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DEVELOPMENT AND VALIDATION OF CHROMATOGRAPHIC METHOD FOR THE SIMULTANEOUS ESTIMATION OF TRANDOLAPRIL AND VERAPAMIL IN PURE FORM AND PHARMACEUTICAL DOSAGE FORM BY USING HPLC

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Abstract:
A new, simple and accurate, precise RP-HPLC method was developed for simultaneous determination of Trandolapril and Verapamil in bulk and in combined pharmaceutical dosage form. The separation of Trandolapril and Verapamil was achieved within 8 minutes on an Agilent Zorbax (C18) (150mm x 4.6mm, 5µm) column using Methanol: Acetate Buffer pH-3.8 (24:76v/v) as the mobile phase. Detection was carried out using wavelength at 262nm. The method showed adequate sensitivity concerning linearity, accuracy and precision over the range 100-500µg/ml and 30-70µg/ml for Trandolapril and Verapamil, respectively. Careful validation proved advantages of high sensitivity, accuracy, precision, selectivity, robust and suitability for quality control laboratories. The developed method was robust as the %RSD was within the range and without effecting system suitability parameters. The proposed method is suitable for simultaneous determination of Trandolapril and Verapamil in bulk and pharmaceutical dosage form.

Keywords: *Trandolapril and Verapamil, RP-HPLC, Validation, Precision, Robustness, ICH Guidelines.*

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1. INTRODUCTION:

Chromatography is a laboratory technique for the separation of a mixture. The mixture is dissolved in a fluid called the *mobile phase*, which carries it through a structure holding another material called the *stationary phase*. The various constituents of the mixture travel at different speeds, causing them to separate. The separation is based on differential partitioning between the mobile and stationary phases. Subtle differences in a compound's partition coefficient result in differential retention on the stationary phase and thus affect the separation.

Chromatography may be preparative or analytical. The purpose of preparative chromatography is to separate the components of a mixture for later use, and is thus a form of purification. Analytical chromatography is done normally with smaller amounts of material and is for establishing the presence or measuring the relative proportions of analytes in a mixture. The two are not mutually exclusive.

Chromatography is based on the principle where molecules in mixture applied onto the surface or into the solid, and fluid stationary phase (stable phase) is separating from each other while moving with the aid of a mobile phase. The factors effective on this separation process include molecular characteristics related to adsorption (liquid-solid), partition (liquid-solid), and affinity or differences among their molecular weights[1, 2]. Because of these differences, some components of the mixture stay longer in the stationary phase, and they move slowly in the chromatography system, while others pass rapidly into mobile phase, and leave the system faster. Based on this approach three components form the basis of the chromatography technique.

- Stationary phase: This phase is always composed of a “solid” phase or “a layer of a liquid adsorbed on the surface a solid support”.
- Mobile phase: This phase is always composed of “liquid” or a “gaseous component.”
- Separated molecules

The type of interaction between stationary phase, mobile phase, and substances contained in the mixture is the basic component effective on separation of molecules from each other. Chromatography methods based on partition are very effective on separation, and identification of small molecules as amino acids, carbohydrates, and fatty acids. However, affinity chromatography’s (ie. ion-exchange chromatography) are more effective in the separation of macromolecules as nucleic acids, and proteins. Paper chromatography is used in the separation of proteins, and in studies related to protein synthesis; gas-liquid chromatography is utilized in the separation of alcohol, esther, lipid, and amino groups, and observation of enzymatic interactions, while molecular-sieve chromatography is employed especially for the determination of molecular weights of proteins. Agarose-gel chromatography is used for the purification of RNA, DNA particles, and viruses.

Stationary phase in chromatography, is a solid phase or a liquid phase coated on the surface of a solid phase. Mobile phase flowing over the stationary phase is a gaseous or liquid phase. If mobile phase is liquid it is termed as liquid chromatography (LC), and if it is gas then it is called gas chromatography (GC). Gas chromatography is applied for gases, and mixtures of volatile liquids, and solid material. Liquid chromatography is used especially for thermal unstable, and non-volatile samples.

The purpose of applying chromatography which is used as a method of quantitative analysis apart from its separation, is to achieve a satisfactory separation within a suitable time interval. Various chromatography methods have been developed to that end. Some of them include column chromatography, thin-layer chromatography (TLC), paper chromatography, gas chromatography, ion exchange chromatography, gel permeation chromatography, high-pressure liquid chromatography, and affinity chromatography.

