

Original Article

## Method development and validation for the simultaneous estimation of Valsartan and Hydrochlorothiazide in tablet dosage form by RP-HPLC

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### ABSTRACT

A simple, reproducible, efficient, rapid, economical and accurate reverse phase high performance liquid chromatography (RP-HPLC) method was developed for simultaneous estimation of Valsartan and Hydrochlorothiazide in tablet dosage form. The separation was achieved on symmetry shield RP-8 column (250 x 4.6 mm internal diameter and 5 µm particle size). The mobile phase having a fixed composition of Acetonitrile: 20 mM Ammonium acetate buffer: Methanol in the ratio of 29:68:03 were used in this study. The flow rate used was 0.9 ml/min. The effluent was monitored with Photodiode array detector at 225 nm. The retention times for Valsartan and Hydrochlorothiazide were found to be 3.61 and 6.14 minutes respectively. The developed method was applied for quantitative determination of above drugs in tablet dosage forms and the method was validated with respect to specificity, precision, linearity, accuracy, system suitability, robustness and solution stability. The method was linear over the range of 40-240 µg/ml and 6.25-37.5 µg/ml for Valsartan and Hydrochlorothiazide respectively. The mean recovery was found to be in the range of 97-103%. The percentage of relative standard deviation was found to be less than critical value. The method was found to be accurate, precise and selective for simultaneous estimation of Valsartan and Hydrochlorothiazide in tablets.

### 1. INTRODUCTION

Valsartan is chemically (S)-N-(1-Oxopentyl)-N-[[2'-(1H-tetrazol-5-yl) [1, 1'biphenyl]-4-yl] methyl]-L-valine [1]. IP [2] describe liquid chromatography method and thin layer chromatography method for its estimation. Literature survey reveals RP-HPLC method, stability, degradation kinetics and *in-vitro* bioequivalence study method, UV-spectrophotometric method, isocratic HPLC method for determination of Valsartan in pharmaceutical dosage form. Literature survey also reveals spectrophotometric method, RP-HPLC method, stability indicating HPLC method, biphasic drug release method, HPTLC method and UPLC method and for the determination of Valsartan with other drugs in combination.

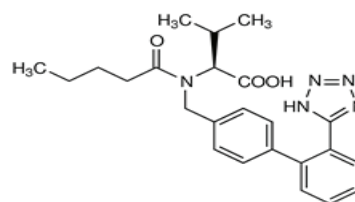


Fig. 1. Chemical structure of Valsartan

Hydrochlorothiazide (HCTZ) is chemically 6-chloro-3, 4-dihydro-2H-1, 2, 4- benzothiadiazine- 7-sulfonamide-1, 1- dioxide [3]. Hydrochlorothiazide is official in IP [4], USP [5] and liquid chromatography method and HPLC method are described for its estimation. Literature survey reveals HPTLC

method with UV absorption densitometry, HPLC method, RP-HPLC method and LC-MS/MS method for the determination of hydrochlorothiazide. Literature survey also reveals RP-HPLC method, UV-spectrophotometric method and HPLC method for the hydrochlorothiazide with combination of other drugs [6-12].

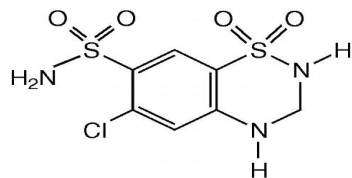


Fig. 2. Chemical structure of Hydrochlorothiazide

## 2. EXPERIMENTAL

### 2.1 Equipment

Chromatographic separation was performed on Waters HPLC system consists of model 2996 having PDA detector. Waters empower pro software was applied for data collecting and processing.

### 2.2 Reagent and chemicals

Acetonitrile and Methanol of HPLC grade were procured from Rankem lab Ltd. Water of HPLC grade were used of Millipore Company. Valsartan and Hydrochlorothiazide working standard were received as gift samples from Jubilant Life Science Ltd. Noida, India, respectively. Tablet (Valent-H) having combination Valsartan (80mg) and Hydrochlorothiazide (12.5mg) was used.

