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Method Development and Validation for Simultaneous Estimation of Propranolol and Valsartan by using RP-HPLC in Bulk and Pharmaceutical Dosage Form

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A B S T R A C T

A simple, sensitive and precise reverse phase high performance liquid chromatographic method has been developed for the simultaneous estimation of Propranolol and Valsartan in pharmaceutical dosage form. The Chromatography was carried out on column ODS inertsil C_{18} (4.6mm x 250mm, 5µm) column using Methanol: Buffer (KH_2PO_4) in the ratio of 60:40, as the mobile phase at the flow rate of 1.0 ml/min with UV detection at 272nm. The retention times of Propranolol and Valsartan 2.955 min and 3.532 min respectively. The method was validated in terms of linearity, precision, accuracy, limit of detection, limit of quantification. The LOD for Propranolol and Valsartan is 0.25mg/ml & 0.34mg/ml and LOQ for Propranolol and Valsartan is 0.77mg/ml & 1.05mg/ml respectively. The coefficient of variance for both the drug was more than 0.999.The proposed method can be used for determination of these drugs.

Keywords: Propranolol and Valsartan, RP-HPLC

A R T I C L E I N F O

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1. Introduction

Propranolol is a chemically named as (RS)-1-(1-methylethylamino-3-(1-naphthyloxy) propan-2-ol [1, 2, 3]. Propranolol competes with sympathomimetic neurotransmitters such as catecholamines for binding at

beta (1)-adrenergic receptors in the heart, inhibits sympathetic stimulation [4,5,6]. This results in a reduction in resting heart rate cardiac output, systolic and diastolic blood pressure, and reflux orthostatic hypotention [7,8].

Valsartan is a chemically named as (S)-3-methyl-2-N-{[2'-(2H-1, 2, 3, 4-tetrazol-5-yl) biphenyl-4-yl] methyl} pentanamido) butanoic acid [9, 10]. Valsartn is an ARB that selectively inhibits the binding of angiotensin II to AT1, which is found in many tissues such as vascular smooth muscle and the adrenal glands [11]. This effectively inhibits the AT1-mediated vasoconstructive and aldosterone-secreting effects of angiotensin II and results in a decrease in vascular resistance and blood pressure [12]. Valsartan is selective for AT1 and has virtually no affinity for AT2. Inhibition of aldosterone secretion may inhibit sodium and water reabsorption in kidneys while decreasing potassium excretion [13,14]. The primary metabolite of valsartan, valeryl 4-hydroxy valsartan, has no pharmacological activity [15].

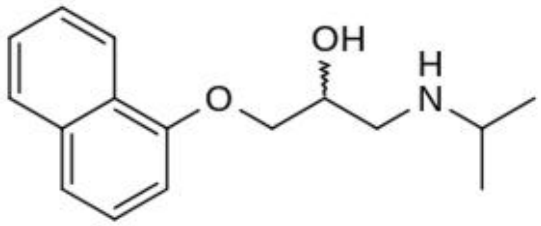

Figure 1: Structure of Propranolol

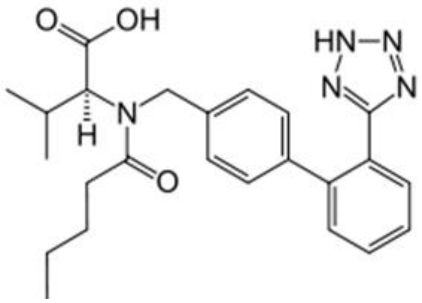

Figure 2: Structure of Valsartan

2. Materials and Methods

Instruments:

HPLC –Waters Model NO.2690/5 series Compact System Consisting of

- Inertsil-C18 ODS column.
- Electronic balance (SARTORIOUS)
- Sonicator(FAST CLEAN)

Chemicals:

- Methanol HPLC Grade.
- Buffer (KH2PO4)Hplc Grade.

Raw Material:

Propranolol and Valsartan Working Standards.

2.1 Method Development For HPLC:

The objective of this experiment was to optimize the assay method for simultaneous estimation of Propranolol and Valsartan on the literature survey made. So here the trials mentioned describes how the optimization was done.

Trial: 1

Mobile Phase: Degassed Acetonitrile 100%.

Preparation of Standard Solution:

Weigh down 10mg's of Propranolol and Valsartan drugs and dissolved in 10ml of Mobile phase taken in two 10ml of volumetric flasks seperately and sonicated for 20 minutes to get 1000ppms and 1 ml was taken from each solution into a 10ml volumetric flask and diluted to 10 ml with mobile phase.

Chromatographic Conditions:

Flow rate : 1.0ml/min
Column : Inertsil -C18, ODS column
Detector wavelength : 272nm
Column temp : Ambient
Injection volume : 20µl
Run time : 10min
Retention time : 3.8min for Propranolol and 4.1 for Valsartan.

Observation:

Two peaks are merged and not separated completely. The trial 1 chromatogram result was shown in Fig: 4.

Trail: 2

Mobile Phase: Degassed Acetonitrile and methanol in the ratio of 90:10 V/V.

