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Jyothsna Modugula
Department of pharmaceutical Analysis, SIMS College of Pharmacy, Mangaldas Nagar, Guntur, Andhra Pradesh, India

Sushma Rachamalla
Bacholar of Pharmacy, SIMS College of Pharmacy, Mangaldas Nagar, Guntur, Andhra Pradesh, India

Manohar Reddy Jakkireddy
Bacholar of Pharmacy, SIMS College of Pharmacy, Mangaldas Nagar, Guntur, Andhra Pradesh, India

Indira Devi P
Bacholar of Pharmacy, SIMS College of Pharmacy, Mangaldas Nagar, Guntur, Andhra Pradesh, India

Venkata Seshagiri Podili
Department of Pharmacology, St. Xavier Institute of Pharmacy, Phirangipuram, Guntur, Andhra Pradesh, India

Krishnaveni Kamepalli
Department of Pharmaceutical Chemistry, St. Xavier Institute of Pharmacy, Phirangipuram, Guntur, Andhra Pradesh, India

Correspondence
Jyothsna Modugula
Department of pharmaceutical Analysis, SIMS College of Pharmacy, Mangaldas Nagar, Guntur, Andhra Pradesh, India

Quantification of valsartan and hydrochlorothiazide in bulk and tablet formulation by RP-HPLC method

Jyothsna Modugula, Sushma Rachamalla, Manohar Reddy Jakkireddy, Indira Devi P, Venkata Seshagiri Podili and Krishnaveni Kamepalli

Abstract
A simple, economic, selective and precise RP-HPLC method has been developed and validated for Valsartan and Hydrochlorothiazide in bulk and tablet dosage form. The isocratic LC analysis was performed on Inertsil BDS C18 column (250 mm x 4.6 mm, 5 µ) using mobile phase composed of Methanol and 0.05M phosphate buffer pH 4.5 (55:45 v/v) at a flow rate of 1.0 ml/min. Quantitation was performed using UV detector at 281 nm. The retention time was found to be 3.541min for Valsartan and 5.221min for Hydrochlorothiazide. The analytical method was validated according to ICH guidelines. The linearity was observed in the range of 20-60 µg/ml for both Valsartan and 99.67 to 99.94 % for Hydrochlorothiazide with correlation coefficients (r^2) are 0.999 for Valsartan and 0.998 for Hydrochlorothiazide. The % recovery was found to be 100.12 - 100.89 % for Valsartan and Hydrochlorothiazide. The relative standard deviation values for repeatability and intermediate precision studies were less than 2%. The proposed method was precise, rapid, accurate, and cost-effective and can be used for the routine estimation for Valsartan and Hydrochlorothiazide in tablet dosage form.

Keywords: Valsartan, Hydrochlorothiazide, RP-HPLC, Tablet dosage form

1. Introduction [1-2]

Valsartan is an angiotensin-receptor blocker (ARB) that may be used to treat a variety of cardiac conditions including hypertension, diabetic nephropathy and heart failure. Valsartan lowers blood pressure by antagonizing the renin-angiotensin-aldosterone system (RAAS); it competes with angiotensin II for binding to the type-1 angiotensin II receptor (AT1) subtype and prevents the blood pressure increasing effects of angiotensin II. Unlike angiotensin-converting enzyme (ACE) inhibitors, ARBs do not have the adverse effect of dry cough. Valsartan may be used to treat hypertension, isolated systolic hypertension, left ventricular hypertrophy and diabetic nephropathy. It may also be used as an alternative agent for the treatment of heart failure, systolic dysfunction, myocardial infarction and coronary artery disease.

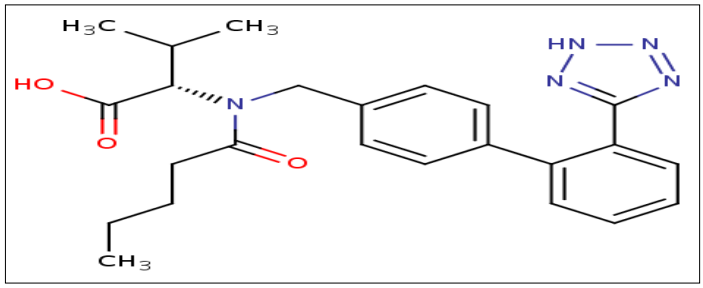

Fig 1: Structure of Valsartan

Hydrochlorothiazide, a thiazide diuretic, inhibits water reabsorption in the nephron by inhibiting the sodium-chloride symporter (SLC12A3) in the distal convoluted tubule, which is responsible for 5% of total sodium reabsorption. Normally, the sodium-chloride symporter transports sodium and chloride from the lumen into the epithelial cell lining the distal convoluted tubule. The energy for this is provided by a sodium gradient established by sodium-potassium ATPases on the basolateral membrane. Once sodium has entered the cell, it is transported out into the basolateral interstitium via the sodium-potassium ATPase,

causing an increase in the osmolarity of the interstitium, thereby establishing an osmotic gradient for water reabsorption. By blocking the sodium-chloride symporter, hydrochlorothiazide effectively reduces the osmotic gradient and water reabsorption throughout nephron.

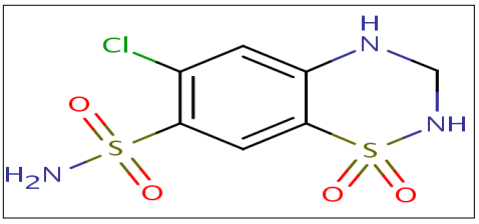

Fig 2: Structure of Hydrochlorothiazide

2. Materials and Methods [3-5]

2.1 Apparatus

HPLC apparatus used in this investigation is waters Alliance HPLC system (Model No.2998) series Compact System Consisting of Waters Empower2 software was used to process the chromatographic data. Electronic balance model ELE 300, Sonicator (Fast Clean), Digital pH meter were used.

2.2 Chemicals

Valsartan and Hydrochlorothiazide Working Standards obtained from local market. Methanol HPLC Grade, Analytical reagent grade Potassium dihydrogen phosphate, orthophosphoric acid were obtained from SD Fine Chemicals Ltd (Mumbai, India). Distilled water purified by a Millipore Milli-Q apparatus (Millipore, France) was used right through the investigation.

2.3 Preparation of Mobile phase

Methanol: 0.05M phosphate buffer PH 4.5 (55:45)

2.3.1 Preparation of 0.05 M phosphate buffer solution pH 4.5

Dissolve 6.80 g of potassium dihydrogen phosphate in 1000.0 mL of water. Adjust the pH of solution to 4.5 if necessary with phosphoric acid.