### 2.3 HPLC conditions

A Symmetry shield RP-8 (250mm x 4.6mm, 5 $\mu$  particle size) column was used as the stationary phase. A mixture of Acetonitrile, Buffer and Water in the ratio of (29:68:03v/v) was used as mobile phase. A mixture of Acetonitrile and Water in the ratio of (40:60v/v) was used as Diluent for the preparation of sample and standard solutions. The mobile phase was pumped at 0.9 ml/min. the injection volumes of sample and standard were used 15 $\mu$ l. The eluents were monitored at 225nm.

### 2.4 Sample solution

Accurately weighed and transferred 514 mg (average weight) of tablet powder of Valsartan & Hydrochlorothiazide sample into a 100ml clean and dry volumetric flask, add about 40ml of Acetonitrile and sonicate it to dissolve completely and make up volume up to the mark with the water(stock solution). Further pipette out 5ml from the above stock solution into a 25ml volumetric flask and dilute up to the mark with water.

### 2.5 Standard solutions

Accurately weighed 16 mg of Valsartan into a 25ml clean dry volumetric flask, add about 10ml of Acetonitrile and sonicate to dissolve it completely and make volume up to the mark with the water (stock-1). Accurately weighed 10 mg of Hydrochlorothiazide into a 100ml clean dry volumetric flask, add about 40ml of Acetonitrile and sonicate to dissolve it completely

and makeup volume up to the mark with the water (stock-2). Take 5ml each from stock-1 and stock-2 into a 20ml clean dry volumetric flask and makeup volume up to the mark with Water.

### 2.6 Assay of tablet formulation

Twenty tablets were taken and weighed (each containing 80 mg of Valsartan and 12.5 mg of Hydrochlorothiazide) and finely powdered. A quantity of powder (average weight) equivalent to 80 mg of Valsartan and 12.5 mg of Hydrochlorothiazide was weighed and transferred to 100 ml standard volumetric flask. The drug was initially dissolved in Acetonitrile and sonicated for 15 minutes. The volume was made up to 100 ml with water. The solution was further diluted to 25 ml by taking 5 ml from above solution with Water. The solution was filtered through PTFE Axiva filter. Then 15 $\mu$ L of this solution was injected into the column and collecting data and record the chromatogram as shown in the figure 1. Concentration of valsartan and hydrochlorothiazide in the tablet formulation were calculated by comparing area of the sample with that of standard. The percentage assay of individual drug was calculated and presented in Table 1.

Table 1. Report for assay

S. No.	Drug	Amount present (mg/tablet)	Amount found (mg/tablet)	Label claim (%)
1.	Valsartan	80	79.81	99.2
2.	Hydrochlorothiazide	12.5	12.47	98.0

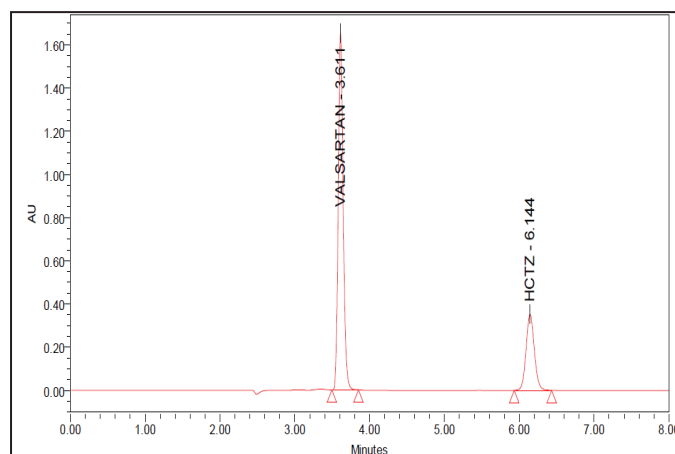


Fig. 3. Chromatogram of the Sample

## 3. VALIDATION OF THE METHOD

### 3.1 System suitability

The resolution and peak symmetry were calculated for the standard solution. The values obtained demonstrated the suitability of the system for the analysis of this drug combination and the system suitability parameters fall within  $\pm 2\%$  relative standard deviation range during performance of the method. Here tailing factor for the peaks of Valsartan and Hydrochlorothiazide was found to be less than 2.0 and the resolution was satisfactory.

The peaks obtained for Valsartan and Hydrochlorothiazide were sharp and have clear base line separation.

