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Reverse phase-high performance liquid chromatography method development and validation for the simultaneous determination of verapamil and trandolapril in pure form and their marketed combined pharmaceutical dosage form

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ABSTRACT

Analytical Method Development and Validation for Verapamil and Trandolapril in bulk and Combined Dosage Form by RP-HPLC. New method was established for simultaneous estimation of Verapamil and Trandolaprilby RP-HPLC method. The chromatographic conditions were successfully developed for the separation of Verapamil and Trandolaprilby using Inertsil C18 (4.6mm ×250mm, 5µm particle size), flow rate was 1.0 ml/min, mobile phase ratio was (55:45% v/v) Methanol: Phosphate buffer pH 4.8 (pH was adjusted with ortho phosphoric acid), detection wavelength was 282nm. The instrument used was WATERS Alliance 2695 separation module, Software: Empower 2, 996 PDA detector. The retention times were found to be 1.688mins and 3.282mins. The % purity of Verapamil and Trandolapril was found to be 99.86%. The system suitability parameters for Verapamil and Trandolaprilsuch as theoretical plates and tailing factor were found to be 7586, 1.69 and 6235 and 1.58, the resolution was found to be 10.85. The analytical method was validated according to ICH guidelines (ICH, Q2 (R1)). The linearity study of Verapamil and Trandolaprilwas found in concentration range of 100µg-500µg and 30µg-70µg and correlation coefficient (r2) was found to be 0.999 and 0.999, % recovery was found to be 100.112% and 100.16%, %RSD for repeatability was 0.1702 and 0.043 respectively. The precision study was precise, robust, and repeatable. The LOD value was found to be 2.1µg/ml and 1.28µg/ml, and LOQ value was 6.3µg/ml and 3.84µg/ml for Verapamil and Trandolapril respectively. Hence the suggested RP-HPLC method can be used for routine analysis of Verapamil and Trandolapril in API and Pharmaceutical dosage form.

Keywords: Verapamil and Trandolapril, Method Development, Validation, Accuracy, ICH Guidelines.

INTRODUCTION

Analytical methods development and validation play important roles in the discovery, development, and manufacture of pharmaceuticals. The current good manufacturing practice (CGMP) and food drug administration (FDA) guidelines insist for adoption of sound methods of analysis with greater sensitivity and reproducibility. Development of a method of analysis is usually based on prior art (or) existing literature, using the same (or) quite similar instrumentation .It is rare today that an HPLC-based method is developed that does not in same way relate (or) compare to existing, literature based approaches. Today HPLC(high performance liquid chromatography) is the method of choice used by the pharmaceutical industry to assay the intact drug and degradation products. The appropriate selection and chromatographic conditions ensure that the HPLC method will have the desired specificity. UV spectroscopy is also a simple analytical tool widely used for routine assay of drugs. Hence for the assay of the selected drugs HPLC and UV spectroscopy has been chosen for these proposed methods.

The developed chromatographic methods further validated as per ICH or USFDA guidelines for all the critical parameters. To access the precision and to evaluate the results of analysis the analyst must use statistical methods. These methods include confidence limit, regression analysis to establish calibration curves. In each analysis the critical response parameters must be optimized and recognized if possible.

Pharmaceutical analysis plays a major role today, and it can be considered as an interdisciplinary subject. Pharmaceutical analysis derives its principles from various branches like chemistry, physics and microbiology etc. Pharmaceutical analytical techniques are applied mainly in two areas, quantitative analysis and qualitative analysis, although there are several other applications.

Drugs and pharmaceuticals are chemicals or like substances, which or of organic inorganic or other origin. Whatever may be the origin, we some property of the medicinal agent to measure them quantitatively or qualitatively.

In recent years, several analytical techniques have been evolved thatcombine two or more methods into one called "hyphenated" technique e.g.GC/MS, LC/MS etc. The complete analysis of a substance consists of four main steps.

The concept of analytical chemistry lies in the simple, precise and accurate measurements. These determinations require highly sophisticated instruments and methods like mass spectroscopy, gas chromatography, high performance thin layer chromatography, high performance liquid chromatography etc. The HPLC method is sensitive, accurate, precise and desirable for routine estimation of drugs in formulations.

Thereby it is advantageous than volumetric methods. Many HPLC methods has been developed and validated for the quantitative determination of various marketed drugs.

Analytical method development and validation places an important role in drug discovery and manufacture of pharmaceuticals. These methods are used to ensure theidentity, purity, potency and performance of drug products majority of analytical developmenteffort goes into validating a stability indicating method. So it is a quantitative analytical methodbased on the structure and chemical properties of each active ingredient of the drug formulation.

Most of the drugs can be analyzed by HPLC method because of several advantages like rapidity, specificity, accuracy, precision, reproducibility, ease of automation and eliminates tedious extraction and isolation procedures.

Method development

Estimation ofVerapamiland Trandolapril in pharmaceutical dosage form:

Procedure

Preparation of mobile phase

Accurately measured 500 ml (50%) of HPLC Methanol and 350 ml of Acetonitrile (35%) and 150 ml of Water (15%) were mixed and degassed in a digital ultrasonicater for 10 minutes and then filtered through 0.45 µ filter under vacuum filter.

Diluent Preparation

Accurately measured 450 ml (45%) of HPLC Methanol and 550 ml of Phosphate Buffer (55%) were mixed and degassed in a digital ultra sonicater for 15 minutes and then filtered through 0.45 µ filter under vacuum filter.

Assay

Preparation of the Verapamil and Trandolaprilstandard solution

Preparation of standard solution: (Verapamil)

Accurately weigh and transfer 10 mg of Verapamil, working standard into a 10ml of clean dry volumetric flasks add about 7ml of diluent and sonicate to dissolve and removal of air completely and make volume up to the mark with the diluent.

Preparation of standard solution: (Trandolapril)

Accurately weigh and transfer 10 mg of Trandolapril working standard into a 10ml of clean dry volumetric flasks add about 7ml of diluent and sonicate to dissolve and removal of air completely and make volume up to the mark with the diluent.

Further pipette 3ml of Verapamil, 0.5ml of Trandolapril from stock solutions in to a 10ml volumetric flask and dilute up to the mark with diluent.

Procedure

Inject the samples by changing the chromatographic conditions and record the chromatograms, note the conditions of proper peak elution for performing validation parameters as per ICH guidelines.

Preparation of Sample Solution

Take average weight of Tablet and crush in a mortar by using pestle and weight 10 mg equivalent weight of Verapamil, Trandolapril sample into a 10ml clean dry volumetric flask and add about 7ml of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

Procedure

Further pipette 1.2ml of Verapamil, Trandolapril from above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Inject the three replicate injections of standard and sample solutions and calculate the assay by using formula:

$$\%ASSAY = \frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100$$

%ASSAY was calculated by using the formula and reported in the Table: 15, 16 (7.1.1)

Analytical Method Validation

Validation

Validation is a process of establishing documented evidence which provide a high degree of assurance that specific activity will consistently produce a desired result or product meeting its predetermined specification and quality characteristics.

A. System Suitability
B. Accuracy
C. Precision

Method precision (Repeatability)

Intermediate precision (Reproducibility)

D. Linearity/Range
E. Limit of Detection (LOD)
F. Limit of Quantification (LOQ)
G. Robustness

System Suitability

System suitability is the evaluation of the components of an analytical system to show that the performance of a system meets the standards required by a method. A system suitability evaluation usually contains its own set of parameters. For chromatographic assays, these may include tailing factor, resolution, precision, capacity factor time and

theoretical plates. System suitability parameter Results were reported in **Table: 23 (7.2.1)**

Accuracy

For preparation of 50% Standard stock solution

Further pipette 1.5ml of Verapamil, 0.25ml of Trandolapril from stock solutions in to a 10ml volumetric flask and dilute up to the mark with diluent.

For preparation of 100% Standard stock solution

Further pipette 3ml ofVerapamil, 0.5ml of Trandolaprilfrom stock solutions in to a 10ml volumetric flask and dilute up to the mark with diluent.

For preparation of 150% Standard stock solution

Further pipette 4.5ml ofVerapamil, 0.75ml of Trandolaprilfrom stock solutions in to a 10ml volumetric flask and dilute up to the mark with diluent.

Procedure

Inject the Three replicate injections of individual concentrations (50%, 100%, 150%) were made under the optimized conditions. Recorded the chromatograms and measured the peak responses. Calculate the Amount found and Amount added for Verapamiland Trandolapril and calculate the individual recovery and mean recovery values. Results were reported in Table: 35, 36 (7.2.2)

Acceptance criteria

The %RSD for each level should not be more than 2

Precision

Repeatability

Preparation of Verapamil, Trandolapril for Precision

Further pipette 3ml of Verapamil, 0.5ml of Trandolapril from stock solutions in to a 10ml volumetric flask and dilute up to the mark with diluent
The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits.
Results were reported in Table: 35 (7.2.3)

Ruggedness

To evaluate the intermediate precision of the method, Precision was performed on different days by maintaining same conditions.

Procedure

Day 1

The standard solution was injected for six times and measured the area for all six injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits.
Results were reported in Table: 36 (7.2.3)

Day 2

The standard solution was injected for six times and measured the area for all six injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits.
Results were reported in Table: 37 (7.2)
The % RSD for the area of five standard injections results should be not more than 2%

Linearity

Preparation of Level – I (100µg/ml of Verapamiland 30µg/ml of Trandolapril)

Further pipette 1ml of Verapamil, 0.3ml of Trandolapril from stock solutions in to a 10ml volumetric flask and dilute up to the mark with diluent.

Preparation of Level – II (200µg/ml of Verapamiland 40µg/ml of Trandolapril)

Further pipette 2ml of Verapamil, 0.4ml of Trandolapril from stock solutions in to a 10ml volumetric flask and dilute up to the mark with diluent.

Preparation of Level – III (300µg/ml of Verapamiland 50µg/ml of Trandolapril)

Further pipette 3ml of Verapamil, 0.5ml of Trandolapril from stock solutions in to a 10ml volumetric flask and dilute up to the mark with diluent.

Preparation of Level – IV (400µg/ml of Verapamiland 60µg/ml of Trandolapril)

Further pipette 4ml of Verapamil, 0.6ml of Trandolapril from stock solutions in to a 10ml volumetric flask and dilute up to the mark with diluent.

Preparation of Level – V (500µg/ml of Verapamiland 70µg/ml of Trandolapril)

Further pipette 5ml of Verapamil, 0.7ml of Trandolapril from stock solutions in to a 10ml volumetric flask and dilute up to the mark with diluent.

Procedure

Inject each level into the chromatographic system and measure the peak area. Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient.
Results were reported in Tables: 38, 39 (6.2.3)
Acceptance Criteria: Correlation coefficient should be not less than 0.999

Limit of Detection

The detection limit is determined by the analysis of samples with known concentration of analyte and by establishing

that minimum level at which the analyte can reliably detected.