- Column chromatography
- Ion-exchange chromatography
- Gel-permeation (molecular sieve) chromatography
- Affinity chromatography
- Paper chromatography
- Thin-layer chromatography
- Gas chromatography
- Dye-ligand chromatography
- Hydrophobic interaction chromatography
- Pseudoaffinity chromatography
- High-pressure liquid chromatography (HPLC)

High-pressure liquid chromatography (HPLC)

Using this chromatography technique it is possible to perform structural, and functional analysis, and purification of many molecules within a short time, This technique yields perfect results in the separation, and identification of amino acids, carbohydrates, lipids, nucleic acids, proteins, steroids, and other biologically active molecules, In HPLC, mobile phase passes through columns under 10–400 atmospheric

pressure, and with a high (0.1–5 cm//sec) flow rate. In this technique, use of small particles, and application of high presure on the rate of solvent flow increases separation power, of HPLC and the analysis is completed within a short time.
Essential components of a HPLC device are solvent depot, high- pressure pump, commercially prepared column, detector, and recorder. Duration of separation is controlled

Essential components of a HPLC device are solvent depot, high- pressure pump, commercially prepared column, detector, and recorder. Duration of separation is controlled with the aid of a computerized system, and material is accrued.

Types of HPLC

There are following variants of HPLC, depending upon the phase system (stationary) in the process:

1. Normal Phase HPLC

This method separates analytes on the basis of polarity. NP-HPLC uses polar stationary phase and non-polar mobile phase. Therefore, the stationary phase is usually silica and typical mobile phases are hexane, methylene chloride, chloroform, diethyl ether, and mixtures of these.
Polar samples are thus retained on the polar surface of the column packing longer than less polar materials.

2. Reverse Phase HPLC

The stationary phase is nonpolar (hydrophobic) in nature, while the mobile phase is a polar liquid, such as mixtures of water and methanol or acetonitrile. It works on the principle of hydrophobic interactions hence the more nonpolar the material is, the longer it will be retained.

3. Size-exclusion HPLC

The column is filled with material having precisely controlled pore sizes, and the particles are separated according to its their molecular size. Larger molecules are rapidly washed through the column; smaller molecules penetrate inside the porous of the packing particles and elute later.

4. Ion-Exchange HPLC

The stationary phase has an ionically charged surface of opposite charge to the sample ions. This technique is used almost exclusively with ionic or ionizable samples.

The stronger the charge on the sample, the stronger it will be attracted to the ionic surface and thus, the longer it will take to elute. The mobile phase is an aqueous buffer, where both pH and ionic strength are used to control elution time.

MATERIALS AND METHODS:

Instruments And Glassware’s Model- HPLCWATERS, Alliance 2695 separation module. Software: Empower 2,996 PDA detector, pH meter- LabIndia, Weighing machine- Sartorius, Volumetric flasks- Borosil, Pipettes and Burettes- Borosil, Beakers- Borosil, Digital ultra sonicator- Labman.

HPLC METHOD DEVELOPMENT:

TRAILS

Preparation of standard solution:

Accurately weigh and transfer 10 mg of Trandolapril and Verapamil working standard into a 10ml of clean dry volumetric flasks add about 7ml of Methanol and sonicate to dissolve and removal of air completely and make volume up to the mark with the same Methanol. Further pipette 3ml of Trandolapril and 0.5ml of Verapamil from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

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.

Mobile Phase Optimization:

Initially the mobile phase tried was Methanol: Water, Acetonitrile and water with varying proportions. Finally, the mobile phase was optimized to Methanol: Acetate Buffer pH-3.8 in proportion 24:76 v/v respectively.

Optimization of Column:

The method was performed with various columns like C18 column, Symmetry and X-Bridge. Agilent Zorbax (C18) (150mm x 4.6mm, 5µm) column was found to be ideal as it gave good peak shape and resolution at 1ml/min flow.