2.4 Preparation of standard solutions

The solution was prepared by dissolving 10.0 mg of accurately weighed Valsartan and 15.0 mg of Hydrochlorothiazide in Mobile phase, in two 50.0 mL volumetric flasks separately and sonicated for 10min. From the above solutions take 5.0 mL from each solution into a 25.0 mL volumetric flask and then makeup with mobile phase and sonicate for 10min. The above solutions were diluted to get a concentrations equivalent to 40 and 60 µg/mL of Valsartan and Hydrochlorothiazide respectively and the solution was filtered through 0.45µ membrane.

2.5 Preparation of sample drug solution for pharmaceutical formulations

Twenty tablets were weighed accurately and a quantity of tablet powder equivalent to 20 mg Valsartan and 30 mg of Hydrochlorothiazide was weighed and dissolved in the 30 mL mobile phase and sonicate for 10 min. The content was diluted to 100 mL with mobile phase. This solution was filtered through a 0.45 µm Nylon syringe filter and 10.0 mL of the filtrate was diluted into a 50.0 mL volumetric flask to give a test solution containing 40 µg/mL of Valsartan and 60 µg/mL of Hydrochlorothiazide.

2.6 Optimized chromatographic conditions

Stationary phase (column): Inertsil -BDS C_{18} (250 x 4.6 mm, 5 µ)
Mobile Phase: Methanol: 0.05M phosphate buffer PH 4.5 (55:45)
Flow rate (ml/min): 1.0 ml/min
Run time (minutes): 10 min
Column temperature (°C): 25°C
Volume of injection loop (µl): 20
Detection wavelength (nm): 281 nm
Drug RT (min): 3.541min for Valsartan and 5.221min for Hydrochlorothiazide

2.7 Detection of absorption maximum

The sensitivity of method that uses UV detector depends upon the proper selection of wavelength is that gives maximum absorbance and good response for the given set of drugs. In setting up the conditions for development of the assay method, the choice of the detection wavelength was based on the scanned absorption spectrum for Valsartan and Hydrochlorothiazide. The UV-spectrum of Valsartan and Hydrochlorothiazide was separately scanned in the wavelength range of 200-400 nm against blank. After correlation of the both spectrums 281nm wavelength was selected for the analysis.

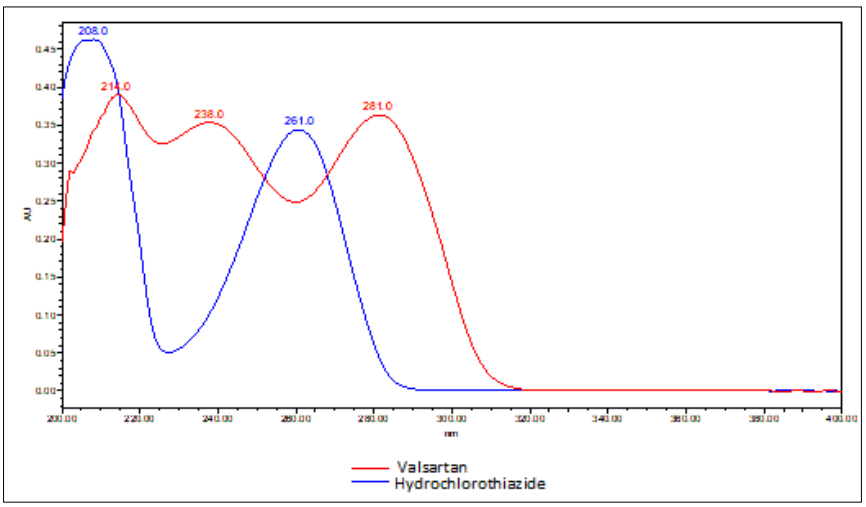

Fig 3: Overlay UV spectrum of Valsartan and Hydrochlorothiazide

3. Results and Discussion

3.1 Method Development

Trials were done to develop a RP-HPLC method which was able to separate and quantify the Valsartan and Hydrochlorothiazide in short time with an adequate sensitivity and selectivity. So as to achieve the good chromatographic separation of Valsartan and Hydrochlorothiazide and to improve peaks symmetry, various parameters like choice of mobile phase, its composition, flow rate and detection wavelength were considered during optimization of method. During trials with different columns, it was observed that Inertsil-BDS C_{18} (250 x 4.6 mm, 5 µ) gave good results (Good symmetric and sharp peaks). Hence the same column is used in the analysis. Different mobile phases in different ratios, different flow rate and different pH were tried. Finally methanol and 0.05M phosphate buffer PH 4.5 in the ratio of (55:45v/v) with a flow rate of 1 ml/min was selected as mobile phase and these chromatographic conditions provided less analysis time, good peak response, symmetric peaks and best resolution. The sensitivity of the method was good at a wavelength of 281 nm. Therefore the same wavelength was selected as analytical wavelength. The chromatogram of Valsartan and Hydrochlorothiazide after optimization is given in fig. 4.

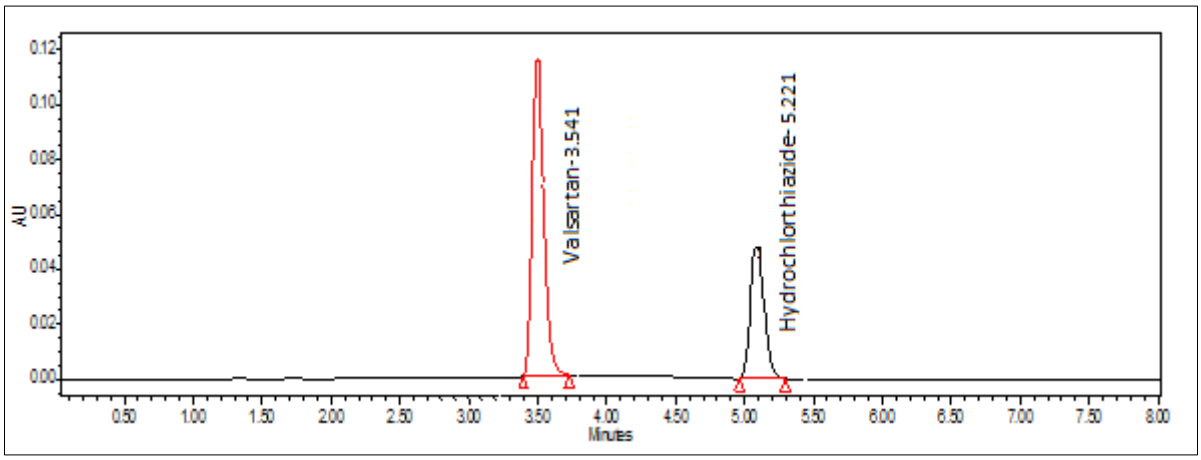

Fig 4: Standard chromatogram for Valsartan and Hydrochlorothiazide

3.2 Method validation [6, 7]

The method validation parameters were done according to Instructions given by ICH guidelines for method validation.