Limit of Quantitation

The quantification limit is generally determined by the analysis of sample with known concentrations of analyte and by establishing the minimum level at which the analyte can be quantified with acceptable accuracy and precision.

Robustness

The analysis was performed in different conditions to find the variability of test results. The following conditions are checked for variation of results. .

Effect of Variation of flow Rate

The sample was analyzed at 0.9 ml/min and 1.1 ml/min instead of 1ml/min, remaining conditions are same. 20µl of the above sample was injected and chromatograms were recorded.

The Results are reported in Table: 42, 43 (7.2.6)

Effect of Variation of mobile phase organic composition

The sample was analyzed by variation of mobile phase i.e. Methanol: Phosphate Buffer was taken in the ratio and 50:50, 40:60 instead of 45:55, remaining conditions are same. 20µl of the above sample was injected and chromatograms were recorded.

The Results are reported in Table: 44, 45 (7.2.6)

RESULTS AND DISCUSSION

Method development

Trials

Trial 1

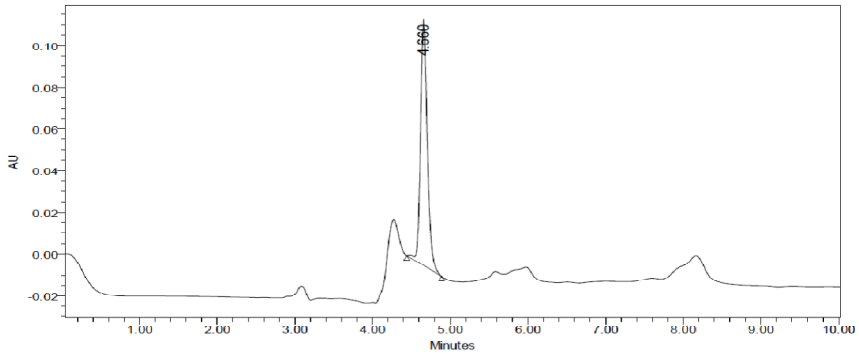

Fig 4: Chromatogram of trial 1

Inference

The Retention Time observed from chromatogram was 4.66, only one peak was eluted.

Trail 2

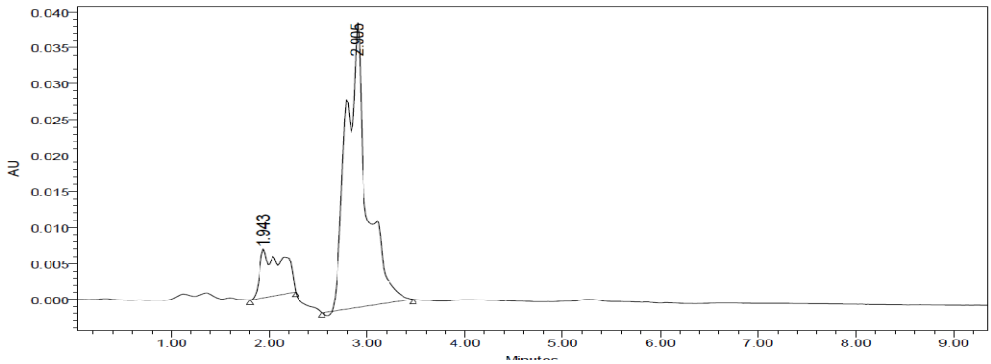

Fig 5: Chromatogram of trial 2

Inference

The Retention Time, plate count decreased observed from the chromatogram by changing column.

Trial 3

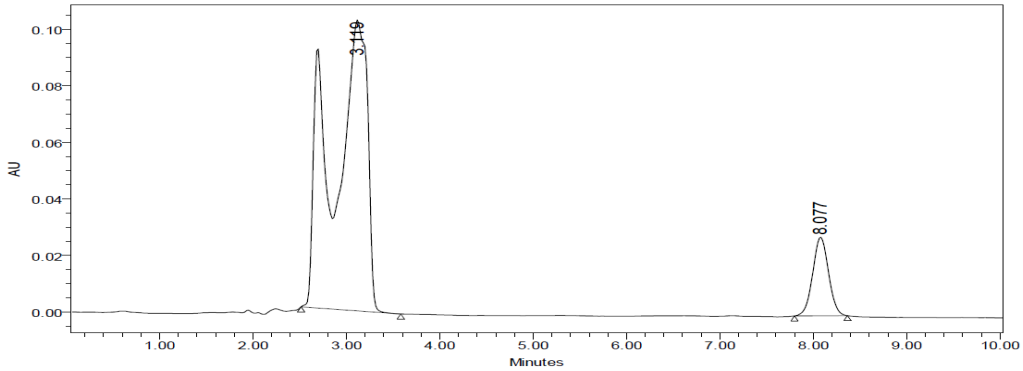

Fig 6: Chromatogram of trial 3

Inference

1. The Retention Time is increased by decreased flow rate from 1.3 to 0.9ml /min.
2. Increased in the plate count observed from chromatogram.

Trial 4

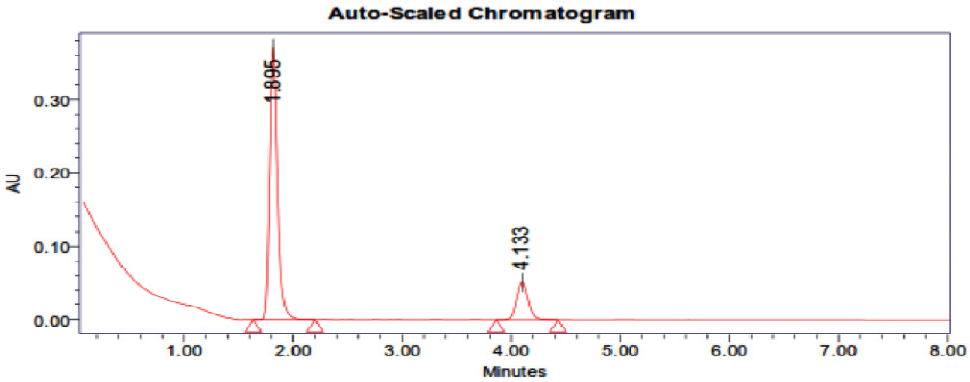

Fig 7: Chromatogram of trial 4

Inference

The Retention Time is decreased observed from chromatogram, for Verapamil 1.895 and Trandolapril 4.133 min.

Trial 5

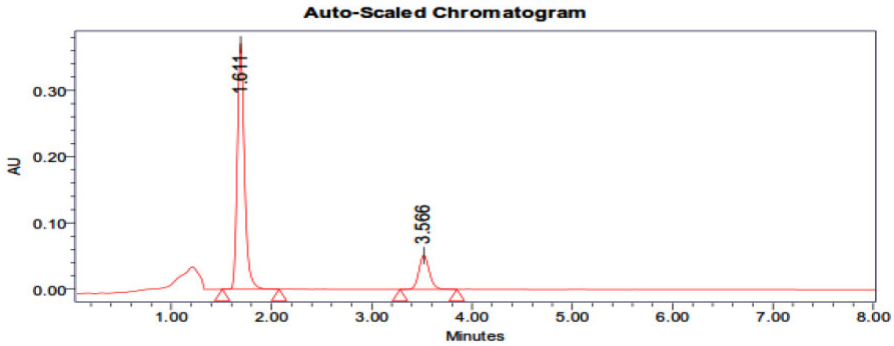

Fig 8:Chromatogram showing the trial5

Inference

The Retention Time is decreased observed from chromatogram by increasing flow rate the retention time was Verapamil and Trandolapril was found to be 1.611and 3.566min respectively.

Optimized Chromatogram

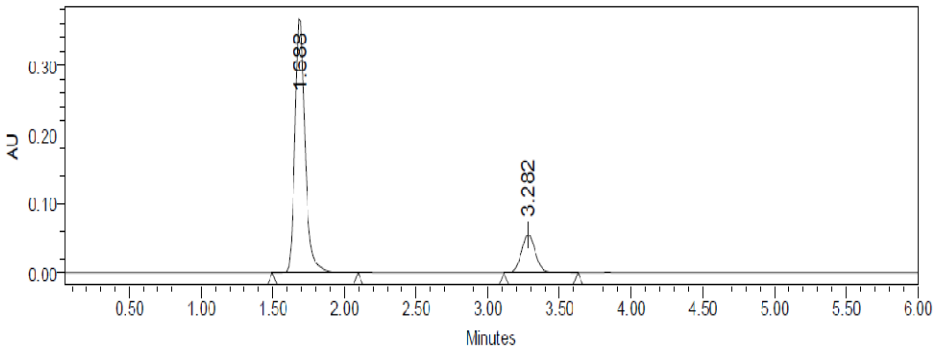

Fig 9: Optimized Chromatogram

Inference

1. The Retention Time is decreased observed from chromatogram by increasing flow rate.
2. The retention time was Verapamil and Trandolapril was found to be 1.688 and 3.282 respectively.
3. The tailing is not more than two and plate count observed is more than 2500. Pass all the system suitability parameters.
4. The peak shapes are good with good resolution and less Retention Time and more theoretical levels, pass the system suitability parameters.

Optimized Chromatographic Conditions

Table 12: Shows Optimized Chromatographic conditions

PARAMETER	OPTIMIZED CHROMATOGRAPHIC CONDITIONS
Mobile phase :	Phosphate Buffer (pH-4.8): Methanol (55:45% v/v)
Column :	Inertsil C18 (4.6mm ×250mm, 5µm particle size)
Flow rate :	1ml/min
Diluent	Phosphate Buffer (pH-4.8): Methanol (55:45% v/v)
Injection Volume	20 µl
Wavelength:	282 nm
Column temp:	35ºC
Run mode	Isocratic
Runtime	6minutes

- From the above experiment it was found that Verapamiland Trandolapril can effectively be analyzed by using the RP-HPLC method with Mobile phase at a flow rate of 1 ml/min and detection wave length of 282nm.
- The retention time of Verapamiland Trandolapril were found to be 1.688 and 3.282 minutes respectively.

