INTERNATIONAL RESEARCH JOURNAL OF PHARMACY **ISSN 2230 – 8407**
Available online http://www.irjponline.com
Research Article

RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF VALSARTAN AND HYDROCHLOROTHIAZIDE IN SOLID DOSAGE FORM

Sheikh Rahila J[1]*, Puranik Manisha P[1], Bavadkar Deepali N[1], Mali Prabha R[2] and Yeole Pramod G[1]
[1]Institute of Pharmaceutical Education and Research,Borgaon (Meghe),Wardha-442 001,India
[2]Dr. D. Y. Patil Institute of Pharmacy,Akurdi, Pune, Maharastra, India

Article Received on: 17/01/2011 Revised on: 04/03/2011 Approved for publication: 11/03/2011

*Sheikh Rahila J., M.Pharma (Quality Assurance), Institute of Pharmaceutical Education and Research, Borgaon (Meghe), Wardha- 442 001, India Email: rahilat26@gmail.com

ABSTRACT
A simple, selective, rapid, precise and economical reverse phase HPLC method has been developed for the simultaneous estimation of Valsartan and Hydrochlorothiazide in solid dosage form. The method was carried out on a C18 Intersil (250 X 4.6 i.d., particle size 10µm) column with a mobile phase consisting of 0.02M Potassium dihydrogen orthophosphate:Methanol:Triethylamine [25:75:0.2 %v/v/v, pH 6.0]at a flow rate of 1.0 mL/min. Detection was carried out at 259 nm. The retention time of Valsartan and Hydrochlorothiazide was found to be 4.15 and 3.20 min, respectively. The developed method was validated in terms of accuracy, precision, linearity, limit of detection and limit of quantitation and can be used for the estimation of these drugs in combined pharmaceutical dosage forms.
KEY WORDS: Valsartan, Hydrochlorothiazide, RP- HPLC.

INTRODUCTION

Valsartan is chemically N-(1-oxopentyl)-N-[[2′-(1H-tetrazol-5-yl) [1, 1′-biphenyl]-4- yl] methyl]-L-valine. It is used mainly as antihypertensive[1]. Hydrochlorothiazide is 6-chloro-3, 4-dihydro-2H-1, 2, 4-benzothiadiazine-7-sulfonamide 1, 1-dioxide. It finds its use as diuretic[2]. Literature survey reveals that spectrophotometric[3], HPLC[4] and protein precipitation[4] methods are available for the estimation of Valsartan and RP-HPLC[5,6,7] and LC-UV[8] methods for the estimation of Hydrochlorothiazide in combination with other drugs. However, there is no HPLC method so far reported for the simultaneous estimation of these drugs in combined dosage forms.

The aim of this work was to develop an RP-HPLC method with ultraviolet detection for the simultaneous determination of Valsartan and Hydrochlorothiazide in solid dosage forms.

MATERIALS AND METHODS

Acetonitrile, HPLC grade; water, HPLC grade; orthophosphoric acid, AR grade; methanol, HPLC grade; Triethylamine, HPLC grade and potassium dihydrogen orthophosphate, GR grade were procured from Loba Chemicals, Mumbai, India. Reference standards of drugs were procured from Cipla Pvt. Ltd, Mumbai, India

Chromatographic separation was performed on a Jasco PU 1580 intelligent pump, variable wavelength UV/VIS detector (Jasco UV 1575), precision loop injector (Rheodyne 20µl) Borwin Software (version 1.21.60). Column C18 Intersil (250 X 4.6 i.d., particle size 10µm) was used for the separation.

Preparation of mobile phase and standard solutions

The mobile phase used was a mixture of 0.02M Potassium dihydrogen orthophosphate: Methanol: Triethylamine [25:75:0.2 %v/v/v, pH 6.0]. It was filtered through Whatman filter paper No. 42. Standard stock solutions of 0.8 mg mL^{-1} of Valsartan and 0.125 mg mL^{-1} of Hydrochlorothiazide were prepared separately in methanol. From the standard stock solution, mixed standard solutions were prepared with mobile phase to contain 32 µg mL^{-1} of Valsartan and 5 µg mL^{-1} of Hydrochlorothiazide. The mobile phase was delivered at a flow rate of 1 mL/min with detection at 259 nm. The injection volume was 20 µl; Analysis was performed at room temperature.