3.2.1 System suitability

A Standard solution was prepared by using Valsartan and Hydrochlorothiazide working standards as per test method and was injected Five times into the HPLC system.

The system suitability parameters were evaluated from standard chromatograms by calculating the % RSD, retention times and peak areas from five replicate injections for Valsartan and Hydrochlorothiazide. Results presented in Table-1 and proved that the values are within the accepted limits indicating suitability of the system for good chromatographic separation and analysis.

Table 1: System Suitability parameters for valsartan and Hydrochlorothiazide

Parameters	Valsartan	Hydrochlorothiazide	Recommended limits
Retention time	3.523	5.214	RSD≤ 2
Peak area	1328793	829379	RSD≤ 2
USP resolution	_	7.951	> 1.5
USP plate count	10453	11647	> 2000
USP tailing factor	0.776	1.468	≤ 2

3.2.2 Specificity

The specificity of the method was demonstrated by comparing the chromatogram of a placebo solution, a mobile phase blank, a tablet sample solution (40 µg/ml Valsartan and 60 µg/ml Hydrochlorothiazide) with a drug standard solution (40 µg/ml Valsartan and 100 µg/ml Hydrochlorothiazide). Chromatograms of placebo solution (Fig. 5a) and mobile phase blank (Fig. 5b) showed that there was no interference from common excipients and components of mobile phase. Chromatogram of tablet sample solution (Fig. 5c) and Chromatogram of standard solution (Fig. 5d) proved that tablet excipients did not interfere as no additional interfering peaks were observed. These results proved that the method was selective.