Blank Chromatogram

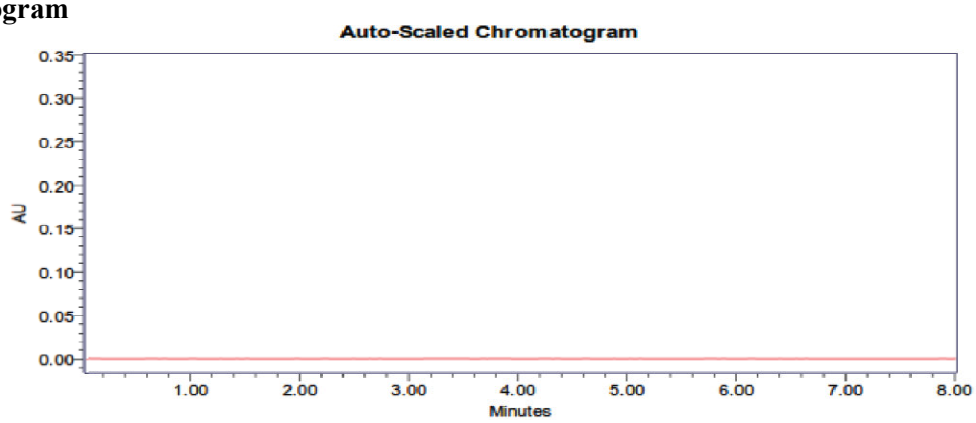

Fig 11: Blank Chromatogram

Standard Chromatogram

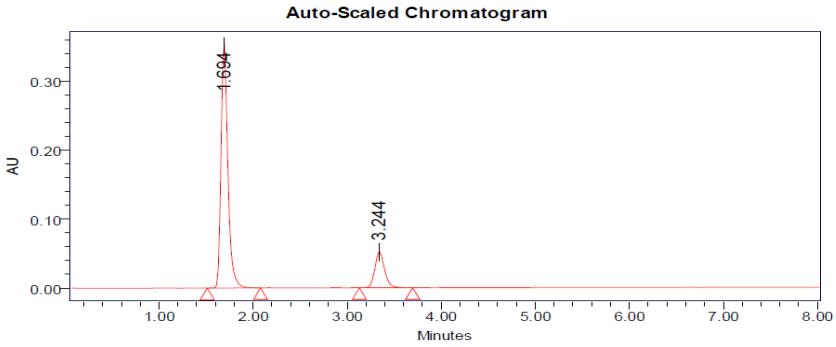

Fig 12: Standard Chromatogram-1

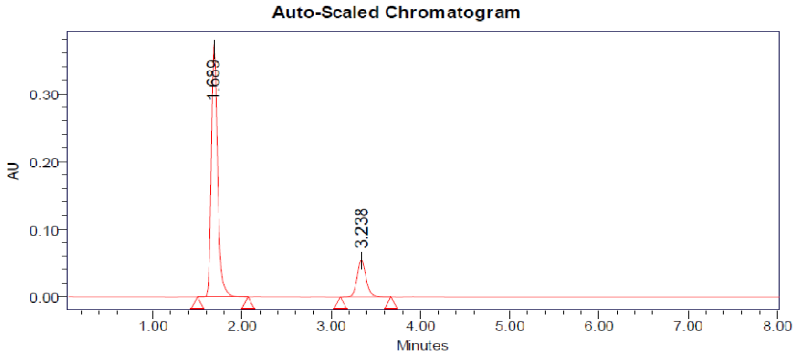

Fig 13: standard Chromatogram-2

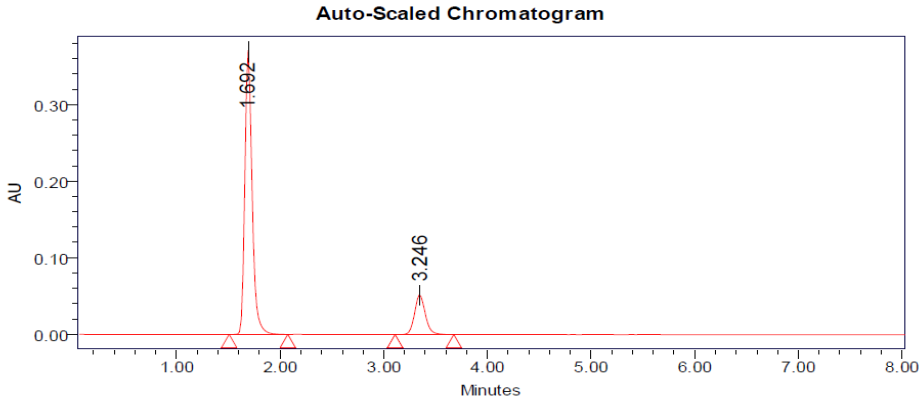

Fig 14: standard Chromatogram-3

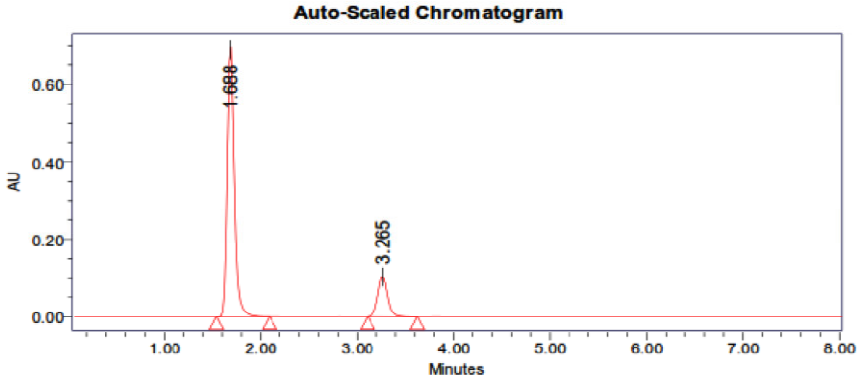

Fig 15: standard Chromatogram-4

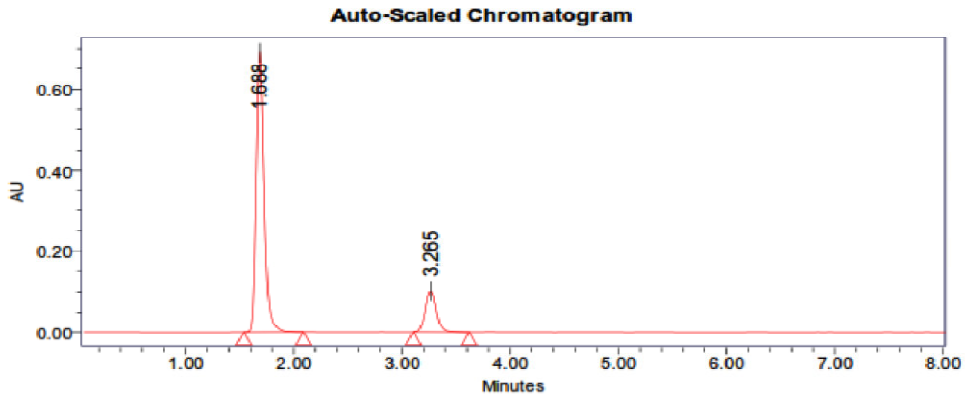

Fig 16: Standard Chromatogram-5

Assay (Sample)

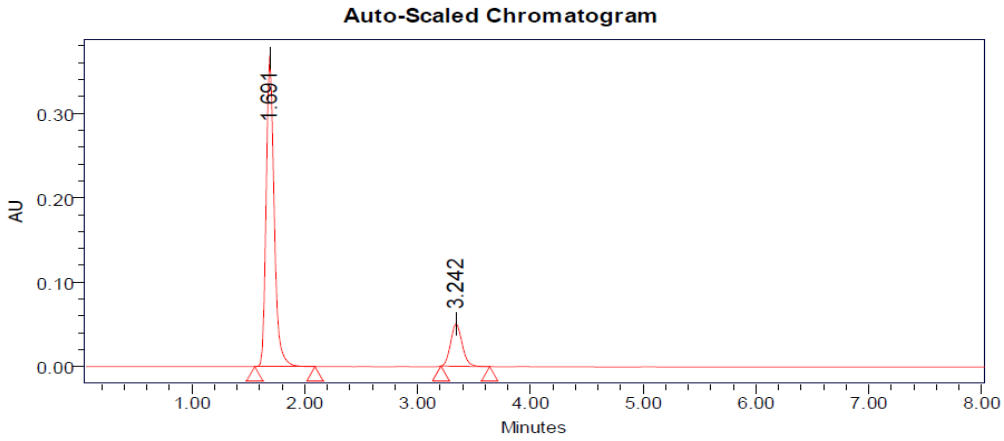

Fig 17: Sample Chromatogram -1

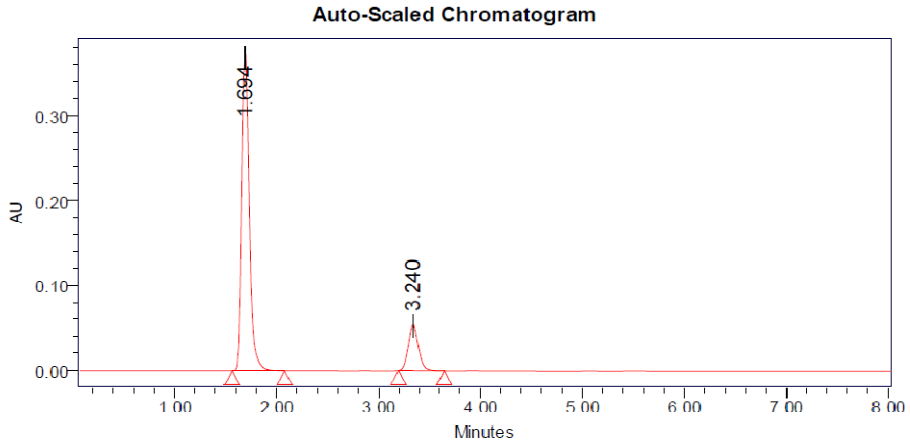

Fig 18: Sample Chromatogram -2

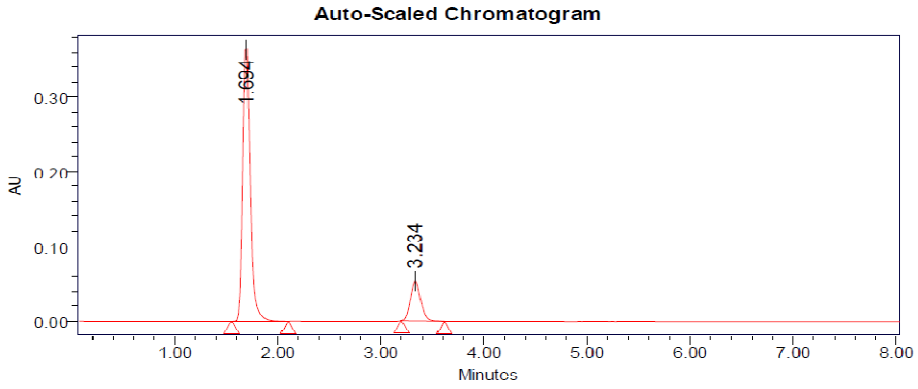

Fig19: Sample Chromatogram -3

$$\%ASSAY = \frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100$$

System Suitability Results

1) Tailing factor obtained from the standard injection is 1.69.
2) Theoretical plates obtained from the standard injection are 7586.

Assay limits for Verapamiland Trandolapril is 98-102%.

