparatory steps involved in the extraction of the drug from the formulation excipients for the estimation of drug contents. The retention times for valsartan and hydrochlorothiazide were 5.59 and 2.36 min, respectively.

Experimental

Chromatographic conditions

The analysis of the drug was carried out on a Waters HPLC system equipped with a reverse phase Xterra C₁₈ column (100mmx4.6mm; 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 a Elico SL-159 UV-Visible spectrophotometer.

Chemicals and Solvents

The reference sample of valsartan and hydrochlorothiazide 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.

Preparation of phosphate buffer (pH 3.0)

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

Preparation of mobile phase and diluents

500 ml of the phosphate buffer was mixed with 500 ml of acetonitrile. The solution was degassed in an ultrasonic water bath for 5 minutes and filtered through 0.45 μ filter under vacuum.

Procedure

A mixture of buffer and acetonitrile in the ratio of 50:50 v/v was found to be the most suitable mobile phase for ideal separation of valsartan and hydrochlorothiazide. 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 0.8 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 225 nm. The run time was set at 8 min. Under these optimized chromatographic conditions the retention time obtained for the drugs valsartan and hydrochlorothiazide was 5.59 min and 2.36 min. A typical chromatogram showing the separation of the drug is given in Fig. 1.

Calibration plot

About 100 mg of valsartan and 100 mg of hydrochlorothiazide was weighed accurately, transferred into a 100 ml volumetric flask and

dissolved in 25 ml of a 50:50 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 $\mu\text{g}/\text{ml}$ solution. From this, a working standard solution of the drugs (20 $\mu\text{g}/\text{ml}$ for valsartan and 20 $\mu\text{g}/\text{ml}$ for hydrochlorothiazide) was prepared by diluting the above solution to 10 ml in a volumetric flask. Further dilutions ranging from 10-50 $\mu\text{g}/\text{ml}$ for valsartan and 10-50 $\mu\text{g}/\text{ml}$ for hydrochlorothiazide 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 0.8 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 10-50 $\mu\text{g}/\text{ml}$ for valsartan and 10-50 $\mu\text{g}/\text{ml}$ for hydrochlorothiazide. The relevant data are furnished in Table 1&2. The regression equations of this curves was computed. This regression equation was later used to estimate the amount of valsartan and hydrochlorothiazide in tablets dosage forms.

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 valsartan and hydrochlorothiazide. Solution containing 20 $\mu\text{g}/\text{ml}$ for valsartan and 20 $\mu\text{g}/\text{ml}$ for hydrochlorothiazide 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 3&4. The accuracy of the HPLC method was assessed by analyzing solutions of valsartan and hydrochlorothiazide at 50, 100 and 150% concentrated levels by the proposed method. The results are furnished in Table 5&6. The system suitability parameters are given in Table 7.

Estimation of valsartan and hydrochlorothiazide in tablet dosage forms

Two commercial brands of tablets were chosen for testing the suitability of the proposed method to

estimate valsartan and hydrochlorothiazide in tablet formulations. Twenty tablets were weighed and powdered. An accurately weighed portion of this powder equivalent to 100 mg of valsartan and 100 mg of hydrochlorothiazide was transferred into a 100 ml volumetric flask and dissolved in 25 ml of a 50:50 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. This solution 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 Table 8&9.

Table 1: Calibration data for Valsartan

Concentration ($\mu\text{g/ml}$)	Mean peak area (n=5)
10	725891
20	1438757
30	2162436
40	2846537
50	3525531

Table 2: Calibration data for Hydrochlorothiazide

Concentration ($\mu\text{g/ml}$)	Mean peak area (n=5)
10	916324
20	1771369
30	2689139
40	3491362
50	4419896

Table 3

Precision of the proposed HPLC method (Valsartan)

Concentration of valsartan(20 $\mu\text{g/ml}$)	Peak area	
	Intra-day	Inter-day
Injection-1	1422634	1446692
Injection-2	1425605	1445092
Injection-3	1426060	1445917
Injection-4	1426345	1449615
Injection-5	1425060	1448526
Average	1425141	1447167
Standard Deviation	1483.1	1866.4
%RSD	0.10	0.13