Preparation of sample solutions

Twenty tablets (VALZAAR-H, Torrent Pharmaceuticals, Baddi, India.) containing 80 mg of Valsartan and 12.5 mg of Hydrochlorothiazide were taken, average weight was determined. Weight equivalent to 80 mg of Valsartan (=12.5 mg of Hydrochlorothiazide) was taken in 100 ml volumetric flask and 40 mL of methanol was added and sonicated for 30 min, finally volume was made up to the mark with methanol. The extracts were filtered through Whatman filter paper No. 42 and required dilutions were made with mobile phase to get

the final concentration containing 32 µg mL^{-1} of Valsartan and 5 µg mL^{-1} of Hydrochlorothiazide, respectively.

Assay method

With the optimized chromatographic conditions, a steady baseline was recorded, the mixed standard solutions were injected and the chromatogram was recorded. The retention time of Valsartan and Hydrochlorothiazide was found to be 4.15 and 3.20 min, respectively. This procedure was repeated for the sample solution obtained from the formulation. The response factor (peak area) of the standard solution and sample solution was recorded. The analyte concentration of the drugs was calculated and presented in Table 1.

RESULT

The method described enables to the quantification of Valsartan and Hydrochlorothiazide. The advantages lie in the simplicity of sample preparation and the low costs of reagents used. The proposed HPLC conditions ensure sufficient resolution and the precise quantification of the compounds. Results from statistical analysis of the experimental results were indicative of satisfactory precision and reproducibility. Hence, this HPLC method can be used for routine drug analysis.

DISCUSSION

Estimation of Valsartan and Hydrochlorothiazide in solid dosage forms by RP-HPLC method was carried out using optimized chromatographic conditions. The standard and sample solutions were prepared. The chromatograms were recorded. The typical chromatogram of sample solution is given in Fig I. The peak area ratio of standard and sample solutions was calculated. The assay procedure was repeated for five determinations. Table 1 shows the result of analysis of pharmaceutical dosage form. The results of analysis shows that the amount of drugs was in good agreement with the label claim of the formulation.

The method was validated as per ICH guidelines. The accuracy of the method was determined by recovery experiments. The recovery studies were carried out at three levels (60%, 80% and 100%) by standard addition method. The percentage recovery and standard deviation were calculated and presented in Table 2. From the data obtained, added recoveries of standard drugs were found to be accurate.

Precision of an analytical method is the degree of agreement among individual test results. It was ascertained by replicate estimation of marketed formulation (five times) and expressed as the S.D. and R.S.D. of the series of measurements. The ruggedness of the method was demonstrated by inter day and intra day variation studies. In the intra day studies, three repeated injections of standard and sample solutions were made and the response factor of drug peaks and percentage RSD were calculated. In the inter day variation studies, three repeated injections of standard and sample solutions were made for three consecutive days and response factor of drug peaks and percentage RSD were calculated. No marked changes in the chromatograms demonstrated that the HPLC method developed are rugged and robust.

The linearity of the method was determined at five concentration levels ranging from 80-120%. According to USP, 80% to 120% of test concentration was taken and dilution was done appropriately. The calibration curve was constructed by plotting response factor against concentration of drugs. The slope and intercept value for calibration curve was $y = 4443.2x$ ($R^2 = 0.9995$) for Valsartan and $y = 696.72x$ ($R^2 = 0.9998$) for Hydrochlorothiazide. The results show that an excellent correlation exists between response factor and concentration of drugs within the concentration range indicated above.

The Limit of Detection (LOD) and Limit of Quantification (LOQ) of the developed method were determined by injecting progressively low concentrations of the standard solutions using the developed RP-HPLC method. The LOD is the smallest concentration of the analyte that gives a measurable response (signal to noise ratio of 3). The LOD for Valsartan and Hydrochlorothiazide was found to be 16 µg mL^{-1} and 2.5 µg mL^{-1}, respectively. The LOQ is the smallest concentration of the analyte, which gives response that can be accurately quantified (signal to noise ratio of 10). The LOQ was 48 µg mL^{-1} and 7.5µg mL^{-1} for Valsartan and Hydrochlorothiazide, respectively.

The column efficiency, resolution and peak asymmetry were calculated for the standard solutions (Table 3). The values obtained demonstrated the suitability of the system for the analysis of this drug combination.

Thus the proposed RP-HPLC method for the simultaneous estimation of Valsartan and Hydrochlorothiazide in combined dosage forms is accurate, precise, linear, rugged, robust, simple and rapid.

ACKNOWLEDGEMENT

Authors are grateful to Cipla Pvt. Ltd, Mumbai, India for gift sample. Above all, authors would like to offer their gratitude to all the authorities of Institute of Pharmaceutical Education and Research, Wardha for providing all facilities.

REFERENCES

1. The United States Pharmacopoeia, 31, National Formulary, 26. Vol 2. US Pharmacopoeial Convention, Inc.; Rockville M; 2008. D- 3496-3498.

