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DEVELOPMENT AND VALIDATION OF RP-HPLC METHD FOR THE ASSAY OF CLARITHROMYCIN IN BULK AND FORMULATION

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ABSTRACT

The present work describes a simple, rapid, and reproducible reverse phase high performance liquid chromatography (RP-HPLC) method for the simultaneous estimation of Clarithromycin (CLA). The flow rate was 1.0 mL/min and the eluents were detected by UV detector at 205 nm. The retention times were found to be 2.21 and 3.73 mins, respectively. The developed method was validated according to ICH guidelines Q2 (R1) and found to be linear within the range of 75–175 µg/mL for both drugs. The developed method was applied successfully for assay of Clarithromycin in their combined in-house developed dosage forms and in vitro dissolution studies.

INTRODUCTION

High performance liquid chromatography (HPLC)

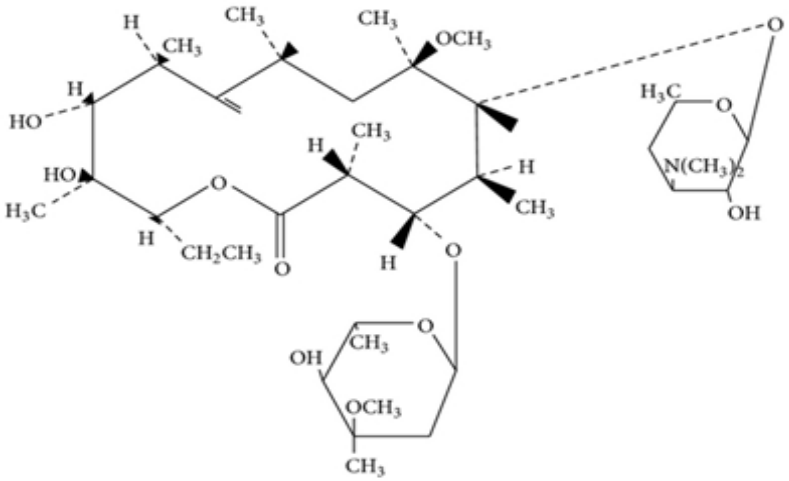
High Performance Liquid Chromatography (HPLC)[1-2] is a simple, fast, specific, precise and highly accurate analytical technique that is used for the separation and determination of organic and inorganic solutes in any samples especially biological, pharmaceutical, food, environmental, industrial etc. In HPLC, separations are achieved by partition, adsorption or ion exchange, according to the nature of the interactions between the solute and the stationary phase, which may arise from hydrogen bonding, Vander walls forces, electrostatic forces or hydrophobic forces or basing on the size of the particles.[3] Reversed phase HPLC (RP-HPLC or RPC) has a non-polar stationary phase and an aqueous, moderately polar mobile phase. One common stationary phase is silica which has been treated with RMe_2SiCl, where

R is a straight chain alkyl group such as $C_{18}H_{37}$ or C_8H_{17}. With these stationary phases, retention time is longer for molecules which are more non-polar, while polar molecules elute more readily. An investigator can increase retention time by adding more water to the mobile phase; thereby making the affinity of the hydrophobic analyte for the hydrophobic stationary phase stronger relative to the now more hydrophilic mobile phase. Similarly, an investigator can decrease retention time by adding more organic solvent to the eluent. Structural properties of the analyte molecule play an important role in its retention characteristics. In general, an analyte with a larger hydrophobic surface area (C-H, C-C, and generally non-polar atomic bonds, such as S-S and others) results in a longer retention time because it increases the molecule's non-polar surface area, which is non-interacting with the water structure. On the other hand, polar groups, such as -OH, $-NH_2$, COO^- or $-NH_3^+$ reduce retention as they are well integrated into water.

