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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF CLARITHROMYCIN BY RP-HPLC METHOD

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ABSTRACT

A novel very rapid, sensitive, reverse phase High Performance Liquid Chromatography (RP-HPLC) technique was developed for the quantitative estimation of Clarithromycin in bulk and tablet dosage form.It was resolved by using a mobile phase methanol: Buffer in the ratio (50:50 v/v) at a flow rate of 1.0 mL/min. using UV - Visible detector at the wavelength of 243 nm for quantification. Efficient separation was achieved for Clarithromycin on used Waters Acquity HSS C_{18} (100 × 2.1 mm, 1.7µm). The retention time Clarithromycin of was 2.754 min. The calibration graphs were linear and the method showed excellent recovery for tablet dosage form. The developed method was validated according to the International Conference on Harmonization (ICH) guidelines with respect to linearity, accuracy, precision, specificity and robustness.

KEY WORDS: CLARITHROMYCIN, HPLC, new method development, validation

1. INTRODUCTION

Clarithromycin is primarily used to treat a number of bacterial infections including pneumonia, Helicobacter pylori, and as an alternative to penicillin in strep throat.[1] Other uses include cat scratch disease and other infections due to bartonella, cryptosporidiosis, as a second line agent in Lyme disease and toxoplasmosis.[1] It

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may also be used to prevent bacterial endocarditis in those who cannot take penicillin.[1] It is effective against upper and lower respiratory tract infections, skin and soft tissue infections and helicobacter pylori infections associated with duodenal ulcers.

Structure of drug

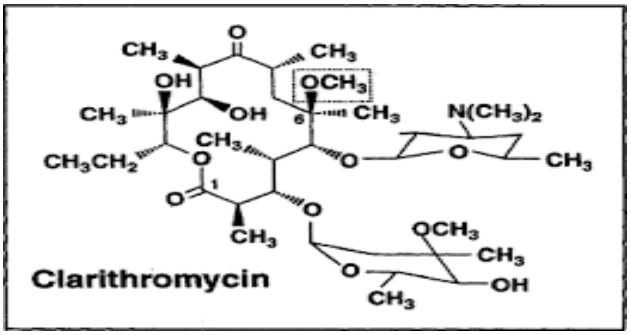

Figure 1: Structure of Clarithromycin

2. EXPERIMENTAL DETAILS

2.1 Materials and Reagents: ClarithromycinWorking Standard was procured from Aurobindo laboratories, Hyderabad, India. Commercially Clarithromycin available purchased from local pharmacy. Methanol HPLC Grade grade water were obtained from Merck chemicals, Mumbai. Water was prepared by using Millipore Milli Q Plus water purification system.

2.2 Chromatographic conditions: Chromatography separation was performed on LC Solution HPLC with UV detector. The output signal was monitored and processed using Empower 2 software. The chromatographic column used Waters Acquity HSS C_{18} (100 × 2.1 mm, 1.7µm). The mobile phase of methanol: buffer in the ratio (50:50 v/v) at a flow rate of 1.0 mL/min. The

detection was monitored at the Wavelength of at 243nm nm. The injection volume was 20.0 µL and the chromatographic runtime of 6 min was used.

2.3 Preparation of solutions

2.3.1 Preparation of Phosphate buffer:

2.3.2 Preparation of mobile phase: Mixed a mixture of above buffer 500mL (50%) and 500 mL of methanol (50%) and degas in ultrasonic water bath for 5 minutes. Filter through 0.45 µ filter under vacuum filtration.

2.4 Preparation of the Clarithromycin Standard and sample Solution:

2.4.1 Standard Solution Preparation:. Accurately transferred 10mg of Clarithromycin working standard into a 10 mL volumetric flask and about 7 mL of diluent added then sonicated to dissolve it completely and the volume was made up to the mark with the same solvent(Stock solution). Further pipetted 0.5 mL of the above stock solution into a 10mL volumetric flask and diluted up to the mark with diluent. Mix well and filter through 0.45µm filter

2.4.2 Sample Solution Preparation: Accurately transferred the sample equivalent to 10 mg of Clarithromycin into a 10 mL volumetric flask. About 7 mL of diluent added and sonicated to dissolve it completely and the volume is made up to the mark with diluent. Mixed well and filtered through 0.45µm filter. Further pipetted 5 mL of the above stock solution into a 50mL volumetric flask and diluted up to the mark with diluent. Mix well and filter through 0.45µm filter.Further pipetted 3 mL of the above stock solution into a 10mL volumetric flask and dilute up to the mark with diluent. Mix well and filter through 0.45µm filter.

2.5 Method validation

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2.5.1 Precision: The precision of the method was evaluated by carrying out six independent asses of test sample against a qualified reference standard and the %RSD of assay was calculated (% RSD should not be more than 2%).