OPTIMIZED CHROMATOGRAPHIC CONDITIONS:

Instrument used : Waters HPLC with auto sampler and PDA Detector 996 model.
Temperature : 37ºC
Column : Agilent Zorbax (C18) (150mm x 4.6mm, 5µm) column
Mobile phase : Methanol: Acetate Buffer pH-3.8 (24:76v/v)
Flow rate : 1ml/min
Wavelength : 262nm
Injection volume : 10 µl
Run time : 8 min

METHOD VALIDATION

PREPARATION OF MOBILE PHASE:

Accurately measured 240 ml (24%) of Methanol and 760 ml of Acetate Buffer (76%) a were mixed and degassed in digital ultra sonicater for 15 minutes and then filtered through 0.45 µ filter under vacuum filtration.

Diluent Preparation:

The Mobile phase was used as the diluent.

RESULTS AND DISCUSSION:

Optimized Chromatogram

Instrument used	:	Waters HPLC with auto sampler and PDA Detector 996 model.
Temperature	:	37ºC
Column	:	Agilent Zorbax (C18) (150mm x 4.6mm, 5µm) column
Mobile phase	:	Methanol: Acetate Buffer pH-3.8 (24:76v/v)
Flow rate	:	1ml/min
Wavelength	:	262nm
Injection volume	:	10 µl
Run time	:	6 min

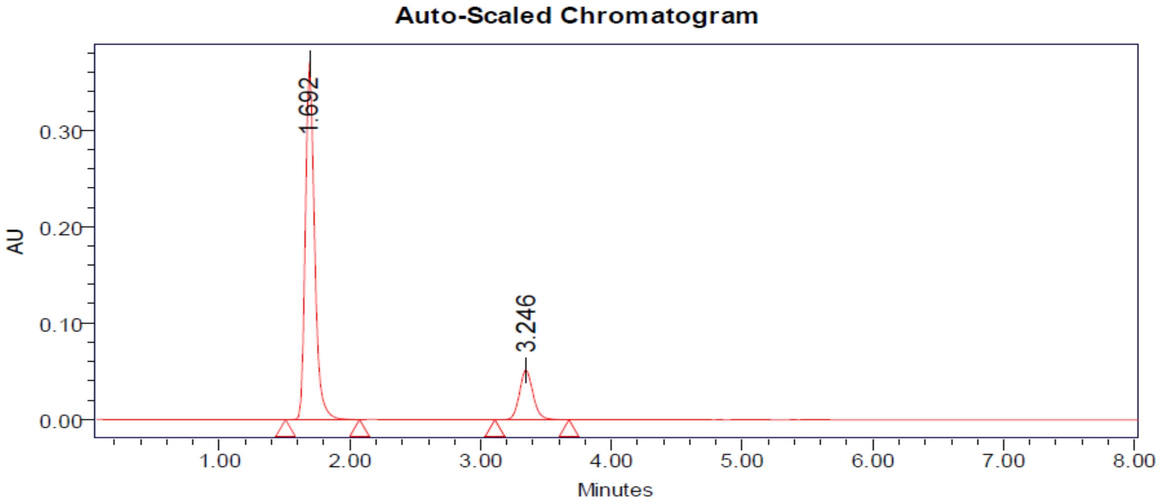

Fig 1: Optimized Chromatogram

Table 1: Observation of Optimized Chromatogram

S.No.	Peak Name	Retention Time	Area	Height	USP Tailing	USP Plate Count	USP Resolution
1	Trandolapril	1.692	1658785	385669	1.69	7586	10.85
2	Verapamil	3.246	425631	65245	1.58	6235	

Inference:

1. The Retention Time is decreased observed from chromatogram by increasing flow rate.
2. The retention time was Trandolapril and Verapamil was found to be 1.692 and 3.246 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 2: Shows Optimized Chromatographic conditions

PARAMETER	OPTIMIZED CHROMATOGRAPHIC CONDITIONS
Mobile phase :	Methanol: Acetate Buffer pH-3.8 (24:76v/v)
Column :	Agilent Zorbax (C18) (150mm x 4.6mm, 5µm) column
Flow rate :	1ml/min
Diluent	Methanol: Acetate Buffer pH-3.8 (24:76v/v)
Injection Volume	10 µl
Wavelength:	262 nm
Column temp:	37ºC
Run mode	Isocratic
Runtime	8minutes

- From the above experiment it was found that Trandolapril and Verapamil 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 262nm.
- The retention time of Trandolapril and Verapamil were found to be 1.692 and 3.246 minutes respectively.