**Table 2.** System suitability parameters

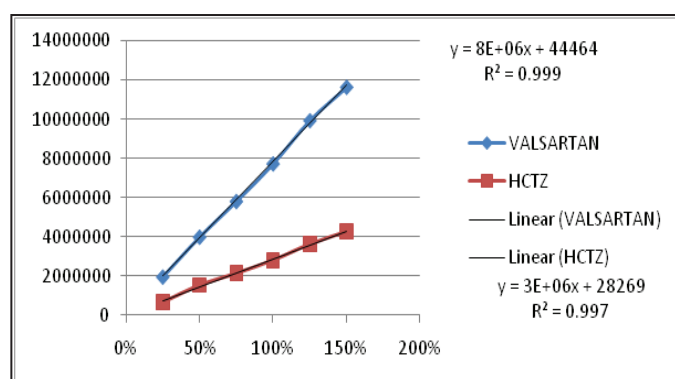
S. No.	Parameters	Valsartan	Hydrochlorothiazide	Acceptance criteria
1	Plate count (USP)	9271	14591	NLT 5000
2	Retention time (min.)	3.56	6.14	NA
3	Tailing factor	1.22	1.09	NMT 2.0
4	Resolution		14.41	NLT 2.0

### 3.2 Linearity

The developed method has been validated as per ICH guidelines. Every 15µL of the working standard solution of Valsartan in the concentration range of 40–240 µg/ml and for Hydrochlorothiazide in the concentration range of 6.25-37.5 µg/ml were injected into the chromatographic system. The chromatograms were developed and the peak area was determined for each concentration of the drug solution. Calibration curve of Valsartan and Hydrochlorothiazide were obtained by plotting the peak area ratio versus the applied concentrations of Valsartan and Hydrochlorothiazide. The linearity curves of Valsartan and Hydrochlorothiazide were shown in Figure 4 and linearity data were shown in Table 3.

**Table 3.** Statistical data of calibration curve of Valsartan and Hydrochlorothiazide

S. No.	Parameters	Valsartan	Hydrochlorothiazide
1.	Linearity range (µg/ml)	40-240µg/ml	6.25-37.5µg/ml
2.	Correlation coefficient	0.999	0.997

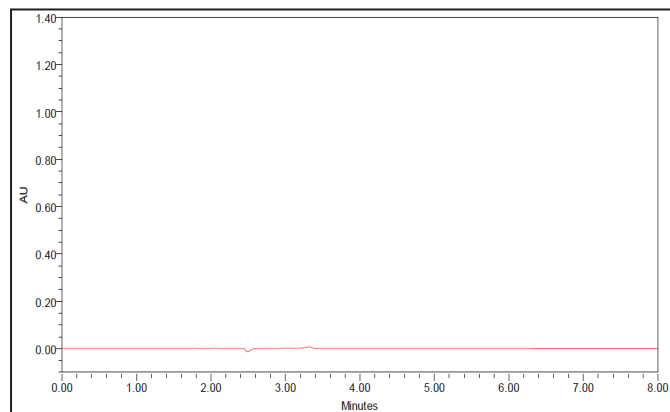


**Fig. 4.** Linearity curve for Valsartan and Hydrochlorothiazide concentration level versus mean area

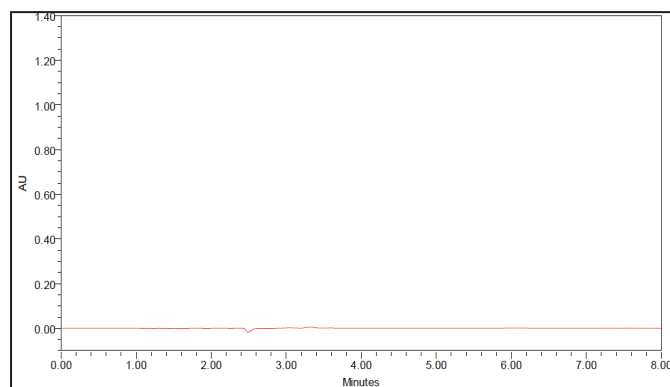
### 3.3 Specificity

Specificity measures only the desired component without interference from other species that might be present. Analyze blanks, sample matrix (placebo) and other known related impurities to determine whether interference occurs or not.