Table 22: Shown Assay Result

Label claim	% purity
Verapamil andTrandolapril	99.86%

The chromatogram for blank, standard and sample ofVerapamiland Trandolapril were shown in Fig: 11-19.The assay limits for Verapamiland Trandolapril was 98-102% and the results obtained for Verapamiland Trandolapril was found to be 99.86%.

Hence the results were within the limits. The results shown in Table: 24.

Method validation

System suitability parameters

Table23: Observation of system suitability parameters

S. NO	Parameter	Verapamil	Trandolapril
1.	Retention Time (min)	1.688	3.282
2.	Theoretical Plates	7586	6235
3.	Tailing factor	1.69	1.58
4.	Area	1658768	426589
5.	Resolution		10.89

The system suitability parameters were found to be within the specified limits for the proposed method.

Accuracy

Accuracy50%

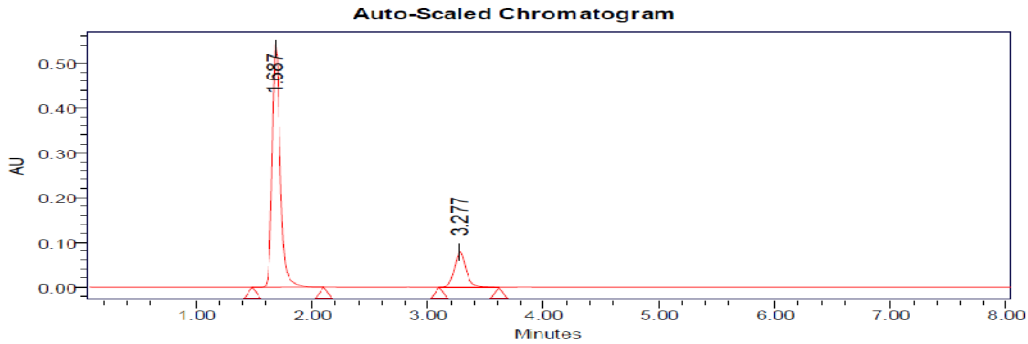

Fig 20: Accuracy 50% Chromatogram-1

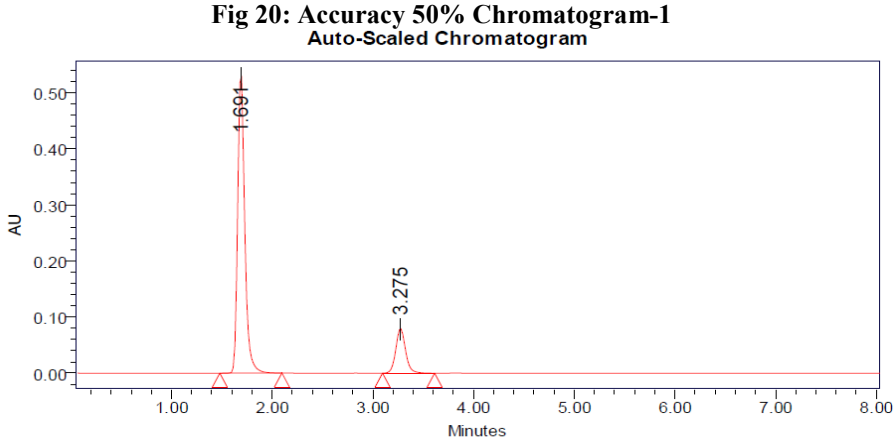

Fig 21: Accuracy 50% Chromatogram-2

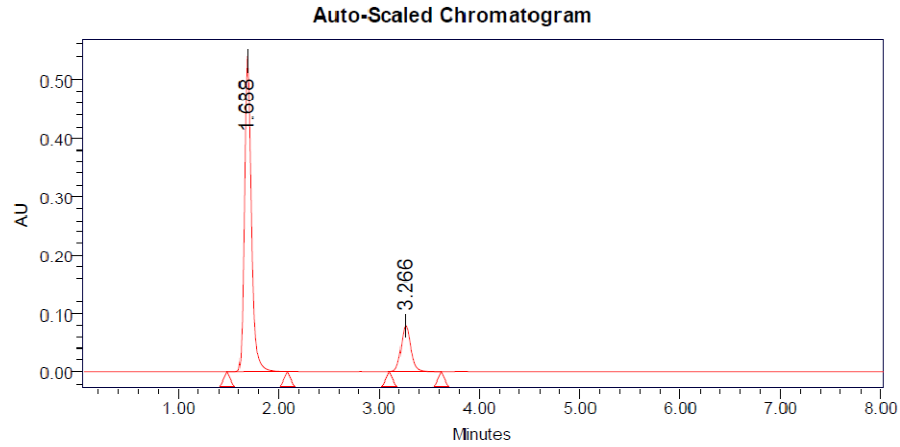

Fig 22: Accuracy 50% Chromatogram-3

Accuracy100%

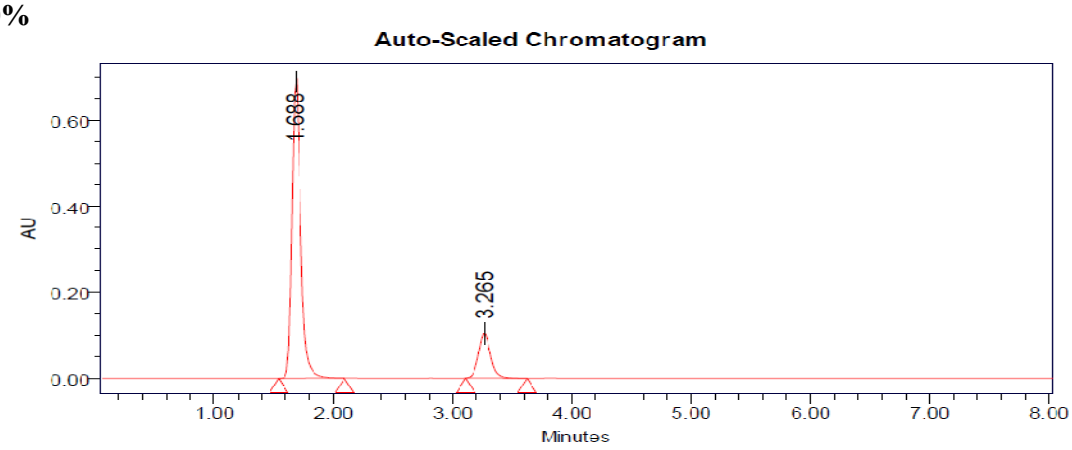

Fig 23: Accuracy100% Chromatogram-1

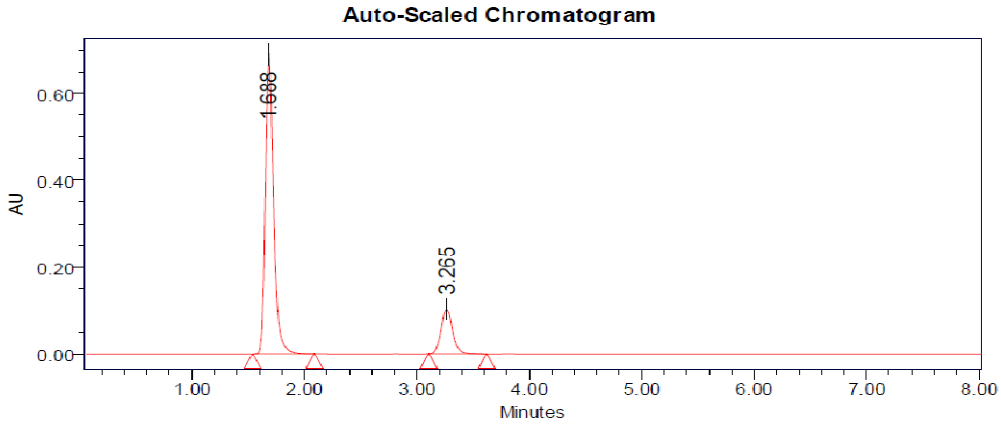

Fig 24: Accuracy100% Chromatogram-2

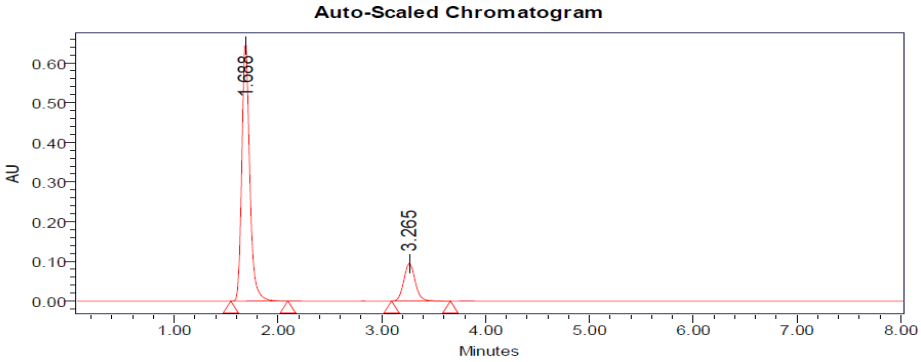

Fig 25: Accuracy100% Chromatogram-3

Accuracy150%

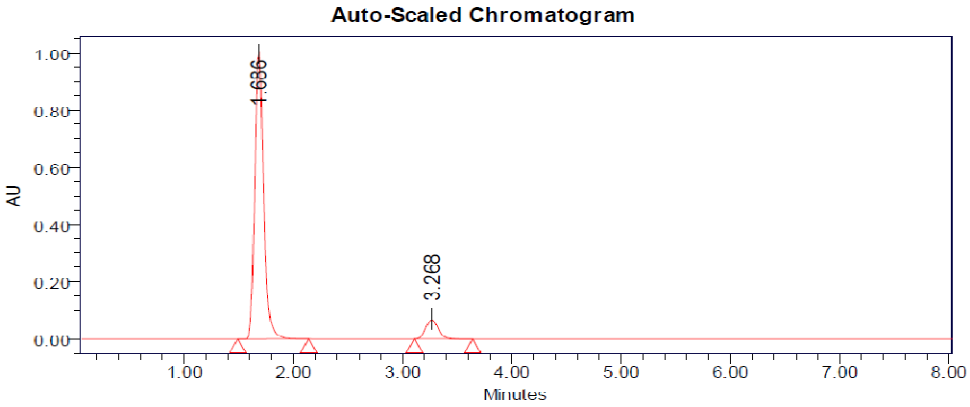

Fig 26: Accuracy150% Chromatogram-1

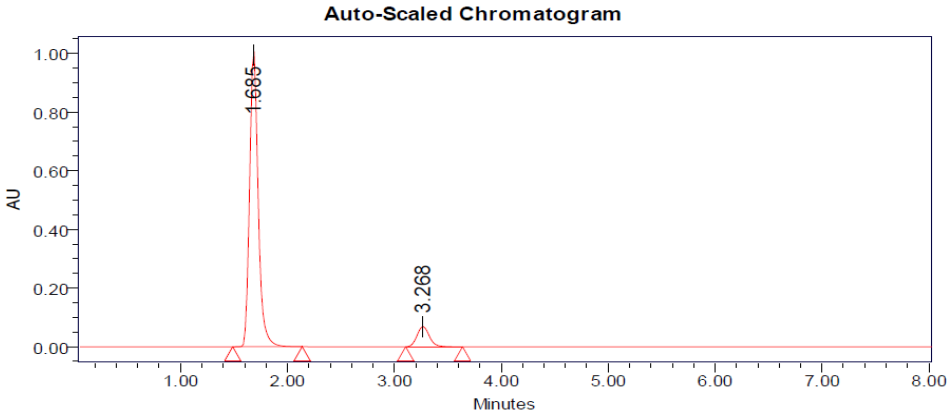

Fig 27: Accuracy150% Chromatogram-2

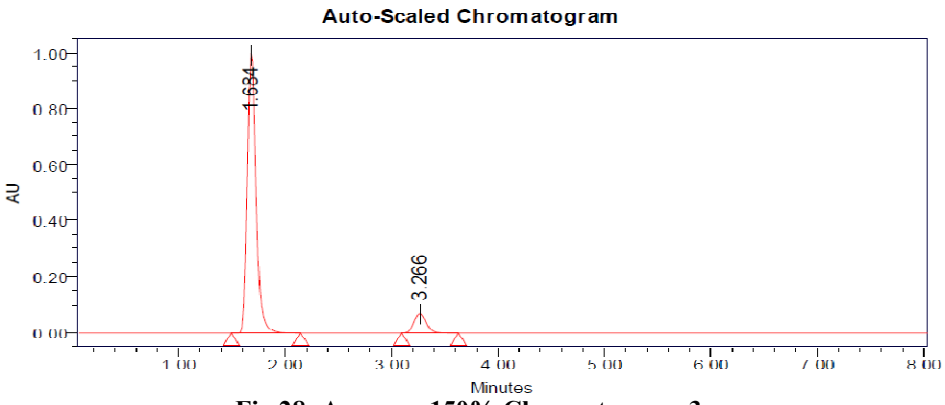

Fig 28: Accuracy150% Chromatogram-3

Verapamil

Table 33: Accuracy Observation ofVerapamil

%Concentration (at specification Level)	Average Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	879537	150	150.048	100.032	100.112%