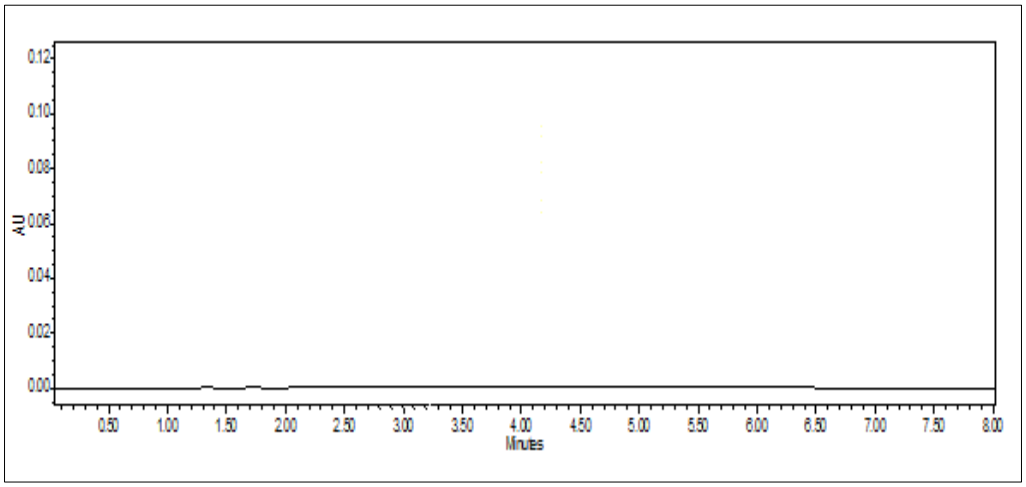

Fig 5 (a): Chromatogram of Placebo

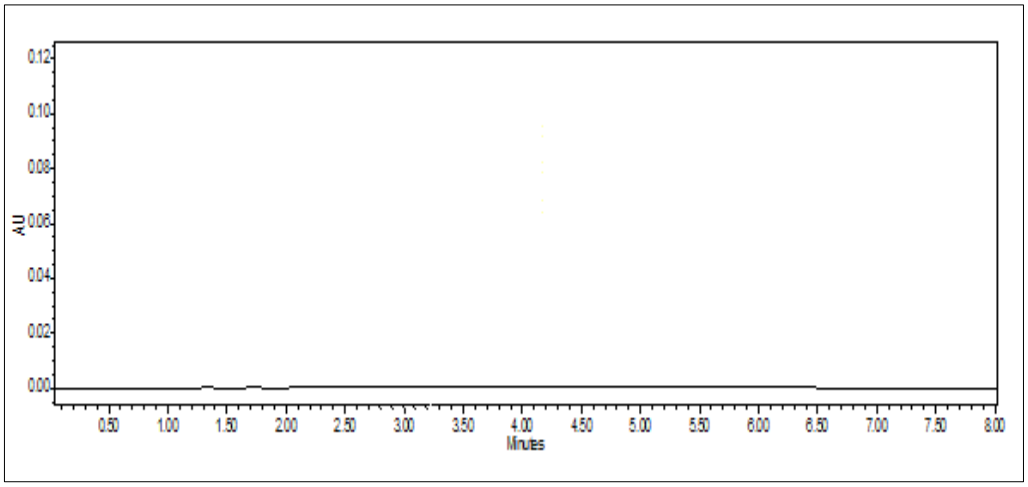

Fig 5 (b): Chromatogram of Mobile phase

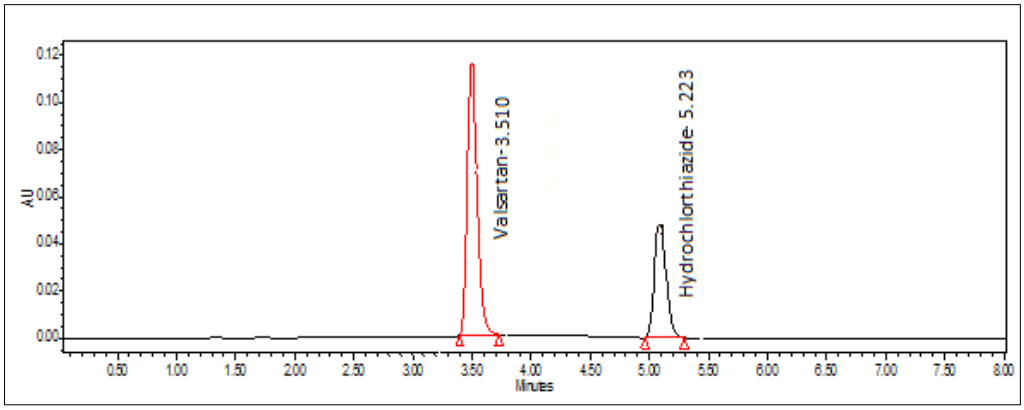

Fig 5 (c): Chromatogram of tablet sample solution

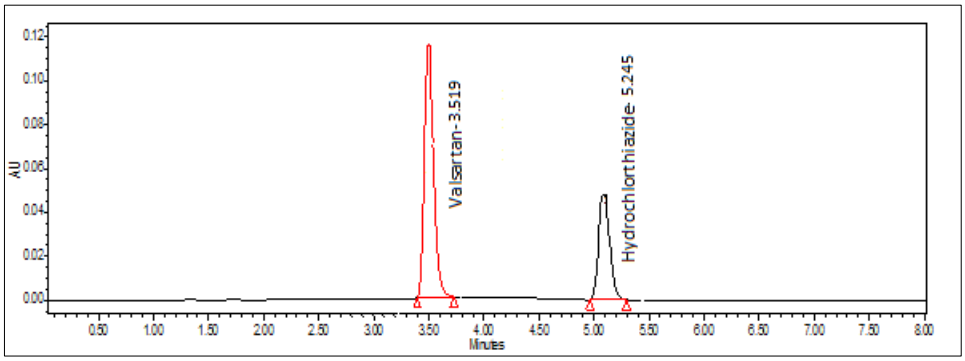

Fig 5 (d): Chromatogram of standard solution

3.2.3 Precision

3.2.3.1 System precision: Standard solution prepared as per test method and injected for five times and the results were given in Table-2.

Table 2: Results for System precision

S. No.	Peak Area		% Assay	
	Valsartan	Hydrochlorothiazide	Valsartan	Hydrochlorothiazide
1	1318805	834360	99.95	98.86
2	1314014	839098	100.24	99.86
3	1315474	855696	100.06	100.56
4	1327655	848289	100.53	99.54
5	1367019	844147	99.98	99.54
Mean	1328593	844318	100.15	99.672
SD	22124.07	8241.164	0.23952	0.61605
% RSD	1.66	0.98	0.24	0.06