Clarithromycin is a second generation macrolide with broad spectrum of antibiotic activity. It is active against the organisms which are responsible for bacterial exacerbations of lower respiratory tract infections.[1] Clarithromycin (6- O-methyl erythromycin) is synthesized by substituting a methoxy group for the C-6 hydroxyl group of erythromycin. This substitution creates a more acid stable antimicrobial and prevents the degradation of the erythromycin base to the hemiketal intermediate. The increased acid stability of clarithromycin results in improved oral bioavailability and reduced gastrointestinal intolerance.[2] Clarithromycin is metabolized in the liver and in the stomach. Approximately 22% of an oral dose is recovered as parent compound, 18% in the urine and 4% in the faeces. Clearance of clarithromycin decreases with increasing dose, probably because of saturable hepatic metabolism. IUPAC Name: (3R,4S,5S,6R,7R,9R,11R,12R,13S,14R)-6-[(2S,3R,4S,6R)-4(dimethylamino)-3-

hydroxy-6-methyloxan-2-yl]oxy-14-ethyl-12,13-dihydroxy-4-[(2R,4R,5S,6S)-5-hydroxy-4-methoxy-4,6-dimethyloxan-2-yl]oxy-7-methoxy-3,5,7,9,11,13-hexamethyl -oxacyclotetradecane-2,10-dione Molecular Formula : $C_{38}H_{69}NO_{13}$ and Molecular weight: 747.95336 g/mol shown in Fig 1.1.

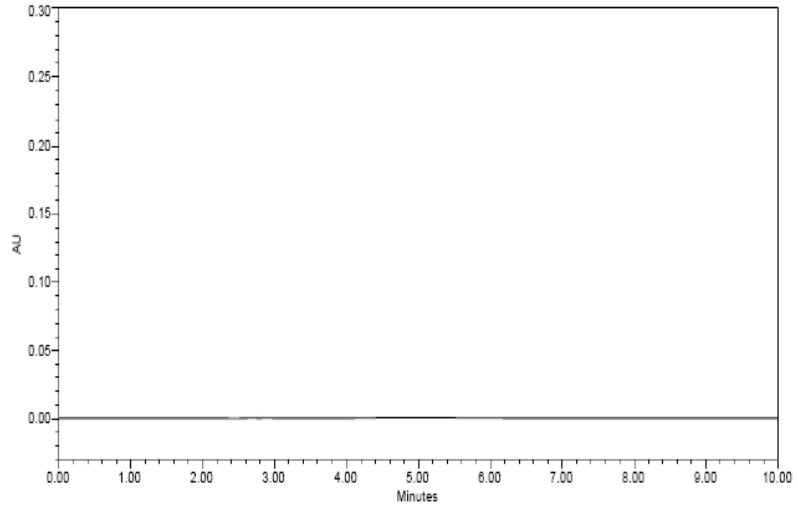
RESULTS AND DISCUSSION



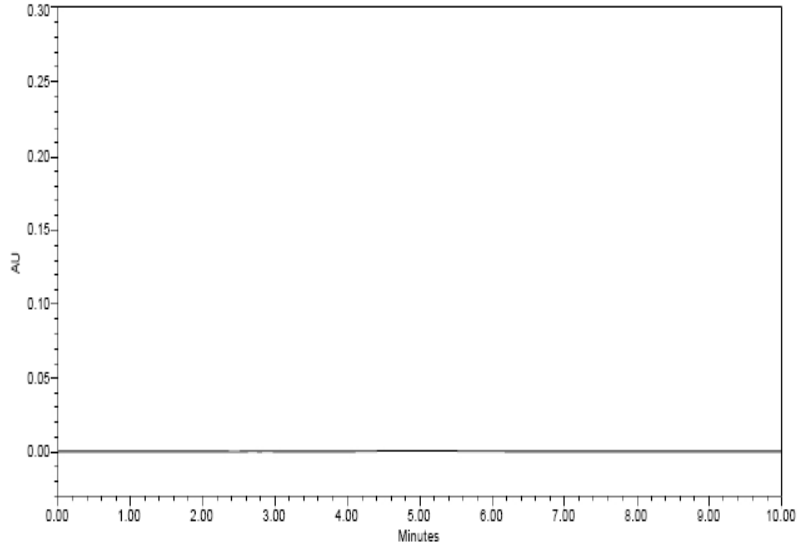
Validation

1) SPECIFICITY

Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. Typically these might include impurities, degradants, matrix, etc. Lack of specificity of an individual analytical procedure may be compensated by other supporting analytical procedure(s). This definition has the following implications: Identification: to ensure the identity of an analyte. Purity Tests: to ensure that all the analytical procedures performed allow an accurate statement of the content of impurities of an analyte, i.e. related substances test, heavy metals, residual solvents content, etc. Assay (content or potency): to provide an exact result which allows an accurate statement on the content or potency of the analyte in a sample.