100%	1743252	300	300.521	100.172	
150%	2609693	450	450.598	100.132	

Trandolapril

Table 34: Accuracy Observation ofTrandolapril

%Concentration (at specification Level)	Average Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	224271	25	25.114	100.456%	100.16%
100%	445748.3	50	49.952	99.904%	
150%	670006.3	75	75.101	100.134%	

The accuracy studies were shown as % recovery for Verapamiland Trandolapril at 50%, 100% and 150% the limits of % recovery should be in range of 98-102%.

The results obtained for Verapamiland Trandolapril were found to be within the limits. Hence the method was found to be accurate.

The accuracy studies showed % recovery of the Verapamil100.112%-and Trandolapril 100.16%.

The limits of % recovery of drugs were98-102 % and from the above results it indicates that the commonly used excipients present in the pharmaceutical formulation do not interfere in the proposed method.

The chromatograms for accuracy shown in Figs 21-29 and results were shown in Tables 26-36.

Precision

System Precision

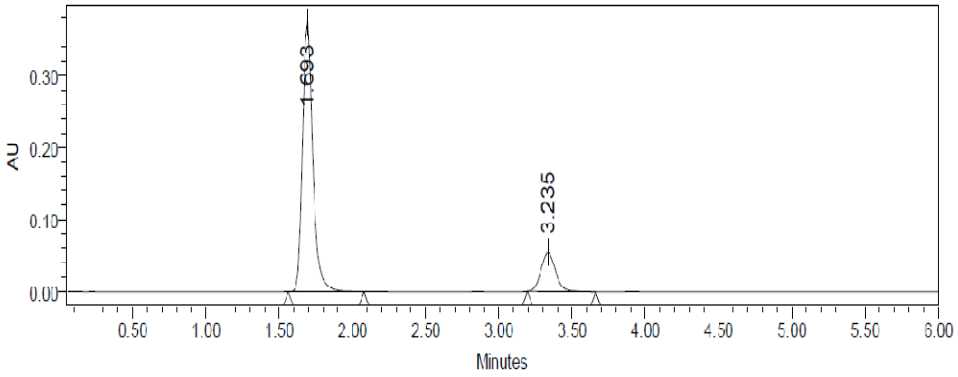

Fig 28: System Precision Chromatogram -1

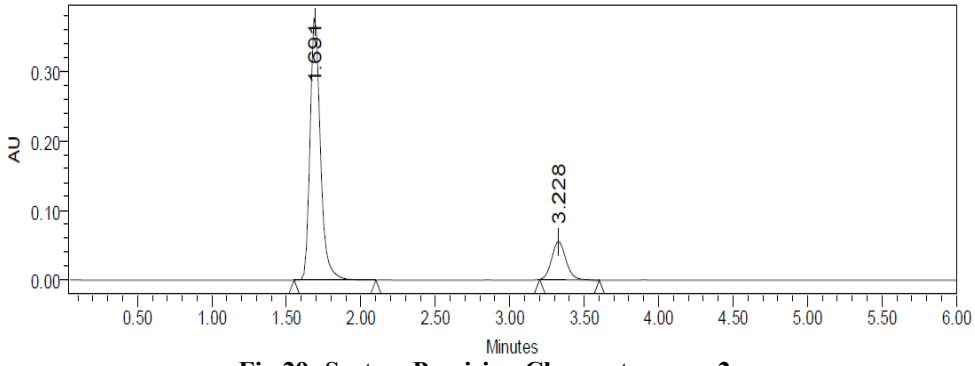

Fig 29: System Precision Chromatogram -2

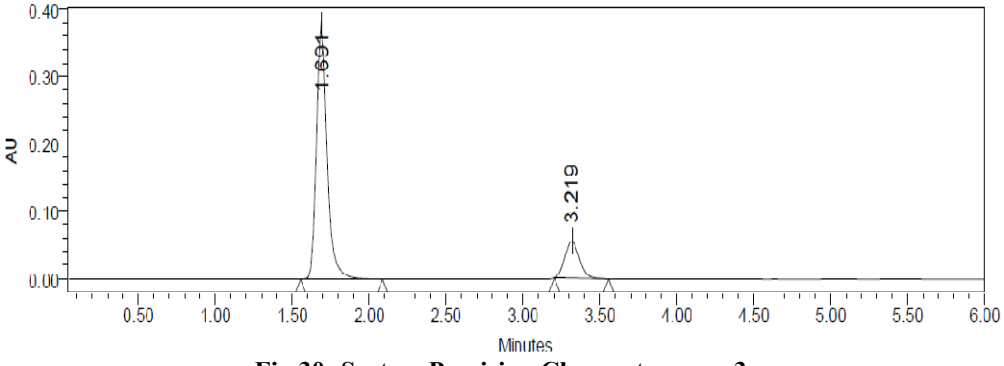

Fig 30: System Precision Chromatogram -3

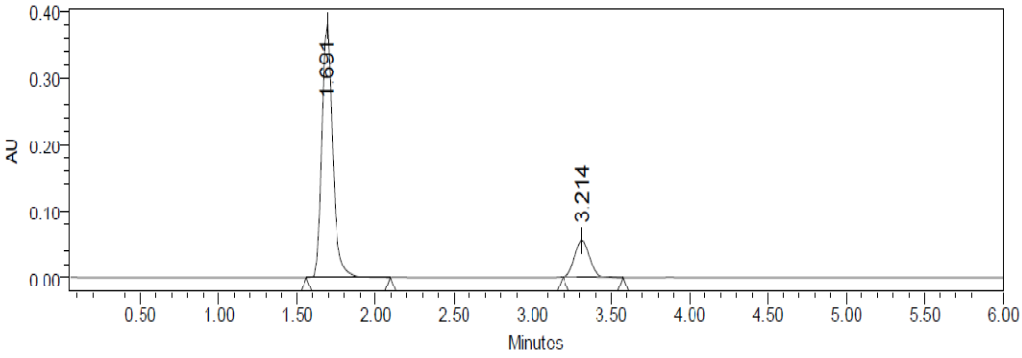

Fig 32: System Precision Chromatogram -4

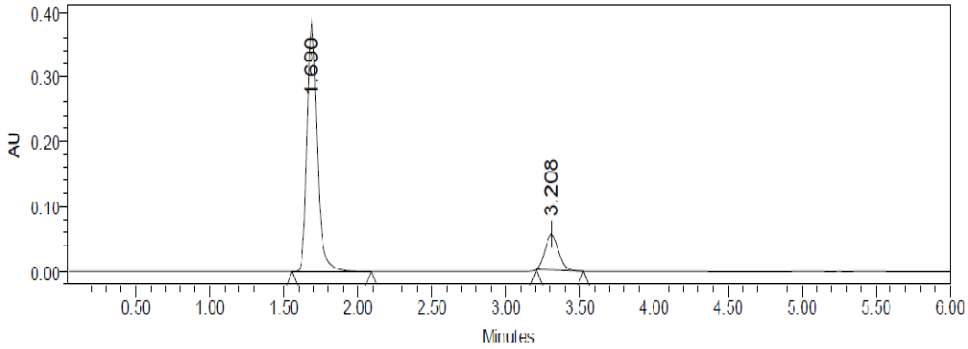

Fig 33: System Precision Chromatogram -5

Acceptance Criteria

In the precision study %RSD was fond to be less than 2%. For Verapamil 0.17% and Trandolapril 0.04% which indicates that the system has good reproducibility.