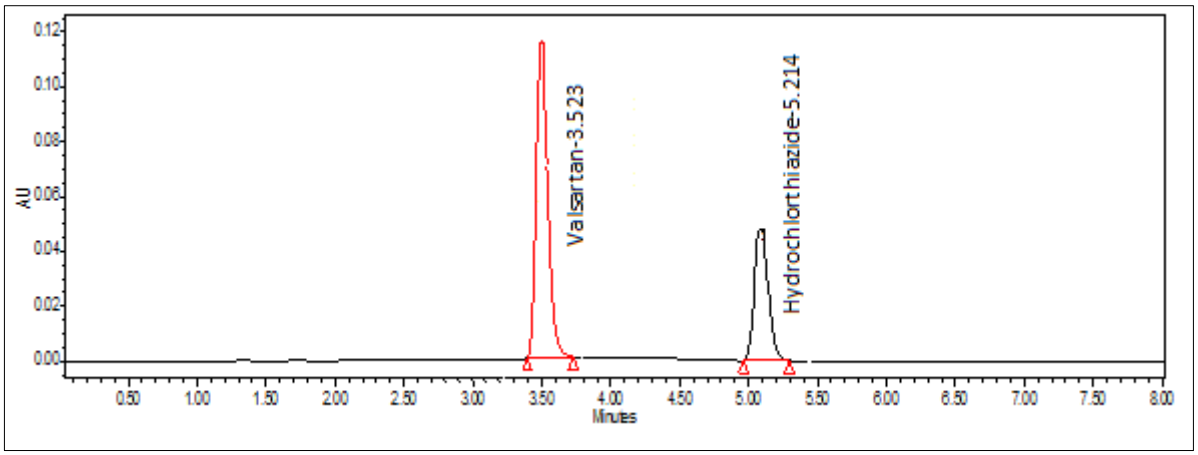

Fig 6(a): Chromatogram for system precision-1

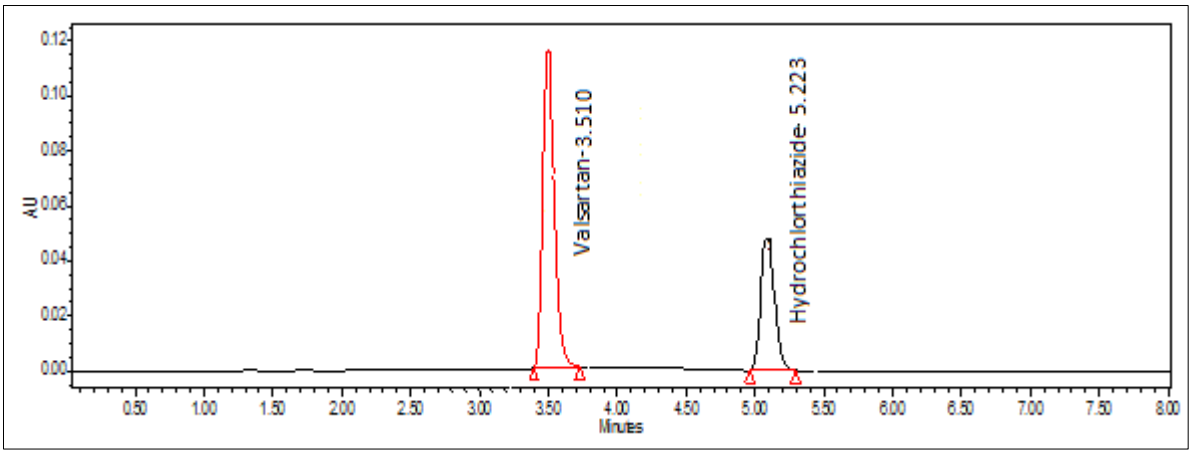

Fig 6(b): Chromatogram for system precision-2

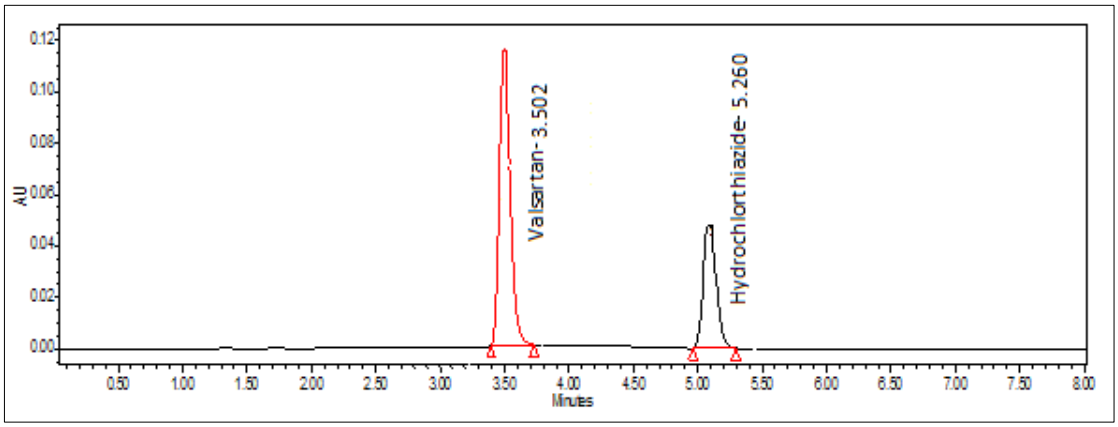

Fig 6(c): Chromatogram for system precision-3

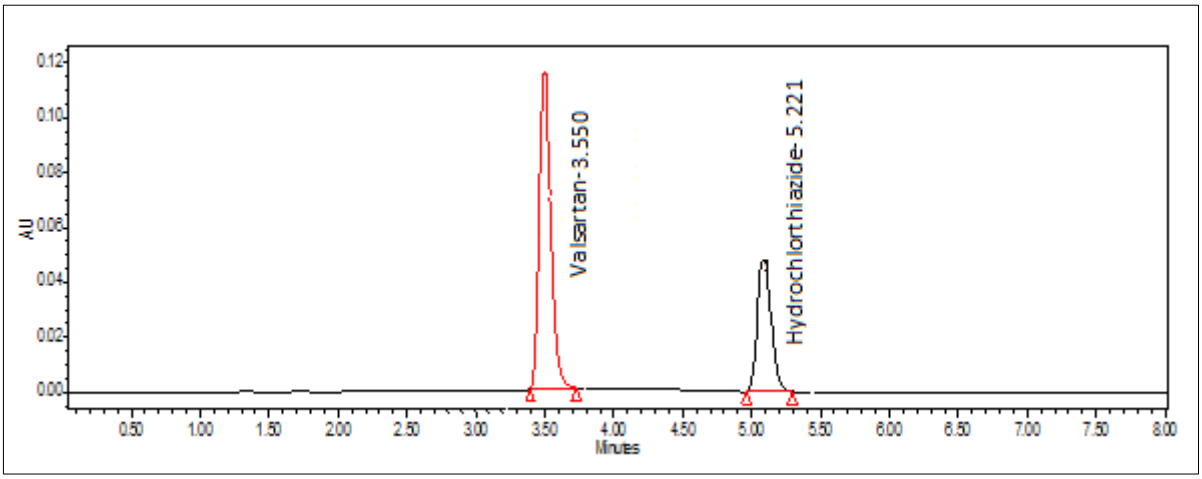

Fig 6(d): Chromatogram for system precision-4

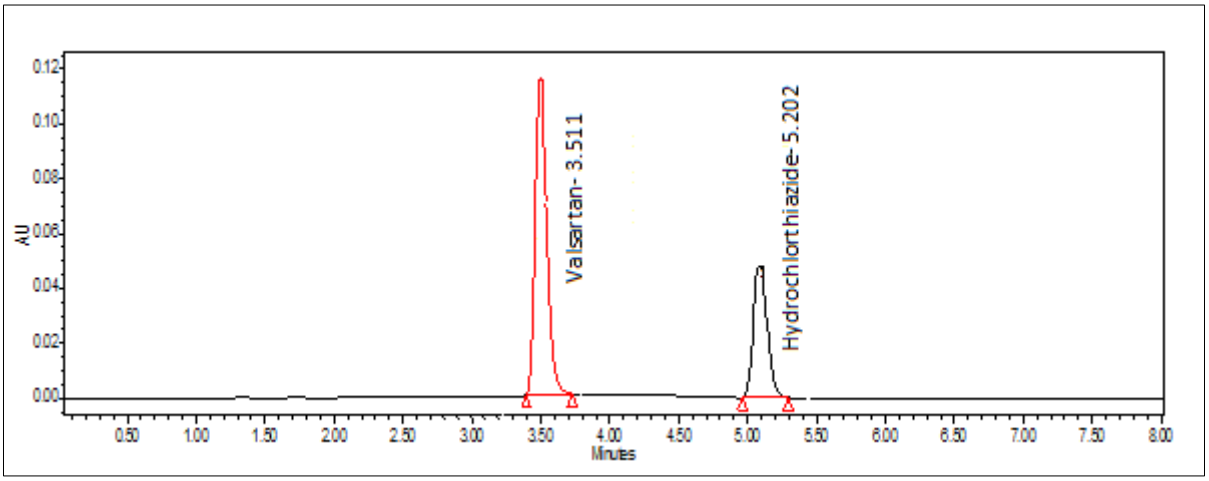

Fig 6(e): Chromatogram for system precision-5

3.2.3.2 Method precision: Prepared six sample preparations individually using single as per test method and injected each solution and the results were given in Table-3.

Table 3: Results for Method precision

S No.	Peak Area		% Assay	
	Valsartan	Hydrochlorothiazide	Valsartan	Hydrochlorothiazide
1	1302410	833495	99.98	98.56
2	1314214	835992	100.32	99.51
3	1315874	839828	100.15	100.21
4	1327655	839098	100.85	99.85
5	1317419	848289	99.99	99.94
Mean	1315514	839340.4	100.25	99.61
SD	9007.876	5607.014	0.35884	0.64010
% RSD	0.68	0.67	0.36	0.64

3.2.3.3 Intermediate precision (analyst to analyst variability)
A study was conducted by two analysts as per test method. The results for analyst-1 were given in Table-3 and analyst-2 was given in Table-4.