Preparation of Standard Solution:

Weigh down 10mg's of Propranolol and Valsartan drugs and dissolved in 10ml of Mobile phase taken in two 10ml of volumetric flasks seperately and sonicated for 20 minutes to get 1000ppms and 1 ml was taken from each solution and diluted to 10 ml with mobile phase.

Chromatographic Conditions:

Flow rate : 1ml/min
Column : Inertsil -C18, ODS column
Detector wavelength : 272nm
Column temp : Ambient
Injection volume : 20µl
Run time : 10min
Retention time : 3.7 min for Propranolol and 4.0 min for Valsartan.

Observation: The two peaks are separated completely but peak shapes are not good. The trial 2 chromatogram result was shown in Fig:5

Trail: 3

Mobile Phase: Degassed Acetonitrile and Methanol in the ratio of 80:20 V/V.

Preparation of Standard Solution:

Weigh down 10mg's of Propranolol and Valsartan drugs and dissolved in 10ml of Mobile phase taken in two 10ml of volumetric flasks separately and sonicated for 20 minutes to get 1000ppms and 1 ml was taken from each solution and diluted to 10 ml with mobile phase.

Chromatographic Conditions:

Flow rate : 1.0ml/min
Column : Inertsil - C18, BDS column
Detector wavelength : 272nm
Column temp : Ambient
Injection volume : 20µl
Run time : 10min
Retention time : 1.7 min for Propranolol and 2.1 min for Valsartan.

Observation:

Valsartangot peak fronting and base line between two peaks is not straight. The trial 3chromatogram result was shown in Fig:6

2.2 Optimized Method

Mobile Phase: Degassed Methanol and Buffer in the ratio of 60:40 V/V.

Preparation of (KH2PO4 0.1M) buffer:

Weight 3.8954g of di-sodium hydrogen phosphate and 3.4023 of potassium dihydrogen phosphate in to a beaker containing 1000ml of distilled water and dissolve completely. Then ph is adjusted with orthophosphoric acid and then filtered through 0.45µm membrane filter.

Preparation of stock solution:

Reference solution:

The solution was prepared by dissolving 20.0 mg of accurately weighed Propranolol and 20.0 mg Valsartan in Mobile phase, in two 100.0 mL volumetric flasks separately and sonicate for 20min. From the above solutions take 10.0 mL from each solution into a 50.0 mL volumetric flask and then makeup with mobile phase and sonicate for 10min.

Preparation of working standard solution:

The stock solutions equivalent to 20ppm to 80ppm with respect to both drugs were prepared in combination of Propranolol and Valsartan above, sonicated and filtered through 0.45µ membrane.

Table 1: Optimized chromatographic conditions

Parameters	Method
Stationary phase (column)	Inertsil -ODS C18(250 x 4.6 mm, 5 µ)
Mobile Phase	Methanol : Buffer (60:40)
Flow rate (ml/min)	1.0 ml/min
Run time (minutes)	10 min
Column temperature (°C)	Ambient
Volume of injection loop (µl)	20
Detection wavelength (nm)	272nm
Drug RT (min)	2.955min for Propranolol and 3.532 for Valsartan.

Method Validation

System Suitability:

A Standard solution was prepared by using Propranolol and Valsartan 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 from five replicate injections for Propranolol and Valsartan , retention times and peak areas.

Acceptance Criteria:

1. The % RSD for the retention times of principal peak from 5 replicate injections of each Standard solution should be not more than 2.0 %
2. The % RSD for the peak area responses of principal peak from 5 replicate injections of each standard Solution should be not more than 2.0%.
3. The number of theoretical plates (N) for the Propranolol and Valsartan peaks is NLT 3000.
4. The Tailing factor (T) for the Propranolol and Valsartan peaks is NMT 2.0

Observation:

The %RSD for retention times and peak areas were found to be within the limit.

2. Specificity:

Propranolol and Valsartan:

Solutions of standard and sample were prepared as per the test method are injected into chromatographic system.

Acceptence Criteria:

Chromatograms of standard and sample should be identical with near Retention time.

Observation:

The chromatograms of Standard and Sample were same identical with same retention time.

3. Precision:

Repeatability:

- System precision: Standard solution prepared as per test method and injected five times.
- Method precision: Prepared six sample preparations individually using single as per test method and injected each solution.4.5.1to 4.5.6

Acceptance Criteria:

The % relative standard deviation of individual Propranolol and Valsartan, from the six units should be not more than 2.0%. The individual assays of Propranolol and Valsartan should be not less than 98% and not more than 102.0%.

Observation:

Test results are showing that the test method is precise. Refer tables 4.5 and 6.7 for system precision and for method precision.

Intermediate precision (analyst to analyst variability):

A study was conducted by two analysts as per test method

Acceptence Criteria:

The individual assays of Propranolol and Valsartan should be not less than 98% and not more than 102% and %RSD of assays should be NMT2.0% by both analysts.