Relative standard deviation and other factors like plate count, tailing factor, and resolution were also determined.



**Fig. 5.** Chromatogram of Blank solution (specificity study)



**Fig. 6.** Chromatogram of Placebo solution (specificity study)

**Table 4.** Specificity study data of Valsartan and Hydrochlorothiazide in standard solution

S. No.	Valsartan Area	Hydrochlorothiazide Area
1	7522777	2891980
2	7544729	2858511
3	7569158	2871415
4	7590134	2881657
5	7579386	2884280
6	7533374	2868847
Mean	7556593	2876115
SD	29252	12115
% RSD	0.39	0.42

**Table 5.** Specificity study data of Valsartan and Hydrochlorothiazide in sample solution

Sample	Valsartan Area	HCTZ Area
Injection-1	7536488	2700240
Injection-2	7542795	2682734
Mean	7539641	2691487
SD	4460	12379
RSD	0.06	0.46
% Assay in tablets	99.2	98.0

### 3.4 Accuracy

Recovery studies were carried out by applying the standard addition method. A known amount of standard valsartan and hydrochlorothiazide corresponding to 50%, 100% and 150% of the label claim was added to pre analyzed sample of tablet dosage form separately. The recovery studies were carried out three times, at each level of recovery. The data's of accuracy were shown in table 6.

**Table 6.** Recovery studies of Valsartan and Hydrochlorothiazide

Drug	Concentration (%)	Amount added (µg)	Amount recovered (µg)	Recovery (%)	Mean recovery (%)
Valsartan	50%	400	409.03	102.26	
	100%	800	799.12	99.89	100.75
	150%	1200	1201.17	100.10	
Hydrochlorothiazide	50%	50	49.98	99.97	
	100%	100	97.23	97.23	98.52
	150%	150	147.57	98.38	

### 3.5 Precision

The precision of the method was demonstrated by System precision, Method precision, Intraday precision and Interday precision studies. In the System precision studies, six replicate injections of the working standard solution prepared as per the proposed method and chromatograms were recorded. Standard deviation and Relative standard deviation for the area was calculated and presented in Table 7. In the Method precision studies, six different samples were prepared in different volumetric flask of similar average weight and injected two runs as per the proposed method and chromatograms were recorded. Standard deviation and relative standard deviation for the area was calculated and presented in Table 8. In Intraday precision, different analyst were prepared the six different samples and injected of same day of method precision as per the proposed method and chromatograms were recorded. Standard deviation and relative standard deviation for the area was calculated and presented in Table 9. In Interday precision, the main analyst were prepared the sample solution on different day and injected as per the proposed method and chromatograms were recorded. Standard deviation and Relative standard deviation for the area was calculated and presented in Table 10.

**Table 7.** System precision report

S. No.	Valsartan Area	Hydrochlorothiazide Area
1	7561932	2882112
2	7517633	2877683
3	7502996	2871655
4	7481992	2864345
5	7527237	2879311
6	7519650	2878032
Mean	7518573	2875523

SD	33853	6460
% RSD	0.45	0.22
% Assay in tablet	101.9	98.2

**Table 8.** Method precision report

S. No.	Valsartan Area	Hydrochlorothiazide Area
1	7727113	2731161
2	7601220	2694821
3	7637966	2726768
4	7654907	2718028
5	7745132	2759368
6	7691787	2745359
Mean	7676354	2729251
SD	54998	22306
% RSD	0.72	0.82
% Assay in tablet	102.0	99.3

**Table 9.** Intraday precision report

Name	S. No.	Valsartan assay	Hydrochlorothiazide assay
Method Precision	1	102.70	99.5
	2	100.90	98
	3	101.50	99.3
	4	101.80	99
	5	102.90	100.4
	6	102.10	99.8
Intraday Precision	7	102.40	99.2
	8	100.90	97.9
	9	101.30	99.5
	10	101.60	98.8
	11	103.20	100.5
	12	101.20	98
	AVERAGE	101.88	99.16
	S.D.	0.78	0.88
	% R.S.D.	0.77	0.88