For precision studies 5 replicated injections of Verapamil and Trandolapril formulation was performed. %RSD was determined for peak areas of Verapamil and Trandolapril.
The acceptance limits should be not more than 2% and the results were found to be within the acceptance limits.
The chromatogram of precision was showed in Figs: 29-33results were reported in Table: 35

Ruggedness
Day 1

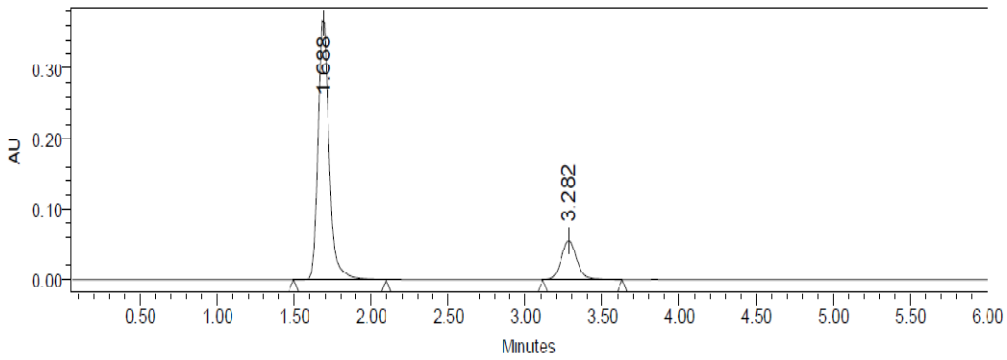

Fig 34: Chromatogram showing Day1 injection -1

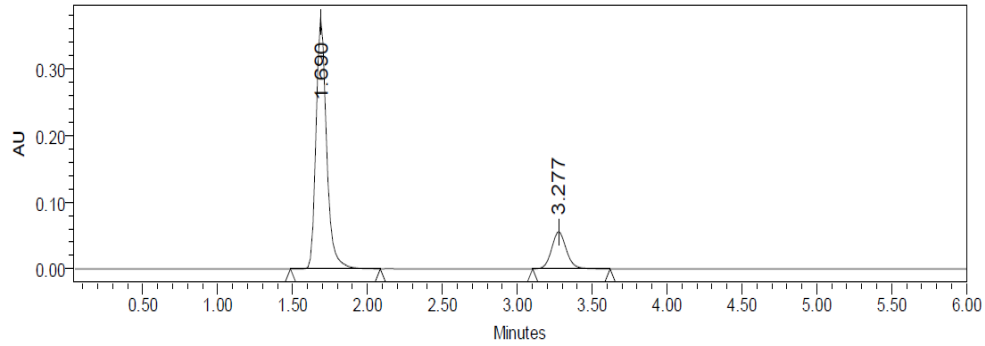

Fig 35: Chromatogram showing Day1 injection -2

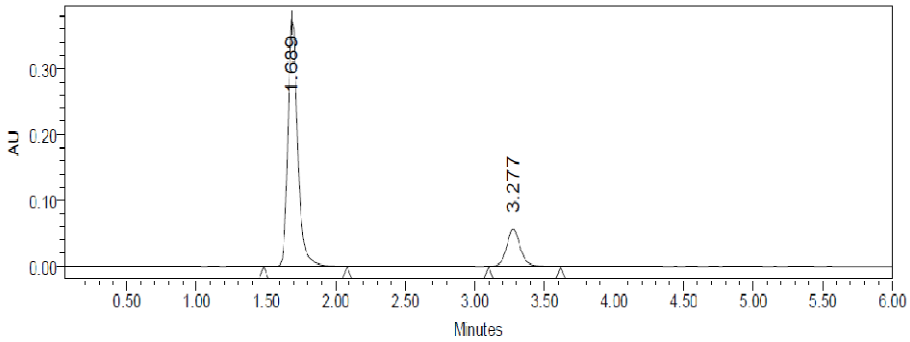

Fig 36: Chromatogram showing Day1 injection -3

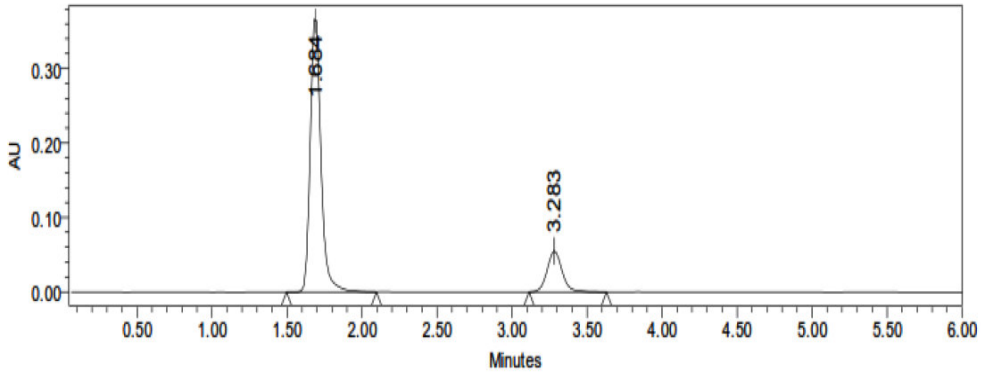

Fig 37: Chromatogram showing Day1 injection -4

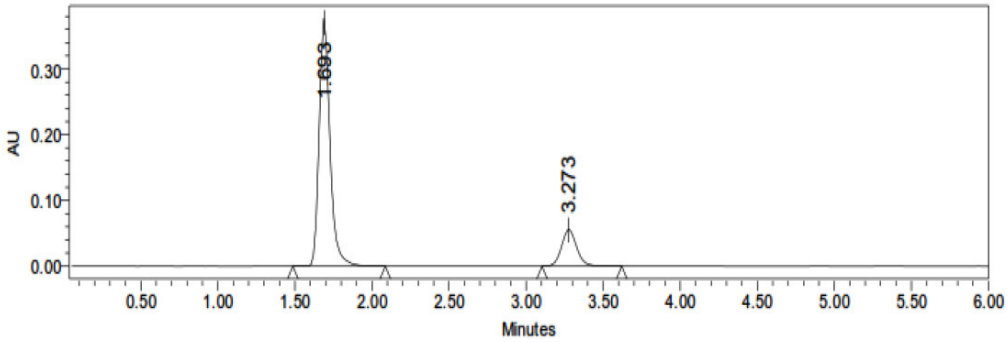

Fig 38: Chromatogram showing Day1 injection -5

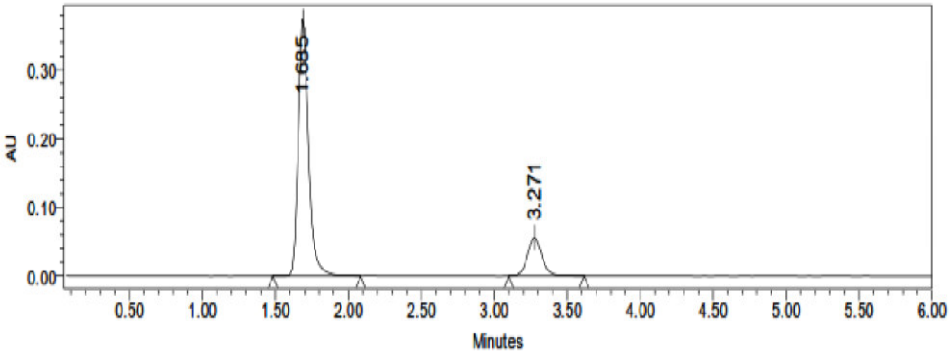

Fig 39: Chromatogram showing Day1 injection -6

Acceptance Criteria

- %RSD of five different sample solutions should not more than 2.