Observation:

Individual %assays and %RSD of Assay are within limit and passes the intermediate precision, Refer table: 8-9

4. Accuracy (Recovery):

A study of Accuracy was conducted. Drug Assay was performed in triplicate as per test method with equivalent amount of Propranolol and Valsartan into each volumetric flask for each spike level to get the concentration of Propranolol and Valsartan equivalent to 50%, 100%, and 150% of the labeled amount as per the test method. The average % recovery of Propranolol and Valsartan were calculated.

Acceptance Criteria:

The mean % recovery of the Propranolol and Valsartan at each spike level should be not less than 98.0% and not more than 102.0% for both the drugs separately.

Observation:

$$\% \text{ Recovery} = \frac{\text{Amount found}}{\text{Amount added}} \times 100$$

The recovery results indicating that the test method has an acceptable level of accuracy. Refer table: 10-11.

Linearity of Test Method:

A Series of solutions are prepared using Propranolol and Valsartan working standards at concentration levels from 20ppm to 80 ppm of target concentration .Measure the peak area response of solution at Level 1 and Level 6 six times and Level 2 to Level 5 two times.

Acceptance Criteria:

Correlation Coefficient should be not less than 0.9990.

% of y- Intercept should be ±2.0.

% of RSD for level 1 and Level 6 should be not more than 2.0%.

Observation:

The linear fit of the system was illustrated graphically. The results are presented in table 2-3.

6. Ruggedness of Test Method:

a) System to system variability:

System to system variability study was conducted on different HPLC systems, under similar conditions at different times. Six samples were prepared and each was analyzed as per test method. Comparison of both the results obtained on two different HPLC systems, shows that the assay test method are rugged for System to system variability.

Acceptance Criteria:

The % relative standard deviation of Propranolol and Valsartan from the six sample preparations should be not more than 2.0%**.** The % assay of Propranolol and Valsartan should be between 98.0%-102.0%.

Observation: The % RSD was found within the limit.

b) Column to column variability:

Column to column variability study was conducted by using different columns. Six samples were prepared and each was analyzed as per test method.

Acceptance Criteria:

The %RSD of Propranolol and Valsartan tablets should be NMT2.0%. The %assay of Propranolol and Valsartan should be between 98.0% and 102.0% for individual drugs.

Observation:

The results obtained by comparing with both two types were within limit.

7. Robustness:

a) 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. Propranolol and Valsartan 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.

Acceptance Criteria:

The Tailing Factor of Propranolol and Valsartan standards should be NMT 2.0 for Variation in Flow.

Observation:

The tailing factor for Propanolol and Valsartan was found to be within the limits.

b) Effect of variation of temperature:

A study was conducted to determine the effect of variation in temperature. Standard solution prepared as per the test method was injected into the HPLC system at 20ºC temperature. The system suitability parameters were evaluated and found to be within the limits for a temperature change of 20ºc. Similarly sample solution was chromatographed at 25ºC temperature. Propranolol and Valsartan were resolved from all other peaks and the retention times were comparable with those

Acceptance Criteria:

The Tailing Factor of Propranolol and Valsartan standard and sample solutions should be NMT 2.0 for Variation in temperature.

Observation: The tailing factor for Propranolol and Valsartan x is found to be within the limits.

8. Limit of Detection and Quantitation (LOD and LOQ):

From the linearity data calculate the limit of detection and quantitation, using the following formula.

$$LOD = \frac{3.3\,\sigma}{S}$$

σ = standard deviation of the response

S = slope of the calibration curve of the analyte.

$$LOQ = \frac{10\,\sigma}{S}$$

σ = standard deviation of the response

S = slope of the calibration curve of the analyte.