Table 4

Precision of the proposed HPLC method (Hydrochlorothiazide)

Concentration of hydrochlorothiazide (20 $\mu\text{g/ml}$)	Peak area	
	Intra-day	Inter-day
Injection-1	1756423	1727358
Injection-2	1757640	1727898
Injection-3	1760081	1731069
Injection-4	1761108	1736914
Injection-5	1762172	1735512
Average	1759489	1732150
Standard Deviation	2397.2	3906.4
%RSD	0.14	0.23

Table 5: Accuracy studies (Valsartan)

Concentration	Amount added (mg)	Amount found (mg)	% Recovery	% Mean recovery
50%	5.0	5.05	101.0%	
100%	10.1	9.99	98.9%	100.1%
150%	15.0	15.06	100.4%	

Table 6: Accuracy studies (Hydrochlorothiazide)

Concentration	Amount added (mg)	Amount found (mg)	% Recovery	% Mean recovery
50%	5.06	5.16	101.9%	
100%	10.02	9.99	99.7%	100.8%
150%	15.02	15.16	100.9%	

Table 7: System suitability parameters

Parameter	Result (Valsartan)	Result (Hydrochlorothiazide)
Linearity ($\mu\text{g/ml}$)	10-50	10-50
Correlation coefficient	0.999	0.999
Theoretical plates (N)	6083	3510
Tailing factor	1.20	1.40
LOD ($\mu\text{g/ml}$)	0.02	0.01
LOQ ($\mu\text{g/ml}$)	0.08	0.03

Table 8: Assay and recovery studies (Valsartan)

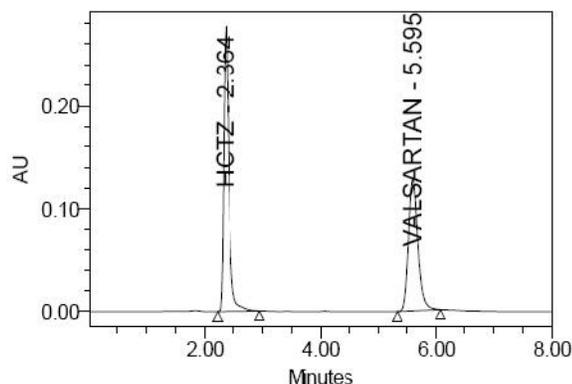
Formulation	Label claim (mg)	Amount found (mg)	% Amount found
Brand 1	80	80.09	100.11
Brand 2	80	80.13	100.16

Table 9: Assay and recovery studies (Hydrochlorothiazide)

Formulation	Label claim (mg)	Amount found (mg)	% Amount found
Brand 1	12.5	12.45	99.60
Brand 2	12.5	12.59	100.72

Results and Discussion

In the proposed method, the retention time of valsartan and hydrochlorothiazide was found to be 5.59 min and 2.36 min. Quantification was linear in the concentration range of 10-50 $\mu\text{g/ml}$ for valsartan and 10-50 $\mu\text{g/ml}$ for hydrochlorothiazide. The regression equation of the linearity plot of concentration of valsartan and hydrochlorothiazide over its peak area was found to be $Y=37712.4+70070.6X$ ($r^2=0.999$) for valsartan and $Y=39476.9+87271.37X$ ($r^2=0.999$) for hydrochlorothiazide, where X is the concentration of valsartan and hydrochlorothiazide ($\mu\text{g/ml}$) and Y is the corresponding peak area. The number of theoretical plates calculated was 6083 for valsartan and 3510 for hydrochlorothiazide, which indicates efficient performance of the column.

**Fig. 1: Typical chromatogram of hydrochlorothiazide and valsartan**

The limit of detection and limit of quantification for valsartan were found to be 0.02 $\mu\text{g/ml}$ and 0.08 $\mu\text{g/ml}$ and for hydrochlorothiazide were found to be 0.01 $\mu\text{g/ml}$ and 0.03 $\mu\text{g/ml}$ respectively, which indicate the sensitivity of the method. The use of phosphate buffer and acetonitrile in the ratio of 50:50 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 valsartan and hydrochlorothiazide and can be reliably adopted for routine quality control analysis of valsartan and hydrochlorothiazide in its tablet dosage forms.

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