VERAPAMIL HCL – ANALYTICAL METHOD DEVELOPMENT AND VALIDATION

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Abstract: *Verapamil is a calcium channel blocker. Verapamil is used for the treatment of high blood pressure and for the control of angina. In the present paper the authors have reviewed the analytical methods published in the literature for the estimation of Verapamil in pharmaceutical formulations and in biological samples.*

Keywords: *Verapamil; Calcium Channel Blocker; Analytical Techniques*

INTRODUCTION

Verapamil is a calcium channel blocker with anti-anginal, antihypertensive and antiarrhythmic activities Verapamil belongs to non-dihydro pyridine class of calcium channel blockers and it is administered as racemic mixture. The S-enantiomer of Verapamil
is approximately 20 folds more potent than R-enantiomer [1,2].
Verapamil (Figure 1) has a molecular formula C27H38N2O4 and molecular weight 454.602 g/mole and is soluble in methanol, ethanol and water. The pKa value of Verapamil is 8.92

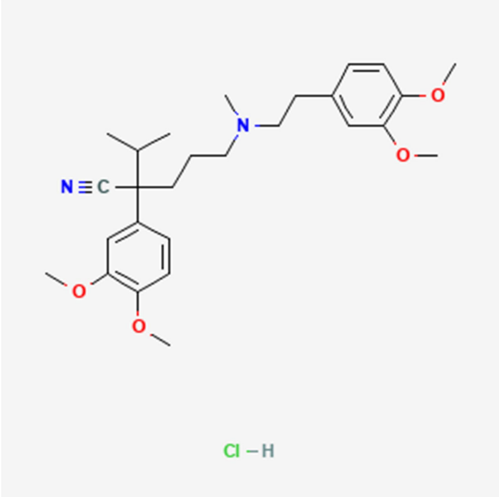

Figure 1: Chemical structure of verapamil

Literature survey revealed that few analytical methods have been reported for estimation of individually oVerapamil HCL in combination with other drugs. The reported methods are spectrophotometric, RP-HPLC and HPTLC methods. The present study was aimed to develop a simple, sensitive, rapid and precise RP-HPLC method for estimation of Verapamil HCL. The analytical method was validated according to ICH validation parameters.

Analytical method development

Verapamil

Determination of Lambda maximum

Preparation of stock solution of Verapamil

Verapamil (100 mg) in a 100mL volumetric flask and 25 mL of Acetonitrile to it and it was vortexed (Eltek) for 2 minutes. This was the main stock accounting for concentrations of 1000 µg/mL. A diluted solution was used to scan in UV-Spectrophotometer in the range of 200-400nm, taking methanol as blank. The lambda maximum for Verapamil was found to be 278.00 nm.

Instrumentation and Chromatographic Conditions

HPLC system used was JASCO system equipped with model PU 4180 RHPLC pump, Rheodyne sample injection port (20 µl), JASCO UV-4075 UV-VIS detector and ChromNAV CFR chromatography software (version 2.0). Separation was carried out on HiQSil C18 (250 mm × 4.6 mm, 5 µm) column using Acetonitrile: Potassium dihydrogen Phosphate buffer in the ratio of 70:30 V/V as mobile phase at flow rate of 1.0 mL/min. Samples were injected using Rheodyne injector with 20µL loop, Detection was carried out at 246nm. All weighing were done on Shimadzu balance (Model AY-120)

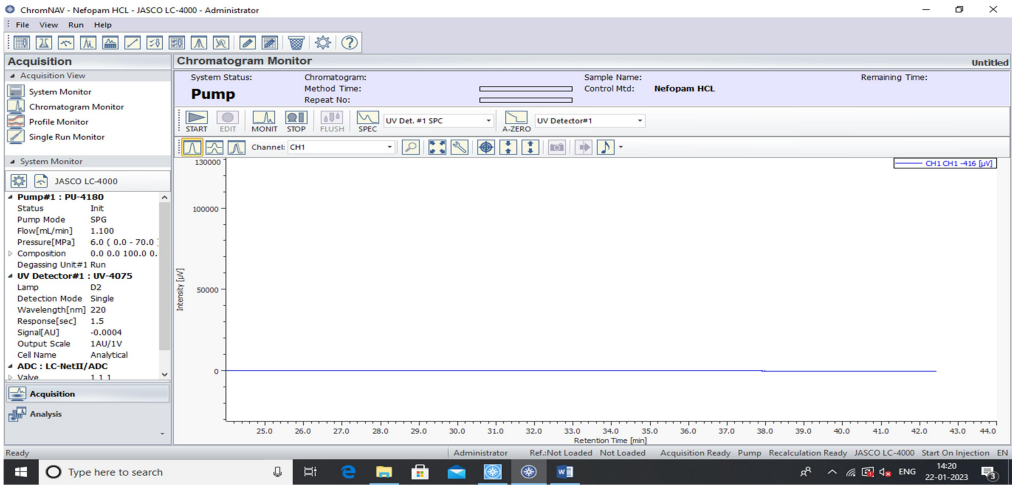

Figure 2: HPLC chromatogram of blank.