2.5.2 Intermediate Precision/Ruggedness:

2.5.2.1 Intra-day precision: The precision of the assay method was evaluated by carrying out six independent assays Clarithromycin (50,100, 150% i.e. 5.0, 10.0, 15.0µg/mL.) test samples against qualified reference standard. The percentage of RSD of six assay values was calculated.

2.5.2.2 Intermediate precision (inter-day): Different analyst from the same laboratory and by using different column of same brand evaluated the intermediate precision of the method. This was performed by assaying the six samples of Clarithromycin against qualified reference standard. The percentage of RSD of six assay values was calculated. The %RSD for the area of six replicate injections was found to be within the specified limits (% RSD should not be more than 2%).

2.5.3 Accuracy: Recovery of the assay method for Clarithromycin was established by three determinations of test sample using tablets at 50%, 100% and 150% of analyte concentration. Each solution was injected thrice (n=3) into HPLC system and the average peak area was calculated from which Percentage recoveries were calculated. (% Recovery should be between 98.0 to 102.0%).

2.5.4 Linearity: Test solutions were prepared from stock solution at 5 concentration levels (10, 20, 30, 40 and 50 µm/mL). The peak area vs. concentration data treated by least square linear regression analysis. (Correlation coefficient should be not less than 0.999.)

2.5.5 Limit of Detection (LOD) Limit of Quantification (LOQ): LOD and LOQ for the were determined at signal to noise ratios of 3:1 and 10:1, respectively by injecting series of dilute solutions with known concentrations

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2.5.6 Robustness: To prove the reliability of the analytical method during normal usage, some small but deliberate changes were made in the analytical method (e.g., flow rate, column temperature, and mobile phase composition). Changes in the chromatographic parameters (i.e., theoretical plates and the tailing factor) were evaluated for the studies.

3. RESULTS

3.1 Method development: Different chromatographic conditions were experimented to achieve better efficiency of the chromatographic system. Parameters such as mobile phase composition, wavelength of detection, column, column temperature, pH of mobile phase, and diluents were optimized. Several proportions of buffer, and solvents (water, Phosphate buffer and acetonitrile) were evaluated in order to obtain suitable composition of the mobile phase. Choice of retention time, tailing, theoretical plates, and run time were the major tasks while developing the method. At 50:50 (buffer:methanol) ratio of the mobile phase, a perfect peak was eluted. Thus the mobile phase ratio was fixed at50:50 (buffer: ACN) in an isocratic mobile phase flow rate. The typical chromatogram obtained for from final HPLC conditions are depicted in Figure2.

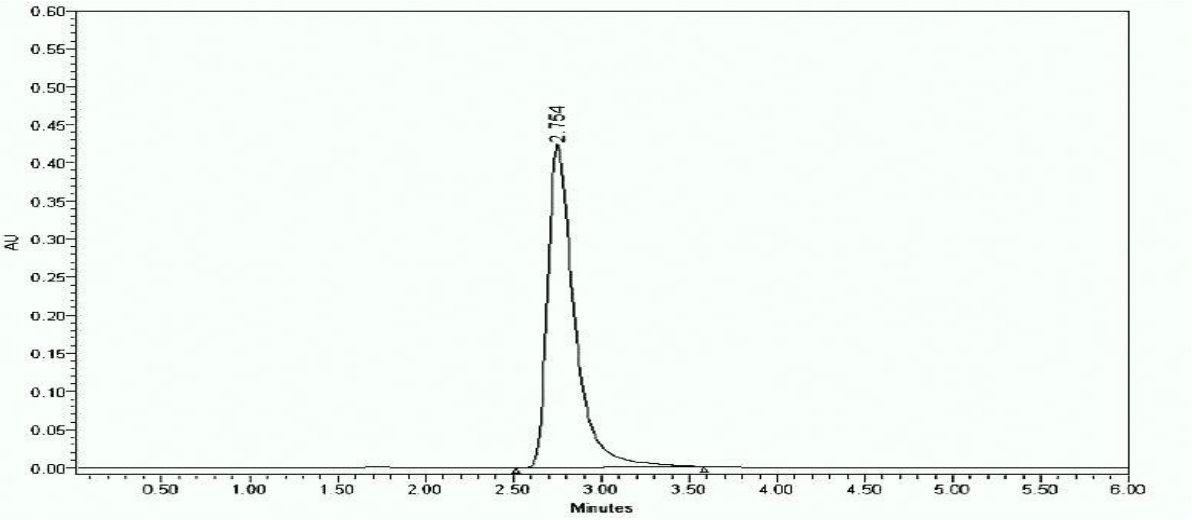

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Figure 2: typical chromatogram of Clarithromycinby proposed method

3.2 Method validation: Based on International Conference on Harmonization (ICH) guidelines, the method is validated with regard to system suitability, linearity, accuracy, precision, LOD, LOQ , robustness and sensitivity as follows.