Table 4: Results for Method precision

S No.	Peak Area		% Assay	
	Valsartan	Hydrochlorothiazide	Valsartan	Hydrochlorothiazide
1	1312110	832295	99.95	99.86
2	1324114	838592	99.24	98.86
3	1318414	838428	100.16	100.16
4	1324615	839458	100.23	99.84
5	1317099	845129	99.88	99.94
Mean	1319270	838780	99.89	99.732

SD	5214.042	4554.77	0.3920	0.5037
% RSD	0.40	0.54	0.39	0.50

3.2.4 Accuracy (recovery)

A study of Accuracy was conducted. Drug Assay was performed in triplicate as per test method with equivalent amount of Valsartan and Hydrochlorothiazide into each volumetric flask for each spike level to get the concentration of Valsartan and Hydrochlorothiazide equivalent to 50%, 100%, and 150% of the labelled amount as per the test method. The average % recovery of Valsartan was tabulated in Table-5(a) and Hydrochlorothiazide was tabulated in Table-5(b).

Table 5(a): Results for Accuracy for Valsartan

S No.	Concentration (%)	Original level (µg/ml)	Amount added (µg/ml)	% Recovery	Mean % Recovery	%RSD
1	50	20	20.24	101.2	100.26	0.81
2	50	20	19.98	99.9		
3	50	20	19.94	99.7		
4	100	40	40.58	101.45	100.89	0.82
5	100	40	40.51	101.27		
6	100	40	39.98	99.95		
7	150	60	60.22	100.36	100.12	0.32
8	150	60	59.86	99.76		
9	150	60	60.16	100.26		

Table 5(b): Results for Accuracy for Hydrochlorothiazide

S No.	Concentration (%)	Original level (µg/ml)	Amount added (µg/ml)	% Recovery	Mean % Recovery	%RSD
1	50	30	30.15	100.50	99.67	0.82
2	50	30	29.96	98.86		
3	50	30	29.90	99.66		
4	100	60	59.88	99.80	99.89	0.28
5	100	60	60.12	100.20		
6	100	60	59.80	99.67		
7	150	90	90.12	100.13	99.94	0.19
8	150	90	89.76	99.73		
9	150	90	90.06	100.07		

3.2.5 Linearity

Linearity is a key parameter of analytical method that demonstrates the limit within which the intended method is to be used for its optimum performance. A Series of solutions are prepared using Valsartan and Hydrochlorothiazide working standards at concentration levels from 20ppm to 60ppm of target concentration. Measure the peak area response of solution and the results were tabulated in Table-6. The graph was plotted by taking concentration of the drug on x-axis and peak area on y-axis. The linearity plots of Valsartan and Hydrochlorothiazide were shown in the fig 7(a) and 7(b) respectively.

Table 6: Results for Linearity of Valsartan and Hydrochlorothiazide

Analyte	Concentration range (µg/mL)	Correlation Coefficient (R2)	Slope	Intercept
Valsartan	20-60	0.999	32827	7179.6
Hydrochlorothiazide	20-60	0.998	13753	2601.9