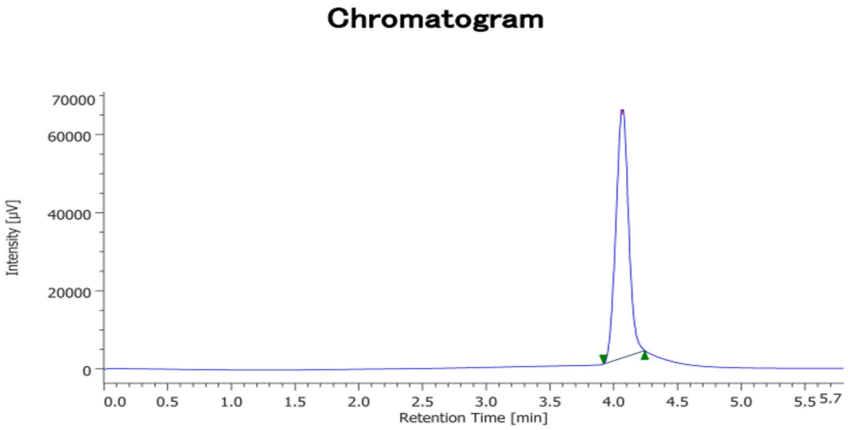

Figure 3: HPLC chromatogram of standard Verapamil.

The retention time was found to be 4.067 with distinct peak.

MATERIALS AND METHODS

Material
Verapamil was procured as a gift sample from Chemicals utilized for method development are of HPLC grade includes Acetonitrile, phosphate buffer were purchased from Merck (India) Ltd.

Preparation of mobile phase
The preparation of mobile phase was done by mixing Acetonitrile: Potassium dihydrogen Phosphate buffer in the ratio of 70:30 V/V. Removal of gases was carried out in ultrasonic water bath for 15 minutes. Filtered the solution through 0.45µ filter.

Diluent preparation
Mobile phase used as diluents.

Preparation of standard stock solution
100mg of Verapamil standard was transferred into 100ml volumetric flask, dissolved & make up to volume with mobile phase. Further dilution was done by transferring 1 ml of the above solution into a 10mlvolumetric flask and make up to volume with mobile phase and performed the subsequent dilutions.

Preparation of test solution
100mg equivalent of Verapamil liposomes was transferred into 100ml volumetric flask, dissolved & make up to volume with mobile phase. Further dilution was done by transferring 1 ml of the above solution into a 10ml volumetric flask and make up to volume with mobile phase and performed the subsequent dilutions.

Selection of analytical wavelength
It is the characteristic of a compound which helps to provide the electronic structure of the compound or analyte. The structural analysis of Verapamil was carried out under UV ranging from 200-400nm using the standard solution.

METHODOLOGY

The optimization of chromatographic conditions was carried out on Agilent zorbax eclipse XDB C18 (4.6 x 250 mm, 5µm) column. The separation was done by utilizing Acetonitrile: Potassium dihydrogen Phosphate buffer in the ratio of 70:30 V/V, the volume of sample was 20µl. The flow rate was maintained at 1.0 ml/min. The detection of drug Verapamil was done at 246nm.

Method Validation
Linearity
The linearity of the developed method was studied over the concentration ranges between 10- 30µg/ml. The aliquots of 10, 15, 20, 25 and 30µg/ml were prepared by diluting standard stock solution of 0.2, 0.3, 0.4, 0.5 and 0.6 ml with mobile phase. The obtained concentrations were injected into the chromatographic system. Calibration curve of Verapamil was constructed by plotting peak area versus used concentration of Verapamil. To assure the concentration range studied is linear the regression equation and correlation coefficient were evaluated.

Accuracy
Accuracy was carried out by % recovery studies at three different concentration levels. To the pre-analysed sample solution of Verapamil, a known amount of standard drug powder of Verapamil was added to 80, 100, 120% level.

Precision method
By studying the changes in the inter-day and intra-day determined the precision of the method. In the intra-day studies, six repeated injections of standard solution was made and % RSD were calculated. In the inter-day variation studies, six repeated injections of standard solution were made for six consecutive days and %RSD were calculated.

Limit of Detection and Limit of Quantitation
Based on the standard deviation of response of the calibration curve the LOD and LOQ of the drug was determined separately.

Robustness
Robustness of the method was tested by small but deliberate variations of flow rate, mobile phase composition and wavelength.

RESULTS AND DISCUSSION

Selection of wavelength maxima
The solution of Verapamil was scanned between ranges 200- 400nm. UV spectra of the drug show maximum absorbance at 278nm.