3. Results and Discussion

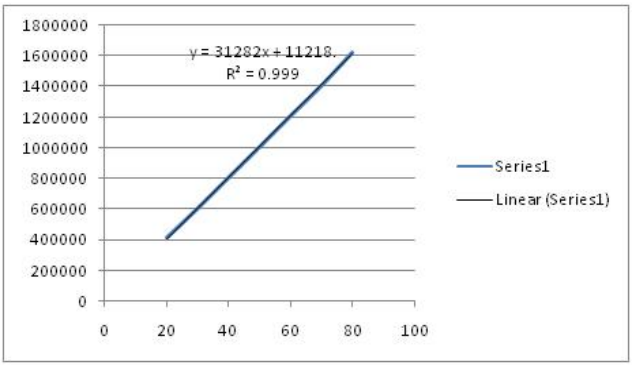

Figure 3: Linearity Plot (Concentration Vs Response) of Propanolol

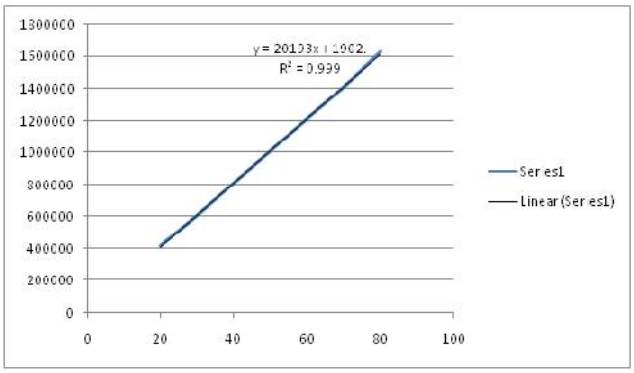

Figure 4: Linearity Plot (Concentration Vs Response) of valsartan

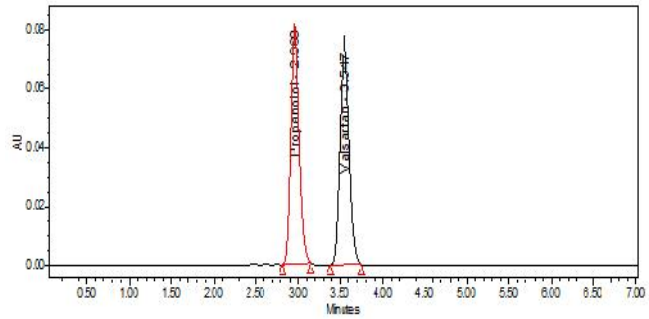

Figure 5: Chromatograms for 20 ppm

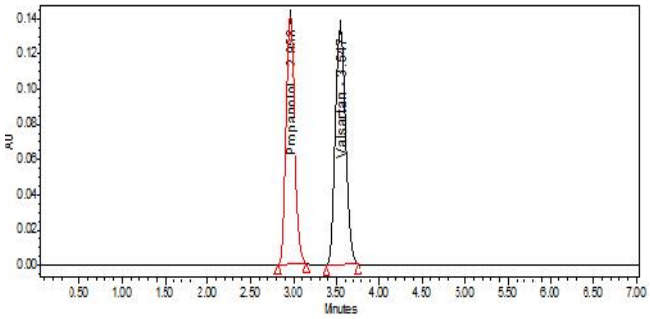

Figure 6: Chromatograms for 30ppm

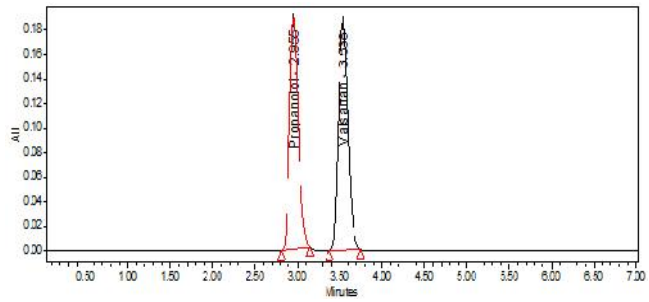

Figure 7: Chromatogram for 40 ppm standard 1

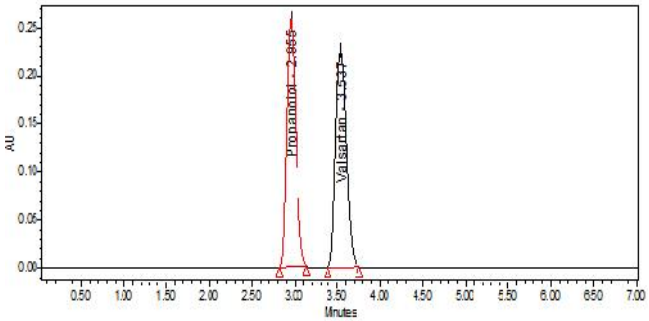

Figure 8: Chromatograms for 50 ppm

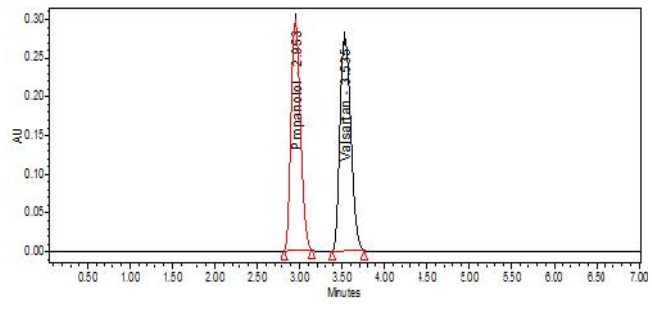

Figure 9: Chromatogram for 60 ppm standard 1

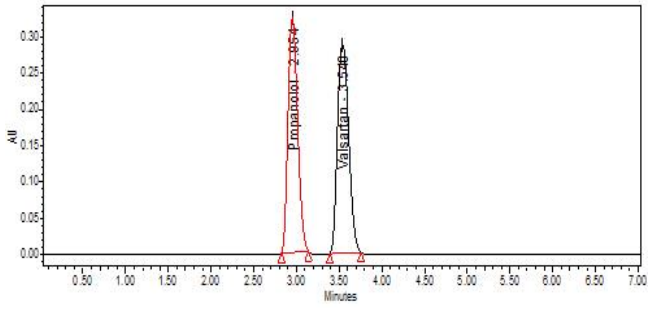