Assay (Sample):

Observation of sample Chromatogram -1

S.No	Peak Name	Retention Time (min)	Area	USP Plate Count	USP Tailing
1	Trandolapril	1.691	1669558	7695	1.70
2	Verapamil	3.242	436589	6359	1.61

Observation of sample Chromatogram -2

S.No	Peak Name	Retention Time (min)	Area	USP Plate Count	USP Tailing
1	Trandolapril	1.694	1678598	7698	1.72
2	Verapamil	3.240	436985	6557	1.59

Observation of sample Chromatogram -3

S.No	Peak Name	Retention Time (min)	Area	USP Plate Count	USP Tailing
1	Trandolapril	1.694	1668985	7659	1.72
2	Verapamil	3.234	436598	6347	1.61

%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 Trandolapril and Verapamil is 98-102%.

Table 3: Shown Assay Result

Label claim	% purity
Trandolapril and Verapamil	99.86%

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

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

TRANDOLAPRIL

Table 4: Accuracy Observation of Trandolapril

%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	

VERAPAMIL

Table 5: Accuracy Observation of Verapamil

%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 Trandolapril and Verapamil at 50%, 100% and 150% the limits of % recovery should be in range of 98-102%.

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

The accuracy studies showed % recovery of the Trandolapril 100.112%- and Verapamil 100.16%.

The limits of % recovery of drugs were 98-102 % 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:

Observation of System Precision

S. No	Sample Area 1	Sample Area 2
1	1658254	426598
2	1658952	426589
3	1654857	426985
4	1659854	426587
5	1653298	426515
Mean	**1657043**	**426654.8**
Std.dev	**2820.29**	**187.5692**
%RSD	**0.1702**	**0.043963**

Acceptance Criteria:

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

For precision studies 5 replicated injections of Trandolapril and Verapamil formulation was performed. %RSD was determined for peak areas of Trandolapril and Verapamil.

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: 29-33 results were reported in Table: 35

Observation of Robustness Day 1

S. No.	Sample Area 1	Sample Area 2
1	1665985	436598
2	1662598	436855
3	1668484	436598
4	1664598	436587
5	1663579	436741
6	1664587	432659
Mean	**1664972**	**436006.3**
Std. Dev.	**2060.327**	**1643.285**
% RSD	**0.123745**	**0.376895**

Acceptance Criteria:

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

Observation of robustness Day 2

S. No.	Sample Area 1	Sample Area 2
1	1648598	415985
2	1642587	415267
3	1649852	415986
4	1648754	415265
5	1645289	415874
6	1647581	415632
Mean	**1647110**	**415668.2**
Std. Dev.	**2699.291**	**337.2106**
% RSD	**0.16388**	**0.081125**

Acceptance Criteria:

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

LINEARITY:

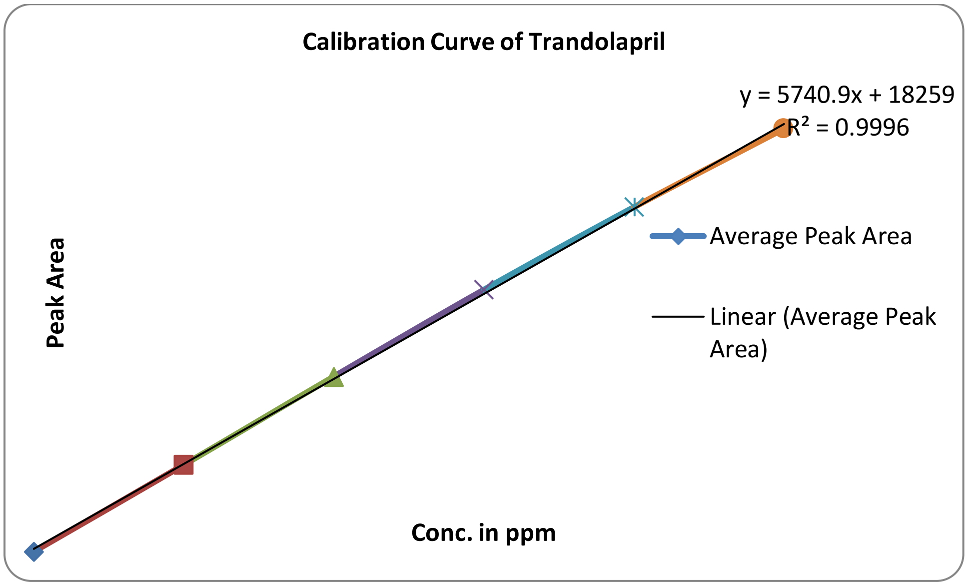

Fig 2: Calibration Curve for Trandolapril

Table 6: Linearity Observation of Trandolapril

S. No	Concentration Level (%)	Concentration µg/ml	Average Peak Area
1.	I	100	585985
2.	II	200	1182468
3.	III	300	1768785
4.	IV	400	2326852
5.	V	500	2856874
Correlation coefficient			0.999