Method development
The proposed chromatographic method was found to be suitable for effective separation of Verapamil with good resolution, peak shape given in the figure. The mobile phase composed of Acetonitrile: Potassium dihydrogen Phosphate buffer in the ratio of 70:30 V/V, at a flow rate of 1.0 ml/min was selected as it gave well resolved peaks of standard Verapamil. The optimum wavelength 246nm selected for detection and quantitation.

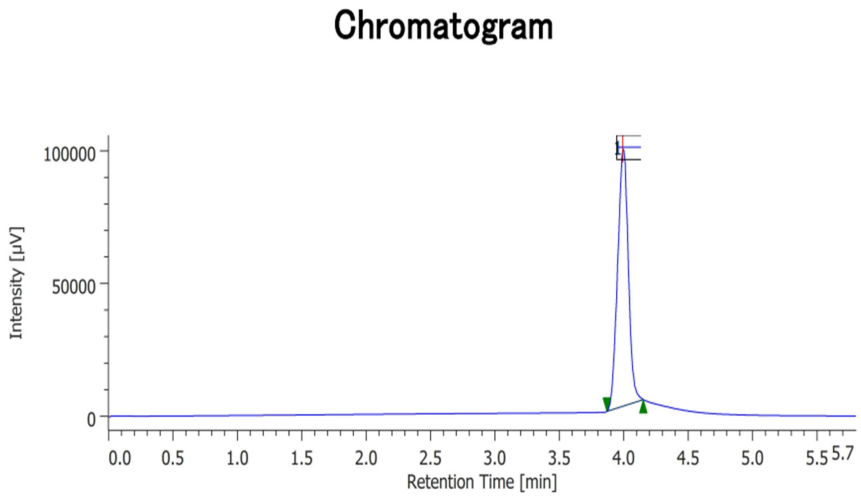

Figure 4: HPLC Chromatogram with resolved peak of Verapamil

Method validation
Linearity
The calibration curves were found be linear for the concentration range of 10-30ppm. The standard working curve equation for drug was found to be y = 2199.5x - 10481 with correlation coefficient value r^2 = 0.9987. The results of linearity are given in Table and Figure.