Figure 10: Chromatograms for 70 ppm

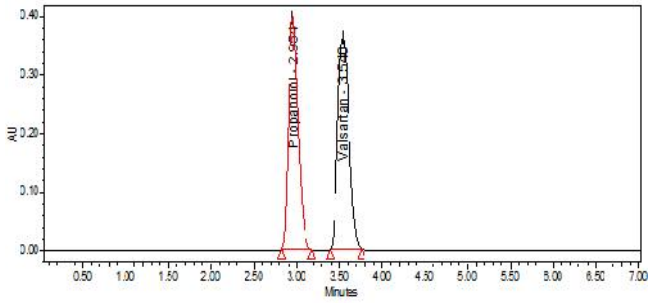

Figure 11: Chromatogram for 80 ppm standard 1

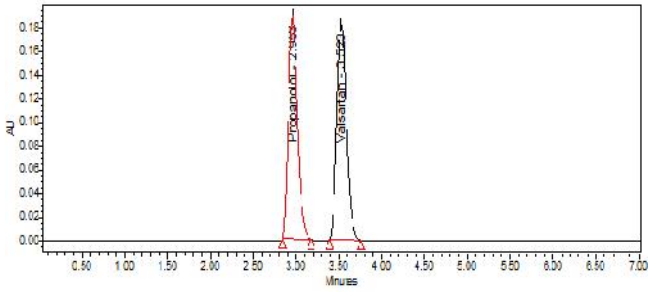

Figure 12: Chromatograms of system precision

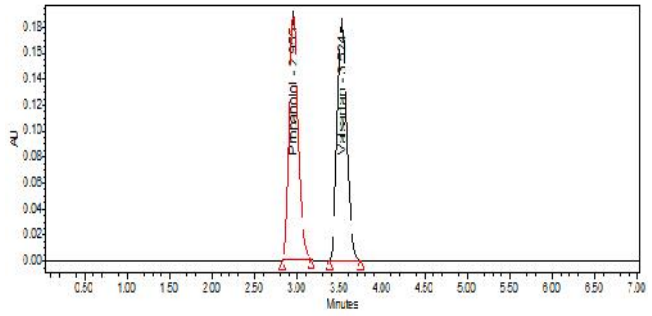

Figure 13: Chromatogram for system precision (std- 2)

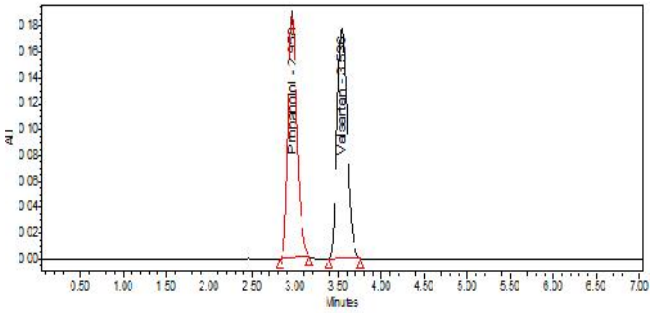

Figure 14: Chromatogram for system precision (std- 3)

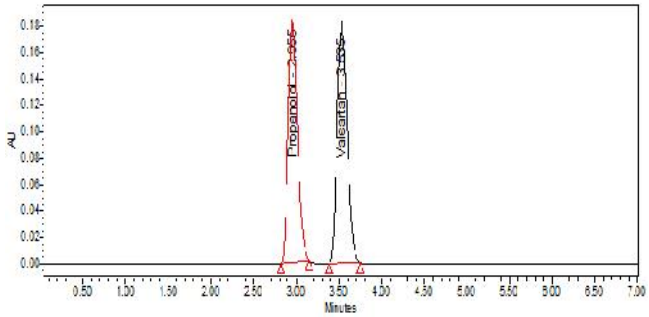

Figure 15: Chromatogram for system precision (std-4)

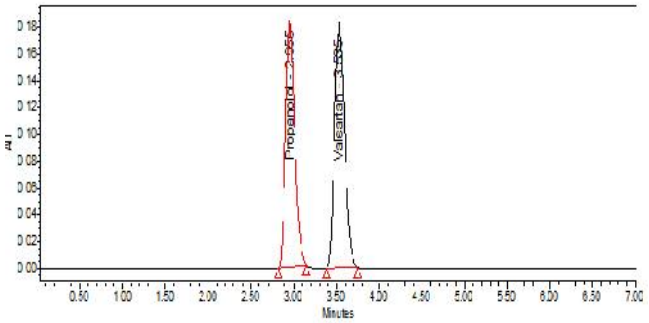

Figure 16: Chromatograms of Repeatability (std-1)

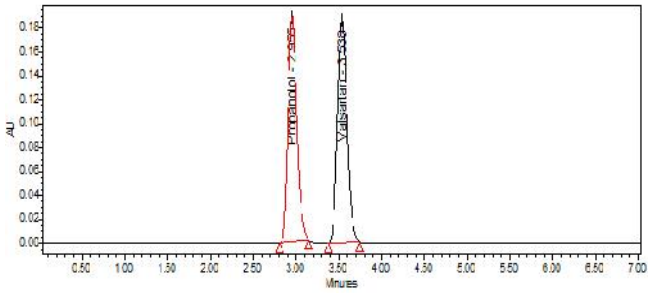

Figure 17: Chromatogram for Repeatability (std-2)

