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CONVENTIONAL AND CONTEMPORARY PERSPECTIVE FOR COMPUTATION OF RANOLAZINE

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ABSTRACT

The current study was focused on giving a simple, accurate and rapid validated analytical method for the estimation of Ranolazine using different analytical methods which is designed to utilize UV-spectroscopy (Conventional) and HPTLC (Contemporary) techniques. Utilizing automatic **HPTLC** (**Aetron**) with **'Spraylin'** software and the equipment which consists of separate sample applicator (**Hamilton** microlitre-100 µL syringe) and separate densitometer (spot detector at 270 nm and 365 nm) can be said as a major asset of this research work. The technique also consists of other software **'JUST TLC'** for the calculation of band width and area of the sample spots developed. Uv-Spectroscopy (**Labindia**) with **'Uv-Win 5'** software was also utilized for the same study on Ranolazine. Buffer has been used as a major diluent in both given methods in sample preparation process where the methods were proved falling under green chemistry, eco-friendly and economical methods. The Calibration plot shown best regression over the concentration range of 10-50 µg/ml which provides the proof that the method is linear and sensitive in estimating lower quantity of the sample. The sample application was done on Silica Gel 60 F_{254} plates and developed using mobile phase Toluene, Ethyl Acetate and Methanol in the ratio of 6: 2: 2 v/v. The developed method was validated as per ICH Q2 (R1) guidelines. System suitability, precision, LOD, LOQ were performed, calculated and reported and were found to be within specified limits. The recovery studies were performed and amount recovered was found between 98.20-102.20%.

Keywords: Ranolazine, HPTLC, UV, method development, Validation

INTRODUCTION

Angina, also known as angina pectoris, is chest pain or pressure, a symptom of coronary heart disease, usually due to insufficient blood flow to the heart muscle (myocardium). Angina is usually due to obstruction or spasm of the arteries that supply blood to the heart muscle. Other causes include anemia, abnormal heart rhythms, and heart failure. The main mechanism of coronary artery obstruction is atherosclerosis as part of coronary artery disease **[1]**.

Ranolazine, sold under the brand name Ranexa among others, is a medication used to treat heart related chest pain **[1]**. Typically it is used together with other medications when those are insufficient **[1, 2]**. It is taken by mouth. Ranolazine was approved for medical use in the United States in 2006 **[1]**. In 2017, it was the 273rd most commonly prescribed medication in the United States, with more than one million prescriptions **[3, 4]**. Chemical structure of the Ranolazine was shown in **Figure 1**.

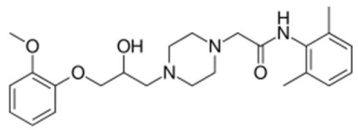

Figure 1: Chemical structure of Ranolazine

EXPERIMENTAL RESOURCES

Chemical Resources

Ranolazine working standard was procured from Biocon., India. Ortho phosphoric acid (OPA), disodium hydrogen ortho phosphate were purchased from LOBA chemical laboratories Pvt. Ltd., HPLC grade water, Toluene, Ethyl Acetate and Methanol were purchased from Thermo Fisher Scientific Pvt. Ltd., India.

Instrument Resources

UV-Spectroscopy and pH meter (Labindia), High performance thin layer chromatography (HPTLC-Aetron)) micro balance (Teraoka Pvt. Ltd), variable range micro pipettes (Cyberpet pro, ANM Amkette Industries), variable size glass bottles, graduated measuring cylinders, volumetric flasks (Borosil), ultrasonic water bath (LOBA Chem Pvt. Ltd., Mumbai), vortexer (Remi equipment Pvt. Ltd.,), refrigerator (Godrej). Pipette tips 10 µL-1000 µL and variable size surgical gloves (Surgicare) were used to complete the research work.

PREPARATION OF SOLUTIONS

Preparation of 0.2 M Disodium hydrogen orthophosphate buffer:

2.85 gms of Disodium hydrogen orthophosphate salt was taken into 100ml volumetric flask and dissolved in 50 ml of HPLC grade water, vertexed, sonicated, filtered and then made up to the mark with HPLC grade water to get 0.2M Disodium hydrogen orthophosphate buffer. pH was adjusted to 6.0 using 0.1% ortho phosphoric acid.