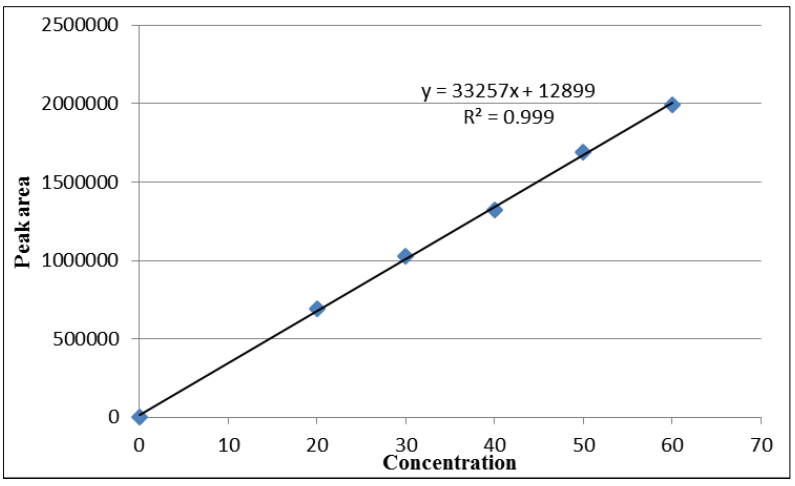

Fig 7(a): Linearity graph for Valsartan

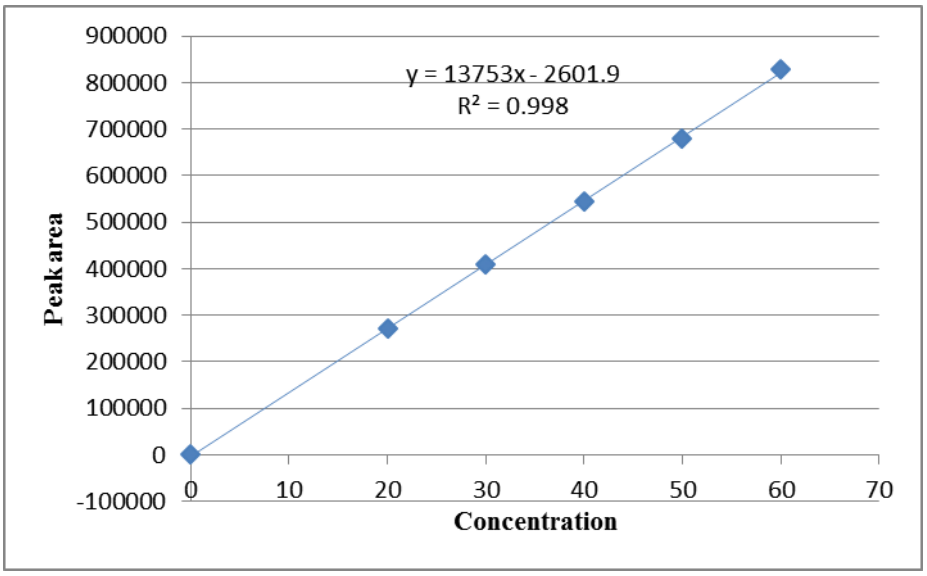

Fig 7(b): Linearity graph for Hydrochlorothiazide

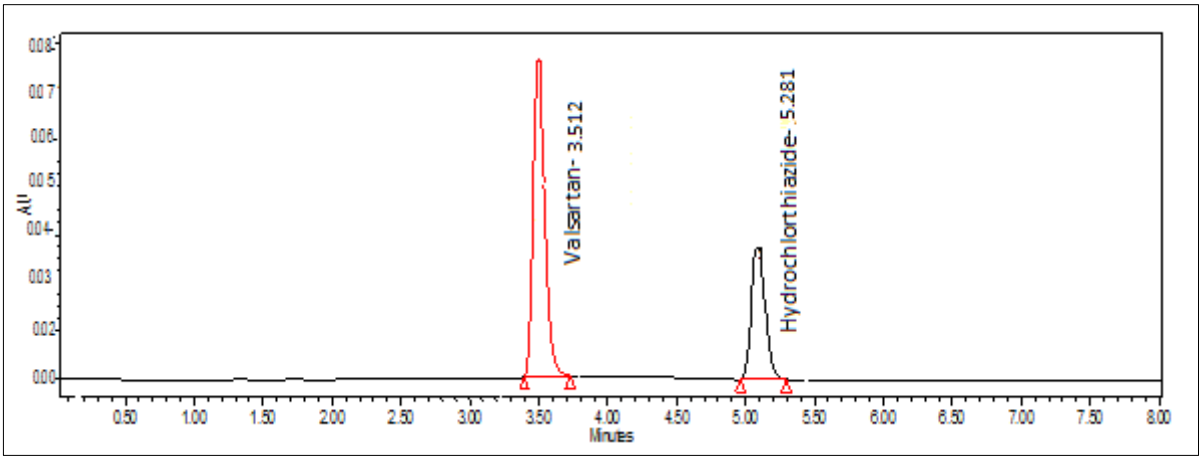

Fig 8(a): Linearity Chromatogram for Valsartan and Hydrochlorothiazide (20µg/ml)

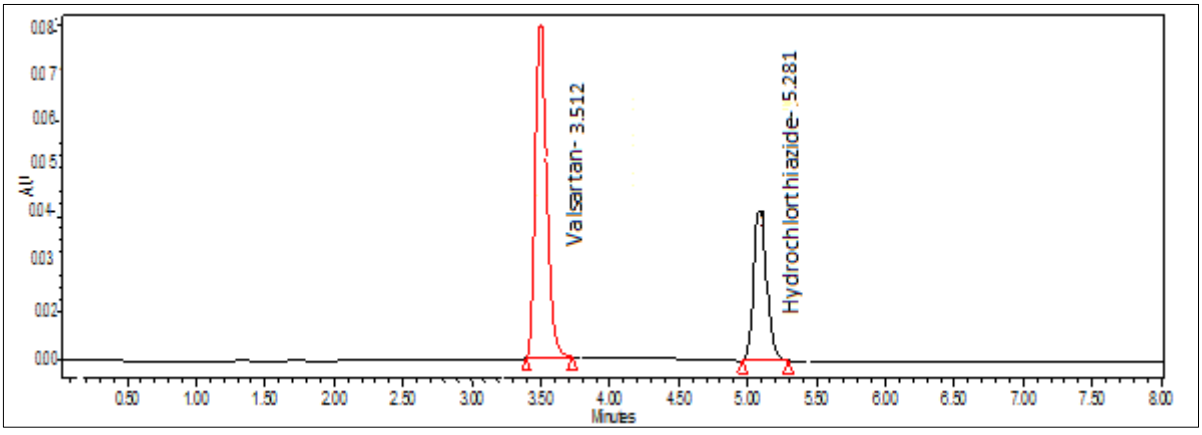

Fig 8(b): Linearity Chromatogram for Valsartan and Hydrochlorothiazide (30µg/ml)

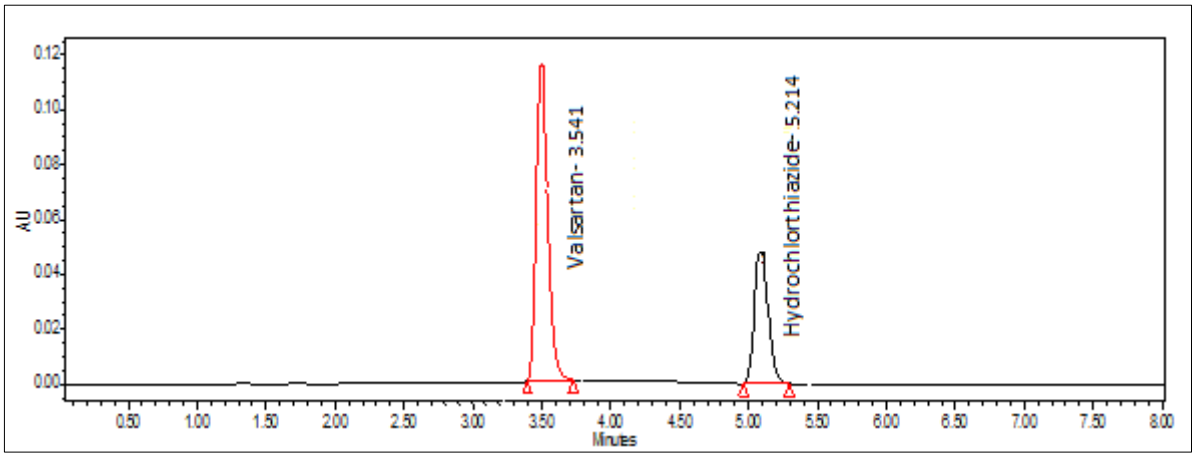

Fig 8(c): Linearity Chromatogram for Valsartan and Hydrochlorothiazide (40µg/ml)

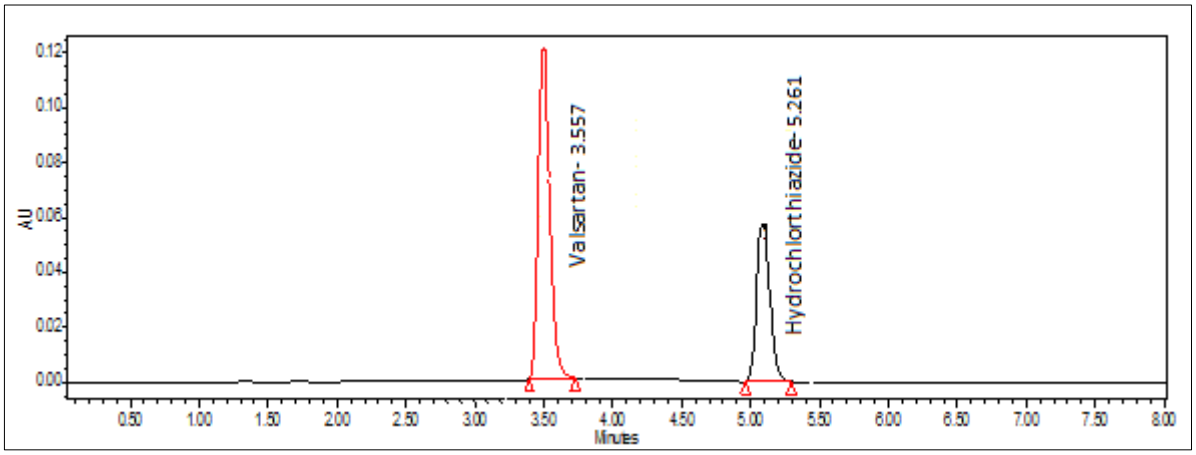

Fig 8(d): Linearity Chromatogram for Valsartan and Hydrochlorothiazide (50µg/ml)