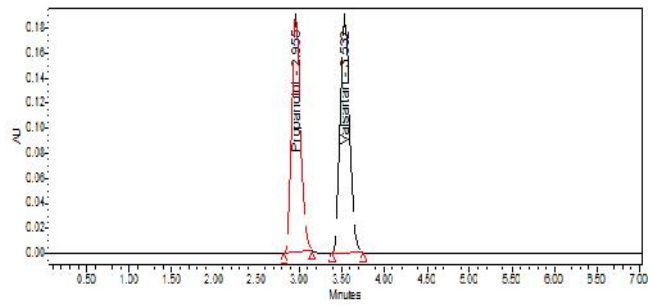

Figure 18: Chromatogram for Repeatability (std-3)

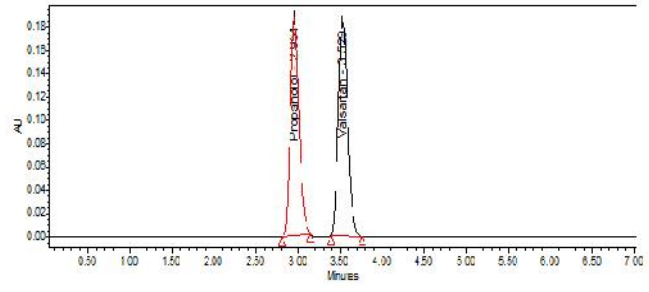

Figure 19: Chromatogram for Repeatability (std-4)

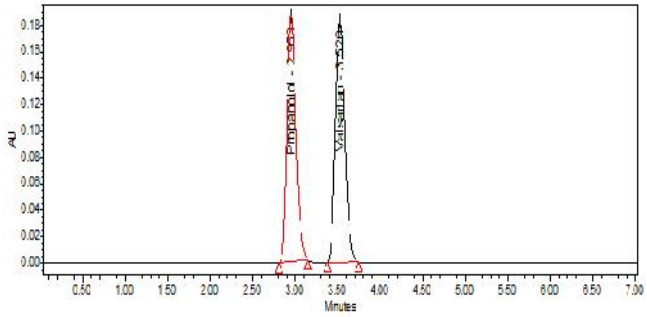

Figure 20: Chromatogram for Repeatability (std-5)

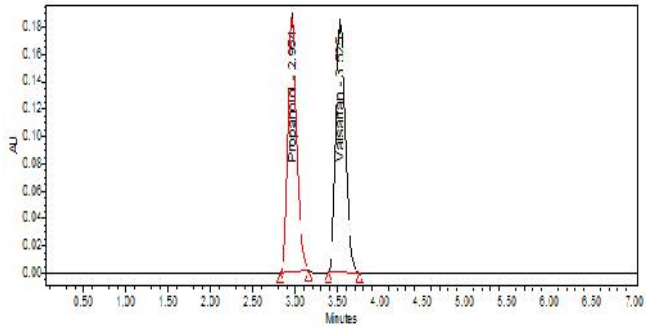

Figure 21: Chromatogram for Repeatability (std-6)

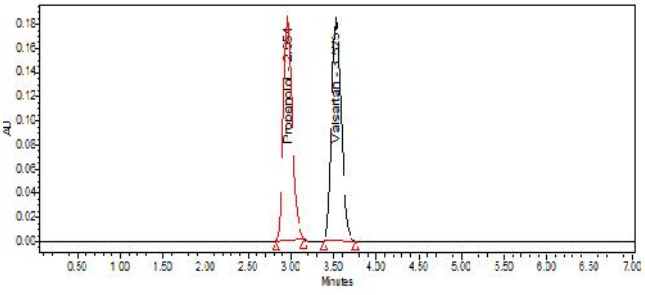

Figure 22: Chromatogram of Intermediate precision

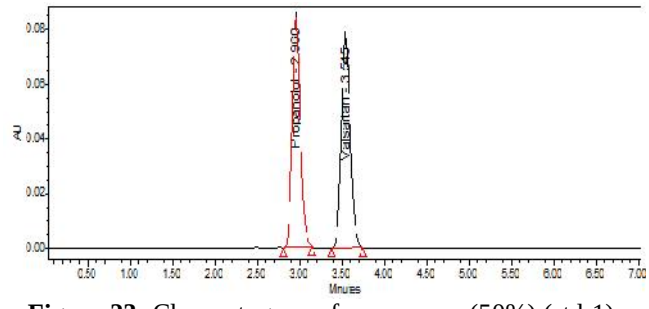

Figure 23: Chromatograms for accuracy (50%) (std-1)