3.2.1 System suitability: The system suitability results for the proposed HPLC method are Tailing factor Obtained from the standard injection is 1.4.Theoretical Plates Obtained from the standard injection is 7582.4. The results proved that the optimized HPLC method fulfils these requirements within the USP accepted limits indicated in the 'Experimental' section.

3.2.2 Precision: The % R.S.D. of Albandazoleassay during the method precision was found to be 0.45%, indicating good precision of the method. The results are summarized in table 1.

Table 1- Results of precision

Injection	**Area**
Injection-1	4796667
Injection-2	4712916
Injection-3	4721422
Injection-4	4771493
Injection-5	4750737
Average	4750647
Standard Deviation	34749.6
%RSD	0.73%

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3.2.4 Limits of detection (LOD) and quantification (LOQ): LOD and LOQ for Clarithromycin were 0.049 and 0.15µg/ml, respectively. Since the LOQ and LOD values of Clarithromycin are achieved at a very low level, this method can be suitable for cleaning validation in the pharmaceutical industry.

3.2.5 Accuracy: Percentage recovery of Clarithromycin samples ranged from 100.0% to 101.2% and the mean recovery is 100.5%, showing the good accuracy of the method. The result is shown in Table 3.

Table 3 - Results of Accuracy

Injection	Area
Injection-1	4796667
Injection-2	4712916
Injection-3	4721422
Injection-4	4771493
Injection-5	4750737
Average	4750647
Standard Deviation	34749.6
%RSD	0.73%

3.2.6 Linearity: The linearity of the calibration plot for the method was obtained over the calibration ranges tested, i.e.10 - 50 µg/ml for three times, and the correlation coefficient

obtained was 0.999, thus indicating excellent correlation between peak areas and concentrations of the analyte.

3.2.7 Robustness: In all the deliberately varied chromatographic conditions in the concentration range for the evaluation of robustness is 20 -60 µg/ml, (n=3). It can be concluded that the variation in flow rate and the variation in 10% Organic composition do not affect the method significantly. Hence it indicates that the method is robust even by change in the flow rate ±10% and change in the Mobile phase ±10%. The results are summarized in table 4.

Table- 4- Results of Robustness

Injection	Area
Injection-1	4796667
Injection-2	4712916
Injection-3	4721422
Injection-4	4771493
Injection-5	4750737
Average	4750647
Standard Deviation	34749.6
%RSD	0.73%

3.2.7 Application of the developed method to commercial Clarithromycin tablets: When the developed method was used to analyze a commercial brand of Clarithromycin tablet formulation,

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the mean recovery of five replicates was 99.69 % with % R.S.D. of 0.45. The % recovery value indicates non-interference from the excipients present in the dosage form.

DISCUSSION:

Method development and optimization: The main aim of the developed method was to achieve separation and quantification of Clarithromycin using an isocratic mobile phase with HPLC system. Developing a HPLC method was to reduce the run time of the method and solvent consumption for routine analysis such as assay, dissolution and content uniformity during quality assurance. Detection of Clarithromycin was adequate at 249 n nm.The initial trial was conducted using HPLC and chromatographic separation was obtained on Cosmosil C_{18} (100 × 2.1 mm, 5µm).The mobile phase was buffer 500mL (50%) and 500 mL of methanol (50%) at a flow rate of 1.0 ml/min. While developing the HPLC method, basic chromatographic conditions such as the used used Waters Acquity HSS C_{18} (100 × 2.1 mm, 1.7µm) column, solvents and UV detection employed in the HPLC method were taken into account. In selecting the HPLC column, its stability at the lower pH was taken into consideration to preserve the long life of the column. Most commercial C_{18} columns are not stable at lower pH on the longer run, thus shortening their life span. Column was found to be more suitable and stable at this pH. The peak was sharp and acceptable. The flow rate also is scaled down from 2.0 to 1.0 ml/min. When these operating conditions were applied to the developed method, a satisfactory peak was achieved for Clarithromycin which eluted at around 2.754min min giving a total run time of 6 min.

4. CONCLUSION: The new, isocratic RP-HPLC method proved to be simple, linear, precise, accurate, robust, rugged and rapid. The developed method was capable of giving faster elution,

maintaining good separation more than that achieved with conventional HPLC. The short retention time of 2.754min min allows the analysis of a large number of samples in a short period of time and is therefore more cost-effective for routine analysis in the pharmaceutical industries. It is suitable for rapid and accurate quality control of Clarithromycin in tablet formulations.

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