**Table 10.** Interday precision report

	S. No.	Valsartan assay	Hydrochlorothiazide assay
Method Precision	1	102.70	99.5
	2	100.90	98.0
	3	101.50	99.3
	4	101.80	99.0
	5	102.90	100.4
	6	102.10	99.8

Interday Precision	7	99.60	99.0
	8	98.30	97.1
	9	98.30	97.0
	10	98.90	97.3
	11	98.40	97.5
	12	99.20	98.1
	AVERAGE	100.38	98.5
	S.D.	1.78	1.10
	% R.S.D.	1.78	1.12

### 3.6 Solution stability

Solution stability was evaluated at room temperature for 24 hours. The percentage difference of relative standard deviation was found to be less than 2.0%. It indicates that both sample and standard solution were stable for 24 hours, which was sufficient to complete the whole analytical process.

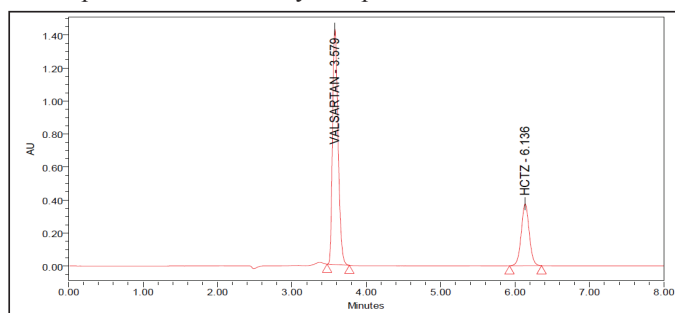


Fig. 7. Chromatogram of Standard solution (solution stability study)

Table 11. System suitability parameters

Name	Area	Retention Time (min)	USP Tailing	USP Plate Count	USP Resolution
1 VALSARTAN	7398836	3.58	1.32	10954	
2 HCTZ	2800740	6.14	1.03	15859	15.14

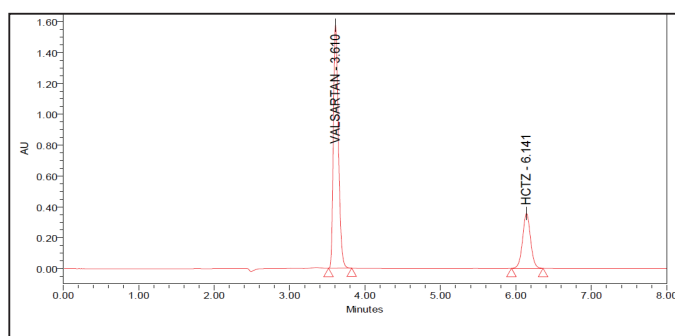


Fig. 8. Chromatogram of Sample solution at 0-hour

Table 12. System suitability parameters

Name	Area	Retention Time (min)	USP Tailing	USP Plate Count	USP Resolution
1 VALSARTAN	7310815	3.61	1.31	13894	
2 HCTZ	2598419	6.14	1.03	16604	15.82

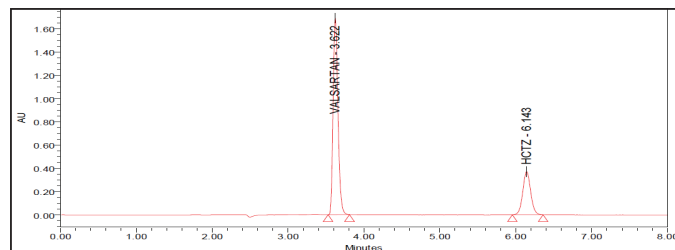


Fig. 9. Chromatogram of Sample solution at 24-hour

Table 13. System suitability parameters

Name	Area	Retention Time (min)	USP Tailing	USP Plate Count	USP Resolution
1 VALSARTAN	7516323	3.62	1.24	15206	
2 HCTZ	2684159	6.14	1.06	16968	16.14

Table 14. Solution stability data for Valsartan and Hydrochlorothiazide

S. No.	Valsartan area	Hydrochlorothiazide area
1	7302562	2596967
2	7331229	2606449
3	7358405	2620820
4	7400640	2637768
5	7422129	2631495
6	7494084	2661763
Mean	7384841	2625877
SD	69162	23236
% RSD	0.94	0.88

### 3.7 Robustness

The robustness of the method was determined by making slight changes in the method conditions such as the changes in the column oven temperature and changes in the buffer concentration and the chromatographic characteristics were evaluated.