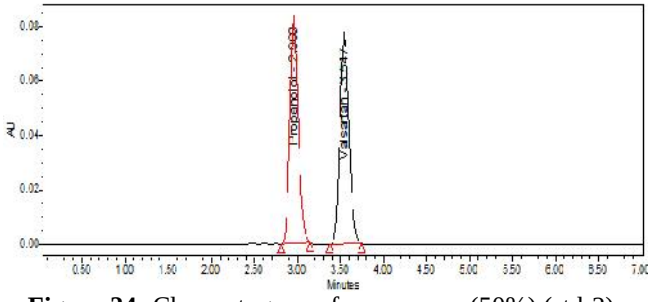

Figure 24: Chromatograms for accuracy (50%) (std-2)

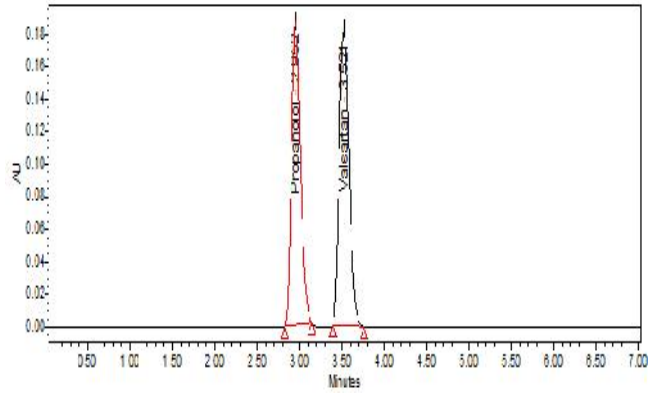

Figure 25: Chromatograms for accuracy (100%) (Std-1)

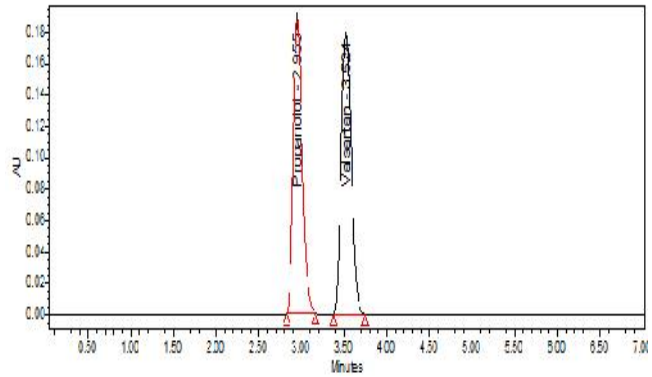

Figure 26: Chromatograms for accuracy (100%) (Std-2)

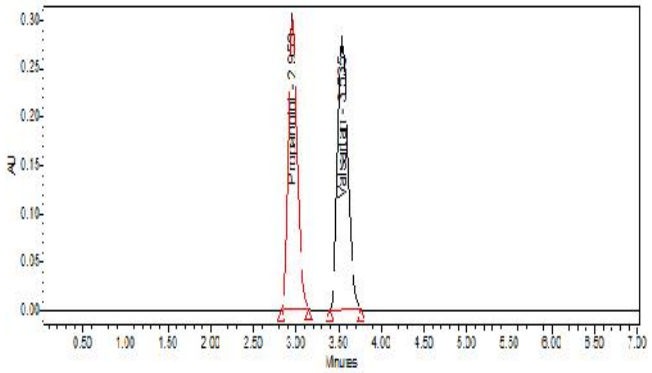

Figure 27: Chromatograms for accuracy (150%) (std-1)

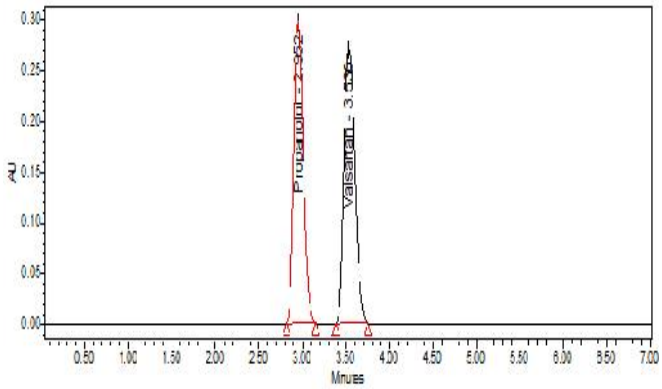

Figure 28: Chromatograms for accuracy (150%) (Std-2)

4. Conclusion

The analytical method was developed by studying different parameters. First of all, maximum absorbance was found to be at 289nm Propranolol for and 265nm for Valsartan. Common wavelength will be 272nm and the peaks purity was excellent. Injection volume was selected to be 20µl which gave a good peak area. The column used for study was Inertsil C_{18}, ODS chosen good peak shape. Ambient temperature was found to be suitable for the nature of drug solution. The flow rate was fixed at 1.0ml/min because of good peak area, satisfactory retention time and good resolution. Different ratios of mobile phase were studied, mobile phase with ratio of 60:40 Methanol : Buffer was fixed due to good symmetrical peaks and for good resolution. So this mobile phase was used for the proposed study. The present recovery was found to be 98.0-101.50 was linear and precise over the same range. Both system and method precision was found to be accurate and well within range. Detection limit was found to be 0.25 Propranolol and 0.34 for Valsartan. Linearity study was, correlation coefficient and curve fitting was found to be.0.999. The analytical method was found linearity over the range of 20-80ppm of the target concentration for both the drugs. The analytical passed both robustness and ruggedness tests. On both cases, relative standard deviation was well satisfactory.