First Stage



Last Stage

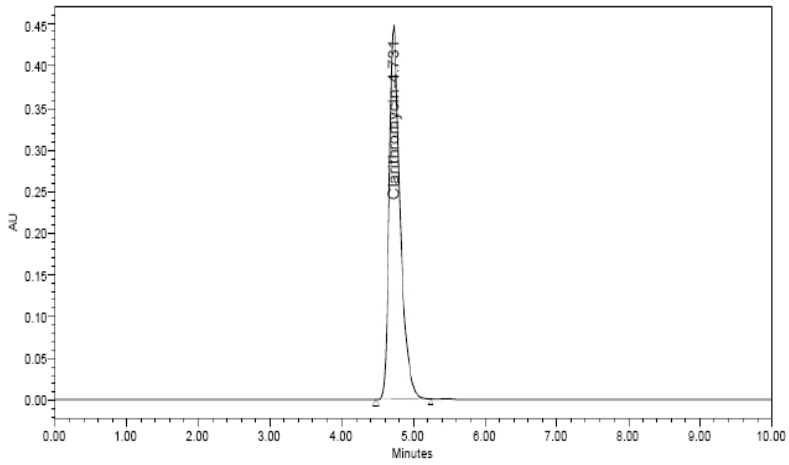
S.No.	Name	RT	Area
1	Blank	0	0
2	Clarithromycin-Standard	4.735	4691461
3	Placebo	0	0
4	Sample	4.731	4692245

Our study was discussed and the following charcters (PRECISION , LINEARITY, ACCURACY , RUGGEDNESS, ROBUSTNESS) untill it was reching the seventh stage

7) Assay

For chromatographic procedures, representative chromatograms should be used to demonstrate specificity and individual components should be appropriately labelled. Similar considerations should be given to other separation techniques. Critical separations in chromatography should be investigated at an appropriate level. For critical separations,

specificity can be demonstrated by the resolution of the two components which elute closest to each other. In cases where a non-specific assay is used, other supporting analytical procedures should be used to demonstrate overall specificity. For example, where a titration is adopted to assay the active substance for release, the combination of the assay and a suitable test for impurities can be used.



	Peak Name	RT	Area	Height	% Area	USP Tailing	USP Plate Count
1	Clarithromycin	4.731	4716412	446575	100.000	1.19	4895

CONCLUSIONS

A validated RP-HPLC method was developed for the separation and quantitative determination of related substances of clarithromycin in clarithromycin powder for oral suspension. All the degradation products were well separated from the drug substance, demonstrating the stability-indicating nature of the proposed method. The RP-HPLC method is simple, robust, accurate and selective. The method was completely validated as per ICH guide lines and results from validation confirm that the method can be used for its intended purpose. The present study was aimed at developing a simple , sensitive , precise and accurate HPLC method for the simultaneous analysis of Clarithromycin in Clarithromycin powder for oral suspension and there tablet dosage forms .Anon –Poly C_{18} analytical Chromatographic column was chosen as the stationary phase for the separation and simultaneous determination of Clarithromycin. Mixtures of commonly used solvents like water, methanol and acetonitrile with or without buffers in different combinations were tested as mobile phases. The choice of the optimum composition is based on the chromatographic response factor, a good peak shape with minimum tailing. A mixture of buffer and acetonitrile in the ratio of 50:50 v/v was proved to be the most suitable of all the combinations since the chromatographic peak obtained was well defined, better resolved and almost free from

tailing. The retention times of the Clarithromycin were found to be 2.21 and 3. 73 min respectively. The linearity was found satisfactory for both the drugs in the range 75 – 175 µg/mL . This reveals that the method is 1472 quite precise. The percent recoveries of the drug solutions were studied at six different concentration levels. The percent individual recovery and the %RSD at each level were within the acceptable limits. This indicates that the method is accurate. The absence of additional peaks in the chromatogram indicates non-interference of the commonly used excipients in the tablets and hence the method is specific. The deliberate changes in the method have not much affected the peak tailing, theoretical plates and the percent assay. This indicates that the present method is robust . The system suitability studies were carried out to check various parameters such as theoretical plates and tailing factor. The lowest values of LOD and LOQ as obtained by the proposed method indicate that the method is sensitive. The solution stability studies indicate that both the drugs were stable up to 24 hours. Therefore, the proposed method was simple, specific and sensitive and can be used for simultaneous analysis of Clarithromycin in bulk samples and its tablet dosage forms.

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