## 4. RESULTS AND DISCUSSION

The proposed method was found to be simple and sensitive with linearity in the concentration range of 40-240 µg/ml and 6.25-37.5 µg/ml of Valsartan and Hydrochlorothiazide respectively in the above 0.99 correlation coefficient. System suitability parameter indicates good resolution of both the peaks. In addition high column efficiency was indicated from the large number of theoretical plates (>5000). The degree of asymmetry was also evaluated using the tailing factor which did not exceed the critical value (2.0) indicating acceptable degree of peak asymmetry. The method was found to be accurate and precise as indicated by results of recovery studies and precision studies in which % RSD not more than 2.0%. There were no marked changes in the chromatograms which confirmed the ruggedness of the method. The standard deviation of % assay for sample was calculated for each parameter in robustness studies and relative standard deviation was found to be less than 2.0%. The low RSD value confirms the robustness of the method.

## 5. CONCLUSION

The proposed RP-HPLC method allows for accurate, precise and reliable measurement of Valsartan and Hydrochlorothiazide simultaneously in tablet dosage form. The method was evaluated in mass of facts, such as best condition, linear relation including coefficient of correlation, robustness, accuracy, reproducibility and precision. The developed method can be used for routine quantitative simultaneous estimation of Valsartan and Hydrochlorothiazide in pharmaceutical preparation. All these factors lead to the conclusion that the proposed method is accurate, precise, simple, sensitive, rugged and rapid and can be applied successfully for the estimation of Hydrochlorothiazide and Valsartan in pharmaceutical formulations without interference and with good sensitivity.

## REFERENCES

- [1] Sweetman C Ed. The Martindale Complete Drug Reference 35th Edition, Pharmaceutical Press, 2002, pp. 938, 964.
- [2] Indian Pharmacopoeia, Vol. III, Government of India, Indian Pharmacopoeia commission, The controller of Publications, Ghaziabad, 2010, pp. 2286-2287.
- [3] J. O'Neil Maryadele ed, Merck Index 14th Edition, Merck & Co. Inc, White House Station NJ, 2006, pp. 4936,6431.
- [4] Indian Pharmacopoeia, Vol. II, Indian Pharmacopoeia Commission, Ghaziabad, 2010, p. 1452.
- [5] The United States pharmacopoeia, Vol. II & III, 30th Revision, NF 25, (2007), The United states Pharmacopoeia Convention, Inc. Rockville, MD, p. 2287-2288.
- [6] Kumar S, Sahu PV, Durgaprasad K, Shekhar M. Development and Validation of Analytical Method for the Estimation of Valsartan in Pure and Tablet Dosage form by RP-HPLC method. International Journal of Research in Pharmacy and Chemistry 2011; 1: 945-949.
- [7] Kendre MD, Banerjee SK. Precise and accurate RP-HPLC method development for quantification of Valsartan in tablet dosage form. International Journal of Pharmaceutical Sciences and Drug Research 2012; 2: 137-139.
- [8] Vinzuda DU, Sailor GU, Sheth NR. RP-HPLC method for determination of Valsartan in tablet dosage form. International Journal of Chemtech Research 2010; 2: 1461-1467.
- [9] Siddiqui N, Husain A, Choudhary L, Mitra M, Bhasin PS. Stability degradation kinetics and invitro bioequivalence studies of Valsartan tablets. Current pharma research 2011; 2: 389-395.
- [10] Gupta KR, Wadodkar AR. UV-spectrophotometric methods for estimation of Valsartan in bulk and tablet dosage form. International Journal of Chemtech Research 2010; 2: 985-989.
- [11] Kalaimagal A, Jerad SA, Niraimathi V. Spectrophotometric methods for the estimation of valsartan in bulk and oral dosage form. International Journal of Pharmacy and Pharmaceutical Sciences 2012; 4: 481-483.
- [12] Tarkase KN, Tajane SR, Jadhav MB. Development and validation of UV-spectrophotometric methods for estimation of valsartan in bulk and tablet dosage form. Journal of Pharmacy Research 2012; 5: 2344-2346.