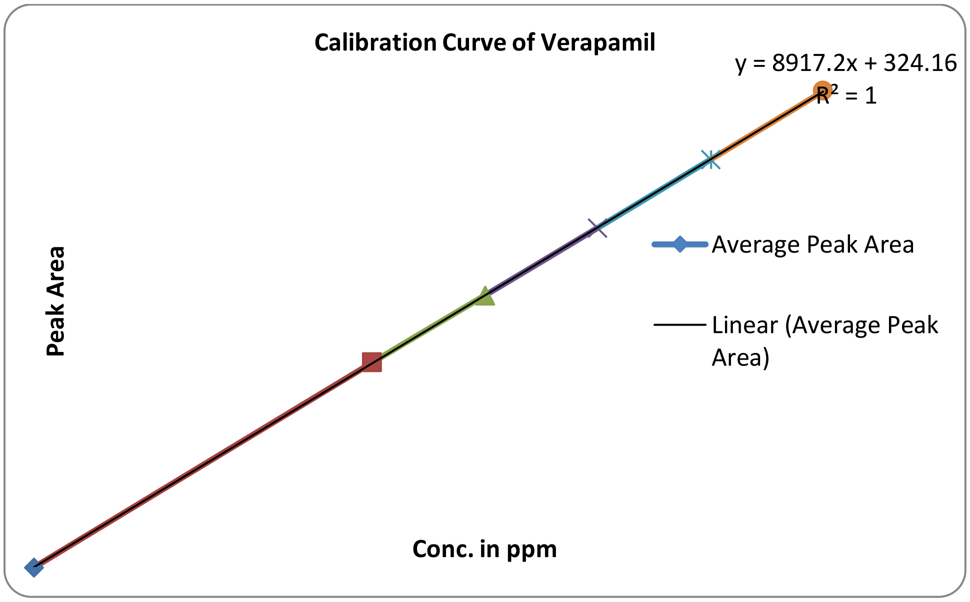

Fig 3: Calibration Curve for Verapamil

Table 7: Linearity Observation of Verapamil

S. No.	Concentration Level (%)	Concentration µg/ml	Average Peak Area
1	I	30	268764
2	II	40	356958
3	III	50	445631
4	IV	60	535186
5	V	70	624698
Correlation coefficient			0.999

The linearity range was found to be 100-500 and 30-70µg/ml for both Trandolapril and Verapamil 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.

ROBUSTNESS

System suitability Results for Trandolapril

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 Verapamil

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:

Table 8: System suitability results Trandolapril

Organic phase		System suitability Results		
		USP Plate	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 9: System suitability result Verapamil

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.

CONCLUSION:

High performance liquid chromatography is at present one of the most sophisticated tool of the analysis. The estimation of Trandolapril and Verapamil was done by RP-HPLC. The separation was optimized with mobile phase consists of Methanol: acetate buffer (pH-3.8) mixed in the ratio of 24:76%v/v. An Agilent Zorbax (C18) (150mm x 4.6mm, 5µm) column or equivalent chemically bonded to porous silica particles were used as stationary phase. The solutions were chromatographed at a constant flow rate of 1.0 ml/min. The linearity range of Trandolapril and Verapamil were found to be from 100-500µg/ml, 30-70µg/ml respectively. Linear regression coefficient was not more than 0.999, 0.999.

The values of % RSD are less than 2% indicating accuracy and precision of the method. The percentage recovery varies from 98-102% of Trandolapril and Verapamil. LOD and LOQ were found to be within limits.

The results obtained on the validation parameters met ICH and USP requirements. It inferred the method found to be simple, accurate, precise and linear. The method was found to be having suitable application in routine laboratory analysis with high degree of accuracy and precision.

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