Table 2: Data of Linearity (Propranolol)

Concentration (ppm)	Average Area	Statistical Analysis	
0	0	Slope	31282
20	523467	y-Intercept	11218
30	829544	Correlation Coefficient	0.999
40	1139272		
50	1448018		
60	1728926		
70	2089505		
80	2407574		

Table 3: Data of Linearity (Valsartan)

Concentration (ppm)	**Average Area**	**Statistical Analysis**	
0	0	Slope	20193
20	412977	y-Intercept	1902
30	605369	Correlation Coefficient	0.999
40	807564		
50	1007428		
60	1210925		
70	1409560		
80	1627087		

Table 4: Data of Repeatability (System precision) for Propranolol

	Injection	**Peak Areas of Propranolol**	**% Assay**
Concentration 40ppm	1	1146923	99.65
	2	1143596	99.08
	3	1158293	99.98
	4	1147283	100.04
	5	1152490	100.16
Statistical Analysis	**Mean**	1149717	99.78
	SD	5754.015	0.435569
	% RSD	0.500472	0.43652

Table 5: Data of Repeatability (System precision) for Valsartan

	Injection	**Peak Areas of** Valsartan	**% Assay**
Concentration 40ppm	1	801690	98.84
	2	797631	99.69
	3	805783	100.05
	4	801496	101.11
	5	806432	100.96
Statistical Analysis	**Mean**	802606.4	100.13
	SD	3590.034	0.937203
	% RSD	0.447297	0.935987

Table 6: Data of Repeatability (Method precision) for Propanolol

	Injection	**Peak Areas of Propranolol**	**% Assay**
Concentration 40ppm	1	1152293	99.55
	2	1146923	99.88
	3	1147283	99.40
	4	1152490	99.56
	5	1139272	99.85
Statistical Analysis	**Mean**	1147283	99.40
	SD	1147591	99.67
	% RSD	4815.615	0.250093

Table 7: Data of Repeatability (Method precision) for Valsartan

	Injection	**Peak Areas of** Valsartan	**% Assay**
Concentration 40ppm	1	805783	99.85
	2	801690	99.96
	3	801496	100.53
	4	806432	100.30
	5	797564	100.08
Statistical Analysis	**Mean**	801496	100.53
	SD	801593	100.20
	% RSD	3262.714	0.290477

Table 8: Data of Intermediate precision (Analyst 2) for Propranolol

	Injection	**Peak Areas of Propranolol**	**% Assay**
Concentration 40ppm	1	1139272	98.80
	2	1140892	99.54
	3	1136601	99.98
	4	1141067	100.02
	5	1136024	101.08
Statistical Analysis	**Mean**	1140892	99.54
	SD	1139125	99.82
	% RSD	2281.417	0.755001

Table 9: Data of Intermediate precision (Analyst 2) for Valsartan

	Injection	**Peak Areas of Valsartan**	**% Assay**
Concentration 40ppm	1	797564	99.85
	2	795138	99.68
	3	795685	100.08
	4	800569	100.01
	5	797049	99.52
Statistical Analysis	**Mean**	795685	100.08
	SD	796948.3	100.37
	% RSD	1998.386	0.337086

Table 10: Data of Accuracy for Propranolol

Concentration % of spiked level	**Amount added (ppm)**	**Amount found (ppm)**	**% Recovery**	**Statistical Analysis of % Recovery**	
50% Injection 1	20	19.85	99.25	**MEAN**	99.88
50% Injection 3	20	20.12	100.6	**%RSD**	0.67
100 % Injection 1	40	39.74	99.35	**MEAN**	99.81
100 % Injection 2	40	40.08	100.2		
100% Injection 3	40	40.24	100.6	**%RSD**	0.399
150% Injection 1	60	59.04	98.40	**MEAN**	99.19
150% Injection 2	60	59.62	99.36		
150% Injection 3	60	59.89	99.81	**%RSD**	0.72

Table 11: Data of Accuracy for Valsartan

Concentration % of spiked level	**Amount added (ppm)**	**Amount found (ppm)**	**% Recovery**	**Statistical Analysis of % Recovery**	
50% Injection 1	20	19.86	99.30	**MEAN**	99.46
50% Injection 3	20	19.84	99.20	**%RSD**	0.38
100 % Injection 1	40	39.54	98.85	**MEAN**	99.76
100 % Injection 2	40	39.82	99.55		
100% Injection 3	40	39.96	99.9	**%RSD**	0.189
150% Injection 1	60	59.92	99.86	**MEAN**	100.0067

150% Injection 2	60	60.08	100.13		
150% Injection 3	60	60.02	100.03	**%RSD**	0.136

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