Day 2

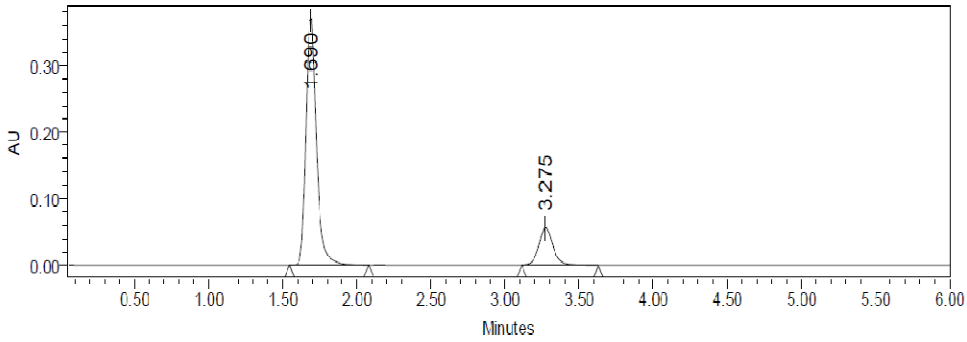

Fig 40: Chromatogram showing Day 2 injection -1

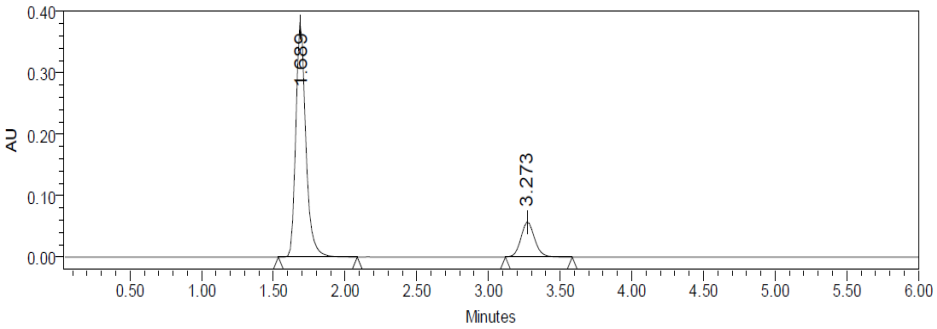

Fig 41: Chromatogram showing Day 2 injection -2

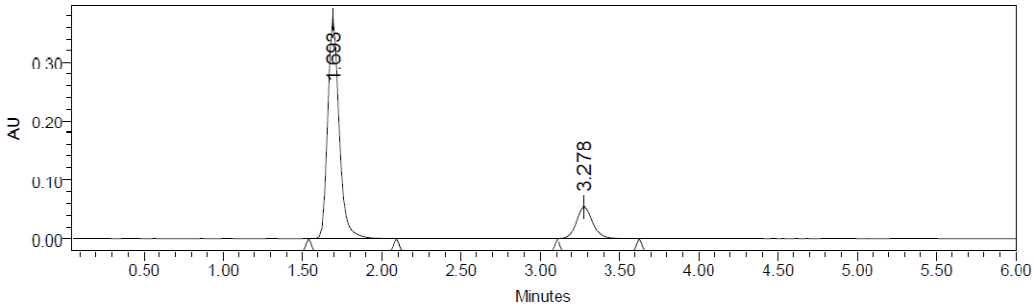

Fig 42: Chromatogram showing Day 2 injection -3

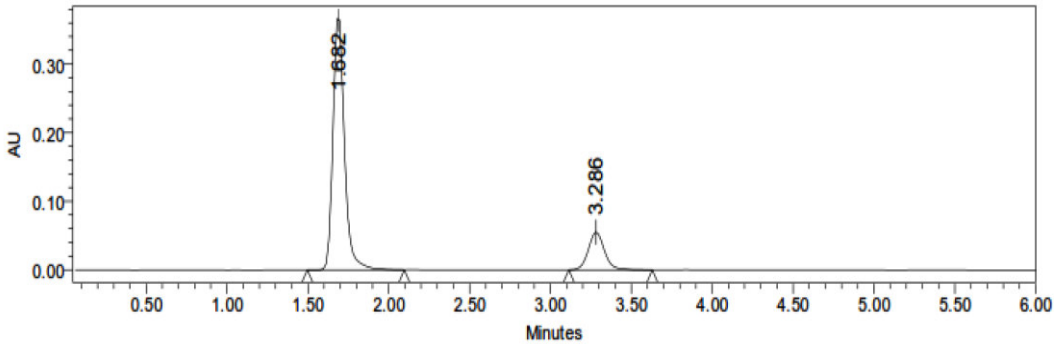

Fig 43: Chromatogram showing Day 2 injection -4

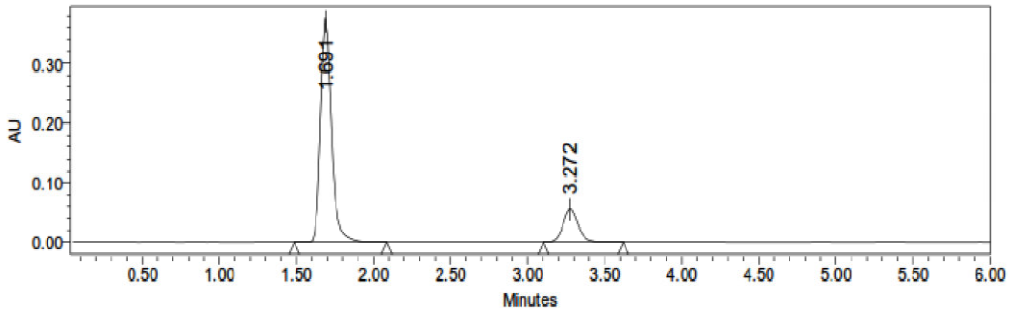

Fig 44: Chromatogram showing Day 2 injection -5

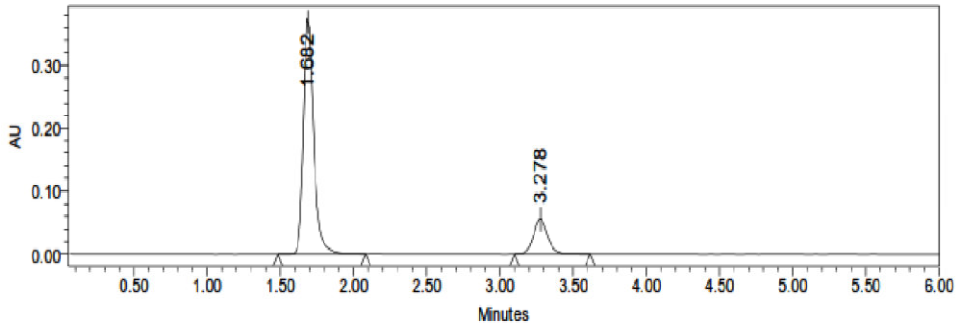

Fig 45: Chromatogram showing Day 2 injection -6

Acceptance Criteria

- %RSD of five different sample solutions should not more than 2.

Linearity

Level I

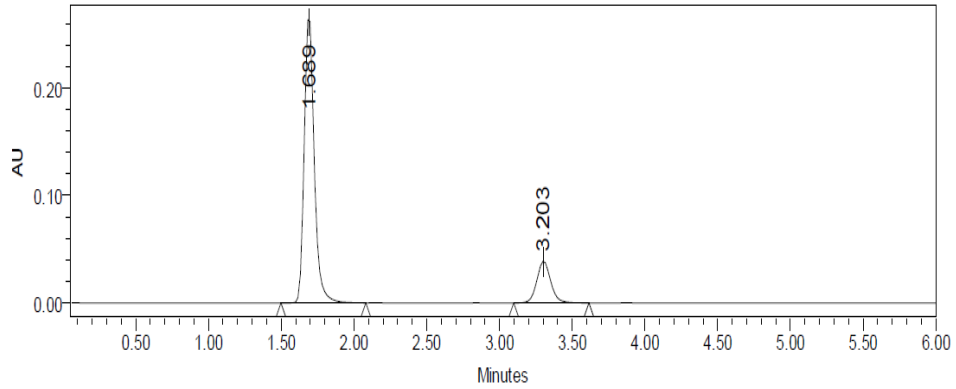

Fig 46: Linearity Chromatogram -1

Level II

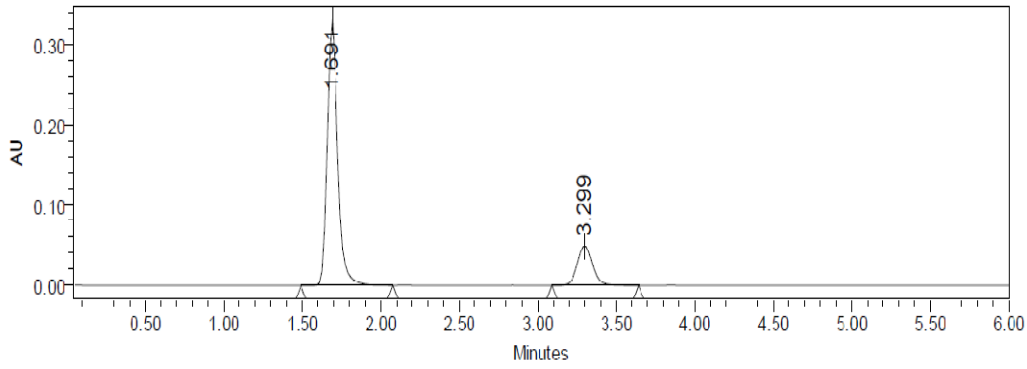

Fig 47: Linearity Chromatogram -2

Level III

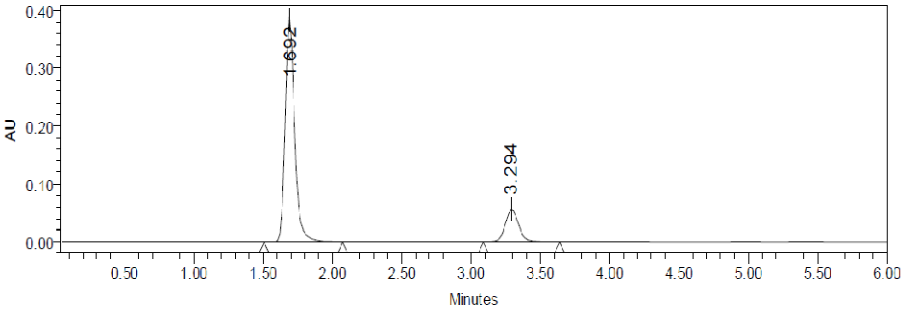

Fig 48: Chromatogram showing linearity level-3

Level IV

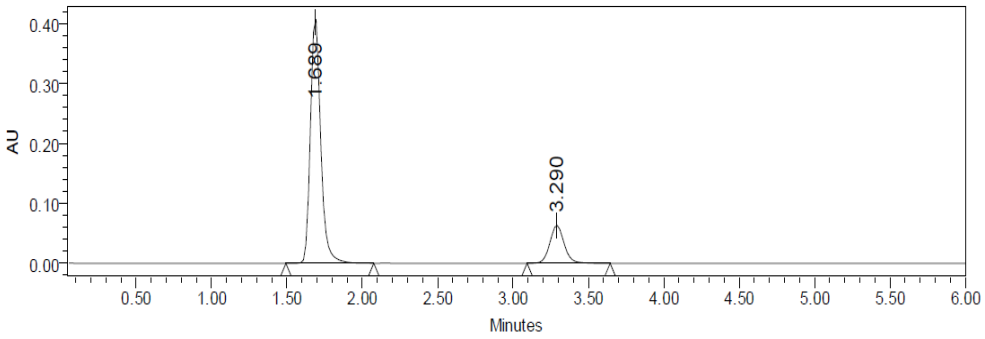

Fig 49: Chromatogram showing linearity level-4

Level V

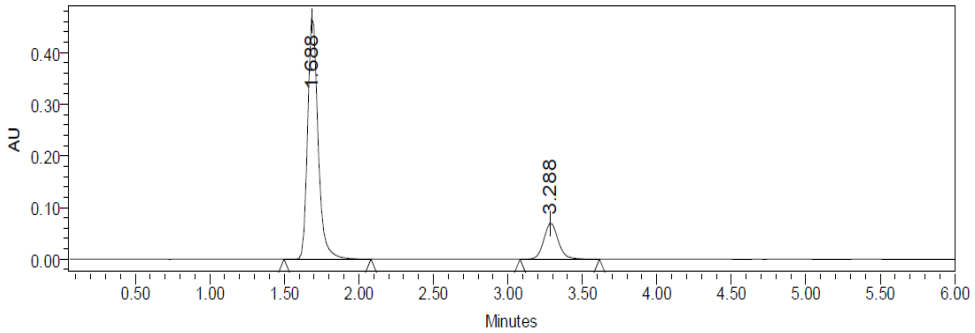

Fig 50: Chromatogram showing linearity level-5

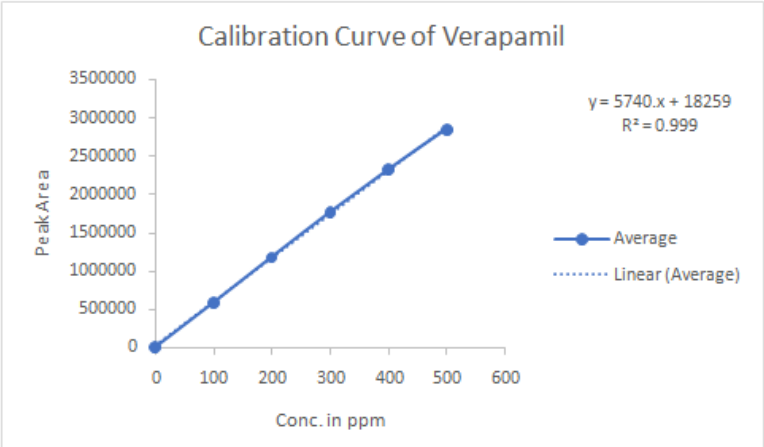

Fig 51: Calibration Curve for Verapamil

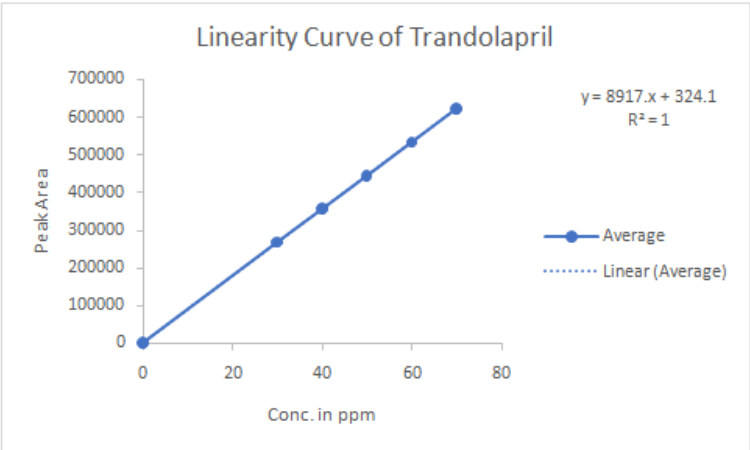

Fig 52: Calibration Curve for Trandolapril

The linearity range was found to be 100-500 and 30-70µg/ml for both Verapamil and Trandolapril respectively. Calibration curve was plotted and correlated Co-efficient for both the drugs found to be 0.999.
Hence the results obtained were within the limits. The linearity curves were shown in Figs: 52, 53.
The linearity chromatograms recorded were shown in Figs: 47-51.The linearity results were reported in Table: 62, 63.