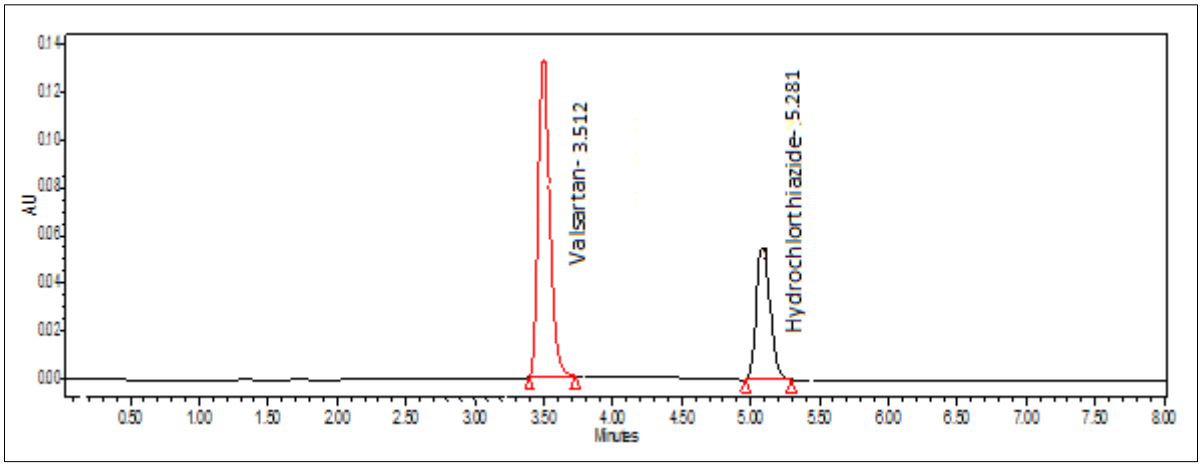

Fig 8(e): Linearity Chromatogram for Valsartan and Hydrochlorothiazide (60µg/ml)

3.2.6 Robustness

3.2.6.1 Effect of variation of flow rate

A study was conducted to determine the effect of variation in flow rate. Standard solution prepared as per the test method was injected into the HPLC system using flow rates, 1.0ml/min and 1.2ml/min. The system suitability parameters were evaluated and found to be within the limits for 1.0ml/min and 1.2ml/min flow.

Valsartan and Hydrochlorothiazide and was resolved from all other peaks and the retention times were comparable with those obtained for mobile phase having flow rates 1.0ml/min.

3.2.6.2 Effect of variation of temperature

A study was conducted to determine the effect of variation in temperature. The sample solution was chromatographed at 25º±2C temperature. Valsartan and Hydrochlorothiazide were resolved from all other peaks and the retention times were comparable and the results were tabulated in Table-7.

Table 7: Results for robustness of valsartan and hydrochlorothiazide

Parameter	Adjusted to	System suitability parameters		
		Mean area	Mean RT	%RSD
Valsartan				
Flow rate(ml/min)	1.0-0.2	1305212	3.514	0.82
	1.0+0.2	1311245	3.120	0.45
Temperature (ºC)	25+2.0	1352100	3.152	0.41
	25-2.0	1325212	3.418	0.52
Hydrochlorothiazide				
Flow rate(ml/min)	1.0+0.2	825411	5.210	0.21
	1.0-0.2	817541	5.225	0.52
Temperature (ºC)	25+2.0	826951	5.218	0.48
	25-2.0	810245	5.236	0.42

4. Summary and Conclusion

In the present investigation, a simple, sensitive, precise and accurate RP-HPLC method was developed for the quantitative estimation of Valsartan and Hydrochlorothiazide in bulk and Pharmaceutical dosage forms.

The advantage of the developed method lies in the simplicity of the solution preparation, accurate and less number of reagents was used. And all the validation parameters were within the limits.

It can be concluded that the proposed method can be used for the routine analysis for the RP-HPLC method development and validation for the estimation of Valsartan and Hydrochlorothiazide in bulk and Pharmaceutical dosage forms.

5. References

1. Ramesh R, Dargad Mahesh, Prajapati Rohit R, Dargad Jai Parekh D. Sacubitril/valsartan: A novel angiotensin receptor-neprilysin inhibitor. Indian Heart Journal. 2018; 70(1):102-110.
2. George C. Roush Domenic A. Sica. Diuretics for Hypertension: A Review and Update. American Journal of Hypertension. 2016; 29(10):1130-1137.
3. Tian DF, Tian XL, Tian T, Wang ZY, Mo FK *et al*. Simultaneous Determination of Valsartan and Hydrochlorothiazide in Tablets by RP-HPLC. Indian J Pharm Sci. 2008; 70(3):372-374.
4. Monika Jadhav L, Manoj Girase V, Shripad Tidme K, Manish Junagade S. Development and Validation of Spectrophotometric Methods for Simultaneous Estimation of Valsartan and Hydrochlorothiazide in Tablet Dosage Form. International Journal of Spectroscopy. 2014; 14(6):1-6.
5. Vivekkumar Redasani K, Pinakin Patel V, Sanjay Surana J *et al*. Spectrophotometric method for simultaneous estimation of Valsartan and Hydrochlorothiazide in combined tablet dosage form. Pelagia Research Library Der Pharmacia Sinica. 2011; 2(3):123-130.
6. International conference on the harmonization. ICH Harmonized Tripartite Guideline. Stability Testing of New Drug Substances and Products Q1A (R2), 2003.
7. International conference on the harmonization. ICH Harmonized Tripartite Guideline. Validation of Analytical Procedures: Text and Methodology Q2 (R1), 2005.