Preparation of diluent:

0.2 M Disodium hydrogen orthophosphate buffer and methanol were used in the ratio of 60:40 v/v, mixed well, sonicated, degassed and filtered via 0.45µ filter using ultra vacuum filtration.

Preparation of standard stock solution:

10 mg of Ranolazine was weighed and transferred into 100 ml of volumetric flask and dissolved in 50 ml of methanol. The resultant solution was vertexed, sonicated, filtered and then made up to the mark with methanol to obtain 100 µg/ml solutions. Further dilutions were made from the stock solution by using diluent to get the concentration solutions from 10-50 µg/ml for both UV-Spectrophotometric and HPTLC methods.

DEVELOPMENT OF SPECTROMETRIC AND CHROMATOGRAPHIC METHODS

In the present investigation Uv-spectroscopic and High performance thin layer chromatography methods were developed, validated and reported. Mixture buffer of methanol was used as diluent and λ_{max} was found as 270 nm. As well as HPTLC method also was developed on Aetron system with Spraylin TLC software and bands were quantified by utilizing JUST TLC software. Twin trough glass chamber (10 cm × 10 cm) was used for silica plate development after application of samples. Hamilton Syringe (100 µL) and densitometer (fluorescence detector at 270 nm and 365 nm) were equipped for application and detection of sample concentration as well as area. Mobile phase consisting of toluene, ethyl acetate and methanol in the ratio of (6:2:2 v/v) was finalized after placing number of trials so it was used for the validation purpose. Silica Gel 60 F_{254} kind of TLC plates were used to fulfill the purpose. 8mm band was applied with 10 mm band space with ascending order of application at a rate of 5 sec µl-1.

METHODOLOGY

The developed method was validated according to ICH Q2 (R1) guidelines for the parameters of specificity, linearity, limit of detection (LOD), limit of quantification (LOQ), precision, robustness and accuracy. The above parameters were discussed below in detail along with data **[5-10]**.

Specificity:

Specificity is the ability of the analytical method to produce a response for the analyte in the presence of other components present in the solution; technically they can be like impurities, degradants or matrix. In this method the specificity is tested for the standard solution and blank and found no interference in the blank injection. Tailing factor and theoretical plates were taken into consideration.

Linearity:

The linearity is validation parameter which confirms the ability of a method (within a

given range) to obtain test results which will be directly proportional to the concentration of analyte in the sample. By giving different concentrations of sample solutions the regression value should be 0.999.

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. The values were determined by calculating from slope and regression line by following the equation.

$LOD = 3.3* \sigma / S$

Limit of Quantification (LOQ):

LOQ is the parameter which will explain about the detection and quantification of lowest amount. In the method the values of LOQ was determined from the following formula.

$LOQ = 10* \sigma / S$

Precision:

Precision is an analytical procedure expresses the closeness of agreement between a series of measurement obtained from multiple sampling of the same homogeneous sample under prescribed conditions. In the current study the % RSD for the sample solution was found below <2.0.

Accuracy:

Accuracy can be defined as the closeness of agreement between accepted reference value and the value found. In this study recovery was calculated by standard weighing method for 50%, 100% and 150%.

Robustness:

A robustness method was performed to confirm whether the method is capable of reproducibility during the deliberate changes taken place in the proposed method.

RESULTS AND DISCUSSION

The current investigation of estimation of Ranolazine by UV and HPTLC was validated as per International Conference on Harmonization ICH Q2 (R1) guidelines. By following the guidelines the parameters of precision, accuracy, LOD, LOQ and linearity were validated and recorded for the data **[9, 10]**. The linear concentration range for UV-Spectroscopy and for HPTLC was found between 10-50 µg/ml. The values were calculated for obtained real time data and % RSD for precision was found to be within limits (%RSD<2%). Recovery studies performed and amount recovered was found between 98.20-102.20%. Results are given in the following **Figures 2-4** and **Table 1**.