Limit of detection (lod)

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value.

LOD= 3.3 × S.D / Slope

Table 40: LOD results of the method

Drug	Amount(µg/ml)
Verapamil	2.1
Trandolapril	1.28

From the above, the LOD values of Verapamil and Trandolapril were found to be 2.1 and 1.28µg/ml respectively.

Limit of quantitation (LOQ)

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined.

LOQ=10× S.D / Slope

Table41: LOQ results of the method

Drug	Amount(µg/ml)
Verapamil	6.3
Trandolapril	3.84

From the above, the LOQ values of Verapamil and Trandolapril were found to be 6.3 and 3.84µg/ml respectively.

Robustness

The robustness was performed for the flow rate variations from 0.9 ml/min to 1.1ml/min and mobile phase ratio variation from more organic phase to less organic phase ratio for Trandolapril and Verapamil. The method is robust only in less flow condition and the method is robust even by change in the Mobile phase ±5%. The standard and samples of Trandolapril, Verapamilwere injected by changing the conditions of chromatography. There was no significant change in the parameters like resolution, tailing factor, asymmetric factor, and plate count.

Flow Rate: (ml/min)
Low Flow Rate: (0.9 ml/min)

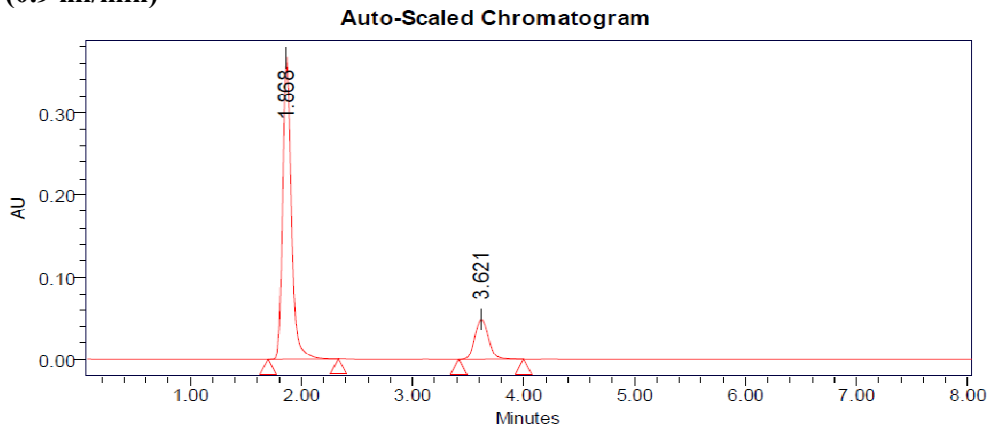

Fig 53: Chromatogram showing less flow of 0.9ml/min

High Flow Rate: (1.1ml/min)

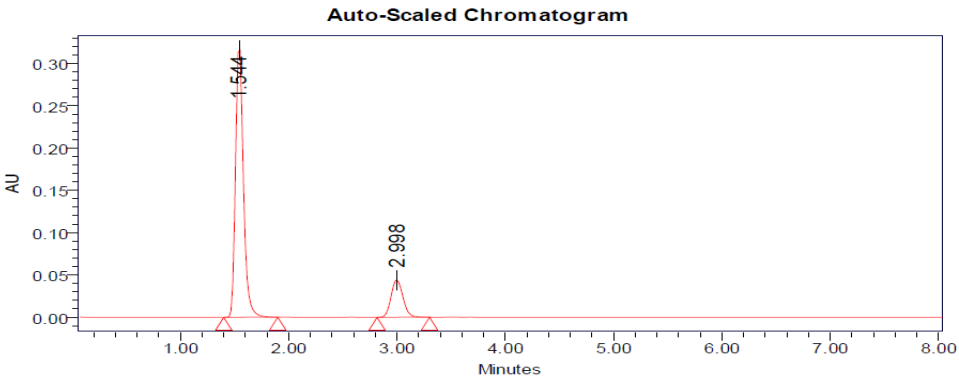

Fig 54: Chromatogram showing more flow of 1.1 ml/min

System suitability Results for Verapamil

Table 42: Flow rate Observation of Verapamil

Flow Rate (ml/min)		System suitability Results		
		USP Plate Count	USP Tailing	Retention Time (min)
Less Flow rate	0.8	7365	1.62	1.868
Actual Flow rate	1	7586	1.69	1.688
More Flow rate	1.2	7254	1.61	1.544

Results for actual flow rate have been considered from assay standard.

System suitability Results for Trandolapril

Table 43: Flow rate Observation of Trandolapril

Flow Rate (ml/min)		System suitability Results		
		USP Plate Count	USP Tailing	Retention Time (min)
Less Flow rate	0.8	6284	1.51	3.621
Actual Flow rate	1	6235	1.58	3.282
More Flow rate	1.2	6168	1.56	2.998

On evaluation of the above results, it can be concluded that the variation in flow rate not affect the method significantly.

Organic Composition
Less organic Composition

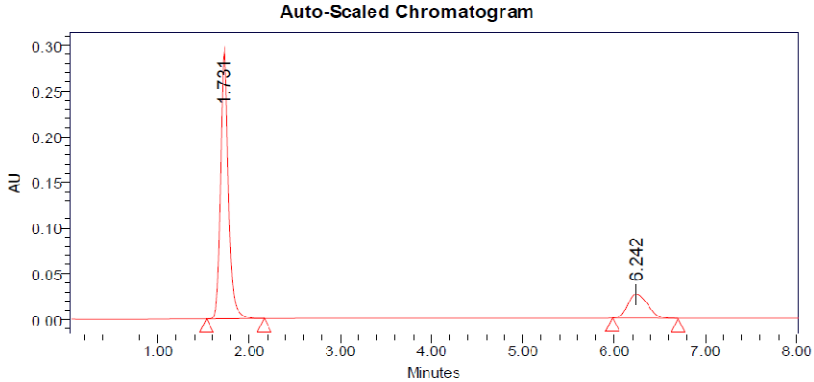

Fig 55: Chromatogram showing less organic composition

More organic composition

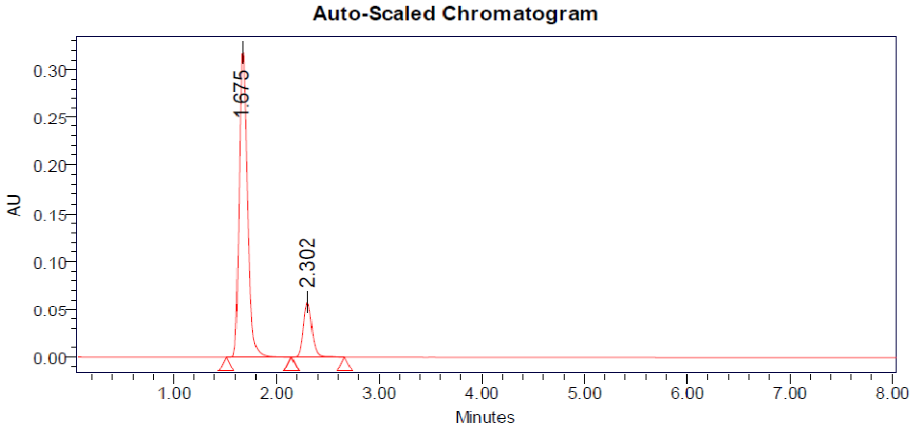

Fig 56: Chromatogram showing more organic composition

Table 44: System suitability results Verapamil

Organic phase		System suitability Results		
		USP Plate Count	USP Tailing	Retention Time (min)
Less organic phase	50:50	7269	1.61	1.868
Actual organic phase	55:45	7586	1.69	1.688
More organic phase	60:40	7496	1.64	1.675

Table 45: System suitability result Trandolapril

Organic phase		System suitability Results		
		USP Plate Count	USP Tailing	Retention Time (min)
Less organic phase	50:50	6182	1.54	3.621
Actual organic phase	55:45	6235	1.58	3.282
More organic phase	60:40	6322	1.56	2.302

Acceptance Criteria

The tailing factor should be less than 2.0 and the number of theoretical plates (N) should be more than 2000.

SUMMARY

The analytical method was developed by studying different parameters.
First of all, maximum absorbance was found to be at 282 nm and the peak purity was excellent.
Injection volume was selected to be 20µl which gave a good peak area.
The column used for study was Inertsil C18 (4.6mm ×250mm, 5µm particle size)particle size because it was giving good peak.
35ºC temperatures 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 and satisfactory retention time.
Mobile phase is Phosphate Buffer (pH-4.8): Methanol (55:45% v/v)was fixed due to good symmetrical peak. So this mobile phase was used for the proposed study.
Run time was selected to be 6 min because analyze gave peak around 1.688, 3.282 ±0.02min respectively and also to reduce the total run time.
The percent recovery was found to be 98.0-102% was linear and precise over the same range. Both system and method precision was found to be accurate and well within range.
The analytical method was found linearity over the range 100-500mg/ml of Verapamiland 30-70mg/ml of Trandolaprilof the target concentration.
The analytical passed both robustness and ruggedness tests. On both cases, relative standard deviation was well satisfactory.

CONCLUSION

In the present investigation, a simple, sensitive, precise and accurate RP-HPLC method was developed for the quantitative estimation of Verapamil and Trandolapril in bulk drug and pharmaceutical dosage forms.
This method was simple, since diluted samples are directly used without any preliminary chemical derivatisation or purification steps.
Verapamil was found to be readily soluble in ethanol. It is slightly soluble in water; freely soluble in sodium hydroxide

solution; sparingly soluble in methanol; insoluble in ether, chloroform, benzene, and dilute mineral acids and Trandolapril was found to be practically insoluble in water; slightly soluble in alcohol and in methyl alcohol; sparingly soluble in acetone and in chloroform; very slightly too slightly soluble in ether.

Phosphate Buffer (pH-4.8): Methanol (55:45% v/v)was chosen as the mobile phase. The solvent system used in this method was economical.

The %RSD values were within 2 and the method was found to be precise.

The results expressed inTables for RP-HPLC method was promising. The RP-HPLC method is more sensitive, accurate and precise compared to the Spectrophotometric methods.

This method can be used for the routine determination of Verapamil and Trandolapril in bulk drug and in Pharmaceutical dosage forms.

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