2. Indian Pharmacopoeia, Govt. of India. Ministry of Health and Family Welfare. Vol 1. Publication by the Indian Pharmacopoeia Commission, Ghaziabad; 2007. p- 1194 – 1195.
3. Dutt R, Sing G, Dutt V, Kapoor RP. Spectrophotometric method for the determination of valsartan residue in swab samples. Indian Drugs 2007; 44 (8):591-596.
4. Macek J, Klima J. Rapid determination of valsartan in human plasma by protein precipitation and high-performance liquid chromatography. J Chromatogr B 2006; 832: 169- 172.
5. Bhanu R, Kanumula GV. Simultaneious determination of hydrochlorothiazide and losartan potassium in pharmaceutical dosage by reverse phase high performance liquid chromatography. Indian Drugs 2000; 37(1):38-41.
6. Rane SH, Zarapakar SS. Reverse phase high-performance liquid chromatographic determination of ramipril and hydrochlorothiazide in tablets. Indian Drugs 2000; 37(12): 589-593.
7. Patel DM, Shah RR, Suhagia BN. Development of a RP-HPLC method for evaluating losartan potassium and hydrochlorothiazide tablets. Indian J of Pharm Sci 2005; 67(1): 37-42.
8. Qutab SS, Ashfaq M, Khan IU. Simple and sensitive LC-UV method for simultaneous analysis of hydrochlorothiazide and candesartan cilexetil in pharmaceutical formulations. ACTA Chromatographica 2007; 19: 119-129

Table 1: Result of tablet assay

Valsartan			Hydrochlorothiazide		
Amount claimed mg/tablet	Amount found mg/tablet	% found*	Amount claimed mg/tablet	Amount found mg/tablet	% found*
80	80.2	100.33	12.5	12.4	99.23
	80.05	100.07		12.3	99.18
	80.1	100.20		12.3	99.15
	79.8	99.75		12.4	99.28
	80.01	100.02		12.4	99.32
Mean	80.05	100.07	Mean	12.4	99.23
S. D.	0.1478	0.2175	S. D.	0.05477	0.0697
R.S.D.	0.0018	0.00217	R.S.D.	0.0044	0.000702

*Mean of five estimations

S.D- Standard Deviation, R.S.D- Relative Standard Deviation

Table 2: Recovery study data of tablet formulation

Valsartan			Hydrochlorothiazide		
Amount added (mg)	Amount found (mg)	% Recovery*	Amount added (mg)	Amount found (mg)	% Recovery*
19.2	19.1	99.62	3	3.00	100.33
25.6	25.4	99.39	4	3.95	98.75
32	32.01	100.05	5	4.98	99.63

*mean of three estimations

Table 3: System suitability parameters

Parameter	Valsartan*	Hydrochlorothiazide*
Retention Time	4.15	3.20
Asymmetry	1.072	1.17
No. of Therotical Plates	2614.12	3781.63
Calibration Range µg mL^{-1}	32-80 µg mL^{-1}	2.5-12.5 µg mL^{-1}
Resolution	1.75	-
Limit of detection µg mL^{-1}	16 µg mL^{-1}	2.5 µg mL^{-1}
Limit of quantitation µg mL^{-1}	48 µg mL^{-1}	7.5 µg mL^{-1}

*mean of seven estimations

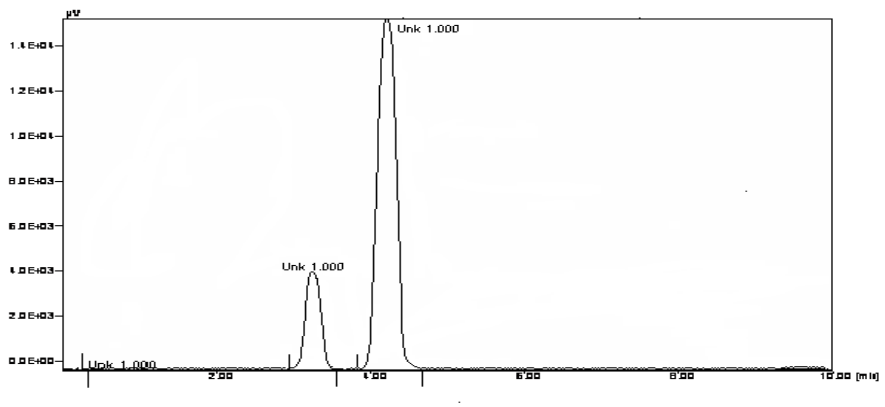

Fig I: Typical Chromatograms of Hydrochlorothiazide (Rt 3.20) with Valsartan (Rt 4.15)

Source of support: Nil, Conflict of interest: None Declared