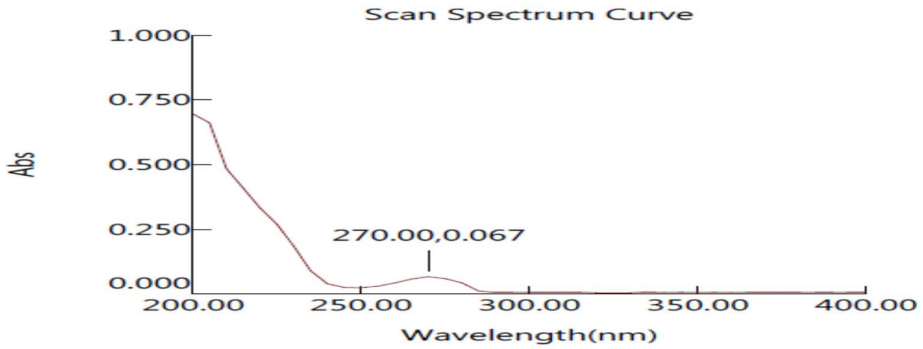

Figure 2: UV spectra of Ranolazine

Figure 3: Densitogram of Ranolazine

Graph

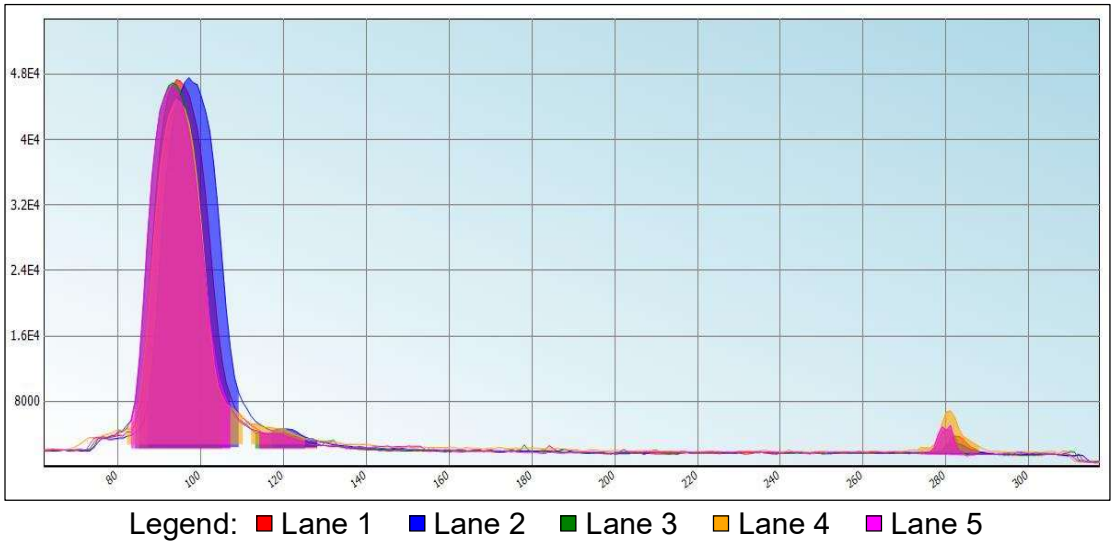

Figure 4: HPTLC Graph for Ranolazine

Table 1: Validation Results for determination of Ranolazine by UV and HPTLC methods

Validation data of reported methods		
Parameters		**UV Spectrophotometric method**
Linearity range		**10-50 µg/ml**
Regression equation		**y = 0.0069x - 0.0009**
Correlation coefficient		**0.9996**
Precision (%RSD)		**1.36**
LOD		**0.43**
LOQ		**1.30**
%Recovery	**50%**	**100.27**
	100%	**100.76**
	150%	**100.27**
Parameters		**HPTLC method**
Correlation coefficient		**0.9991**
Linearity		**10-50 µg/ml**
Precision (% RSD)		**0.92**
LOD		**1.45**
LOQ		**4.40**
%Recovery	**50%**	**99.96**
	100%	**100.10**
	150%	**100.04**
Specificity		**Method is Specific**
Robustness		**Method is robust**
Rf		**0.84±0.03**
SD=standard deviation, RSD=relative standard deviation, y=concentration of Ranolazine in µg/mL x= absorbance of Ranolazine.		

CONCLUSION

New and simple standard UV, HPTLC methods were given for the estimation of Ranolazine in the current study. As there is no official compendial method available for Ranolazine this proved, proposed method can be utilized for the routine analysis which is applicable for industrial and academic purposes. By giving all the real time data and its validated values it was proved that the proposed method is more sensitive, precise, accurate, cost effective and rapid one and suitable for commercial estimation of Ranolazine. Buffer mixture has been used as a diluent throughout entire study in the both methods so that it can be said that the method is simple to perform and eco-friendly.

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