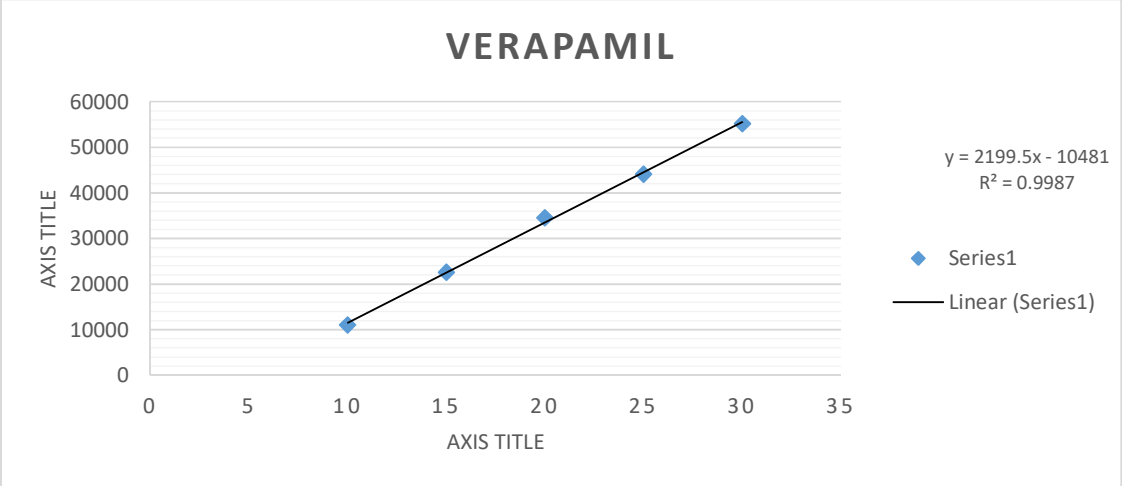

Figure 5: Linearity curve of standard Verapamil

Table 1: Linearity data of Verapamil

Concentration µg/mL	Area
10	11023
15	22589
20	34557
25	44152
30	55230

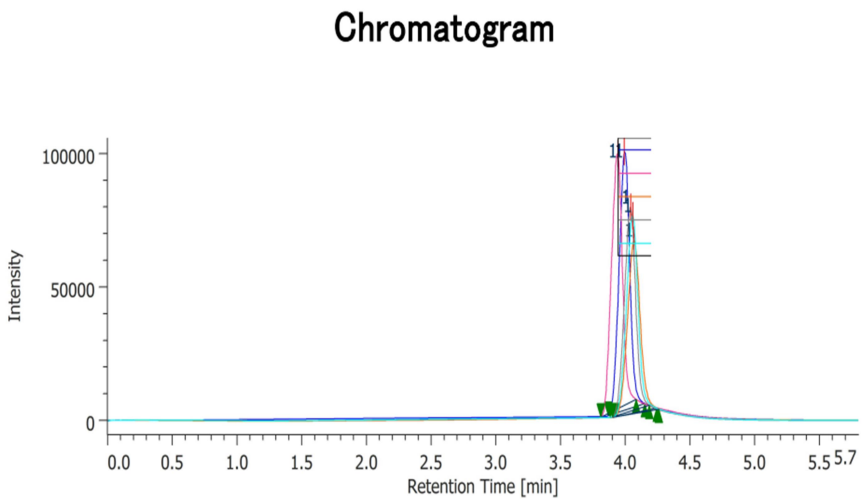

Figure 6: Overlay Graph of Verapamil- (10-30µg/mL)

Recovery studies

The mean % recovery at 80, 100, 120 % of the test concentration along with its statistical validation for drug Verapamil given in Table. The % recovery at 80, 100, and 120 % is given below. It was confirmed that the developed method was accurate as the percent recovery was in the range of 100%.

Table 2: Recovery data of Verapamil

Level (%)	Drug Conc (mg)	Amt recovered (mg)	% Recovery
100%	10	9.8	98.56
150%	15	19.85	99.85
200%	20	19.80	99.80

Precision

The repeatability of sample application and measurement of peak area were expressed in terms of % RSD and was found to be less than 2.0%. The % RSD of intra-day precision is given below. The results of precision studies are shown in Table.

Table 3: Precision study (intra- day) of Verapamil

Conc µg/mL	Area	AVG	SD	%RSD
10	11457	11516.333	53.8175932	0.46731535
	11562			
	11530			
15	22635	22221.667	359.212101	1.61649487
	21985			
	22045			
20	34559	34380.667	319.331072	0.92881
	34012			
	34571			

Conc, Concentration; AVG, average; SD, Standard deviation; RSD, Relative standard deviation

Table 4: Precision study (inter-day) of Verapamil

Conc µg/mL	Area	AVG	SD	%RSD
10	12459	11766.667	601.785953	5.11432821
	11472			
	11369			
15	22356	22367	225.70113	1.00908092
	22147			
	22598			
20	35601	35099.333	437.045001	1.2451661
	34896			
	34801			

Conc, Concentration; AVG, average; SD, Standard deviation; RSD, Relative standard deviation

Limit of Detection (LOD) and Limit of Quantification (LOQ)

This data showed that the sensitivity of method to determine the drug Verapamil. The Minimum concentration level at which the analyte can be reliable detected (LOD) & quantified (LOQ) were found to be 0.54 & 1.15 µg/m/ respectively.

Robustness

Robustness of method was measured by multiple injections of a homogenous sample containing Verapamil by changing flow rate 0.9 mL/min and 1.1 mL/min, mobile phase composition Acetonitrile: Potassium dihydrogen Phosphate buffer in the ratio of 69:31 and 71:29, wavelength i.e 245nm and 247nm. The method was found to be robust in the range of deliberate changes made.

Table 5: Robustness study with change in flow rate of Verapamil

Flow rate mL/min	Conc µg/mL	Area	AVG	%RSD
0.9	20	35412	35404	0.84194
0.9		35698		
0.9		35102		
1.1	20	35741	35704.67	0.77384
1.1		35961		
1.1		35412		

Conc, Concentration; AVG, average; SD, Standard deviation; RSD, Relative standard deviation

Table 6: Robustness study with change in concentration of mobile phase of Verapamil

Mobile phase (Methanol: 01% OPA)	Conc µg/mL	Area	AVG	%RSD
69:31	20	34512	34917.67	1.91635
69:31		35690		
69:31		34551		
71:29	20	35621	35207.33	1.54966
71:29		35412		
71:29		34589		

Conc, Concentration; AVG, average; SD, Standard deviation; RSD, Relative standard deviation

Table 7: Robustness study with change in Wavelength of Verapamil.

Wavelength nm	Conc µg/mL	Area	AVG	%RSD
245	20	35102	35170	1.20307
245		35623		
245		34785		
247	20	35698	35226	1.16418
247		34957		
247		35023		

CONCLUSION

In the present study, an attempt was made to develop a simple, accurate, selective and sensitive HPLC method of Rivaroxaban in pharmaceutical analysis. This method was validated for selectivity, accuracy, linearity, precision (inter-day and intra-day), sensitivity, robustness and ruggedness in accordance with ICH guidelines. A simple mobile phase without preparation of any buffer solution and a short run time are advantageous and make this method suitable for routine analysis of large number of samples per day.

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